Neuroprotective Natural Products

Neuroprotective Natural Products

Clinical Aspects and Mode of Action

Edited by Goutam Brahmachari

WILEY-VCH

Editor

Dr. Goutam Brahmachari
Visva-Bharati (a Central University)
Department of Chemistry, Laboratory
of Natural Products and Organic Synthesis,
Santiniketan
West Bengal 731 235
India

Cover
Neurons: © fotolia_ktsdesign

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Bibliographic information published by the Deutsche Nationalbibliothek
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at http://dnb.d-nb.de.

Print ISBN: 978-3-527-34186-3
ePDF ISBN: 978-3-527-80379-8
ePub ISBN: 978-3-527-80380-4
Mobi ISBN: 978-3-527-80381-1
oBook ISBN: 978-3-527-80378-1

Cover Design Adam-Design, Weinheim, Germany
Typesetting SPi Global Private Limited, Chennai, India
Printing and Binding

Printed on acid-free paper

Contents

List of Contributors

Javed Ali
Jamia Hamdard
Department of Pharmaceutics
Hamdard Nagar
New Delhi 110062
India

Mario Amore
University of Genoa
Department of Neuroscience,
Rehabilitation, Ophthalmology,
Genetics, Maternal and Child Health
Section of Psychiatry
IRCCS San Martino
Largo Rosanna Benzi 10
16132 Genoa
Italy

Sanjula Baboota
Jamia Hamdard
Department of Pharmaceutics
Hamdard Nagar
New Delhi 110062
India

Manveen Bhardwaj
Panjab University
UGC Centre of Advanced Study
University Institute of Pharmaceutical
Sciences
Department of Pharmacology
Pharmacology Division
Chandigarh 160014
India

Anupom Borah
Assam University
Department of Life Science
and Bioinformatics
Cellular and Molecular Neurobiology
Laboratory
Silchar 788011
Assam
India

Goutam Brahmachari
Visva-Bharati (a Central University)
Department of Chemistry
Laboratory of Natural Products and
Organic Synthesis
Santiniketan
West Bengal 731 235
India

Alicia Brusco
Universidad de Buenos Aires
Consejo Nacional de lnvestigaciones
Científicas y Técnicas
Instituto de Biología Celular
y Neurociencia (IBCN)
Facultad de Medicina
Paraguay 2155
Ciudad Autónoma de Buenos Aires
Buenos Aires 1114
Argentina

Laura R. Caltana
Universidad de Buenos Aires
Consejo Nacional de lnvestigaciones Científicas y Técnicas
Instituto de Biología Celular y Neurociencia (IBCN)
Facultad de Medicina
Paraguay 2155
Ciudad Autónoma de Buenos Aires
Buenos Aires, 1114
Argentina

Swapnali Chetia
Assam University
Department of Life Science and Bioinformatics
Cellular and Molecular Neurobiology Laboratory
Silchar 788011
Assam
India

Amarendranath Choudhury
Assam University
Department of Life Science and Bioinformatics
Cellular and Molecular Neurobiology Laboratory
Silchar 788011
Assam
India

Kapil Dev
Academy of Scientific and Innovative Research
CSIR-Central Drug Research Institute
Medicinal and Process Chemistry Division
Sector 10
Jankipuram Extension
Sitapur Road
Lucknow 226031
India

Abhijit Dey
Presidency University
Department of Life Sciences
Ethnopharmacology and Natural Products Research Laboratory
86/1 College Street
Kolkata 700073
India

Ranjan Dutta
Department of Neurosciences
Lerner Research Institute
Cleveland Clinic
9500 Euclid Avenue, NC-30
Cleveland, OH 44195
USA

Carlos Fernández-Moriano
University Complutense of Madrid
School of Pharmacy
Department of Pharmacology
Plaza Ramón y Cajal s/n
28040 Madrid
Spain

Bharti Gaba
Jamia Hamdard
Department of Pharmaceutics
Hamdard Nagar
New Delhi 110062
India

Mehdi Ghasemi
University of Massachusetts Medical Center
Department of Neurology
55 Lake Avenue North
Worcester, MA 01655
USA

Maria Pilar Gómez-Serranillos
University Complutense of Madrid
School of Pharmacy
Department of Pharmacology
Plaza Ramón y Cajal s/n
28040 Madrid
Spain

Elena González-Burgos
University Complutense of Madrid
School of Pharmacy
Department of Pharmacology
Plaza Ramón y Cajal s/n
28040 Madrid
Spain

Shawn Hayley
Carleton University
Department of Neuroscience
1125 Colonel By Drive
Ottawa, K1S 5B6 ON
Canada

Hossein Hosseinzadeh
Mashhad University of Medical Sciences
Pharmaceutical Research Center
School of Pharmacy
Department of Pharmacodynamics and Toxicology
Vakilabad Blvd.
Mashhad 1365-91775
Iran

Harshpreet Kaur
Panjab University
UGC Centre of Advanced Study
University Institute of Pharmaceutical Sciences
Department of Pharmacology
Pharmacology Division
Chandigarh 160014
India

Hadi M. Khanli
Olive View UCLA Medical Center
Department of Neurology
14445 Olive View Drive
Sylmar, CA 91342
USA

Anil Kumar
Panjab University
UGC Centre of Advanced Study
University Institute of Pharmaceutical Sciences
Department of Pharmacology
Pharmacology Division
Chandigarh 160014
India

Shobhit Kumar
Jamia Hamdard
Department of Pharmaceutics
Hamdard Nagar
New Delhi 110062
India

Rakesh Maurya
Academy of Scientific and Innovative Research
CSIR-Central Drug Research Institute
Medicinal and Process Chemistry Division
Sector 10
Jankipuram Extension
Sitapur Road
Lucknow 226031
India

Muhammed K. Mazumder
Assam University
Department of Life Science and Bioinformatics
Cellular and Molecular Neurobiology Laboratory
Silchar 788011
Assam
India

Shadab Md
International Medical University (IMU)
School of Pharmacy
Department of Pharmaceutical Technology
Kuala Lumpur 57000
Malaysia

Shri K. Mishra
University of Southern California
Keck School of Medicine
1100 North State Street
Clinic Tower
Los Angeles, CA 90033
USA

Jasjeet. K. Narang
Khalsa College of Pharmacy
Department of Pharmaceutics
Amritsar
India

Marjan Nassiri-Asl
Qazvin University of Medical Sciences
Cellular and Molecular Research Centre
School of Medicine
Department of Pharmacology
Bahonar Blvd.
Qazvin 341197-598
Iran

Rajib Paul
Assam University
Department of Life Science and Bioinformatics
Cellular and Molecular Neurobiology Laboratory
Silchar 788011
Assam
India

Pritam Sadhukhan
Bose Institute
Division of Molecular Medicine
P-1/12, CIT Scheme VII M
Kolkata 700054
India

Sukanya Saha
Bose Institute
Division of Molecular Medicine
P-1/12, CIT Scheme VII M
Kolkata 700054
India

Gianluca Serafini
University of Genoa
Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health
Section of Psychiatry
IRCCS San Martino
Largo Rosanna Benzi 10
16132 Genoa
Italy

Parames C. Sil
Bose Institute
Division of Molecular Medicine
P-1/12, CIT Scheme VII M
Kolkata 700054
India

Bharathi A. Venkatachalapathy
Medical Ayurveda Rejuvenation Center
Newport Beach, CA 92660
USA

Christina Volsko
Department of Neurosciences
Lerner Research Institute
Cleveland Clinic
9500 Euclid Avenue, NC-30
Cleveland, OH 44195
USA

Dedication

Dr. Arnold L. Demain (Drew University, USA).

Preface

Neuroprotective Natural Products: Clinical Aspects and Mode of Action is an endeavor to offer an account on the recent cutting-edge research advances in the field of bioactive natural products with neuroprotective potential against various neurological diseases and disorders, particularly focusing on their clinical aspects and mode of action, and also to underline how natural product research continues to make significant contributions in the domain of discovery and development of new medicinal entities. This book consists of a total of 13 chapters contributed by eminent researchers from several countries in response to my personal invitation. I am most grateful to the contributors for their generous and timely response in spite of their busy and tight schedules with academics, research, and other responsibilities.

The term neuroprotection refers to strategies able to defend the nervous system against neuronal injury and/or death when exposed to trauma and surgery and that developed due to both acute and chronic neurodegenerative disorders. Among central nervous system (CNS) disorders, neurodegenerative disorders affect majority of population worldwide and are a major health problem in the twenty-first century. Neurodegenerative disorders such as Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) are currently incurable pathologies with huge social and economic impacts closely related to the increasing of life expectancy in modern times. In the course of time, a number of neurotransmitters and signaling molecules have been identified, which have been considered as therapeutic targets against these devastating disorders, and conventional and newer molecules have been tried against these targets. Still the progress is too limited. Neuroprotection is, thus, an important part of care for all types of neurological disorders. Treatment of neurological disorders should not be merely symptomatic, but an effort should be made to prevent the progression of the underlying disease and to develop therapies for regeneration.

The history of neuroprotection dates back to ancient Greek physicians who used hypothermia as treatment of head injury. Neuroprotection has been used in medical practice for more than the past half century. The earliest agents were barbiturates and nonpharmacological approaches such as hypothermia and hyperbaric oxygen. Neuroprotection has now been placed on a firm scientific basis due to an improved understanding of the molecular basis of neurological diseases. The concept of neuroprotection has found increased acceptance in

neurology during the past decade and is linked initially to the role of free radicals in the etiology of neurological disorders, particularly stroke and degenerative neurological symptoms. Considerable work has been performed to elucidate the pathomechanism of various neurological disorders; consequently, a number of neurotoxic phenomena have been identified.

Nature stands as an inexhaustible source of novel chemotypes and pharmacophores; natural products present in the plant and animal kingdoms offer a huge diversity of chemical structures, which are the result of biosynthetic processes that have been modulated over the millennia through genetic efforts. Natural products continue to provide useful drugs in their own right and also provide templates for the development of other useful compounds. A major advantage of natural products approach to drug delivery is that it is capable of providing complex molecules that are not accessible by other routes. Many of such bioactive molecules are found to play a vital role in maintaining the brain's chemical balance by influencing the function of receptors for the major inhibitory neurotransmitters. In traditional medicinal practice, several plants have been reported to treat cognitive disorders. Plant secondary metabolites include an array of bioactive constituents from both medicinal and food plants that are able to improve human health. The exposure to these phytochemicals, including phenylpropanoids, isoprenoids, and alkaloids, through proper dietary habits may promote health benefits, protecting against chronic degenerative disorders. Recently, it has been suggested that drug discovery should not always be limited to the discovery of a single molecule, and the current belief is that rationally designed polyherbal formulation could also be investigated as an alternative in multitargeted therapeutics and prophylaxis. Development of standardized, safe, and effective herbal formulation with proven scientific evidence can also provide an economical alternative in several disease areas.

It is regarded that herbal medicine may represent a valuable resource in prevention rather than in therapy of some CNS diseases, in association with a healthy lifestyle including beneficial dietary habits and moderate physical activities. Nutritional therapy is a healing system using functional foods and nutraceuticals as therapeutics. This complementary therapy is based on the assumption that food not only is a source of nutrients and energy but also can provide health benefits. In particular, the reported health-promoting effects of plant foods and beverages can be ascribed to the numerous bioactive chemicals present in plant tissues and, consequently, occurring in foods. Consumed as part of a normal diet, plant foods are thus a source of nutrients and energy. It may additionally provide health benefits beyond basic nutritional functions by virtue of their dietary therapeutics. Thus, neuroprevention appears to be an important target and strategy in overcoming neurodegenerative disorders! Prevention coupled with curing therapy for various neurodegenerative diseases is of demanding importance in modern medicinal chemistry.

This book, which comprises 13 chapters written by active researchers and leading experts working in the field of neuroprotective natural products, brings together an overview of current discoveries and trends in this remarkable field. Chapter 1 presents an overview of the book and summarizes the contents of other chapters so as to offer glimpses of the subject matter covered to the readers

before they go in for a detailed study. Chapters 2–13 are devoted to exploring the ongoing chemical, biological, and pharmacological advances in naturally occurring neuroprotective agents with a focus on their clinical aspects and mode of action. This timely volume encourages interdisciplinary work among chemists, biologists, pharmacologists, botanists, and agronomists with an interest in bioactive natural products. It is also an outstanding source of information with regard to the industrial application of natural products for medicinal purposes. The broad interdisciplinary approach dealt with in this book would surely make the work much more interesting for scientists deeply engaged in the research and/or use of neuroprotective natural products.

Representation of facts and their discussions in each chapter are exhaustive, authoritative, and deeply informative; hence, the book would serve as a key reference for recent developments in the frontier research on neuroprotective natural products at the interface of chemistry and biology and would also be of much utility to scientists working in this area. I would like to express my sincere thanks once again to all the contributors for their excellent reviews on the chemistry, biology, and pharmacology of these medicinally promising agents. It is their participation that makes my effort to organize such a book possible. Their masterly accounts will surely provide the readers with a strong awareness of current cutting-edge research approaches being followed in some of the promising fields of biologically active natural products.

Finally, I would like to express my deep sense of appreciation to all of the editorial and publishing staff–members associated with Wiley-VCH, Weinheim, Germany, for their keen interest in publishing the work and also for their all-round help so as to ensure that the highest standards of publication are maintained in bringing out this book.

Goutam Brahmachari
Visva-Bharati University, Chemistry Department, Santiniketan, India
November 2016

Editor Biography

Professor (Dr) Goutam Brahmachari currently holds the position of full professor of chemistry at the Department of Chemistry, Visva-Bharati University, Santiniketan, India. He was born at Barala in the district of Murshidabad (West Bengal, India) in 1969. He received B.Sc. (Honours) in Chemistry and M.Sc. with specialization in organic chemistry from Visva-Bharati University, India, in 1990 and 1992, respectively. Thereafter, he received his Ph.D. in organic chemistry in 1997 from the same university. In 1998, he joined his alma mater as an assistant professor. He became an associate professor in 2008 and was promoted to full professor in 2011. At present, he is responsible for teaching courses in organic chemistry, natural products chemistry, and physical methods in organic chemistry. Several students received their Ph.D. degree under the supervision of Prof. Brahmachari during this period, and couples of research fellows are presently working with him in the fields of both natural products and synthetic organic chemistry. Prof. Brahmachari's research is supported by several funding organizations including SERB-DST (New Delhi), CSIR (New Delhi), DBT (New Delhi), and UGC (New Delhi). He is a 2015 and 2016 *Who's Who in the World* listee and also a recipient of the 2015 Academic Brilliance Award (Excellence in Research). He is the series editor of the book series *Natural Product Drug Discovery*.

Prof. Brahmachari's research interests include (i) isolation, structural determination, and/or detailed NMR study of new natural products from medicinal plants; (ii) synthetic organic chemistry with special emphasis on green chemistry; (iii) semisynthetic studies with natural products; and (iv) evaluation of biological activities and pharmacological potential of natural and synthetic compounds. With more than eighteen years of teaching experience, he has also produced so far nearly 160 publications including original research papers, review articles, and invited book chapters in edited books in the field of natural products and organic synthesis from internationally reputed presses. Prof. Brahmachari has authored/edited a number of textbooks and reference books, including *Organic Name Reactions: A Unified Approach* (Narosa Publishing House, New Delhi; copublished by Alpha Science International, Oxford, 2006), *Chemistry of Natural Products: Recent Trends & Developments* (Research Signpost, 2006), *Organic Chemistry Through Solved Problems* (Narosa Publishing House, New Delhi; copublished by Alpha Science International, Oxford, 2007), *Natural Products: Chemistry, Biochemistry and Pharmacology* (Narosa Publishing House, New Delhi; copublished by Alpha Science International, Oxford, 2009),

Handbook of Pharmaceutical Natural Products—2-Volume Set (Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 2010), *Bioactive Natural Products: Opportunities & Challenges in Medicinal Chemistry* (World Scientific Publishing Co. Pte. Ltd, Singapore, 2011), *Chemistry and Pharmacology of Naturally Occurring Bioactive Compounds* (CRC Press, Taylor & Francis group, USA, 2013), *Natural Bioactive Molecules: Impacts & Prospects* (Narosa Publishing House, New Delhi; copublished by Alpha Science International, Oxford, 2014), *Green Synthetic Approaches for Biologically Relevant Heterocycles* (Elsevier Inc., USA, 2014), *Bioactive Natural Products—Chemistry & Biology* (Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 2015), *Room Temperature Organic Synthesis* (Elsevier Inc., USA, 2015), *Biotechnology of Microbial Enzymes: Production, Biocatalysis and Industrial Applications* (Academic Press, London, 2016), and *Discovery and Development of Antidiabetic Agents from Natural products* (*Natural Product Drug Discovery Series*; Elsevier Inc., USA, 2016), and few are forthcoming.

Prof. Brahmachari serves as a member of the Indian Association for the Cultivation of Science (IACS) and Indian Science Congress Association (ISCA), Kolkata, and as an editor-in-chief of *Signpost Open Access Journal of Organic and Biomolecular Chemistry*. He also serves as an editorial advisory board member for several international journals. He is regularly consulted as a referee by leading international journals including Elsevier, Royal Society of Chemistry, American Chemical Society, Wiley, Taylor & Francis, Springer, Bentham Science, Indian Chemical Society, Indian Journal of Chemistry (Sec. B), Korean Chemical Society, Pakistan Chemical Society, Brazilian Chemical Society, Bulgarian Academy of Sciences, and so on and also by various financial commissions.

Goutam Brahmachari enjoys songs of Rabindranath Tagore and finds interests in literature as well!

1

Neuroprotective Natural Products: Clinical Aspects and Modes of Action – An Overview

Goutam Brahmachari

Visva-Bharati (a Central University), Department of Chemistry, Laboratory of Natural Products and Organic Synthesis, Santiniketan, West Bengal 731 235, India

1.1 Introduction

The book titled *Neuroprotective Natural Products: Clinical Aspects and Modes of Action* is an endeavor to the present cutting-edge research in the neuroprotective natural products and helps the reader understand how natural product research continues to make significant contributions in the discovery and development of new medicinal entities. The reference is meant for phytochemists, synthetic chemists, combinatorial chemists, biologists, pharmacologists, clinicians, as well as other practitioners and advanced students in related fields. This book, comprising 12 technical chapters, highlights the clinical aspects and modes of action of potential neuroprotective natural products with an intention to unravel their pharmaceutical applicability in modern drug discovery processes in the field of neurodegenerative diseases.

This introductory chapter presents an overview of the book and summarizes the contents and subject matter of each chapter so as to offer certain glimpses of the coverage of discussion to the readers before they go for detailed study.

1.2 An Overview of the Book

This book contains a total of 12 technical chapters – Chapters 2–13; this section summarizes the contents and subject matter of each of these chapters.

1.2.1 Chapter 2

In Chapter 2, Volsko and Dutta have offered an overview on the general modes of action of neuroprotective agents in several neurodegenerative disorders as studied in various animal models. The results suggest that administration of such therapeutic candidates postpones disease progression and increases survival rate. Neuroprotective agents act through certain key pathways associated with

Neuroprotective Natural Products: Clinical Aspects and Mode of Action, First Edition.
Edited by Goutam Brahmachari.

development, maturation, and repair in abnormal pathological environments during neurodegenerative diseases, thereby resulting in the reduction of cellular distress and slowing disease development in the nervous system. Specific trophic factors, polypeptides, and heterodimers activate or block the receptors during pathogenesis to slow disease progression. Natural neuroprotective agents that are effective in humans and suppress symptoms and delay disease progression are regarded as promising lead candidates in the drug discovery process in treating neurodegenerative diseases. Modifying treatments based on neuropathology of each such disease is essential, and this chapter boosts the ongoing research in this remarkable field.

1.2.2 Chapter 3

Sil and his group have furnished a thorough discussion on the beneficial effects of different classes of naturally occurring antioxidant compounds against various neurological disorders in Chapter 3. Oxidative stress (elevation of intracellular reactive oxygen species level) is a major cause in the development and progression of neurological diseases such as neurodegenerative diseases, movement disorders, and so on. The brain in particular is prone to this oxidative stress phenomenon, and impairment in memory and cognition are hallmarks of progressive neurodegenerative diseases. Therefore, targeting these diseases with antioxidants may be expected to be a fruitful solution. Antioxidant molecules combat oxidative stress by neutralizing excessively produced free radicals and inhibiting them from initiating the signaling cascades and chain reactions that result in various diseases and premature aging. Several natural compounds with antioxidant property have been found to be greatly effective in treating these diseases as they effectively scavenged free radicals and inhibited their generation. This chapter covers the sources of such antioxidants and the general mechanism by which they play a protective role in different cognitive and movement-related neurological disorders. This illuminating review on natural antioxidants would obviously enrich the readers and would motivate them in undertaking in-depth further research.

1.2.3 Chapter 4

Chapter 4 is dedicated to natural neuroprotectives for the management of Parkinson's disease (PD) by Ali and his group. PD is regarded as the second most general neurodegenerative disorder that involves a decreased nigrostriatal availability of dopamine, resulting in motor impairment including bradykinesia, rigidity, and tremor. Currently, the exact cause of this devastating disease is unclear with no single factor accountable for neurodegeneration. It shows that several factors may contribute to its development, such as formation of reactive oxygen species (ROS), protein misfolding, and neuroinflammation. The deficiency of dopamine occurs due to loss of dopaminergic neurons and degradation of dopamine. It has been evidenced that oxidative stress is critically involved in the pathogenesis of PD, and thus antioxidants may find beneficial role in treating the disease. This chapter deals with the literature covering the use of various natural antioxidative neuroprotective agents including naringenin, curcumin,

vitamin E, vitamin C, resveratrol, coenzyme Q10, and melatonin, which may find application in PD. In addition, the authors have discussed on the mechanism of actions and *in vitro* and *in vivo* application of natural neuroprotectives in experimental animal models and in patients with PD. This chapter offers an up-to-date development in this field.

1.2.4 Chapter 5

In Chapter 5, Borah and his group have discussed the role and therapeutic efficacy of Ayurvedic preparations in treating Parkinson's disease (PD). A prospective clinical trial on the effectiveness of an Ayurvedic formulation, composed of *Mucuna pruriens*, *Withania somnifera*, *Hyoscyamus niger*, and *Sida cordifolia*, on PD patients demonstrated significant improvement of the symptoms. The authors have elaborated the potentials of such natural products used in Ayurvedic formulations as alternative/adjuvant to the dopamine replenishment therapy for PD and also highlighted their molecular mechanisms of action.

1.2.5 Chapter 6

Chapter 6 deals with the role of natural products as cytoprotective agents against lipid peroxidation and mitochondrial dysfunction in Alzheimer's and Parkinson's diseases by Gómez-Serranillos and her group. Among their pathological hallmarks, increased lipid peroxidation and mitochondrial dysfunction appear to be relevant from the early events of these age-related disorders. Neurodegenerative diseases in humans are strongly associated with oxidative stress generated by ROS, which can cause oxidative damage to cell structures, including alterations in membrane lipids, proteins, and DNA. In turn, it may trigger cellular organelle dysfunction that finally leads to cell death. Lipid peroxidation is a process that takes place along the cell membrane by effect of free radical oxidation of polyunsaturated fatty acids, and as a consequence of this chain reaction, it results in the formation of reactive products with toxic effects. Mitochondria are cytoplasmic organelles that regulate both metabolic and apoptotic signaling pathways, including energy generation; thus, they exhibit special susceptibility to oxidative stress, which eventually provokes the mitochondrial dysregulation. Herein, the authors have provided a detailed overview of the involvement of lipid peroxidation and mitochondrial dysfunction in Parkinson's and Alzheimer's diseases, with special consideration to natural products exerting beneficial effects on neurodegeneration models through an amelioration of these molecular disorders.

1.2.6 Chapter 7

Dev and Maurya have presented an exhaustive review on potential marine-derived anti-Alzheimer's agents in Chapter 7. Marine secondary metabolites develop under very adverse conditions and, thus, may contain very unusual structural skeletons; such chemical entities with new and varying scaffolds and interesting biological activity have created a new hope of drug discovery and development for various disease areas including neurodegenerative disorders. The main hurdle in drug discovery for Alzheimer's disease is associated with the

permeability of blood–brain barrier (BBB) to exhibit drug's effective activity. A number of marine natural products and their synthetic analogs showed efficacy with good bioavailability against Alzheimer's disease. This chapter includes 163 compounds and some extracts from different marine sources such as algae, sponges, coelenterates, bryozoans, molluscs, tunicates, and echinoderms together with their pharmacological activity in the treatment of Alzheimer's disease. This informative review would act as a stimulus in this direction.

1.2.7 Chapter 8

Huntington's disease (HD) is a neurological disorder characterized by abnormal body movements (chorea) associated with cognitive and motor dysfunctions, neuropsychiatric disturbances, and striatal damage. Therapeutic advancement in screening of natural products against HD suffers from constraints such as limited animal models and giving maximum emphasis on cellular models during experimentations. However, recent progress in animal HD transgenic models expressing mutant proteins may reveal the therapeutic efficacy of natural products against HD, a disease with less elucidated pathogenesis and inadequate treatment strategies. In Chapter 8, Dey has offered an illuminating and comprehensive account on the anti-HD efficacy of a number of plant extracts, fractions, and isolated compounds investigated in various neurotoxic animal models and transgenics highlighting their ability to influence signaling pathways, leading to neuromodulation and probable neuroprotection.

1.2.8 Chapter 9

Chapter 9 by Kumar and his group deals with the possible role of neuroprotectants and natural products in epilepsy, a common neurological problem with complex pathology and uncured treatment. The roles of oxidative stress, mitochondrial dysfunction, and neuroinflammation have been well suggested to explain its pathophysiology and related complications, particularly cognitive dysfunction. Several antiepileptic drugs have been in use for the treatment and management of epilepsy, but majority of them are often associated with the problems due to either side effects, drug interactions, or treatment resistance. Different neuroprotectants of diverse nature are being tried with limited success. In search of new and more efficacious drugs, researchers have been engaged to explore therapeutic potentials of plant-based bioactive molecules, particularly belonging to the alkaloid, flavonoid, terpenoid, saponin, and coumarin skeletons, which have been found responsible for their anticonvulsants properties. In this chapter, the authors have made a significant attempt to highlight the potential role of various natural neuroprotectants, their modes of action, and clinical aspects/status for the treatment of epilepsy and related problems.

1.2.9 Chapter 10

Hosseinzadeh and Nassiri-Asl have presented an account on the neuroprotective effects of flavonoids in epilepsy in Chapter 10. Flavonoids are present in foods such as fruits and vegetables, and these natural polyphenolics are reported to

possess beneficial effects against many neurological disorders including epilepsy, a serious but common problem in our society. It seems that many of these compounds are ligands for γ-aminobutyric acid type A (GABA-A) receptors in the central nervous system. Furthermore, flavonoids have well-established antioxidants and free radical scavenging activities. The authors have discussed such effects in their presentation.

1.2.10 Chapter 11

Chapter 11 is devoted to the role of noncompetitive antagonists of the *N*-methyl-D-aspartate (NMDA) receptors in treatment-resistant depression by Serafini and coauthors. The authors have discussed the pros and cons of using NMDA antagonists in treating the disease manifestation. As mentioned, ketamine (an NMDA antagonist) exhibits good response as a useful clinical agent in cases of severe intractable depression and suicidal risk; the drug works rapidly in many such patients but is not devoid of adverse effects. Hence, it is of critical importance to develop alternate NMDA or other novel antidepressants as well as possible combinatorial drug approaches to treat depression. For instance, novel agents that target AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors are currently being explored; it has been found that compounds able to exert neurotrophic effects and anti-inflammatory drugs might be useful as add-on (or adjuvants) with traditional antidepressants. The authors are in opinion that more personalized approaches will be the way of the future.

1.2.11 Chapter 12

Mishra and coauthors have presented an overview of the safety and efficacy of Ashwagandha (*Withania somnifera*), an important medicinal plant used in Ayurvedic preparations to treat various diseases including neurological disorders, in Chapter 12. Medicinal herbs in Ayurveda have been widely used for thousands of years to promote health and treat diseases. However, limited evidence is available to testify the safety and efficacy of Ayurvedic herbs. An integrated approach for safety assessment focused on the hazard identification is imperative. Under this purview, this chapter highlighting the safety and efficacy of Ashwagandha plant is of interest.

1.2.12 Chapter 13

Chapter 13 deals with the neuroprotective properties of cannabinoids by Laura and Alicia. The cannabinoid system is well characterized, and abundant research supports their role in ameliorating neuropathologies such as ischemia, Alzheimer's diseases (AD), Parkinson's disease (PD), multiple sclerosis, retinal diseases, and psychiatric disorders. Hence, the manipulation of the endocannabinoid system, using phytocannabinoids or synthetic cannabinoids, could lighten the processing in the treatment and evolution of cerebral diseases. Certain clinical trials have also demonstrated promising results; however, cannabinoid adverse effects still remain to be elucidated. The authors have discussed all such considerations in this chapter.

1.3 Concluding Remarks

This introductory chapter summarizes each technical chapter of the book for which representation of facts and their discussions are exhaustive, authoritative, and deeply informative. The readers would find interest in each of the chapters, which practically cover a wide area of neuroprotective natural product research, particularly on their clinical aspects and modes of action. The reference encourages interdisciplinary works among chemists, pharmacologists, clinicians, biologists, botanists, and agronomists with an interest in these bioactive natural products. Hence, this book would surely serve as a key reference for recent developments in the frontier research on neuroprotective natural products and also would find much utility to the scientists working in this area.

2

Neuroprotective Agents: An Overview on the General Modes of Action

Christina Volsko and Ranjan Dutta

Department of Neurosciences, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Avenue, NC-30, Cleveland, OH 44195, USA

2.1 Introduction

Neuroprotection is defined as the protection of neurons from principle mechanisms that results due to cell loss within the central nervous system (CNS) or peripheral nervous system (PNS). Common mechanisms that have been linked to neurodegeneration within the CNS include neurotoxicity, inflammation, oxidative stress, accumulation of iron, excitotoxicity, and dysregulation of gene expression. While a considerable amount of research has been conducted into the mechanisms underlying neurodegeneration, the majority of diseases, there are no treatment options that can either stop or reverse the degenerative process. Researchers are turning to neuroprotective agents such as hepatocyte growth factor (HGF) and/or trophic factors that increase cell and neuron survival [1, 2] as a possible therapeutic treatment to relieve symptoms and delay progression of these diseases. In this chapter, we discuss some of the available neuroprotective agents, their mechanisms of action, and clinically derived neuroprotective treatment for some common neurodegenerative diseases.

2.2 Neuroprotective Agents

2.2.1 Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP)

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuroprotective peptide that is abundant and present in the nervous system during development to adulthood [3]. Within the CNS, PACAP is located in the hippocampus, amygdala, substantia nigra, and cerebellar granule. PACAP can also be found in PNS structures including sensory neurons in the dorsal root ganglia, sympathetic and parasympathetic nervous system, and some somatomotor neurons [4]. PACAP is derived from 38 amino acid hypothalamic neuropeptides and a derivative of vasoactive intestinal peptide/secretin/glucagon peptide superfamily. There are two forms, PACAP27 (PAC_1) and PACAP38 (PAC_2), that are dependent on binding to

Neuroprotective Natural Products: Clinical Aspects and Mode of Action, First Edition.
Edited by Goutam Brahmachari.

three different G-protein-coupled receptors [2, 5]. $VPAC_1$ and $VCAP_2$ are vasoactive intestinal polypeptide receptors, yet they have around 70% homology with PACAP. Once activated, these receptors do not yield high intensity for stimulation unlike PAC_1 [2]. When activated, PACAP receptors play a significant role in synaptic plasticity, memory, hippocampal neurogenesis, and neuroprotection. Similar to BDNF, PACAP acts as a neurotransmitter, neuromodulator, and neurotrophic factor [3]. The functional mechanism of PACAP binding to G-protein-coupled receptors activates cAMP-dependent protein kinase A (PKA) pathway. This pathway modulates ion channel properties, neuronal excitability, and synaptic strength.

PAC_1 has been found to be an endogenous ligand that is a treatment target for neurodegenerative and neuropathic diseases [5]. PACAP promotes cortical neurogenesis, protects from necrosis, and inhibits neuronal death by the induction of excitotoxin *N*-methyl-D-aspartate (NMDA) [2]. NMDA receptors are glutamate receptors that have the ability to prevent degeneration from occurring when glutamate and kainate are receptor bound. NMDAR has been found to prevent the commonly occurring degradations such as excitotoxic and ischemic injuries, degeneration from UV-A, optic nerve transection, and streptozotocin-induced diabetic retinopathy [5]. Studies suggest that the NMDA receptors and PAC_1 receptors can control the nociception signal being sent through ERK phosphorylation and JNK pathway [6], leading to increased neuroprotection. Recent studies are investigating the effects of administrating PACAP to wild-type mice after middle cerebral artery occlusion (MCAO). These mice became PACAP deficient but revealed a lower lesion volume and improvement of neurological deficiencies after PACAP treatments in striatal lesions [2]. In addition to the examples mentioned earlier, the role of PACAP is also under investigation in improving neurological deficits in models of stroke, traumatic brain injury, and Parkinson's disease (PD) [7].

2.2.2 Hepatocyte Growth Factor (HGF)

HGF is a heterodimer that induces favorable protective responses within the brain, especially following a stroke, and could have an impact in the pathogenesis of PD [1, 8]. The heterodimer consists of 728 amino acids forming 69 kDa α-chains and 34 β-sheets [8, 9]. HGF is secreted from stromal cells and activates a signal transduction cascade through tyrosine phosphorylating proto-oncogenic c-Met receptors. This pathway generates differentiation, proliferation, and regeneration of multiple different cell types [1, 8]. Activation of the c-Met tyrosine causes survival and proliferation not only in the brain but also in the kidneys and lungs while playing a critical role in embryonic development [1, 2]. Within the brain, HGF is believed to be a key factor for motor and sensory neuron survival [1]. Neuroprotective characteristics include preventing nuclear translocation of apoptosis-inducing factors [2]. Ongoing clinical studies involve the administration of HGF into the brain. The treatment has been determined to delay neuronal death within the hippocampus following ischemia. Accumulation of HGF blocks additional pathways preventing oxidative DNA damage and stimulates polymerase/p53/apoptosis-inducing factors. This results in protection within the CA1 region of the hippocampus and transient forebrain of ischemic rats [2]. HGF administration has shown to improve motor coordination in 3 days'

post-stroke-induced rat models. HGF induces neuroprotective responses up to 28 days after induced-stroke model in both rat and mouse; it has the ability to initiate regeneration within PD cell models by regulating intracellular Ca^{2+} levels through gene expression of CaBP-D28k. This treatment also improves axotomized retinal ganglion cells, but postischemic proliferation of NPCs has not been researched [1, 8]. Further investigations are underway to decipher the downstream pathways that regulate HGF-mediated protection.

2.2.3 Trophic Factors

Within the CNS, trophic factors are characterized as neurotrophin clusters entailing BDNF, neurotrophin-3, neurotrophin-4, and nerve growth factor (NGF). These proteins stimulate axonal growth, synaptic plasticity, and neurotransmitter synthesis and release [2, 10]. In addition, two other neurotrophic factors that have been widely studied include ciliary neurotrophic factor (CNTF) and glial cell line-derived neurotrophic factor (GDNF) [2].

Each trophic factor molecule is activated by many different molecular pathways, ultimately leading to neuroprotection. BDNF and NGF are stimulated through the phosphatidylinositol-3-kinase Akt pathway by Trk receptors [2]. Signaling through these receptors has been shown to protect hippocampal progenitor cells from staurosporine-induced apoptosis [2]. BDNF and NGF, when used to treat cultured hippocampal neurons, protects neurons from glutamate-induced neurotoxicity [2] through enhanced antioxidant enzyme activation and blockage of intracellular calcium. Similar to BDNF and NGF, CNTF also shows neuroprotection potential by protecting retinal ganglion cells when activated through Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway [2]. Lastly, mesenchymal stem cells have been shown to release NT3, NT4, and BDNF, which bind to specific Trk receptors on sensory neurons located on dorsal root ganglia, leading to increased cell survival following spinal cord injury [11]. In an attempt to use these trophic factors in a clinical settings, experimentations have been conducted with the administration of CNTF and BDNF with some success in promoting neuroprotective functions in Huntington's disease [2]. Treatments involving CNTF conclude survival of striatal output neurons, whereas BDNF and neurotrophin-3 administration results in survival of immature neurons within the cerebellum in neurotrophin-deficient models. Researchers recently discovered that Alzheimer's disease (AD) mouse models treated with a TrkB agonist, 7,8-dihydroxyflavone, showed improvement in hippocampus-dependent learning and memory [2]. Transplantation of mesenchymal stem cells that express trophic factors has shown initial promise. When these stem cells express GDNF, it has been shown to improve motor performance as well as protects dopaminergic neurons in the striatum [2] and lower expression of apoptotic markers in Friedreich's ataxia (FRDA) mouse models [11].

2.2.4 Apolipoprotein E (apoE)–Containing Lipoproteins

This 35 kDa protein is primarily found in the liver but is also highly expressed in the brain [2, 12] by the glial cells [13]. Apolipoprotein E (apoE)-containing lipoproteins have endocytosis receptors known to interact with members of a low-density

lipoprotein receptor-related protein 1 (LRP1) [2]. It is an important component in the cerebrospinal fluid (CSF) and acts as a ligand for LDL receptor. This receptor is expressed on the outer membrane of glia and neurons and stimulates the transportation of cholesterol and phospholipids [12]. LRP1 mediates the accumulation of apoE-containing lipoprotein. The expression of apoE controls the regulation of lipid metabolism and transport. Once the LRP1 receptor is activated, it initiates intracellular signaling pathway involving phospholipase Cγ1, protein kinase Cδ, and glycogen synthase kinase 3β. If LRP1 is bound to an NMDA receptor, activation from apoE blocks intracellular calcium from entering and allows calcium to accumulate within the intracellular membrane [2]. The buildup of intracellular calcium inhibits calcineurin and the dephosphorylation of BAD and caspase-3, which blocks apoptosis from occurring. ApoE must therefore be bound to lipoproteins in order to execute its antiapoptotic properties. This molecule also has the ability to prevent retinal ganglion cells from degeneration in the retina of glutamate/aspartate transporter-deficient mice [2, 14].

Neuroprotective research has focused on the role of LRP1 ligands due to its involvement in neuritic outgrowth, neuronal development, and survival. Structurally, LRP1, embedded into the membrane, are attached to the NMDA receptor. ApoE lipoprotein binds to LRP1 to activate phospholipase Cγ1, resulting in the production of diacylglycerol and triphosphate from PIP_2 [2]. Protein kinase Cγ activation signals proapoptotic kinase and glycogen synthase kinase to be phosphorylated, causing them to be deactivated. Based on these findings, glycogen synthase kinase inhibitors are currently undergoing clinical research as a potential therapeutic treatment for neurodegeneration [14].

2.2.5 Prothymosin α (PTMA)

Prothymosin α (PTMA) is a nuclear protein with acidic and hydrophilic characteristics highly expressed in mammalian cells [2, 15]. PTMA was formally extracted from a thymosin α1 precursor; however, it could also be isolated from cultured cortical neurons. While the function of PTMA is not well understood, it is considered to contain hormone-like factors [2], which may be important in its involvement in several key biological processes such as cell proliferation, cell survival, regulating antioxidative stress genes, stimulating immune response, stimulation or inhibition of specific pathways, and activation of receptor or transcription factors [16].

PTMA induces cell proliferation through inhibition of estrogen receptor stimulation. This action is linked to the proliferation of cancer cells if PTMA is highly expressed in the intracellular membrane [16]. Additionally, through interaction of PTMA with Keap1, anti-necrosis factors are also stimulated [15]. Mechanistically, PTMA binding to Keap1 inhibits formation of the Keap1-Nrf2 (nuclear factor erythroid 2-related factor 2) complex, leading to Nrf2-mediated increase of antioxidative stress genes, resulting in protection from apoptosis-mediated cell death [16]. Lastly, PTMA has also been shown to alter inflammation due to increased signaling through Toll-like receptors on monocytes [15]. This mechanism can protect neurons from necrosis during ischemic stress once PTMA is mediated by S100A13, a cargo protein, in order to block caspase-3 activation [16].

PTMA is considered to be a promising candidate for the treatment of stroke and Huntington's disease. In stroke-induced models and cultured cortical neurons, PTMA expressed the downregulation of necrosis at the ischemic core damage [2, 16]. In treated culture cells expressing mutant huntingtin protein (mHtt), PTMA suppressed mHtt-caused cytotoxicity by binding to the mutated protein with its central acidic domain [17]. Research is still evaluating the tendencies of this nuclear protein for future therapeutic treatments.

2.2.6 Erythropoietin (EPO)

Erythropoietin (EPO) (a 34 kDa glycoprotein composed of 165 amino acids) is known as humoral regulator of erythropoiesis during maturation and proliferation of erythroid progenitor cells and synthesized from the fetal liver, kidney, and brain after birth [2, 18]. EPO is a multifunctional molecule for enhancing neuroprotective mechanisms. Through different molecular pathways, EPO stimulates cytoprotective, antiapoptotic, antioxidant, anti-inflammatory behaviors, improves tissue oxygenation, and stimulates neurogenesis and angiogenesis [18]. During the activation of neurogenesis, histological functions are improved after hypoxic ischemia episodes [19]. Two EPO molecules form a dimer in order to activate JAK-2 pathway through autophosphorylation, which ultimately leads to suppression of apoptosis. This also triggers a cascade of signaling to branch out, creating a domino effect and stimulating multiple intracellular transductions factors [2]. In addition, using paracrine and autocrine mechanism within the brain, EPO produces antiapoptotic signals to protect neurons against ischemic damages and inflammation [2, 15, 20].

EPO administration is becoming highly used in treating hypothermia. Research has also shown that EPO administration improves long-term motor and cognitive responses along with cerebellar growth. Administration of EPO also protects cultured hippocampal and cortical neurons from glutamate-induced neurotoxicity and nitric oxide-induced neuronal death [2]. Administration of EPO in conjunction with other neuroprotective agents could therefore help neurogenesis and lower long-term neurological deficits [19].

2.2.7 Neuregulin-1 (NRG1)

Neuregulin-1 (NRG1) is a complex growth factor that transcribes more than 20 different transmembrane proteins with a single membrane-spanning domain. This molecule belongs to a multipotential neuroprotective and anti-inflammatory growth factor family and generates large numbers of isoforms in tissue- and cell-type-specific patterns. There are different types of NRG1 (type I, II, or III) based on the molecules of amino acid sequence. Type I NRG1 are secreted immunoglobulin-like antigens with carbohydrate-spacer regions along the extracellular regions. Type II NRG1 are similar isoforms as type I but lack a carbohydrate-spacer region. Lastly, type III NRG1 are membrane-bound isoforms with cysteine-rich domains. Each NGR1 type consists of similar epidermal growth factor (EGF) domains. These domains entail formations of alpha units and beta sheets. The beta domain contains a higher potential of activity compared with alpha domains, which defines their functional contribution [21].

NRG1 binds to a simple extracellular amino terminus with two cysteine-rich domains of the ErbB receptor protein tyrosine kinases. NGR1 bound to the selected receptor leads to suppression of specific genes through selected canonical pathways including immune cell trafficking, hepatic fibrosis/hepatic stellate cell activation, acute phase responses, and IL-6 signaling [21, 22]. Functional implication of this inhibition leads to beneficial outcomes toward neurons, astrocytes, oligodendrocyte precursor cells, endothelial cells, and microglia. The structure of NGR1 receptors include transmembrane and intracellular tyrosine kinase domain with a carboxyl-terminal tail. When attaching to ligands, signaling cascades activate for neuronal migration, dendritic spine maturation, cellular differentiation and proliferation, and inhibitory synapses onto excitatory pyramidal neurons [21]. Studies have provided information of NGR1 stimulation signals for blocking microglia activation and increasing permeability of endothelial cells and blood–brain barrier after traumatic brain injury [22, 23]. NGR1, however, does not contain neuroprotective characteristics to completely inactivate microglia in amygdala, medial dorsal thalamus, reunion of thalamus or piriform cortex within neurotoxic diisopropylfluorophosphate rat model [22]. Ongoing studies are looking into how NRG1 can be used as a neuroprotective therapy.

2.3 Neurodegenerative Diseases

Neurodegeneration is the progressive and irreversible loss of structure or function in neurons within the CNS [24] culminating in some form of neurological disorder. In a recent epidemiological study, statistics revealed that 1 in every 400 individuals develops some form of neurological disorder [25]. Individuals diagnosed with these diseases experience a range of motor or sensory deficits to cognitive impairment [24]. Such plethora of neurological deficits are derived from multifactorial mechanisms that are caused by neuronal death. Intense research activity is directed toward exposing new possible causes for neurodegenerative disease development. Among these, major focus has been on factors that control gene regulation and protein expression, which cause major disturbances in cellular homeostasis.

Tight regulation of gene expression is needed to control the amount of transcribed RNA and translated proteins. Duplication of a gene in the genetic sequence causes disruption in genetic splicing, which determines gene expression. Proximal spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disease in infants. It is derived by the duplication of chromosome 5p13 within the survival motor neuron (SMN) gene locus. This duplication causes a disruption in exon 7 which splices SMN1 and SMN2; the second copy of SMN gene remains in the genetic coding. The number of copies of SMN2 defines disease severity due to the production of unstable proteins that lack functional aspects needed for survival [26]. Looking at a different angle, genes can also form a mutation within its sequence. This phenomenon has been seen in a number of diseases such as FRDA and amyotrophic lateral sclerosis (ALS). For example, in ALS, the repeated G_4C_2 sequence in the chromosome 9 open reading frame 72 (C9orf72) gene can be translated into toxic dipeptide repeat proteins. This mechanism is detrimental to the nucleocytoplasmic transport. C9orf72 proteins

are able to self-bind to nuclear pore complexes in order to relocate from the nuclear membrane to the plasma membrane in neurons [27].

Apart from gene sequences, misfolded or unfolded proteins present a high risk for energetic dysfunction, molecular damages, metabolic changes, and dysregulation of ion homeostasis [28]. These changes within the body can translate into an unbalanced environment, oxidative stress, mitochondrial dysfunction, neuroinflammation, bioenergetics, disruption of cellular/axonal transport, and so on, thereby leading to neuronal death [28]. An example of this is Htt protein where mutation leads to expansion of polyglutamine in the N-terminal region of Htt and alters the protein–protein interaction, leading to toxicity within the neurons of the striatum and cortex [17].

Researchers commonly use animal models as well as postmortem human studies to explore mechanisms underlying neurodegeneration in various diseases. Transgenic mouse models have been developed to mimic disease pathogenesis. For example, human amyloid precursor protein(hAPP) transgenic mouse models have been generated to cause high levels of amyloid-β peptides in the brain [29]. These Alzheimer's mouse models generate excitability or inhibitory mechanism within the hippocampus to contribute to Aβ-induced neurological deficits similar to AD patients. Apart from genetic modifications, different chemicals are also used to replicate the human disease characteristics in animal models. For example, to study multiple sclerosis (MS), an autoimmune demyelinating disease, transgenic animal models are not available due to the difficulty in replicating the immune and demyelinating disease components. Experimental autoimmune encephalomyelitis (EAE)-induced mouse models are also used to mimic autoimmune responses similar to MS pathological environment within the spinal cord. To reproduce the demyelinating aspect of the disease, researchers treat mice with cuprizone (a copper chelator) to kill oligodendrocytes. As oligodendrocytes are the cells that make myelin, these mice develop demyelination.

While animal models are useful to help scientists discover unknown mechanisms that cause pathogenesis to occur, genomic structure differs between animals and humans, which makes the translations between the species difficult to read. Recent analysis shows the comparison between mouse and human myelin fraction in MS mouse models. After converting mouse proteins for human identification, only ~40% of the proteins matched. Limitation divided the two models due to the differences in amino acid sequences that result in different physiochemical characteristics. Overall observation underestimates protein genetic overlap between human and mouse proteomes [29]. Studies related to human samples therefore are essential to unravel the pathogenesis associated with neurodegenerative diseases and development of neuroprotective therapies.

2.4 Neuroprotection in Common Neurodegenerative Diseases

ALS, also known as Lou Gehrig's disease, is a fatal neurodegenerative disease classified as a progressive loss of upper and lower somatomotor neurons, leading to physical manifestations such as muscle weakness, muscle atrophy, stiffening,

and/or twitching of muscles [30]. The life span of diagnosed individuals is between 3 and 5 years. A small portion of the patients (10%) have been shown to survive for 10 or more years since diagnosis. The pathological pathway that causes neuronal death within this disease is still unknown. Several pathways such as autoimmunity, mitochondrial dysfunction, disruption of neurofilament network, oxidative stress, excitotoxicity, protein aggregation, and neural inflammation have been linked to disease pathogenesis [31, 32].

Two common types of ALS that differentiate patients are sporadic and familial. Approximately 90% of all ALS cases are characterized as sporadic ALS (sALS), whereas the other 10% are familial ALS (fALS) [4]. sALS is considered spontaneous since it is not fully connected to genetic inheritance and associated with environmental factors or DNA transcription with epigenetic modifications. fALS, on the other hand, is characterized by its genetic inheritance with single genomic DNA mutations [33]. About 2% of all ALS cases are generated from a mutation within the gene encoding superoxide dismutase 1, SOD1 [4]. SOD1 mutations are linked to oxidation changes and posttranslational modifications [34]. C9orf72 is another genetic site that could also impact disease onset when mutated. fALS occurs when noncoding GGGGCC hexanucleotide repeats from 2 to 23 within the C9orf72 gene on chromosome 9p21 [27].

Even though there is no true treatment to cure ALS, most neuroprotective treatment options are directed to extend life expectancy [30]. One of the well-known FDA-approved treatments for ALS patients is riluzole [35]. This particular pharmaceutical drug is characterized as a glutamate blocker and activates small-conductance calcium-activated potassium channels [32, 35]. Riluzole has shown to improve motor function and expand life expectancy by a few months [32]. A new clinically derived drug known as *deferiprone* (DFP) is a metal chelator and blood–brain barrier iron siderophore [36]. Iron accumulation has been seen within the CNS of fALS and sALS, indicating the causation of mitochondrial dysfunction. In animal and multiple cell line models, DFP has shown its ability to decrease mitochondrial redox potential and ROS production, prevent iron accumulation, improve motor ability, and increase patients' survival rate [36] and is currently undergoing FDA-approved clinical trials.

PD is a neurological movement disorder classified as progressive neurodegeneration of dopaminergic pathway within the CNS, considered as the 14th leading cause of death among the elderly in the United States. It is characterized by loss of dopaminergic neurons in the substantia nigra region and the loss of protein α-synuclein in Lewy bodies [36]. Symptoms begin to appear approximately when 60–80% of dopamine-producing cells are damaged. All PD patients go through physical and motor experiences including tremor, muscle rigidity, bradykinesia, and postural instability [1]. Recent studies have shown that accumulation of iron within the substantia nigra leads to expression of mitochondrial complex I inhibitors and inhibition of complex I, leading to stress-induced dopaminergic cell death [36]. To limit onset symptoms, patients have been treated with levodopa directly after medical diagnoses. Levodopa (also known as *L-DOPA*) is metabolized by the body to produce dopamine. Giving dopamine directly is ineffective because the brain's natural defense blocks it from being used by the body.

Levodopa prevents early symptoms, yet does not stop disease progression because of its short plasma half-life [1, 37].

Additional experimental treatments are now being tested through clinical trials to explore their neuroprotective effects by targeting dopamine receptors. A dopaminergic agonist, rotigotine, has been tested to help reduce nocturnal disability and suppress tremor [37]. Rotigotine was first treated in chronic PD mouse models and shown to delay dyskinesia [38]. Administered as a single or a dual treatment with levodopa, rotigotine offers a promising future in limiting some PD symptoms by activating dopamine receptor 2 and dopamine receptor 3 [39]. A second neuroprotective treatment, known as *dexpramipexole*, is being used for symptomatic treatments in PD, and new clinical studies are being performed with ALS patients as well [40]. Dexpramipexole is an *S*-enantiomer of mitochondrial protector pramipexole (PPX) that reduces proapoptotic pathways and produces dopamine receptor activity [40]. Majority of PD treatments are gradually transitioning from preclinical trials to human clinical trials.

FRDA is classified as a progressive loss of motor function and coordination from low levels of frataxin (FXN) protein expression. This disease is developed when a mutation occurs in the FXN gene, leading to a guanine–adenine–adenine repeat expansion in intron 1 of this 210-amino acid mitochondrial protein. This mutation disrupts mRNA transcription, dysregulation of iron metabolism, lack of detoxification, and increase in iron bioavailability [36, 41]. FRDA is also known as a *mitochondrial disease* due to FXN being a critical protein that regulates mitochondrial iron–sulfur clusters that catalyze for oxidative phosphorylation and the Krebs cycle [42]. Patients eventually experience loss in voluntary muscle coordination, speech impairment, loss of reflexes, and muscle weakness [36]. Many therapeutic studies are in development to increase FXN expression levels. Among these interestingly, EPO has been shown to increase FXN expression within the peripheral nervous tissue. This increase however seems to be non-transcriptional and does not sustain over long periods within the CNS [41].

Similarly to ALS, no clinical treatment has been shown to eliminate the progression of FRDA. Evidence of inflammation has been detected in FRDA patients from autopsy studies and alteration of immune pathways through microarray. Idebenone is an anti-inflammatory treatment used to postpone the progression of FRDA, prevents neurotoxicity from occurring, and is shown to improve patients' strength and simple motor movements [43]. A FDA-approved clinical trial is recruiting patients with FRDA to examine how methylprednisolone, a steroid administered as an anti-inflammatory, diminishes patients' symptoms [44]. Researchers seek to see if there will be improvement in ataxia symptoms and if this treatment prevents complications responding from extensive inflammation. Finally, resveratrol is an antioxidant with neuroprotective tendencies and increased expression of FXN in very high doses. In a two-phase clinical human trial, experimental patients taking a relatively high dose of resveratrol (5 g daily) for a 12-week span showed improvement in their neurological function, significantly in their speech and hearing [45]. Clinical trials are still uncovering the neuroprotective effects resveratrol produces.

Stroke is the third leading cause of death and adult disability in the United States. It is known as a *cerebrovascular accident* caused by a sudden blockage in the cerebral artery [46]. There are two types of stroke: hemorrhagic and ischemic. Approximately 15% of strokes are characterized as hemorrhagic, but close to 40% of all stroke deaths are derived from brain aneurysm burst or the blood vessel leak. The other 85% experience an ischemic stroke. This is when a blood clot blocks blood from circulating through the brain. After an individual has an ischemic stroke, they experience irreversible neurological deficits and even death. These neurological deficits include hemiplegia, numbness, balance complications, ptosis, decline in reflexes, visual field defects, apraxia, and aphasia from neuronal damage [47].

Recent studies are taking a close look into how oxidative stress factors play a role in the pathology after ischemic stroke has occurred. The upregulation of oxidative stress factors stimulate inflammation, neuronal apoptosis, and necrosis in the CNS. Within the last couple of years, researchers have thrived to find therapeutic treatments that target the regulation of oxidative stress factors [46]. The most recent pharmaceutical drug in clinical trials is ebselen. Ebselen is a selenoorganic compound that acts similarly to glutathione peroxidase by removing hydroperoxides and lipoperoxides from the intercellular membrane. Its functional role oxidizes NMDA receptors through NR1 redox sites, thereby reversing the dithiothreitol potential to act as an oxidant within collecting free radicals. An additional drug being administrated after ischemic stroke is acetaminophen. Acetaminophen is used as an over-the-counter antiapoptotic agent and can be found in common drugs such as Excedrin and Tylenol. Through intrinsic mitochondrial pathways, it gives the potential to protect against mitochondrial dysfunction by preventing inflammation and maintaining homeostasis. Lastly, an anti-inflammatory drug known as *melanocortin* has been recently shown to regulate the activation of CNS melanocortin MC_4 receptors in ischemia-induced rat models. These receptors have the ability to modulate interleukin-10 in the same ischemic stroke models and are under investigation as neuroprotective agents to ameliorate the damage caused by stroke [48].

2.5 Concluding Remarks

Modifying treatments based on neuropathology of each disease is essential. Studies on animal models have shown that administration of neuroprotective agents postpones disease progression and increases survival rate. Each agent acts through key pathways associated with development, maturation, and repair in abnormal pathological environments during neurodegenerative diseases [2]. These studies on animal models have, however, been difficult to replicate in human trials. An increased number of studies involving human tissue and blood samples need to be undertaken to reduce the translational redundancy that exists between species. Use of naturally occurring neuroprotective agents that are effective in humans and suppress symptoms and delay disease progression should be considered a research priority in the drug discovery process in treating neurodegenerative diseases.

Acknowledgments

Work carried out in our laboratory is supported in part by research grants from the National Multiple Sclerosis Society (USA) (RG-5298) and National Institute of Health (R01NS096148).

Abbreviations

AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
apoE	apolipoprotein E
CNS	central nervous system
CNTF	ciliary neurotrophic factor
DFP	deferiprone
EAE	experimental autoimmune encephalomyelitis
EPO	erythropoietin
fALS	familial ALS
FRDA	Friedreich's ataxia
FXN	frataxin
GDNF	glial cell line-derived neurotrophic factor
HGF	hepatocyte growth factor
LRP1	lipoprotein receptor-related protein 1
mHtt	mutant huntingtin protein
MS	multiple sclerosis
NGF	nerve growth factor
NMDA	*N*-Methyl-D-aspartate
NRG1	neuregulin-1
PACAP	pituitary adenylate cyclase-activating polypeptide
PD	Parkinson's disease
PNS	peripheral nervous system
PTMA	prothymosin α
sALS	sporadic ALS
SMA	spinal muscular atrophy

References

1 Lui, X.-S., Li, J.-F., Wang, S.-S., Wang, Y.-T., Zhang, Y.-Z., Yin, H.-L., Geng, S., Gong, H.-C., Han, B., and Wang, Y.-L. (2014) Human umbilical cord mesenchymal stem cells infected with adenovirus expressing HGF promote regeneration of damage neuron cells in a Parkinson's disease model. *Biomed Res. Int.*, **2014**, 1–7.

2 Hayashi, H. and Takagi, N. (2015) Endogenous neuroprotective molecule and their mechanisms in the central nervous system. *Biol. Pharm. Bull.*, **38** (8), 1104–1108.

3 Ogata, K., Shintani, N., Hayata-Takano, A., Kamo, T., Higashi, S., Seiriki, K., Momosaki, H., Vaudry, D., Vaudry, H., Galas, L., Kasai, A., Nagayasu, K., Nakazawa, T., Hashimoto, R., Yukio, A., Matsuda, T., Baba, A., and Hashimoto, H. (2015) ACAP enhances axon outgrowth in cultured hippocampal neurons to a comparable extent as BNDF. *PLoS One*, **10** (3), 1–13.
4 Ringer, C., Büning, L.-S., Schäfer, M.K.H., Eiden, L.E., Eberhard, W., and Schütz, B. (2013) PACAP signaling exerts opposing effects on neuroprotection and neuroinflammation during disease progression in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis. *Neurol. Dis.*, **54**, 32–42.
5 Cheng, H., Ding, Y., Yu, R., Chen, J., and Wu, C. (2014) Neuroprotection of a novel cyclopeptide C*HSDGIC* from the cyclization of PACAP (1-5) in cellular and rodent models of retinal ganglion cell apoptosis. *PLoS One*, **9** (10), 1–11.
6 Ohnou, T., Yokai, M., Kurihara, T., Hasegawa-Moriyama, M., Shimizu, T., Inoue, K., Kambe, Y., Kanmura, Y., and Miyata, A. (2016) Pituitary adenylate cyclase-activating polypeptide type 1 receptor signaling evokes long-lasting nociceptive behaviors through the activation of spinal astrocytes in mice. *J. Pharm. Sci.*, **130** (4), 194–203.
7 Baxter, P.S., Martel, M.-A., McMahon, A., Kind, P.C., and Hardingham, G.E. (2011) Pituitary adenylate cyclase-activating peptide induces long-lasting neuroprotection through the induction of activity-dependent signaling via the cyclic AMP response element-binding protein-regulated transcription co-activator 1. *J. Neurochem.*, **118** (3), 365–378.
8 Doeppner, T.R., Kaltwasser, B., ElAli, A., Zecharish, A., Hermann, D.M., and Bähr, M. (2011) Acute hepatocyte growth factor treatment induces long-term neuroprotection and stroke recovery via mechanisms involving neural precursor cell proliferation and differentiation. *J. Cereb. Blood Flow Metab.*, **31** (5), 1251–1262.
9 Matsumoto, K. and Nakamura, T. (1992) Hepatocyte growth factor: molecular structure, role in liver regeneration, and other biological functions. *Crit. Rev. Oncog.*, **3** (2), 27–54.
10 Pilliai, D.R., Shanbhag, N.C., Dittmar, M.S., Bogdahn, U., and Schlachetzki, F. (2013) Neurovascular protection by targeting early blood-brain barrier disruption with neurotrophic factors after ischemia-reperfusion in rats. *J. Cereb. Blood Flow Metab.*, **33** (4), 557–566.
11 Jones, J., Estirado, A., Redondo, C., Pacheco-Torres, J., Sirerol-Piquer, M.-S., Garcia-Verdugo, J.M., and Martinez, S. (2015) Mesenchymal stem cells improve motor functions and disease neurodegeneration in ataxic mice. *Mol. Ther.*, **23** (1), 130–138.
12 Kim, S.H., Adhirkari, B.B., Cruz, S., Schramm, M.P., Vinson, J.A., and Narayanaswami, V. (2015) Targeted intracellular delivery of resveratrol to glioblastoma cells using apolipoprotein E-containing reconstituted HDL as a nanovehicle. *PLoS One*, **10** (8), 1–17.
13 Vance, J.E. and Hayashi, H. (2010) Formation and function of apolipoprotein E-containing lipoproteins in the nervous system. *Biochim. Biophys. Acta*, **1801** (8), 806–818.
14 Hayashi, H., Eguchi, Y., Fukunchi-Nakaishi, Y., Takeya, M., Nakagata, N., Tanaka, K., Vance, J.E., and Tanihara, H. (2012) A potential neuroprotective role of apolipoprotein E-containing lipoproteins through low density lipoprotein

receptor-related protein 1 in normal tension glaucoma. *J. Biol. Chem.*, **287** (30), 25395–25406.

15 Halder, S.K., Matsunaga, H., Yamaguchi, H., and Uedo, H. (2013) Novel neuroprotective action of prothymosin alpha-derived peptide against retinal and brain ischemic damages. *J. Neurochem.*, **125** (5), 713–723.

16 Kijogi, C.M., Kehayeka-Wandabwa, C., Sasaki, K., Tanaka, Y., Kurosu, H., Matsunaga, H., and Udea, H. (2016) Subcellular dissemination of prothymosin alpha at normal physiology: immunohistochemical vis-a-vis western blotting perspective. *BMC Physiol.*, **16** (2), 1–13.

17 Dong, G., Callegari, E.A., Gloeckner, C.J., Ueffing, M., and Wang, H. (2012) Prothymosin-a interacts with mutant huntingtin and suppresses its cytotoxicity in cell culture. *J. Biol. Chem.*, **287** (2), 1279–1289.

18 Navarro, J.C., Pillai, S., Ponce, L.L., Van, M., Goodman, J.C., and Robertson, C.S. (2014) Endothelial nitric oxide synthase mediates the cerebrovascular effects of erythropoietin in traumatic brain injury. *Front. Immunol.*, **5** (494), 1–7.

19 Rodgers, E.E., Bonifacio, S.L., Glass, H.C., Juul, S.E., Chang, T., Mayock, D.E., Durand, D., Song, D., Backovich, A.J., Ballard, R.A., and Wu, Y.W. (2014) Erythropoietin and hypothermia for hypoxic-ischemic encephalopathy. *Pediatric Neurol.*, **51** (5), 657–662.

20 Rangarajan, V. and Juul, S. (2014) Erythropoietin: emerging role of erythropoietin in neonatal neuroprotection. *Pediatric Neurol.*, **51** (8), 1–15.

21 Wu, L., Walas, S.J., Leung, W., Lo, E.H., and Lok, J. (2015) Neuregulin1-β decreases IL-1β-induced neutrophil adhesion to human brain microvascular endothelial cells. *Transl. Stroke Res.*, **6** (2), 116–124.

22 Li, Y., Lein, P.J., Ford, G.D., Liu, C., Stovall, K.C., White, T.E., Bruun, D., Tewolde, T., Gates, A.S., Distel, T.J., Surles-Zeigler, M.C., and Ford, B. (2015) Neuregulin-1 inhibits neuroinflammatory responses in a rat model of organophosphate-nerve agent-induced delayed neuronal injury. *J. Neuroinflammation*, **12** (64), 1–13.

23 Lok, J., Zhao, S., Leung, W., Seo, J.H., Navaratna, D., Wang, X., Whalen, M.J., and Lo, E.H. (2012) Neuregulin-1 effects on endothelial and blood-brain-barrier permeability after experimental injury. *Transl. Stroke Res.*, **3** (1), 119–124.

24 Holvoet, B., De Waele, L., Quattrocelli, M., Gheysens, O., Sampaolesi, M., Verfaillie, C.M., and Deroose, C.M. (2016) Increase understanding of stem cell behavior in neurodegenerative and neuromuscular disorders by use of noninvasive cell imaging. *Stem Cells Int.*, **2016** (2), 1–20.

25 Yu-Wai-Man, P. (2016) Genetic manipulation for inherited neurodegenerative disease: myth or reality? *Br. J. Ophthalmol.*, **10** (6), 1–10.

26 Butchbach, M.E.R. (2016) Implications for spinal muscular atrophy and other neurodegenerative diseases. *Front. Mol. Biosci.*, **3** (7), 1–10.

27 Niblock, M., Smith, B.N., Lee, Y.-B., Sardone, V., Topp, S., Troakes, C., Al-Sarraj, S., Leblond, C.S., Dion, P.A., Rouleau, G.A., Shaw, C.E., and Gallo, J.-M. (2016) Retention of hexanucleotide repeat-containing intron in C9orf72 mRNA: implications for the pathogenesis of ALS/FTD. *Acta Neuropathol. Commun.*, **4** (18), 1–12.

28 Kovacs, G.G. (2016) Molecular pathological classification of neurodegenerative diseases: turning towards precision medicine. *Int. J. Mol. Sci.*, **17** (2), 189–222.

29 Zheng, X., Boyer, L., Jin, M., Kim, Y., Fan, W., Brady, C., Berggren, T., Evans, R.M., Gage, F.H., and Hunter, T. (2016) Alleviation of neuronal energy deficiency by mTOR inhibition as a treatment for mitochondria-related neurodegeneration. *eLife*, **5** (e13378), 1–23.

30 Ishii, A., Dutta, R., Wark, G.M., Hwang, S.-I., Han, D.K., Trapp, B.D., Pfeiffer, S.E., and Bansal, R. (2009) Human myelin proteome and comparative analysis with mouse myelin. *Proc. Natl. Acad. Sci. U.S.A.*, **106** (34), 14605–14610.

31 Bartus, R.T., Alexandre, B., Basile, A., Peterson, B.L., Glass, J., and Bouis, N.M. (2016) β_2-Adrenoceptor agonists as novel, safe and potentially effective therapies for amyotrophic lateral sclerosis (ALS). *Neurobiol. Dis.*, **85**, 11–24.

32 Leis, A.A., Ross, M.A., Verheijde, J.L., and Leis Jose, L. (2016) Immunoablation and stem cell transplantation in amyotrophic lateral sclerosis: the ultimate test for the autoimmune pathogenesis hypothesis. *Front. Neurol.*, **7** (12), 1–4.

33 Nagata, E., Ogino, M., Iwamoto, K., Kitagawa, Y., Iwasaki, Y., Yoshii, F., Ikeda, J.-E., and ALS Consortium Investigators (2016) Bromocriptine mesylate attenuates amyotrophic lateral sclerosis: a phase 2a, randomized, double-blind, placebo-controlled research in Japanese patients. *PLoS One*, **11** (2), 1–16.

34 Moujalled, D. and White, A.R. (2016) Advances in the development of disease-modifying treatments for amyotrophic lateral sclerosis. *CNS Drugs*, **30** (285), 227–243.

35 Nizzardo, M., Simone, C., Rizzo, F., Ulzi, G., Ramirez, A., Rizzuti, M., Bucchia, M., Gatti, S., Bresolin, N., Comi, G.P., and Corti, S. (2016) Morpholino-mediated SOD1 reduction ameliorates an amyotrophic lateral sclerosis disease phenotype. *Sci. Rep.*, **6**, 21301, 1–13.

36 Thompson, J.M., Ji, G., and Neugebauer, V. (2015) Small-conductance calcium-activated potassium (SK) channels in the amygdala mediate pain-inhibiting effects of clinically available riluzole in a rat model of arthritis pain. *Mol. Pain*, **11** (51), 1–10.

37 Grubman, A., White, A.R., and Liddell, J.R. (2014) Mitochondrial metals as a potential therapeutic target in neurodegeneration. *Br. J. Pharmacol.*, **171** (8), 2159–2173.

38 Vallderiola, F., Compta, Y., Aparicio, J., Tarradellas, J., Salazar, G., Oliver, J.M., Callén, A., Delgado, T., and Nobbe, F. (2015) Effects of night-time use of rotigotine on nocturnal symptoms in Parkinson's disease. *J. Parkinson's Dis.*, **2015**, 1–6.

39 Yu, X., Yao, J.-Y., He, J., and Tian, J.-W. (2015) Protection of MPTP-induced neuroinflammation and neurodegeneration by rotigotine-loaded microspheres. *Life Sci.*, **124** (3), 136–143.

40 Kim, J.-M., Chung, S.J., Kim, J.W., Jeon, B.S., Singh, P., Thierfelder, S., Ikeda, J., and Bauser, L. (2015) Rotigotine transdermal system as add-on oral dopamine agonist in advanced Parkinson's disease: an open label study. *BMC Neurol.*, **15** (17), 1–9.

41 Corcia, P. and Gordon, P.H. (2012) Amyotrophic lateral sclerosis and the clinical potential of dexpramipexole. *Ther. Clin. Risk Manag.*, **8**, 359–366.

42 Lazaropoulos, M., Dong, Y., Clark, E., Greeley, N.R., Seyer, L.A., Brigatti, K.W., Christie, C., Perlmen, S.L., Wilmot, G.R., Gomez, C.M., Mathews, K.D., Yoon, G., Zesiewicz, T., Hoyle, C., Subramony, S.H., Brocht, A.F., Framer, J.M., Wilson,

R.B., Deutsch, E.C., and Lynch, D.R. (2015) Frataxin levels in peripheral tissue in Friedreich ataxia. *Ann. Clin. Transl. Neurol.*, **2** (8), 831–842.

43 Shinnick, J.E., Isaacs, C.J., Vivaldi, S., Schadt, K., and Lynch, D.R. (2016) Friedrich Ataxia and nephrotic syndrome: a series of two patients. *BMC Neurol.*, **16** (3), 1–6.

44 Parkinson, M.H., Schulz, J.B., and Giunti, P. (2013) Co-enzyme Q_{10} and idebenone use in Friedreich's ataxia. *J. Neurochem.*, **126** (Suppl. 1), 125–141.

45 Wei, Z., Deng, X., Hong, M., Su, Q., Lui, A., Huang, Y., Yu, H., and Peng, Z. (2015) Icariin has synergistic effects with methylprednisolone to ameliorate EAE via modulating HPA function, promoting anti-inflammatory and anti-apoptotic effect. *Int. J. Clin. Exp. Med.*, **8** (11), 20188–20197.

46 Yiu, E.M., Tai, G., Peverill, R.E., Lee, K.J., Croft, K.D., Mori, T.A., Scheiber-Mojdehkar, B., Sturm, B., Prashberger, M., Vogel, A.P., Rance, G., Stephenson, S.E., Sarsero, J.P., Stockley, C., Lee, C.-Y.J., Churchyard, A., Evans-Galea, M.V., Ryan Monique, M., Lockhart, P.J., Corben, L.A., and Delatycki, M.B. (2015) An open-label trial in Friedreich ataxia suggests clinical benefit with high-dose resveratrol, without effect on frataxin levels. *J. Neurol.*, **262** (5), 1344–1353.

47 Zhang, L., Wu, J., Duan, X., Tian, X., Shen, H., Sun, Q., and Chen, G. (2016) NADPH oxidase: a potential target for treatment of stroke. *Oxid. Med. Cell. Longevity*, **2016**, 1–9.

48 Pandya, R.S., Mao, L., Zhou, H., Zhou, S., Zeng, J., Popp, A.J., and Wang, X. (2011) Central nervous system agents for ischemic stroke: neuroprotection mechanisms. *Cent. Nerv. Syst. Agents Med. Chem.*, **11** (2), 81–97.

3

Beneficial Upshots of Naturally Occurring Antioxidant Compounds against Neurological Disorders

Sukanya Saha[*], *Pritam Sadhukhan*[*], *and Parames C. Sil*

Bose Institute, Division of Molecular Medicine, P-1/12, CIT Scheme VII M, Kolkata 700054, India

3.1 Introduction

Free radicals are highly reactive molecules with an unpaired electron in their outermost electronic shell and are identified to play an important role in the origin of life and its evolution [1]. They have different beneficial effects in normal cellular physiology but when produced in surplus have some fatal effects as well [2]. Living organisms get exposed to free radicals for different causes, but the problem arises due to excessive exposure to these free radicals, and this is can be life threatening [3–5]. Due to increasing environmental pollution caused by unsustainable developments and different addictive activities such as cigarette smoking, consumption of alcohols, and so on, humans are constantly exposed to exogenous free radicals [6–9]. There are some natural sources of free radicals such as different cosmic radiations, normal cellular metabolism, and so on [10, 11]. The free radical population present in the cell chiefly constitute oxygen-containing reactive species broadly termed as *reactive oxygen species* (ROS). These reactive species mostly originate from oxygen, formed in mitochondria, endoplasmic reticulum (ER), and peroxisomes during cellular metabolism. These include different radicals (superoxide ($O_2^{\bullet -}$) and hydroxyl ($HO^{\bullet}$)) and non-radical species (hydrogen peroxide (H_2O_2)). Apart from ROS, there exist some reactive nitrogen species (RNS) such as nitric monoxide ($NO^{\bullet}$), peroxynitrite ($ONOO^-$), and so on [4]. The cellular concentration of these reactive chemical species is crucial for normal cellular homeostasis. ROS can induce several biological processes such as proliferation, differentiation of cells, and so on. It can mediate production and activity of several inflammatory cytokines to induce stress-responsive molecular pathways for survival [10, 12]. Being an important signaling molecule, nitric oxide (NO) essentially regulates a number of physiological processes, namely, leukocyte adhesion, platelet aggregation, angiogenesis, thrombosis, the relaxation and proliferation of vascular smooth muscle cells, vascular tone, and hemodynamics [13].

[*] These authors contributed equally.

Neuroprotective Natural Products: Clinical Aspects and Mode of Action, First Edition.
Edited by Goutam Brahmachari.

To regulate the level of these reactive species, there exist several endogenous antioxidant defense mechanisms [14–16]. Different enzymes (e.g., heme oxygenase (HMOX1), nicotinamide adenine dinucleotide phosphate (NAD(P)H), quinone oxidoreductase 1 (NQO1), glutathione-S-transferases (GSTs), and UDP-glucuronosyltransferases (UGTs)), with potential antioxidative functions, act mainly by scavenging ROS [11, 17, 18]. The production of these enzymes is regulated by a transcription factor, nuclear factor erythroid 2-related factor 2 (NRF2) [19]. Along with this enzymatic antioxidant machinery, cells also possess nonenzymatic antioxidative system constituted by cellular metabolites, glutathione (GSH), and NADPH [20].

Under perturbed metabolism of these reactive species as discussed earlier, cellular metabolism gets affected. Excessive production of ROS and RNS can lead to cellular damage by oxidation of macromolecules, that is, proteins, lipids, and carbohydrates [21–23]. This damage causes permanent tissue injury and eventually leads to many chronic diseases including diabetes, cancer, cardiovascular disorders, aging, and chronic inflammation and also neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease [24–26]. The central nervous system (CNS) of higher organisms requires a significantly higher amount of oxygen compared with other organs of the body to carry out its normal physiological functions, that is, the activity of excitatory amino acids and neurotransmitters [27, 28]. This leads to the production of excessive free radicals in the brain. Post-mitotic neurons and glial cells in the brain are noted to be highly susceptible to oxidative stress due to the high amount of ROS in the brain [5]. Different research reports suggest that the intracellular antioxidant defense mechanism is the lowest in the brain compared with other organs present in a human body [29]. Besides, it also possesses a large amount of polyunsaturated fatty acids (PUFA) [30]. The selective nature of the blood–brain barrier restricts the distribution of some antioxidant molecules like vitamin E in the brain. It has been found that the activity of the antioxidative enzyme, for example, catalase is only 10% compared to that in the liver [31, 32]. Progressive oxidative damage results in the loss of functions in the neurons and eventually leads to different neurodegenerative disorders. Oxidative stress associated with aging is also responsible for neurodegeneration. This is because of the high mutation rates of mitochondrial DNA [33–36]. Increasing evidences suggest that the physiological decline occurring with age and age-related neurodegeneration is because of the mutations acquired during aging by mtDNA [37].

Therefore, we can conclude that oxidative stress is a crucial aspect in the development of different neurodegenerative diseases. It has been found that the neuronal cell damage could be due to either hyperoxidative insult or insufficient antioxidative machinery or both. Different therapeutic approaches that are aimed to increase the antioxidant capacity or relieve the oxidative load by radical scavengers, that is, antioxidants, are taken under consideration for ameliorating or preventing different neurological disorders [32, 38]. Several experimental and clinical reports found this approach of administering natural antioxidants beneficial against common neurological disorders. Both medicinal and edible plants contain a number of secondary metabolites, including an array of bioactive constituents, which possess the ability to improve human health [20, 39–41]. This benefit may be promoted by the exposure to these phytochemicals (including phenylpropanoids, isoprenoids, and alkaloids) through correct dietary habits,

resulting in protection against chronic degenerative disorders such as cancer and cardiovascular, neurodegenerative diseases, and so on [2, 42–44].

There are more benefits of using natural antioxidants over chemically synthesized drugs. These naturally occurring antioxidants are available in our daily diet; most of the antioxidants are polyphenolic in nature and are found in different fruits and vegetables. Several studies confirm that most of these antioxidants are not toxic, that is, these molecules have no or few side effects compared with the synthetic drugs. Most of the natural antioxidants are pleiotropic in nature and will help to maintain normal physiological conditions [45]. Moreover, since these molecules are easily available in natural products, the therapeutic approach may be cost effective as well [46]. In this chapter, we comprehensively describe the relationship between oxidative stress and neurodegenerative disorders and highlight the current status and future prospects of different effective naturally occurring antioxidant molecules against these kinds of pathophysiological conditions.

3.2 Oxidative Stress

One of the most primitive groups of molecules having a very vital role in the biological evolution and origin of life are free radicals, molecules with an unpaired electron in their outermost orbit. Long back in nature's progression, another very essential component molecule that evolved as a blessing to mankind was the survival gas oxygen. However, this blessing is sometimes potentially dangerous for living organisms when they produce excessive free radicals. Free radicals like ROS, even though are imperative for life, are disastrous when excessively produced. So an intricate system of checks and balances prevails for making proper use of this essential element [7].

3.2.1 Oxidants and Its Types

ROS are generally of many types. They are chemically reactive free radicals containing oxygen. Among them superoxide radical, hydroxyl radical, and hydroperoxyl radical are common [47]. Though not a free radical in nature, H_2O_2 (hydrogen peroxide) is one of the predominant types of ROS. Sometimes these ROS react with other free radicals such as NO and give rise to another class of oxidants, namely, the RNS. A common example of such RNS is the peroxynitrite molecule.

3.2.2 Oxidants: Its Production, Consumption, and Way to Oxidative Stress

In humans, during oxidative phosphorylation in mitochondria, the leakage of activated oxygen from it is one of the primary sources for reactive oxygen. This leakage takes place under normal conditions in the body. However, some other enzymes such as xanthine oxidase, NADPH oxidases, and cytochrome P450 are capable of producing superoxides. Again, hydrogen peroxide is extensively produced by a wide variety of enzymes comprising several oxidases as well. Moreover, research on *Escherichia coli* indicates that there are other enzymes that contribute to the bulk of oxidants produced in them. The researchers observed that the

mutant bacteria (those which lack an active electron transport chain (ETC)) produced the same amount of hydrogen peroxide as the wild-type organisms [48, 49]. One possible phenomenon that led to the formation of the same amount of oxidants may be the production of small amounts of oxidants by multiple redox-active flavoproteins under normal conditions, all of which together contributed in small portions to the total production of oxidants [50].

Certain amount of ROS is essential and exhibits beneficiary roles. By a process named mitohormesis, ROS, in small amounts, play a key role as antiaging agents [17]. In the immune system, they exhibit protective effects by attacking and killing pathogens [16]. Further, certain reactive species also play significant roles in cell signaling by acting as messengers in cellular redox signaling. However, ROS overproduction leads to the deleterious phenomenon known as *oxidative stress*. The causes of oxidative stress can be both extrinsic (environmental pollutants, toxins, heavy metals, UV radiation, etc.) and intrinsic (metabolic activities, emotional stress, etc.). Oxidative stress reveals an inequity between the production of reactive species (ROS, RNS) and the body's ability to simply detoxify reactive intermediates or to heal the resulting damages. Thus, a balance between reactive species production and consumption is gravely essential to maintain proper cellular homeostasis.

The well-known amino acid methionine is very much prone to oxidation, but its oxidized form can be reversed. Its oxidation normally inhibits the phosphorylation of the adjacent Ser/Thr/Tyr sites in the protein it resides [51]. This fact hints a probable mechanism for the cells to couple oxidative stress signals with phosphorylation, a mainstream cellular signaling.

The cellular antioxidant enzymes are the first line of cellular defense against oxidative stress. The widely studied cellular antioxidants are the antioxidant enzymes superoxide dismutase (SOD), catalase, GSH reductase, and GSH peroxidase. SODs (specifically its isoforms, CuZnSOD and MnSOD) are very important antioxidants in the brain as their depletion causes neuronal degeneration and overexpression delays cell death during oxidative insult in the brain. Another important antioxidant enzyme (not so much well known) is the peroxiredoxins. One more recently discovered vital enzymatic antioxidant is sulfiredoxin. Some other enzymes having divergent primary roles but also possessing antioxidant properties are GST, paraoxonase, and aldehyde dehydrogenases. Other than the aforementioned enzymes, there are some other molecules such as GSH and melatonin that play critical roles as antioxidants. GSH is a cysteine-containing peptide synthesized by the cells from the constituent amino acids. Owing to its high concentration and the central role in sustaining the cell's redox state, GSH is one of the most important cellular antioxidants in the brain as well as all the other major organs [50]. It possesses antioxidant properties because the thiol group present in its cysteine moiety acts as a reducing agent, and thus it can be reversibly oxidized and/or reduced. In normal conditions, glutathione is maintained in its reduced form, GSH, with the help of the enzyme GSH reductase, and this GSH in turn reduces other enzyme systems as well as metabolites [52]. However, during oxidative stress, it is converted to its oxidized form, glutathione disulfide (GSSG). The ratio of GSH:GSSG is commonly studied as a marker of oxidative stress within an

organism. Another powerful antioxidant that easily crosses cell membranes and the blood–brain barrier is melatonin [53, 54]. Unlike GSH and other antioxidants, melatonin does not undergo redox cycling, the ability of a molecule to undergo reduction and oxidation in a cyclic manner. It has also been referred to as *terminal* or *suicidal antioxidant* because once after it gets oxidized it cannot go back to its former reduced state (as upon reacting with free radicals many stable end products are formed) [55].

However, the imbalanced defense mechanism of cellular antioxidants causes a serious penalty in normal mechanisms, leading to oxidative damage to all the components of the cell including biomolecules (protein, lipids, and DNA). It causes peroxidation and carbonylation of lipids and proteins, respectively. ROS-induced damage to the DNA results in its strand breaks and base damages. This disruption of prooxidant/antioxidant balance or normal redox state of a cell due to interference in the primary cellular defense leads to a wide array of pathophysiological conditions.

3.2.3 Oxidative Stress Affecting Various Organs Leads to Different Diseases

Oxidative stress nearly affects all the systems of the body. Organs such as the brain, kidney, liver, heart, skin, spleen, and pancreas and even the reproductive organs can be its target. By monitoring the levels of markers such as ROS, RNS, and antioxidant defense molecules, indirect evidence indicates oxidative damage as the culprit in the pathogenesis of different kinds of diseases. Human diseases such as metabolic ailments, organ dysfunctions, and cancers have oxidative stress as their common etiology [56], which is probably involved in the age-related growth of cancer. In oxidative stress, the reactive species produced are mutagenic as they can cause direct damage to the DNA and may also suppress apoptosis, thereby promoting the steps of cancer, namely, proliferation, invasion, and metastasis. In the development of gastric cancer, *Helicobacter pylori*, by increasing the production of ROS and RNS in the human stomach, cause the infection [53].

In the brain, cumulative effects of oxidative stress along with disrupted mitochondrial respiration as well as mitochondrial damage are directly related to various neurological diseases. Thus, oxidative stress is the triggering factor behind common neuronal disorders such as Parkinson's disease [57–60], AD [61–65], autism spectrum disorders [66, 67], attention deficit hyperactivity disorder (ADHD) [68], schizophrenia [69–72], chronic fatigue syndrome [73–75], Lafora disease, and even depression [76–79]. The oxidation of low-density lipoproteins in the vascular endothelium is the precursor to plaque formation, and so oxidative stress is assumed to be linked to certain cardiovascular disease. During hypoxia, where lack of oxygen supply takes place, oxidative stress plays a detrimental role in the heart by initializing the ischemic cascade following ischemia/reperfusion injury. Oxidative stress is also thought to be involved in the progress of heart failure [80–82], myocardial infarction [83, 84], atherosclerosis [85, 86], multiple sclerosis [87–89], sickle cell disease [90, 91], and even infections [56, 92].

3.3 Neurological Disorders

The networks within the body that connect its different parts and synchronize voluntary as well as involuntary actions comprise the nervous system. This complex system controlling all the workings of the body consists of mainly two parts: the CNS and the peripheral nervous system (PNS). While the brain and spinal cord are parts of the CNS, the PNS primarily contains nerves connecting the CNS to the other parts of the body. Thus, abnormalities in any part of this system that can be structural, biochemical, or electrical lead to different pathophysiological conditions not only within the nervous system but also in other systems of the body, affecting the body as a whole. Damage to the nervous system leads to a multitude of diseases all together, termed as *neurological disorders*. With increasing changes in today's lifestyle, these diseases have become rampant in the society. Habits such as drinking, smoking, inadequate sleep, odd working hours, stress, depression, and so on are the biggest contributors to neurological disorders. The interesting fact is that these entire ranges of habits result in a common outcome, oxidative stress, and these act as the etiology and triggering factors behind most of neurological disorders. Till date, there are more than 600 types of neurological disorders whose causes vary. Other than injuries to the brain, spinal cord, and nerve, abnormalities such as genetic disorders, infections, congenital defects, and neuropsychiatric illnesses contribute greatly to the pathophysiological conditions in the nervous system. Moreover, abnormalities in other organs or systems that interrelate with the nervous system also lead to neurological deteriorations such as cerebrovascular disorders, autoimmune disorders, and so on. The most prominent neurological disorders include neurodegenerative diseases, which further can be categorized as cognitive and movement disorders.

3.3.1 ROS and Its Way to Oxidative Stress Results in Neurotoxicity

Activation of molecular oxygen as ROS by the interactions between oxygen and redox reactive metal ions via reactions such as the Fenton and Haber–Weiss reactions or via indirect pathways involving the calcium activation of metalloenzymes such as phospholipases, NO synthase, and xanthine oxidase is an intrinsic part of normal metabolism.

The two cellular organelles, mitochondria and plasma membrane, serve as principal generators of ROS production. In mitochondria, alteration of the ETC due to partial inhibition of complex I and III leads to ROS generation by unpaired electrons that escape the ETC and react with molecular oxygen generating superoxide radical, leading to oxidative stress and formation of toxic species, such as peroxides, alcohols, and so on. These simultaneously and sequentially affect various organs, ultimately leading to dysfunction and thereby causing many pathophysiological conditions. Again, increased free fatty acids in metabolic disorders modulate the decreased NADPH oxidase activity in the plasma membrane, resulting in ROS formation and oxidative stress. ROS on reacting with NO leads to the production of RNS. RNS are also formed by mechanisms independent of ROS.

As ROS are both important and toxic, cells have developed highly elaborate means of regulating mitochondrial functioning, metal ion interactions, and the generation of ROS. Breakdown of any of these harnessed processes is inherently destructive.

Oxidative stress causes a decrease in mitochondrial membrane potential (MMP), a significant increase in malondialdehyde (MDA) and protein carbonyl levels, and dysregulation of intracellular calcium signaling features widely observed in neurological diseases [93]. Thus oxidative stress with its different downstream phenomenon contributes to neurotoxicity (Figure 3.1). Important downstream events that occur in response to ROS are an alteration in the signaling molecules of BDNF–Trk cascade and calcium influx-induced excitotoxic response (activation of glutamate receptors) triggering a cascade of events leading to cell death [94, 95]. Such responses have been implicated in several neuropathies such as epilepsy, stroke, AD, Parkinson's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, and much more.

3.3.2 Why Is the Brain Particularly Prone to Oxidative Stress?

The brain is composed of high levels of lipids such as PUFA, excellent substrates for free radical reaction (catalyzed by the rich reserves of iron in the brain), and targets for the initiation of lipid peroxidation. This leads to the formation of reactive lipid species such as 4-hydroxynonenal and MDA, which in turn are capable of modifying proteins [96]. These covalent modifications of proteins appear to accumulate in the brain during aging and in neurodegenerative diseases [97–101].

The human brain requires a huge amount of oxygen due to the high amount of adenosine triphosphate (ATP) consumption by its neurons. The brain entails

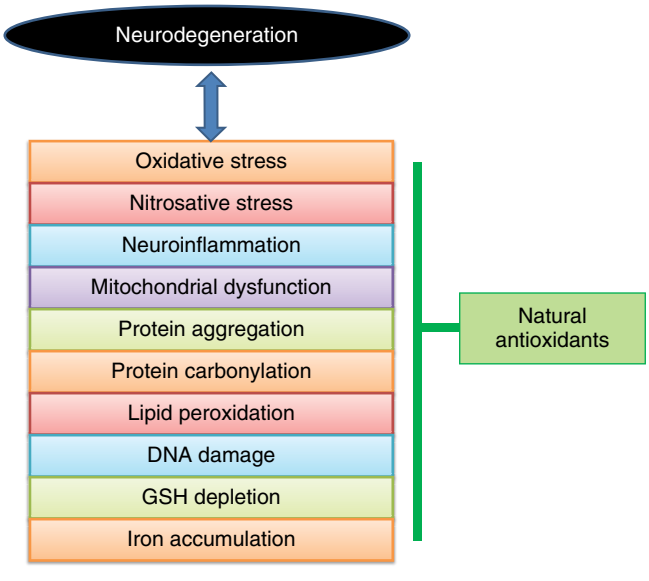


Figure 3.1 Causes and downstream phenomenon associated with different neurodegenerative diseases.

about 20% basal oxygen of the total oxygen required by the body. Hence, the brain needs a large amount of O_2 in its small tissue mass, as the brain accounts for only a small percentage of the total body weight. Again in the neurons, the ATP is essential for proper release and storage of neurotransmitters to maintain a stable membrane potential. Proper functioning of the mitochondria is essential for normal brain functioning, and its damage or disruption leads to increased formation of free radicals due to disoriented ETC. Moreover, brains have a high propensity to generate ROS/RNS such as H_2O_2, NO, superoxide, and peroxynitrite [102–104] from different sources due to the steady supply and high rate of metabolic turnover of mitochondria, contributing to the prooxidant environment [105, 106]. Again, most of the neurotransmitters are autooxidizable, and they react with oxygen to generate free radicals (O_2^-, H_2O_2) and reactive quinones/semiquinones that can exhaust GSH [107, 108]. Unnecessary formation of ROS damages the mitochondria, and these damaged mitochondria produce more reactive species, leading to a consequential vicious cycle. Chemically, ROS originates by the reaction of oxygen with the redox-active metals copper and iron. An important consequence of normal aging is that the levels of redox-active metals copper and iron in the brain increase, becoming a dominant risk factor in different neurological, especially neurodegenerative, diseases. Moreover, as implicated in several age-dependent neurodegenerative disorders, there is an accumulation of proteins (Aβ in AD, α-synuclein in Parkinson's disease, SOD1 in ALS). These increases in accumulated proteins could lead to hypermetalation of proteins by adventitial binding, increasing the likelihood that ROS are generated inappropriately in neurological diseases. All these factors contribute in making the brain more prone to oxidative stress damage than other organs.

3.3.3 The Different Types of Neurological Diseases

The types of neurological diseases and persons affected by such diseases are increasing rapidly in the society. With the progression of mankind, the diseases associated with the brain have propagated hugely, grasping a great percentage of the population. These diseases can be categorized depending on either the primary part of the nervous system affected or the primary dysfunctions involved or the common etiology in them. One major class of neuronal diseases, very well known in the contemporary world and having the common cause, is neurodegenerative diseases. As the name suggests, the underlying pathophysiology of neurodegenerative diseases is neurodegeneration, that is, the degeneration of the neuron. These affect millions each year and the incidence augments in the aged population. It has been projected that by 2030, about one in five Americans over the age of 65 will be diagnosed with neurodegenerative diseases [109]. Finding some effective therapeutic strategy is, therefore, increasingly essential. Different reports over decades demonstrated that the progression of neurodegeneration is associated with decreased endogenous antioxidants and increased oxidative stress, and metal binding is facilitated by persistent oxidative protein aggregation [110]. There are many kinds of neurodegenerative diseases, but the most popular neurodegenerative diseases are AD, Parkinson's disease, and ALS. These three diseases can again be subcategorized under the group cognitive disorders (AD)

Table 3.1 Neurodegenerative diseases: proteins and associated pathology.

Disease	Etiology	Diseased protein deposited	Characteristic pathology
Alzheimer's disease	Sporadic (ApoE risk factor)	Aβ peptide (from APP) and hyperphosphorylated tau	Neurite plaques and neurofibrillary tangles
	APP (dominant) and presenilin 1 and 2 (dominant)	—	—
Parkinson's disease	Sporadic	α-Synuclein	Lewy bodies and Lewy nitrites
	α-Synuclein (dominant)	—	—
	Parkin (also DJ-1, PINK1) (some dominant)	—	Lewy bodies less frequent or absent
Amyotrophic lateral sclerosis	Sporadic Superoxide dismutase-1 (dominant)	Unknown proteins deposited in neurofilaments	Bunina bodies and axonal spheroids

and movement disorders (Parkinson's disease, ALS). AD is the most common neurodegenerative disease affecting the world population followed by Parkinson's disease and ALS in the second and third position. Herein, a brief phenotypical illustration of these diseases along with the brain regions mostly affected is depicted. The associated pathologies are shown in Table 3.1.

In this chapter, we are classifying the neurological disorders based upon common deleterious effects that they show. Here the classification will be under two broad sections, namely, the cognitive disorders (problem with cognition) and the movement disorders (defect in movements). One common thing is that oxidative injury plays a crucial deleterious role in all these classes of neuropathies. Moreover, the accumulated abnormal proteins in most of these diseases act synergistically with oxidative stress to accelerate the further propagation and severity of these diseases.

3.3.4 Cognitive Diseases

Individuals having significant impairment of cognition or memory are known to be suffering from cognitive diseases. In them, if not congenital, a marked deterioration from the previous level of cognitive function takes place. Cognitive disorders are a category of neurological disorders consisting mainly of amnesia, dementia, and delirium where the mental health gets affected. It leads to cognition inabilities such as problem in learning, memory, perception, and problem-solving ability in the affected individuals. These diseases mainly originate from the interruption in the basic cognitive abilities and functions such as memory

processing, language, perception, and so on. Primarily damage to the memory portions of the brain occurs along with other variable causes in different cognitive disorders. Therapies and medications are common treatments depending on the etiology of a particular disease. However, in certain types of diseases, there is no permanent cure as treatments only temporarily relieve the suffering by suppressing the symptoms of the disease. In individuals suffering from delirium, having the situational awareness and processing of any new information is very difficult. Though it usually does not last for a very long time, generally the rate of onset is really high, sometimes accompanied with hallucinations, attention shifts, mood swings, and anomalies in behavior. In humans, when the patient's memory gets erased partially or completely, mainly due to some genetic disorder or trauma, they are said to be suffering from dementia. Usually, other cognitive dysfunctions also accompany dementia. It is believed to have a direct association with age, that is, generally with age, the chance of its onset increases. The severity of dementia is also directly proportional to the age of onset and decline of memory. The sufferings due to mental disabilities are usually lifelong. Among its different causes (genetics, brain diseases, trauma, stroke, and heart problems), the major causes include neurodegenerative diseases, such as AD and Parkinson's disease, as they show decline in brain functions [111–116] and compensate for normal living of affected individuals. Individuals having trouble in retaining long-term memories (due to damage to the hippocampus, the part of the brain mainly responsible for memory) suffer from amnesia. Amnesia can be of two types, namely, the anterograde amnesia (a problem in the creation of new memories) or retrograde amnesia (memories in the process of being encoded to long-term memories get erased) [117, 118]. But whichever may the type, it is very difficult to treat amnesia. The prominent causes of amnesia include anxiety disorders and AD. Thus in both the subtypes of cognitive disorders, dementia and amnesia, AD plays a causal role though it can also be debated that these disorders help in the acceleration of the AD progression. AD is the most common neurodegenerative disease affecting the world population. This is characterized by cognitive disabilities such as a late-onset progressive dementia affecting memory, task performance, speech, and recognition of people and objects. Thus, it can also be said as a cognitive disorder with neurodegeneration as the etiology. Degeneration and altered connections of neurons in the forebrain and hippocampus are found precisely. AD is caused by the formation of extracellular senile plaques consisting of beta-amyloid (Aβ) from amyloid precursor protein (APP) and oxidative stress phenomenon (Figure 3.2).

3.3.5 Movement Diseases

Movement disorders are a kind of neurologic diseases where the movements of an individual get affected. These diseases are not related to weakness or spasticity [119]. Either an excess of movements or a decrease in movements or difficulty in voluntary and automatic movements takes place in movement disorders. Broadly there are two types of movement disorders: primary and secondary. The type of movement disorders where the abnormal movement is the prime manifestation are primary movement disorders, whereas the secondary movements are demonstrated

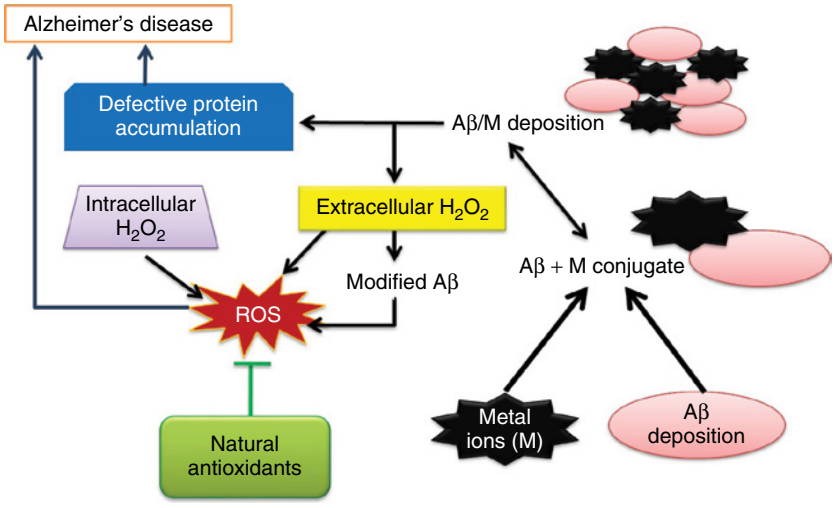


Figure 3.2 Various molecular events leading to the Alzheimer's disease neurodegeneration.

due to other systemic or neurological disorders [120]. According to conventional classification, movement disorders are broadly classified into hyperkinetic movement disorders or dyskinesias (where excessive, frequently repetitive, involuntary movements take place that interrupt normal motor activities) and hypokinetic movement disorders. These hypokinetic movement disorders are again of four types: akinesia (where there is a lack of movement), hypokinesia (where the amplitudes of movements are reduced), bradykinesia (where slow movement takes place), and rigidity. The famous neurodegenerative disorder, Parkinson's disease, falls under the hypokinetic class of movement disorders. Parkinson's disease, the second common neurodegenerative disease, is characterized by movement-related disorders such as resting tremor (shaking), rigidity, slow movements, walking disabilities, gait, and different postural as well as autonomic instability. Later, in the course of the disease, problems in thought and behaviors may begin. In advanced stages of the disease, psychiatric symptoms such as dementia and depression occur. Some suffer even from problems in sleep and emotions. The motor deficits collectively are known as *parkinsonian syndrome*. It is caused by degeneration of dopaminergic neurons in the substantia nigra of the midbrain and monoaminergic neurons in the brain stem. There are deposits of mutated α-synuclein protein. Accumulation of neuromelanin with accumulated iron and Lewy bodies is a hallmark of Parkinson's disease [121–125].

Again motor neuron disease (MND) is another kind of neurological disease where the movement of the affected individuals gets affected due to the problem in the motor neurons (the cells of the body that control the voluntary muscles). Generally, they are neurodegenerative in nature and cause growing disability and finally death. A common type of such kind of MND is ALS. ALS is a progressive fatal disease caused by degeneration of the lower motor neurons in the lateral horn of the spinal cord and upper motor neurons of the cerebral cortex, resulting in increasing motor weakness [126–129]. In these movement

disorders (Parkinson's disease, ALS) that are more prominent in the society, the oxidative stress phenomenon prevails as a side effect of the disease and increases its severity. Sometimes this oxidative stress can also be the primary cause of the onset of these diseases as it can destroy the cellular integrity and regulate numerous signaling molecules [130–132].

3.4 Beneficial Effects of Different Antioxidants against Various Neurological Disorders

In recent times, a part of therapeutic researches is focused on the prophylactic role of a large number of medicinal plants against various pathophysiological conditions starting from diabetes, cancer, or even neurodegenerative disorders [42, 133–136]. Different reports and epidemiological evidence suggest that simple extracts or purified small molecules are effective in the prevention or cure of neurodegeneration [137–140]. The medicinal properties of various plant extracts are mainly due to the presence of several polyphenols, alkaloids, and terpenes in different parts like fruits, leaves, stem, roots, and so on [141–143]. Among these nutraceuticals or micronutrients, substantial experimental reports from our laboratory and others indicate that polyphenols possess promising activity against several pathophysiological conditions [144–148]. It has been found that polyphenolic compounds have well-defined antioxidative properties [149–151]. Other than the free radical scavenging activity, these compounds can also modulate intracellular gene expression. In different neurodegenerative disorders, polyphenolic compounds mainly act as mediators of different signaling pathways and modulators of several neurotransmitter enzymes or by exerting anti-inflammatory effects [152]. These compounds possess an exceptional free radical scavenging activity due to its diversity in the substitutions in the molecular backbone, including the presence of multiple hydroxyl groups [153]. Moreover, these molecules can also act as efficient metal chelators, which suppress the formation of hydroxyl radical from hydrogen peroxide [154, 155]. To investigate the neuroprotective activity of different naturally occurring polyphenolic compounds such as catechin, epicatechin, gallic acid, quercetin, genistein, caffeic acid, and so on, different animal models with cognitive impairment were designed in the past few years, and the activity of these polyphenolic compounds was compared with placebo [156–161]. Some biologically relevant polyphenolic compound with their natural sources and major metabolites are summarized in Table 3.2. Cognitive impairment in the animals was generally estimated by different experiments such as water maze test, step-down test, step-through test, open field test, and so on [162, 163]. Finally, the animals were sacrificed, and samples of the brain were subjected to microscopic and molecular analysis. In Figure 3.3, a line diagram is presented to depict a common signaling mechanism stimulated by different naturally occurring antioxidants against neurodegenerative disorders. Here, the activity of different plant extracts or isolated compounds, identified as effective antioxidants in various neurodegenerative disorders, is comprehensively discussed.

Table 3.2 Some biologically relevant polyphenolic compounds with their natural sources and major metabolites.

Plant-derived polyphenolic compound	Natural source	Main nutritional metabolites in human
Apocynin	*Picrorhiza kurroa*	Diapocynin
Catechin and Epicatechin	Green tea, grape, chocolate	3′,4′-Dihydroxyphenyl-γ-valerolactone
Gallic acid	Blackberry, black tea, pomegranate	4-*O*-Methylgallic acid
Curcumin	Turmeric, cruciferous vegetables	Curcumin glucuronides
Quercetin	Citrus fruits, apples, onions	2-(3-Methoxy-4-hydroxyphenyl) acetic acid
Genistein	Lupin, fava beans, soybeans	Orobol
Chlorogenic acid	Coffee, tea, blueberry	Ferulic acid
Tannic acid	Grapes, tea	Resorcinol
Mangiferin	Mango	Norathyriol
Caffeic acid	Broccoli, apple, coffee	3- and 4-Coumaric acid

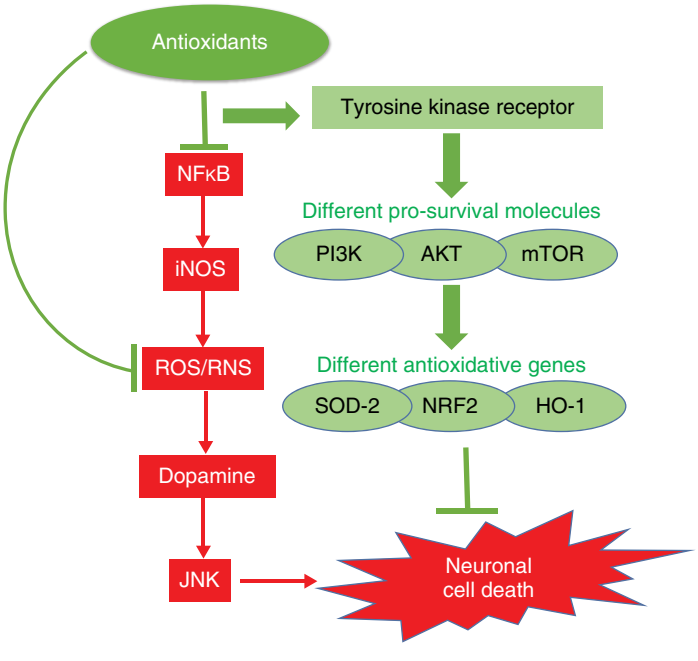


Figure 3.3 Schematic representation of a common signaling mechanism stimulated by different naturally occurring antioxidants against neurodegenerative disorders.

3.4.1 Role of Natural Antioxidants in the Amelioration of Different Cognitive Disorders

Different clinical and experimental data confirm that oxidative stress is one of the major culprits in the manifestation of several neurodegenerative diseases including cognitive disorders. Till date scientists are still in search for an efficient therapeutic approach to prevent the development and progression or to cure different cognitive disorders such as amnesia, dementia, delirium, and AD. However, different reports suggest that administration of plant polyphenols effectively decreases the risk of development and decelerates the progression of cognitive impairment [164, 165]. Plant polyphenols exhibit promising antioxidant activity, and along with this, it is known from different experimental data of our lab and others that these nutraceuticals and their metabolites can induce more intricate and precise effects than scavenging of intracellular ROS [2, 20, 43, 166–168]. Polyphenolic compounds such as mangiferin, genistein, resveratrol, curcumin, and so on can modulate neuronal cell functions by modulating the activity of intracellular kinases [169]. In experimental models of AD and other similar pathology, these polyphenols were found to decrease the deposition of amyloid in the CNS, and improved behavioral performances were observed. Plant-derived polyphenolic compounds can directly react with ROS or can indirectly stimulate signaling cascades to combat oxidative stress-mediated pathophysiological conditions.

Several studies have been conducted to observe the effect of different edible food grains against cognitive disorders. Effective results have been obtained in studies with blueberry, mulberry, strawberry, grape seed, pomegranate, papaya, apple, green tea, coffee, walnut, saffron, turmeric, pepper, cinnamon, ginger, and so on [170, 171]. All of these edible foods have been found to neutralize the effect of free radicals and prevent the development and progression of different cognitive disorders in experimental animal models. Different herbs such as *Ginkgo biloba*, *Withania somnifera*, *Poncirus trifoliata*, *Huperzia serrata*, *Scrophularia buergeriana*, *Hedyotis diffusa*, *Rosmarinus officinalis*, *Paeonia suffruticosa*, and so on have shown significant neuroprotective effects against various disorders [142, 172, 173]. All studies interpreted that the extracts obtained from these herbs contain a high concentration of bioactive alkaloids and polyphenols. The nutraceuticals have inhibitory properties against the accumulation of Aβ peptides, and they prevent the activity of acetylcholinesterase (AChE) enzyme [174].

In an experimental transgenic mouse model of early familial AD where amyloid plaques were formed in the cerebral cortex, it has been found that consumption of wine can effectively prevent the formation of Aβ peptides [175]. Wine contains high concentration of gallic acid, caffeic acid, catechin, and gallotannins, and experimental data was compared with the animals treated with water and ethanol. This confirmed the effect of polyphenolic compounds against AD. Another study with a murine model of AD showed that dietary administration of pomegranate extract prevents the deposition of soluble Aβ42 and amyloid accretion in the hippocampus [176]. Pomegranate juice is found to be rich in several flavonoids such as ellagic acid, gallic acid, tannins, and anthocyanins [177]. Similar results were observed when experimental animals were treated with the extract from grape seeds for 5 months. Grape seed extract is also rich in catechin and epicatechin [178].

It effectively reduces the cognitive impairment and high molecular weight Aβ peptides in the murine brain [178]. A study by the same research group showed that grape seed extract can potentially inhibit the oligomerization of synthetic Aβ1–42 and Aβ1–40 peptides [178]. In an another study, oral administration of grape seed extract at 200 mg kg^{-1} body weight per day subdued the accumulation of the tau protein in the brain of an experimental model of tau pathology associated with numerous cognitive disorders including AD [179]. Further molecular studies with the experimental brain sample observed that expression of phosphorylated extracellular signal-regulated kinases 1/2 (ERK1/2) was suppressed in the tissue. From this data, it can be concluded that grape seed extract inhibited the accumulation of tau by suppressing the activity of ERK1/2 since hyperphosphorylation of tau protein is catalyzed by ERK1/2 [180]. In a study by Moghbelinejad *et al*., it has been shown that polyphenolic compounds from green tea can potentially decelerate the decline in the ability to learn and memorize in AD model [181]. In the study, AD model was established by intraperitoneal injection of D-galactose and intracerebroventricular injection of Aβ 25–35 peptide. In line with these experimental data, it was concluded by Davinelli *et al*. that supplementation of green tea extract in regular diet can improve the total antioxidant capacity of the brain and simultaneously decrease different oxidative stress markers, which results in the improvement of cognitive disorders [182]. Different studies were carried out with green tea extract, and it has been found that this catechin- and epicatechin-rich mixture has significant antidepressant effect in adult mice [183]. It can also suppress the activity of AChE in the cerebrum of aged rats [156, 184].

Different reports suggest that overexpression of presenilin-1 (PS-1) in the human brain indicates the onset of ROS generation and Aβ peptide accumulation. It has been found that administration of 0.5% apple juice with drinking water prevented the onset of AD in experimental animals [185]. Apple-derived antioxidants and *S*-adenosylmethionine in the juice prevent the oligomerization of Aβ peptide, and particularly *S*-adenosylmethionine stabilizes the DNA. Another antioxidant molecule, tannic acid, is found to prevent the accumulation of Aβ1–40 and Aβ1–42 peptides in the brain parenchyma of experimental PSAPP mouse model of cognitive disorders. In that particular experiment, tannic acid was orally administered at a dose of 30 mg kg^{-1} body weight for 6 months. Further molecular analysis with the parenchymatic tissue revealed that tannic acid specifically suppressed the activity of β-secretase in the experimental animals compared with the control set [186]. In a study by Sakurai *et al*., it has been found that administration of oligonol, an antioxidant derived from catechin and proanthocyanidins, is effective in SAMP8 mice. This SAMP8 mouse has disabilities with learning and memory. Administration of oligonol at 60 mg kg^{-1} body weight is found to be effective in suppression of inflammatory response associated with cognitive disorders [187].

The ethanolic extract of *Cassia obtusifolia* is identified as an inhibitor of AchE and is found to be effective in scopolamine-induced memory loss [188, 189]. Similarly, methoxsalen, extracted from *P. trifoliata*, also possesses the same activity that was found to be effective in experimental animal model of AD induced by trimethyltin [190]. Apart from polyphenolic compounds, an alkaloid,

piperine, isolated from *Piper longum* is also found to be effective against hippocampal neurodegeneration and also significantly lowered the cognitive deficits in experimental animal model [191]. In an *in vitro* study with primary neuronal culture, it has been observed that Sanmjuanhwan (Sjh) showed significant neuroprotective effect under the exposure of Aβ25–35. Sjh effectively inhibited the intrinsic apoptotic pathway by upregulating the antiapoptotic Bcl-2 family proteins and preventing the loss of MMP [192]. Apart from flavonoids or alkaloids, few reports also suggest that treatment with vitamin E (alpha-tocopherol) effectively decelerates the progression of AD in early to mid-stage. A clinical trial has also been carried out, which indicates promising results [192].

From the aforementioned studies, it appears that various pathophysiological situations (neurodegenerative diseases and resulting dementia) in organisms can be protected by plant polyphenols. However, the mechanisms of their protective action are not because of direct unspecific antioxidant activity and seem to be the involvement of molecular interactions with various peptides, leading to amyloid formation and cross-talks with different signaling molecules.

3.4.2 Role of Natural Antioxidants in the Amelioration of Different Movement Disorders

Oxidative stress and mitochondrial dysfunction play central roles in complex multifactorial movement disorders in nigrostriatal dopaminergic neurodegeneration [193, 194], and antioxidants have become attractive therapeutic agents targeting these factors in the treatment of different movement disorders [195]. We would like to summarize the current evidence for preventing or slowing the development of movement disorders by neuroprotective antioxidant agents in this particular section. In a variety of pathology (including stroke and trauma) and diseases (including ALS and Parkinson's disease), oxidative stress has been implicated to cause nerve cell death. Use of natural antioxidants should, therefore, be reasonable to prevent these oxidative stress-induced diseases.

Dietary antioxidants (such as vitamins A, C, and E) and several phytochemicals (such as carotenoids, flavonoids, and alkaloids) are available in our daily consumables and found to be effective against the development and progression of different movement disorders. Reports from some case studies suggest that supplementation of vitamin E with diet or intake of vitamin E-rich foods lowers the possibility of the development of neurodegenerative movement disorders [196]. Alternatively, other antioxidant molecules are chosen for experimental purposes on the basis of their free radical scavenging properties, intracellular antioxidant enhancing power, ion chelation activity, and the molecule that can affect the metabolism of dopamine. A naturally occurring antioxidant compound, apocynin, structurally related to vanillin has been found to be effective against several movement-associated neurodegenerative disorders. Apocynin can be isolated from a variety of plant sources; laboratory sample was isolated from the roots of *Picrorhiza kurroa* [197]. Several researches suggest that apocynin is effective against several other neurological disorders such as ischemia, intracerebral hemorrhage, and stroke other than movement-related neurodegenerative disorders [198]. Apocynin could effectively suppress the expression of NOX-2 and prevent

microglia-mediated neurotoxicity [199]. In a study with SOD-1 mutant mice, oral administration of apocynin was found to be effective to withstand antioxidant deficiency. Again apocynin treatment found to prevent the onset of motor neuron disorder in experimental animal model of ALS. It has been also found that orally administered apocynin can easily be accumulated in the brain parenchyma of the experimental animal. This supports the activity of apocynin to mitigate the effect and production of ROS in the brain [200]. Apart from ALS, the protective effect of apocynin was also proved in experimental model of Parkinson's disease mediated by microglia [201]. Moreover, apocynin was found to be effective in "nonhuman primate Parkinson's disease model." Administration of apocynin at $100\,mg\,kg^{-1}$ 1 week before the induction of Parkinson's disease maintains the normal body physiology including body weight. Moreover, in apocynin-treated animals, the survival percentage of dopamine receptor is found to be increased, which establishes its neuroprotective efficacy.

Another pleiotropic polyphenolic drug, curcumin, is found to be effective in the management of Parkinson's disease. Unlike apocynin, curcumin was also found to increase the number of dopaminergic neurons in the substantia nigra [202]. Along with this, curcumin protects the CNS from exogenous or endogenous oxidative insult, increases the total antioxidant capacity, and reduces neuronal apoptosis. Accumulation of α-synuclein is a characteristic feature of Parkinson's disease, and different *in vitro* data suggest that curcumin significantly inhibited the accumulation of α-synuclein in SH-SY5Y cells [203]. Curcumin also protects PC12 cell death from oxidative stress induced by A53T α-synuclein. It maintains mitochondrial homeostasis and downregulates the activation of caspases [204]. Other than Parkinson's disease, curcumin has shown the ameliorative effect on mutant TDP-43-induced experimental animal model of ALS [205].

In a study with the polyphenolic antioxidant molecule mangiferin, it has been found that oral administration significantly attenuated the effect of MPTP-induced pathophysiology in the brain mediated by oxidative stress. The results from that particular study confirm that mangiferin not only maintains intracellular homeostasis but also prevents behavioral deficits [206]. In an another study with MPTP-induced experimental mouse model of Parkinson's disease, mangiferin improved the behavioral deficits in mice tested by using different tests such as open field test and catwalk test. Moreover, mangiferin also ameliorated the oxidative stress condition in the tissue sample and maintained homeostasis in the level of dopamine [207].

A study with an estrogen such as soy plant-derived antioxidant molecule, genistein, showed the ameliorative effect of the neuron in the substantia nigra pars compacta (SNC) against neuronal degeneration induced by 6-hydroxydopamine. Since genistein possesses structural similarity with estrogens, it may have estrogen-like properties and can activate different signaling cascades to protect the neuronal cells through mitochondria-dependent manner [208, 209] and enhance the intracellular antioxidant status and prevent lipid peroxidation and protein carbonylation [210].

Along with the aforementioned neuroprotective properties of several natural antioxidants, resveratrol has been shown to be effective against the development and progression of Parkinson's disease. It can modulate one of the main neuroprotective

signaling cascades mediating SIRT1/AMPK/PGC1-α [211, 212]. In another study by Price *et al*., it has been shown that high dose of resveratrol can induce AMPK activity in an SIRT1-independent manner and decrease levels of NAD^+ and ATP; however at lower doses, resveratrol showed SIRT1-dependent activation of AMPK and found to increase the levels of both metabolites [213, 214].

3.5 Concluding Remarks

Neuronal diseases are an imperative reason for morbidity and mortality of mankind. The suffering of individuals affected by these diseases is very painful. These diseases lead to fatality and may eventually result in human death. Though the etiology of these diseases may differ, a common cause generally prevails in these types of diseases, especially in neurodegenerative diseases. The cause is the excessive generation of free radicals leading to the phenomenon commonly known as *oxidative stress*. Oxidative injury causes acute damages to the nervous system along with increased ROS formation; there is a huge depletion of endogenous antioxidants in the brain. Moreover, due to its high oxygen consumption, huge lipid levels, and high metal content in the brain, it becomes more prone to oxidative diseases compared to the other organs. Antioxidant molecules combat oxidative stress by neutralizing excessively produced free radicals and inhibiting them from initiating signaling cascades and chain reactions that result in various diseases and premature aging. In diseases such as AD, Parkinson's disease, and ALS, oxidative stress plays a persuasive role. Studies have revealed that a number of activities linked to AD potentially stimulate the generation of free radicals and exhaustion of endogenous antioxidant molecules. Again in individuals suffering from Parkinson's disease, reduced levels of GSH and increased lipid peroxidation as well as DNA oxidation as a result of free radical damage are reported; thus, confronting the free radical participation is a good therapeutic target in such diseases. So exogenous antioxidants would be a substantial therapeutic strategy for treating neuronal disorders as they would limit free radical production and slow the progression and severity of these diseases. Several natural compounds or more precisely "phytomolecules" are already selected and extensively studied concerning their antioxidant property. They have been found to be greatly effective in treating these diseases as they effectively scavenged free radicals and inhibited their generation. Moreover, the detrimental signaling cascades activated by these free radicals are also significantly inhibited by these natural compounds. This chapter covered the sources of antioxidants and the general mechanism by which they play a protective role in different cognitive and movement disorders. Major emphasis has been given on the role of various natural molecules such as apocyanin, curcumin, and so on in major neurodegenerative disorders, namely, AD, Parkinson's disease, and ALS.

So far in this chapter, we have discussed the pathogenesis of some clinically relevant neurodegenerative disorders and ameliorative effect of some naturally occurring antioxidant molecules, which exhibited promising neuroprotective role against the neurological pathophysiology. It has been found that many naturally

occurring antioxidant and plant extracts showed prophylactic activities against several cognitive and movement-related neurological disorders, but future research should be focused on the dose of these compounds. Secondly researches must also focus on the bioavailability and biodistribution of those particular plant compounds because most of these compounds are not soluble in aqueous medium. It should also be kept in mind that free radicals are important for normal growth and development of any living organisms; however, excessive administration of such compounds can be responsible for different fatal stress conditions. Future studies must also focus on the elucidation of different molecular pathways activated due to the pathogenesis of neurodegeneration and different signaling pathways activated by natural compounds to overcome those pathophysiological conditions. A better comprehension of the molecular pathway would help scientists to design and synthesize different synthetic derivative from the promising natural compounds.

Abbreviations

Aβ	beta-amyloid
AchE	acetylcholinesterase
ADHD	attention deficit hyperactivity disorder
ALS	amyotrophic lateral sclerosis
APP	amyloid precursor protein
ATP	adenosine triphosphate
BDNF	brain-derived neurotrophic factor
CNS	central nervous system
ER	endoplasmic reticulum
ERK1/2	extracellular signal-regulated kinases 1/2
ETC	electron transport chain
GSH	glutathione
GSSG	glutathione disulfide
GST	glutathione-S-transferase
HMOX1	Heme oxygenase
H2O2	hydrogen peroxide
$HO^{\bullet}$	hydroxyl
MDA	malondialdehyde
MMP	mitochondrial membrane potential
MND	motor neuron disease
NAD(P)H	nicotinamide adenine dinucleotide phosphate
NO	nitric oxide
$NO^{\bullet}$	nitric monoxide
NQO1	quinone oxidoreductase 1
Nrf2	nuclear factor-like 2
$O_2^{\bullet-}$	superoxide
ONOO–	peroxynitrite
PNS	peripheral nervous system

PS-1	presenilin-1
PUFA	polyunsaturated fatty acids
RNS	reactive nitrogen species
ROS	reactive oxygen species
SOD	superoxide dismutase
UGT	UDP-glucuronosyltransferases

References

1 Kandola, K., Bowman, A., and Birch-Machin, M.A. (2015) Oxidative stress-a key emerging impact factor in health, ageing, lifestyle and aesthetics. *Int. J. Cosmet. Sci.*, **37** (Suppl. 2), 1–8.

2 Sadhukhan, P., Saha, S., and Sil, P. (2015) Targeting oxidative stress: a novel approach in mitigating cancer. *Biochem. Anal. Biochem.*, **4** (236), 1000236.

3 Gorrini, C., Harris, I.S., and Mak, T.W. (2013) Modulation of oxidative stress as an anticancer strategy. *Nat. Rev. Drug Discovery*, **12** (12), 931–947.

4 Kim, G.H., Kim, J.E., Rhie, S.J., and Yoon, S. (2015) The role of oxidative stress in neurodegenerative diseases. *Exp. Neurobiol.*, **24** (4), 325–340.

5 Chen, X., Guo, C., and Kong, J. (2012) Oxidative stress in neurodegenerative diseases. *Neural Regener. Res.*, **7** (5), 376–385.

6 Gong, J., Zhu, T., Kipen, H., Wang, G., Hu, M., Ohman-Strickland, P., Lu, S.E., Zhang, L., Wang, Y., Zhu, P. *et al.* (2013) Malondialdehyde in exhaled breath condensate and urine as a biomarker of air pollution induced oxidative stress. *J. Exposure Sci. Environ. Epidemiol.*, **23** (3), 322–327.

7 Barnham, K.J., Masters, C.L., and Bush, A.I. (2004) Neurodegenerative diseases and oxidative stress. *Nat. Rev. Drug Discovery*, **3** (3), 205–214.

8 Kingwell, K. (2009) Lung disease: antioxidant protection against cigarette smoke. *Nat. Rev. Drug Discovery*, **8** (2), 110.

9 Allison, S.J. (2016) Hypertension: oxidative stress and immune activation in hypertension. *Nat. Rev. Nephrol.*, **12** (1), 4.

10 Lobo, V., Patil, A., Phatak, A., and Chandra, N. (2010) Free radicals, antioxidants and functional foods: impact on human health. *Pharmacogn. Rev.*, **4** (8), 118–126.

11 Jomova, K., Vondrakova, D., Lawson, M., and Valko, M. (2010) Metals, oxidative stress and neurodegenerative disorders. *Mol. Cell. Biochem.*, **345** (1-2), 91–104.

12 Weinberg, F., Hamanaka, R., Wheaton, W.W., Weinberg, S., Joseph, J., Lopez, M., Kalyanaraman, B., Mutlu, G.M., Budinger, G.R., and Chandel, N.S. (2010) Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. *Proc. Natl. Acad. Sci. U.S.A.*, **107** (19), 8788–8793.

13 Rosselli, M., Keller, P.J., and Dubey, R.K. (1998) Role of nitric oxide in the biology, physiology and pathophysiology of reproduction. *Hum. Reprod. Update*, **4** (1), 3–24.

14 Katsuyama, Y., Tsuboi, T., Taira, N., Yoshioka, M., and Masaki, H. (2016) 3-*O*-Laurylglyceryl ascorbate activates the intracellular antioxidant system through the contribution of PPAR-gamma and Nrf2. *J. Dermatol. Sci.*, **82** (3), 189–196.

15 Qaisiya, M., Coda Zabetta, C.D., Bellarosa, C., and Tiribelli, C. (2014) Bilirubin mediated oxidative stress involves antioxidant response activation via Nrf2 pathway. *Cell. Signalling*, **26** (3), 512–520.

16 Chaudiere, J. and Ferrari-Iliou, R. (1999) Intracellular antioxidants: from chemical to biochemical mechanisms. *Food Chem. Toxicol.*, **37** (9-10), 949–962.

17 Couto, N., Wood, J., and Barber, J. (2016) The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. *Free Radical Biol. Med.*, **95**, 27–42.

18 Lu, J. and Holmgren, A. (2014) The thioredoxin antioxidant system. *Free Radical Biol. Med.*, **66**, 75–87.

19 Dodson, M., Redmann, M., Rajasekaran, N.S., Darley-Usmar, V., and Zhang, J. (2015) KEAP1-NRF2 signalling and autophagy in protection against oxidative and reductive proteotoxicity. *Biochem. J.*, **469** (3), 347–355.

20 Rashid, K., Sinha, K., and Sil, P.C. (2013) An update on oxidative stress-mediated organ pathophysiology. *Food Chem. Toxicol.*, **62**, 584–600.

21 Bhattacharya, S., Gachhui, R., and Sil, P.C. (2013) Effect of Kombucha, a fermented black tea in attenuating oxidative stress mediated tissue damage in alloxan induced diabetic rats. *Food Chem. Toxicol.*, **60**, 328–340.

22 Sarkar, A., Ghosh, M., and Sil, P.C. (2014) Nanotoxicity: oxidative stress mediated toxicity of metal and metal oxide nanoparticles. *J. Nanosci. Nanotechnol.*, **14** (1), 730–743.

23 Shrivastava, R., Raza, S., Yadav, A., Kushwaha, P., and Flora, S.J. (2014) Effects of sub-acute exposure to TiO_2, ZnO and Al_2O_3 nanoparticles on oxidative stress and histological changes in mouse liver and brain. *Drug Chem. Toxicol.*, **37** (3), 336–347.

24 Grimm, A., Mensah-Nyagan, A.G., and Eckert, A. (2016) Alzheimer, mitochondria and gender. *Neurosci. Biobehav. Rev.*, **67**, 89–101.

25 Onyango, I.G., Dennis, J., and Khan, S.M. (2016) Mitochondrial dysfunction in Alzheimer's disease and the rationale for bioenergetics based therapies. *Aging Dis.*, **7** (2), 201–214.

26 Park, M.H., Jo, M., Kim, Y.R., Lee, C.K., and Hong, J.T. (2016) Roles of peroxiredoxins in cancer, neurodegenerative diseases and inflammatory diseases. *Pharmacol. Ther.*, **163**, 1–23.

27 Radak, Z., Marton, O., Nagy, E., Koltai, E., and Goto, S. (2013) The complex role of physical exercise and reactive oxygen species on brain. *J. Sport Health Sci.*, **2** (2), 87–93.

28 Wagner, P.A., Mitran, S., Sivanesan, S., Chang, E., and Buga, A.-M. (2013) ROS and brain diseases: the good, the bad, and the ugly. *Oxid. Med. Cell Longev.*, **2013**, (963520).

29 Anthonymuthu, T.S., Kenny, E.M., and Bayir, H. (2016) Therapies targeting lipid peroxidation in traumatic brain injury. *Brain Res.*, **1640**, 57–76.

30 Liu, J.J., Green, P., John Mann, J., Rapoport, S.I., and Sublette, M.E. (2015) Pathways of polyunsaturated fatty acid utilization: implications for brain function in neuropsychiatric health and disease. *Brain Res.*, **1597**, 220–246.

31 Wilhelm, I., Nyul-Toth, A., Suciu, M., Hermenean, A., and Krizbai, I.A. (2016) Heterogeneity of the blood-brain barrier. *Tissue Barriers*, **4** (1), e1143544.

32 Sandhir, R., Yadav, A., Sunkaria, A., and Singhal, N. (2015) Nano-antioxidants: an emerging strategy for intervention against neurodegenerative conditions. *Neurochem. Int.*, **89**, 209–226.

33 Keogh, M.J. and Chinnery, P.F. (2015) Mitochondrial DNA mutations in neurodegeneration. *Biochim. Biophys. Acta*, **1847** (11), 1401–1411.

34 Cha, M.Y., Kim, D.K., and Mook-Jung, I. (2015) The role of mitochondrial DNA mutation on neurodegenerative diseases. *Exp. Mol. Med.*, **47**, e150.

35 Calabrese, V., Lodi, R., Tonon, C., D'Agata, V., Sapienza, M., Scapagnini, G., Mangiameli, A., Pennisi, G., Stella, A.M., and Butterfield, D.A. (2005) Oxidative stress, mitochondrial dysfunction and cellular stress response in Friedreich's ataxia. *J. Neurol. Sci.*, **233** (1-2), 145–162.

36 Barrera, G., Gentile, F., Pizzimenti, S., Canuto, R.A., Daga, M., Arcaro, A., Cetrangolo, G.P., Lepore, A., Ferretti, C., Dianzani, C. *et al.* (2016) Mitochondrial dysfunction in cancer and neurodegenerative diseases: spotlight on fatty acid oxidation and lipoperoxidation products. *Antioxidants* (Basel), **5** (1), 7.

37 Calabrese, V., Cornelius, C., Mancuso, C., Lentile, R., Stella, A.M., and Butterfield, D.A. (2010) Redox homeostasis and cellular stress response in aging and neurodegeneration. *Methods Mol. Biol.*, **610**, 285–308.

38 Silva, B.J., Seca, A.M., Barreto, M.C., and Pinto, D.C. (2015) Recent breakthroughs in the antioxidant and anti-inflammatory effects of morella and myrica species. *Int. J. Mol. Sci.*, **16** (8), 17160–17180.

39 Ghosh, S., Banerjee, S., and Sil, P.C. (2015) The beneficial role of curcumin on inflammation, diabetes and neurodegenerative disease: a recent update. *Food Chem. Toxicol.*, **83**, 111–124.

40 Bhattacharyya, S., Sinha, K., and Sil, P.C. (2014) Cytochrome P450s: mechanisms and biological implications in drug metabolism and its interaction with oxidative stress. *Curr. Drug Metab.*, **15** (7), 719–742.

41 Saha, S., Sadhukhan, P., and Sil, P.C. (2014) Genistein: a phytoestrogen with multifaceted therapeutic properties. *Mini. Rev. Med. Chem.*, **14** (11), 920–940.

42 Sinha, K., Sadhukhan, P., Saha, S., Pal, P.B., and Sil, P.C. (2015) Morin protects gastric mucosa from nonsteroidal anti-inflammatory drug, indomethacin induced inflammatory damage and apoptosis by modulating NF-kappaB pathway. *Biochim. Biophys. Acta*, **1850** (4), 769–783.

43 Saha, S., Sadhukhan, P., Sinha, K., Agarwal, N., and Sil, P.C. (2016) Mangiferin attenuates oxidative stress induced renal cell damage through activation of PI3K induced Akt and Nrf-2 mediated signalling pathways. *Biochem. Biophys. Rep.*, **5**, 313–327.

44 Agarwal, N., Sadhukhan, P., Saha, S., and Sil, P.C. (2015) Therapeutic insights against oxidative stress induced diabetic nephropathy: a review. *J. Autoimmune Disord.*, **1** (1:2), 1–16.

45 Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., and Telser, J. (2007) Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.*, **39** (1), 44–84.

46 Brahmachari, G. (2015) Sulfamic acid-catalyzed one-pot room temperature synthesis of biologically relevant bis-lawsone derivatives. *ACS Sustainable Chem. Eng.*, **3** (9), 2058–2066.

47 Chandra, K., Salman, A.S., Mohd, A., Sweety, R., and Ali, K.N. (2015) Protection against FCA induced oxidative stress induced DNA damage as a model of arthritis and in vitro anti-arthritic potential of costus speciosus rhizome extract. *Inter. J. Pharma. Phyto. Res.*, **7** (2), 383–389.

48 Seaver, L.C. and Imlay, J.A. (2004) Are respiratory enzymes the primary sources of intracellular hydrogen peroxide? *J. Biol. Chem.*, **279** (47), 48742–48750.

49 Messner, K.R. and Imlay, J.A. (2002) Mechanism of superoxide and hydrogen peroxide formation by fumarate reductase, succinate dehydrogenase, and aspartate oxidase. *J. Biol. Chem.*, **277** (45), 42563–42571.

50 Imlay, J.A. (2003) Pathways of oxidative damage. *Annu. Rev. Microbiol.*, **57**, 395–418.

51 Hardin, S.C., Larue, C.T., Oh, M.H., Jain, V., and Huber, S.C. (2009) Coupling oxidative signals to protein phosphorylation via methionine oxidation in Arabidopsis. *Biochem. J.*, **422** (2), 305–312.

52 Meister, A. (1994) Glutathione-ascorbic acid antioxidant system in animals. *J. Biol. Chem.*, **269** (13), 9397–9400.

53 Tan, D.X., Manchester, L.C., Terron, M.P., Flores, L.J., and Reiter, R.J. (2007) One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? *J. Pineal Res.*, **42** (1), 28–42.

54 Reiter, R.J., Paredes, S.D., Manchester, L.C., and Tan, D.X. (2009) Reducing oxidative/nitrosative stress: a newly-discovered genre for melatonin. *Crit. Rev. Biochem. Mol. Biol.*, **44** (4), 175–200.

55 Tan, D.X., Manchester, L.C., Reiter, R.J., Qi, W.B., Karbownik, M., and Calvo, J.R. (2000) Significance of melatonin in antioxidative defense system: reactions and products. *Biol. Signals Recept.*, **9** (3-4), 137–159.

56 den Hartog, G., Chattopadhyay, R., Ablack, A., Hall, E., Butcher, L., Bhattacharyya, A., Eckmann, L., Harris, P., Das, S., and Ernst, P. (2016) Regulation of rac1 and reactive oxygen species production in response to infection of gastrointestinal epithelia. *PLoS Pathog.*, **12** (1), e1005382.

57 Hirsch, E. (1993) Does oxidative stress participate in nerve cell death in Parkinson's disease? *Eur. Neurol.*, **33** (Suppl. 1), 52–59.

58 Hwang, O. (2013) Role of oxidative stress in Parkinson's disease. *Exp. Neurobiol.*, **22** (1), 11–17.

59 Dias, V., Junn, E., and Mouradian, M.M. (2013) The role of oxidative stress in Parkinson's disease. *J. Parkinson's Dis.*, **3** (4), 461–491.

60 Lin, M.T. and Beal, M.F. (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature*, **443** (7113), 787–795.

61 Behl, C. (1999) Alzheimer's disease and oxidative stress: implications for novel therapeutic approaches. *Prog. Neurobiol.*, **57** (3), 301–323.

62 Pohanka, M. (2014) Alzheimer s disease and oxidative stress: a review. *Curr. Med. Chem.*, **21** (3), 356–364.

63 Scheff, S.W., Ansari, M.A., and Mufson, E.J. (2016) Oxidative stress and hippocampal synaptic protein levels in elderly cognitively intact individuals with Alzheimer's disease pathology. *Neurobiol. Aging*, **42**, 1–12.

64 Fang, D., Zhang, Z., Li, H., Yu, Q., Douglas, J.T., Bratasz, A., Kuppusamy, P., and Yan, S.S. (2016) Increased electron paramagnetic resonance signal correlates with mitochondrial dysfunction and oxidative stress in an Alzheimer's disease mouse brain. *J. Alzheimers Dis.*, **51** (2), 571–580.

65 Kamat, P.K., Kalani, A., Rai, S., Swarnkar, S., Tota, S., Nath, C., and Tyagi, N. (2016) Mechanism of oxidative stress and synapse dysfunction in the pathogenesis of Alzheimer's disease: understanding the therapeutics strategies. *Mol. Neurobiol.*, **53** (1), 648–661.

66 Parellada, M., Moreno, C., Mac-Dowell, K., Leza, J.C., Giraldez, M., Bailón, C., Castro, C., Miranda-Azpiazu, P., Fraguas, D., and Arango, C. (2012) Plasma antioxidant capacity is reduced in Asperger syndrome. *J. Psychiatr. Res.*, **46** (3), 394–401.

67 Ng, F., Berk, M., Dean, O., and Bush, A.I. (2008) Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. *Int. J. Neuropsychopharmacol.*, **11** (6), 851–876.

68 Joseph, N., Zhang-James, Y., Perl, A., and Faraone, S.V. (2013) Oxidative stress and ADHD: a meta-analysis. *J. Attention Disord.*, **19** (11), 915–924.

69 Prabakaran, S., Swatton, J., Ryan, M., Huffaker, S., Huang, J.J., Griffin, J., Wayland, M., Freeman, T., Dudbridge, F., and Lilley, K. (2004) Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. *Mol. Psychiatry*, **9** (7), 684–697.

70 Mahadik, S.P., Evans, D., and Lal, H. (2001) Oxidative stress and role of antioxidant and ω-3 essential fatty acid supplementation in schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **25** (3), 463–493.

71 Tosic, M., Ott, J., Barral, S., Bovet, P., Deppen, P., Gheorghita, F., Matthey, M.L., Parnas, J., Preisig, M., and Saraga, M. (2006) Schizophrenia and oxidative stress: glutamate cysteine ligase modifier as a susceptibility gene. *Am. J. Hum. Genet.*, **79** (3), 586–592.

72 Bitanihirwe, B.K. and Woo, T.U.W. (2011) Oxidative stress in schizophrenia: an integrated approach. *Neurosci. Biobehav. Rev.*, **35** (3), 878–893.

73 Kennedy, G., Spence, V.A., McLaren, M., Hill, A., Underwood, C., and Belch, J.J. (2005) Oxidative stress levels are raised in chronic fatigue syndrome and are associated with clinical symptoms. *Free Radical Biol. Med.*, **39** (5), 584–589.

74 Logan, A.C. and Wong, C. (2001) Chronic fatigue syndrome: oxidative stress and dietary modifications. *Altern. Med. Rev.*, **6** (5), 450–460.

75 Jammes, Y., Steinberg, J., Mambrini, O., Bregeon, F., and Delliaux, S. (2005) Chronic fatigue syndrome: assessment of increased oxidative stress and altered muscle excitability in response to incremental exercise. *J. Internal. Med.*, **257** (3), 299–310.

76 Romá-Mateo, C., Aguado, C., García-Giménez, J.L., Ibáñez-Cabellos, J.S., Seco-Cervera, M., Pallardó, F.V., Knecht, E., and Sanz, P. (2015) Increased oxidative stress and impaired antioxidant response in Lafora disease. *Mol. Neurobiol.*, **51** (3), 932–946.

77 Sarandol, A., Sarandol, E., Eker, S.S., Erdinc, S., Vatansever, E., and Kirli, S. (2007) Major depressive disorder is accompanied with oxidative stress: short-term antidepressant treatment does not alter oxidative–antioxidative systems. *Hum. Psychopharmacol.*, **22** (2), 67–73.

78 Ozcan, M.E., Gulec, M., Ozerol, E., Polat, R., and Akyol, O. (2004) Antioxidant enzyme activities and oxidative stress in affective disorders. *Int. Clin. Psychopharmacol.*, **19** (2), 89–95.

79 Eren, İ., Nazıroğlu, M., and Demirdaş, A. (2007) Protective effects of lamotrigine, aripiprazole and escitalopram on depression-induced oxidative stress in rat brain. *Neurochem. Res.*, **32** (7), 1188–1195.

80 Wu, Y., Reece, E.A., Zhong, J., Dong, D., Shen, W.B., Harman, C.R., and Yang, P. (2016) Type 2 diabetes mellitus induces congenital heart defects in murine embryos by increasing oxidative stress, endoplasmic reticulum stress, and apoptosis. *Am. J. Obstetrics Gynecology*, **215** (3), 366.e1–366.e10.

81 Santin, Y., Sicard, P., Vigneron, F., Guilbeau-Frugier, C., Dutaur, M., Lairez, O., Couderc, B., Manni, D., Korolchuk, V.I., and Lezoualc'h, F. (2016) Oxidative stress by monoamine oxidase-A impairs transcription factor EB activation and autophagosome clearance, leading to cardiomyocyte necrosis and heart failure. *Antioxid. Redox Signaling*, **25** (1), 10–27.

82 Emelyanova, L., Petrovic, M., Holmuhamedov, E., Rizvi, F., Ross, G., Kress, D.C., Tajik, A.J., and Jahangir, A. (2016) Diabetes mellitus is associated with impaired mitochondrial oxidative phosphorylation system and increased oxidative stress in human atria. *J. Am. Coll. Cardiol.*, **67** (13), 1394.

83 Yang, B., Cao, F., Zhao, H., Zhang, J., Jiang, B., and Wu, Q. (2016) Betanin ameliorates isoproterenol-induced acute myocardial infarction through iNOS, inflammation, oxidative stress-myeloperoxidase/low-density lipoprotein in rat. *Int. J. Clin. Exp. Pathol.*, **9** (3), 2777–2786.

84 Hassan, M., Akhtar, M., Akhtar, M., Ali, J., Haque, S., and Najmi, A. (2016) Edaravone, a potent free radical scavenger and a calcium channel blocker attenuate isoproterenol induced myocardial infarction by suppressing oxidative stress, apoptotic signaling and ultrastructural damage. *Ther. Adv. Cardiovasc. Dis.*, **10** (4), 214–223.

85 Sugiura, T., Dohi, Y., Yamashita, S., Hirowatari, Y., Fujii, S., and Ohte, N. (2016) Serotonin in peripheral blood reflects oxidative stress and plays a crucial role in atherosclerosis: novel insights toward holistic anti-atherothrombotic strategy. *Atherosclerosis*, **246**, 157–160.

86 Mehta, N.U., Grijalva, V., Hama, S., Wagner, A., Navab, M., Fogelman, A.M., and Reddy, S.T. (2016) Apolipoprotein E–/– mice lacking hemopexin develop increased atherosclerosis via mechanisms that include oxidative stress and altered macrophage function. *Arterioscler. Thromb. Vasc. Biol.*, **36** (6), 1152–1163.

87 Maes, M., Kallaur, A.P., Lopes, J., Oliveira, S.R., Ramon, D., and Reiche, E.M.V. (2016) Immune-inflammatory and oxidative and nitrosative stress (IO & NS) pathways in depression and multiple sclerosis (MS): shared IO & NS pathways but less hyper-acute neuro-inflammation explain the increased incidence of depression in MS. *Neurol. Psychiatry Brain Res.*, **1** (22), 16–17.

88 Kallaur, A.P., Reiche, E.M.V., Oliveira, S.R., Simão, A.N.C., Pereira, W.L.C.J., Alfieri, D.F., Flauzino, T., de Meleck Proença, C., Lozovoy, M.A.B., Kaimen-Maciel, D.R., and Maes, M. (2016) Genetic, immune-inflammatory, and oxidative stress biomarkers as predictors for disability and disease progression in multiple sclerosis. *Mol. Neurobiol.*, 1–14.

89 Ohl, K., Tenbrock, K., and Kipp, M. (2016) Oxidative stress in multiple sclerosis: central and peripheral mode of action. *Exp. Neurol.*, **277**, 58–67.

90 Belcher, J.D., Chen, C., Nguyen, J., Zhang, P., Abdulla, F., Nguyen, P., Killeen, T., Xu, P., O'Sullivan, G., and Nath, K.A. (2016) Control of oxidative stress and inflammation in sickle cell disease with the Nrf2 activator dimethyl fumarate. *Antioxid. Redox Signaling*. doi: 10.1089/ars.2015.6571.

91 Hermann, P.B., Pianovski, M.A.D., Henneberg, R., do Nascimento, A.J., and Leonart, M.S.S. (2016) Erythrocyte oxidative stress markers in children with sickle cell disease. *J. Pediatr.*, **92** (4), 394–399.

92 Mathys, L. and Balzarini, J. (2016) The role of cellular oxidoreductases in viral entry and virus infection-associated oxidative stress: potential therapeutic applications. *Expert Opin. Ther. Targets*, **20** (1), 123–143.

93 St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J.M., Rhee, J., Jäger, S., Handschin, C., Zheng, K., Lin, J., and Yang, W. (2006) Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell*, **127** (2), 397–408.

94 Jiang, J.M., Zhou, C.F., Gao, S.L., Tian, Y., Wang, C.Y., Wang, L., Gu, H.F., and Tang, X.Q. (2015) BDNF-TrkB pathway mediates neuroprotection of hydrogen sulfide against formaldehyde-induced toxicity to PC12 cells. *PLoS One*, **10** (3), 1–16.

95 Song, X., Zhou, B., Zhang, P., Lei, D., Wang, Y., Yao, G., Hayashi, T., Xia, M., Tashiro, S.I., Onodera, S. *et al.* (2016) Protective effect of silibinin on learning and memory impairment in LPS-treated rats via ROS-BDNF-TrkB pathway. *Neurochem. Res.*, **41** (7), 1662–1672.

96 Zhong, Z., Zeng, T., Xie, K., Zhang, C., Chen, J., Bi, Y., and Zhao, X. (2013) Elevation of 4-hydroxynonenal and malondialdehyde modified protein levels in cerebral cortex with cognitive dysfunction in rats exposed to 1-bromopropane. *Toxicology*, **306**, 16–23.

97 Wolozin, B. and Apicco (2015) RNA binding proteins and the genesis of neurodegenerative diseases. *Adv. Exp. Med. Biol.*, **822**, 11–15.

98 Ciryam, P., Kundra, R., Morimoto, R.I., Dobson, C.M., and Vendruscolo, M. (2015) Supersaturation is a major driving force for protein aggregation in neurodegenerative diseases. *Trends Pharmacol. Sci.*, **36** (2), 72–77.

99 Jucker, M. and Walker, L.C. (2013) Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature*, **501** (7465), 45–51.

100 Takalo, M., Salminen, A., Soininen, H., Hiltunen, M., and Haapasalo, A. (2013) Protein aggregation and degradation mechanisms in neurodegenerative diseases. *Am. J. Neurodegener. Dis.*, **2** (1), 1–14.

101 Walker, L.C., Diamond, M.I., Duff, K.E., and Hyman, B.T. (2013) Mechanisms of protein seeding in neurodegenerative diseases. *J. Am. Med. Assoc. Neurol.*, **70** (3), 304–310.

102 Kalyanaraman, B. (2013) Teaching the basics of redox biology to medical and graduate students: oxidants, antioxidants and disease mechanisms. *Redox Biol.*, **1** (1), 244–257.

103 Beckman, J.S. (2009) Understanding peroxynitrite biochemistry and its potential for treating human diseases. *Arch. Biochem. Biophys.*, **484** (2), 114–116.

104 Szabo, C., Ischiropoulos, H., and Radi, R. (2007) Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. *Nat. Rev. Drug Discov.*, **6** (8), 662–680.

105 Tieu, K., Ischiropoulos, H., and Przedborski, S. (2003) Nitric oxide and reactive oxygen species in Parkinson's disease. *Int. Union Biochem. Mol. Biol. life*, **55** (6), 329–335.

106 Brown, G.C. and Neher, J.J. (2010) Inflammatory neurodegeneration and mechanisms of microglial killing of neurons. *Mol. Neurobiol.*, **41** (2-3), 242–247.

107 Lushchak, V.I. (2014) Free radicals, reactive oxygen species, oxidative stress and its classification. *Chem. Biol. Interact.*, **224**, 164–175.

108 Halliwell, B. (1984) Manganese ions, oxidation reactions and the superoxide radical. *Neurotoxicology*, **5** (1), 113–117.

109 Dorsey, E.R., George, B.P., Leff, B., and Willis, A.W. (2013) The coming crisis: obtaining care for the growing burden of neurodegenerative conditions. *Neurology*, **80** (21), 1989–1996.

110 Lee, J., Giordano, S., and Zhang, J. (2012) Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling. *Biochem. J.*, **441** (2), 523–540.

111 Fu, A.K., Hung, K.W., Yuen, M.Y., Zhou, X., Mak, D.S., Chan, I.C., Cheung, T.H., Zhang, B., Fu, W.Y., and Liew, F.Y. (2016) IL-33 ameliorates Alzheimer's disease-like pathology and cognitive decline. *Proc. Natl. Acad. Sci. U.S.A.*, **113** (19), E2705–E2713, 201604032.

112 Torpy, J.M., Burke, A.E., and Glass, R.M. (2008) Delirium. *J. Am. Med. Assoc.*, **300** (24), 2936.

113 Rodriguez-Perdigon, M., Solas, M., Moreno-Aliaga, M.J., and Ramirez, M.J. (2016) Lipoic acid improves neuronal insulin signalling and rescues cognitive function regulating VGlut1 expression in high-fat-fed rats: implications for Alzheimer's disease. *Biochim. Biophys. Acta*, **1862** (4), 511–517.

114 Deak, F., Freeman, W.M., Ungvari, Z., Csiszar, A., and Sonntag, W.E. (2016) Recent developments in understanding brain aging: implications for Alzheimer's disease and vascular cognitive impairment. *J. Gerontology A Biol. Sci. Med. Sci.*, **71** (1), 13–20.

115 Fjell, A.M., Sneve, M.H., Grydeland, H., Storsve, A.B., and Walhovd, K.B. (2016) The disconnected brain and executive function decline in aging. *Cereb. Cortex*, bhw082, **26**. doi: 10.1093/cercor/bhw082.

116 Aarsland, D. (2016) Cognitive impairment in Parkinson's disease and dementia with lewy bodies. *Parkinsonism Rel. Dis.*, **22**, S144–S148.

117 Umegaki, H. (2016) Therapeutic potential of antidiabetic medications in the treatment of cognitive dysfunction and dementia. *Drugs Aging*. doi: 10.1007/s40266-016-0375-0.

118 Koder, D. (2016) The use of cognitive behaviour therapy in the management of BPSD in dementia (innovative practice). *Dementia*. doi: 10.1177/1471301216636261.

119 Tremblay, L., Worbe, Y., Thobois, S., Sgambato-Faure, V., and Féger, J. (2015) Selective dysfunction of basal ganglia subterritories: from movement to behavioral disorders. *Mov. Dis.*, **30** (9), 1155–1170.

120 Abbruzzese, G. and Berardelli, A. (2003) Sensorimotor integration in movement disorders. *Mov. Dis.*, **18** (3), 231–240.

121 Álvarez-Avellón, T., Arias-Carrión, Ó., and Menéndez, M. (2015) Neuropsychiatric symptoms and associated caregiver stress in geriatric patients with Parkinson's disease. *Neurol. Neurosci.*, **1**, 5.

122 Zaltieri, M., Longhena, F., Pizzi, M., Missale, C., Spano, P., and Bellucci, A. (2015) Mitochondrial dysfunction and-synuclein synaptic pathology in Parkinson's disease: who's on first? *Parkinson's Dis.*, **2015**, 108029.

123 Leroi, I., Pantula, H., McDonald, K., and Harbishettar, V. (2012) Neuropsychiatric symptoms in Parkinson's disease with mild cognitive impairment and dementia. *Parkinson's Dis.*, **2012**, 308097.

124 Irwin, D.J., Lee, V.M.Y., and Trojanowski, J.Q. (2013) Parkinson's disease dementia: convergence of [alpha]-synuclein, tau and amyloid-[beta] pathologies. *Nat. Rev. Neurosci.*, **14** (9), 626–636.

125 Maetzler, W., Pilotto, A., Apel, A., Deuschle, C., Kuebart, G., Heinzel, S., Liepelt-Scarfone, I., Schulte, C., Reusch, D., and Schleicher, E. (2014) In vivo markers of Parkinson's disease and dementia with Lewy bodies: current value of the 5G4 [alpha]-synuclein antibody. *Acta Neuropathol.*, **128** (6), 893–895.

126 Rowland, L.P. and Shneider, N.A. (2001) Amyotrophic lateral sclerosis. *N. Engl. J. Med.*, **344** (22), 1688–1700.

127 Wijesekera, L.C. and Leigh, P.N. (2009) Amyotrophic lateral sclerosis. *Orphanet J. Rare Dis.*, **4** (1), 1–22.

128 Ray, R., Juranek, J.K., and Rai, V. (2016) RAGE axis in neuroinflammation, neurodegeneration and its emerging role in the pathogenesis of amyotrophic lateral sclerosis. *Neurosci. Biobehav. Rev.*, **62**, 48–55.

129 Hooten, K.G., Beers, D.R., Zhao, W., and Appel, S.H. (2015) Protective and toxic neuroinflammation in amyotrophic lateral sclerosis. *Neurotherapeutics*, **12** (2), 364–375.

130 Hauser, D.N. and Hastings, T.G. (2013) Mitochondrial dysfunction and oxidative stress in Parkinson's disease and monogenic parkinsonism. *Neurobiol. Dis.*, **51**, 35–42.

131 Pollari, E., Goldsteins, G., Bart, G., Koistinaho, J., and Giniatullin, R. (2014) The role of oxidative stress in degeneration of the neuromuscular junction in amyotrophic lateral sclerosis. *Front. Cell. Neurosci.*, **8**, 131.

132 D'Amico, E., Factor-Litvak, P., Santella, R.M., and Mitsumoto, H. (2013) Clinical perspective on oxidative stress in sporadic amyotrophic lateral sclerosis. *Free Radical Biol. Med.*, **65**, 509–527.

133 Pal, P.B., Sinha, K., and Sil, P.C. (2014) Mangiferin attenuates diabetic nephropathy by inhibiting oxidative stress mediated signaling cascade, TNFalpha related and mitochondrial dependent apoptotic pathways in streptozotocin-induced diabetic rats. *PLoS One*, **9** (9), e107220.

134 Sinha, K., Pal, P.B., and Sil, P.C. (2014) Cadmium (Cd(2+)) exposure differentially elicits both cell proliferation and cell death related responses in SK-RC-45. *Toxicol. in Vitro*, **28** (2), 307–318.

135 Saha, S., Rashid, K., Sadhukhan, P., Agarwal, N., and Sil, P.C. (2016) Attenuative role of mangiferin in oxidative stress-mediated liver dysfunction in arsenic-intoxicated murines. *Biofactors* (Oxford). doi: 10.1002/biof.1276.

136 Rashid, K. and Sil, P.C. (2015) Curcumin enhances recovery of pancreatic islets from cellular stress induced inflammation and apoptosis in diabetic rats. *Toxicol. Appl. Pharmacol.*, **282** (3), 297–310.

137 Auddy, B., Ferreira, M., Blasina, F., Lafon, L., Arredondo, F., Dajas, F., Tripathi, P., Seal, T., and Mukherjee, B. (2003) Screening of antioxidant activity of three

Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. *J. Ethnopharmacol.*, **84** (2), 131–138.

138 Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., and Mérillon, J.M. (2009) Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *J. Agric. Food. Chem.*, **57** (5), 1768–1774.

139 Zhang, S., Shao, S.Y., Song, X.Y., Xia, C.Y., Yang, Y.N., Zhang, P.C., and Chen, N.H. (2016) Protective effects of Forsythia suspense extract with antioxidant and anti-inflammatory properties in a model of rotenone induced neurotoxicity. *Neurotoxicology*, **52**, 72–83.

140 Brizi, C., Santulli, C., Micucci, M., Budriesi, R., Chiarini, A., Aldinucci, C., and Frosini, M. (2016) Neuroprotective effects of castanea sativa mill. Bark extract in human neuroblastoma cells subjected to oxidative stress. *J. Cell. Biochem.*, **117** (2), 510–520.

141 Kuete, V., Djeussi, D.E., Mbaveng, A.T., Zeino, M., and Efferth, T. (2016) Cytotoxicity of 15 cameroonian medicinal plants against drug sensitive and multi-drug resistant cancer cells. *J. Ethnopharmacol.*, **186**, 196–204.

142 Pérez-Hernández, J., Zaldívar-Machorro, V.J., Villanueva-Porras, D., Vega-Ávila, E., and Chavarría, A. (2016) A potential alternative against neurodegenerative diseases: phytodrugs. *Oxid. Med. Cell. Longev.*, **2016**, 8378613.

143 Shahpiri, Z., Bahramsoltani, R., Hosein Farzaei, M., Farzaei, F., and Rahimi, R. (2016) Phytochemicals as future drugs for Parkinson's disease: a comprehensive review. *Rev. Neurosci.*, **27** (6), 651–668.

144 Bhattacharya, S., Manna, P., Gachhui, R., and Sil, P.C. (2011) Protective effect of kombucha tea against tertiary butyl hydroperoxide induced cytotoxicity and cell death in murine hepatocytes. *Indian J. Exp. Biol.*, **49** (7), 511–524.

145 Ghosh, J., Das, J., Manna, P., and Sil, P.C. (2011) The protective role of arjunolic acid against doxorubicin induced intracellular ROS dependent JNK-p38 and p53-mediated cardiac apoptosis. *Biomaterials*, **32** (21), 4857–4866.

146 Das, J., Ghosh, J., Manna, P., and Sil, P.C. (2012) Taurine protects rat testes against doxorubicin-induced oxidative stress as well as p53, Fas and caspase 12-mediated apoptosis. *Amino Acids*, **42** (5), 1839–1855.

147 Pal, S., Pal, P.B., Das, J., and Sil, P.C. (2011) Involvement of both intrinsic and extrinsic pathways in hepatoprotection of arjunolic acid against cadmium induced acute damage in vitro. *Toxicology*, **283** (2-3), 129–139.

148 Das, J., Ghosh, J., Manna, P., and Sil, P.C. (2011) Taurine suppresses doxorubicin-triggered oxidative stress and cardiac apoptosis in rat via up-regulation of PI3-K/Akt and inhibition of p53, p38-JNK. *Biochem. Pharmacol.*, **81** (7), 891–909.

149 Manna, P., Das, J., Ghosh, J., and Sil, P.C. (2010) Contribution of type 1 diabetes to rat liver dysfunction and cellular damage via activation of NOS, PARP, IkappaBalpha/NF-kappaB, MAPKs, and mitochondria-dependent pathways: prophylactic role of arjunolic acid. *Free Radic. Biol. Med.*, **48** (11), 1465–1484.

150 Bhattacharya, S., Manna, P., Gachhui, R., and Sil, P.C. (2013) d-Saccharic acid 1,4-lactone protects diabetic rat kidney by ameliorating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines via NF-kappaB and PKC signaling. *Toxicol. Appl. Pharmacol.*, **267** (1), 16–29.

151 Pal, P.B., Sinha, K., and Sil, P.C. (2013) Mangiferin, a natural xanthone, protects murine liver in Pb(II) induced hepatic damage and cell death via MAP kinase, NF-kappaB and mitochondria dependent pathways. *PLoS One*, **8** (2), e56894.

152 Feng, Y., Cui, Y., Gao, J.L., Li, R., Jiang, X.H., Tian, Y.X., Wang, K.J., Li, M.H., Zhang, H.A., and Cui, J.Z. (2016) Neuroprotective effects of resveratrol against traumatic brain injury in rats: involvement of synaptic proteins and neuronal autophagy. *Mol. Med. Rep.*, **13** (6), 5248–5254.

153 Ghosh, J. and Sil, P.C. (2013) Arjunolic acid: a new multifunctional therapeutic promise of alternative medicine. *Biochimie*, **95** (6), 1098–1109.

154 Chen, B., Rao, J., Ding, Y., McClements, D.J., and Decker, E.A. (2016) Lipid oxidation in base algae oil and water-in-algae oil emulsion: impact of natural antioxidants and emulsifiers. *Food Res. Int.*, **85**, 162–169.

155 Valko, M., Jomova, K., Rhodes, C.J., Kuča, K., and Musílek, K. (2016) Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. *Arch. Toxicol.*, **90** (1), 1–37.

156 Ortiz-López, L., Márquez-Valadez, B., Gómez-Sánchez, A., Silva-Lucero, M., Torres-Pérez, M., Téllez-Ballesteros, R., Ichwan, M., Meraz-Ríos, M., Kempermann, G., and Ramírez-Rodríguez, G. (2016) Green tea compound epigallo-catechin-3-gallate (EGCG) increases neuronal survival in adult hippocampal neurogenesis in vivo and in vitro. *Neuroscience*, **322**, 208–220.

157 Santamaría-del Ángel, D., Labra-Ruíz, N.A., García-Cruz, M.E., Calderón-Guzmán, D., Valenzuela-Peraza, A., and Juárez-Olguín, H. (2016) Comparative effects of catechin, epicatechin and N-Ω-nitroarginine on quinolinic acid-induced oxidative stress in rat striatum slices. *Biomed. Pharmacother.*, **78**, 210–215.

158 Alonso, C., Lucas, R., Barba, C., Marti, M., Rubio, L., Comelles, F., Morales, J.C., Coderch, L., and Parra, J.L. (2015) Skin delivery of antioxidant surfactants based on gallic acid and hydroxytyrosol. *J. Pharm. Pharmacol.*, **67** (7), 900–908.

159 Irshad, S., Pragyadeep, S., Rawat, A.K.S., Misra, P., and Khatoon, S. (2015) Comparative pharmacognostical studies of two medicinally important Indian evolvulus species. *Indian J. Tradit. Knowl.*, **14** (4), 564–570.

160 Menze, E.T., Esmat, A., Tadros, M.G., Abdel Naim, A.B., and Khalifa, A.E. (2015) Genistein improves 3-NPA-induced memory impairment in ovariectomized rats: impact of its antioxidant, anti-inflammatory and acetylcholinesterase modulatory properties. *PLoS One*, **10** (2), e0117223.

161 Coelho, V.R., Vieira, C.G., de Souza, L.P., Moysés, F., Basso, C., Papke, D.K.M., Pires, T.R., Siqueira, I.R., Picada, J.N., and Pereira, P. (2015) Antiepileptogenic, antioxidant and genotoxic evaluation of rosmarinic acid and its metabolite caffeic acid in mice. *Life Sci.*, **122**, 65–71.

162 Yang, R.Y., Zhao, G., Wang, D.M., Pang, X.C., Wang, S.B., Fang, J.S., Li, C., Liu, A.L., Wu, S., and Du, G.H. (2015) DL0410 can reverse cognitive impairment, synaptic loss and reduce plaque load in APP/PS1 transgenic mice. *Pharmacol. Biochem. Behav.*, **139**, 15–26.

163 Shen, L., Yan, M., and He, L. (2016) D5 receptor agonist 027075 promotes cognitive function recovery and neurogenesis in a Aβ 1-42-induced mouse model. *Neuropharmacology*, **105**, 72–83.

164 Gong, Y.S., Guo, J., Hu, K., Gao, Y.Q., Xie, B.J., Sun, Z.D., Yang, E.N., and Hou, F.L. (2016) Ameliorative effect of lotus seedpod proanthocyanidins on

cognitive impairment and brain aging induced by d-galactose. *Exp. Gerontol.*, **74**, 21–28.

165 Almeida, S., Alves, M.G., Sousa, M., Oliveira, P.F., and Silva, B.M. (2016) Are polyphenols strong dietary agents against neurotoxicity and neurodegeneration? *Neurotox. Res.*, 1–22.

166 Banerjee, S., Ghosh, J., and Sil, P.C. (2016) Drug metabolism and oxidative stress: cellular mechanism and new therapeutic insights. *Biochem. Anal. Biochem.*, **5** (255). doi: 10.4172/2161-1009.1000255

167 Pal, P.B., Sinha, K., and Sil, P.C. (2014) Mangiferin attenuates diabetic nephropathy by inhibiting oxidative stress mediated signaling cascade, TNFα related and mitochondrial dependent apoptotic pathways in streptozotocin-induced diabetic rats. *PLoS One*, **9** (9), e107220.

168 Sadhukhan, P., Saha, S., and Sil, P.C. (2016) Anti-oxidative effect of genistein and mangiferin on sodium fluoride induced oxidative insult of renal cells: a comparative study. *Biomark. J.*, **2**, 1.

169 Ebrahimi, A. and Schluesener, H. (2012) Natural polyphenols against neurodegenerative disorders: potentials and pitfalls. *Ageing Res. Rev.*, **11** (2), 329–345.

170 Bhandari, P.R. (2013) Dietary approach for cognitive and behavioral problems in dementia. *Int. J. Nutr. Pharmacol. Neurol. Dis.*, **3** (4), 398–400.

171 Subash, S., Essa, M.M., Al-Adawi, S., Memon, M.A., Manivasagam, T., and Akbar, M. (2014) Neuroprotective effects of berry fruits on neurodegenerative diseases. *Neural Regener. Res.*, **9** (16), 1557–1566.

172 Qian, Z.M. and Ke, Y. (2013) Huperzine A: is it an effective disease-modifying drug for Alzheimer's disease? *Front. Aging Neurosci.*, **6**, 216.

173 Kim, H.G., Park, G., Piao, Y., Kang, M.S., Pak, Y.K., Hong, S.P., and Oh, M.S. (2014) Effects of the root bark of *Paeonia suffruticosa* on mitochondria-mediated neuroprotection in an MPTP-induced model of Parkinson's disease. *Food Chem. Toxicol.*, **65**, 293–300.

174 He, D., Wu, H., Wei, Y., Liu, W., Huang, F., Shi, H., Zhang, B., Wu, X., and Wang, C. (2015) Effects of harmine, an acetylcholinesterase inhibitor, on spatial learning and memory of APP/PS1 transgenic mice and scopolamine-induced memory impairment mice. *Eur. J. Pharmacol.*, **768**, 96–107.

175 Doig, A.J. and Derreumaux, P. (2015) Inhibition of protein aggregation and amyloid formation by small molecules. *Curr. Opin. Struct. Biol.*, **30**, 50–56.

176 Ahmed, A., Subaiea, G., Eid, A., Li, L., Seeram, N., and Zawia, N. (2014) Pomegranate extract modulates processing of amyloid-β precursor protein in an aged Alzheimer's disease animal model. *Curr. Alzheimer Res.*, **11** (9), 834–843.

177 Williams, R.J. and Spencer, J.P. (2012) Flavonoids, cognition, and dementia: actions, mechanisms, and potential therapeutic utility for Alzheimer disease. *Free Radical Biol. Med.*, **52** (1), 35–45.

178 Hayden, E.Y., Yamin, G., Beroukhim, S., Chen, B., Kibalchenko, M., Jiang, L., Ho, L., Wang, J., Pasinetti, G.M., and Teplow, D.B. (2015) Inhibiting amyloid β-protein assembly: size–activity relationships among grape seed-derived polyphenols. *J. Neurochem.*, **135** (2), 416–430.

179 Spillantini, M.G. and Goedert, M. (2013) Tau pathology and neurodegeneration. *Lancet Neurol.*, **12** (6), 609–622.

180 Santa-Maria, I., Varghese, M., Księżak-Reding, H., Dzhun, A., Wang, J., and Pasinetti, G.M. (2012) Paired helical filaments from Alzheimer disease brain induce intracellular accumulation of tau protein in aggresomes. *J. Biol. Chem.*, **287** (24), 20522–20533.

181 Moghbelinejad, S., Nassiri-Asl, M., Farivar, T.N., Abbasi, E., Sheikhi, M., Taghiloo, M., Farsad, F., Samimi, A., and Hajiali, F. (2014) Rutin activates the MAPK pathway and BDNF gene expression on beta-amyloid induced neurotoxicity in rats. *Toxicol. Lett.*, **224** (1), 108–113.

182 Davinelli, S., Sapere, N., Zella, D., Bracale, R., Intrieri, M., and Scapagnini, G. (2012) Pleiotropic protective effects of phytochemicals in Alzheimer's disease. *Oxid. Med. Cell. Longev.*, **2012**, 386527.

183 Park, S.K., Jung, I.C., Lee, W.K., Lee, Y.S., Park, H.K., Go, H.J., Kim, K., Lim, N.K., Hong, J.T., Ly, S.Y., and Rho, S.S. (2011) A combination of green tea extract and l-theanine improves memory and attention in subjects with mild cognitive impairment: a double-blind placebo-controlled study. *J. Med. Food*, **14** (4), 334–343.

184 Vauzour, D. (2014) Effect of flavonoids on learning, memory and neurocognitive performance: relevance and potential implications for Alzheimer's disease pathophysiology. *J. Sci. Food Agric.*, **94** (6), 1042–1056.

185 Cheng, D., Xi, Y., Cao, J., Cao, D., Ma, Y., and Jiang, W. (2014) Protective effect of apple (Ralls) polyphenol extract against aluminum-induced cognitive impairment and oxidative damage in rat. *Neurotoxicology*, **45**, 111–120.

186 Mori, T., Rezai-Zadeh, K., Koyama, N., Arendash, G.W., Yamaguchi, H., Kakuda, N., Horikoshi-Sakuraba, Y., Tan, J., and Town, T. (2012) Tannic acid is a natural β-secretase inhibitor that prevents cognitive impairment and mitigates Alzheimer-like pathology in transgenic mice. *J. Biol. Chem.*, **287** (9), 6912–6927.

187 Sakurai, T., Kitadate, K., Nishioka, H., Fujii, H., Ogasawara, J., Kizaki, T., Sato, S., Fujiwara, T., Akagawa, K., and Izawa, T. (2013) Oligomerised lychee fruit-derived polyphenol attenuates cognitive impairment in senescence-accelerated mice and endoplasmic reticulum stress in neuronal cells. *Br. J. Nutr.*, **110** (9), 1549–1558.

188 Yi, J.H., Park, H.J., Lee, S., Jung, J.W., Kim, B.C., Lee, Y.C., Ryu, J.H., and Kim, D.H. (2016) *Cassia obtusifolia* seed ameliorates amyloid β-induced synaptic dysfunction through anti-inflammatory and Akt/GSK-3β pathways. *J. Ethnopharmacol.*, **178**, 50–57.

189 Semwal, P., Kapoor, T., Anthwal, P., Sati, B., and Thapliyal, A. (2014) Herbal extract as potential modulator and drug for synaptic plasticity and neurodegenerative disorders. *Int. J. Pharm. Sci. Rev. Res.*, **25** (1), 69–79.

190 Jo, Y.N., Jin, D.E., Jeong, J.H., Kim, H.J., Kim, D.O., and Heo, H.J. (2015) Effect of anthocyanins from rabbit-eye blueberry (*Vaccinium virgatum*) on cognitive function in mice under trimethyltin-induced neurotoxicity. *Food Sci. Biotechnol.*, **24** (3), 1077–1085.

191 Mao, Q.Q., Huang, Z., Zhong, X.M., Xian, Y.F., and Ip, S.P. (2014) Piperine reverses the effects of corticosterone on behavior and hippocampal BDNF expression in mice. *Neurochem. Int.*, **74**, 36–41.

192 Pérez, H.J., Zaldívar-Machorro, V.J., Villanueva-Porras, D., Vega-Ávila, E., and Chavarría, A. (2016) A potential alternative against neurodegenerative diseases: phytodrugs. *Oxid. Med. Cell. Longev.*, **2016**, 8378613.

193 Andersen, J.K. (2004) Oxidative stress in neurodegeneration: cause or consequence? *Nat. Rev. Neurosci.*, **5**, S18–S25.

194 Deng, Y., Jiao, C., Mi, C., Xu, B., Li, Y., Wang, F., Liu, W., and Xu, Z. (2015) Melatonin inhibits manganese-induced motor dysfunction and neuronal loss in mice: involvement of oxidative stress and dopaminergic neurodegeneration. *Mol. Neurobiol.*, **51** (1), 68–88.

195 Lei, X.G., Zhu, J.H., Cheng, W.H., Bao, Y., Ho, Y.S., Reddi, A.R., Holmgren, A., and Arnér, E.S. (2016) Paradoxical roles of antioxidant enzymes: basic mechanisms and health implications. *Physiol. Rev.*, **96** (1), 307–364.

196 Coyle, J.T. and Puttfarcken, P. (1993) Oxidative stress, glutamate, and neurodegenerative disorders. *Science*, **262** (5134), 689–695.

197 Engels, F., Renirie, B.F., 't Hart, B.A., Labadie, R.P., and Nijkamp, F.P. (1992) Effects of apocynin, a drug isolated from the roots of *Picrorhiza kurroa*, on arachidonic acid metabolism. *FEBS Lett.*, **305** (3), 254–256.

198 Stefanska, J. and Pawliczak, R. (2008) Apocynin: molecular aptitudes. *Mediators Inflammation*, **2008**, 106507.

199 Nayernia, Z., Jaquet, V., and Krause, K.H. (2014) New insights on NOX enzymes in the central nervous system. *Antioxid. Redox Signaling*, **20** (17), 2815–2837.

200 Simonyi, A., Serfozo, P., Lehmidi, T.M., Cui, J., Gu, Z., Lubahn, D.B., Sun, A.Y., and Sun, G.Y. (2012) The neuroprotective effects of apocynin. *Front. Biosci.*, **4**, 2183–2193.

201 Gao, H.M., Hong, J.S., Zhang, W., and Liu, B. (2002) Distinct role for microglia in rotenone-induced degeneration of dopaminergic neurons. *J. Neurosci.*, **22** (3), 782–790.

202 Vajragupta, O., Boonchoong, P., Watanabe, H., Tohda, M., Kummasud, N., and Sumanont, Y. (2003) Manganese complexes of curcumin and its derivatives: evaluation for the radical scavenging ability and neuroprotective activity. *Free Radical Biol. Med.*, **35** (12), 1632–1644.

203 Wang, M.S., Boddapati, S., Emadi, S., and Sierks, M.R. (2010) Curcumin reduces α-synuclein induced cytotoxicity in Parkinson's disease cell model. *BMC Neurosci.*, **11**, 57.

204 Liu, Z., Yu, Y., Li, X., Ross, C.A., and Smith, W.W. (2011) Curcumin protects against A53T alpha-synuclein-induced toxicity in a PC12 inducible cell model for Parkinsonism. *Pharmacol. Res.*, **63** (5), 439–444.

205 Lu, J., Duan, W., Guo, Y., Jiang, H., Li, Z., Huang, J., Hong, K., and Li, C. (2012) Mitochondrial dysfunction in human TDP-43 transfected NSC34 cell lines and the protective effect of dimethoxy curcumin. *Brain Res. Bull.*, **89** (5), 185–190.

206 Kavitha, M., Nataraj, J., Essa, M.M., Memon, M.A., and Manivasagam, T. (2013) Mangiferin attenuates MPTP induced dopaminergic neurodegeneration and improves motor impairment, redox balance and Bcl-2/Bax expression in experimental Parkinson's disease mice. *Chem. Biol. Interact.*, **206** (2), 239–247.

207 Zhang, Z., Bao, Q., Gao, G., Li, S., and Jiang, J. (2014) The post-treatment action of chinonin on MPTP-induced mice model of Parkinson's disease. *J. Pharm. Biomed. Sci.*, **6** (4), 536–544.

208 Baluchnejadmojarad, T., Roghani, M., Nadoushan, M.R.J., and Bagheri, M. (2009) Neuroprotective effect of genistein in 6-hydroxydopamine Hemi-parkinsonian rat model. *Phytother. Res.*, **23** (1), 132–135.

209 Liu, L.X., Chen, W.F., Xie, J.X., and Wong, M.S. (2008) Neuroprotective effects of genistein on dopaminergic neurons in the mice model of Parkinson's disease. *Neurosci. Res.*, **60** (2), 156–161.

210 Gupta, S.P., Patel, S., Yadav, S., Singh, A.K., Singh, S., and Singh, M.P. (2010) Involvement of nitric oxide in maneb-and paraquat-induced Parkinson's disease phenotype in mouse: is there any link with lipid peroxidation? *Neurochem. Res.*, **35** (8), 1206–1213.

211 Ferretta, A., Gaballo, A., Tanzarell, P., Piccoli, C., Capitanio, N., Nico, B., Annese, T., Di Paola, M., Dell'Aquila, C., and De Mari, M. (2014) Effect of resveratrol on mitochondrial function: implications in parkin-associated familiar Parkinson's disease. *Biochim. Biophys. Acta*, **1842** (7), 902–915.

212 Hart, N., Sarga, L., Csende, Z., Koltai, E., Koch, L.G., Britton, S.L., Davies, K.J., Kouretas, D., Wessner, B., and Radak, Z. (2013) Resveratrol enhances exercise training responses in rats selectively bred for high running performance. *Food Chem. Toxicol.*, **61**, 53–59.

213 Price, N.L., Gomes, A.P., Ling, A.J.Y., Duarte, F.V., Martin-Montalvo, A., North, B.J., Agarwal, B., Ye, L., Ramadori, G., Teodoro, J.S. *et al.* (2012) SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. *Cell Metab.*, **15** (5), 675–690.

214 Sin, T.K., Yung, B.Y., and Siu, P.M. (2015) Modulation of SIRT1-Foxo1 signaling axis by resveratrol: implications in skeletal muscle aging and insulin resistance. *Cell. Physiol. Biochem.*, **35** (2), 541–552.

4

Natural Neuroprotectives for the Management of Parkinson's Disease

Bharti Gaba[1], Shobhit Kumar[1], Shadab Md[2], Sanjula Baboota[1], Jasjeet. K. Narang[3], and Javed Ali[1]

[1] *Jamia Hamdard, Department of Pharmaceutics, Faculty of Pharmacy, Hamdard Nagar, New Delhi 110062, India*
[2] *International Medical University (IMU), School of Pharmacy, Department of Pharmaceutical Technology, Kuala Lumpur 57000, Malaysia*
[3] *Khalsa College of Pharmacy, Department of Pharmaceutics, Amritsar, India*

4.1 Introduction

Parkinson's disease (PD) is an age-related neurodegenerative disorder epitomized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the midbrain and the presence of proteinaceous deposits within eosinophilic intracytoplasmic inclusions known as *Lewy bodies* [1, 2]. These deposits are composed of molecular chaperones, α-synuclein, neurofilaments, and ubiquitin [3]. The dopaminergic neurons play an imperative role in the control of multiple brain functions, together with voluntary movement, and their degeneration leads to prominent clinical symptoms. There is a decrease in levels of dopamine (DA) in the striatum, and severe motor symptoms include resting tremor, bradykinesia, rigidity, akinesia, and postural imbalance [4]. Lewy body deposition is also coupled with non-motor features such as cognitive impairment, sleep disturbances, depression, and autonomic dysfunction [5]. The numbers of PD cases in the United States were projected to be 340 000 in 2005 and are envisaged to be double by 2030 [6]. Taking into consideration the number of undiagnosed and misdiagnosed patients, the total number of cases is estimated to be as high as 1 million [6]. The cause of the neurodegenerative processes found in PD is not entirely implicit. The cellular cause of both sporadic and genetic cases of PD is strongly considered to be multifactorial being related to protein misfolding, brain aging, mitochondrial dysfunction, iron homeostasis, environmental toxins, neuroinflammation, and formation of reactive oxygen species (ROS) [7]. Although most of PD cases are sporadic, about 5–10% are caused by genetic mutations, giving rise to rare familial forms of PD [8]. Oxidative stress consequential from the overgeneration of ROS can be derived by more than one factor; for example, autoxidation of DA leads to production of neuromelanin in a pathway that generates ROS, and oxidative stress leads to the loss of nigrostriatal dopaminergic neurons in PD [9]. Higher concentration of iron observed in dopaminergic neurons also contributes to the production of

Neuroprotective Natural Products: Clinical Aspects and Mode of Action, First Edition.
Edited by Goutam Brahmachari.

free radicals via the Fenton–Haber–Weiss reaction, which is implicated in the neurodegeneration in the substantia nigra (SN) [10]. Moreover, oxidative stress can cause protein aggregation, leading to proteolytic stress [11]. Several specific proteins have been implicated to contribute to pathogenesis of PD, including leucine-rich repeat kinase 2 (LRRK2), α-synuclein, parkin, HTRA2, DJ-1, and PTEN-induced kinase 1 (PINK1) [12, 13]. These proteins contribute to the generation of ROS through mechanisms such as ubiquitin–proteasome system impairment, altered DA storage, activated microglia-dependent neuroinflammation, and mitochondrial dysfunction [14]. The consequence of ROS generation and subsequent accumulative oxidative damage is ultimately the decrease of the dopaminergic neurons [14]. Latest reports indicate that neurodegeneration in PD has also been associated with dietary habits, where insufficiency of antioxidant components such as folic acid [15], vitamins (A, C, E, and niacin) and selenium may increase the risk of PD [16, 17]. Several conventional treatments with a variety of medications and therapies for PD are available. The most widely used pharmacotherapies that help to counteract DA loss include DA precursors (levodopa (L-DOPA)), DA receptor agonists (pramipexole, bromocriptine, ropinirole), monoamine oxidase inhibitors (selegiline, rasagiline), and catechol-*O*-methyltransferase inhibitors (entacapone, tolcapone). However, these drugs act only to rescue the remaining DA or to mimic DA loss as a consequence of cell death. They only alleviate the symptoms and do not target the underlying cause or halt the progression of the disease. The drugs are thus only temporarily effective as further loss of DA neurons is inevitable [18, 19].

There is increasing corroboration that oxidative stress is critically implicated in the pathogenesis of PD. It is therefore vital to develop a pharmacological approach to combat oxidative stress, which may reduce the risk of the disease. Exploitation of the natural neuroprotective agents as antioxidants may be a useful approach. This chapter explores the efficacy of various natural neuroprotective agents as antioxidants either as crude extracts or as isolated compounds in PD. The chapter will also focus on the mechanisms of action and the *in vitro* and *in vivo* application of natural neuroprotectives as antioxidants in experimental animal models and in patients with PD.

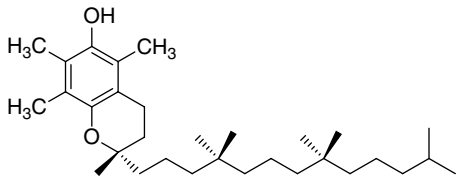
4.2 Role of Antioxidants/Natural Neuroprotectives in PD

Oxidative stress is described as an excessive production of ROS and is characterized by the reduced capacity of endogenous systems to counter the oxidative attack directed toward target biomolecules [20]. ROS are characterized by superoxide peroxynitrite; anion, hydroxyl, alkoxy, and lipid peroxyl radicals; and nitric oxide (NO). Oxidative stress damages the lipids, proteins, enzymes, carbohydrates, and DNA in cells and tissues, leading to membrane damage, fragmentation, or random cross-linking of molecules such as DNA, enzymes, and structural proteins and may even lead to cell death provoked by DNA fragmentation and lipid peroxidation [21]. These consequences of oxidative stress have been confirmed as contributory factor to the pathogenesis and pathophysiology of neurodegenerative disorders [21]. Human antioxidant defense system includes various

scavengers such as lipophilic radical scavengers (tocopherols, carotenoids, and ubiquinol), hydrophilic scavengers (urate, ascorbate, glutathione (GSH), and flavonoids), and enzymatic scavengers (superoxide dismutase (SOD), catalase (CAT), and GSH peroxidase). The defense also encompasses enzymes involved in the diminution of oxidized forms of molecular antioxidants such as GSH reductase and dehydroascorbate reductase [22]. Aside from these scavengers, cellular machinery retains a reducing environment such as the regeneration of nicotinamide adenine dinucleotide phosphate oxidase (NADPH) by glucose-6-phosphate dehydrogenase [21, 22]. A few of these agents are synthesized endogenously, whereas the majority of these such as polyphenols, ascorbic acid, carotenoids, and lipoic acid are derived from dietary sources [23]. In disease conditions, the defense against ROS is undermined, simultaneously amplifying the oxidant load. Under such circumstances, an external supply of antioxidants is necessary to counteract the lethal consequences of oxidative stress. Currently, antioxidants have attracted attention because of their ability to act as good prophylactic and therapeutic agents in numerous diseases. Nutraceuticals are those antioxidant compounds that are derived from natural origins. They established neuroprotective activity in many *in vitro* or *in vivo* models of a number of diseases such as neurodegeneration, cancer, and ischemia [24, 25]. Majority of the compounds derived from natural products may scavenge free radicals either directly or indirectly by upregulation of endogenous cellular antioxidant defense systems. This occurs via upregulation of transcription factors such as nuclear factor erythroid-derived 2-related factor 2 (Nrf2), which controls the expression of critical genes, such as thioredoxin, synuclein, sirtuin, parkin, GSH synthase, and other such proteins [26–28]. The progression and pathogenesis of PD are attributed to the low levels of the fundamental genes in the PD brain [29, 30]. Hence, an upregulation of vital genes may add on to the prevention of PD [28]. Natural products having antioxidant properties are grouped into four main categories based on their chemical structures: (i) flavonoid polyphenols (e.g., epigallocatechin 3-gallate (EGCG) found in green tea and quercetin from apples), (ii) non-flavonoid polyphenols (e.g., resveratrol from grapes and curcumin from turmeric), (iii) phenolic acids (e.g., rosmarinic acid from rosemary and sage), and (iv) organosulfur compounds (e.g., thiosulfinate allicin derived from garlic) [27]. Presently, the active ingredients from these natural products are extracted, purified, and tested for their activities. Outcomes of the studies suggest their benefits in the impediment and therapy in many of the aforementioned diseases. However a very few antioxidants are listed in pharmacopoeias, extensive research is being carried out worldwide on these agents, and majority of them have proven to be pharmacologically active.

4.2.1 Vitamin E (α-Tocopherol)

Contributions of dietary antioxidants are widely studied especially in beta-carotene, vitamin C, and vitamin E. Vitamin E is majorly found in whole grains and high-quality vegetable oils. It is implicated in the fortification of nervous membranes [31]. A dose-dependent inverse association with α-tocopherol dietary consumption related to the incidence of PD is seen [32]. It is a lipid-soluble antioxidant that effectively breaks the chain within the cell membrane, ultimately



Vitamin E (α-tocopherol)

protecting the membrane fatty acids from lipid peroxidation [33–35]. Vitamin E has shown protective effects against toxin-induced devastation of striatal DA terminals [36, 37]. Studies have shown that it prevents neuronal damage from reactive NO species and plays an important role in preventing neurodegenerative diseases such as PD that are related to oxidative stress [38–40]. Also, clinical trials were conducted, which showed that vitamin E therapy has slowed down the progression of neurodegeneration in subjects having PD [41, 42]. Increased levels of vitamin E and GSH in the brain have been shown to have reduced oxidative stress in PD patients [42–44].

4.2.1.1 Animal Studies with Vitamin E

A study in rat neurons subjected to hypoxia leading to reperfusion injury showed that vitamin E prevented neuronal damage and successive apoptosis caused by reactive nitrogen species (RNS) [45]. Another study showed that ethanol-induced oxidative stress can be managed by both vitamin E and beta-carotene on rat neurons [46]. This animal work appeared to be corroborated by a study showing that the progression of PD was slowed by 2.5 years in subjects by the administration of large doses of vitamin C and synthetic vitamin E that required L-DOPA treatment [47]. However, although high doses of vitamin E elevated plasma levels of the vitamin, there was no elevation in concentrations of the vitamin in the cerebrospinal fluid (CSF). On the other hand, it was reported that high doses of vitamin E did result in increase in CSF concentrations of vitamin E levels, as well as possibly an increase in brain vitamin E levels [48].It was shown that the protein responsible for the uptake of vitamin E is present in brain cells of patients suffering from oxidative stress associated with vitamin E deficiency [49].

Rotenone-induced decline in tyrosine hydroxylase (TH) protein and TH immunohistochemical alterations were prevented by α-tocopherol supplementation. Other results showed that 1-month vitamin E pretreatment was accredited to the neuroprotective effect of an elevated dose of vitamin E against 6-hydroxydopamine (6-OHDA)-induced toxicity, which significantly increases the TH-positive neurons [50, 51]. A study was carried out by Sharma and Nehru [52] to explore the effectiveness of vitamin E. The study was divided into four groups of Wistar rats: one control group, the second group treated with rotenone only, the third group injected with vitamin E only, and the fourth group injected with rotenone plus vitamin E. Rotenone reproduces the behavioral features of PD in rats as it is a potent inhibitor of mitochondrial complex I. It works by destroying the dopaminergic neurons, which caused deficiency of DA in the striatum leading to impaired motor functions, causing oxidative stress, which is an important factor in causing PD [53]. Following rotenone exposure, biochemical oxidative stress markers accounted a noteworthy

change. Most vigorous and considerable variation in the antioxidant defense system in PD, that is, the decline in GSH level, was observed [54, 55], with the levels decreasing significantly (47%) at the end of fifth week of rotenone exposure. Also, a decline in SOD activity was noted. In addition, significant hike in the level of malondialdehyde (MDA) generation (an indicator of lipid peroxidation) was detected at the end of third week, which increased further at fifth week [56]. This suggested that neuronal abnormalities in PD produced by rotenone are due to free radical generation and oxidative damage [57–60]. Vitamin E (100 IU kg^{-1} per body weight, i.m.) was co-administered with rotenone. Dose was estimated on the basis of body weight of the species. The results illustrated that vitamin E was able to avoid the damage of dopaminergic neurons caused by oxidative stress by acting as a free radical scavenger, thereby inhibiting cell damage [61, 62]. In addition, there was lesser consumption of GSH and SOD due to vitamin E supplementation [42].

Employing both *in vitro* and *in vivo* experimental model systems, consistent results were obtained for vitamin E-intervened protection in PD. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced experimental PD model in C57/B1 mice was used; it was observed that in stipulation of lethality and DA metabolite reduction in the SN, vitamin E-deficient mice were much more vulnerable to MPTP toxicity than control mice [63]. In contrast, a study was also reported, suggesting that α-tocopherol (100 mg kg^{-1} body weight, i.p.) given every day, 2 days before, 1 day with and 4 days after MPTP administration, did not prevent the noticeable striatal DA reduction produced by MPTP [64]. Using the same MPTP-induced experimental PD model, Perry and coworkers demonstrated an incomplete protection against the loss of dopaminergic neurons and striatal DA content in the SN, when mice were pretreated with an everyday subcutaneous injection of very high levels of vitamin E (2350 mg kg^{-1} body weight) for 48 h before and 72 h after MPTP administration [65]. In a study it was revealed that rats were protected against experimental PD when administering low dose of D-α-tocopheryl succinate (TS) (20 mg TS kg^{-1} body weight, i.m.) three times a week for 1 month simultaneously injecting intrastriatal 6-OHDA. However, the exact mechanism for this protection is unknown [66].

4.2.1.2 Human Studies with Vitamin E

The largest and the longest controlled study conducted in the world is Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP), a study that was led by Ira Shoulson, MD, and Stanley Fahn, MD, and sponsored by NINDS. It was the first of Parkinson Study Group's (PSG) multicenter trials. Use of dietary vitamin E supplementation as an approach to treat PD was investigated in clinical studies [67]. DATATOP trial was conducted by the PSG in 1989. It was a double-blinded, placebo-controlled study that involved the oral administration of 2000 IU *dl*-α-tocopherol per day to 400 PD patients. The patients were diagnosed with PD, and the stage when antiparkinson's medication needs to be started refers to the end point of the study [68, 69]. This 2-year study gave the conclusion that vitamin E supplementation did not interrupt the onset of disability related with PD as compared with the placebo. In comparison, findings from another study, which was conducted in New York City, supports the use of vitamin E in the management of clinical PD. Fahn [67] explored the effect of vitamin E

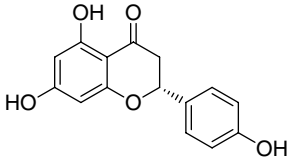
up to 12 years in 21 diagnosed PD patients, as well as that of daily consumption of 3200 IU α-tocopherol and 3000 mg of ascorbic acid along with concomitant amantadine and anticholinergics. The study revealed the ability of vitamin E/C to postpone the need for use of L-DOPA typically of 2.5 years compared to the patients not consuming these vitamins.

Farris and Zhang from the aforementioned study accomplished that following the administration of high doses, the progression of PD might be slowed down. The confliction of the two reports results may be due to the following:

1) In the Fahn trial, vitamin C might have intensified the effect of vitamin E.
2) There was a dose-dependent protection against PD following vitamin E administration, where 60% higher dose (2000 IU vs. 3200 IU) was used in the Fahn trial. The *dl* form of α-tocopherol was used in the DATATOP trial, which was as active as 50% of the *d* form.
3) Administration of high doses of α-tocopherol in PD patients from the diagnosis emerged to have been longer in the DATATOP trial (The Parkinson Study Group, 1989) than in the Fahn study.
4) The Fahn trial was not blinded or controlled; therefore, in the reported data, only 21 treated PD patients were allowed to take amantadine and anticholinergics.

4.2.2 Naringenin

Flavonoids by shielding vulnerable neurons, protection of motor control, and reduction in motor impediment showed the capability to improve the cognitive function in recent therapeutic advancement in PD [70]. Multiple factors triggered the neurodegeneration in PD [71], and current report reinforces the defensive role of flavonoids that are able to work against neuronal injury [72].



Naringenin

Naringenin (4′,5,7-trihydroxyflavanone), a citrus flavanone, occurs abundantly in herbs, plant food beverages, and juicy fruits such as lemons, grapes, pummel, blood orange, tangerines, and grapefruit [73, 74]. It has been reported to possess several effects on biological systems such as antiviral, antioxidant, anti-inflammatory, antifibrogenic, antiatherogenic, and anticancer activities [74–79]. It has the ability to scavenge oxygen free radical, prevent oxidation of low-density lipoproteins (LDL), chelate metals, and inhibit enzymes [80, 81]. It demonstrated high permeability across *in vitro* and *in situ* blood–brain barrier (BBB) models [82, 83]. The uptake of naringenin into the cerebral cortex and the striatum suggested that naringenin should play a role in neuroprotection within the central nervous system (CNS) [84, 85]. Pharmacological investigations of the naringenin show that it possesses powerful antioxidant activity and is effective as a scavenger of H_2O_2 and free radicals [86–89]. The overall antioxidant activity of naringenin might be

attributed to the polyphenolic contents and other phytochemical constituents. Due to its potent antioxidant, free radical scavenging, and neuroprotective properties, it may prevent the progression of PD. It has the benefit of acting at various sites in the brain and muscle regions and possesses neurorestorative activity along with neuroprotective.

Naringenin suffers from low bioavailability when given by oral route, which restricts its therapeutic application. However, several attempts such as salt formation, use of surfactant, particle size reduction, complexation with cyclodextrins, prodrug formation, solid dispersions, nanoparticles, nanocarriers, and self-emulsifying drug delivery system, and so on have been made to surmount the problems connected with oral absorption and bioavailability issues. Employing solvent evaporation and kneading methods, solid dispersions of naringenin were prepared by Khan and coworkers. The formulation showed a significantly higher solubility and drug dissolution rate than pure naringenin [90].

Khan and coworkers employed self-nanoemulsifying drug delivery system (SNEDDS) to improve the solubility and bioavailability of naringenin. Furthermore, globule size distribution, zeta potential, and surface morphology of SNEDDS were evaluated. There is a considerable increase in drug release and bioavailability in comparison with the drug suspension, which may be accredited to the nanosize of the SNEDDS [91].

4.2.2.1 Animal Studies with Naringenin

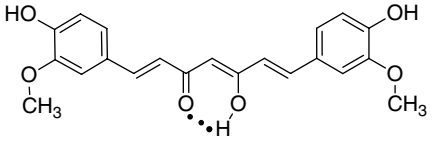
Phenolic compounds resulting from diets are considered as potential agents to be used in PD, as they have the ability to overcome oxidative stress. Zbarsky and coworkers investigated the same with naringenin, curcumin, quercetin, and fisetin in the 6-OHDA model of PD. Considerable decrease of TH-positive cells in the SN along with the decrease in DA content in the striata was seen in the animals treated with unilateral infusion of 6-OHDA into the medial forebrain bundle. Significant protection was seen in animals treated with curcumin or naringenin, the effect being attributed to their antioxidant competence and ability to go through into the brain. In contrast, the animals pretreated with quercetin or fisetin failed to show any effects on TH-positive cells or DA levels [92].

It showed moderate neuroprotection against 6-OHDA-induced toxicity, attributed to its antioxidant activity due to the existence of the 2,3-double bond in conjugation with a 4-oxo group. Neuronal protecting effects against oxidative cell death induced by Ab peptide in the PC12 cells was also shown by naringenin, which can partially subdue the Fenton reaction, which is a characteristic of Fe-adenosine triphosphate (ATP) [93, 94]. Angeline and coworkers showed the role of naringenin (rotenone-induced PD model) individually in neuroprotection upon the exposure of the mitochondrial toxin rotenone. Rotenone mimics complex I inhibitor, which causes degeneration of dopaminergic neurons and motor dysfunction [95]. Neuronal damage was induced in the SN and striatum via rotenone-induced PD-like model [96, 97]. Induced abnormalities such as behavioral changes, motor skill impairment, and loss of body weight along with altered muscle morphology were reversed using the neuroprotective flavonoid naringenin.

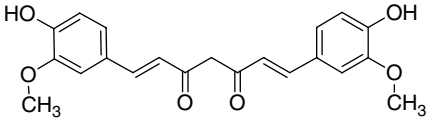
Naringenin can partially suppress the Fenton reaction protective proteins parkin, CHIP, and DJ1 in the brain regions, whereas a clear downregulation has been

observed in the ubiquitin level, which revealed that the flavonoid helps in clearing harmful proteins. Neuroprotective effect on the rotenone-induced rodent model of PD was shown by the oral administration of naringenin ($10\,mg\,kg^{-1}$). Rotenone behaves as a complex I inhibitor causing degeneration of dopaminergic neurons and motor dysfunction [95]. Naringenin potentially inhibits apoptosis activated by neurotoxic species and endorses neuronal survival together with refurbishing the muscle morphology, thereby improving muscular impairment in models. It also efficiently refurbished the level of protective proteins CHIP, parkin, TH, and DJ1 and simultaneously inhibits caspase activation, thereby dropping cell death in the brain and muscle regions along with their antiapoptotic property with a low down level of ubiquitin. On treating with naringenin, the differential expression of Hsp60, Hsp70, and Hsp90 was found, thereby enhancing their cytoprotective role in PD [98].

4.2.3 Curcumin



Enol form of curcumin



Keto form of curcumin

Curcumin is a diarylheptanoid. It is a unique yellow-colored spice derived from the rhizome of *Curcuma longa* (Zingiberaceae). It is widely cultivated in India and Southeast Asia. Studies revealed that curcumin, the active compound in turmeric, is the key ingredient responsible for the major therapeutic activities of turmeric [99, 100]. Turmeric (*C. longa*) is a fascinating ingredient that has a rich history as a dietary spice and herbal supplement. Turmeric was used way back more than 2000 years where it was used in cooking, medicine, cosmetics, and fabric dyes [101]. Ayurveda uses turmeric for the treatment of common eye infections, burns, acne, wound dressing, sprains, and swelling; to enhance the immune system; as a cure for different respiratory diseases such as asthma and for allergy; and for the treatment of diabetes, cough, sinusitis, flu, rheumatism, and liver disorders [102]. Developments in modern medicine have discovered many medicinal properties of turmeric such as antioxidant, antimutagenic, anticancer, antimicrobial, and anticardiovascular activities [103–110]. Several literature reports showed that curcumin protects DA neurons [111]. Curcumin is of versatile pharmacology, and rigorous efforts have been made to evaluate the leeway of using it for the treatment or prevention of neurodegenerative diseases such as Alzheimer's disease (AD) [112], PD [113, 114], and brain tumors [115–118].

4.2.3.1 Animal Studies with Curcumin

Pandey and coworkers demonstrated the neuroprotective effect of curcumin in PD and established the dose-dependent influence of curcumin on α-synuclein aggregation [104]. DA neuron's loss in the SN in a 6-OHDA rat model is greatly reduced by the administration of natural phenolic antioxidants including curcumin, as it was shown that pretreatment of curcumin in the model resulted in reduced DA neuron loss (21%) compared with control group (about 50%), which was dose dependent. Thus, it was concluded that the antioxidant activity is responsible for the neuroprotective action of curcumin against 6-OHDA-intervening toxicity [92, 119]. Chiu and coworkers [120] investigated the neuroprotection of liposomal-formulated curcumin: Lipocurc™ targeting HDAC inhibitor in the DJ-1(Park 7)-gene knockout rat model of PD. Groups made were as follows:

Group I: (DJ-1-KO-Lipocurc™) received Lipocurc™ 20 mg kg^{-1} i.v. three times weekly for 8 weeks.
Group II: DJ-1 KO controls (DJ-1 KO-PBS) received i.v. phosphate-buffered saline (PBS).
Group III: DJ-1-Wild Type (DJ-1 WT-PBS) received PBS.

Results were made on the basis of rotarod, motor behavior, open field behaviors, and dyskinesia, equally at baseline and at regular intervals. At the end of 8 weeks, by immunohistochemistry after postmortem, neuronal apoptosis and DA neuron-specific TH levels were measured. Results showed that DJ-KO Group I and Group II demonstrated judicious degree of motor impairment on the rotarod test, as compared with DJ-1 WT group. Motor behavior impairment was enhanced to a larger extent as compared to the PBS treatment with the Lipocurc™ treatment. Also, distinct apoptosis was seen in the DJ-1 WT-PBS group. The apoptotic index of DJ-1-KO-Lipocurc™ group was strikingly reduced as compared with the DJ-KO-PBS group (3.3 vs. 25.0, $p < 0.001$), indicating that Lipocurc™ extensively blocked neuronal apoptosis. Also, DA neurons in the SN were found to be stimulated by Lipocurc™, as the ratio of immature to mature DA neurons was statistically elevated in the DJ-1-KO-Lipocurc™ group ($p < 0.025$).

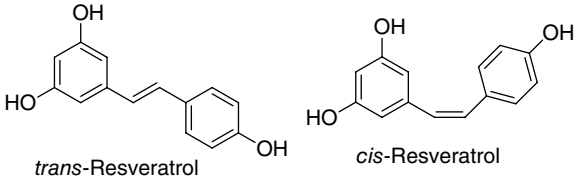
Du and coworkers [121] demonstrated the iron-chelating properties of curcumin, in which it represses the iron-induced degeneration of nigral dopaminergic neurons, ultimately the neuroprotective effect that was shown in *in vivo* 6-OHDA-lesioned rat model of PD. Curcumin was ingested intragastrically in rats for 24 days. 6-OHDA lesioning was conducted on day 4 of curcumin treatment. After 6-OHDA treatment, DA content in the striatum and the number of TH-immunoreactive neurons decreased, which on pretreatment with curcumin reversed. Also, iron-staining cells, which were increased by the 6-OHDA treatment, were spectacularly diminished by curcumin pretreatment. Studies revealed the neuroprotective effect of curcumin in 6-OHDA model PD owing to its antioxidant potential and its competence to pierce into the brain [92]. Curcumin has already shown its neuroprotective and antiaging properties, thus extending the life span in mice and *Caenorhabditis elegans* [122, 123].

In another study, Siddique and coworkers [124] worked on *Drosophila* male PD flies, expressing the human wild-type α-synuclein in the neuron of fly and resultant locomotor dysfunction [125]. In *Drosophila*, based on the gender and

genotype, an extended life span has been reported [126, 127]. Tetrahydrocurcumin extended the life span of *Drosophila* and reduces the oxidative stress by the regulation of O-type forkhead domain transcription factor (FOXO) [128]. For 24 days, the flies were exposed to 25, 50, and 100 μM of curcumin mixed in the diet. *Drosophila* activity monitors (DAMs) were used to monitor the activity of PD model flies. This study revealed that based upon the administered dose of curcumin, a considerable delay in the loss of activity pattern, protein carbonyl content, reduction in lipid peroxidation, increase in the life span, and apoptosis were seen as compared with the unexposed PD flies. The activity shown is accredited to the antioxidant properties of curcumin, because of the unique conjugated structure including two methoxylated phenols [129–131].

CNB-001, a new pyrazole derivative of curcumin, showed various neuroprotective properties. Jayaraj and coworkers [132] designed a study to examine the neuroprotective mechanism of CNB-001 in a subacute MPTP rodent model of PD. 30 mg kg^{-1} for 4 consecutive days of MPTP administration has intensified the oxidative stress and motor impairment, thereby reducing the TH, DA transporter, and vesicular monoamine transporter 2 (VMAT2) expressions. Models that were pretreated with CNB-001 (24 mg kg^{-1}) improved the behavioral irregularities along with enhanced monoamine transporter expressions and confined mitochondria by asset of its antioxidant activity.

4.2.4 Resveratrol



Resveratrol is a non-flavonoid polyphenolic, a naturally occurring powerful antioxidant compound that was firstly discovered by a Japanese named Gao Gang in 1993 from the roots of white hellebore (*Veratrum grandiflorum* O. Leos). It exists in *cis* and *trans* isomeric forms. The stilbene structure of the resveratrol was associated with the synthetic estrogen diethylstilbestrol where 2 phenol rings are coupled with a styrene double bond, generating 3,4,5-trihydroxystilbene [133]. It is a potential antioxidant that acts by inhibiting ROS mainly by activating AMP-activated kinase (AMPK). It also suppresses cyclooxygenase-2 (COX-2) and lipid peroxidation [134]. The antioxidant activity is because of the 3-OH groups in the structure [135]. Being a potential antioxidant, it shows anticancer, antiangiogenic, antioxidant, antitumor, cardioprotective, antidiabetic, radioprotective, and cytogenetic and antiviral activities [136–144].

Current studies revealed that resveratrol showed neuroprotective effects through the instigation of sirtuin 1 (SIRT1) and production of vitagenes [145]. Resveratrol has the ability to activate sirtuins belonging to the family of NAD^+-dependent deacetylases, which increases the life span of *Saccharomyces cerevisiae* as a special effect [146, 147]. This outcome of the drug appears to imitate that of dietary caloric constraints, coupled with activation of sirtuin proteins [146–152].

Its capability to amplify SIRT1 activity is associated with the deacetylation of PGC-1α (protein factor implicated in mitochondrial biogenesis) [29, 153, 154]. Also, effect on SIRT1-dependent deacetylation of PGC-1α and activation of peroxisome proliferator-activated receptor gamma (PPAR-γ) uphold resveratrol's ability to attenuate tissue injury in the brain and refurbish mitochondrial function [155, 156].

The transcription of SOD and CAT genes by increasing the Nrf2/keap 1 pathway may also be targeted by the activation of PPAR-γ [157, 158]. Various studies established the ability for resveratrol to lessen Aβ secretion from different cell lines and inhibit NADPH oxidase, thus suppressing neuroinflammation and attenuating NF-κB-induced expression of iNOS, COX-2, and sPLA2 [119, 159–162]. Hormetic pathway is also activated via resveratrol, which involves the initiation of SOD and CAT genes by rising the level of the PI3K/Nrf2/keap 1 pathway. Also, an increase in MnSOD expression and activity in mouse brain was seen through the dietary administration of resveratrol [157]. Indeed, resveratrol was also shown to upregulate the expression of HO-1 by activating Nrf2 [163]. Ischemia-stimulated oxidative brain damage was protected by the antioxidant pathway resveratrol [164]. AMPK-reliant mitochondrial biogenesis was also shown to be stimulated by resveratrol [155, 156]. These studies indicated the resveratrol's neuroprotective effects through activation of SIRT1 and amplified production of vitagenes [165, 166].

4.2.4.1 Animal Studies with Resveratrol

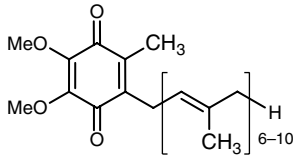
Pangeni and coworkers formulated resveratrol's kinetically stable nanoemulsion (o/w) using Tween 80 as the surfactant and Transcutol P as the cosurfactant and vitamin E:sefsol (1 : 1) as the oil phase for the enhanced management of PD. Spontaneous emulsification method was employed following high-pressure homogenization technique to prepare the nanoemulsion. The prepared formulation was studied for refractive index, viscosity, globule size and surface morphology, zeta potential, and *in vitro* and *ex vivo* release. DPPH assay was used to verify the antioxidant activity, which demonstrated high scavenging competence for the optimized formulation. Pharmacokinetic studies showed higher concentration of the drug in the brain (brain/blood ratio: 2.86 ± 0.70) following intranasal administration of the optimized nanoemulsion. Histopathological studies revealed the decrease in degenerative changes in the groups administered with the resveratrol nanoemulsion. Also, in the same group, the GSH and SOD levels were notably higher, and the level of MDA was considerably lesser [167].

The neuroprotective role of resveratrol was also demonstrated by Okaware and coworkers on dopaminergic neurons of Wistar rats. MPTP model of neurotoxicity was used and the neurodegeneration was due to the cytotoxicity of metabolic conversion of MPTP into MPP^+. When slice culture was applied with 30 μM of MPP^+ only, numbers of viable dopaminergic neurons were significantly decreased, but when in combination with resveratrol, dose-dependent protection of neurons was observed. Similar cytotoxicity was shown by thrombin as MPP^+ with the decrease in dopaminergic neurons in slice culture and resveratrol inhibiting the loss of neuronal numbers, which was due to the antioxidative activity. These results showed neuroprotective activity of resveratrol in dopaminergic neurons [168].

Neuroprotective effect of resveratrol on 6-OHDA-induced PD in rats was demonstrated by Khan and coworkers. Wistar rats (male) were pretreated intraperitoneally with resveratrol ($20\,mg\,kg^{-1}$) once daily for 15 days and then were subjected to unilateral intranasal injection of 6-OHDA. Neurobehavioral observation on circling behavior, muscle coordination using rotarod, and stepping test were carried out, and then rats were killed for the estimation of lipid peroxidation, GSH content, and activity of antioxidant enzymes such as GSH peroxidase, GSH reductase, CAT, and SOD. The results demonstrated that resveratrol induced successful upregulation of antioxidant status and lowered the DA loss. Administration of resveratrol significantly attenuated the level of DA and dihydroxyphenylacetic acid in lesion group compared with treated group from $4.65 \pm 0.17\,ng\,mg^{-1}$ tissue. Conclusively, results demonstrated the potential of resveratrol in PD [169].

The effect of resveratrol in autophagy induction through activation of AMPK/SIRT1 pathway was studied by Wu and coworkers. PD caused by misfolding of proteins and injury of mitochondria was cleared via autophagy using resveratrol as the model drug. The study showed that the modulation of SIRT1 and AMPK was actively involved in autophagy caused by resveratrol treatment leading to neuronal survival. The ability of resveratrol to induce autophagy was confirmed by the increased LC3-II protein levels. Also, resveratrol was found to attenuate rotenone-induced increase of cleaved PARP, which was blocked when Beclin 1 was suppressed [170].

4.2.5 Coenzyme Q10



Coenzyme Q10

Coenzyme Q10 is a naturally occurring lipophilic compound having antioxidant activity that shows neuroprotective action. It consists of a quinoid moiety and a lipophilic tail. In mitochondrial respiratory mechanism, it acts as an electron carrier. It accepts electron for complex I and II and thus helps in improving the level of ATP [171, 172]. In PD patients, there is reduced complex I in the SN. This impairment may result in destruction of dopaminergic neurons [173–177]. Homocysteine has toxic actions in the brain whenever there is alteration in redox equilibrium. High content of homocysteine causes neuronal death [178]. There is a resistance shown by the body's defense system against ROS in response to the changes in coenzyme Q10 redox equilibrium. It also causes changes in membrane electron transport. Coenzyme Q10 prevents peroxidation of membrane phospholipids. As in PD, there is elevation in lipid peroxidation in the brain. Hence this compound has potential for the prevention of PD. It helps in avoiding DNA damage from free radicals. Findings suggested that coenzyme Q10 acts at the nerve terminals. Several published reports support the neuroprotective role

of coenzyme Q10 in preventing the degeneration of dopaminergic neurons. It also helps in restoring the functions of dopaminergic neurons [171, 172, 178, 179]. Coenzyme Q10 is able to suppress the amount of carbonyl in the brain. With the progression of human age, there is reduction in the level of ubiquinol and ubiquinone in body tissues, especially in the brain, which is one of the causes for neurodegenerative disease. These cause motor dysfunctions. In humans coenzyme Q10 is comprised of 10 isoprenoids moieties, but in rodents, nine isoprenoid moieties are present. The CNS, cardiac tissues, hepatic system, and kidney contain more level of coenzyme Q10 in comparison with rest of the organs. Coenzyme Q10 is produced endogenously and is also obtained from diet. Mechanisms by which coenzyme Q10 exhibits antioxidant activity include stimulation of expression of mitochondrial uncoupling proteins. These proteins are responsible for suppression of free radical production [173, 180].

Coenzyme Q10 delayed the mitochondrial dysfunction in the brain, increased the DA level, and prevented dopaminergic axons against excitotoxin-induced destruction [174]. Coenzyme Q10 decreases the protein sulfhydryl content in the brain by activating the complex I action [178]. It is reported that it inhibits oxidation-induced apoptosis. This is due to inhibition of NF-κB, which helps in the maintenance of the integrity of mitochondrial membrane, thus restricting cytochrome C release, which is responsible for cell damage [179]. Patients have been found with decreased complex I activity in the CNS and platelets. In PD patients, the deficiency of coenzyme Q10 occurs in the cerebral cortex than in the striatum and SN. Coenzyme Q10 shows its activity in the cytosol and membrane, where it scavenges the ROS.

4.2.5.1 Animal Studies with Coenzyme Q10

Nigrostriatal dopaminergic neuronal protective action of coenzyme Q10 has been reported in both animal and human disease models. Oral administration of this compound restores the activity of mitochondrial transport chain and thus helps in preventing neuronal destruction. In 1-year-old mice (induced PD by MPTP), coenzyme Q10 showed neuroprotective mechanism and thus proved its important role in the prevention of cellular dysfunction in PD [180].

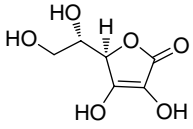
4.2.5.2 Human Studies with Coenzyme Q10

Shults *et al.* demonstrated that there was reduced coenzyme Q10 content in platelet mitochondria of PD patients. This leads to alteration in redox equilibrium within tissues. They also investigated the relationship between the complexes of electron transport chain and coenzyme Q10 content [180]. Muller *et al.* investigated the effect of coenzyme Q10 (360 mg) therapy in the management of PD patients ($n = 28$). They found that it provided a beneficial effect on reducing the disease symptoms, especially visual dysfunction and nigral complex I deficiency [174]. Recently, Seet and coworkers investigated the effect of high coenzyme Q10 dose in preventing oxidative stress. Their study involved 16 PD patients. For 14 days, doses of 400, 800, 1200, and 2400 mg were given per day to the patients. From these results it was found that the dose of 2400 mg was well tolerated and results were improved when studied in the scale known as *Unified Parkinson's Disease Rating Scale* (UPDRS). There was increase in plasma F2-isoprostanes. In

same manner the serum phospholipase A2 activities were observed. There was no alteration in plasma tocopherol and cholesterol even with high dose. Patients who received symptomatic relief with coenzyme Q10 showed lower plasma ubiquinol. From the study it was hypothesized that coenzyme Q10 response depends on the level of reduced form [175].

Gotz and colleagues evaluated the amount of coenzyme Q10 in reduced and oxidized state (known as redox ratio) in PD patients. In their study they found a decreased coenzyme Q10 redox ratio in diseased patients. From their experimental observations, they suggested that there was more need for coenzyme Q10 in reduced form to avoid severity of the disease [181]. Shults *et al.* investigated the safety of high dose (3000 mg day^{-1}) of coenzyme Q10. In their study 17 PD patients were treated with vitamin E (1200 IU day^{-1}) along with escalating dose of coenzyme Q10. Four doses of coenzyme Q10 were 1200, 1800, 2400, and 3000 mg day^{-1} administered orally, and systematic availability of coenzyme Q10 was evaluated at different times. They found that C_{max} reached at the dose of 2400 mg [180]. Zhenguang *et al.* determined the benefits of coenzyme Q10 and creatine combined therapy on cognitive dysfunction system in PD. In their study, 75 patients with PD were enrolled. The severity of disease was measured by employing Montreal Cognitive Assessment (MoCA) and UPDRS. The influence of combination therapy was also investigated on plasma phospholipid level. After 1 year of treatment, the control group and combination therapy group showed significant result in MoCA scores. However, the phospholipid levels of therapy group were reduced as compared with that of control. They concluded that CoQ10 and creatine combination therapy would be helpful in the management of PD, as this combination possessed neuroprotective action [182].

4.2.6 Vitamin C



Vitamin C

Vitamin C is a water-soluble vitamin and comprises a six-carbon lactone. It plays an important role in growth and development of the body. It is an antioxidant and thus helps in reducing the damage caused by ROS. Citrus fruits, mango, pineapple, watermelon, raspberries, blueberries, and so on are some rich sources of vitamin C. Many mammals (except humans) synthesize this vitamin in their liver. Humans can take vitamin C exogenously. This vitamin acts as reducing agent and electron donor. It donates two electrons from a double bond between C2 and C3 of molecule and hence prevents oxidation of other compounds. It is a naturally occurring vitamin and prevents peroxidation of fats and cell membrane damage by scavenging ROS. Fernandez-Calle *et al.* suggested that serum level of vitamin C does not differ with age progression [183]. In the brain this vitamin is highly concentrated. In the striatum it provides neuroprotection against stimulation of glutamate. Additionally it helps in neutralizing ROS. As already mentioned in the literature, oxidative stress and glutamatergic hyperactivity play an

important role in the pathophysiology of PD. Both are responsible for the degradation of dopaminergic neurons [184, 185]. Years ago it was believed that vitamin C is only used for the treatment of scurvy and to enhance collagen synthesis in body. In the last decades, researchers revealed neuroprotective action of vitamin C. The level of this vitamin in the brain is about 16–25 times more as compared to its blood level. Generally in PD, there is autoxidative destruction of dopaminergic neurons by quinine derivatives of DA (which are toxic) and by ROS. Vitamin C provides protection against this autotoxicity [186]. In dopaminergic neurons, it is also called as *neuromodulator*.

In reports it is suggested that high level of L-DOPA is toxic. This is because of high levels of quinines. Additionally enhanced activity of mitochondrial respiratory chain complex II and III is also responsible. Vitamin C helps out in lowering quinone production and toxicity of L-DOPA. Pardo and associates investigated that vitamin C and selegiline prevent the neurotoxicity caused by L-DOPA. Both of these antioxidants are responsible for slowing PD progression [187].

4.2.6.1 *In Vitro* Studies with Vitamin C

Ballaz and coworkers evaluated the role of ascorbate in avoiding cell death. For this purpose they provided a prolonged exposure of glutamate to the dopaminergic neurons. These neurons were of human origin. The exposure caused adverse effects on dopaminergic cells because of metabotropic receptor stimulation. In the experiment they found that the treatment of cells with ascorbate provides protection against glutamate excitotoxicity generally by inhibition of oxidative stress. They suggested that ascorbate has a neuroprotective action and has an accepted role in PD [188]. Choi *et al.* determined the influence of ascorbate on the DA oxidation. For this purpose they employed PC12 cell line and observed a DA oxidation-mediated cytotoxicity. DA cytotoxicity was lowered by ascorbic acid. They found less cell death after more rapid treatment with ascorbic acid [189].

4.2.6.2 Animal Studies with Vitamin C

Lazzarini and associates investigated vitamin C effect on cataleptic rodents. The catalepsy was induced by haloperidol. It is believed that in humans, haloperidol lowers DA neurotransmission and in rodents it induces catalepsy. They carried out hanging-bar test to evaluate the effect of vitamin on catalepsy. They found that ascorbic acid improved the catalepsy produced by haloperidol [190].

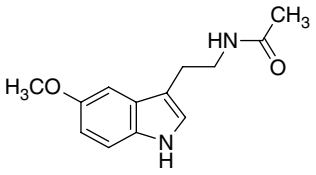
4.2.6.3 Human Studies with Vitamin C

Scheider and colleagues carried out a case–control experiment to evaluate the protective action of vitamin C in 45–79-year-old males suffering from PD. From experimental observations they suggested that vitamin C intake decreases the risk of PD by scavenging the ROS, hence avoiding injury to dopaminergic neurons [191]. Recently, Ide and coworkers evaluated the relationship between ascorbic acid levels in different stages of PD by employing 62 PD patients. Blood samples were taken out and the amount of ascorbic acid in the lymphocyte and plasma was determined. They found low level of ascorbic acid in both lymphocyte and plasma. They concluded that lymphocyte vitamin C levels might be used as biomarker for PD [192].

Nagayama and coworkers estimated the effect of ascorbic acid on the pharmacokinetic profile of L-DOPA. They employed 67 PD patients in their study and allowed them to take 100 mg tablet of L-DOPA and 10 mg carbidopa. The plasma drug level was calculated at different time periods by using HPLC with electrochemical detector. The different pharmacokinetic parameters were determined. After this, patients were allowed to take 200 mg ascorbic acid tablets, and again pharmacokinetic parameters were estimated. Results from all patients did not show significant alterations in area under curve) (i) , C_{max}, and t_{max}. However, by giving ascorbic acid in treatment, about 25 patients showed reduction in t_{max} and increase in AUC and C_{max} of L-DOPA. From the observations Nagayama *et al.* suggested that ascorbic acid could improve absorption of L-DOPA. According to them L-DOPA along with ascorbic acid may be one of the successful therapies for the treatment of PD [193].

Paraskevas *et al.* carried out an experimental study to evaluate the level of ascorbic acid in PD patients. In their study among 44 patients, low level of ascorbic acid was found, which indicated the importance of ascorbic acid in diet especially in elders [194]. DA can trigger apoptosis. It was suggested that improper stimulation of apoptosis by DA or its derivatives causes nigral cell loss. They found that vitamin C avoided the autoxidation of DA. It also prevents DA-induced apoptosis and DNA fragmentation [195]. Fahn carried out a trial involving PD patients to investigate the effect of combination of vitamin C and vitamin E on PD progression. Experimental results showed that the combination of these antioxidants delayed the progression of PD [67].

4.2.7 Melatonin



Melatonin

Melatonin is derived from tryptophan. Its production takes place in the pineal gland, retina, Harderian glands, gastrointestinal tract, gonads, bone marrow, and lens. It is reported that during dark phase, its synthesis takes place from serotonin [62, 196]. One important point regarding this antioxidant is that it has the ability to pass across all biological membranes and barriers including BBB. This is the basic reason of its presence in each and every cell or tissue of the body. In the brain, the endogenously produced melatonin (from pineal gland) gets distributed to the CSF and in neurons [197]. In nuclei and mitochondria, about 100 nmol of melatonin normally gains access. There are many marketed preparations of melatonin that are sold without prescription. Human studies showed that melatonin is very effective for the treatment of neurodegenerative diseases [198]. It is an indoleamine and responsible for the alteration of neural and endocrine functions. Presently a wide range of experimental research proved the substantial antioxidant activity of melatonin. Moreover it stimulates GSH redox

enzyme activity, which provides beneficial effect in reducing oxidative stress. This molecule gets access to subcellular compartment due to its small molecular size [199].

The literature demonstrated the antioxidative action of melatonin [200–203]. It regulates the activity of GSH peroxidase and reductase and SOD [204]. Studies showed the capability of melatonin in retaining cell integrity after ROS damage. Its structure comprises an indole ring that acts as electron donor and is responsible for neutralizing ROS. In this way melatonin prevents amino acids, fats, and DNA from ROS damage [205]. It is also responsible for the downregulation of some enzymes especially lipoxygenase and NO synthases [206, 207]. Studies suggested that melatonin suppresses the Fenton reaction, hence preventing oxidative stress [208]. CYP450 monooxygenases are responsible for the metabolism of melatonin [209]. Two metabolites of melatonin, namely, N^1-acetyl-N^2-formyl-5-methoxykynuramine and N^1-acetyl-5-methoxykynuramine, are produced in the body tissues after metabolism of melatonin [62]. Both these metabolites are ROS scavengers [210]. These metabolites can easily enter the brain through the BBB. N^1-acetyl-5-methoxykynuramine has NO scavenger activity along with COX-2 inhibition action, hence preventing the formation of prostaglandin-2 [211]. Moreover, it has different pharmacological activities such as sedative, antidepressant, and antiepileptic. Breen and associates reported that patients with PD have decreased level of circulating melatonin [212]. Reports suggested that if there is declination of endogenous level of melatonin along with low level of GSH and antioxidants, then it would be managed and restored by the oral intake of melatonin. This shows the importance of melatonin for the maintenance of antioxidant system in the body. In mitochondrial homeostasis, melatonin plays an important role as it inhibits alpha-synuclein protein aggregation, thus preventing the production of toxins such as protofibrils and oligomers [213]. Emre *et al.* suggested that melatonin acts as a useful therapeutic agent for the management of rapid eye movement behavior disorder [214].

In general, NO is responsible for the production of RNS. These species may also lead to oxidative stress by inhibiting complex I, phospholipid oxidation, and increased iron release [215]. Leon and coworkers suggested that melatonin is responsible for the inhibition of NO synthase (mainly in the brain), which catalyzes the production of NO [216]. In studies NO was found to stimulate NMDA receptors and cause damage to the neurons [217, 218].

4.2.7.1 Animal Studies with Melatonin

Naskar and associates evaluated the effect of melatonin (10–30 mg kg^{-1}) on potentiating L-DOPA therapeutic effect. They induced PD in mice by MPTP. A dose of 5–8 mg kg^{-1} of L-DOPA was ineffective to reduce MPTP-induced catalepsy. But co-administration of melatonin with it was helpful in reducing this behavior [219]. Kotler and coworkers investigated the enhancement of mRNA for endogenous antioxidants by treating rats with melatonin [220]. ATP production is also increased by improving the activity of complex I and IV of mitochondrial respiratory chain [221].

Okatani and associates determined the effect of melatonin intake on the level of complex I and IV. They employed mice for their experimental study.

They observed that daily intake of melatonin by animals improved the activities of mitochondrial complexes and GSH peroxidase [222]. It was reported that melatonin has a role in the protection of NADH and NADPH, which would be helpful in the maintenance of GSH reductase activity. It has the capability to convert NAD radicals (by-product of oxidation) to NADH. In this way it also plays a key role in producing ATP by maintaining the NADH level. Mitochondrial complex I is generally inhibited by NO in GSH deficiency. It results in improper respiratory cycle, which leads to reduction in ATP production, thus causing apoptotic cell death [223, 224].

Nogues and coworkers studied the protective action of melatonin against oxidative stress. They employed senescence-accelerated mice (SAMP8) as animal model. Melatonin was administered orally to 1-month-old mice in a dose of $10\,mg\,kg^{-1}\,day^{-1}$ for 9 months. In the 10th month various oxidative stress markers were evaluated by taking plasma samples. Results showed increased level of thiobarbituric acid reactive substances and oxidized GSH. Experimental results showed stimulation of GSH peroxidase and GSH reductase [225]. In a study, oxidative stress was induced in rats by administering ochratoxin A. The effect of melatonin in minimizing oxidative stress was evaluated by dividing 15 animals in four groups. One group was used as control, the second group was treated with melatonin, and the third group was administered only with ochratoxin A. The fourth group received both melatonin and ochratoxin A. One month later, animals were sacrificed and the levels of different oxidative markers were evaluated. Third group animals showed elevated level of lipid peroxidation products as compared with control group. Furthermore, there were decreased activities of antioxidant enzymes including SOD, CAT, and GSH peroxidase, and GSH reductase. The results were reversed in the case of fourth group, with the animals showing no significant difference in the level of lipid peroxidation product. Additionally there was stimulation of antioxidant enzyme activities. They concluded that melatonin possessed neuroprotective activity and has therapeutic applications for the management and prevention of CNS disorders [226].

4.3 Concluding Remarks

PD is a neurodegenerative disorder of the CNS, chiefly affecting the motor system. It is an age-related disease that leads to protein aggregation, ROS production, mitochondrial dysfunction, and cell death. Clinical trials' data has revealed serious impediments in the treatment of PD with synthetic compounds attributed to their high toxicity and carcinogenic potential. Consequently, treatments with natural antioxidants through diet or dietary supplements have turn out to be an attractive substitute. The fate of natural antioxidants has been intricately explored in this chapter. Therefore, it can finally be concluded that these natural sources may be used as first-line therapy in the treatment of PD due to their high efficacy and minimal side effects. Many clinical trials have been conducted as discussed previously, which demonstrated the effectiveness of the natural antioxidants and their potential to minimize side effects.

Abbreviations

MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
AD	Alzheimer's disease
AUC	area under curve
ATP	adenosine triphosphate
BBB	blood–brain barrier
CAT	catalase
CNS	central nervous system
CSF	cerebrospinal fluid
DATATOP	Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism
DA	dopamine
TS	D-α-tocopheryl succinate
GSH	glutathione
LRRK2	leucine-rich repeat kinase
LDLs	low-density lipoproteins
MDA	malondialdehyde
NADPH	nicotinamide adenine dinucleotide phosphate oxidase
PSG	Parkinson Study Group
PD	Parkinson's disease
PPAR-γ	peroxisome proliferator-activated receptor gamma
PINK 1	PTEN-induced kinase 1
ROS	reactive oxygen species
SNEDDS	self-nanoemulsifying drug delivery system
SIRT1	sirtuin 1
SNpc	substantia nigra pars compacta
SOD	superoxide dismutase
TH	tyrosine hydroxylase

References

1 Youdim, M.B.H., Geldenhuys, W.J., and Van der Schyf, C.J. (2007) Why should we use multifunctional neuroprotective and neurorestorative drugs for Parkinson's disease? *Parkinsonism Relat. Disord.*, **13**, S281–S291.

2 Tufekci, K.U., Bayin, E.C., Genc, S., and Genc, K. (2011) The Nrf2/ARE pathway: a promising target to counteract mitochondrial dysfunction in Parkinson's disease. *Parkinsons Dis.*, 314082, 1–14.

3 Lees, A.J., Hardy, J., and Revesz, T. (2009) Parkinson's disease. *Lancet*, **373** (9680), 2055–2066.

4 Brichta, L., Greengard, P., and Flajolet, M. (2013) Advances in the pharmacological treatment of Parkinson's disease: targeting neurotransmitter systems. *Trends Neurosci.*, **36**, 543–554.

5 Braak, H., Rub, U., Gai, W.P., and Tredici, K.D. (2003) Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *J. Neural Transm.*, **110** (5), 517–536.

6 Dorsey, E.R., Constantinescu, R., Thompson, J.P., Biglan, K.M., Holloway, R.G., and Kieburtz, K. (2007) Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology*, **68** (5), 384–386.

7 Md, S., Khan, R.A., Mustafa, G., Chuttani, K., Baboota, S., Sahni, J.K., and Ali, J. (2013) Bromocriptine loaded chitosan nanoparticles intended for direct nose to brain delivery: pharmacodynamic, pharmacokinetic and scintigraphy study in mice model. *Eur. J. Pharm. Sci.*, **48** (3), 393–405.

8 Bekris, L.M., Mata, I.F., and Zabetian, C.P. (2010) The genetics of Parkinson disease. *J. Geriatr. Psychiatry Neurol.*, **23**, 228–242.

9 Sulzer, D. (2007) Multiple hit hypotheses for dopamine neuron loss in Parkinson's disease. *Trends Neurosci.*, **30**, 244–250.

10 Jahromi, S.R., Haddadi, M., Shivanandappa, T., and Ramesh, S.R. (2015) Attenuation of neuromotor deficits by natural antioxidants of *Decalepis hamiltonii* in transgenic *Drosophila* model of Parkinson's disease. *Neuroscience*, **293**, 136–150.

11 Olanow, C.W. (2007) The pathogenesis of cell death in Parkinson's disease. *Mov. Disord.*, **17**, S335–S342.

12 Lin, M.T. and Beal, M.F. (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature*, **443**, 787–795.

13 Dias, V., Junn, E., and Mouradian, M.M. (2013) The role of oxidative stress in Parkinson's disease. *J. Parkinsons Dis.*, **3** (4), 461–491.

14 Zhang, Z. (2015) Examining the neuroprotective effects of protocatechuic acid and chrysin on in vitro and in vivo models of Parkinson's disease. *Free Radical Biol. Med.*, **84**, 331–343.

15 Zhu, B.T. (2004) CNS dopamine oxidation and catechol-O-methyltransferase: importance in the etiology, pharmacotherapy, and dietary prevention of Parkinson's disease. *Int. J. Mol. Med.*, **13** (3), 343–353.

16 Chaturvedi, R.K., Shukla, S., Seth, K., Chauhan, S., Sinha, C., Shukla, Y., and Agrawal, A.K. (2006) Neuroprotective and neurorescue effect of black tea extract in 6-hydroxydopamine-lesioned rat model of Parkinson's disease. *Neurobiol. Dis.*, **22** (2), 421–434.

17 Hritcu, L., Foyet, H.S., Stefan, M., Mihasan, M., Asongalem, A.E., and Kamtchouing, P. (2011) Neuroprotective effect of the methanolic extract of Hibiscus as per leaves in 6-hydroxydopamine-lesioned rat model of Parkinson's disease. *J. Ethnopharmacol.*, **137** (1), 585–591.

18 Olanow, C.W., Watts, R.L., and Koller, W.C. (2001) An algorithm (decision tree) for the management of Parkinson's disease (2001): treatment guidelines. *Neurology*, **56**, S1–S88.

19 Rascol, O., Payoux, P., Ory, F., Ferreira, J.J., Brefel-Courbon, C., and Montastruc, J.L. (2003) Limitations of current Parkinson's disease therapy. *Ann. Neurol.*, **53**, S3–S12.

20 Pisoschi, A.M. and Pop, A. (2015) The role of antioxidants in the chemistry of oxidative stress: a review. *Eur. J. Med. Chem.*, **97**, 55–74.

21 Ratnam, D.V., Ankola, D.D., Bhardwaj, V., Sahana, D.K., and Kumar, M.N. (2006) Role of antioxidants in prophylaxis and therapy: a pharmaceutical perspective. *J. Controlled Release*, **113**, 189–207.

22 Singh, M., Kaur, M., and Silakari, O. (2014) Flavones: an important scaffold for medicinal chemistry. *Eur. J. Med. Chem.*, **84**, 206–239.

23 Gilgun-sherki, Y., Melamed, E., and Offen, D. (2001) Oxidative stress induced neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier. *Neuropharmacology*, **40**, 959–975.

24 Minor, R.K., DeCabo, R., Mouton, P.R., Shukitt-Hale, B., Joseph, J.A., and Ingram, D.K. (2008) A blueberry-enriched diet provides cellular protection against oxidative stress and reduces a kainate-induced learning impairment in rats. *Neurobiol. Aging*, **29**, 1680–1689.

25 Kelsey, N.A., Wilkins, H.M., and Linseman, D.A. (2010) Nutraceutical antioxidants as novel neuroprotective agents. *Molecules*, **15**, 7792–7814.

26 Ren, J., Fan, C., Chen, N., Huang, J., and Yang, Q. (2011) Resveratrol pretreatment attenuates cerebral ischemic injury by upregulating expression of transcription factor Nrf2 and HO-1 in rats. *Neurochem. Res.*, **36** (12), 2352–2362.

27 Sultana, R. (2012) Ferulic acid ethyl ester as a potential therapy in neurodegenerative disorders. *Biochim. Biophys. Acta*, **1822** (5), 748–752.

28 Lou, H., Jing, X., Wei, X., Shi, H., Ren, D., and Zhang, X. (2014) Naringenin protects against 6-OHDA-induced neurotoxicity via activation of the Nrf2/ARE signaling pathway. *Neuropharmacology*, **79**, 380–388.

29 Calabrese, V., Cornelius, C., Mancuso, C., Barone, E., Calafato, S., Bates, T., Rizzarelli, E., and Kostova, A.T. (2009) Vitagenes, dietary antioxidants and neuroprotection in neurodegenerative diseases. *Front. Biosci.*, **14**, 376–397.

30 Voelker, R. (2006) Parkinson disease guidelines aid diagnosis, management. *J. Am. Med. Assoc.*, **295**, 2126–2128.

31 Jain, K.K. (2010) Vitamin E in neurologic disorders, in *MedLink Neurology* (ed. S. Gilman), Medlink Publishing Corporation, San Diego, CA.

32 Yacoubian, T.A. and Standaert, D.G. (2009) Targets for neuroprotection in Parkinson's disease. *Biochim. Biophys. Acta*, **1792**, 676–687.

33 Block, G., Patterson, B., and Subar, A. (1992) Fruits, vegetables and cancer prevention: a review of epidemiological evidence. *Nutr. Cancer*, **18**, 1–29.

34 Halliwell, B. (1994) Free radicals, antioxidants and human disease: curiosity, cause or consequences? *Lancet*, **344**, 721–724.

35 Jacob, R.A. (1995) The integrated antioxidant system. *Nutr. Res.*, **15** (5), 755–766.

36 Fujimoto, S., Mizoi, K., Yoshimoto, T., and Suzuki, J. (1984) The protective effect of vitamin E on cerebral ischemia. *Surg. Neurol.*, **22**, 449–454.

37 Ishihara, L. and Brayne, C. (2005) A systematic review of nutritional risk factors of Parkinson's disease. *Nutr. Res. Rev.*, **18**, 259–282.

38 Butterfield, D.A., Castegna, A., Drake, J., Scapagnini, G., and Calabrese, V. (2002) Vitamin E and neurodegenerative disorders associated with oxidative stress. *Nutr Neurosci.*, **5**, 229–239.

39 Peyser, C.E., Folstein, M., Chase, G.A., Starkstein, S., Brandt, J., Cockrell, J.R., Bylsma, F., Coyle, J.T., McHugh, P.R., and Folstein, S.E. (1995) Trial of D-alpha-tocopherol in Huntington's disease. *Am. J. Psychiatry*, **152**, 1771–1775.

40 Golbe, L.I., Farrell, T.M., and Davis, P.H. (1998) Case-control study of early life dietary factors in Parkinson's disease. *Arch. Neurol.*, **45**, 1350–1353.

41 Ebadi, M., Srinivasan, S.K., and Baxi, M.D. (1996) Oxidative stress and antioxidant therapy in Parkinson's disease. *Prog. Neurobiol.*, **48**, 1–19.

42 Reiter, R.J. (1998) Oxidative damage in the central nervous system: protection by melatonin. *Prog. Neurobiol.*, **56**, 359–384.

43 Stoof, J.C., Vermeulen, R.J., Van Royen, E.A., Drukarch, B., Voorn, P., Wolters, E.C., and Groenewegen, H.J. (1996) Dopaminergic systems and Parkinson's disease: some latest developments in pathogenetic, diagnostic and pharmacotherapeutic investigations. *Neurosci. Res. Commun.*, **18**, 133–142.

44 Greene, J.G., Noorian, A.R., and Srinivasan, S. (2009) Delayed gastric emptying and enteric nervous system dysfunction in the rotenone model of Parkinson's disease. *Exp. Neurol.*, **218**, 154–161.

45 Yu, Z.F., Bruce-Keller, A.J., Goodman, Y., and Mattson, M.P. (1998) Uric acid protects neurons against excitotoxic and metabolic insults in cell culture, and against focal ischemic brain injury in vivo. *J. Neurosci. Res.*, **53** (5), 613–625.

46 Copp, R.P., Wisniewski, T., Hentatin, F., Larnaout, A., Ben Hamida, M., and Kayden, H.J. (1999) Localisation of alpha tocopherol transfer protein in the brains of patients with ataxia with vitamin E deficiency and other oxidative stress related neurodegenerative disorders. *Brain Res.*, **822** (1-2), 80–87.

47 Prasad, K.N., Cole, W.C., and Kumar, B. (1999) Multiple antioxidants in the prevention and treatment of Parkinson's disease. *J. Am. Coll. Nutr.*, **18** (5), 413–423.

48 Pappert, E.J., Tangney, C.C., Goetz, C.G., Ling, Z.D., Lipton, J.W., Stebbins, G.T., and Carvey, P.M. (1996) Alpha tocopherol in the ventricular cerebrospinal fluid of Parkinson's disease patients: dose-response study and correlations with plasma levels. *Neurology*, **47** (4), 1037–1042.

49 Hellenbrand, W., Boeing, H., Robra, B.P., Seidler, A., Vieregge, P., Nischan, P., Joerg, J., Oertel, W.H., Schneider, E., and Ulm, G. (1996) Diet and Parkinson's disease. II: a possible role for the past intake of specific nutrients. Results from a self-administered food-frequency questionnaire in a case control study. *Neurology*, **47**, 644–650.

50 Perumal, A.S., Gopal, V.B., Tordzro, W.K., Cooper, T.B., and Cadet, J.L. (1992) Vitamin E attenuates the toxic effects of 6 hydroxydopamine on free radical scavenging systems in rat brain. *Brain Res. Bull.*, **29**, 699–701.

51 de Rijk, M.C., Breteler, M.M., den Breeijen, J.H., Launer, L.J., Grobbee, D.E., van der Meche, F.G., and Hofman, A. (1997) Dietary antioxidants and Parkinson disease. The Rotterdam Study. *Arch. Neurol.*, **54**, 762–765.

52 Sharma, N. and Nehru, B. (2013) Beneficial effect of vitamin E in rotenone induced model of PD: behavioural, neurochemical and biochemical study. *Exp. Neurobiol.*, **22** (3), 214–223.

53 Nehru, B., Verma, R., Khanna, P., and Sharma, S.K. (2008) Behavioral alterations in rotenone model of Parkinson's disease: attenuation by co-treatment of centrophenoxine. *Brain Res.*, **1201**, 122–127.

54 Pearce, R.K., Owen, A., Daniel, S., Jenner, P., and Marsden, C.D. (1997) Alterations in the distribution of glutathione in the substantia nigra in Parkinson's disease. *J. Neural Transm.*, **104**, 661–677.

55 Kaur, H., Chauhan, S., and Sandhir, R. (2011) Protective effect of lycopene on oxidative stress and cognitive decline in rotenone induced model of Parkinson's disease. *Neurochem. Res.*, **36**, 1435–1443.

56 Koutsilieri, E., Sopper, S., Scheller, C., ter Meulen, V., and Riederer, P. (2002) Parkinsonism in HIV dementia. *J. Neural Transm.*, **109**, 767–775.

57 Adams, J.D. Jr. and Odunze, I.N. (1991) Oxygen free radicals and Parkinson's disease. *Free Radical Biol. Med.*, **10**, 161–169.

58 Singh, C., Ahmad, I., and Kumar, A. (2007) Pesticides and metals induced Parkinson's disease: involvement of free radicals and oxidative stress. *Cell Mol. Biol. (Noisy-le-grand)*, **53**, 19–28.

59 Farber, J.L. (1995) *In: Pathology of Environmental and Occupational Disease* (ed. J.E. Craighead), Mosby, St. Louis, MO, pp. 287–302.

60 Burton, G.W. and Traber, M.G. (1990) Vitamin E: antioxidant activity, biokinetics, and bioavailability. *Annu. Rev. Nutr.*, **10**, 357–382.

61 Selby, G. (1990) in *Parkinson's Disease* (ed. G.M. Stern), The Johns Hopkins University Press, Baltimore, MD, pp. 333–388.

62 Reiter, R.J. (1991) Pineal melatonin: cell biology of its synthesis and of its physiological interactions. *Endocr. Rev.*, **12**, 151–180.

63 Odunze, I.N., Klaidman, L.K., and Adams, J.D. Jr. (1990) MPTP toxicity in the mouse brain and vitamin E. *Neurosci. Lett.*, **108**, 346–349.

64 Martinovits, G., Melamed, E., Cohen, O., Rosenthal, J., and Uzzan, A. (1986) Systemic administration of antioxidants does not protect mice against the dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridin (MPTP). *Neurosci. Lett.*, **69**, 192–197.

65 Perry, T.L., Wee Yong, V., Clavier, R.M., Jones, K., Wright, J.M., Foulks, J.G., and Wall, R.A. (1985) Partial protection from the dopaminergic neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by four different antioxidants in the mouse. *Neurosci. Lett.*, **60**, 109–114.

66 Roghani, M. and Behzadi, G. (2001) Neuroprotective effect of vitamin E on the early model of Parkinson's disease in rat: behavioral and histochemical evidence. *Brain Res.*, **892**, 211–217.

67 Fahn, S. (1992) A pilot trial of high-dose alpha-tocopherol and ascorbate in early Parkinson's disease. *Ann. Neurol.*, **32**, S128–S132.

68 The Parkinson Study Group, Vatassery, G.T., Fahn, S., and Kuskowski, M.A. (1998) Alpha tocopherol in CSF of subjects taking high-dose vitamin E in the DATATOP study. *Neurology*, **50**, 1900–1902.

69 Vatassery, G.T., Brin, M.F., Fahn, S., Kayden, H.J., and Traber, M.G. (1998) Effect of high dose of dietary vitamin E on the concentration of vitamin E in several brain regions, plasma, liver, and adipose tissue of rats. *J. Neurochem.*, **51**, 621–623.

70 Vauzour, D., Vafeiadou, K., Rodriguez-Mateos, A., Rendeiro, C., and Spencer, J.P. (2008) The neuroprotective potential of flavonoids: a multiplicity of effects. *Genes Nutr.*, **3** (3-4), 115–126.

71 Barzilai, A. and Melamed, E. (2003) Molecular mechanisms of selective dopaminergic neuronal death in Parkinson's disease. *Trends Mol. Med.*, **9** (3), 126–132.

72 Spencer, B., Rockenstein, E., Crews, L., Marr, R., and Masliah, E. (2007) Novel strategies for Alzheimer's disease treatment. *Expert Opin. Biol. Ther.*, **7** (12), 1853–1867.

73 Jayaraman, J., Veerappan, M., and Namasivayam, N. (2009) Potential beneficial effect of naringenin on lipid peroxidation and antioxidant status in rats with ethanol-induced hepatotoxicity. *J. Pharm. Pharmacol.*, **61**, 1383–1390.

74 Rice-Evans, C.A., Miller, N.J., and Paganga, G. (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.*, **20**, 933–956.

75 Scalbert, A. and Williamson, G. (2000) Dietary intake and bioavailability of polyphenols. *J. Nutr.*, **130**, 2073S–2085S.

76 Cao, G., Sofic, E., and Prior, R.L. (1997) Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radical Biol. Med.*, **22**, 749–760.

77 Manthey, J.A. (2000) Biological properties of flavonoids pertaining to inflammation. *Microcirculation*, **7**, S29–S34.

78 Lee, M.H., Yoon, S., and Moon, J.O. (2004) The flavonoid naringenin inhibits dimethylnitrosamine induced liver damage in rats. *Biol. Pharm. Bull.*, **27**, 72–76.

79 Lee, C.H., Jeong, T.S., Choi, Y.K., Hyun, B.H., Oh, G.T., Kim, E.H., Kim, J.R., Han, J.I., and Bok, S.H. (2001) Anti-atherogenic effect of citrus flavonoids, naringin and naringenin, associated with hepatic ACAT and aortic VCAM-1 and MCP-1 in high cholesterol-fed rabbits. *Biochem. Biophys. Res. Commun.*, **84**, 681–688.

80 Madsen, H.L., Andersen, C.M., Jorgensen, L.V., and Skibsted, L.H. (2000) Radical scavenging by dietary flavonoids. A kinetic study of antioxidant efficiencies. *Eur. Food Res. Technol.*, **211**, 240–246.

81 Yu, J., Wang, L., Walzem, R.L., Miller, E.G., Pike, L.M., and Patil, B.S. (2005) Antioxidant activity of citrus limonoids, flavonoids, and coumarins. *J. Agric. Food. Chem.*, **53**, 2009–2014.

82 Tsai, T.H. (2002) Determination of naringin in rat blood, brain, liver, and bile using microdialysis and its interaction with cyclosporine A, a P-glycoprotein modulator. *J. Agric. Food. Chem.*, **50**, 6669–6674.

83 Youdim, K.A., Dobbie, M.S., Kuhnle, G., Proteggente, A.R., Abbott, N.J., and Rice-Evans, C. (2003) Interaction between flavonoids and the blood-brain barrier: in vitro studies. *J. Neurochem.*, **85**, 180–192.

84 Peng, H.W., Cheng, F.C., Huang, Y.T., Chen, C.F., and Tsai, T.H. (1998) Determination of naringenin and its glucuronide conjugate in rat plasma and brain tissue by high-performance liquid chromatography. *J. Chromatogr. B Biomed. Sci. Appl.*, **714**, 369–374.

85 Youdim, K.A., Qaiser, M.Z., Begley, D.J., Rice-Evans, C.A., and Abbott, N.J. (2004) Flavonoid permeability across an in situ model of the blood-brain barrier. *Free Radical Biol. Med.*, **36**, 592–604.

86 Akki, K.S., Krishnamurthy, G., and Bhojanaik, H.S. (2004) Phytochemical investigation and in vitro evaluation of Nyctanthes arbor-tristis leaf extracts for antioxidant property. *J. Pharm. Res.*, **2** (4), 752–755.

87 Mani, S. and Krishnaswamy, M. (2010) Evaluation of *Nyctanthes arbor-tristis* leaf extract for antioxidant, antibacterial and anticancer potentials in vitro, Medicinal Plants. *Int. J. Phytomed. Related Ind.*, **2** (3), 205–214.

88 Thangavelu, N.R. and Thomas, S. (2010) In vitro antioxidant studies on ethanolic extracts of leaves and stems of *Nyctanthes arbor-tristis*.L (Night flowering Jasmine). *Int. J. Biol. Med. Res.*, **1** (4), 188–192.

89 Rathee, J.S., Hassarajani, S.A., and Chattopadhyay, S. (2007) Antioxidant activity of *Nyctanthes arbor-tristis* leaf extract. *Food Chem.*, **103** (4), 1350–1357.

90 Khan, A.W., Kotta, S., Ansari, S.H., Sharma, R.K., and Ali, J. (2015) Enhanced dissolution and bioavailability of grapefruit flavonoid Naringenin by solid dispersion utilizing fourth generation carrier. *Drug Dev. Ind. Pharm.*, **41** (5), 772–779.

91 Khan, A.W., Kotta, S., Ansari, S.H., Sharma, R.K., and Ali, J. (2015) Self-nanoemulsifying drug delivery system (SNEDDS) of the poorly water-soluble grapefruit flavonoid Naringenin: design, characterization, *in vitro* and *in vivo* evaluation. *Drug Deliv.*, **22** (4), 552–561.

92 Zbarsky, V., Datla, K.P., Parkar, S., Rai, D.K., Aruoma, O.I., and Dexter, D.T. (2005) Neuroprotective properties of the natural phenolic antioxidants curcumin and naringenin but not quercetin and fisetin in a 6-OHDA model of Parkinson's disease. *Free Radical Res.*, **39**, 1119–1125.

93 Cheng, F. and Breen, K. (2000) On the ability of four flavonoids, baicilein, luteolin, naringenin and quercetin, to suppress the Fenton reaction of the iron ATP complex. *Biometals*, **13**, 77–83.

94 Heo, H.J., Kim, D.O., Shin, S.C., Kim, M.J., Kim, B.G., and Shin, D.H. (2004) Effect of antioxidant flavanone, naringenin, from *Citrus junos* on neuroprotection. *J. Agric. Food. Chem.*, **52**, 1520–1525.

95 Kim, H.J., Song, J.Y., Park, H.J., Park, H.K., Yun, D.H., and Chung, J.H. (2009) Naringin protects against rotenone-induced apoptosis in human neuroblastoma SH-SY5Y cells. *Korean J. Physiol. Pharmacol.*, **13**, 281–285.

96 Lapointe, N., St-Hilaire, M., Martinoli, M.G., Blanchet, J., Gould, P., Rouillard, C., and Cicchetti, F. (2004) Rotenone induces non-specific central nervous system and systemic toxicity. *FASEB J.*, **18**, 717–719.

97 Sonia Angeline, M., Chaterjee, P., Anand, K., Ambasta, R.K., and Kumar, P. (2012) Rotenone induced Parkinsonism elicit the behavioral impairments along with differential expression of parkin, heat shock proteins and caspase in rat. *Neuroscience*, **220**, 291–301.

98 Sonia Angeline, M., Sarkar, A., Anand, K., Ambasta, R.K., and Kumar, P. (2013) Sesamol and naringenin reverse the effect of rotenone-induced PD rat model. *Neuroscience*, **254**, 379–394.

99 Pal, S., Choudhuri, T., Chattopadhyay, S., Bhattacharya, A., Datta, G.K., Das, T., and Sa, G. (2001) Mechanisms of curcumin-induced apoptosis of Ehrlich's ascites carcinoma cells. *Biochem. Biophys. Res. Commun.*, **288**, 658–665.

100 Mukundan, M.A., Chacko, M.C., Annapurna, V.V., and Krishnaswamy, K. (1993) Effect of turmeric and curcumin on Bp-DNA adducts. *Carcinogenesis*, **14**, 493–496.

101 Aggarwal, B.B., Sundaram, C., Malani, N., and Ichikawa, H. (2007) Curcumin: the Indian solid gold (molecular targets and therapeutic uses of curcumin in health and disease). *Adv. Exp. Med. Biol.*, **595**, 1–75.

102 Araujo, M.C.P., Antunes, L.M.G., and Takahashi, C.S. (2001) Protective effect of thiourea, a hydroxy/radical scavenger, on curcumin-induced chromosomal aberrations in an in vitro mammalian cell system. *Teratog. Carcinog. Mutagen*, **21**, 175–180.

103 Gupta, S.C., Patchva, S., Koh, W., and Aggarwal, B.B. (2012) Discovery of curcumin, a component of golden spice, and its miraculous biological activities. *Clin. Exp. Pharmacol. Physiol.*, **39**, 283–299.

104 Pandey, A., Gupta, R.K., Bhargava, A., and Agrawal, B. (2011) Antibacterial activities of curcumin bioconjugates. *Int. J. Pharmacol.*, **7**, 874–879.

105 Kim, S.R., Park, H.J., Bae, Y.H., Ahn, S.C., Wee, H.J., Yun, I., Jang, H.O., Bae, M.K., and Bae, S.K. (2012) Curcumin down-regulates visfatin expression and inhibits breast cancer cell invasion. *Endocrinology*, **153**, 554–563.

106 Rahmani, A.H., Al Zohairy, M.A., Aly, S.M., and Khan, M.A. (2014) Curcumin: a potential candidate in prevention of cancer via modulation of molecular pathways. *Biomed Res. Int.*, 1–15.

107 Shehzad, A., Khan, S., and Lee, Y.S. (2012) Curcumin molecular targets in obesity and obesity-related cancers. *Future Oncol.*, **8**, 179–190.

108 Brinkevich, S.D., Ostrovskaya, N.I., Parkhach, M.E., Samovich, S.N., and Shadyro, O.I. (2012) Effects of curcumin and related compounds on processes involving alpha-hydroxyethyl radicals. *Free Radical Res.*, **46**, 295–302.

109 Moon, H.J., Ko, W.K., Han, S.W., Kim, D.S., Hwang, Y.S., Park, H.K., and Kwon, I.K. (2012) Antioxidants, like coenzyme Q10, selenite, and curcumin, inhibited osteoclast differentiation by suppressing reactive oxygen species generation. *Biochem. Biophys. Res. Commun.*, **418**, 247–253.

110 Shin, S.K., Ha, T.Y., McGregor, R.A., and Choi, M.S. (2011) Long-term curcumin administration protects against atherosclerosis via hepatic regulation of lipoprotein cholesterol metabolism. *Mol. Nutr. Food Res.*, **55**, 1829–1840.

111 Cole, G.M., Teter, B., and Frautschy, S.A. (2007) Neuroprotective effects of curcumin. *Adv. Exp. Med. Biol.*, **595**, 197–212.

112 Mutsuga, M., Chambers, J.K., Uchida, K., Tei, M., Makibuchi, T., Mizorogi, T., Takashima, A., and Nakayama, H. (2012) Binding of curcumin to senile plaques and cerebral amyloid angiopathy in the aged brain of various animals and to neurofibrillary tangles in Alzheimer's brain. *J. Vet. Med. Sci.*, **74**, 51–57.

113 Mythri, R.B., Harish, G., Dubey, S.K., Misra, K., and Bharath, M.M.S. (2011) Glutamoyl diester of the dietary polyphenol curcumin offers improved protection against peroxynitrite-mediated nitrosative stress and damage of brain mitochondria in vitro: implications for Parkinson's disease. *Mol. Cell. Biochem.*, **347**, 135–143.

114 Mansouri, A. and Perry, C.A. (1982) Alteration of platelet aggregation by cigarette smoke and carbon monoxide. *Thromb Haemost.*, **48**, 286–288.

115 Lim, K.J., Bisht, S., Bar, E.E., Maitra, A., and Eberhart, C.G. (2011) A polymeric nanoparticle formulation of curcumin inhibits growth, clonogenicity and stem-like fraction in malignant brain tumors. *Cancer Biol. Ther.*, **11**, 464–473.

116 Lee, S.J., Krauthauser, C., Maduskuie, V., Fawcett, P.T., Olson, J.M., and Rajasekaran, S.A. (2011) Curcumin-induced HDAC inhibition and attenuation of medulloblastoma growth *in vitro* and *in vivo*. *BMC Cancer*, **11**, 144.

117 Purkayastha, S., Berliner, A., Fernando, S.S., Ranasinghe, B., Ray, I., Tariq, H., and Banerjee, P. (2009) Curcumin blocks brain tumor formation. *Brain Res.*, **1266**, 130–138.

118 Bangaru, M.L.Y., Woodliff, J., Raff, H., and Kansra, S. (2010) Growth suppression of mouse pituitary corticotroph tumor AtT20 cells by curcumin: a model for treating Cushing's disease. *PLoS One*, **5**, e9893.

119 Wang, J., Du, X.X., Jiang, H., and Xie, J.X. (2009) Curcumin attenuates 6-hydroxydopamine-induced cytotoxicity by anti-oxidation and nuclear factor-kappaB modulation in MES23.5 cells. *Biochem. Pharmacol.*, **78**, 178–183.

120 Chiu, S., Terpstra, K.J., Bureau, Y., Hou, J., Raheb, H., Cernvosky, Z., Badmeav, V., Copen, J., Husni, M., and Woodbury-Farina, M. (2013) Liposomal-formulated curcumin Lipocurc™ targeting HDAC (histone deacetylase) prevents apoptosis and improves motor deficits in Park 7 (DJ-1)-knockout rat model of Parkinson's

disease: implications for epigenetics-based nanotechnology-driven drug platform. *J. Complement Integr. Med.*, **10** (1), 75–88.

121 Du, X.X., Xu, H.M., Jiang, H., Song, N., Wang, J., and Xie, J.X. (2012) Curcumin protects nigral dopaminergic neurons by iron-chelation in the 6-hydroxydopamine rat model of Parkinson's disease. *Neurosci. Bull.*, **28** (3), 253–258.

122 Strong, R., Miller, R.A., Astle, C.M., Baur, J.A., de Cabo, R., Fernandez, E., Guo, W., Javors, M., Kirkland, J.L., Nelson, J.F., Sinclair, D.A., Teter, B., Williams, D., Zaveri, N., Nadon, N.L., and Harrison, D.E. (2013) Evaluation of resveratrol, green tea extract, curcumin, oxaloacetic acid and medium chain triglyceride oil on life span of genetically heterogeneous mice. *J. Gerontol. A Bio. Sci. Med. Sci.*, **68**, 6–16.

123 Liao, V.H.C., Yu, C.W., Chu, Y.J., Li, W.H., Hsieh, Y.C., and Wang, T.T. (2011) Curcumin-mediated lifespan extension in *Caenorhabditis elegans*. *Mech. Ageing Dev.*, **132** (10), 480–487.

124 Siddique, Y.H., Naz, F., and Jyoti, S. (2014) Effect of curcumin on lifespan, activity pattern, oxidative stress, and apoptosis in the brains of transgenic Drosophila model of Parkinson's disease. *Biomed. Res. Int.*, 606928.

125 Feany, M.B. and Bender, W.W. (2000) A drosophila model of Parkinson's disease. *Nature*, **404** (6776), 394–398.

126 Lee, K.S., Lee, B.S., Semnani, S., Avanesian, A., Um, C.Y., Jeon, H.J., Seong, K.M., Yu, K., Min, K.J., and Jafari, M. (2010) Curcumin extends life span, improves health span, and modulates the expression of age-associated aging genes in Drosophila melanogaster. *Rejuvenation Res.*, **13** (5), 561–570.

127 Soh, J.W., Marowsky, N., Nichols, T.N., Rahman, A.M., Miah, T., Sarao, P., Khasawneh, R., Unnikrishnan, A., Heydari, A.R., Silver, R.B., and Arking, R. (2013) Curcumin is an early-acting stage-specific inducer of extended functional longevity in Drosophila. *Exp. Gerontol.*, **48**, 229–239.

128 Xiang, L., Nakamura, Y., Lim, Y.M., Yamasaki, Y., Kurokawa-Nose, Y., Maruyama, W., Osawa, T., Matsuura, A., Motoyama, N., and Tsuda, L. (2011) Tetrahydrocurcumin extends life span and inhibits the oxidative stress response by regulating the FOXO forkhead transcription factor. *Aging*, **3** (11), 1098–1109.

129 Qualls, Z., Brown, D., Ramlochansingh, C., Hurley, L.L., and Tizabi, Y. (2014) Protective effects of curcumin against rotenone and salsolinol-induced toxicity: implications for Parkinson's disease. *Neurotox. Res.*, **25**, 81–89.

130 Prasad, S.N. and Muralidhara (2014) Neuroprotective effect of geraniol and curcumin in an acrylamide model of neurotoxicity in Drosophilamelanogaster: relevance to neuropathy. *J. Insect Physiol.*, **60**, 7–16.

131 Masuda, T., Maekawa, T., Hidaka, K., Bando, H., Takeda, Y., and Yamaguchi, H. (2001) Chemical studies on antioxidant mechanism of curcumin: analysis of oxidative coupling products from curcumin and linoleate. *J. Agric. Food. Chem.*, **49** (5), 2539–2547.

132 Jayaraj, R.L., Elangovan, N., Manigandan, K., Singh, S., and Shukla, S. (2014) CNB-001 a novel curcumin derivative, guards dopamine neurons in MPTP model of Parkinson's disease. *Biomed. Res. Int.*, 236182.

133 Lastra, C.A. and Villegas, I. (2005) Resveratrol as an anti-inflammatory and anti-aging agent: mechanisms and clinical implications. *Mol. Nutr. Food Res.*, **49**, 405–430.

134 Szabo, G. (2009) A glass of red wine to improve mitochondrial biogenesis? Novel mechanisms of resveratrol. *Am. J. Physiol. Heart Circ. Physiol.*, **297**, H8–H9.

135 Wang, Y., Xu, H., Fu, Q., Ma, R., and Xiang, J. (2011) Protective effect of resveratrol derived from Polygonum cuspidatum and its liposomal form on nigral cells in Parkinsonian rats. *J. Neurol. Sci.*, **304**, 29–34.

136 Cal, C., Garban, H., Jazirehi, A., Yeh, C., Mizutani, Y., and Bonavida, B. (2003) Resveratrol and cancer: chemoprevention, apoptosis and chemoimmunosensitizing activities. *Curr. Med. Chem. Anticancer Agents*, **3**, 77–93.

137 Cao, Y., Fu, Z.D., Wang, F., Hong, Y.L., and Han, R. (2005) Anti-angiogenic activity of resveratrol, a natural compound from medicinal plants. *J. Asian Nat. Prod. Res.*, **7** (3), 205–213.

138 Kim, H.J., Chang, E.J., Cho, S.H., Chung, S.K., Park, H.D., and Choi, S.W. (2002) Resveratrol attenuates 6-hydroxydopamine-induced oxidative damage and dopamine depletion in rat model of Parkinson's disease. *Biosci. Biotechnol., Biochem.*, **66** (9), 1990–1993.

139 Harikumar, K.B., Kunnumakkara, A.B., Sethi, G., Diagaradjane, P., Anand, P., Pandey, M.K., Gelovani, J., Krishnan, S., Guha, S., and Aggarwal, B.B. (2010) Resveratrol, a multitargeted agent, can enhance antitumor activity of gemcitabine in vitro and in orthotopic mouse model of human pancreatic cancer. *Int. J. Cancer*, **127** (2), 257–268.

140 Hung, L.M., Chen, J.K., Huang, S.S., Lee, R.S., and Su, M.J. (2000) Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. *Cardiovasc. Res.*, **47**, 549–555.

141 Sahu, S.S., Madhyastha, S., and Rao, G.M. (2013) Neuroprotective effect of resveratrol against prenatal stress induced cognitive impairment and possible involvement of Na^+, K^+-ATPase activity. *Pharmacol. Biochem. Behav.*, **103**, 520–525.

142 Palsamy, P. and Subramanian, S. (2008) Resveratrol, a natural phytoalexin, normalizes hyperglycemia in streptozotocin-nicotinamide induced experimental diabetic rats. *Biomed. Pharmacother.*, **62**, 598–605.

143 Sebastia, N., Almonacid, M., Villaescusa, J.I., Cervera, J., Such, E., Silla, M.A., Soriano, J.M., and Montoro, A. (2013) Radioprotective activity and cytogenic effect of resveratrol in human lymphocytes: an in-vitro evaluation. *Food Chem. Toxicol.*, **51**, 391–395.

144 Docherty, J.J., Fu, M.M., Hah, J.M., Sweet, T.J., Faith, S.A., and Booth, T. (2005) Effect of resveratrol on herpes simplex virus vaginal infection in the mouse. *Antiviral Res.*, **67** (3), 155–162.

145 Rasouri, S., Lagouge, M., and Auwerx, J. (2007) SIRT1/PGC-1: a neuroprotective axis? *Med. Sci. (Paris)*, **23**, 840–844.

146 Howitz, K.T., Bitterman, K.J., Cohen, H.Y., Lamming, D.W., Lavu, S., Wood, J.G., Zipkin, R.E., Chung, P., Kisielewski, A., Zhang, L.L., Scherer, B., and Sinclair, D.A. (2003) Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature*, **425**, 191–196.

147 Baur, J.A., Pearson, K.J., Price, N.L., Jamieson, H.A., Lerin, C., Kalra, A., Prabhu, V.V., Allard, J.S., Lopez-Lluch, G., Lewis, K., Pistell, P.J., Poosala, S., Becker, K.G., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K.W.,

Spencer, R.G., Lakatta, E.G., Le Couteur, D., Shaw, R.J., Navas, P., Puigserver, P., Ingram, D.K., de Cabo, R., and Sinclair, D.A. (2006) Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*, **444**, 337–342.

148 Sinclair, D.A. (2005) Toward a unified theory of caloric restriction and longevity regulation. *Mech. Ageing Dev.*, **126**, 987–1002.

149 Allard, J.S., Perez, E., Zou, S., and de Cabo, R. (2009) Dietary activators of Sirt1. *Mol. Cell Endocrinol.*, **299**, 58–63.

150 Lamming, D.W., Wood, J.G., and Sinclair, D.A. (2004) Small molecules that regulate lifespan: evidence for xenohormesis. *Mol. Microbiol.*, **53**, 1003–1009.

151 Wood, J.G., Rogina, B., Lavu, S., Howitz, K., Helfand, S.L., Tatar, M., and Sinclair, D. (2004) Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature*, **430**, 686–689.

152 Baur, J.A. and Sinclair, D.A. (2006) Therapeutic potential of resveratrol: the *in vivo* evidence. *Nat. Rev. Drug Discov.*, **5**, 493–506.

153 Dasgupta, B. and Milbrandt, J. (2007) Resveratrol stimulates AMP kinase activity in neurons. *Proc. Natl. Acad. Sci. U.S.A.*, **104**, 7217–7222.

154 Pallas, M., Casadesus, G., Smith, M.A., Coto-Montes, A., Pelegri, C., Vilaplana, J., and Camins, A. (2009) Resveratrol and neurodegenerative diseases: activation of SIRT1 as the potential pathway towards neuroprotection. *Curr. Neurovasc. Res.*, **6**, 70–81.

155 Sundararajan, S., Gamboa, J.L., Victor, N.A., Wanderi, E.W., Lust, W.D., and Landreth, G.E. (2005) Peroxisome proliferator-activated receptor-gamma ligands reduce inflammation and infarction size in transient focal ischemia. *Neuroscience*, **130**, 685–696.

156 Wu, J.S., Cheung, W.M., Tsai, Y.S., Chen, Y.T., Fong, W.H., Tsai, H.D., Chen, Y.C., Liou, J.Y., Shyue, S.K., Chen, J.J., Chen, Y.E., Maeda, N., Wu, K.K., and Lin, T.N. (2009) Ligand-activated peroxisome proliferator-activated receptor-gamma protects against ischemic cerebral infarction and neuronal apoptosis by 14-3-3 epsilon upregulation. *Circulation*, **119**, 1124–1134.

157 Rubiolo, J.A., Mithieux, G., and Vega, F.V. (2008) Resveratrol protects primary rat hepatocytes against oxidative stress damage: activation of the Nrf2 transcription factor and augmented activities of antioxidant enzymes. *Eur. J. Pharmacol.*, **591**, 66–72.

158 Tang, B.L. and Chua, C.E. (2008) SIRT1 and neuronal diseases. *Mol. Aspects Med.*, **29**, 187–200.

159 Marambaud, P., Zhao, H., and Davies, P. (2005) Resveratrol promotes clearance of Alzheimer's disease amyloid beta peptides. *J. Biol. Chem.*, **280**, 37377–37382.

160 Kim, Y.A., Lim, S.Y., Rhee, S.H., Park, K.Y., Kim, C.H., Choi, B.T., Lee, S.J., Park, Y.M., and Choi, Y.H. (2006) Resveratrol inhibits inducible nitric oxide synthase and cyclooxygenase-2 expression in beta-amyloid-treated C6 glioma cells. *Int. J. Mol. Med.*, **17**, 1069–1075.

161 Lin, T.N., Wang, Q., Simonyi, A., Chen, J.J., Cheung, W.M., He, Y.Y., Xu, J., Sun, A.Y., Hsu, C.Y., and Sun, G.Y. (2004) Induction of secretory phospholipase A2 in reactive astrocytes in response to transient focal cerebral ischemia in the rat brain. *J. Neurochem.*, **90**, 637–645.

162 Bi, X.L., Yang, J.Y., Dong, Y.X., Wang, J.M., Cui, Y.H., Ikeshima, T., Zhao, Y.Q., and Wu, C.F. (2005) Resveratrol inhibits nitric oxide and TNF-alpha

production by lipopolysaccharide-activated microglia. *Int. Immunopharmacol.*, **5**, 185–193.

163 Robb, E.L., Winkelmolen, L., Visanji, N., Brotchie, J., and Stuart, J.A. (2008) Dietary resveratrol administration increases MnSOD expression and activity in mouse brain. *Biochem. Biophys. Res. Commun.*, **372**, 254–259.

164 Chen, C.Y., Jang, J.H., Li, M.H., and Surh, Y.J. (2005) Resveratrol upregulates heme oxygenase-1 expression via activation of NF-E2-related factor 2 in PC12 cells. *Biochem. Biophys. Res. Commun.*, **331**, 993–1000.

165 Anekonda, T.S. (2006) Resveratrol-a boon for treating Alzheimer's disease? *Brain Res. Rev.*, **52**, 316–326.

166 Mancuso, C., Bates, T.E., Butterfield, D.A., Calafato, S., Cornelius, C., De Lorenzo, A., Dinkova Kostova, A.T., and Calabrese, V. (2007) Natural antioxidants in Alzheimer's disease. *Expert Opin. Investig. Drugs*, **16**, 1921–1931.

167 Pangeni, R., Sharma, S., Mustafa, G., Ali, J., and Baboota, S. (2014) Vitamin E loaded resveratrol nanoemulsion for brain targeting for the treatment of Parkinson's disease by reducing oxidative stress. *Nanotechnology*, **25** (48), 485102.

168 Okaware, M., Katsuki, H., Kurimoto, E., Shibata, H., Kume, T., and Akaike, A. (2007) Resveratrol protects dopaminergic neurons in midbrain slice culture from multiple insults. *Biochem. Pharmacol.*, **73**, 550–560.

169 Khan, M.M., Ahmad, A., Ishrat, T., Khan, M.B., Hoda, M.N., Khuwaja, G., Raza, S.S., Khan, A., Javed, H., Vaibhav, K., and Islam, F. (2010) Resveratrol attenuates 6-hydroxydopamine-induced oxidative damage and dopamine depletion in rat model of Parkinson's disease. *Brain Res.*, **1328**, 139–151.

170 Wu, Y., Li, X., Zhu, J.X., Xie, W., Le, W., Fan, Z., Jankovie, J., and Pan, T. (2011) Resveratrol–activated ampk/sirt1/autophagy in cellular models of Parkinson's disease. *Neurosignals*, **19**, 163–174.

171 Spindler, M., Beal, M.F., and Henchcliffe, C. (2009) Coenzyme Q10 effects in neurodegenerative disease. *Neuropsychiatr. Dis. Treat.*, **5**, 597–610.

172 Matthews, R.T., Yang, L., Browne, S., Baik, M., and Beal, M.F. (1998) Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects. *Proc. Natl. Acad. Sci. U.S.A.*, **95** (15), 8892–8897.

173 Liu, J., Wang, L., and Zhan, S.Y. (2011) Coenzyme Q10 for Parkinson's disease. *Cochrane Database Syst. Rev.*, **12**, CD008150.

174 Muller, T., Buttner, T., Gholipour, A.F., and Kuhn, W. (2003) Coenzyme Q10 supplementation provides mild symptomatic benefit in patients with Parkinson's disease. *Neurosci. Lett.*, **341** (3), 201–204.

175 Seet, R.C., Lim, E.C., Tan, J.J., Quek, A.M., Chow, A.W., Chong, W.L., Ng, M.P., Ong, C.N., and Halliwell, B. (2014) Does high-dose coenzyme Q10 improve oxidative damage and clinical outcomes in Parkinson's disease? *Antioxid. Redox Signal.*, **21** (2), 211–217.

176 Ebadi, M., Govitrapong, P., Sharma, S., Muralikrishnan, D., Shavali, S., Pellett, L., Schafer, R., Albano, C., and Eken, J. (2001) Ubiquinone (coenzyme q10) and mitochondria in oxidative stress of Parkinson's disease. *Biol. Signals Recept.*, **10** (3-4), 224–253.

177 Gorgone, G., Curro, M., Ferlazzo, N., Parisi, G., Parnetti, L., Belcastro, V., Tambasco, N., Rossi, A., Pisani, F., Calabresi, P., Ientile, R., and Caccamo, D. (2012) Coenzyme Q10, hyperhomocysteinemia and MTHFR C677T

polymorphism in levodopa-treated Parkinson's disease patients. *Neuromolecular Med.*, **14** (1), 84–90.

178 Esteves, A.R., Arduino, D.M., Swerdlow, R.H., Oliveira, C.R., and Cardoso, S.M. (2009) Oxidative stress involvement in alpha-synuclein oligomerization in Parkinson's disease cybrids. *Antioxid. Redox Signal.*, **11** (3), 439–448.

179 Sharma, S., Kheradpezhou, M., Shavali, S., El Refaey, H., Eken, J., Hagen, C., and Ebadi, M. (2004) Neuroprotective actions of coenzyme Q10 in Parkinson's disease. *Methods Enzymol.*, **382**, 488–509.

180 Shults, C.W., Flint Beal, M., Song, D., and Fontaine, D. (2004) Pilot trial of high dosages of coenzyme Q10 in patients with Parkinson's disease. *Exp. Neurol.*, **188** (2), 491–494.

181 Gotz, M.E., Gerstner, A., Harth, R., Dirr, A., Janetzky, B., Kuhn, W., Riederer, P., and Gerlach, M. (2000) Altered redox state of platelet coenzyme Q10 in Parkinsons disease. *J. Neural Transm.*, **107** (1), 41–48.

182 Li, Z., Wang, P., Yu, Z., Cong, Y., Sun, H., Zhang, J., Zhang, J., Sun, C., Zhang, Y., and Ju, X. (2015) The effect of creatine and coenzyme Q10 combination therapy on mild cognitive impairment in Parkinson's disease. *Eur. Neurol.*, **73**, 205–211.

183 Fernandez-Calle, P., Jimenez-Jimenez, F.J., Molina, J.A., Cabrera-Valdivia, F., Vazquez, A., Garcia Urra, D., Bermejo, F., Cruz, Matallana, M., and Codoceo, R. (1993) Serum levels of ascorbic acid (vitamin C) in patients with Parkinson's disease. *J. Neurol. Sci.*, **118** (1), 25–28.

184 Quiroga, M.J., Carroll, D.W., and Brown, T.M. (2014) Ascorbate- and zinc-responsive parkinsonism. *Ann. Pharmacother.*, **48** (11), 1515–1520.

185 Sutachan, J.J., Casas, Z., Albarracin, S.L., Stab, B.R., Samudio, I., Gonzalez, J., Morales, L., and Barreto, G.E. (2012) Cellular and molecular mechanisms of antioxidants in Parkinson's disease. *Nutr. Neurosci.*, **15** (3), 120–126.

186 Smythies, J.R. (1999) The role of ascorbate in brain: therapeutic implications. *J. R. Soc. Med.*, **89** (5), 241.

187 Pardo, B., Mena, M.A., Fahn, S., and Garcia de Yébenes, J. (1993) Ascorbic acid protects against levodopa-induced neurotoxicity on a catecholamine-rich human neuroblastoma cell line. *Mov. Disord.*, **8** (3), 278–284.

188 Ballaz, S., Morales, I., Rodriguez, M., and Obeso, J.A. (2013) Ascorbate prevents cell death from prolonged exposure to glutamate in an in vitro model of human dopaminergic neurons. *J. Neurosci. Res.*, **91** (12), 1609–1617.

189 Choi, H.Y., Song, J.H., Park, D.K., and Ross, G.M. (2000) The effects of ascorbic acid on dopamine-induced death of PC12 cells are dependent on exposure kinetics. *Neurosci. Lett.*, **296** (2-3), 81–84.

190 Lazzarini, M., Salum, C., and Del Bel, E.A. (2005) Combined treatment of ascorbic acid or alpha-tocopherol with dopamine receptor antagonist or nitric oxide synthase inhibitor potentiates cataleptic effect in mice. *Psychopharmacology (Berl)*, **181** (1), 71–79.

191 Scheider, W.L., Hershey, L.A., Vena, J.E., Holmlund, T., Marshall, J.R., and Freudenheim (1997) Dietary antioxidants and other dietary factors in the etiology of Parkinson's disease. *Mov. Disord.*, **12** (2), 190–196.

192 Ide, K., Yamada, H., Umegaki, K., Mizuno, K., Kawakami, N., Hagiwara, Y., Matsumoto, M., Yoshida, H., Kim, K., Shiosaki, E., Yokochi, T., and Harada, K.

(2015) Lymphocyte vitamin C levels as potential biomarker for progression of Parkinson's disease. *Nutrition*, **31** (2), 406–408.

193 Nagayama, H., Hamamoto, M., Ueda, M., Nito, C., Yamaguchi, H., and Katayama, Y. (2004) The effect of ascorbic acid on the pharmacokinetics of levodopa in elderly patients with Parkinson disease. *Clin. Neuropharmacol.*, **27** (6), 270–273.

194 Paraskevas, G.P., Kapaki, E., Petropoulou, O., Anagnostouli, M., Vagenas, V., and Papageorgiou, C. (2003) Plasma levels of antioxidant vitamins C and E are decreased in vascular parkinsonism. *J. Neurol. Sci.*, **215** (1-2), 51–55.

195 Offen, D., Ziv, I., Sternin, H., Melamed, E., and Hochman, A. (1996) Prevention of dopamine-induced cell death by thiol antioxidants: possible implications for treatment of Parkinson's disease. *Exp. Neurol.*, **141** (1), 32–39.

196 Menendez-Pelaez, A., Howes, K.A., Gonzalez-Brito, A., and Reiter, R.J. (1987) N-acetyltransferase activity, hydroxyindole-O-methyltransferase activity, and melatonin levels in the Harderian glands of the female Syrian hamster: changes during the light:dark cycle and the effect of 6-parachlorophenylalanine administration. *Biochem. Biophys. Res. Commun.*, **145**, 1231–1238.

197 Tricoire, H., Locatelli, A., Chemineau, P., and Malpaux, B. (2002) Melatonin enters the cerebrospinal fluid through the pineal recess. *Endocrinology*, **143**, 84–90.

198 Miller, E., Morel, A., Saso, L., and Saluk, J. (2014) Melatonin redox activity. Its potential clinical application in neurodegenerative disorders. *Curr. Top. Med. Chem.*

199 Garcia, J.J., Lopez-Pingarron, L., Almeida-Souza, P., Tres, A., Escudero, P., García-Gil, F.A., Tan, D.X., Reiter, R.J., Ramírez, J.M., and Bernal-Perez, M. (2014) Protective effects of melatonin in reducing oxidative stress and in preserving the fluidity of biological membranes: a review. *J. Pineal Res.*, **56** (3), 225–237.

200 Reiter, R.J., Tan, D.X., Cabrera, J., D'Arpa, D., Sainz, R.M., Mayo, J.C., and Ramos, S. (1999) The oxidant/antioxidant network: role of melatonin. *Biol. Signals Recept.*, **8**, 56–63.

201 Rosen, J., Than, N.N., Koch, D., Poeggeler, B., Laatsch, H., and Hardeland, R. (2006) Interactions of melatonin and its metabolites with the ABTS cation radical: extension of the radical scavenger cascade and formation of a novel class of oxidation products, C2-substituted 3-indolinones. *J. Pineal Res.*, **41**, 374–381.

202 Manda, K., Ueno, M., and Anzai, K. (2007) AFMK, a melatonin metabolite, attenuates X-ray-induced oxidative damage to DNA, proteins and lipids in mice. *J. Pineal Res.*, **42**, 386–393.

203 Tan, D.X., Manchester, L.C., Terron, M.P., Flores, L.J., and Reiter, R.J. (2007) One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? *J. Pineal Res.*, **42**, 28–42.

204 Tomas-Zapico, C. and Coto-Montes, A. (2005) A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. *J. Pineal Res.*, **39**, 99–104.

205 Martinez, G.R., Almeida, E.A., Klitzke, C.F., Onuki, J., Prado, F.M., Medeiros, M.H., and Di Mascio, P. (2005) Measurement of melatonin and its metabolites: importance for the evaluation of their biological roles. *Endocrine*, **27**, 111–118.

206 Radogna, F., Diederich, M., and Ghibelli, L. (2010) Melatonin: a pleiotropic molecule regulating inflammation. *Biochem. Pharmacol.*, **80**, 1844–1852.

207 Teixeira, A., Morfim, M.P., de Cordova, C.A., Charao, C.C., de Lima, V.R., and Creczynski-Pasa, T.B. (2003) Melatonin protects against pro-oxidant enzymes and reduces lipid peroxidation in distinct membranes induced by the hydroxyl and ascorbyl radicals and by peroxynitrite. *J. Pineal Res.*, **35**, 262–268.

208 Ferry, G., Ubeaud, C., Lambert, P.H., Bertin, S., Coge, F., Chomarat, P., Delagrange, P., Serkiz, B., Bouchet, J.B., Truscott, R.J., and Boutin, J.A. (2005) Molecular evidence that melatonin is enzymatically oxidized in a different manner than tryptophan: investigations with both indoleamine 2,3-dioxygenase and myeloperoxidase. *Biochem. J.*, **388**, 205–215.

209 Claustrat, B., Brun, J., and Chazot, G. (2005) The basic physiology and pathophysiology of melatonin. *Sleep Med. Rev.*, **9**, 11–24.

210 Leon, J., Escames, G., Rodriguez, M.I., Lopez, L.C., Tapias, V., Entrena, A., Camacho, E., Carrion, M.D., Gallo, M.A., Espinosa, A., Tan, D.X., Reiter, R.J., and Acuna-Castroviejo, A. (2006) Inhibition of neuronal nitric oxide synthase activity by N1-acetyl-5-methoxykynuramine, a brain metabolite of melatonin. *J. Neurochem.*, **98**, 2023–2033.

211 Rios, E.R., Venancio, E.T., Rocha, N.F., Woods, D.J., Vasconcelos, S., Macedo, D., Sousa, F.C., and Fonteles, M.M. (2010) Melatonin: pharmacological aspects and clinical trends. *Int. J. Neurosci.*, **120**, 583–590.

212 Breen, D.P., Vuono, R., Nawarathna, U., Fisher, K., Shneerson, J.M., Reddy, A.B., and Barker, R.A. (2014) Sleep and circadian rhythm regulation in early Parkinson disease. *JAMA Neurol.*, **71** (5), 589–595.

213 Ono, K. and Yamada, M. (2014) Alpha-Synuclein in blood and cerebrospinal fluid of patients with alpha-synucleinopathy. *Rinsho Byori*, **62** (3), 241–245.

214 Emre, M., Ford, P.J., Bilgic, B., and Uc, E.Y. (2014) Cognitive impairment and dementia in Parkinson's disease: practical issues and management. *Mov. Disord.*, **29** (5), 663–672.

215 Brown, G.C. and Borutaite, V. (2004) Inhibition of mitochondrial respiratory chain complex I by nitric oxide, peroxynitrite and S-nitrosothiols. *Biochim. Biophys. Acta*, **1658**, 44–49.

216 Leon, J., Vives, F., Crespo, E., Camacho, E., Espinosa, A., Gallo, M.A., Escames, G., and Acuna-Castroviejo, D. (1998) Modification of nitric oxide synthase activity and neuronal response in rat striatum by melatonin and kynurenine derivatives. *J. Neuroendocrinol.*, **10**, 297–302.

217 Schulz, J.B., Matthews, R.T., Muqit, M.M., Browne, S.E., and Beal, M.F. (1995) Inhibition of neuronal nitric oxide synthase by 7-nitroindazole protects against MPTP-induced neurotoxicity in mice. *J. Neurochem.*, **64** (2), 936–939.

218 Phillipson, O.T. (2014) Management of the aging risk factor for Parkinson's disease. *Neurobiol. Aging*, **35** (4), 847–857.

219 Naskar, A., Prabhakar, V., Singh, R., Dutta, D., and Mohanakumar, K.P. (2015) Melatonin enhances L-DOPA therapeutic effects, helps to reduce its dose, and protects dopaminergic neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in mice. *J. Pineal Res.*, **58** (3), 262–274.

220 Kotler, M., Rodriguez, C., Sainz, R.M., Antolin, I., and Menendez-Pelaez, A. (1998) Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. *J. Pineal Res.*, **24**, 83–89.

221 Lopez, A., Garcia, J.A., Escames, G., Venegas, C., Ortiz, F., Lopez, L.C., and Acuna-Castroviejo, D. (2009) Melatonin protects the mitochondria from oxidative damage reducing oxygen consumption, membrane potential, and superoxide anion production. *J. Pineal Res.*, **46**, 188–198.

222 Okatani, Y., Wakatsuki, A., and Reiter, R.J. (2002) Melatonin protects hepatic mitochondrial respiratory chain activity in senescence-accelerated mice. *J. Pineal Res.*, **32**, 143–148.

223 Antunes, F., Han, D., Rettori, D., and Cadenas, E. (2002) Mitochondrial damage by nitric oxide is potentiated by dopamine in PC12 cells. *Biochim. Biophys. Acta*, **1556**, 233–238.

224 Yamamoto, T., Maruyama, W., Kato, Y., Yi, H., Shamoto-Nagai, M., Tanaka, M., Sato, Y., and Naoi, M. (2002) Selective nitration of mitochondrial complex I by peroxynitrite: involvement in mitochondria dysfunction and cell death of dopaminergic SHSY5Y cells. *J. Neural Transm.*, **109**, 1–13.

225 Nogues, M.R., Giralt, M., Romeu, M., Mulero, M., Sanchez-Martos, V., Rodriguez, E., Acuna-Castroviejo, D., and Mallol, J. (2006) Melatonin reduces oxidative stress in erythrocytes and plasma of senescence-accelerated mice. *J. Pineal Res.*, **41** (2), 142–149.

226 Meki, A.R. and Hussein, A.A. (2001) Melatonin reduces oxidative stress induced by ochratoxin A in rat liver and kidney. *Comp. Biochem. Physiol. C: Toxicol. Pharmacol.*, **130** (3), 305–313.

5

Neuroprotective Effect of Ayurvedic Preparations and Natural Products on Parkinson's Disease

Anupom Borah[*], *Amarendranath Choudhury*[*], *Rajib Paul, Muhammed K. Mazumder, and Swapnali Chetia*

Cellular and Molecular Neurobiology Laboratory, Department of Life Science and Bioinformatics, Assam University, Silchar 788011, Assam, India

5.1 Introduction

Parkinson's disease (PD), the most common neurodegenerative motor disorder, has been diagnosed in 6 million people worldwide with a twofold increasing probability in the next two decades [1]. Classically, PD is characterized by motor abnormalities, namely, bradykinesia, resting tremor, rigidity and postural instability, and freezing or pause phenomenon [2, 3]. In addition, some non-motor symptoms, including depression, dementia, and sleep disorder, are reported in PD patients [4]. The reason behind motor abnormalities is the degeneration or selective loss of dopaminergic (DAergic) neurons of the *substantia nigra* (SN) *pars compacta* region of the midbrain [5, 6] and the resultant decrease in the level of dopamine (DA) in the striatum [7, 8]. Both genetic and environmental factors have been documented as risk factors of the disease [1], while a number of endogenous molecules have also been reported to potentially influence the pathology of PD [9–12]. It has been reported that the mechanism of DAergic neurodegeneration involves the pathogenic accumulation of α-synuclein and other misfolded proteins as Lewy bodies – the hallmark pathological signature of PD [13–15]. Oxidative stress, inflammation, mitochondrial defects, and excitotoxicity are regarded as the major underlying mechanisms for DAergic neurodegeneration [16, 17]. Moreover, in PD patients, abnormal brain acetylcholine level and cortical serotonin level have been reported to be associated with tremor [18] and mood swing [19], respectively. However, the frontier treatment strategy for PD focuses on normalizing the DA level through DA replenishment therapy [2, 20, 21].

5.1.1 Therapy for Parkinson's Disease

Although no cure for PD is available till date, the available treatment options confer symptomatic rectification and provide partial relief from pain and slacken

[*] Contributed equally to the authors

Neuroprotective Natural Products: Clinical Aspects and Mode of Action, First Edition.
Edited by Goutam Brahmachari.

progression of the disease [22]. Among all therapeutic strategies, oral administration of a DA precursor, L-3,4-dihydroxyphenylalanine (L-DOPA), to replenish the DA level in the striatum is widely practiced [2, 3, 23]. However, chronic use of L-DOPA is known to cause adverse side effects at both behavioral and molecular levels, including motor fluctuations, dyskinesia, and elevation in the levels of endogenous molecules that have been implicated to be cytotoxic [3, 24–26]. Other treatment strategies are the use of DA agonist and inhibitors of DA/L-DOPA-catabolizing enzymes, such as monoamine oxidase-B (MAO-B) and catechol-*O*-methyltransferase (COMT) [27–29]. Use of amantadine – a DA agonist – has promising therapeutic potency against parkinsonism [30]. However, use of amantadine has limitations, including lessening of the effects over time, adverse behavioral side effects (visual hallucinations, confusion, livedo reticularis), and ankle edema [31, 32]. Moreover, inhibitors of COMT and MAO-B also have side effects, such as onset/aggravation of dyskinesia, nausea, vomiting, anorexia, insomnia, hallucination, headache, diarrhea, hepatic toxicity, etc. [33, 34]. Use of such treatment provides temporary symptomatic relief, and in a longer treatment window, "off-time" decreases rapidly, which indicates the resistance of the disease toward the drug [35, 36]. Anticholinergic therapy also provides temporary improvement [37, 38], but in chronic use, it showed the same limitations [39].

Eastern strategies for PD therapy include meditation and exercise [40–43]. An earlier report has shown that physical exercise and medication elevate the level of DA in the brain [43]. Deep brain stimulation has been regarded as the most effective strategy for symptomatic treatment of PD [44, 45]. However, it also has side effects such as treatment-resistant depression (TRD) [46] as well as the disadvantages of high-cost surgery and maintenance [47], transient relief from pain and suffering, and failure in protecting the remnant neurons from progressive degeneration [45].

Thus, the need for a better therapy is the prime focus of the current PD research. Since monotherapy with L-DOPA fails to prevent disease progression [20], combination drug therapy has got ample of rationale [48–50]. Many drug combinations are under clinical trial, but unfortunately some of them have shown lesser efficacy [51]. Comparatively, natural products used in the treatment of the disease have shown better response [52]. Therefore, efficacy of natural products in combination with conventional drugs may open up a new era of PD therapy.

5.2 Parkinsonian Symptoms and Ayurveda

5.2.1 Equivalent Parkinsonian Symptoms in Ayurveda

A literary evidence of parkinsonism was highlighted from the medical article of Galen who wrote the monogram in and around 175 AD [53]. The same disease symptoms were elaborated by James Parkinson under the name "shaking palsy" [54, 55]. Honoring the contribution of James Parkinson to the disease, Jean Martin (1917) coined the disease as "Parkinson's disease" [56]. Descriptions of equivalent parkinsonian symptoms are found in ancient Indian medical system, Ayurveda [53]. One such literature, compiled by Suśruta (a surgeon in ancient India who described slowness and akinesia (*Cestasanga* and *Cestahani* in

Sanskrit) for the first time), is *Susruta Samhita*, the most ancient literature on medical science written in 600 BC [53, 57, 58]. As early as 300 BC, Charaka, in his *Charaka Samhita* (another Ayurvedic literature), described a coherent picture of parkinsonism where head tremor (*Sirakampa*) and generalized tremor were described [59]. The fifteenth-century Ayurvedic classic *Bhasava Rajyam* described a disease, *Kampavata*, that may be regarded as an Ayurvedic analog of parkinsonism [57]. The primary symptoms of *Kampavata* are *Kampa* (tremor) and *Stambha* (rigidity or stiffness), which could be *Ekanga* (localized) or *Sarvanga* (generalized), and were regarded as abnormal patterns of the *Cala* (moving), a property of *Vata* humor [57]. In Ayurveda it is described that the balance between healthy and unhealthy state depends on variable proportion of three humors: *Vata*, *Pitta*, and *Kapha* present in the human body [60]. *Vata* regulates movement; *Pitta* is responsible for the regulation of heat, metabolism, and energy production, while *Kapha* regulates physical structure and fluid balance [57, 60]. Descriptions of other symptoms of parkinsonism, such as slowness (*Cestasanga*), akinesia (*Cestahani*), gait disturbances (*Gatisanga*), postural instability (*Skhalanam Gatau*), dementia (*Smrtikshaya*), and depression (*Vishada*), are also found in Ayurveda [57, 60]. In modern Ayurvedic literature, parkinsonism is described in various names: *Kampavata* (tremors due to *Vata*), *Vepathu* (shaking), *Prevepana* (excessive shaking), *Sirakampa* (head tremor), *Spandin* (quivering), and *Kampana* (tremors) [61].

5.2.2 Treating Parkinsonian Symptoms with Ayurvedic Preparations

Phytochemical ingredients are the gift of nature and also a blessing to all those who are suffering from diseases and pain [62]. From the ancient times of human history, humans got their primary remedy from natural products [62, 63]. Depending upon the available fauna, different ethnic groups have set up their own kind of medicinal practice [64]. In the case of therapeutic interventions for PD, the effect of phytochemicals has been documented in ancient scripts of Egypt and India [53]. In Ayurvedic perspective, *Bhasava Rajyam* described the therapy of *Kampavata* with Ayurvedic recipes [61]. The recipe includes a cocktail of powdered seeds of Atmagupta (*Mucuna pruriens*) and Paraseekayavanee (*Hyoscyamus reticulatus*) with roots of Ashwagandha (*Withania somnifera*) and Bala (*Sida cordifolia*) with cow's milk [53]. Use of these medicinal plants by different ethnic groups showed the possibility of an alternative medicine for PD [65]. It seems to be rational that due to unavailability of histopathological and molecular techniques at ancient times, most of the studies were based on empirical observation [59, 61]. *Bhasava Rajyam* mentioned the use of some potential antiparkinsonian herbs, namely, *M. pruriens*, *H. reticulatus*, *W. somnifera*, *Bacopa monnieri*, *Centella asiatica*, and *S. cordifolia* [66–68]. Among them, the seeds of *M. pruriens* contain L-DOPA, which has more half-life in the human body compared with synthetic L-DOPA [65, 69]. The presence of neuroactive ingredients (see Table 5.1), in addition to L-DOPA, adds to the therapeutic potency of Ayurvedic formulations in PD [61, 95]. It has also been reported that Ayurvedic formulations without *M. pruriens* also improve PD [65, 92], which warrants determination of the specific role of the neuroactive components in the formulation (see Table 5.1) [57, 61].

5.3 Medicinal Plants in the Ayurvedic Formulation for Parkinson's Disease Therapy

The most important and crucial herb in the Ayurvedic formulation is *M. pruriens*, commonly known as "velvet bean." Studies have shown that use of powdered seeds of *M. pruriens* successfully reduced the Hoehn and Yahr stage of the disease and improved the Unified Parkinson's Disease Rating Scale (UPDRS) score in a double-blind clinical trial with PD patients [68]. Similar outcome was evident from a clinical study of Nagashayana *et al.* [65], where the authors have documented the contribution of powdered seeds of *M. pruriens* (4.5 g) and *Hyoscyamus niger* (0.75 g) along with the roots of *W. somnifera* (14.5 g) and *S. cordifolia* (14.5 g) added in 200 ml cow's milk in 18 clinically diagnosed parkinsonian patients [65]. The study [65] has shown the effectiveness of *M. pruriens* in symptomatic improvement from tremor, bradykinesia, stiffness, and cramps, compared to the synthetic L-DOPA. In toxin-induced animal model of PD, it was found that L-DOPA from *M. pruriens* is more efficacious as compared with those produced synthetically [74]. *M. pruriens*-derived L-DOPA shows no adverse effect at the behavioral level, such as dyskinesia [20]. Thus, *M. pruriens* is a better alternative to synthetic L-DOPA and has promising therapeutic potency, alone or in combination with peripheral decarboxylase inhibitors, in PD therapy.

H. niger L., commonly known as henbane, is another component plant of antiparkinsonian Ayurvedic formulation [65, 96]. Although L-DOPA is not a constituent of *H. niger* [65], it is rich in tropane alkaloids, that is, hyoscine and hyoscyamine, which are well known for their anticholinergic effects [97]. Anticholinergic therapy, to correct the neuromuscular anomalies, is used as a remedy of tremor [98]. Moreover, aqueous methanolic seed extract of *H. niger* attenuates motor disabilities and enhances DA level in the striatum in PD animal models [92] and thus assists in the therapeutic success of Ayurvedic formulation in PD.

W. somnifera L. (Dunal), commonly known as Ashwagandha, is an important constituent of several Ayurvedic preparations. The root extract of the plant has been reported to be neuroprotective in animal model of PD [99]. Other relevant studies have shown that root extract of this plant significantly reverses parkinsonian behavioral abnormalities in toxin-induced animal models [99–101]. Root extract of *W. somnifera* has been reported to reverse haloperidol-induced catalepsy [102], reserpine-induced dyskinesia, and cognitive dysfunction [103] in animal models, and the drug BR-16A derived from it has been reported to reverse pentobarbitone-induced sleep, analgesia, and reduction in locomotor activity [104].

S. cordifolia L., commonly called Bala, is an important Ayurvedic medicinal herb and the component of the antiparkinsonian Ayurvedic formulation and is also used for the treatment of asthma, nasal congestion, and blennorrhea [86]. Ethyl acetate root and shoot extract of the plant has anti-inflammatory and analgesic activities, while the methanolic extract has hypoglycemic activity [89]. The plant has several neuroactive antioxidant and anti-inflammatory molecules ([86]; Table 5.1), which explains its usefulness in PD therapy [65]. Aqueous extracts of the plant have been reported to be protective against toxin-induced parkinsonism [87].

Table 5.1 Active ingredients present in the plants used in antiparkinsonian Ayurvedic formulation.

Name of the plant	Name of antiparkinsonian active ingredients	Function	References
Atmagupta (*Mucuna pruriens*)	L-DOPA	Increases DA level	[5, 70]
	Serotonin	Mood and behavior regulation	[71]
	5-Methoxy-*N*,*N*-dimethyltryptamine	Increases serotonin	[72]
	5-Hydroxytryptophan	Increases serotonin	[71]
	Nicotine	Antioxidant, anti-inflammatory, improvement of synaptic plasticity, protects dopaminergic neurons	[73]
	Coenzyme Q10, NADH	Antioxidant	[74]
Ashwagandha (*Withania somnifera*)	Alkaloids (ashwagandhine, cuscohygrine, tropine, pseudotropine, isopelletierine, anaferine, withanamides, etc.)	Antioxidant Anti-inflammatory	[64, 75–80]
	Estrogen-like steroids (withanolides A-Y, withanoside IV/VI, withasomniferin A, dehydrowithanolide R, withasomidienone, withasomniferol A–C, withaferin A, withanone, etc.)	Dendritic spine outgrowth, axonal outgrowth, synaptic reconstruction Anti-inflammatory	[64, 81, 82]
	Phytosterols (sitoindosides VII–X, beta-sitosterol)	Cognitive improvement Enhances acetylcholinesterase activity	[83–85]
Bala (*Sida cordifolia*)	Ephedrine	Antioxidant Anti-inflammatory	[86–88]
	Hypaphorine	Antioxidant	[87]
	Vasicinone	Anti-inflammatory	[89]
	Betaine	Antioxidant Anti-inflammatory	[90, 91]
Paraseekayavanee (*Hyoscyamus reticulatus*)	Hyoscyamine	Antioxidant	[92, 93]
	Scopolamine	Antioxidant	[94]
	Salicylic acid	Antioxidant	[92]

5.3.1 Mechanism of Action of Ayurvedic Preparation in PD

Ayurvedic formulation uses both DA replenishment and stress minimization strategies for treating PD [50, 105, 106], with limited side effects [66, 107]. Antioxidant and neuroactive components (Table 5.1) of the formulation have been reported to minimize stress, which adds an adjuvant benefit in PD therapy [65, 95, 108]. Active ingredients of the formulation have neuroprotective nature when given solely or in combinatorial practice.

5.3.1.1 *Mucuna pruriens*

It replenishes DA level owing to its L-DOPA content that has longer half-life and better pharmacokinetic properties compared with synthetic L-DOPA [68, 109, 110]. Moreover, *M. pruriens* improves mitochondrial complex I activity without altering MAO activity and also restores the level of endogenous monoamine neurotransmitters levels (L-DOPA, DA, norepinephrine, serotonin, and their metabolites) in the SN [74]. Interestingly, few other neuroprotective entities such as nicotinamide adenine dinucleotide and coenzyme Q10, which are used in the therapeutic intervention of PD, are also found in *M. pruriens* [68, 74, 111–113]. On the contrary, Manyam *et al.* [111] have shown that 52 weeks' oral administration of the drug HP-200 derived from endocarp of *M. pruriens* did not show any effect on monoamine neurotransmitter levels in the nigrostriatal pathway, although an increase in cortical DA has been found [111]. Thus, it is speculated that the role of *M. pruriens* in restoring DA and other neurotransmitter levels in different brain regions cannot be solely attributed to the presence of the active molecule (HP-200), and hence, the study warrants determination of the role of other neuroactive constituents of *M. pruriens*. Study on animal model of PD has shown that seed extract of *M. pruriens* is more potent in recovering behavioral anomalies in comparison to equivalent dose of synthetic L-DOPA [113]. L-DOPA from *M. pruriens* has longer half-life and better clinical and pharmacokinetic effects in comparison to synthetic L-DOPA [68]. In the presence of copper ion (Cu^{2+}), L-DOPA damages genomic DNA [114]. *M. pruriens* chelates Cu^{2+} and thereby reduces genotoxicity of L-DOPA [115]. Dhanasekaran *et al.* [112] have reported that *M. pruriens* inhibits lipid peroxidation and oxidation of deoxyribose sugar, which has been attributed to its metal chelating and free radical scavenging properties [112].

5.3.1.2 *Hyoscyamus niger*

Aqueous methanolic seed extract of *H. niger* restores striatal loss of DA in toxin-induced PD model through inhibition of MAO and also shows hydroxyl radical scavenging activity [92]. It acts as an anticholinergic agent and helps to correct the neuromuscular and behavioral anomalies in PD patient [92]. Thus, anticholinergic, MAO inhibitory, and antioxidant effects of *H. niger* play major role in the therapeutic success of PD.

5.3.1.3 *Withania somnifera*

W. somnifera provides protection against oxidative stress and inflammation by the virtue of its constituent phytochemicals, namely, glycowithanolides, tannin,

somnine, somniferiene, anaferine, and withanolides [101, 116, 117]. Moreover, administration of estrogen-like steroids from *W. somnifera* promotes dendritic spine outgrowth, neuritic regeneration, synaptic reconstruction, and axonal outgrowth [81, 82]. Other relevant studies have shown that root extract of this plant significantly improves behavioral abnormalities, catecholamine levels, reduced DAergic D2 receptor binding, and tyrosine hydroxylase (TH) expression in toxin-induced PD [99–103]. The extract ameliorates oxidative stress by inhibiting lipid peroxidation and alterations in the activities of antioxidant enzymes (glutathione-*S*-transferase, glutathione reductase, glutathione peroxidase, superoxide dismutase, and catalase) as well as replenishes the level of antioxidant molecule (reduced glutathione) in PD model [99–101].

5.3.1.4 *Sida cordifolia*

Aqueous extract of *S. cordifolia* attenuates motor abnormalities and eosinophilic lesions in toxin-induced animal model of PD [87]. Also, the extracts reversed lipid peroxidation, generation of superoxide anion, decrease in reduced glutathione, and catalase activity in the cortex, midbrain, and cerebellum [87]. Moreover, the extracts also attenuated decrease in the DA level in the midbrain [87]. Ephedrine, hypaphorine, vasicinone, and betaine from *S. cordifolia* have been reported to be effective in preventing apoptosis and inflammation in PD model [60, 86–88]. In summary, Ayurvedic formulations show robust therapeutic efficacy against different pathophysiological aspects of PD.

5.4 Concluding Remarks

Ayurvedic preparation for the therapeutic intervention of PD is reported to have better response to correct the behavioral anomalies of the disease. The Ayurvedic formulation used to treat PD contains *M. pruriens*, *W. somnifera*, *H. niger*, and *S. cordifolia*. While *M. pruriens* contains L-DOPA, thereby replenishing DA level, *H. niger* has MAO inhibitory and anticholinergic effect, and thus they are helpful in ameliorating the motor and non-motor behavioral abnormalities of PD. In addition, each component of the formulation has been independently found to have neuroprotective properties in PD, owing to their antioxidant and anti-inflammatory effect. The formulation is also appraised for better efficacy, limited side effects, and cost-effectiveness. It is astonishing that Ayurvedic practitioners of ancient India formulated such a unique composition that serves probably the best therapy for PD in comparison to the presently prescribed drugs.

Abbreviations

BC	Before Christ
COMT	catechol-*O*-methyltransferase
DA	dopamine
DAergic	dopaminergic
DNA	deoxyribonucleic acid

L-DOPA	3,4-dihydroxyphenylalanine
PD	Parkinson's disease
MAO-B	monoamine oxidase-B
SN	substantia nigra
TH	tyrosine hydroxylase
TRD	treatment-resistant depression

References

1 Nass, R. and Przedborski, S. (eds) (2011) *Parkinson's Disease: Molecular and Therapeutic Insights from Model Systems*, Elsevier Academic Press.
2 Fahn, S. (2015) The medical treatment of Parkinson disease from James Parkinson to George Cotzias. *Mov. Disord.*, **30** (1), 4–18.
3 Fahn, S. and Poewe, W. (2015) Levodopa: 50 years of a revolutionary drug for Parkinson disease. *Mov. Disord.*, **30** (1), 1–3.
4 Chaudhuri, K.R., Healy, D.G., and Schapira, H. (2006) Non-motor symptoms of Parkinson's disease: diagnosis and management. *Lancet Neurol.*, **5** (3), 235–245.
5 Tolosa, A., Zhou, X., Spittau, B., and Krieglstein, K. (2013) Establishment of a survival and toxic cellular model for Parkinson's disease from chicken mesencephalon. *Neurotox. Res.*, **24** (2), 119–129.
6 Bové, J., Prou, D., Perier, C., and Przedborski, S. (2005) Toxin-induced models of Parkinson's disease. *NeuroRx*, **2** (3), 484–494.
7 Senthilkumar, K.S., Saravanan, K.S., Chandra, G., Sindhu, K.M., Jayakrishnan, A., and Mohanakumar, K.P. (2007) Unilateral implantation of dopamine-loaded biodegradable hydrogel in the striatum attenuates motor abnormalities in the 6-hydroxydopamine model of hemi-Parkinsonism. *Behav. Brain Res.*, **184** (1), 11–18.
8 Sedelis, M., Schwarting, R.K.W., and Huston, J.P. (2001) Behavioral phenotyping of the MPTP mouse model of Parkinson's disease. *Behav. Brain Res.*, **125** (1-2), 109–125.
9 Borah, A. and Mohanakumar, K.P. (2010) L-DOPA-induced 6-hydroxydopamine production in the striata of rodents is sensitive to the degree of denervation. *Neurochem. Int.*, **56** (2), 357–362.
10 Paul, R., Choudhury, A., and Borah, A. (2015) Cholesterol – A putative endogenous contributor towards Parkinson's disease. *Neurochem. Int.*, **90**, 125–133.
11 Borah, A., Paul, R., Mazumder, M.K., and Bhattacharjee, N. (2013) Contribution of beta-phenethylamine, a component of chocolate and wine, to dopaminergic neurodegeneration: implications for the pathogenesis of Parkinson's disease. *Neurosci. Bull.*, **29** (5), 655–660.
12 Bhattacharjee, N., Paul, R., Giri, A., and Borah, A. (2016) Chronic exposure of homocysteine in mice contributes to dopamine loss by enhancing oxidative stress in nigrostriatum and produces behavioral phenotypes of Parkinson's disease. *Biochem. Biophys. Rep.*, **6**, 47–53.
13 Breydo, L., Wu, J.W., and Uversky, V.N. (2012) Alpha-Synuclein misfolding and Parkinson's disease. *Biochim. Biophys. Acta*, **1822** (2), 261–285.

14 Spillantini, M.G., Schmidt, M.L., Lee, V.M., Hasegawa, M., and Goedert, M. (1997) Alpha-synuclein in Lewy bodies. *Nature*, **388** (6645), 839–840.

15 Di Maio, R., Barrett, P.J., Hoffman, E.K., Barrett, C.W., Zharikov, A., Borah, A., Hu, X., McCoy, J., Chu, C.T., Burton, E.A., Hastings, T.G., and Greenamyre, J.T. (2016) α-Synuclein binds to TOM20 and inhibits mitochondrial protein import in Parkinson's disease. *Sci. Transl. Med.*, **8** (342), 342–378.

16 Jellinger, K. (2014) The pathomechanisms underlying Parkinson's disease. *Expert Rev. Neurother.*, **14**, 199–215.

17 Dauer, W. and Przedborski, S. (2003) Parkinson's disease: mechanisms and models. *Neuron*, **39** (6), 889–909.

18 Lester, D.B., Rogers, T.D., and Blaha, C.D. (2010) Acetylcholine-dopamine interactions in the pathophysiology and treatment of CNS disorders. *CNS Neurosci. Ther.*, **16** (3), 137–162.

19 Scatton, B., Javoy-Agid, F., Rouquier, L., Dubois, B., and Agid, Y. (1983) Reduction of cortical dopamine, noradrenaline, serotonin and their metabolites in Parkinson's disease. *Brain Res.*, **275** (2), 321–328.

20 Nagatsu, T. and Sawada, M. (2007) L-dopa therapy for Parkinson's disease: past, present, and future. *Parkinsonism Relat. Disord.*, **15** (Suppl. 1), S3–S8.

21 Kalia, L.V., Kalia, S.K., and Lang, A.E. (2015) Disease-modifying strategies for Parkinson's disease. *Mov. Disord.*, **30** (11), 1442–1450.

22 Boll, M.-C., Alcaraz-Zubeldia, M., and Rios, C. (2011) Medical management of Parkinson's disease: focus on neuroprotection. *Curr. Neuropharmacol.*, **9** (2), 350–359.

23 Lipski, J., Nistico, R., Berretta, N., Guatteo, E., Bernardi, G., and Mercuri, N.B. (2011) L-DOPA: a scapegoat for accelerated neurodegeneration in Parkinson's disease? *Prog. Neurobiol.*, **94** (4), 389–407.

24 Borah, A. and Mohanakumar, K.P. (2012) L-DOPA induced-endogenous 6-hydroxydopamine is the cause of aggravated dopaminergic neurodegeneration in Parkinson's disease patients. *Med. Hypotheses*, **79** (2), 271–273.

25 Bhattacharjee, N., Mazumder, M.K., Paul, R., Choudhury, A., Choudhury, S., and Borah, A. (2016) L-DOPA treatment in MPTP-mouse model of Parkinson's disease potentiates homocysteine accumulation in substantia nigra. *Neurosci. Lett.*, **628**, 225–229.

26 Paul, R. and Borah, A. (2016) L-DOPA-induced hyperhomocysteinemia in Parkinson's disease: elephant in the room. *Biochim. Biophys. Acta*, **1860** (9), 1989–1997.

27 Mazumder, M.K., Bhattacharjee, N., and Borah, A. (2016) Garcinol prevents hyperhomocysteinemia and enhances bioavailability of L-DOPA by inhibiting catechol-*O*-methyltransferase: an in silico approach. *Med. Chem. Res.*, **25** (1), 116–122.

28 Muller, T. (2015) Catechol-*O*-methyltransferase inhibitors in Parkinson's disease. *Drugs*, **75** (2), 157–174.

29 Riederer, P. and Laux, G. (2011) MAO-inhibitors in Parkinson's disease. *Exp. Neurobiol.*, **20** (1), 1–17.

30 Macchio, G.J., Ito, V., and Sahgal, V. (1993) Amantadine-induced coma. *Arch. Phys. Med. Rehabil.*, **74** (10), 1119–1120.

31 Cook, P.E., Dermer, S.W., and McGurk, T. (1986) Fatal overdose with amantadine. *Can. J. Psychiatry*, **31** (8), 757–758.

32 Hayden, F.G., Hoffman, H.E., and Spyker, D.A. (1983) Differences in side effects of amantadine hydrochloride and rimantadine hydrochloride relate to differences in pharmacokinetics. *Antimicrob. Agents Chemother.*, **23** (3), 458–464.

33 Lamberti, P., Zoccolella, S., Iliceto, G., Armenise, E., Fraddosio, A., de Mari, M., and Livrea, P. (2005) Effects of levodopa and COMT inhibitors on plasma homocysteine in Parkinson's disease patients. *Mov. Disord.*, **20** (1), 69–72.

34 Ferry, P., Johnson, M., and Wallis, P. (2002) Use of complementary therapies and non-prescribed medication in patients with Parkinson's disease. *Postgrad. Med. J.*, **78** (924), 612–614.

35 Garcia-Ruiz, P.J., Martinez Castrillo, J.C., Alonso-Canovas, A., Herranz Barcenas, A., Vela, L., Sanchez Alonso, P., Mata, M., Olmedilla Gonzalez, N., and Mahillo Fernandez, I. (2014) Impulse control disorder in patients with Parkinson's disease under dopamine agonist therapy: a multicentre study. *J. Neurol. Neurosurg. Psychiatr.*, **85** (8), 840–844.

36 Jankovic, J. and Aguilar, L.G. (2008) Current approaches to the treatment of Parkinson's disease. *Neuropsychiatr. Dis. Treat.*, **4** (4), 743–757.

37 Baba, Y., Higuchi, M.A., Abe, H., Fukuyama, K., Onozawa, R., Uehara, Y., Inoue, T., and Yamada, T. (2012) Anti-cholinergics for axial symptoms in Parkinson's disease after subthalamic stimulation. *Clin. Neurol. Neurosurg.*, **114** (10), 1308–1311.

38 Fox, C., Smith, T., Maidment, I., Chan, W.Y., Bua, N., Myint, P.K., Boustani, M., Kwok, C.S., Glover, M., Koopmans, I., and Campbell, N. (2014) Effect of medications with anti-cholinergic properties on cognitive function, delirium, physical function and mortality: a systematic review. *Age Ageing*, **43** (5), 604–615.

39 Rochester, L., Yarnall, A.J., Baker, M.R., David, R.V., Lord, S., Galna, B., and Burn, D.J. (2012) Cholinergic dysfunction contributes to gait disturbance in early Parkinson's disease. *Brain*, **135** (9), 2779–2788.

40 Rodrigues-de-Paula, F. and Oliveira, L.L. (2010) Physical therapy – exercise and Parkinson's disease, in *International Encyclopedia of Rehabilitation* (eds J.H. Stone and M. Blouin), Center for International Rehabilitation Research Information and Exchange. http://cirrie.buffalo.edu/encyclopedia/en/article/336 (accessed 01 May 2016).

41 Newberg, A.B., Serruya, M., Wintering, N., Moss, A.S., Reibel, D., and Monti, D.A. (2014) Meditation and neurodegenerative diseases. *Ann. N.Y. Acad. Sci.*, **1307** (1), 112–123.

42 Brown, R.P. and Gerbarg, P.L. (2009) Yoga breathing, meditation, and longevity. *Ann. N.Y. Acad. Sci.*, **1172**, 54–62.

43 Kjaer, T.W., Bertelsen, C., Piccini, P., Brooks, D., Alving, J., and Lou, H.C. (2002) Increased dopamine tone during meditation-induced change of consciousness. *Cognit. Brain Res.*, **13** (2), 255–259.

44 Frank, M.J., Samanta, J., Moustafa, A.A., and Sherman, S.J. (2007) Hold your horses: impulsivity, deep brain stimulation, and medication in Parkinsonism. *Science*, **18** (5854), 1309–1312.

45 Miocinovic, S., Somayajula, S., Chitnis, S., and Vitek, J.L. (2013) History, applications, and mechanisms of deep brain stimulation. *JAMA Neurol.*, **70** (2), 163–171.

46 Pinsker, M., Amtage, F., Berger, M., Nikkhah, G., and van Elst, L.T. (2013) Psychiatric side-effects of bilateral deep brain stimulation for movement disorders. *Acta Neurochir. Suppl.*, **117** (Suppl. 1), 47–51.

47 Dams, J., Siebert, U., Bornschein, B., Volkmann, J., Deuschl, G., Oertel, W.H., Dodel, R., and Reese, J.P. (2013) Cost-effectiveness of deep brain stimulation in patients with Parkinson's disease. *Mov. Disord.*, **28** (6), 763–771.

48 Imamura, A., Uitti, R.J., and Wszolek, Z.K. (2006) Dopamine agonist therapy for Parkinson disease and pathological gambling. *Parkinsonism Relat. Disord.*, **12** (8), 506–508.

49 Caraceni, T., Geminiani, G., and Tamma, F. (2008) Current approaches in the treatment of Parkinson disease. *Neuropsychiatr. Dis. Treat.*, **4** (4), 743–757.

50 Chen, S. and Le, W. (2006) Neuroprotective therapy in Parkinson disease. *Am. J. Ther.*, **13** (5), 445–457.

51 Poewe, W. (2009) Treatments for Parkinson disease-past achievements and current clinical needs. *Neurology*, **72** (7, Suppl. 2), S65–S73.

52 Mythri, R., Harish, G., and Bharath, M. (2012) Therapeutic potential of natural products in Parkinson's disease. *Recent Pat. Endocr. Metab. Immune Drug Discov.*, **6** (3), 181–200.

53 Garcia Ruiz, P.J. (2004) Prehistory of Parkinson's disease. *Neurología*, **19** (10), 735–737.

54 Lakke, J.P. (1996) Wilhelm von Humboldt and James Parkinson. An appraisal of observation and creativity. *Parkinsonism Relat. Disord.*, **2** (4), 225–229.

55 Goedert, M., Spillantini, M.G., Del Tredici, K., and Braak, H. (2013) 100 years of Lewy pathology. *Nat. Rev. Neurol.*, **9** (1), 13–24.

56 Lees, A.J. (2007) Unresolved issues relating to the shaking palsy on the celebration of James Parkinson's 250th birthday. *Mov. Disord.*, **22** (Suppl. 17), S327–S334.

57 Halpern, M. (2015) Parkinson's Disease (Kampavata): understanding the Ayurvedic Approach, California College of Ayurveda, https://www.ayurvedacollege.com/articles/drhalpern/clinical/Parkinson (accessed 22 June 2016).

58 Loukas, M., Lanteri, A., Ferrauiola, J., Tubbs, R.S., Maharaja, G., Shoja, M.M., Yadav, A., and Rao, V.C. (2010) Anatomy in ancient India: a focus on the Susruta Samhita. *J. Anat.*, **217** (6), 646–650.

59 Galib, Mashru, M., Patgiri, B.J., Barve, M., Jagtap, C., and Prajapati, P.K. (2011) Therapeutic potentials of metals in ancient India: a review through *Charaka Samhita*. *J. Ayurveda Integr. Med.*, **2** (2), 55–63.

60 Manyam, B.V. and Kumar, A. (2013) Ayurvedic constitution (prakruti) identifies risk factor of developing Parkinson's disease. *J. Altern. Complement. Med.*, **19** (7), 644–649.

61 Ovallath, S. and Deepa, P. (2013) The history of Parkinsonism: descriptions in ancient Indian medical literature. *Mov. Disord.*, **28** (5), 566–568.

62 Murray, M.T. (2012) *Textbook of Natural Medicine*, 4th edn, Elsevier.

63 Cody, G. (1985) History of naturopathic medicine, in *A Textbook of Natural Medicine* (eds J.E. Pizzorno Jr., and M.T. Murray), John Bastyr College Publications, Seattle.

64 Adams, M., Gmünder, F., and Hamburger, M. (2007) Plants traditionally used in age related brain disorders – A survey of ethnobotanical literature. *J. Ethnopharmacol.*, **113** (3), 363–381.

65 Nagashayana, N., Sankarankutty, P., Nampoothiri, M.R.V., Mohan, P.K., and Mohanakumar, K.P. (2000) Association of L-DOPA with recovery following ayurveda medication in Parkinson's disease. *J. Neurol. Sci.*, **176** (2), 124–127.

66 Chopra, A. and Doiphode, V.V. (2002) Ayurvedic medicine: core concept, therapeutic principles, and current relevance. *Med. Clin. North Am.*, **86** (1), 75–89.

67 Prioreschi, P. (1991) A history of medicine: primitive and ancient medicine. *Mellen Hist. Med.*, **1**, 1–642.

68 Katzenschlager, R., Evans, A., Manson, A., Patsalos, P.N., Ratnaraj, N., Watt, H., Timmermann, L., Van der Giessen, R., and Lees, A.J. (2004) *Mucuna pruriens* in Parkinson's disease: a double blind clinical and pharmacological study. *J. Neurol. Neurosurg. Psychiatr.*, **75** (12), 1672–1677.

69 Chaudhri, R.D. (ed.) (1996) *Herbal Drug Industry. A Practical Approach to Industrial Pharmacognosy*, Eastern Publishers, New Delhi.

70 Misra, L. and Wagner, H. (2004) Alkaloidal constituents of *Mucuna pruriens* seeds. *Phytochemistry*, **65** (18), 2565–2567.

71 Lampariello, L.R., Cortelazzo, A., Guerranti, R., Sticozzi, C., and Valacchi, G. (2012) The magic velvet bean of *Mucuna pruriens*. *J. Tradit. Complement. Med.*, **2** (4), 331–339.

72 Sotnikova, T.D., Beaulieu, J.M., Barak, L.S., Wetsel, W.C., Caron, M.G., and Gainetdinov, R.R. (2005) Dopamine-independent locomotor actions of amphetamines in a novel acute mouse model of Parkinson disease. *PLoS Biol.*, **3** (8), e271.

73 Park, H.J., Lee, P.H., Ahn, Y.W., Choi, Y.J., Lee, G., Lee, D.Y., Chung, E.S., and Jin, B.K. (2007) Neuroprotective effect of nicotine on dopaminergic neurons by anti-inflammatory action. *Eur. J. Neurosci.*, **26** (1), 79–89.

74 Manyam, B.V., Dhanasekaran, M., and Hare, T.A. (2004a) Neuroprotective effects of the antiparkinson drug *Mucuna pruriens*. *Phytother. Res.*, **18** (9), 706–712.

75 Das, P.K., Malhotra, C.L., and Prasad, K. (1964) Cardiotonic activity of Ashwagandhine and ashwagandhinine, two alkaloids from Withania ashwagandha, Kaul. *Arch. Int. Pharmacodyn. Ther.*, **150**, 356–362.

76 Jayaprakasam, B., Padmanabhan, K., and Nair, M.G. (2010) Withanamides in *Withania somnifera* fruit protect PC-12 cells from beta-amyloid responsible for Alzheimer's disease. *Phytother. Res.*, **24** (6), 859–863.

77 Kumar, S., Seal, C.J., Howes, M.J.R., Kite, G.C., and Okello, E.J. (2010) In vitro protective effects of *Withania somnifera* (L.) dunal root extract against hydrogen peroxide and β-amyloid(1-42)-induced cytotoxicity in differentiated PC12 cells. *Phytother. Res.*, **24** (10), 1567–1574.

78 Jayaprakasam, B., Strasburg, G.A., and Nair, M.G. (2004) Potent lipid peroxidation inhibitors from *Withania somnifera* fruits. *Tetrahedron*, **60** (13), 3109–3121.

79 Alam, N., Hossain, M., Mottalib, M.A., Sulaiman, S.A., Gan, S.H., and Khalil, M.I. (2012) Methanolic extracts of *Withania somnifera* leaves, fruits and roots possess antioxidant properties and antibacterial activities. *BMC Complement. Altern. Med.*, **12** (1), 175.

80 Bhattacharya, A., Ghosal, S., and Bhattacharya, S.K. (2001) Antioxidant effect of *Withania somnifera* glycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. *J. Ethnopharmacol.*, **74** (1), 1–6.

81 Matsuda, H., Murakami, T., Kishi, A., and Yoshikawa, M. (2001) Structures of withanosides I, II, III, IV, V, VI, and VII, new withanolide glycosides, from the roots of Indian *Withania somnifera* DUNAL. and inhibitory activity for tachyphylaxis to clonidine in isolated guinea-pig ileum. *Bioorg. Med. Chem.*, **9** (6), 1499–1507.

82 Tohda, C., Kuboyama, T., and Komatsu, K. (2005) Search for natural products related to regeneration of the neuronal network. *Neurosignals*, **14** (1-2), 34–45.

83 Ghosal, S., Lal, J., Srivastava, R., Bhattacharya, S.K., Upadhyay, S.N., Jaiswal, A.K., and Chattopadhyay, U. (1989) Immunomodulatory and CNS effects of sitoindosides IX and X, two new glycowithanolides from *Withania somnifera*. *Phytother. Res.*, **3** (5), 201–206.

84 Schliebs, R., Liebmann, A., Bhattacharya, S.K., Kumar, A., Ghosal, S., and Bigl, V. (1997) Systemic administration of defined extracts from *Withania somnifera* (Indian Ginseng) and Shilajit differentially affects cholinergic but not glutamatergic and GABAergic markers in rat brain. *Neurochem. Int.*, **30** (2), 181–190.

85 Bhattacharya, S.K., Bhattacharya, D., Sairam, K., and Ghosal, S. (2002) Effect of *Withania somnifera* glycowithanolides on a rat model of tardive dyskinesia. *Phytomedicine*, **9** (2), 167–170.

86 Franco, C.I.F., Morais, L.C.S.L., Quintans, L.J., Almeida, R.N., and Antoniolli, A.R. (2005) CNS pharmacological effects of the hydroalcoholic extract of *Sida cordifolia* L. leaves. *J. Ethnopharmacol.*, **98** (3), 275–279.

87 Khurana, N. and Gajbhiye, A. (2013) Ameliorative effect of *Sida cordifolia* in rotenone induced oxidative stress model of Parkinson's disease. *Neurotoxicology*, **39**, 57–64.

88 Momin, M.A.M., Bellah, S.F., Rahman, S.M.R., Rahman, A.A., Murshid, G.M.M., and Emran, T.B. (2014) Phytopharmacological evaluation of ethanol extract of *Sida cordifolia* L. roots. *Asian Pac. J. Trop. Biomed.*, **4** (1), 18–24.

89 Ravi Kanth, V. and Diwan, P.V. (1999) Analgesic, antiinflammatory and hypoglycaemic activities of *Sida cordifolia*. *Phytother. Res.*, **13** (1), 75–77.

90 Çoban, J., Bingül, I., Yesil-Mizrak, K., Dogru-Abbasoglu, S., Oztezcan, S., and Uysal, M. (2013) Effects of carnosine plus vitamin E and betaine treatments on oxidative stress in some tissues of aged rats. *Curr. Aging Sci.*, **6** (2), 199–205.

91 Tonekaboni, S.H. and Mollamohammadi, M. (2014) Neurodegeneration with brain iron accumulation: an overview. *Iran. J. Child Neurol.*, **8** (4), 1–8.

92 Sengupta, T., Vinayagam, J., Nagashayana, N., Gowda, B., Jaisankar, P., and Mohanakumar, K.P. (2011) Antiparkinsonian effects of aqueous methanolic extract of *Hyoscyamus niger* seeds result from its monoamine oxidase inhibitory and hydroxyl radical scavenging potency. *Neurochem. Res.*, **36** (1), 177–186.

93 Mohammad, M.K., Almasri, I.M., Tawaha, K., Issa, A., Al-Nadaf, A., Hudaib, M., AlKhatib, H.S., Abu-Gharbieh, E., and Bustanji, Y. (2010) Antioxidant, antihyperuricemic and xanthine oxidase inhibitory activities of *Hyoscyamus reticulatus*. *Pharm. Biol.*, **48** (12), 1376–1383.

94 Singh, U.P., Singh, N.P., Busbee, B., Guan, H., Singh, B., Price, R.L., Taub, D.D., Mishra, M.K., Nagarkatti, M., and Nagarkatti, P.S. (2012) Alternative medicines as emerging therapies for inflammatory bowel diseases. *Int. Rev. Immunol.*, **31** (1), 66–84.

95 Manyam, B.V. (1990) Paralysis agitans and levodopa in "ayurveda": ancient Indian medical treatise. *Mov. Disord.*, **5** (1), 47–48.

96 Begum, A.S. (2010) Bioactive non-alkaloidal secondary metabolites of *Hyoscyamus niger* linn. Seeds: a review. *Res. J. Seed Sci.*, **3** (4), 210–217.

97 Gilani, A.H., Khan, A.U., Raoof, M., Ghayur, M.N., Siddiqui, B.S., Vohra, W., and Begum, A.S. (2008) Gastrointestinal, selective airways and urinary bladder relaxant effects of *Hyoscyamus niger* are mediated through dual blockade of muscarinic receptors and Ca^{2+} channels. *Fundam. Clin. Pharmacol.*, **22** (1), 87–99.

98 Rezak, M. (2007) Current pharmacotherapeutic treatment options in Parkinson's disease. *Disease-a-Month*, **53** (4), 214–222.

99 Ahmad, M., Saleem, S., Ahmad, A.S., Ansari, M.A., Yousuf, S., Hoda, M.N., and Islam, F. (2005) Neuroprotective effects of *Withania somnifera* on 6-hydroxydopamine induced Parkinsonism in rats. *Hum. Exp. Toxicol.*, **24** (3), 137–147.

100 Sankar, S.R., Manivasagam, T., Krishnamurti, A., and Ramanathan, M. (2007) The neuroprotective effect of *Withania somnifera* root extract in MPTP-intoxicated mice: an analysis of behavioral and biochemical variables. *Cell. Mol. Biol. Lett.*, **12** (4), 473–481.

101 RajaSankar, S., Manivasagam, T., Sankar, V., Prakash, S., Muthusamy, R., Krishnamurti, A., and Surendran, S. (2009) *Withania somnifera* root extract improves catecholamines and physiological abnormalities seen in a Parkinson's disease model mouse. *J. Ethnopharmacol.*, **125** (3), 369–373.

102 Nair, V., Arjuman, A., Gopalakrishna, H.N., and Nandini, M. (2008) Effect of *Withania somnifera* root extract on haloperidol-induced catalepsy in albino mice. *Phytother. Res.*, **22** (2), 243–246.

103 Naidu, P.S., Singh, A., and Kulkarni, S.K. (2006) Effect of *Withania somnifera* root extract on reserpine-induced orofacial dyskinesia and cognitive dysfunction. *Phytother. Res.*, **20** (2), 140–146.

104 Kumar, A. and Kulkarni, S.K. (2006) Protective effect of BR-16A, a polyherbal preparation against social isolation stress: possible GABAergic mechanism. *Phytother. Res.*, **20** (7), 538–541.

105 Connolly, B.S. and Lang, A.E. (2014) Pharmacological treatment of Parkinson disease: a review. *JAMA*, **311** (16), 1670–1683.

106 Mukherjee, S., Pawar, N., Kulkarni, O. *et al.* (2011) Evaluation of free-radical quenching properties of standard ayurvedic formulation *Vayasthapana Rasayana*. *BMC Complement. Altern. Med.*, **11** (1), 38.

107 Sharma, H., Chandola, H.M., Singh, G., and Basisht, G. (2007) Utilization of ayurveda in health care: an approach for prevention, health promotion, and treatment of disease. Part 2 – Ayurveda in primary health care. *J. Altern. Complement. Med.*, **13** (10), 1135–1150.

108 Manyam, B.V. and Sánchez-Ramos, J.R. (1999) Traditional and complementary therapies in Parkinson's disease. *Adv. Neurol.*, **80**, 565–574.

109 Contin, M., Lopane, G., Passini, A., Poli, F., Iannello, C., and Guarino, M. (2015) *Mucuna pruriens* in Parkinson disease: a kinetic-dynamic comparison with levodopa standard formulations. *Clin. Neuropharmacol.*, **38** (5), 201–203.

110 Pras, N., Woerdenbag, H.J., Batterman, S., Visser, J.F., and Van Uden, W. (1993) *Mucuna pruriens*: improvement of the biotechnological production of the anti-Parkinson drug L-DOPA by plant cell selection. *Pharm. World Sci.*, **15** (6), 263–268.

111 Manyam, B.V., Dhanasekaran, M., and Hare, T.A. (2004b) Effect of antiparkinson drug HP-200 (*Mucuna pruriens*) on the central monoaminergic neurotransmitters. *Phytother. Res.*, **18** (2), 97–101.

112 Dhanasekaran, M., Tharakan, B., and Manyam, B.V. (2008) Antiparkinson drug – *Mucuna pruriens* shows antioxidant and metal chelating activity. *Phytother. Res.*, **22** (1), 6–11.

113 Kasture, S., Pontis, S., Pinna, A., Schintu, N., Spina, L., Longoni, R., Simola, N., Ballero, M., and Morelli, M. (2009) Assessment of symptomatic and neuroprotective efficacy of *Mucuna pruriens* seed extract in rodent model of Parkinson's disease. *Neurotox. Res.*, **15** (2), 111–122.

114 Spencer, J.P., Jenner, A., Aruoma, O.I., Evans, P.J., Kaur, H., Dexter, D.T., Jenner, P., Lees, A.J., Marsden, D.C., and Halliwell, B. (1994) Intense oxidative DNA damage promoted by L-DOPA and its metabolites. Implications for neurodegenerative disease. *FEBS Lett.*, **353** (3), 246–250.

115 Tharakan, B., Dhanasekaran, M., Mize-Berge, J., and Manyam, B.V. (2007) Anti-Parkinson botanical *Mucuna pruriens* prevents levodopa induced plasmid and genomic DNA damage. *Phytother. Res.*, **21** (12), 1124–1126.

116 Maheswari, T., Vijayraja, D., Natchiyar, R.K., Kalaivania, K., Rajasankar, S., Tamilselvama, K., and Manivasagama, T. (2010) Synergistic neuroprotective effect of Withania somnifera root powder and *Mucuna pruriens* seed powder in Parkinsonian mice model. *J. Herb. Med. Toxicol.*, **4** (2), 63–69.

117 Konar, A., Shah, N., Singh, R., Saxena, N., Kaul, S.C., Wadhwa, R., and Thakur, M.K. (2011) Protective role of Ashwagandha leaf extract and its component withanone on scopolamine-induced changes in the brain and brain-derived cells. *PLoS One*, **6** (11), e3425–e3429.

6

Lipid Peroxidation and Mitochondrial Dysfunction in Alzheimer's and Parkinson's Diseases: Role of Natural Products as Cytoprotective Agents

Carlos Fernández-Moriano, Elena González-Burgos, and Maria Pilar Gómez-Serranillos

University Complutense of Madrid, School of Pharmacy, Department of Pharmacology, Plaza Ramón y Cajal s/n 28040, Madrid, Spain

6.1 Introduction

6.1.1 Oxidative Stress

Oxidative stress is the result of an imbalance in prooxidant/antioxidant homeostasis that occurs when there is an increase in the levels of reactive oxygen species (ROS) and/or a reduction in the activity of the endogenous antioxidant defense system. Oxidative stress induces modifications to biomolecules including proteins, lipids, DNA, and RNA (Figure 6.1), and this may trigger cellular organelle dysfunction that finally leads to cell death [1–3].

In the case of proteins, ROS may provoke the formation of protein–protein cross-linkages, the oxidation of the protein backbone, and the oxidation of amino acid side chains, among which cysteine, methionine, and aromatic amino acid residues are the most sensitive to ROS attack [4]. The oxidative protein modification can alter the electric charge of proteins, fragment the peptide chain, modify the site-specific amino acid, and promote proteolysis [5].

Concerning lipids, the double bonds (C═C) between carbon atoms and the ester linkage between glycerol and the fatty acid are key targets for the ROS action in the lipid peroxidation reactions. The end products of lipid peroxidation are reactive aldehydes such as acrolein, 3-aminopropanal (3-AP), 4-oxononenal (4-ONE), 4-hydroxynonenal (4-HNE), and malondialdehyde (MDA) that can lead to the disruption of cellular membrane structure and function [5, 6].

For DNA, there have been more than 20 oxidatively modified DNA bases of interaction with ROS identified through addition to double bonds of DNA bases, abstraction of hydrogen atoms, and deamination reactions, among other mechanisms. Some examples of DNA base lesions are 8-hydroxyguanine, 5-hydroxycytosine, 5-formyluracil, and 8-oxo-2′-deoxyguanosine [7]. As it has been found for DNA, more than 20 ROS-induced alterations in RNA molecule have been identified. The most frequent is RNA oxidation, whose product is the 8-hydroxyguanosine (8-OHG) [8].

Neuroprotective Natural Products: Clinical Aspects and Mode of Action, First Edition.
Edited by Goutam Brahmachari.

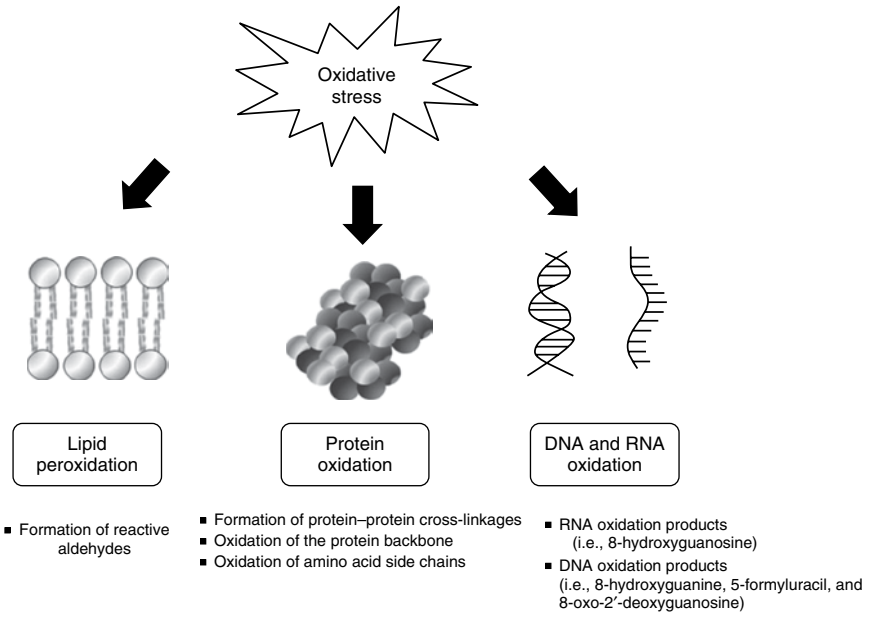


Figure 6.1 Oxidative stress induces modifications to biomolecules including proteins, lipids, DNA, and RNA.

Oxidative stress is a major pathogenic factor that contributes to the progression of aging and many disorders including Parkinson's disease (PD) and Alzheimer's disease (AD). The brain is an organ especially vulnerable to the action of ROS, and this is partly due to its high levels of transition metals (iron and copper), its high metabolic rate, its low concentration in antioxidant enzymes (particularly catalase), and its high content of polyunsaturated lipids. The involvement of oxidative stress in the pathogenesis of PD and AD stems primarily from the brain autopsy or postmortem examinations and from the identification and quantification of oxidative stress biomarkers in the blood, urine, and cerebrospinal fluid of patients with these neurodegenerative disorders. These biochemical and histopathological data are supported by *in vitro* studies (i.e., glioma and neuroblastoma cell lines, cultured cortical neurons) and experimental animal models (i.e., transgenic animals such as transgenic mice with human pre-amyloid protein for Alzheimer's model) in which oxidative stress damage is caused by using different agents such as β-amyloid peptide and hydrogen peroxide, among others [9–13].

ROS include both free radicals (molecules that possess one impaired electron) and non-free radicals, such as superoxide anion (O_2^-), hydroxyl radical ($HO^\bullet$), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), and peroxyl, among others. These molecules can be directly formed from secondary reactions or as a product of them [14]. Moreover, ROS can react with other ROS to continue the chain reaction, thus propagating and increasing the level of these compounds. Among the ROS formation mechanisms, one may find ionizing radiation, metal ion catalysis (i.e., the Fenton reaction $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^\bullet OH + {}^-OH$ and the Haber–Weiss reaction $H_2O_2 + O_2^- \rightarrow O_2 + OH^- + OH^-$), and enzymatic catalysis

Table 6.1 Types of reactive oxygen species (ROS).

Reactive oxygen species	Properties	Lifetime	Reaction of formation
Hydroxyl radical ($OH^{\bullet}$)	• The most reactive among all ROS • Particularly unstable • React rapidly and nonspecifically with most biological molecules	1 ns	$O_2^{\bullet -} + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + O_2$
Superoxide anion radical ($O_2^{\bullet -}$)	• Moderately reactive	1 μs	It is formed by the single electron reduction of molecular oxygen
Hydrogen peroxide (H_2O_2)	• Moderately reactive • Highly diffusible • Traverse the cell membrane easily	1 ms	Via dismutation of superoxide anion
Peroxyl radicals ($ROO^{\bullet}$)	• Highly reactive	10 ms	Via direct reaction of oxygen with alkyl radicals ($R^{\bullet}$) Via decomposition of alkyl peroxides (ROOH)
Nitric oxide ($NO^{\bullet}$)	• Moderately reactive • Small size, high diffusion rate, and lipophilicity	Few seconds	Via the enzymatic conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS)
Peroxynitrite ($OONO^-$)	• Moderately strong reactive	1 s	$NO + O_2^{\bullet -} \rightarrow ONOO^-$

(i.e., NADPH oxidase, myeloperoxidase, monoamine oxidase, xanthine oxidoreductase, and cytochrome P_{450} (CYP_{450}) oxidase) (Table 6.1) [15].

Endogenous antioxidant defense system comprises both enzymatic and nonenzymatic molecules, which play a crucial role in the detoxification and protection of cellular ROS-induced damages. These chemical compounds can be classified as primary, secondary, and tertiary antioxidants based on their different defensive modes of action (Figure 6.2):

- *Primary antioxidants* (also called chain-breaking antioxidants). These compounds convert free radicals into less harmful molecules, and thus, they prevent oxidant formation by interfering with the chain propagation step:
  - The enzyme superoxide dismutase (SOD) catalyzes the conversion of superoxide into hydrogen peroxide and oxygen. There have been three SOD types identified: SOD-1 (Cu-Zn SOD), which is located in the cytoplasm; SOD-2 (Mn SOD), which is located in the mitochondria; and the extracellularly located SOD-3 (Cu-Zn SOD) [16].
  - The enzyme catalase is a heme-containing homo-tetrameric protein that transforms hydrogen peroxide (H_2O_2) to water and oxygen. This enzyme is found in the peroxisomes (80%) and in the cytosol (20%) [17].

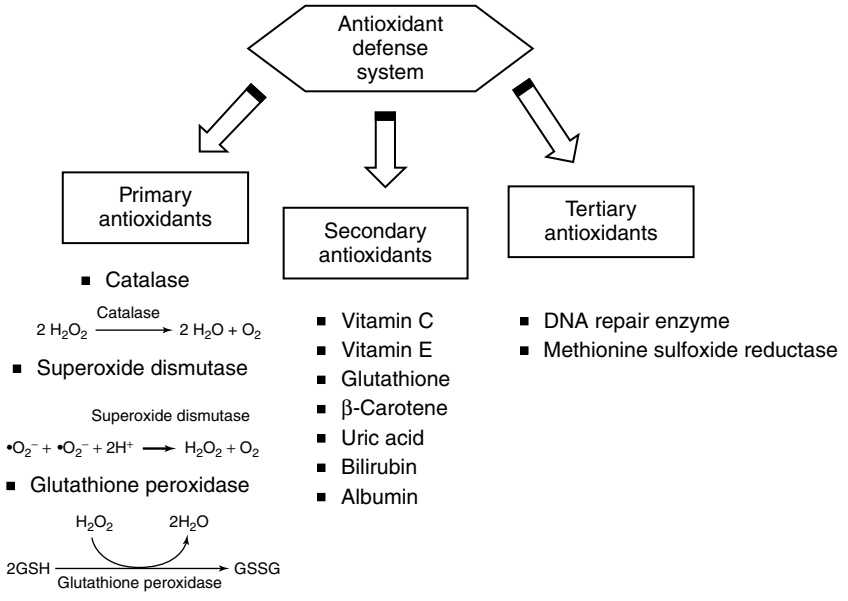


Figure 6.2 Antioxidant defense system.

  - The enzyme glutathione peroxidase (GPx) catalyzes the reduction of hydroperoxides with reduced glutathione (GSH) as cofactor. There have been two GPx types identified [18–20]:
    - Selenium-dependent glutathione peroxidases (Se-D GPxs), which are tetrameric enzymes with selenium groups at their active site. The family of selenium-dependent GPxs is comprised of GPx-1, which is presented in the cytosol; GPx-2, which is expressed in the epithelium of the gastrointestinal tract; GPx-3, with an extracellular location; and GPx-4, which is in the membrane fraction.
    - Selenium-independent glutathione peroxidases (Se-I GPxs), which are present in the mitochondria, cytosol, and membranes.
  - The enzyme GSH reductase is a homodimeric flavoenzyme found in the cytoplasm and in the mitochondria, which catalyzes the reduction of GSH disulfide by NADPH to yield two molecules of GSH [21].
  - Glutathione-S-transferases (GSTs) are phase II enzymes that are involved in the detoxification of different endogenous and exogenous electrophilic compounds by catalyzing their conjugation to GSH [22].
  - Metal-binding proteins such as ferritin, transferrin, metallothionein, and ceruloplasmin [23].
- *Secondary antioxidants*: These molecules are efficient scavengers of ROS. Examples are vitamin C, vitamin E, GSH, β-carotene, uric acid, bilirubin, and albumin [24–27].
- *Tertiary antioxidants*: This type of antioxidants, in which DNA repair enzymes and methionine sulfoxide reductases are included, repair the oxidized biomolecules damaged by ROS [28].

6.1.2 Lipid Peroxidation

As previously commented, uncontrolled oxidative stress causes injury to biomolecules, including lipids. Lipid peroxidation is the oxidative deterioration process of lipids under which ROS attack those lipids containing carbon–carbon double bond(s) such as polyunsaturated fatty acids (PUFAs), glycolipids, phospholipids, and cholesterol, leading to a structural and functional damage of cellular membranes. The higher the degree of unsaturation, the higher the rate of oxidation of lipids is. For example, the rate of oxidation of linolenic acid (18-carbon chain and 3 double bonds) is estimated to be about 10 and 100 times higher than the rate of oxidation of linoleic acid (18-carbon chain and 2 double bonds) and oleic acid (18-carbon chain and 1 double bond), respectively [29–31].

The lipid peroxidation process may occur via different mechanisms including autoxidation, photooxidation, and enzymatic lipid oxidation:

1) *Autoxidation:* The autoxidation is the most frequent mechanism leading to oxidative deterioration of lipids. This is a radical chain process that consists of three reactions: initiation, propagation, and termination [29–31]:
   a) *Initiation* (production of free radicals)

$$RH + O_2 \rightarrow R^{\bullet} + {}^{\bullet}OH$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} + {}^{\bullet}(e)$$

   b) *Propagation* (free radical chain reactions, conversion of free radicals into other radicals)

$$ROO^{\bullet} + RH \rightarrow R^{\bullet} + ROOH$$

$$ROOH \rightarrow RO^{\bullet} + HO^{\bullet}$$

   c) *Termination* (formation of hydroperoxides)

$$R^{\bullet} + R^{\bullet} \rightarrow RR$$

$$R^{\bullet} + ROO^{\bullet} \rightarrow ROOR$$

$$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOR + O_2$$

2) *Photooxidation:* In the photooxidation process, the singlet molecule oxygen ($^1O_2$), promoted by sensitizers (i.e., riboflavin, chlorophyll, myoglobin, bilirubin), reacts with unsaturated lipids, yielding the formation of hydroperoxides. The photooxidation process is a quicker mechanism of lipid peroxidation than the autoxidation process [29, 32].
3) *Enzymatic lipid oxidation:* The oxidative enzymes cyclooxygenase (COX), lipoxygenase (LOX), and CYP_{450} catalyze the reactions between oxygen and PUFAs via regio-, stereo-, and enantio-specific mechanisms:
   a) The enzyme COX catalyzes the bis-dioxygenation and reduction of the arachidonic acid (ω-6 polyunsaturated fatty acid (ω-6 PUFA) 20:4) to prostaglandin H2 (PGH2). There have been two COX isoforms identified, named as COX-1 and COX-2, which have structural similarities: both enzymes are homodimers with similar number of amino acids, near-identical catalytic

sites, and over 60% of identical amino acid sequence. The COX-1 is the constitutive form, and it is presented in most tissues under homeostatic conditions, whereas the COX-2 is the inducible form, and its expression is induced by cytokines, mitogens, growth factors, and hormones [33, 34].

b) LOX are non-heme iron-containing dioxygenases that catalyze the dioxygenation of PUFAs containing (*Z*,*Z*)-1,4-pentadiene structural units (i.e., eicosapentaenoic acid, docosapentaenoic acid, and arachidonic acid) to yield 1-hydroperoxy-2*E*,4*Z*-pentadiene products (i.e., lipoxins, hydroxyeicosatetraenoic acid (HETE), hydroperoxyoctadecadienoic acid (HpODE), hydroxyoctadecadienoic acid (HODE), hepoxilins, hydroperoxyeicosatetraenoic acids (HpETEs), and leukotrienes) [35, 36].

c) The CYP_{450} is a large family of heme-containing enzymes responsible for the formation of epoxyeicosatrienoic acids (EETs) and HETEs through the NADPH-dependent oxidation of arachidonic acid [37, 38].

The end products of lipid peroxidation are 4-hydroxynonenal (4-HNE), acrolein, isoprostanes, and MDA, among others.

1) *4-HNE*: The 4-HNE (α,β-unsaturated hydroxyalkenal) (Figure 6.3) is an aldehydic product of lipid peroxidation generated during the decomposition of oxidized ω-6 PUFA such as arachidonic acid and linoleic acid at the sn-2 position of glycerophospholipids in cellular membranes. 4-HNE was first discovered by Schauenstein *et al.* in the early 1960s. Under physiological conditions, the cellular concentration of 4-HNE is 0.1–0.3 mM; these concentration values increase 50–100 times under oxidative stress conditions, which makes this product a key marker of an altered redox status. On the other hand, this compound is also a potential inducer of cellular oxidative stress. This by-product of lipid peroxidation can react with DNA and proteins (cysteine, histidine, or lysine residues of the protein) across its carbon–carbon double bond by Michael addition reaction. This reaction represents more than 99% of all 4-HNE-induced structural modifications. Moreover, although to a lesser extent, this α,β-unsaturated hydroxyalkenal can form Schiff bases with ε-NH_2 groups of lysine residues. Furthermore, 4-HNE can also deplete cellular sulfhydryl compounds such as the antioxidant GSH, altering the cellular balance between oxidants and antioxidants. Consistent evidences involve 4-HNE in many disease processes including neurodegenerative disorders, cancer, atherogenesis, and diabetes. In addition, this compound has demonstrated to perform other biological activities including chemotactic properties, genotoxic and cytotoxic actions, and growth inhibitory activity [39–43].
2) *Acrolein*: Acrolein (propenal) (Figure 6.4) is an α,β-unsaturated aldehydic compound originated from a wide range of exogenous and endogenous

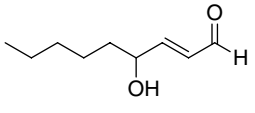


Figure 6.3 Chemical structure of 4-hydroxynonenal.

Figure 6.4 Chemical structure of acrolein.

sources. This compound is an environmental pollutant found as the major component of cigarette smoke. Moreover, it is presented in the gasoline and the diesel exhaust and in the vapors of overheated cooking oil. Endogenously, this is a by-product derived from lipid peroxidation. During lipid peroxidation process, acrolein is formed in less quantity than other aldehydic products such as 4-HNE and MDA; however, its reactivity exceeds over 100 times that of these compounds. This strong electrophile forms covalent adducts with proteins through its cysteine, histidine, and lysine residues and free amino-terminus via Michael addition and Schiff base formation, consequently generating protein-linked carbonyl derivative compounds. Moreover, acrolein can induce mutagenicity by reacting with 20-deoxyguanosine producing DNA adducts (i.e., alpha-hydroxy-1,*N*(2)-propano-2′-deoxyguanosine (alpha-HOPdG) and gamma-hydroxy-1,*N*(2)-propano-2′-deoxyguanosine (gamma-HOPdG) adducts). Acrolein is implicated in the pathogenesis of many different diseases including diabetes mellitus, cardiovascular disease, hepatic disorders, and neurodegenerative diseases [44–47].

3) *Isoprostanes*: Isoprostanes are prostaglandin analog compounds that are derived from the lipid peroxidation of PUFAs via nonenzymatic reaction. For instance, the oxidation of the arachidonic acid yields the production of 64 different isoforms of F2-isoprostanes such as 8-isoprostane F2α (8-iso-PGF2α). Isoprostanes are very useful markers to assess oxidative stress status, especially highlighting the F2-isoprostanes. This class of isoprostanes is the best biomarker of redox condition because it has higher chemical stability than other classes of isoprostanes such as D2- and E2-isoprostanes. Isoprostanes can be detected in their free form in biological fluids, particularly in plasma and urine, and in their esterified form in tissue specimens. These by-products of lipid peroxidation have been identified as major causative agents of oxidative stress-related diseases including pulmonary, vascular, liver, renal, and neurodegenerative diseases, among others [48–51].
4) *MDA*: MDA (Figure 6.5) is an end product of lipid peroxidation formed during the oxidative decomposition of PUFAs. This secondary product is a biomarker and an inductor of oxidative stress. MDA can react with nucleophilic groups (SH or NH_2) of amino acids (i.e., lysine, cysteine, and histidine) to form adducts and cross-links on proteins. Moreover, this lipid peroxidation by-product can also modify DNA by forming mutagenic and carcinogenic adducts of deoxyguanosine (M1G, M1dG), deoxyadenosine (M1A, OPdA), and deoxycytidine (M1C, OPdC). This product has been involved in the pathogenesis of multiple disorders including chronic liver disease, chronic

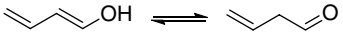


Figure 6.5 Chemical structure of malondialdehyde.

obstructive pulmonary disease, retinal diseases, neurodegenerative diseases, coronary heart diseases, and cancer [52–55].

6.1.3 Mitochondrial Dysfunction

Mitochondria were first discovered and described by the German pathologist and histologist Richard Altmann in 1890 under the name of bioblasts. It was in 1898 when the microbiologist Carl Benda coined the term *mitochondria*, derived from the Greek words "mitos," which means thread, and "chondros," which means granule [56, 57].

Mitochondria are rod-shaped or kidney-shaped cytoplasmic organelles, ranging from 1 to 10 μm in size, and are present in all eukaryotic cells (i.e., mammalian cells contain around 800–2500 mitochondria per cell). Structurally, mitochondria have a phospholipid double membrane (inner and outer membranes separated by an intermembrane space) and a mitochondrial matrix, which contains ribosomes, mitochondrial DNA (mtDNA), and enzymes responsible for metabolic function [58, 59].

Mitochondria play a key role in several metabolic functions. One of their main functions is to synthesize adenosine triphosphate (ATP). The production of this energetic molecule occurs within the inner mitochondrial membrane via electron transport and oxidative phosphorylation reactions. Besides energy production, other functions have been described for mitochondria, such as thermogenesis, maintenance of intracellular Ca^{2+} homeostasis, ROS generation, and regulation of the cell cycle – signaling, differentiation, growth, and cell death [60, 61].

Because of the damaging actions of ROS, mitochondrial structure and function can be affected, leading to mitochondrial dysfunction. Numerous studies on postmortem AD and PD brain tissues, and also investigations using experimental animals and cell-based models, have evidenced the pathogenic role of oxidative stress in mitochondrial damage:

- Evidences in postmortem brain tissues of the association between mitochondrial dysfunction and PD and AD:
  - *PD*: The first evidence of a mitochondrial defect in the pathogenesis of PD was clinically demonstrated in the twentieth century when researchers examined the *postmortem* brains in a number of intravenous 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) drug abusers. This neurotoxin is metabolized into the compound 1-methyl-4-phenylpyridine (MPP^+) by the enzyme glial monoamine oxidase B (MAO-B). It has been demonstrated that the toxic cation MPP^+ inhibits the complex I (NADH/quinone oxidoreductase) of the electron transport chain, causing ATP depletion and increase in ROS level. Moreover, further studies have confirmed deficiency or impairment in the complex I activity in different samples of brain areas from people affected from PD [62–64].
  - *AD*: *Postmortem* brain studies (cortex, hippocampus, and amygdala regions) have shown an accumulation of nonglycosylated full-length and C-terminal truncated amyloid precursor protein (APP) in the protein import channel of mitochondria in patients suffering from AD. This APP accumulation in

mitochondria leads to the formation of complexes with the outer membrane translocase and/or the inner membrane translocase and consequently to the inhibition of cytochrome c oxidase protein activity and the increased of hydrogen peroxide levels. Moreover, analyses of the *postmortem* brain tissues have demonstrated a significant reduction in the activity of the mitochondrial complex II, III, and IV in AD patients [65, 66].

- Evidences in animal and cell-based experimental models of the association between mitochondrial dysfunction and PD and AD:
  - *PD*: Experimentally, several different compounds that mimic the clinical features of PD have been employed in animal models and cell-based assays. Among these compounds, one may highlight rotenone, 6-hydroxydopamine (6-OHDA), paraquat, and MPTP. The insecticide rotenone interferes with the complex I of the electron transport chain in the mitochondria. The intracerebral administration of the catecholaminergic neurotoxin 6-OHDA causes the inhibition of mitochondrial complexes I and IV, induces ROS formation, and leads to the irreversible loss of nigrostriatal dopaminergic neurons. The herbicide paraquat induces α-synuclein fibril formation and ROS generation. Finally, 1-the neurotoxin MPTP inhibits complex I of the electron transport chain, as previously mentioned [62, 67–69].
  - *AD*: As for PD, there are different *in vitro* and *in vivo* models widely used to experimentally reproduce AD features. In cell-based assays, the $A\beta_{1-42}$ oligomers mimic the generation of opening of mitochondrial permeability transition pore (mPTP) and cause cytochrome c release, decline in mitochondrial membrane potential (MMP), and an excess of calcium ion. On the other hand, the amyloid precursor protein mouse transgenic models, the 3×Tg-AD mouse model and the APP/PS1 transgenic model, have shown to present defective mitochondria as evidenced in MMP loss, lower ATP levels, increased ROS generation, and reduction of mitochondrial respiratory rate [70–73].

6.1.4 Lipid Peroxidation and Mitochondrial Dysfunction in PD

The first clinical detailed description of PD was published by the English doctor James Parkinson in 1817. Around 6.3 million of people worldwide usually over the age of 60 suffer from this disease. PD constitutes the second most common neurodegenerative disorder after AD. From an epidemiological point of view, as it occurs with AD, the prevalence and incidence of PD increase with age. Due to the ageing of the population, the number of patients suffering from PD is estimated to increase and it will be doubled by the following 25 years [74, 75].

Clinical features of patients with PD are mainly motor symptoms including bradykinesia, rigid muscles, tremor, dystonia, impaired posture and balance, and loss of automatic movements. Moreover, there have also been non-motor manifestations identified, such as depression, psychosis, cognitive impairment, sleep problems, sexual dysfunction, and anxiety. The major pathologic findings are loss of dopaminergic neurons in the substantia nigra pars compacta, globus pallidus, caudate, and putamen and the presence of Lewy bodies and Lewy neurites [76–79].

The causes of PD involve both genetic and environmental factors. Regarding genetic causes, around 15% of all PD cases are familial; many mutations in genes have been identified, such as those for parkin (PARK2), PTEN-induced putative kinase 1 (PINK1), DJ-1 (PARK7), leucine-rich repeat kinase 2 (LRRK2), alpha-synuclein (SNCA), and glucocerebrosidase. Besides, research data suggest that several environmental agents including insecticides (i.e., beta-hexachlorocyclohexane and permethrin), herbicides (i.e., paraquat), fungicides (i.e., maneb), and tobacco and caffeine consumption may increase the risk of suffering from this disease [80, 81].

6.1.4.1 Lipid Peroxidation

Increased levels of lipid peroxidation products have been largely evidenced in PD patients. Shamoto-Nagai *et al.* demonstrated that the end product of lipid peroxidation acrolein may modify the protein alpha-synuclein in people with PD; acrolein may even alter the proteolytic systems by direct inhibition of the proteasome activity [82]. On the other hand, some authors have determined high levels of the MDA in the plasma of PD patients [83]. Moreover, Dexter *et al.* analyzed the levels of MDA in different brain regions, and they demonstrated that the concentration of this lipid peroxidation product is higher in parkinsonian substantia nigra than in other parkinsonian brain regions and control tissues [84].

6.1.4.2 Mitochondrial Dysfunction

Mitochondrial dysfunction has been found to occur in both familial and sporadic PD. For instance, for familial PD, it has been determined that mutations in the DJ1 gene of chromosome 1p36, in PINK1 gene, and in the protein α-synuclein can cause autosomal recessive early-onset PD by modification of the structure and function of mitochondria in dopaminergic cells, particularly defects in mitochondrial complex I [85–88]. In addition, the pathological mechanism of an early-onset familial PD that is associated with mutations in the parkin gene seems to be related to the damage in mitochondrial function, affecting recognition, transportation, and ubiquitination activities [89]. Furthermore, the autosomal dominant PD form is related to mutations in the gene encoding LRRK2 that lead to a mitochondrial fragmentation and a decrease in ATP production and MMP activity [90]. For sporadic PD, it has been described to be generated by an overproduction of ROS that alters mitochondrial respiratory chain complex function and leads to mitochondrial dysfunction [91, 92].

6.1.5 Lipid Peroxidation and Mitochondrial Dysfunction in AD

AD was first described in the early twentieth century by the German neurologist Alois Alzheimer. AD is the most common neurodegenerative disorder that affects more than 44 million people worldwide, usually over the age of 65. The incidence and prevalence of this disease exponentially increase with age from 3% among individuals aged 65–74 to almost 50% among those 85 or older. As the worldwide population gets older and older, it is expected that the number of men and women who will suffer from AD will quadruple by 2050 [93, 94].

The clinical manifestations of AD are memory loss, difficulty in completing familiar tasks, confusion with time or place, and problems with speaking or

writing, among others. The neuropathological features of this central nervous system disorder are the formation of β-amyloid protein (Aβ) in extracellular plaques and the deposit of the microtubule-associated protein tau in intracellular neurofibrillary tangles (NFTs) in the hippocampal region. These hallmarks mainly affect cholinergic neurons and cause a reduction in the levels of the neurotransmitter acetylcholine [95, 96].

Both environmental factors and hereditary risk factors have been identified as causal agents of AD. Molecular genetic investigations have revealed that over 15–25% of all AD cases are late-onset familial AD (onset age >60–65 years) and they are due to mutations in a wide range of genes including APOE e4 allele, pArg47His allelic variant in TREM2, SORL1 on chromosome 11q23, and clusterin (CLU, APOJ). Moreover, less than 2% of all AD cases correspond to early-onset familial AD (EOFAD) (onset age <60–65 years): Alzheimer's disease type 1 (AD1), caused by mutation of APP (10–15% of EOFAD); Alzheimer disease's type 3 (AD3), caused by mutation of PSEN1 (30–70% of EOFAD); and Alzheimer disease's type 4 (AD4), caused by mutation of PSEN2 (<5% of EOFAD). Furthermore, research data have demonstrated that, by the age of 40, people with Down syndrome (trisomy 21) develop AD. Regarding the involvement of environmental factors in this neurodegenerative disease, Grant *et al.* found a causal association between diet (dietary fat and caloric intake), aluminum and viral infections, and the risk of suffering AD [97, 98].

Oxidative stress and mitochondrial dysfunction have also been implicated in the etiology of AD. The study of *postmortem* brains and the analysis of the blood, urine, and cerebrospinal fluid of people with AD have evidenced the presence of ROS-induced oxidative products and biomarkers of mitochondrial injury and failure [99].

6.1.5.1 Lipid Peroxidation

Different biomarkers of lipid peroxidation have been detected in high levels in AD patients. In fact, the by-product of the peroxidation of fatty acids named acrolein has been found to be increased in plasma and in brain regions of people suffering from this disease [99, 100]. Moreover, the lipid peroxidation product 4-HNE has been detected to be significantly elevated in the ventricular fluid from AD patients [101]. Furthermore, increased levels of F2-isoprostanes are present in the cerebrospinal fluid in late-onset cases of AD [102, 103].

6.1.5.2 Mitochondrial Dysfunction

There are consistent evidences of the role of mitochondrial dysfunction in the pathogenesis of AD. Research studies on brain tissue, fibroblasts, and lymphocytes of AD patients have demonstrated alterations in mitochondrial respiration, MMP, and calcium ion signal transduction [104, 105].

6.2 History and Context

There are no current treatments available for the cure of AD and PD, and the pharmaceutical drugs used so far aim to counteract signs and symptoms such as dementia in AD and bradykinesia in PD.

For AD, the most common drugs used are acetylcholinesterase inhibitors including donepezil, rivastigmine, and galantamine and *N*-methyl-D-aspartate (NMDA) receptor antagonists such as memantine. Donepezil, rivastigmine, and galantamine increase the concentration of the neurotransmitter acetylcholine at sites of neurotransmission by inhibiting the enzyme acetylcholinesterase, and these treatments are indicated for managing mild to moderate AD. Memantine is a noncompetitive NMDA receptor antagonist that normalizes the glutamatergic system and has been approved for the treatment of moderate to severe AD. Among all these drugs employed for AD treatment, rivastigmine and galantamine present a natural origin. Rivastigmine is a semisynthetic derivative of the alkaloid physostigmine, and galantamine is an alkaloid originally obtained from members of the Amaryllidaceae family such as *Galanthus woronowii* [106, 107].

In PD therapy, clinical therapy includes the following drugs: levodopa, dopamine agonists, MAO-B inhibitors, and catechol-*O*-methyltransferase (COMT) inhibitors. Levodopa is the most prescribed and one of the most effective drugs for PD. Levodopa is a chemical precursor of dopamine; this prodrug is actively transported into the brain where it is converted into dopamine by DOPA decarboxylase. Among the dopamine agonists, one may highlight the ergoline agonists such as pergolide, lisuride, and cabergoline and the non-ergoline agonists ropinirole and pramipexole, which act on dopamine D2-type receptors. The MAO-B inhibitors selegiline and rasagiline inhibit the enzyme MAO-B, blocking the breakdown of the neurotransmitter dopamine and consequently increasing its concentration in the brain. The COMT inhibitors such as entacapone and tolcapone are clinically beneficial to use in conjunction with levodopa in the therapy of PD since they prolong the half-life of levodopa and, therefore, increase its action [108, 109].

In addition to these pharmacological treatments for AD and PD, the neuroprotective therapy is undergoing investigation. The use of exogenous antioxidants constitutes one of the most investigated therapeutic approaches for preventing and slowing down the progression of these neurodegenerative disorders by reducing the oxidative stress damage and therefore the directly related lipid peroxidation and mitochondrial failure. Antioxidants are defined as compounds with capability to counteract ROS-induced harmful effects in biomolecules, thus being molecules with the ability to protect cells against oxidative damage. These kind of compounds may act through different mechanisms including the scavenging of free radicals and reactive species, chelation of transition metals (i.e., iron and copper ions), and upregulation of endogenous antioxidant enzymatic system (i.e., catalase, SOD, and GPx). Through those and other mechanisms, antioxidants may protect the nervous system cells against neurodegeneration events and exert beneficial effects in the potential treatment of oxidative stress-related conditions [110, 111].

Concerning antioxidant agents with pharmacological interest, those coming directly from nature are attracting growing research due to their more favorable properties than synthetic compounds; natural products from medicinal plants and foods are being widely investigated as potential antioxidants to be used in the therapeutic of the two neurodegenerative diseases. Among them, one could

highlight compounds such as flavonoids, carotenoids, phenolic compounds and organic acids, terpenes, and tocopherols [112].

As discussed in the next section of this chapter, in the scientific literature, there are numerous *in vitro* and *in vivo* studies using models of AD and PD that evaluate and demonstrate the antioxidant properties of many kinds of natural products and their protective effect against ROS-induced oxidative damage, which may prevent the progression of central nervous system diseases. However, the numbers of clinical trials that demonstrate the potential efficacy of these natural antioxidants are very scarce and present certain limitations that do not allow establishing definitive conclusions. The limitations found in the clinical trials may be due to the poor and low variability of antioxidants tested (most of the studies were performed with vitamin E and EGb 761 extract from *Ginkgo biloba*) and methodological restrictions (i.e., number of patients, time period and/or monitoring in each of the clinical trial, and the inconsistency in the measured clinical parameters for assessing the effectiveness of treatments). Besides, we have to consider the difficulty of compounds crossing through the blood–brain barrier (BBB), due to their physical, metabolic, and BBB transport characteristics; therefore, many natural antioxidant compounds are unable to exert their neuropharmacological activity. It is estimated that over 98% of small molecules and almost 100% of large molecules that are discovered with the central nervous system activity cannot penetrate the BBB. For this reason, one of the pillars in the neuropharmacological research dealing with the antioxidant activity of natural products is focused on the study of the ability of active compounds to cross the BBB and, moreover, on the search of new galenic formulations (i.e., nanoparticles) capable to cross the BBB and thus facilitate the action of these neurocompounds [113].

6.3 Potential Therapeutic Agents with Natural Origin: Current Knowledge on the Discovery of Newer Drugs

Throughout this part of the chapter, we will focus on the potential therapeutic agents for neurodegenerative disorders that possess natural origin and have been demonstrated to exert neuroprotective effects by acting through the attenuation of mitochondrial dysfunction and/or reduction of abnormally enhanced lipid peroxidation; as previously mentioned, such cellular alterations are largely documented to occur in the brains of patients with the two most prevalent neurodegenerative disorders: AD and PD [114, 115].

Herein, we collect the available and current information on relevant compounds, identified mainly in plants, which afforded the most promising results on *in vitro* and *in vivo* models of neurodegeneration, including some clinical studies. For a better comprehension, the referred compounds have been gathered in relation to their chemical structures.

6.3.1 Flavonoids

They are a class of widely occurring natural compounds that represent the single most group of phenolic phytochemicals. Structurally, all flavonoids present a

benzo-γ-pyrone system containing a heterocyclic pyran and phenolic aromatic rings. They are described as hydrogen-donating antioxidants due to the reducing properties of their hydroxyl groups present in the aromatic rings. Therefore, these types of molecules are considered as potential chain-breaking antioxidants, as they are able to easily delocalize the resulting phenoxyl radical within the structure. Their capability to scavenge different oxygen and nitrogen radicals (such as $SO^{\bullet -}$, $OH^{\bullet}$, $ONOO^{-}$, etc.) may be explained by the following mechanism: flavonoid (OH) + $R^{\bullet} \rightarrow$ flavonoid ($O^{\bullet}$) + RH [116].

To date, more than 4000 flavonoids have been identified with structural variations (flavonols, flavandiols, flavanones, etc.), the compound quercetin being acknowledged as one of the most promising and ubiquitous antioxidants (Figure 6.6). Quercetin has displayed protective effects on mitochondrial deficits in preclinical models; it protected against deleterious effects of rotenone in a rat model of PD by upregulating the mitochondrial complex I activity in a dose-dependent manner, thus maintaining MMP and reducing mitochondrial ROS generation [117]. *In vitro*, the amelioration of mitochondrial integrity and function was also demonstrated in a glial–neuronal model of MPP(+)-induced PD, where quercetin treatment reduced the expression of inducible nitric oxide synthase (iNOS0 and the generation of superoxide radicals [118]. Furthermore, the mitochondrial protective activity of quercetin was evidenced together with a reducing effect in lipid peroxidation in a model of oxidative stress-related neurodegeneration induced by aluminum intake in rats [119].

Another structurally related flavonoid such as kaempferol, very common in numerous foods and plants, has recently demonstrated a protective action against rotenone-mediated toxicity in SH-SY5Y human dopaminergic cells with the preservation of functional mitochondria via enhancement of mitochondrial turnover by autophagy [120]. Its neuroprotective activities may also be mediated by an ameliorative effect toward excessive lipid peroxidation, as shown *in vivo* in

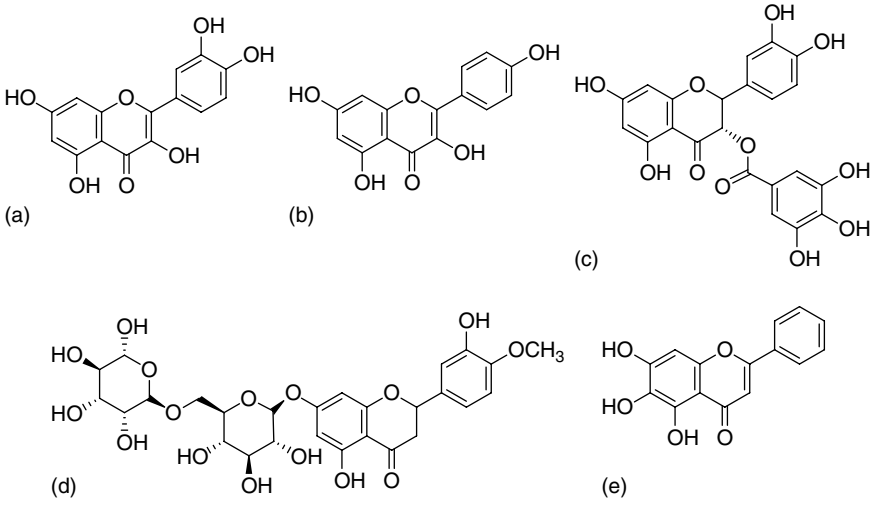


Figure 6.6 Chemical structures of flavonoid compounds quercetin (a), kaempferol (b), epigallocatechin gallate (c), hesperidin (d), and baicalein (e).

a MPTP-induced mouse model of PD; an oral treatment of 50 and 100 mg kg^{-1} day^{-1} for 14 days with kaempferol significantly reduced the content of MDA in the substantia nigra of MPTP-treated mice [121].

The green tea flavonoid (–)-epigallocatechin gallate (EGCG) has also largely been evaluated for its neuroprotective potential. On an Aβ-induced AD model of cultured rat hippocampal neurons, a co-treatment with 10 μM EGCG prevented an increase in membrane lipid peroxidation, which was evidenced by a decrease in MDA levels to a basal range, and diminished the abnormal activity of mitochondrial enzyme caspase-3 [122]. A reducing effect in mitochondrial-mediated apoptosis was later confirmed for EGCG against an $A\beta_{1-42}$-induced insult in rat primary cortical neurons; a short-term treatment with EGCG significantly attenuated neurotoxicity through a decrease in mitochondrial ROS generation and a downregulation in caspase-3 levels, with a proven involvement for the α7 nicotinic acetylcholine receptor signaling cascade [123].

Hesperidin, a flavanone glycoside that is frequently found in oranges and lemons, has exerted mitochondrial-targeted neuroprotection in an *in vitro* model of rotenone-induced PD. It actually inhibited the mitochondrial apoptotic pathway and preserved mitochondrial function in the human neuroblastoma SK-N-SH cell line, as revealed by increased Bcl2 levels and decreased Bax, caspase-3 and caspase-9 activities, reduction in cytochrome c release and mitochondrial ROS production, and maintenance of MMP [124]. Regarding the effects on lipid peroxidation, Naseem and Parvez demonstrated that it is able to restore the experimentally CCl_4-induced neurotoxicity *in vivo*; the antioxidant potency of hesperidin (200 mg kg^{-1} day^{-1}, 8 days, orally) was reflected in the significant reduction in thiobarbituric acid reactive substances (TBARS) levels in the brain tissue of rats [125]. Similar results have been found in the striatum of mice with MPTP-induced PD [126].

Baicalein is another flavonoid compound that should be highlighted as a potential therapeutic antioxidant, which has already been proposed as a neuroprotective candidate agent for the therapy of AD [127] and PD [128]. Regarding its mechanisms of action, both the mitochondrial stabilization and the reduction in lipid peroxidation may be considered. For instance, a co-treatment with 1 and 10 μM baicalein maintained the MMP and mitochondrial normal function and inhibited the mitochondrial apoptotic pathway in a human neuroblastoma SH-SY5Y cell model of 6-OHDA-induced PD; baicalein was also capable of reducing the enhanced lipid peroxidation in such model [129] as well as decreasing the level of MDA in the striatum of mice exposed to MPTP and its PD-like deleterious effects [130].

6.3.2 Vitamins

Vitamin E (Figure 6.7) is a lipophilic and phenolic compound that is obtained mostly from plant sources. There are several naturally occurring vitamin E homologs (α-, β-, and γ-tocopherols and tocotrienols), but α-tocopherol is the most abundant, active, and potent antioxidant form, whose antioxidant capacity relies on the donating capacity of the H atom of the hydroxyl group present in the chromanol ring [131]. It is the natural antioxidant that has shown the

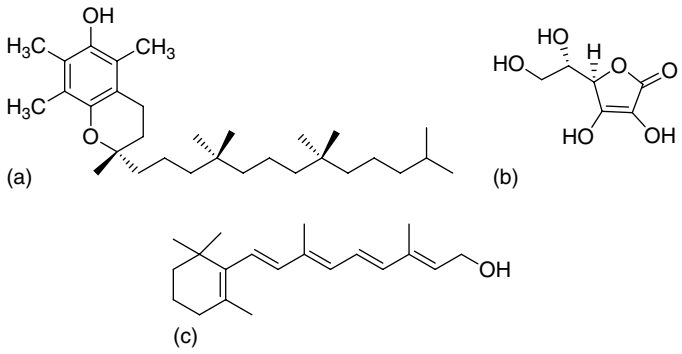


Figure 6.7 Chemical structures of vitamin E as alpha-tocopherol (a), vitamin C (b), and vitamin A as retinol (c).

most promising results in the treatment of AD; a 2-year-long clinical trial concluded that patients with moderately severe impairment from AD could be benefited from a treatment with α-tocopherol, which slows the progression of disease [132]. It was also shown to prevent or slow the progression of PD [133], and a vitamin E deficiency contributed to the characteristic nigral neurodegeneration in PD, whereas pretreatments with α-tocopherol attenuated the PD-like signs and symptoms in 6-OHDA-treated rats [134]. A reduction in lipid peroxidation has been suggested as a plausible mechanism for the neuroprotection afforded by vitamin E; it significantly reduced the TBARS levels in several brain regions of mice exposed to lindane-induced neurodegeneration [135]. Similarly, α-tocopherol demonstrated an enhancer activity of mitochondria functionality with depletion of ROS generation in neuronal cells *in vitro*, thus protecting against glutamate-induced neurotoxicity [136]. Furthermore, drug delivery systems have been investigated, and Shea *et al.* proposed that a nanosphere-mediated delivery of vitamin E increases its efficacy against oxidative stress resulting from exposure to amyloid beta in SH-SY5Y human neuroblastoma cells [137].

Vitamin C (L-ascorbic acid) (Figure 6.7) is commonly recognized as a natural antioxidant in our daily diet, essentially obtained from fruits and vegetables. Owing to its high bioavailability, it acts as one of the most potent water-soluble antioxidants within the cells, especially those in the brain (it easily crosses the BBB), and has been reported to efficiently scavenge diverse ROS [138]. Vitamin C has shown a potent mitochondrial-targeted antioxidant effect both in *in vitro* and *in vivo* models of AD, as it could decrease the MMP disruption in Aβ-threatened human HCN-1A cells and prevented abnormal mitochondrial morphology and function in 5XFAD knockout transgenic mouse model [139, 140]. In addition, one study suggested that a high-dose supplement intake of vitamin C and E may lower the risk of AD [141]. Concerning lipid peroxidation process, ascorbic acid co-treatment ($40\,mg\,kg^{-1}$) demonstrated an inhibitory action in a model of enhanced lipid peroxidation by gentamicin in rabbits: it prevented the increase of MDA and 4-HNE levels in the blood [142].

Vitamin A (Figure 6.7) can be found in various forms in the body, including retinol, retinal, and retinoic acid, and thanks to the presence of a hydrophobic

chain of the polyene units, it is also capable of acting as a chain-breaking antioxidant by combining with peroxyl radicals. Vitamin A is mainly obtained from green and yellow vegetables and fruits, and both retinol and retinal are the most active forms of the vitamin [143]. Although its future clinical value has not been fully determined regarding neuroprotection [144], it should be mentioned here for its capacity to inhibit lipid peroxidation. Various forms of vitamin A (0.1–10 mM) exerted interesting activities at reducing peroxidation in rat brain mitochondria, in terms of iron-induced MDA production [145].

6.3.3 Carotenoids

They are a family of pigmented compounds that are mainly found in plants, where they are involved in photosynthesis and photoprotection. All carotenoids are structurally related to vitamin A as they possess polyisoprenoid units joined by a long chain of conjugated double bonds; some carotenoids are able to convert into vitamin A in the body. They are regarded as powerful antioxidants, although their antioxidant behavior depends on the length of the conjugated C═C chain and the nature, number, and position of substituent groups in the molecule [110] (Figure 6.8).

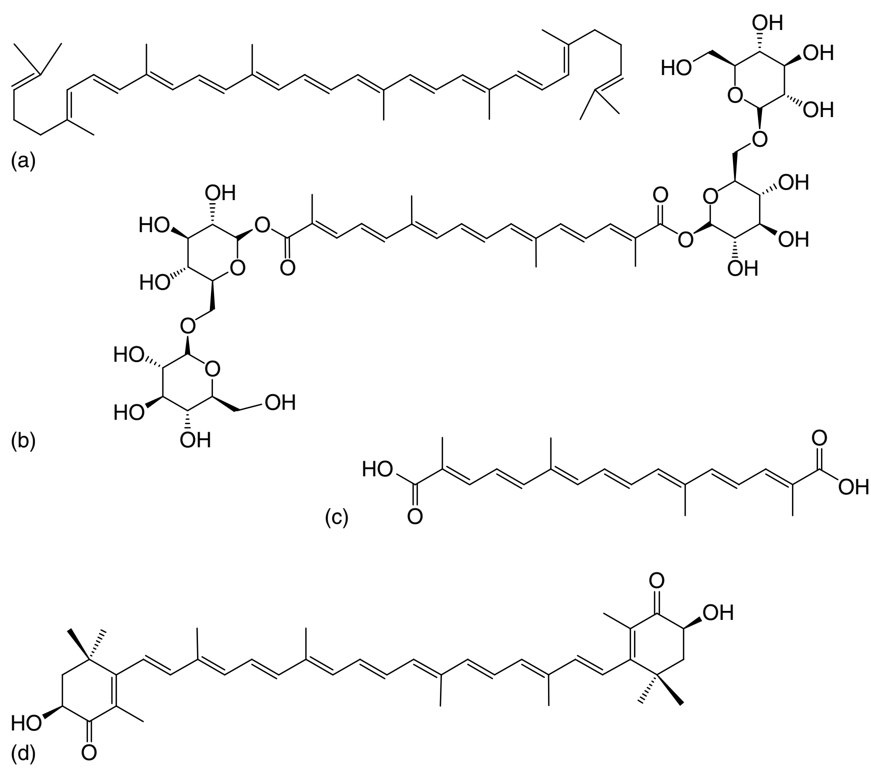


Figure 6.8 Chemical structures of carotenoid compounds lycopene (a), crocin (b), crocetin (c), and astaxanthin (d).

Among carotenoids obtained from vegetables, and mostly found in tomatoes, lycopene has been studied as one of the most potent antioxidants and as a neuroprotective compound in several models of neurodegenerative diseases, in which a mitochondrial protective effect was evidenced [146–150]. In an *in vitro* model of MPP(+)-induced PD in human SH-SY5Y cell line, lycopene displayed a promising effect on several mitochondrial markers of normal function: it maintained an appropriate MMP, reduced ROS generation, and increased intracellular ATP production and mtDNA copies and RNA transcripts levels; in such model, it also reduced MDA levels in cell membranes, suggesting that lipid peroxidation reduction may also be involved in its cytoprotective properties [149]. *In vivo* studies confirmed the interest of lycopene as a candidate therapeutic drug for PD. Kaur *et al.* demonstrated ameliorative properties on oxidative stress and cognitive decline in a rotenone-induced model in mice; lycopene supplementation ($10\,mg\,kg^{-1}$, orally for 30 days) protected against mitochondrial failure by reducing cytochrome c release and upregulating SOD activity and also decreased MDA and lipid peroxidation levels in the striatum [150]. Concerning AD models, lycopene provided *in vitro* cytoprotection against $A\beta_{25-35}$-induced neurotoxicity in cultured rat cortical neurons mainly via mitochondrial-mediated defense; 2 μM lycopene decreased apoptotic rate (restored normal levels of proapoptotic Bax, antiapoptotic Bcl-2, and caspase-3 activation) and inhibited MMP depolarization and ROS generation [151].

Crocin, the main pharmacologically active compound of *Crocus sativus* L. (saffron), is another natural carotenoid that showed effective antioxidant activity, even stronger than α-tocopherol, and has been studied for neuroprotective potential. In models of cytotoxicity induced in PC12 rat pheochromocytoma cells, treatments with crocin displayed cytoprotection through inhibitory actions of mitochondrial-mediated apoptosis and decrease in the formation of membrane-peroxidized lipids [152, 153]. Similar effects were extrapolated to *in vivo* models of streptozotocin-induced neurodegeneration in rats, where crocin administration improved learning and memory impairments with a marked reduction in MDA levels [154, 155]. Another similar carotenoid from saffron, crocetin, afforded antioxidant-mediated neuroprotection in a 6-OHDA-induced hemi-parkinsonian rat model, in which a pretreatment with crocetin (25, 50, and $75\,\mu g\,kg^{-1}$ for 7 days) evidenced lower TBARS content in neurons from the striatum [156]. These results suggest that both crocin and crocetin should merit further research as beneficial compounds in the treatment or prevention of neurodegenerative diseases.

Astaxanthin, a red pigment present in numerous marine organisms, is another antioxidant carotenoid that deserves to be mentioned, since its neuroprotective properties are attracting growing research [157–160]. This natural product showed protective effects on *in vitro* and *in vivo* models of PD and may provide a valuable therapeutic strategy for the treatment of such progressive diseases. As example, pretreatment of SH-SY5Y cells with astaxanthin suppressed 6-OHDA-induced apoptosis in a dose-dependent manner, counteracting mitochondrial dysfunctions such as lowered MMP, and abnormal cleavage of caspase-9 and caspase-3 and poly-ADP-ribose polymerase, through the blockade of p38 MAPK activation [157]. Lee *et al.* also suggested that the protective

effects of astaxanthin on MPP(+)- and MPTP-induced apoptosis (mediated by mitochondria) in *in vitro* and *in vivo* PD models may be due to its antioxidative properties and the normalization of the expression and activity of mitochondrial proteins such as Bcl-2 and Bax and the enzyme caspase-3 [158]. Astaxanthin also acted as a potential neuron protectant in Aβ-induced model of AD *in vitro*, and although ROS scavenging activities were suggested, its mechanism of neuroprotection needs to be further investigated for a better understanding [160].

6.3.4 Alkaloids

Alkaloids are a group of naturally occurring compounds that are present primarily as a class of nitrogen-containing organic compounds in plants, fungi, and bacteria and possess significant biological activities (Figure 6.9). In fact, they have been frequently used as important active ingredients in traditional medicines, and some of them are currently included in clinical therapy for numerous diseases due to their antitumor, analgesic, and anti-inflammatory effects, among others [161]. Some alkaloids have been reported for their therapeutic potentials in neurodegenerative diseases (reviewed in [162]), and even two US FDA-approved acetylcholinesterase inhibitors for AD, galantamine and rivastigmine, are actually alkaloids.

Besides those compounds, berberine is an isoquinoline alkaloid that has been demonstrated as an interesting butyl and acetylcholinesterase inhibitor with favorable neuroprotective results in *in vitro* and *in vivo* models of AD [163]. It is capable of reducing lipid peroxidation *in vitro* [164] and *in vivo* in a rat model of aluminum-induced neurodegeneration, where a supplementation with berberine (100 mg kg^{-1}) significantly blunted the increase in MDA in hippocampal neurons; in such model, berberine presented a mitochondrial-targeted effect and normalized the altered expression of mitochondrial enzymes SOD and MAO-B [165]. Regarding its actions on PD models, berberine protected against 6-OHDA-induced human dopaminergic neuronal cell death through the induction of heme oxygenase-1 and a marked reduction in mitochondrial-mediated ROS

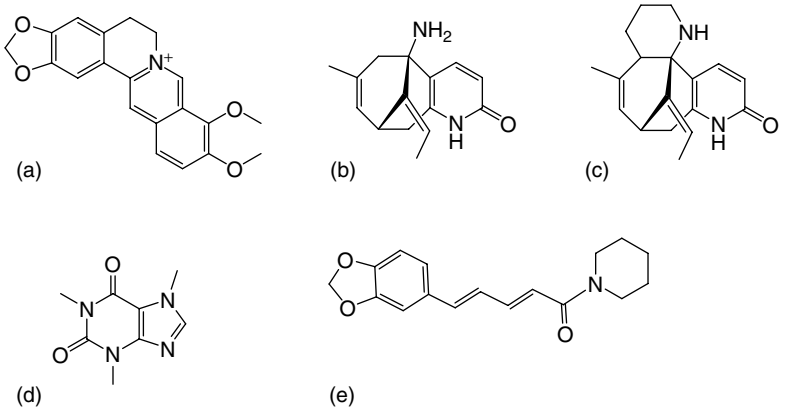


Figure 6.9 Chemical structures of alkaloid compounds berberine (a), huperzine A (b), huperzine B (c), caffeine (d), and piperine (e).

generation and caspase-3 activation [166]. Furthermore, berberine has been studied for the development of novel mitochondrial-targeted formulations for improving its activities [167].

Huperzine A, a sesquiterpene alkaloid isolated from *Huperzia serrata*, has similarly been revealed as a potent cholinesterase inhibitor, with potency even higher than that of other approved drugs for AD, and presents good pharmacokinetic properties such as oral bioavailability and BBB penetration. A clinical trial showed promising amelioration of cognitive and functional impairments in patients with mild to moderate AD. Huperzine A possesses the ability to protect neuron cells against neurotoxicity, which is in part mediated by regulation of oxidative stress and its protective action on mitochondrial integrity and function; it regulates the expression of apoptotic proteins Bcl-2, Bax, P53, and caspase-3 and prevents an increased cytochrome c release and reduced ATP generation and MMP loss. The improving effects of abnormal lipid peroxidation have also been suggested to mediate the neuroprotection afforded by huperzine A. Thus, this compound could be a lead candidate for the discovery of new treatments of AD [168–172]. The closely related alkaloid huperzine B, with similar acetylcholinesterase inhibitory activity, displayed *in vitro* diminution of lipid peroxidation; pretreatment with huperzine B (10–100 μM) decreased the level of MDA in H_2O_2-treated PC12 cells by approximately 25% [173].

The well-known stimulating compound caffeine is another methylxanthine alkaloid that deserves to be discussed in this chapter for its neuroprotective properties. Caffeine reduces levels of Aβ and alleviates Aβ-induced neurotoxicity *in vitro* and *in vivo*, as well as improves cognitive performance in Aβ-induced AD mouse models; its beneficial effects in the treatment of AD have even been reported as results of clinical trials [172, 174]. Caffeine reverses and prevents cognitive impairment in Swedish mutant APP transgenic mice and promotes neuron survival through the maintenance of normal mitochondrial function by restoring MMP, diminishing ROS production, and increasing ATP levels; those and other inhibitory effects in caspase-3 activity (mitochondrial apoptotic pathway) were confirmed in cell models of AD [175, 176]. Comparable potential has been reported for caffeine in relation to PD [177, 178]. As an adenosine A_{2A} antagonist, this drug completely reversed 6-OHDA-induced lipid peroxidation in rat mesencephalic cells [179]. However, a recent meta-analysis of observational epidemiological studies on the association between caffeine intake and the risk of cognitive disorders found no association between both factors [180]. Therefore, additional studies with longer follow-up periods are required to clarify that relationship.

Finally, piperine, the main active alkaloid in black pepper, has also exerted positive effects in animal models of AD [181] and PD [182], and those effects were proven to be partially associated with a decrease in lipid peroxidation and an inhibition of mitochondrial apoptotic pathway.

6.3.5 Non-flavonoid Phenolic Compounds

Apart from the aforementioned flavonoid compounds, there are other phenolic compounds that have displayed neuroprotective potential, mediated by mitochondrial protection and reduction in lipid peroxidation (Figure 6.10).

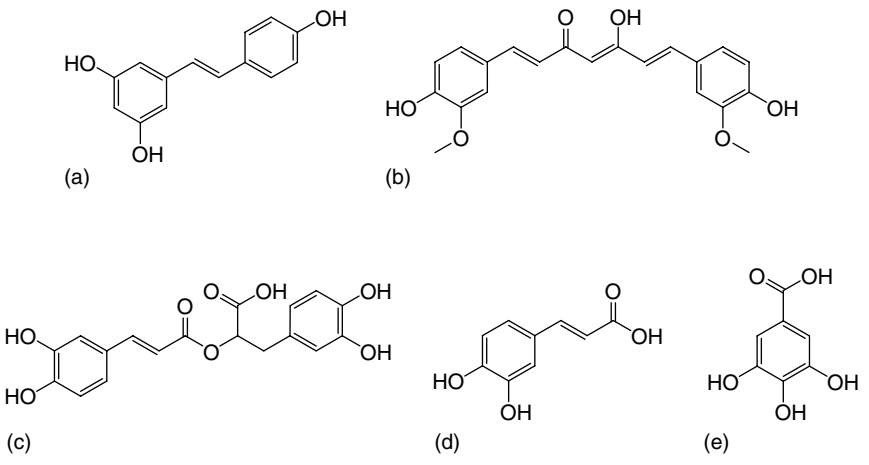


Figure 6.10 Chemical structures of phenolic compounds resveratrol (a), curcumin (b), rosmarinic acid (c), caffeic acid (d), and gallic acid (e).

Among them, we would firstly highlight the stilbenoid compound resveratrol, a constituent of red wine that has been largely studied for its therapeutic potential due to the reported biological effects, such as antioxidation, cardioprotection, or chemoprevention [183]. Resveratrol is able to improve mitochondrial function [184], and this capacity has been shown to be associated with neuroprotective properties, which were demonstrated in research models of both AD and PD. In Aβ-induced mouse neuroblastoma N2a cell AD model, pretreatment with resveratrol maintained normal expression of mitochondrial peroxiredoxins and structural genes, and it normalized the number of mitochondria; in AβPP transgenic mice, it could reduce the expression of cyclophilin D [185]. On the other hand, addition of resveratrol to cultured fibroblast from PD patients with parkin mutations (PARK2) also manifested the capacity of this compound to regulate mitochondrial energy homeostasis: it enhanced mitochondrial complex I activity, basal oxygen consumption, and ATP production [186]. Other studies reported an ameliorative activity of resveratrol on H_2O_2-induced lipid peroxidation in rat cortical cells [187].

Curcumin is a polyphenol derived from the spice turmeric, which is used as a food coloring additive. Curcumin and other curcuminoid compounds (including synthetic analogs) have shown biological activities with pharmacological interest. Evidence indicates that curcumin presents a potent neuroprotective activity in AD and PD models. As example, curcumin lowers amyloid deposits and inhibits tau protein aggregation in a transgenic model of AD and reduces oxidative injury and neuroinflammatory response, as well as the cognitive deficit after infusion of amyloid into the brain [188–190]. It was shown to exert a mitochondrial antiapoptotic action in PD models of neuronal cells in culture through the inhibition of caspase-3 and caspase-9 activities and the cytochrome c release; it also protected mitochondrial integrity and function via inhibitory action of ROS production and an enhancement of mitochondrial complex I activity [191, 192]. Similarly, the glutamoyl diester of curcumin

maintained the MMP and inhibited ROS generation in peroxynitrite-induced PD model in mouse brain mitochondria [193]. Furthermore, a synthetic pyrazole derivative compound (CNB-001) has been studied and demonstrated to avoid rotenone-induced mitochondrial damage in the human neuroblastoma SK-N-SH cell line by inhibiting mitochondrial apoptotic pathway and maintaining normal structure [194, 195]. Curcumin also displayed an inhibitory effect of lipid peroxidation *in vivo*; in a 3-nitropropionic acid-induced neurodegeneration rat model, curcumin administration (20 and 50 mg kg^{-1} for 8 days) significantly reversed the increase in MDA levels [196]. However, due to its low oral bioavailability, several drug delivery systems have been tested for better targeting of curcumin, such as liposomes, polymeric nanoparticles, etc.; solid lipid nanoparticles showed a great recovery in membrane lipids, as well as acetylcholinesterase activity in $AlCl_3$-treated mice, with results comparable with those achieved by rivastigmine [197].

Herein, some carboxylic acids with phenolic groups should be highlighted. Among them, rosmarinic acid is a polyphenolic antioxidant that exists in many Lamiaceae herbs commonly used for culinary purposes, such as rosemary and oregano; it possesses many biological activities including anti-inflammatory, anticancer, antiviral, antibacterial, and neuroprotective effects, and no severe side effect has been reported for it [198]. It has been revealed as a potent free radical scavenger and a protective agent against memory impairment induced by $A\beta_{25-35}$ *in vivo* [199]. The neuroprotective action of rosmarinic acid in AD models is suggested to be mediated by its mitochondrial-targeted activity; against Aβ peptide insults, it exerts an antiapoptotic mechanism evidenced by upregulation of Bax and downregulation of Bcl-2 gene expression and caspase-3 activity and also a reduced ROS generation. The decrease in lipid peroxidation has been proposed as another underlying mechanism of the referred neuroprotection [200, 201]. Comparable effects have been shown in 6-OHDA-induced models of PD [202, 203].

Caffeic acid is another phenolic compound with promising therapeutic applications in this field, and several works have reported its antioxidant and neuroprotective properties [204–207]. As example, caffeic acid was able to reverse the deleterious effects induced by 6-OHDA in both *in vitro* and *in vivo* models of PD, in which it maintained the normal function of mitochondria and inhibited apoptotic pathway, as evidenced by decreases in caspase-3 activity and in the release of cytochrome c [208, 209].

Finally, an abundant bioactive compound in grapes and berries, gallic acid, has been reported to provide protective effects in the brain tissue against AD and PD, as evidenced in several research models [210]. Concerning the mechanisms discussed in this chapter, gallic acid exerted neuroprotection in 6-OHDA-induced PD model in rats where an involvement for lipid peroxidation diminution was suggested; besides motor memory improvements, different doses of oral gallic acid pretreatment markedly decreased MDA levels in the hippocampus and striatum of rats [211]. On the other hand, Sun *et al.* recently demonstrated a specific protective effect of gallic acid on neuronal mitochondrial dysfunction under neurotoxic damage. Using both *in vitro* and *in vivo* models, they showed that gallic acid was capable of modulating the

MMP, mitochondrial ROS generation, oxygen consumption, ATP levels, mitochondrial transition pore viability, and cytochrome c release in favor of a greater neuron survival [212].

6.3.6 Terpenes

Various terpenoid compounds have demonstrated ameliorative effects in *in vitro* and/or *in vivo* models of neurodegenerative diseases (Figure 6.11). Unlike other groups of compounds, a quantitative structure–activity relationship has already been proposed for neuroprotective activity of terpenoids, indicating that the activity is mainly reliant on lipophilicity, shape index, and electrostatic property of molecules [213]. Herein, we will highlight the recent studies that dealt with the neuroprotection of natural terpenoids.

For instance, the triterpenoid xyloketal B is a novel marine compound with a unique chemical structure isolated from the mangrove fungus *Xylaria* sp. and has been proven to exert neuroprotective action against MPP(+)-induced neurotoxicity in *Caenorhabditis elegans* and PC12 cells as research models of PD; it displayed a dose-dependent protection via maintenance of MMP and decrease in ROS generation [214].

Another remarkable terpenoid compound is the sesquiterpene trilactone bilobalide, the main active principle of *G. biloba* leaves, which has been largely studied for its neuroprotective actions and showed potent protective effects on neurons and Schwann cells [89]. Through both *in vivo* and *in vitro* works, authors have suggested various mechanisms of action associated with neuroprotection by bilobalide, including preservation of mitochondrial ATP synthesis, inhibition

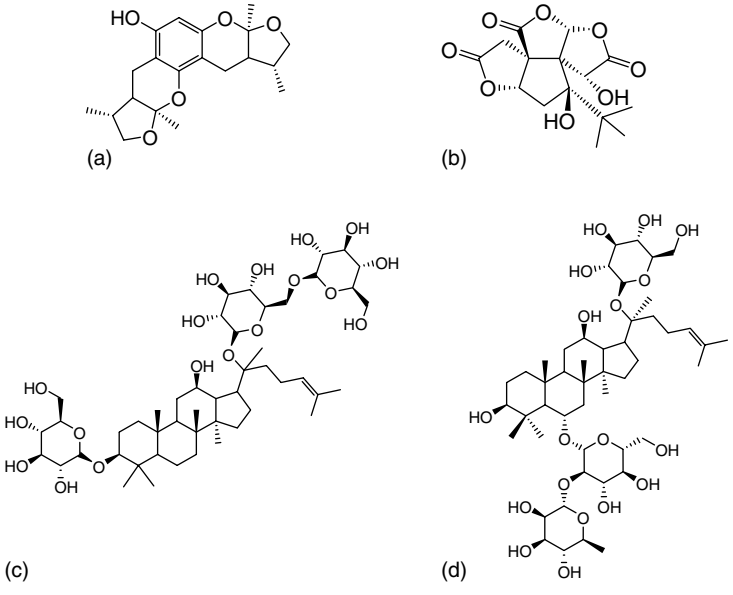


Figure 6.11 Chemical structures of phenolic compounds xyloketal b (a), bilobalide (b), gypenoside XVII (c), and ginsenoside Re (d).

of apoptotic damage, and capacity to increase the expression of mtDNA-encoded COX III subunit of cytochrome c oxidase and the ND1 subunit of NADH dehydrogenase [215, 216]. As example, bilobalide (25–100 μM) blocked ROS-induced apoptosis in early stages and decreased the elevated levels of p53, Bax, and caspase-3 in PC12 cells [217].

Gypenoside XVII (GP-17) is a novel triterpene glycoside isolated from *Gynostemma pentaphyllum* or *Panax notoginseng* that, due to its structure, was found to confer neuroprotection against $A\beta_{25-35}$-induced neurotoxicity in PC12 cells via estrogen receptor-dependent activation of PI3K/Akt and Nrf2/ARE/HO-1 pathways; regarding mitochondrial failures, pretreatment with GP-17 (10 μM) for 12 h is demonstrated to restore normal MMP and reduced cytochrome c release and the enhanced apoptosis by inhibiting caspase-3 activation and cleavage [218].

Terpenoid metabolites from ginseng (*Panax* sp.) are other promising compounds regarding neuroprotection [219]. For instance, ginsenoside Re has been proposed as an interesting natural product candidate that acts via the two mechanisms referred to in this chapter [220]. It has recently been demonstrated as a neuroprotector agent in a model of human neuroblastoma dopaminergic SH-SY5Y cell lines subjected to a neurodegenerative insult with methamphetamine. Treatment with ginsenoside Re exhibited significant protection against cytosolic damage, as evidenced by a decrease in 4-HNE levels (marker of lipid peroxidation). Still, the main protective mechanism was the alleviation of mitochondrial burden and proapoptotic effects; it favored the induction of mitochondrial GPx, caused a genetic inhibition of protein kinase C δ, and reduced the mitochondrial dysfunction (mitochondrial transmembrane potential and intramitochondrial Ca^{2+}) and apoptotic events (cytochrome c release and cleavage of caspase-3) [221].

Finally, González-Burgos *et al.* suggested interesting neuroprotective properties for diterpenes isolated from *Sideritis* sp. Similarly, both the prevention from mitochondrial dysfunction and the reduction of lipid peroxidation were demonstrated against ROS-induced neurodegeneration *in vitro* [222, 223].

6.3.7 Other Compounds

There are several compounds of interest as neuroprotective drugs that, regarding chemical features, were not classified in any of the previously discussed main groups and will be mentioned here, as they also deserve a reference in this chapter (Figure 6.12).

Wang *et al.* evaluated the potential activity of acteoside, an antioxidant phenylethanoid glycoside first extracted from *Verbascum sinuatum* and named "verbascoside" on $A\beta_{25-35}$-induced cell injury in an SH-SY5Y cell model of AD. They concluded that a pretreatment with acteoside for 15 h was effective for inhibiting ROS production, mitochondrial dysfunction, and apoptotic pathway; this modulation involved preservation of normal MMP and decrease in Bax/Bcl-2 ratio, cytochrome c release, and cleavage of caspase-3 [224].

Similarly, Sun *et al.* demonstrated *in vitro* neuroprotective effects via antioxidant potential for the steroidal saponin nolinospiroside F, isolated from the plant

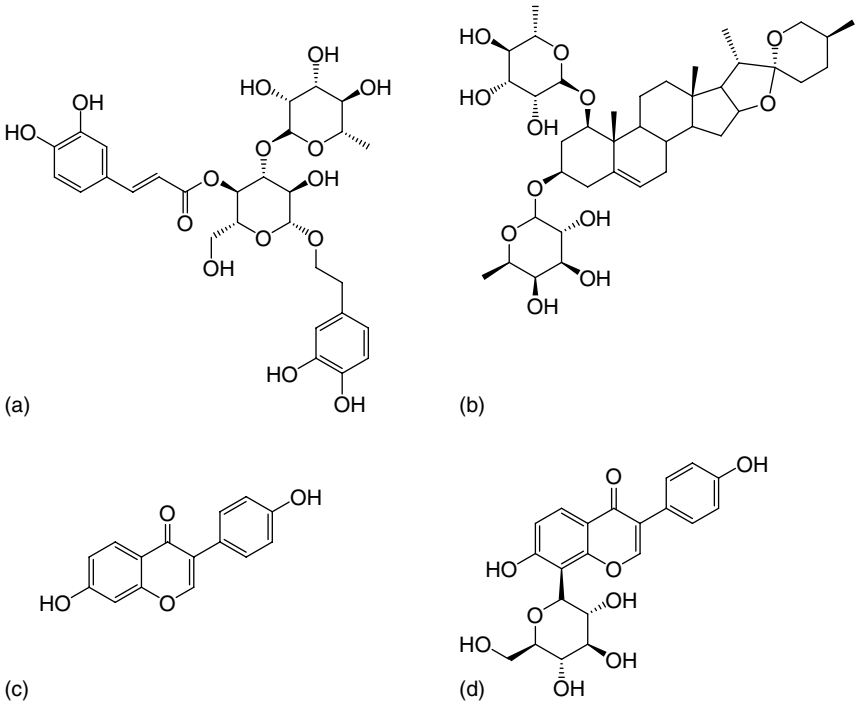


Figure 6.12 Chemical structures of acteoside (a), nolinospiroside F (b), genistein (c), and puerarin (d).

Ophiopogon japonicus. In a model of H_2O_2-induced oxidative stress in K6001 yeast strain, treatment with different doses of the compound (1, 3, and 10 μM) extended the life span of yeasts and avoided the enhancement of MDA production, and it was able to increase the expression and activity of mitochondrial SOD [225].

Finally, there are natural compounds with flavonoid structure that are considered as phytoestrogens and have exerted positive effects in models of AD and/or PD. The phytoestrogen genistein showed its potency to upregulate the mitochondrial Na/K-ATPase activity in human AD brains, which is proved to be disturbed in the early phase of the disease; thus, by ameliorating the energy metabolism in the initiation of AD pathology, genistein is presented as an interesting neuroprotective drug [226]. In the same way, the phytoestrogen puerarin displayed anti-Alzheimer's effect *in vivo* and *in vitro.* In rats subjected to intrahippocampal injection of $A\beta_{1-42}$, puerarin treatment ameliorated the induced cognitive impairment and reversed the increase of apoptosis in the hippocampus, as shown by the reduced mitochondrial RNA levels and caspase-9 activity. Also, in mitochondrial transgenic neuronal cell cybrid models of sporadic AD, Zhang *et al.* confirmed the mitochondrial protective effect of puerarin, where it reduced mitochondrial ROS accumulation and the subsequent apoptotic pathway (downregulation of Bax/Bcl-2 ratio and reduction of caspase-3 activity) [227, 228].

6.3.8 Plant Extracts

As mixtures of several bioactive principles, plant extracts have also been the issue of many studies dealing with the neuroprotective potentials of natural products [229, 230]. Some of them displayed mitochondrial-targeted actions as well as a reducing effect on lipid peroxidation as major processes involved in the mechanism of action.

For instance, the standardized extract EGb761 from *G. biloba* (composed of flavoglycosides, terpene lactones, and ginkgolic acid) displayed protective actions on an Aβ-induced rat model of AD; when injected intraperitoneally at a concentration of 20 mg kg^{-1} day^{-1} for 15 days, it was able to ameliorate oxidative stress markers, including lipid peroxidation, as indicated by a reduction of hippocampal levels of MDA [231]. On the other hand, a green tea leaf extract rich in epicatechin and EGCG was able to reverse AD-like damaging effects of $AlCl_3$ in a rat model, suggesting that it might be beneficial in the therapy of AD; it reduced the aluminum-induced neurotoxicity via antioxidant and mitochondrial protective effects, such as an augment in cytochrome c oxidase activity [232].

The beneficial effects on AD- and PD-related neuronal disturbances demonstrated by some inhaled volatile essential oils are also remarkable; they have attracted scientific research on disease models [233, 234]. Among them, it has been proven that the cognitive-enhancing properties exerted by the essential oil of the traditionally used *Coriandrum sativum* in $A\beta_{1-42}$-induced rat model of AD. Inhalation of 1% and 3% (daily, for 21 days) of that linalool-rich volatile oil by rats improved spatial memory performance as well as oxidative stress markers; its action was actually correlated with an attenuation in the increased MDA levels in hippocampal neurons and an enhancement of the activity of mitochondrial antioxidant enzymes GPx and SOD [235]. Similarly, Hancianu *et al.* evidenced neuroprotective effects for the essential oil of *Lavandula* spp. on scopolamine-induced dementia in rats via antioxidative activities. Subacute exposures (200 μl, 60 min day^{-1} for 7 days) of rats to inhaled lavender oil decreased the levels of lipid peroxidation in rat temporal lobe homogenates, presenting contents of MDA even lower than those in control animals [236].

6.4 Future Trends in Research

Among all natural compounds investigated for their potential as neuroprotective antioxidants, only a few of them have shown limited efficiency in animal models or in small clinical studies (those mentioned in this chapter), but none of them have still proven efficacious in a large-scale controlled study.

First of all, the unavailability of the adequate animal models for preclinical assessment limits the scientific research in this field and subsequent successes in clinical trials. One line of research should be therefore aimed to obtain better animal models that faithfully reproduce the pathological hallmarks of AD and PD. Moreover, regarding clinical trials, it would be interesting to increase the number of patients and the duration of the trials as well as to study in depth a larger number of potential antioxidants.

On the other hand, several mitochondrial-targeted antioxidants have been designed so far by conjugating lipophilic cations to an antioxidant moiety (e.g., MitoQ, SS tetrapeptides, etc.), also with limited value when tested in patients [237]. In this sense, if the mitochondrial dysfunction is reproduced in detail as it occurs in nervous system cells of patients, the search of mitochondrial-targeted antioxidants might be facilitated. The application of nanomaterials is a new attracting field in the research on these mitochondrial-directed compounds that may suppose the development of innovative therapeutic molecules.

The current therapeutic use of most of these natural compounds is limited owing to their incapacity to cross the BBB. Therefore, any novel antioxidant molecules designed as potential neuroprotective treatment in acute or chronic neurological disorders should have the mandatory prerequisite that they can cross the BBB after systemic administration [238]. For instance, combination of vitamins C and E is found to be efficacious in both AD and cognitive disorders; however, they are not proved to be up to the mark as they are also unable to cross the BBB efficiently. Similarly, flavonoids are considered as potent antioxidants, but they are highly polar molecules due to the presence of many phenolic groups and restricted to blood–brain permeability. Hence, those problems should be considered in future research, as there is a need for equilibrium between the antioxidant properties of a certain molecule and its BBB-crossing capacity. On the other hand, to increase the permeability of antioxidants of the BBB, it would be also interesting to deepen the investigation of different galenic forms such as liposomes that enable these potential active compounds to reach the brain.

Some scientific articles discussed the relationship between molecular structure and antioxidant activity of quercetin, the importance of phenolic groups in flavonoids, the importance of conjugated double bonds in carotene in capturing free electrons, and so on. The most challenging goal in this field will be to construct a molecule that is effective in both ways as a drug candidate for the neurodegenerative diseases and a potential antioxidant that has the ability of neuroprotection (implying good pharmacokinetic properties). The natural compounds referred throughout this chapter could be utilized as lead molecules for the development of new and better therapeutics. But considering the polar nature of most of the antioxidants used and their little penetration into the central nervous system, chemical strategies such as substitution of the bulky groups (alkyl groups, aromatic rings, aromatic alkyl groups, or heterocyclic aromatic ring systems) in order to increase the lipophilicity of the molecule need to be investigated.

6.5 Concluding Remarks

Neurodegenerative diseases in humans are strongly associated with oxidative stress generated by ROS, and strong scientific evidence supports this idea regarding the very much prevalent AD and PD. Although individual neurodegenerative diseases manifest in distinct neuronal cell types, oxidative stress and suppression of neuronal survival signals are common to many of these pathological conditions and appear to be highly relevant targets for treatment. In these

pathologic states, the mitochondrial dysfunction and the peroxidation of membrane lipids are important contributors to the degeneration of nervous cells in both conditions.

In general, neurodegenerative diseases currently lack effective treatment options for patients, and even though AD and PD receive the most attention through extensive funding and research, these diseases have only palliative therapies available and none that significantly slow or halt the underlying pathology of the disease. Considering that our endogenous antioxidant defenses are not always completely effective to counteract oxidative stress processes, and due to the limitations of the current therapy, several antioxidants of natural origin and widely varying chemical structures have been investigated for use as therapeutic agents. Many of these natural antioxidants are not only active scavengers of free radicals but also modulators of pro-survival or proapoptotic signaling pathways; their capacity to mitigate oxidative damage and promote neuron survival underlies their effectiveness in many *in vitro* and *in vivo* models of neuronal degeneration. In this chapter, we mainly aimed to report the available information on the basic and clinical research of natural compounds of interest as potential therapeutic antioxidants, with special focus on those compounds that specifically act via reduction of mitochondrial deficits and abnormal lipid peroxidation.

Besides their multiple modes of action, there is also a belief of a lower risk of unexpected side effects and safety profiles in the use of antioxidants from natural sources. However, a very few of them have shown effective results in clinical trials, and some antioxidant-based therapeutic approaches have disappointed the researchers regarding the neuroprotective nature of the agents, which is usually due to problems related to biopharmaceutical properties.

Concerning the most promising compounds, one should highlight the recent initiation of several clinical trials with EGCG, which is currently being tested in phase II trials for PD (Xuanwu Hospital, Beijing, China) and early-stage AD (Charite University, Berlin, Germany). Similarly, resveratrol is being tested in a phase II trial to improve memory performance in the elderly (McKnight Brain Institute, University of Florida), and finally, the safety and tolerability of curcumin are being investigated in patients with AD [239].

In conclusion, there is still a need for the discovery of novel antioxidants that can be better utilized as therapeutic agents with minimal side effects, having no or less biopharmaceutical problems.

Abbreviations

AD	Alzheimer's disease
APP	amyloid precursor protein
Aβ	amyloid beta peptide
BBB	blood–brain barrier
COMT	catechol-*O*-methyltransferase
COX	cyclooxygenase
EGCG	epigallocatechin gallate
4-HNE	4-hydroxynonenal

6-OHDA	6-hydroxydopamine
GPx	glutathione peroxidase
GSTs	glutathione-S-transferases
LOX	lipoxygenase
MAO	monoamine oxidase
MDA	malondialdehyde
MMP	mitochondrial membrane potential
MPP^+	1-methyl-4-phenylpyridine
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NMDA	*N*-methyl-D-aspartate
PD	Parkinson's disease
ROS	reactive oxygen species
SOD	superoxide dismutase
TBARS	thiobarbituric acid reactive substances

References

1 Jones, D.P. (2008) Radical-free biology of oxidative stress. *Am. J. Physiol. Cell Physiol.*, **295** (4), C849–C868.
2 Emerit, J., Edeas, M., and Bricaire, F. (2004) Neurodegenerative diseases and oxidative stress. *Biomed. Pharmacother.*, **58** (1), 39–46.
3 Chen, Z. and Zhong, C. (2014) Oxidative stress in Alzheimer's disease. *Neurosci. Bull.*, **30** (2), 271–281.
4 Berlett, B.S. and Stadtman, E.R. (1997) Protein oxidation in aging, disease, and oxidative stress. *J. Biol. Chem.*, **272** (33), 20313–20316.
5 Sharma, P., Jha, A.B., Dubey, R.S., and Pessarakli, M. (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.*, **2012**, 1–26.
6 Singh, S., Brocker, C., Koppaka, V., Chen, Y., Jackson, B.C., Matsumoto, A., Thompson, D.C., and Vasiliou, V. (2013) Aldehyde dehydrogenases in cellular responses to oxidative/electrophilic stress. *Free Radical Biol. Med.*, **56**, 89–101.
7 Cooke, M.S., Evans, M.D., Dizdaroglu, M., and Lunec, J. (2003) Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J.*, **17** (10), 1195–1214.
8 Kong, Q. and Lin, C.L. (2010) Oxidative damage to RNA: mechanisms, consequences, and diseases. *Cell. Mol. Life Sci.*, **67** (11), 1817–1829.
9 Friedman, J. (2011) in *Oxidative Stress and Free Radical Damage in Neurology* (eds N. Gadoth and H.H. Göbel VIII), Humana Press, pp. 19–22.
10 Humpel, C. (2011) Identifying and validating biomarkers for Alzheimer's disease. *Trends Biotechnol.*, **29** (1), 26–32.
11 Rosén, C., Hansson, O., Blennow, K., and Zetterberg, H. (2013) Fluid biomarkers in Alzheimer's disease-current concepts. *Mol. Neurodegener.*, **8**, 20–31.
12 García-Matas, S., de Vera, N., Aznar, A.O., Marimon, J.M., Adell, A., Planas, A.M., Cristòfol, R., and Sanfeliu, C. (2010) In vitro and in vivo activation of astrocytes by amyloid-beta is potentiated by pro-oxidant agents. *J. Alzheimers Dis.*, **20** (1), 229–245.

13 Butterfield, D.A., Howard, B.J., and LaFontaine, M.A. (2001) Brain oxidative stress in animal models of accelerated aging and the age-related neurodegenerative disorders, Alzheimer's disease and Huntington's disease. *Curr. Med. Chem.*, **8** (7), 815–828.

14 D'Autreaux, B. and Toledano, M.B. (2007) ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat. Rev. Mol. Cell Biol.*, **8** (10), 813–824.

15 Ames, B.N., Shigenaga, M., and Hagen, T. (1993) Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.*, **90** (17), 7915–7922.

16 McCord, J.M. and Fridovich, I. (1969) Superoxide Dismutase an enzymic function for erythrocuprein (Hemocuprein). *J. Biol. Chem.*, **244** (22), 6049–6055.

17 Matés, J.M. (2000) Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology*, **153** (1-3), 83–104.

18 Mills, G.C. (1957) Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. *J. Biol. Chem.*, **229** (1), 189–197.

19 Tosatto, S.C., Bosello, V., Fogolari, F., Mauri, P., Roveri, A., Toppo, S., Flohé, L., Ursini, F., and Maiorino, M. (2008) The catalytic site of glutathione peroxidases. *Antioxid. Redox Signaling*, **10** (9), 1515–1526.

20 Arthur, J.R. (2000) The glutathione peroxidases. *Cell. Mol. Life Sci.*, **57** (13-14), 1825–1835.

21 Rescigno, M. and Perham, R.N. (1994) Structure of the NADPH-binding motif of glutathione reductase: efficiency determined by evolution. *Biochemistry*, **33** (19), 5721–5727.

22 Mazzetti, A.P., Fiorile, M.C., Primavera, A., and Lo, B.M. (2015) Glutathione transferases and neurodegenerative diseases. *Neurochem. Int.*, **82**, 10–18.

23 Storey, K.B. (ed.) (2004) *Functional Metabolism: Regulation and Adaptation*, John Wiley & Sons, Inc, Hoboken, NJ.

24 Townsend, D.M., Tew, K.D., and Tapiero, H. (2003) The importance of glutathione in human disease. *Biomed. Pharmacother.*, **57** (3-4), 145–155.

25 Nicolet, B.H. (1930) The structure of glutathione. *Science*, **71** (1849), 589–590.

26 Cobley, J.N., McHardy, H., Morton, J.P., Nikolaidis, M.G., and Close, G.L. (2015) Influence of vitamin C and vitamin E on redox signaling: implications for exercise adaptations. *Free Radical Biol. Med.*, **84**, 65–76.

27 Cao, G., Alessio, H.M., and Cutler, R.G. (1993) Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biol. Med.*, **14** (3), 303–311.

28 Kim, H.Y. and Gladyshev, V.N. (2007) Methionine sulfoxide reductases: selenoprotein forms and roles in antioxidant protein repair in mammals. *Biochem. J.*, **407** (3), 321–329.

29 El-Beltagi, H.S. and Mohamed, H.I. (2013) Reactive oxygen species, lipid peroxidation and antioxidative defense mechanism. *Not. Bot. Horti Agrobo.*, **41**, 44–57.

30 Ayala, A., Muñoz, M.F., and Argüelles, S. (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid. Med. Cell. Longevity*, **2014**, 360438–360469.

31 Repetto, M., Semprine, J., and Boveris, A. (2012) in *Biochemistry, Genetics and Molecular Biology* (ed. A. Catala), InTech, Rijeka, pp. 1–28.

32 Yasaei, P.M., Yang, G.C., Warner, C.R., Daniels, D.H., and Ku, Y. (1996) Singlet oxygen oxidation of lipids resulting from photochemical sensitizers in the presence of antioxidants. *J. Am. Oil Chem. Soc.*, **73**, 1177–1181.

33 Schneider, C., Pratt, D.A., Porter, N.A., and Brash, A.R. (2007) Control of oxygenation in lipoxygenase and cyclooxygenase catalysis. *Chem. Biol.*, **14** (5), 473–488.

34 Marnett, L.J., Rowlinson, S.W., Goodwin, D.C., Kalgutkar, A.S., and Lanzo, C.A. (1999) Arachidonic acid oxygenation by COX-1 and COX-2. Mechanisms of catalysis and inhibition. *J. Biol. Chem.*, **274** (33), 22903–22906.

35 Yamamoto, S. (1991) "Enzymatic" lipid peroxidation: reactions of mammalian lipoxygenases. *Free Radical Biol. Med.*, **10** (2), 149–159.

36 Maccarrone, M., Melino, G., and Finazzi-Agrò, A. (2001) Lipoxygenases and their involvement in programmed cell death. *Cell Death Differ.*, **8** (8), 776–784.

37 Chen, J.K., Capdevila, J., and Harris, R.C. (2001) Cytochrome p450 epoxygenase metabolism of arachidonic acid inhibits apoptosis. *Mol. Cell. Biol.*, **21** (18), 6322–6331.

38 Edin, M.L., Wang, Z., Bradbury, J.A., Graves, J.P., Lih, F.B., DeGraff, L.M., Foley, J.F., Torphy, R., Ronnekleiv, O.K., Tomer, K.B., Lee, C.R., and Zeldin, D.C. (2011) Endothelial expression of human cytochrome P450 epoxygenase CYP2C8 increases susceptibility to ischemia-reperfusion injury in isolated mouse heart. *FASEB J.*, **25** (10), 3436–3447.

39 Uchida, K. and Stadtman, E.R. (1992) Modification of histidine residues in proteins by reaction with 4-hydroxynonenal. *Proc. Natl. Acad. Sci. U.S.A.*, **89** (10), 4544–4548.

40 Zhong, H. and Yin, H. (2015) Role of lipid peroxidation derived 4-hydroxynonenal (4-HNE) in cancer: focusing on mitochondria. *Redox Biol.*, **4**, 193–199.

41 Uchida, K. (2003) 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. *Prog. Lipid Res.*, **42** (4), 318–343.

42 Spickett, C.M. (2013) The lipid peroxidation product 4-hydroxy-2-nonenal: advances in chemistry and analysis. *Redox Biol.*, **1** (1), 145–152.

43 Esterbauer, H., Schaur, R.J., and Zollner, H. (1991) Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biol. Med.*, **11** (1), 81–128.

44 Uchida, K. (1999) Current status of acrolein as a lipid peroxidation product. *Trends Cardiovasc. Med.*, **9** (5), 109–113.

45 Lovell, M.A., Xie, C., and Markesbery, W.R. (2000) Acrolein, a product of lipid peroxidation, inhibits glucose and glutamate uptake in primary neuronal cultures. *Free Radical Biol. Med.*, **29** (8), 714–720.

46 Cai, J., Bhatnagar, A., and Pierce, W.M. Jr. (2009) Protein modification by acrolein: formation and stability of cysteine adducts. *Chem. Res. Toxicol.*, **22** (4), 708–716.

47 Liu, X.Y., Zhu, M.X., and Xie, J.P. (2010) Mutagenicity of acrolein and acrolein-induced DNA adducts. *Toxicol. Mech. Methods*, **20** (1), 36–44.

48 Cracowski, J.L., Durand, T., and Bessard, G. (2002) Isoprostanes as a biomarker of lipid peroxidation in humans: physiology, pharmacology and clinical implications. *Trends Pharmacol. Sci.*, **23** (8), 360–366.

49 Janicka, M., Kot-Wasik, A., Kot, J., and Namieśnik, J. (2010) Isoprostanes-biomarkers of lipid peroxidation: their utility in evaluating oxidative stress and analysis. *Int. J. Mol. Sci.*, **11** (11), 4631–4659.

50 Morrow, J.D. and Roberts, L.J. (1997) The isoprostanes: unique bioactive products of lipid peroxidation. *Prog. Lipid Res.*, **36** (1), 1–21.

51 Roberts, L.J. and Morrow, J.D. (2002) Products of the isoprostane pathway: unique bioactive compounds and markers of lipid peroxidation. *Cell. Mol. Life Sci.*, **59** (5), 808–820.

52 Esterbauer, H., Dieber-Rotheneder, M., Waeg, G., Striegl, G., and Jurgens, G. (1990) Biochemical, structural, and functional properties of oxidized low-density lipoprotein. *Chem. Res. Toxicol.*, **3** (2), 77–92.

53 Esterbauer, H. (1993) Cytotoxicity and genotoxicity of lipid-oxidation products. *Am. J. Clin. Nutr.*, **57** (Suppl. 5), 779S–785S.

54 Marnett, L.J. (1999) Lipid peroxidation-DNA damage by malondialdehyde. *Mutat. Res.*, **424** (1-2), 83–95.

55 Del Rio, D., Stewart, A.J., and Pellegrini, N. (2005) A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr. Metab. Cardiovasc. Dis.*, **15** (4), 316–328.

56 O'Rourke, B. (2010) From bioblasts to mitochondria: ever expanding roles of mitochondria in cell physiology. *Front. Physiol.*, **1** (7), 1–4.

57 Dorn, G.W. (2013) Mitochondrial dynamics in heart disease. *Biochim. Biophys. Acta*, **1833** (1), 233–241.

58 Schenkel, L.C. and Bakovic, M. (2014) Formation and regulation of mitochondrial membranes. *Int. J. Cell. Biol.*, **2014**, 709828–709841.

59 Horvath, S.E. and Daum, G. (2013) Lipids of mitochondria. *Prog. Lipid Res.*, **52** (4), 590–614.

60 Dudkina, N.V., Kouřil, R., Peters, K., Braun, H.P., and Boekema, E.J. (2010) Structure and function of mitochondrial supercomplexes. *Biochim. Biophys. Acta*, **1797** (6-7), 664–670.

61 Brdiczka, D.G., Zorov, D.B., and Sheu, S.S. (2006) Mitochondrial contact sites: their role in energy metabolism and apoptosis. *Biochim. Biophys. Acta*, **1762** (2), 148–163.

62 Langston, J.W., Ballard, P., Tetrud, J.W., and Irwin, I. (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science*, **219** (4587), 979–980.

63 Schapira, A.H.V., Cooper, J.M., Dexter, D., Clark, J.B., Jenner, P., and Marsden, C.D. (1990) Mitochondrial complex I deficiency in Parkinson's disease. *J. Neurochem.*, **54** (3), 823–827.

64 Subramaniam, S.R. and Chesselet, M.F. (2013) Mitochondrial dysfunction and oxidative stress in Parkinson's disease. *Prog. Neurobiol.*, **106–107**, 17–32.

65 Devi, L., Prabhu, B.M., Galati, D.F., Avadhani, N.G., and Anandatheerthavarada, H.K. (2006) Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. *J. Neurosci.*, **26** (35), 9057–9068.

66 Reichmann, H., Flörke, S., Hebenstreit, G., Schrubar, H., and Riederer, P. (1993) Analyses of energy metabolism and mitochondrial genome in post-mortem brain from patients with Alzheimer's disease. *J. Neurol.*, **240** (6), 377–380.

67 Hien, P.P., Gortnizka, H., and Kraemer, R. (2003) Development of a new binary solvent system using ionic liquids as additives to improve rotenone extraction yield from Malaysia Derris sp. *Omonrice*, **11**, 468917–468924.

68 Manning-Bog, A.B., McCormack, A.L., Li, J., Uversky, V.N., Fink, A.L., and Di Monte, D.A. (2002) The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha-synuclein. *J. Biol. Chem.*, **277** (3), 1641–1644.

69 Simola, N., Morelli, M., and Carta, A.R. (2007) The 6-hydroxydopamine model of Parkinson's disease. *Neurotox. Res.*, **11** (3-4), 151–167.

70 Feng, Z., Qin, C., Chang, Y., and Zhang, J.T. (2006) Early melatonin supplementation alleviates oxidative stress in a transgenic mouse model of Alzheimer's disease. *Free Radical Biol. Med.*, **40** (1), 101–109.

71 Dragicevic, N., Copes, N., O'Neal-Moffitt, G., Jin, J., Buzzeo, R., Mamcarz, M., Tan, J., Cao, C., Olcese, J.M., Arendash, G.W., and Bradshaw, P.C. (2011) Melatonin treatment restores mitochondrial function in Alzheimer's mice: a mitochondrial protective role of melatonin membrane receptor signaling. *J. Pineal Res.*, **51** (1), 75–86.

72 McManus, M.J., Murphy, M.P., and Franklin, J.L. (2011) The mitochondria-targeted antioxidant MitoQ prevents loss of spatial memory retention and early neuropathology in a transgenic mouse model of Alzheimer's disease. *J. Neurosci.*, **31** (44), 15703–15715.

73 Sanz-Blasco, S., Valero, R.A., Rodríguez-Crespo, I., Villalobos, C., and Nuñez, L. (2008) Mitochondrial Ca^{2+} overload underlies Aβ oligomers neurotoxicity providing an unexpected mechanism of neuroprotection by NSAIDs. *PLoS One*, **3** (7), e2718.

74 Gazewood, J.D., Richards, D.R., and Clebak, K. (2013) Parkinson disease: an update. *Am. Fam. Physician*, **87** (4), 267–273.

75 Muangpaisan, W., Mathews, A., Hori, H., and Seidel, D. (2011) A systematic review of the worldwide prevalence and incidence of Parkinson's disease. *J. Med. Assoc. Thai.*, **94** (6), 749–755.

76 Pandey, S. (2012) Parkinson's disease: recent advances. *J. Assoc. Physicians India*, **60**, 30–32.

77 Jenner, P. (2003) Oxidative stress in Parkinson's disease. *Ann. Neurol.*, **53** (Suppl. 3), S26–S36.

78 Worth, P.F. (2013) How to treat Parkinson's disease in 2013. *Clin. Med.*, **13** (1), 93–96.

79 Ziemssen, T. and Reichmann, H. (2007) Non-motor dysfunction in Parkinson's disease. *Parkinsonism Relat. Disord.*, **13** (6), 323–332.

80 Schiesling, C., Kieper, N., Seidel, K., and Krüger, R. (2008) Review: familial Parkinson's disease-genetics, clinical phenotype and neuropathology in relation to the common sporadic form of the disease. *Neuropathol. Appl. Neurobiol.*, **34** (3), 255–271.

81 Chin-Chan, M., Navarro-Yepes, J., and Quintanilla-Vega, B. (2015) Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. *Front. Cell. Neurosci.*, **9**, 124.

82 Shamoto-Nagai, M., Maruyama, W., Hashizume, Y., Yoshida, M., Osawa, T., Riederer, P., and Naoi, M. (2007) In parkinsonian substantia nigra, alpha-synuclein is modified by acrolein, a lipid-peroxidation product, and accumulates in the dopamine neurons with inhibition of proteasome activity. *J. Neural Transm.*, **114** (12), 1559–1567.

83 Sanyal, J., Bandyopadhyay, S.K., Banerjee, T.K., Mukherjee, S.C., Chakraborty, D.P., Ray, B.C., and Rao, V.R. (2009) Plasma levels of lipid peroxides in patients with Parkinson's disease. *Eur. Rev. Med. Pharmacol. Sci.*, **13** (2), 129–132.

84 Dexter, D.T., Carter, C.J., Wells, F.R., Javoy-Agid, F., Agid, Y., Lees, A., Jenner, P., and Marsden, C.D. (1989) Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *J. Neurochem.*, **52** (2), 381–389.

85 Wang, X., Petrie, T.G., Liu, Y., Liu, J., Fujioka, H., and Zhu, X. (2012) Parkinson's disease-associated DJ-1 mutations impair mitochondrial dynamics and cause mitochondrial dysfunction. *J. Neurochem.*, **121** (5), 830–839.

86 Song, S., Jang, S., Park, J., Bang, S., Choi, S., Kwon, K.Y., Zhuang, X., Kim, E., and Chung, J. (2013) Characterization of PINK1 (PTEN-induced putative kinase 1) mutations associated with Parkinson disease in mammalian cells and *Drosophila*. *J. Biol. Chem.*, **288** (8), 5660–5672.

87 Lashuel, H.A., Petre, B.M., Wall, J., Simon, M., Nowak, R.J., Walz, T., and Lansbury, P.T. Jr. (2002) Alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils. *J. Mol. Biol.*, **322** (5), 1089–1102.

88 Stefanis, L. (2012) α-Synuclein in Parkinson's disease. *Cold Spring Harbor Perspect. Med.*, **2** (2), a009399–a009422.

89 Lee, J.Y., Nagano, Y., Taylor, J.P., Lim, K.L., and Yao, T.P. (2010) Disease-causing mutations in parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy. *J. Cell Biol.*, **189** (4), 671–679.

90 Li, J.Q., Tan, L., and Yu, J.T. (2014) The role of the LRRK2 gene in Parkinsonism. *Mol. Neurodegener.*, **9** (47), 1–17.

91 Hauser, D.N. and Hastings, T.G. (2013) Mitochondrial dysfunction and oxidative stress in Parkinson's disease and monogenic parkinsonism. *Neurobiol. Dis.*, **51**, 35–42.

92 Ebrahimi-Fakhari, D., Wahlster, L., and McLean, P.J. (2012) Protein degradation pathways in Parkinson's disease: curse or blessing. *Acta Neuropathol.*, **124** (2), 153–172.

93 Barnes, D. and Yaffe, K. (2011) The projected effect of risk factor reduction on Alzheimer's disease prevalence. *Lancet Neurol.*, **10** (9), 819–828.

94 Alzheimer, A. (1907) Über einen eigenartigen schweren Erkrankungsprozeβ der Hirnrincle. *Allg. Z. Psychiatr.*, **25** (64), 1134.

95 Liu, C.Y., Wang, P.N., Lin, K.N., and Liu, H.C. (2007) Behavioral and psychological symptoms in Taiwanese patients with Alzheimer's disease. *Int. Psychogeriatr.*, **19** (3), 605–613.

96 Honjo, K., Black, S.E., and Verhoeff, N.P. (2012) Alzheimer's disease, cerebrovascular disease, and the β-amyloid cascade. *Can. J. Neurol. Sci.*, **39** (6), 712–728.

97 Chouraki, V. and Seshadri, S. (2014) Genetics of Alzheimer's disease. *Adv. Genet.*, **87**, 245–294.

98 Grant, W.B., Campbell, A., Itzhaki, R.F., and Savory, J. (2002) The significance of environmental factors in the etiology of Alzheimer's disease. *J. Alzheimers Dis.*, **4** (3), 179–189.

99 Williams, T.I., Lynn, B.C., Markesbery, W.R., and Lowell, M.A. (2006) Increased levels of 4-hydroxynonenal and acrolein, neurotoxic markers of lipid peroxidation, in the brain in Mild Cognitive Impairment and early Alzheimer's disease. *Neurobiol. Aging*, **27** (8), 1094–1099.

100 Waragai, M., Yoshida, M., Mizoi, M., Saiki, R., Kashiwagi, K., Takagi, K., Arai, H., Tashiro, J., Hashimoto, M., Iwai, N., Uemura, K., and Igarashi, K. (2012) Increased protein-conjugated acrolein and amyloid-β40/42 ratio in plasma of patients with mild cognitive impairment and Alzheimer's disease. *J. Alzheimers Dis.*, **32** (1), 33–41.

101 Lovell, M.A., Ehmann, W.D., Mattson, M.P., and Markesbery, W.R. (1997) Elevated 4-hydroxynonenal in ventricular fluid in Alzheimer's disease. *Neurobiol. Aging*, **18** (5), 457–461.

102 Irizarry, M.C., Yao, Y., Hyman, B.T., Growdon, J.H., and Praticò, D. (2007) Plasma F2A isoprostane levels in Alzheimer's and Parkinson's disease. *Neurodegener. Dis.*, **4** (6), 403–405.

103 Montine, T.J., Peskind, E.R., Quinn, J.F., Wilson, A.M., Montine, K.S., and Galasko, D. (2011) Increased cerebrospinal fluid F2-isoprostanes are associated with aging and latent Alzheimer's disease as identified by biomarkers. *Neuromolecular Med.*, **13** (1), 37–43.

104 Leuner, K., Schulz, K., Schütt, T., Pantel, J., Prvulovic, D., Rhein, V., Savaskan, E., Czech, C., Eckert, A., and Müller, W.E. (2012) Peripheral mitochondrial dysfunction in Alzheimer's disease: focus on lymphocytes. *Mol. Neurobiol.*, **46** (1), 194–204.

105 Gibson, G., Martins, R., Blass, J., and Gandy, S. (1996) Peripheral mitochondrial dysfunction in Alzheimer's disease: focus on lymphocytes. *Life Sci.*, **46** (1), 194–204.

106 Kumar, A., Singh, A., and Ekavali (2014) A review on Alzheimer's disease pathophysiology and its management: an update. *Pharmacol. Rep.*, **67** (2), 195–203.

107 Zemek, F., Drtinova, L., Nepovimova, E., Sepsova, V., Korabecny, J., Klimes, J., and Kuca, K. (2014) Outcomes of Alzheimer's disease therapy with acetylcholinesterase inhibitors and memantine. *Expert Opin. Drug Saf.*, **13** (6), 759–774.

108 Agúndez, J.A., García-Martín, E., Alonso-Navarro, H., and Jiménez-Jiménez, F.J. (2013) Anti-Parkinson's disease drugs and pharmacogenetic considerations. *Expert Opin. Drug Metab. Toxicol.*, **9** (7), 859–874.

109 Sprenger, F. and Poewe, W. (2013) Management of motor and non-motor symptoms in Parkinson's disease. *CNS Drugs*, **27** (4), 259–272.

110 Danta, C.C. and Piplani, P. (2014) The discovery and development of new potential antioxidant agents for the treatment of neurodegenerative diseases. *Expert Opin. Drug Discovery*, **9** (10), 1205–1222.

111 Pisoschi, M. and Pop, A. (2015) The role of antioxidants in the chemistry of oxidative stress: a review. *Eur. J. Med. Chem.*, **97**, 55–74.

112 Guerra-Araiza, C., Álvarez-Mejía, A.L., Sánchez-Torres, S., Farfan-García, E., Mondragón-Lozano, R., Pinto-Almazán, R., and Salgado-Ceballos, H. (2013) Effect of natural exogenous antioxidants on aging and on neurodegenerative diseases. *Free Radical Res.*, **47** (6-7), 451–462.

113 Pardridge, W.M. (2005) The blood-brain barrier: bottleneck in brain drug development. *NeuroRx*, **2** (1), 3–14.

114 Adibhatla, R.M. and Hatcher, J.F. (2010) Lipid oxidation and peroxidation in CNS health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxid. Redox Signaling*, **12** (1), 125–169.

115 Yan, M.H., Wang, X., and Zhu, X. (2013) Mitochondrial defects and oxidative stress in Alzheimer disease and Parkinson disease. *Free Radical Biol. Med.*, **62**, 90–101.

116 Williams, R.J., Spencer, J.P.E., and Rice-Evans, C. (2004) Flavonoids: antioxidants or signaling molecules? *Free Radical Biol. Med.*, **36**, 838–849.

117 Karuppagounder, S.S., Madathil, S.K., Pandey, M., Haobam, R., Rajamma, U., and Mohanakumar, K.P. (2013) Quercetin up-regulates mitochondrial complex-I activity to protect against programmed cell death in rotenone model of Parkinson's disease in rats. *Neuroscience*, **236**, 136–148.

118 Bournival, J., Plouffe, M., Renaud, J., Provencher, C., and Martinoli, M.G. (2012) Quercetin and sesamin protect dopaminergic cells from MPP+-induced neuroinflammation in a microglial (N9)-neuronal (PC12) coculture system. *Oxid. Med. Cell. Longevity*, **2012**, 921941.

119 Sharma, D.R., Wani, W.Y., Sunkaria, A., Kandimalla, R.J., Verma, D., Cameotra, S.S., and Gill, K.D. (2013) Quercetin protects against chronic aluminum-induced oxidative stress and ensuing biochemical, cholinergic, and neurobehavioral impairments in rats. *Neurotox. Res.*, **23** (4), 336–357.

120 Filomeni, G., Graziani, I., de Zio, D., Dini, L., Centonze, D., Rotilio, G., and Ciriolo, M.R. (2012) Neuroprotection of kaempferol by autophagy in models of rotenone-mediated acute toxicity: possible implications for Parkinson's disease. *Neurobiol. Aging*, **33** (4), 767–785.

121 Li, S. and Pu, X.P. (2011) Neuroprotective effect of kaempferol against a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced mouse model of Parkinson's disease. *Biol. Pharm. Bull.*, **34** (8), 1291–1296.

122 Choi, Y.T., Jung, C.H., Lee, S.R., Bae, J.H., Baek, W.K., Suh, M.H., Park, J., Park, C.W., and Suh, S.I. (2001) The green tea polyphenol (−)-epigallocatechin gallate attenuates β-amyloid-induced neurotoxicity in cultured hippocampal neurons. *Life Sci.*, **70** (5), 603–614.

123 Zhang, X., Wu, M., Lu, F., Luo, N., He, Z.P., and Yang, H. (2004) Involvement of α7 nAChR signaling cascade in epigallocatechin gallate suppression of β-amyloid-induced apoptotic cortical neuronal insults. *Mol. Neurobiol.*, **49** (1), 66–77.

124 Tamilselvam, K., Braidy, N., Manivasagam, T., Essa, M.M., Prasad, N.R., Karthikeyan, S., Thenmozhi, A.J., Selvaraju, S., and Guillemin, G.J. (2013) Neuroprotective effects of hesperidin, a plant flavanone, on rotenone-induced oxidative stress and apoptosis in a cellular model for Parkinson's disease. *Oxid. Med. Cell. Longevity*, **2013**, 102741.

125 Naseem, M. and Parvez, S. (2014) Hesperidin restores experimentally induced neurotoxicity in Wistar rats. *Toxicol. Mech. Methods*, **24** (7), 512–519.

126 Tamilselvam, K., Nataraj, J., Janakiraman, U., Manivasagam, T., and Essa, M.M. (2013) Antioxidant and anti-inflammatory potential of hesperidin against 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced experimental Parkinson's disease in mice. *Int. J. Nut. Pharmacol. Neurol. Dis.*, **3** (3), 294–302.

127 Choi, R.C., Zhu, J.T., Yung, A.W., Lee, P.S., Xu, S.L., Guo, A.J., Zhu, K.Y., Dong, T.T., and Tsim, K.W. (2013) Synergistic action of flavonoids, baicalein, and daidzein in estrogenic and neuroprotective effects: a development of potential health products and therapeutic drugs against Alzheimer's disease. *Evid. Based Complement. Alternat. Med.*, **2013**, 635694.

128 Mu, X., He, G., Cheng, Y., Li, X., Xu, B., and Du, G. (2009) Baicalein exerts neuroprotective effects in 6-hydroxydopamine-induced experimental parkinsonism *in vivo* and *in vitro*. *Pharmacol. Biochem. Behav.*, **92** (4), 642–648.

129 Wang, Y.H., Yu, H.T., Pu, X.P., and Du, G.H. (2013) Baicalein prevents 6-hydroxydopamine-induced mitochondrial dysfunction in SH-SY5Y cells via inhibition of mitochondrial oxidation and up-regulation of DJ-1 protein expression. *Molecules*, **18** (12), 14726–14738.

130 Cheng, Y., He, G., Mu, X., Zhang, T., Li, X., Hu, J., Xu, B., and Du, G. (2008) Neuroprotective effect of baicalein against MPTP neurotoxicity: behavioral, biochemical and immunohistochemical profile. *Neurosci. Lett.*, **441** (1), 16–20.

131 Traber, M.G. and Atkinson, J. (2007) Vitamin E, antioxidant and nothing more. *Free Radical Biol. Med.*, **43** (1), 4–15.

132 Sano, M., Erresto, C., Thomas, R.G., Klauber, M.R., Schafer, K., Grundman, M., Woodbury, P., Growdon, J., Cotman, C.W., Pfeiffer, E., Schneider, L.S., and Thal, L.J. (1997) A controlled trial of selegiline, alpha tocopherol or both as treatment for Alzheimer's disease. *N. Engl. J. Med.*, **336** (17), 1216–1222.

133 Etminan, M., Gill, S.S., and Samii, A. (2005) Intake of vitamin E, vitamin C, and carotenoids and the risk of Parkinson's disease: a meta-analysis. *Lancet Neurol.*, **4** (6), 362–365.

134 Cadet, J.L., Katz, M., Jackson-Lewis, V., and Fahn, S. (1989) Vitamin E attenuates the toxic effects of intrastriatal injection of 6-hydroxydopamine (6-OHDA) in rats: behavioral and biochemical evidence. *Brain Res.*, **476** (1), 10–15.

135 Bist, R. and Bhatt, D.K. (2009) The evaluation of effect of alpha-lipoic acid and vitamin E on the lipid peroxidation, gamma-amino butyric acid and serotonin level in the brain of mice (*Mus musculus*) acutely intoxicated with lindane. *J. Neurol. Sci.*, **276** (1-2), 99–102.

136 Rati Selvaraju, T., Khaza'ai, H., Vidyadaran, S., Sokhini Abd Mutalib, M., Ramachandran, V., and Hamdan, Y. (2014) Cytoprotective effect of tocotrienol-rich fraction and α-tocopherol vitamin E isoforms against glutamate-induced cell death in neuronal cells. *Int. J. Vitam. Nutr. Res.*, **84** (3-4), 140–151.

137 Shea, T.B., Ortiz, D., Nicolosi, R.J., Kumar, R., and Watterson, A.C. (2005) Nanosphere-mediated delivery of vitamin E increases its efficacy against oxidative stress resulting from exposure to amyloid beta. *J. Alzheimers Dis.*, **7** (4), 297–301.

138 Oudemans-van Straaten, H.M., Spoelstra-de Man, A.M., and de Waard, M.C. (2014) Vitamin C revisited. *Crit. Care*, **18** (4), 460.

139 Medina, S., Martínez, M., and Hernanz, A. (2002) Antioxidants inhibit the human cortical neuron apoptosis induced by hydrogen peroxide, tumor necrosis factor alpha, dopamine and beta-amyloid peptide 1-42. *Free Radical Res.*, **36** (11), 1179–1184.

140 Kook, S.Y., Lee, K.M., Kim, Y., Cha, M.Y., Kang, S., Baik, S.H., Lee, H., Park, R., and Mook-Jung, I. (2014) High-dose of vitamin C supplementation reduces amyloid plaque burden and ameliorates pathological changes in the brain of 5XFAD mice. *Cell Death Dis.*, **5** (2), e1083.

141 Morris, M.C., Bechett, L.A., Scherr, P.A., Hebert, L.E., Bennett, D.A., Field, T.S., and Evans, D.A. (1998) Vitamin E and vitamin C supplement use and risk of incident Alzheimer's disease. *Alzheimer Dis. Assoc. Disord.*, **12**, 121–126.

142 Devbhuti, P., Saha, A., and Sengupta, C. (2009) Gentamicin induced lipid peroxidation and its control with ascorbic acid. *Acta Pol. Pharm.*, **66** (4), 363–369.

143 Palace, V.P., Khaper, N., Qin, Q., and Singal, P.K. (1999) Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. *Free Radical Biol. Med.*, **26** (5-6), 746–761.

144 Obulesu, M., Dowlathabad, M.R., and Bramhachari, P.V. (2011) Carotenoids and Alzheimer's disease: an insight into therapeutic role of retinoids in animal models. *Neurochem. Int.*, **59** (5), 535–541.

145 Patmanatha, N.D. (1989) Effects of vitamin A and its analogs on nonenzymatic lipid peroxidation in rat brain mitochondria. *J. Neurochem.*, **52** (2), 585–588.

146 Di Mascio, P., Kaiser, S., and Sies, H. (1989) Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.*, **274** (2), 532–538.

147 Sandhir, R., Mehrotra, A., and Kamboj, S.S. (2010) Lycopene prevents 3-nitropropionic acid-induced mitochondrial oxidative stress and dysfunctions in nervous system. *Neurochem. Int.*, **57** (5), 579–587.

148 Qu, M., Zhou, Z., Chen, C., Li, M., Pei, L., Chu, F., Yang, J., Wang, Y., Li, L., Liu, C., Zhang, L., Zhang, G., Yu, Z., and Wang, D. (2011) Lycopene protects against trimethyltin-induced neurotoxicity in primary cultured rat hippocampal neurons by inhibiting the mitochondrial apoptotic pathway. *Neurochem. Int.*, **59** (8), 1095–1103.

149 Yi, F., He, X., and Wang, D. (2013) Lycopene protects against MPP+-induced cytotoxicity by maintaining mitochondrial function in SH-SY5Y cells. *Neurochem. Res.*, **38** (8), 1747–1757.

150 Kaur, H., Chauhan, S., and Sandhir, R. (2011) Protective effect of lycopene on oxidative stress and cognitive decline in rotenone induced model of Parkinson's disease. *Neurochem. Res.*, **36** (8), 1435–1443.

151 Qu, M., Li, L., Chen, C., Li, M., Pei, L., Chu, F., Yang, J., Yu, Z., Wang, D., and Zhou, Z. (2011) Protective effects of lycopene against amyloid β-induced neurotoxicity in cultured rat cortical neurons. *Neurosci. Lett.*, **505** (3), 286–290.

152 Ochiai, T., Ohno, S., Soeda, S., Tanaka, H., Shoyama, Y., and Shimeno, H. (2004) Crocin prevents the death of rat pheochromocytoma (PC-12) cells by its antioxidant effects stronger than those of alpha-tocopherol. *Neurosci. Lett.*, **362** (1), 61–64.

153 Mehri, S., Abnous, K., Mousavi, S.H., Shariaty, V.M., and Hosseinzadeh, H. (2012) Neuroprotective effect of crocin on acrylamide-induced cytotoxicity in PC12 cells. *Cell. Mol. Neurobiol.*, **32** (2), 227–235.

154 Tamaddonfard, E., Farshid, A.A., Asri-Rezaee, S., Javadi, S., Khosravi, V., Rahman, B., and Mirfakhraee, Z. (2013) Crocin improved learning and

memory impairments in streptozotocin-induced diabetic rats. *Iran J. Basic Med. Sci.*, **16** (1), 91–100.

155 Naghizadeh, B., Mansouri, M.T., Ghorbanzadeh, B., Farbood, Y., and Sarkaki, A. (2013) Protective effects of oral crocin against intracerebroventricular streptozotocin-induced spatial memory deficit and oxidative stress in rats. *Phytomedicine*, **20** (6), 537–542.

156 Ahmad, A.S., Ansari, M.A., Ahmad, M., Saleem, S., Yousuf, S., Hoda, M.N., and Islam, F. (2005) Neuroprotection by crocetin in a hemi-parkinsonian rat model. *Pharmacol. Biochem. Behav.*, **81** (4), 805–813.

157 Ikeda, Y., Tsuji, S., Satoh, A., Ishikura, M., Shirasawa, T., and Shimizu, T. (2008) Protective effects of astaxanthin on 6-hydroxydopamine-induced apoptosis in human neuroblastoma SH-SY5Y cells. *J. Neurochem.*, **107** (6), 1730–1740.

158 Lee, D.H., Kim, C.S., and Lee, Y.J. (2011) Astaxanthin protects against MPTP/MPP+-induced mitochondrial dysfunction and ROS production *in vivo* and *in vitro*. *Food Chem. Toxicol.*, **49** (1), 271–280.

159 Ye, Q., Huang, B., Zhang, X., Zhu, Y., and Chen, X. (2012) Astaxanthin protects against MPP($^{+}$)-induced oxidative stress in PC12 cells via the HO-1/NOX2 axis. *BMC Neurosci.*, **13**, 156.

160 Chang, C.S., Chang, C.L., and Lai, G.H. (2013) Reactive oxygen species scavenging activities in a chemiluminescence model and neuroprotection in rat pheochromocytoma cells by astaxanthin, beta-carotene, and canthaxanthin. *Kaohsiung J. Med. Sci.*, **29** (8), 412–421.

161 Qiu, S., Sun, H., Zhang, A.H., Xu, H.Y., Yan, G.L., Han, Y., and Wang, X.J. (2014) Natural alkaloids: basic aspects, biological roles, and future perspectives. *Chin. J. Nat. Med.*, **12** (6), 401–406.

162 Ng, Y.P., Or, T.C., and Ip, N.Y. (2015) Plant alkaloids as drug leads for Alzheimer's disease. *Neurochem. Int.*, **89**, 260–270.

163 Haghani, M., Shabani, M., and Tondar, M. (2015) The therapeutic potential of berberine against the altered intrinsic properties of the CA1 neurons induced by Aβ neurotoxicity. *Eur. J. Pharmacol.*, **758**, 82–88.

164 Abd El-Wahab, A.E., Ghareeb, D.A., Sarhan, E.E., Abu-Serie, M.M., and El Demellawy, M.A. (2013) *In vitro* biological assessment of *Berberis vulgaris* and its active constituent, berberine: antioxidants, anti-acetylcholinesterase, anti-diabetic and anticancer effects. *BMC Complement. Altern. Med.*, **13**, 218.

165 Zhang, J., Yang, J.Q., He, B.C., Zhou, Q.X., Yu, H.R., Tang, Y., and Liu, B.Z. (2009) Berberine and total base from rhizoma coptis chinensis attenuate brain injury in an aluminum-induced rat model of neurodegenerative disease. *Saudi Med. J.*, **30** (6), 760–766.

166 Bae, J., Lee, D., Kim, Y.K., Gil, M., Lee, J.Y., and Lee, K.J. (2013) Berberine protects 6-hydroxydopamine-induced human dopaminergic neuronal cell death through the induction of heme oxygenase-1. *Mol. Cells*, **35** (2), 151–157.

167 Chernyak, B.V., Antonenko, Y.N., Galimov, E.R., Domnina, L.V., Dugina, V.B., Zvyagilskaya, R.A., Ivanova, O.Y., Izyumov, D.S., Lyamzaev, K.G., Pustovidko, A.V., Rokitskaya, T.I., Rogov, A.G., Severina, I.I., Simonyan, R.A., Skulachev, M.V., Tashlitsky, V.N., Titova, E.V., Trendeleva, T.A., and Shagieva, G.S. (2012)

Novel mitochondria-targeted compounds composed of natural constituents: conjugates of plant alkaloids berberine and palmatine with plastoquinone. *Biochemistry (Moscow)*, **77** (9), 983–995.

168 Shang, Y.Z., Ye, J.W., and Tang, X.C. (1999) Improving effects of huperzine A on abnormal lipid peroxidation and superoxide dismutase in aged rats. *Acta Pharmacol. Sin.*, **20**, 824–828.

169 Zhang, Z., Wang, X., Chen, Q., Shu, L., Wang, J., and Shan, G. (2002) Clinical efficacy and safety of huperzine Alpha in treatment of mild to moderate Alzheimer disease, a placebo-controlled, double-blind, randomized trial. *Zhonghua Yi Xue Za Zhi*, **82** (14), 941–944.

170 Wang, R. and Tang, X.C. (2005) Neuroprotective effects of huperzine A. A natural cholinesterase inhibitor for the treatment of Alzheimer's disease. *Neurosignals*, **14** (1-2), 71–82.

171 Yang, L., Ye, C.Y., Huang, X.T., Tang, X.C., and Zhang, H.Y. (2012) Decreased accumulation of subcellular amyloid-β with improved mitochondrial function mediates the neuroprotective effect of huperzine A. *J. Alzheimers Dis.*, **31** (1), 131–142.

172 Ratia, M., Giménez-Llort, L., Camps, P., Muñoz-Torrero, D., Pérez, B., Clos, M.V., and Badia, A. (2013) Huprine X and huperzine A improve cognition and regulate some neurochemical processes related with Alzheimer's disease in triple transgenic mice (3xTg-AD). *Neurodegener. Dis.*, **11** (3), 129–140.

173 Zhang, H.Y. and Tang, X.C. (2000) Huperzine B, a novel acetylcholinesterase inhibitor, attenuates hydrogen peroxide induced injury in PC12 cells. *Neurosci. Lett.*, **292** (1), 41–44.

174 Maia, L. and de Mendonça, A. (2002) Does caffeine intake protect from Alzheimer's disease? *Eur. J. Neurol.*, **9** (4), 377–382.

175 Dragicevic, N., Delic, V., Cao, C., Copes, N., Lin, X., Mamcarz, M., Wang, L., Arendash, G.W., and Bradshaw, P.C. (2012) Caffeine increases mitochondrial function and blocks melatonin signaling to mitochondria in Alzheimer's mice and cells. *Neuropharmacology*, **63** (8), 1368–1379.

176 Chu, Y.F., Chang, W.H., Black, R.M., Liu, J.R., Sompol, P., Chen, Y., Wei, H., Zhao, Q., and Cheng, I.H. (2012) Crude caffeine reduces memory impairment and amyloid β 1–42 levels in an Alzheimer's mouse model. *Food Chem.*, **135** (3), 2095–2102.

177 Prediger, R.D. (2010) Effects of caffeine in Parkinson's disease: from neuroprotection to the management of motor and non-motor symptoms. *J. Alzheimers Dis.*, **20** (1), S205–S220.

178 Kalia, L.V., Kalia, S.K., and Lang, A.E. (2015) Disease-modifying strategies for Parkinson's disease. *Mov. Disord.*, **30** (11), 1442–1450.

179 Nobre, H.V. Jr., Cunha, G.M., de Vasconcelos, L.M., Magalhães, H.I., Oliveira Neto, R.N., Maia, F.D., de Moraes, M.O., Leal, L.K., and Viana, G.S. (2010) Caffeine and CSC, adenosine A2A antagonists, offer neuroprotection against 6-OHDA-induced neurotoxicity in rat mesencephalic cells. *Neurochem. Int.*, **56** (1), 51–58.

180 Kim, Y.S., Kwak, S.M., and Myung, S.K. (2015) Caffeine intake from coffee or tea and cognitive disorders: a meta-analysis of observational studies. *Neuroepidemiology*, **44** (1), 51–63.

181 Chonpathompikunlert, P., Wattanathorn, J., and Muchimapura, S. (2010) Piperine, the main alkaloid of Thai black pepper, protects against neurodegeneration and cognitive impairment in animal model of cognitive deficit like condition of Alzheimer's disease. *Food Chem. Toxicol.*, **48** (3), 798–802.

182 Shrivastava, P., Vaibhav, K., Tabassum, R., Khan, A., Ishrat, T., Khan, M.M., Ahmad, A., Islam, F., Safhi, M.M., and Islam, F. (2013) Anti-apoptotic and anti-inflammatory effect of Piperine on 6-OHDA induced Parkinson's rat model. *J. Nutr. Biochem.*, **24** (4), 680–687.

183 Baur, J.A. and Sinclair, D.A. (2006) Therapeutic potential of resveratrol: the *in vivo* evidence. *Nat. Rev. Drug Discovery*, **5** (6), 493–506.

184 Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P., Elliott, P., Geny, B., Laakso, M., Puigserver, P., and Auwerx, J. (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell*, **127** (6), 1109–1122.

185 Manczak, M., Mao, P., Calkins, M.J., Cornea, A., Reddy, A.P., Murphy, M.P., Szeto, H.H., Park, B., and Reddy, H.H. (2010) Mitochondria-targeted antioxidants protect against amyloid-beta toxicity in Alzheimer's disease neurons. *J. Alzheimers Dis.*, **20** (2), S609–S631.

186 Lu, X., Xu, H., Sun, B., Zhu, Z., Zheng, D., and Li, X. (2013) Enhanced neuroprotective effects of resveratrol delivered by nanoparticles on hydrogen peroxide-induced oxidative stress in rat cortical cell culture. *Mol. Pharm.*, **10** (5), 2045–2053.

187 Ferretta, A., Gaballo, A., Tanzarella, P., Piccoli, C., Capitanio, N., Nico, B., Annese, T., Di Paola, M., Dell'aquila, C., De Mari, M., Ferranini, E., Bonifati, V., Pacelli, C., and Cocco, T. (2014) Effect of resveratrol on mitochondrial function: implications in parkin-associated familiar Parkinson's disease. *Biochim. Biophys. Acta*, **1842** (7), 902–915.

188 Esatbeyoglu, T., Huebbe, P., Ernst, I.M., Chin, D., Wagner, A.E., and Rimbach, G. (2012) Curcumin – from molecule to biological function. *Angew. Chem. Int. Ed.*, **51** (22), 5308–5332.

189 Pluta, R., Bogucka-Kocka, A., Ułamek-Kozioł, M., Furmaga-Jabłońska, W., Januszewski, S., Brzozowska, J., Jabłoński, M., and Kocki, J. (2015) Neurogenesis and neuroprotection in postischemic brain neurodegeneration with Alzheimer phenotype: is there a role for curcumin? *Folia Neuropathol.*, **53** (2), 89–99.

190 Ishrat, T., Hoda, M.N., Khan, M.B., Yousuf, S., Ahmad, M., Khan, M.M., Ahmad, A., and Islam, F. (2009) Amelioration of cognitive deficits and neurodegeneration by curcumin in rat model of sporadic dementia of Alzheimer's type (SDAT). *Eur. Neuropsychopharmacol.*, **19**, 636–647.

191 Jagatha, B., Mythri, R.B., Vali, S., and Bharath, M.M.S. (2008) Curcumin treatment alleviates the effects of glutathione depletion *in vitro* and *in vivo*: therapeutic implications for Parkinson's disease explained via *in silico* studies. *Free Radical Biol. Med.*, **44** (5), 907–917.

192 Liu, Z., Yu, Y., Li, X., Ross, C.A., and Smith, W.W. (2011) Curcumin protects against A53T alpha-synuclein-induced toxicity in a PC12 inducible cell model for Parkinsonism. *Pharmacol. Res.*, **63** (5), 439–444.

193 Mythri, R.B., Harish, G., Dubey, S.K., Misra, K., and Srinivas Bharath, M.M. (2011) Glutamoyl diester of the dietary polyphenol curcumin offers improved protection against peroxynitrite-mediated nitrosative stress and damage of brain mitochondria *in vitro*: implications for Parkinson's disease. *Mol. Cell. Biochem.*, **347** (1-2), 135–143.

194 Jayaraj, R.L., Tamilselvam, K., Manivasagam, T., and Elangovan, N. (2013) Neuroprotective effect of CNB-001, a novel pyrazole derivative of curcumin on biochemical and apoptotic markers against rotenone-induced SK-N-SH cellular model of Parkinson's disease. *J. Mol. Neurosci.*, **51** (3), 863–870.

195 Jayaraj, R.L., Elangovan, N., Manigandan, K., Singh, S., and Shukla, S. (2014) CNB-001 a novel curcumin derivative, guards dopamine neurons in MPTP model of Parkinson's disease. *Biomed. Res. Int.*, **2014**, 236182.

196 Kumar, P., Padi, S.S., Naidu, P.S., and Kumar, A. (2007) Possible neuroprotective mechanisms of curcumin in attenuating 3-nitropropionic acid-induced neurotoxicity. *Methods Find. Exp. Clin. Pharmacol.*, **29** (1), 19–25.

197 Alam, S., Panda, J.J., and Chauhan, V.S. (2012) Novel dipeptide nanoparticles for effective curcumin delivery. *Int. J. Nanomed.*, **7**, 4207–4221.

198 Ansari, N. and Khodagholi, F. (2013) Natural products as promising drug candidates for the treatment of Alzheimer's disease: molecular mechanism aspect. *Curr. Neuropharmacol.*, **11** (4), 414–429.

199 Alkam, T., Nitta, A., Mizoguchi, H., Itoh, A., and Nabeshima, T. (2007) A natural scavenger of peroxynitrites, rosmarinic acid, protects against impairment of memory induced by Abeta(25-35). *Behav. Brain Res.*, **180** (2), 139–145.

200 Iuvone, T., De Filippis, D., Esposito, G., D'Amico, A., and Izzo, A.A. (2006) The spice sage and its active ingredient rosmarinic acid protect PC12 cells from amyloid-beta peptide-induced neurotoxicity. *J. Pharmacol. Exp. Ther.*, **317** (3), 1143–1149.

201 Lee, H.J., Cho, H.S., Park, E., Kim, S., Lee, S.Y., Kim, C.S., Kim do, K., Kim, S.J., and Chun, H.S. (2008) Rosmarinic acid protects human dopaminergic neuronal cells against hydrogen peroxide-induced apoptosis. *Toxicology*, **250** (2-3), 109–115.

202 Ren, P., Jiang, H., Li, R., Wang, J., Song, N., Xu, H.M., and Xie, J.X. (2009) Rosmarinic acid inhibits 6-OHDA-induced neurotoxicity by anti-oxidation in MES23.5 cells. *J. Mol. Neurosci.*, **39** (1-2), 220–225.

203 Wang, J., Xu, H., Jiang, H., Du, X., Sun, P., and Xie, J. (2012) Neurorescue effect of rosmarinic acid on 6-hydroxydopamine-lesioned nigral dopamine neurons in rat model of Parkinson's disease. *J. Mol. Neurosci.*, **47** (1), 113–119.

204 Sul, D., Kim, H.S., Lee, D., Joo, S.S., Hwang, K.W., and Park, S.Y. (2009) Protective effect of caffeic acid against beta-amyloid-induced neurotoxicity by the inhibition of calcium influx and tau phosphorylation. *Life Sci.*, **84** (9-10), 257–262.

205 Jeong, C.H., Jeong, H.R., Choi, G.N., Kim, D.O., Lee, U., and Heo, H.J. (2011) Neuroprotective and anti-oxidant effects of caffeic acid isolated from *Erigeron annuus* leaf. *Chin. Med.*, **6**, 25.

206 Khan, K.A., Kumar, N., Nayak, P.G., Nampoothiri, M., Shenoy, R.R., Krishnadas, N., Rao, C.M., and Mudgal, J. (2013) Impact of caffeic acid on

aluminium chloride-induced dementia in rats. *J. Pharm. Pharmacol.*, **65** (12), 1745–1752.

207 Silva, T., Oliveira, C., and Borges, F. (2014) Caffeic acid derivatives, analogs and applications: a patent review (2009-2013). *Expert Opin. Ther. Pat.*, **24** (11), 1257–1270.

208 Noelker, C., Bacher, M., Gocke, P., Wei, X., Klockgether, T., Du, Y., and Dodel, R. (2005) The flavonoid caffeic acid phenethyl ester blocks 6-hydroxydopamine-induced neurotoxicity. *Neurosci. Lett.*, **383** (1-2), 39–43.

209 Barros Silva, R., Santos, N.A.G., Martins, N.M., Ferreira, D.A., Barbosa, F. Jr., Oliveira Souza, V.C., Kinoshita, A., Baffa, O., Del-Bel, E., and Santos, A.C. (2013) Caffeic acid phenethyl ester protects against the dopaminergic neuronal loss induced by 6-hydroxydopamine in rats. *Neuroscience*, **233**, 86–94.

210 Daglia, M., Di Lorenzo, A., Nabavi, S.F., Talas, Z.S., and Nabavi, S.M. (2014) Polyphenols: well beyond the antioxidant capacity: gallic acid and related compounds as neuroprotective agents: you are what you eat!. *Curr. Pharm. Biotechnol.*, **15** (4), 362–372.

211 Mansouri, M.T., Farbood, Y., Sameri, M.J., Sarkaki, A., Naghizadeh, B., and Rafeirad, M. (2012) Neuroprotective effects of oral gallic acid against oxidative stress induced by 6-hydroxydopamine in rats. *Food Chem.*, **138** (2-3), 1028–1033.

212 Sun, J., Li, Y.Z., Ding, Y.H., Wang, J., Geng, J., Yang, H., Ren, J., Tang, J.Y., and Gao, J. (2014) Neuroprotective effects of gallic acid against hypoxia/reoxygenation-induced mitochondrial dysfunctions *in vitro* and cerebral ischemia/reperfusion injury *in vivo*. *Brain Res.*, **1589**, 126–139.

213 Chang, H.J., Kim, H.J., and Chun, H.S. (2007) Quantitative structure-activity relationship (QSAR) for neuroprotective activity of terpenoids. *Life Sci.*, **80** (9), 835–841.

214 Lu, X.L., Yao, X.L., Liu, Z., Zhang, H., Li, W., Li, Z., Wang, G.L., Pang, J., Lin, Y., Xu, Z., Chen, L., Pei, Z., and Zeng, J. (2010) Protective effects of xyloketal B against MPP+-induced neurotoxicity in *Caenorhabditis elegans* and PC12 cells. *Brain Res.*, **1332**, 110–119.

215 Defeudis, F.V. (2002) Bilobalide and neuroprotection. *Pharmacol. Res.*, **46** (6), 565–568.

216 Shi, C., Zou, J., Li, G., Ge, Z., Yao, Z., and Xu, J. (2011) Bilobalide protects mitochondrial function in ovariectomized rats by up-regulation of mRNA and protein expression of cytochrome c oxidase subunit I. *J. Mol. Neurosci.*, **45** (2), 69–75.

217 Zhou, L.J. and Zhu, X.Z. (2000) Reactive oxygen species-induced apoptosis in PC12 cells and protective effect of bilobalide. *J. Pharmacol. Exp. Ther.*, **293** (3), 982–988.

218 Meng, X., Wang, M., Sun, G., Ye, J., Zhou, Y., Dong, X., Wang, T., Lu, S., and Sun, X. (2014) Attenuation of Aβ25-35-induced parallel autophagic and apoptotic cell death by gypenoside XVII through the estrogen receptor-dependent activation of Nrf2/ARE pathways. *Toxicol. Appl. Pharmacol.*, **279** (1), 63–75.

219 Ong, W.Y., Farooqui, T., Koh, H.L., Farooqui, A.A., and Ling, E.A. (2015) Protective effects of ginseng on neurological disorders. *Front. Aging Neurosci.*, **7**, 129.

220 Zhou, Y.Z., Chen, H., Qiao, L., Xu, N., Cao, J.Q., and Pei, Y.H. (2008) Two new compounds from *Carthamus tinctorius*. *J. Asian Nat. Prod. Res.*, **10** (5-6), 429–433.

221 Nam, Y., Wie, M.B., Shin, E.J., Nguyen, T.T., Nah, S.Y., Ko, S.K., Jeong, J.H., Jang, C.G., and Kim, H.C. (2015) Ginsenoside Re protects methamphetamine-induced mitochondrial burdens and proapoptosis via genetic inhibition of protein kinase C δ in human neuroblastoma dopaminergic SH-SY5Y cell lines. *J. Appl. Toxicol.*, **35** (8), 927–944.

222 González-Burgos, E., Carretero, M.E., and Gómez-Serranillos, M.P. (2013) *In vitro* permeability study of CNS-active diterpenes from *Sideritis* spp. using cellular models of blood-brain barrier. *Planta Med.*, **79** (16), 1545–1551.

223 González-Burgos, E., Carretero, M.E., and Gómez-Serranillos, M.P. (2013) Kaurane diterpenes from *Sideritis* spp. exert a cytoprotective effect against oxidative injury that is associated with modulation of the Nrf2 system. *Phytochemistry*, **93**, 116–123.

224 Wang, H., Xu, Y., Yan, J., Zhao, X., Sun, X., Zhang, Y., Guo, J., and Zhu, C. (2009) Acteoside protects human neuroblastoma SH-SY5Y cells against beta-amyloid-induced cell injury. *Brain Res.*, **1283**, 139–147.

225 Sun, K., Cao, S., Pei, L., Matsuura, A., Xiang, L., and Qi, J. (2013) A steroidal saponin from *Ophiopogon japonicus* extends the lifespan of yeast via the pathway involved in SOD and UTH1. *Int. J. Mol. Sci.*, **14** (3), 4461–4475.

226 Kairane, C., Mahlapuu, R., Ehrlich, K., Zilmer, M., and Soomets, U. (2014) The effects of different antioxidants on the activity of cerebrocortical MnSOD and Na,K-ATPase from post mortem Alzheimer's disease and age-matched normal brains. *Curr. Alzheimer Res.*, **11** (1), 79–85.

227 Li, J., Wang, G., Liu, J., Zhou, L., Dong, M., Wang, R., Li, X., Li, X., Lin, C., and Niu, Y. (2010) Puerarin attenuates amyloid-beta-induced cognitive impairment through suppression of apoptosis in rat hippocampus *in vivo*. *Eur. J. Pharmacol.*, **649** (1–3), 195–201.

228 Zhang, H., Liu, Y., Lao, M., Ma, Z., and Yi, X. (2011) Puerarin protects Alzheimer's disease neuronal cybrids from oxidant-stress induced apoptosis by inhibiting pro-death signaling pathways. *Exp. Gerontol.*, **46** (1), 30–37.

229 Aruoma, O.I., Bahorun, T., and Jen, L.S. (2003) Neuroprotection by bioactive components in medicinal and food plant extracts. *Mutat. Res.*, **544** (2–3), 203–215.

230 Kim, S.T., Kim, J.D., Lyu, Y.S., Lee, M.Y., and Kang, H.W. (2006) Neuroprotective effect of some plant extracts in cultured CT105-induced PC12 cells. *Biol. Pharm. Bull.*, **29** (10), 2021–2024.

231 Tian, X., Zhang, L., Wang, J., Dai, J., Shen, S., Yang, L., and Huang, P. (2012) The protective effect of hyperbaric oxygen and *Ginkgo biloba* extract on Aβ25-35-induced oxidative stress and neuronal apoptosis in rats. *Behav. Brain Res.*, **242**, 1–8.

232 Jelenković, A., Jovanović, M.D., Stevanović, I., Petronijević, N., Bokonjić, D., Zivković, J., and Igić, R. (2014) Influence of the green tea leaf extract on neurotoxicity of aluminium chloride in rats. *Phytother. Res.*, **28** (1), 82–87.

233 Cho, J., Kong, J.Y., Jeong, D.Y., Lee, K.D., Lee, D.U., and Kang, B.S. (2001) NMDA receptor-mediated neuroprotection by essential oils from the rhizomes of *Acorus gramineus*. *Life Sci.*, **68** (13), 1567–1573.

234 Dobetsberger, C. and Buchbauer, G. (2011) Actions of essential oils on the central nervous system: an updated review. *Flavour Fragrance J.*, **26** (5), 300–316.

235 Cioanca, O., Hritcu, L., Mihasan, M., and Hancianu, M. (2013) Cognitive-enhancing and antioxidant activities of inhaled coriander volatile oil in amyloid β(1-42) rat model of Alzheimer's disease. *Physiol. Behav.*, **120**, 193–202.

236 Hancianu, M., Cioanca, O., Mihasan, M., and Hritcu, L. (2013) Neuroprotective effects of inhaled lavender oil on scopolamine-induced dementia via anti-oxidative activities in rats. *Phytomedicine*, **20** (5), 446–452.

237 Fernández-Moriano, C., González-Burgos, E., and Gómez-Serranillos, M.P. (2015) Mitochondria-targeted protective compounds in Parkinson's and Alzheimer's diseases. *Oxid. Med. Cell. Longevity*, **2015**, 408927.

238 Gilgun-Sherki, Y., Melamed, E., and Offen, D. (2001) Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier. *Neuropharmacology*, **40** (8), 959–975.

239 Kelsey, N.A., Wilkins, H.M., and Linseman, D.A. (2010) Nutraceutical antioxidants as novel neuroprotective agents. *Molecules*, **15** (11), 7792–7814.

7

Marine-Derived Anti-Alzheimer's Agents of Promise

Kapil Dev and Rakesh Maurya

Medicinal and Process Chemistry Division and Academy of Scientific and Innovative Research, CSIR-Central Drug Research Institute, Sector 10, Jankipuram Extension, Sitapur Road, Lucknow-226031, India

7.1 Introduction

The ocean occupies nearly 70% of the Earth's surface and possesses approximately 80% of all living species [1]. In fact, some part of the sea such as coral reefs has more biodiversity than that of tropical rainforests [1–3]. A study on the diversity, distribution, and abundance of marine life was assessed by about 2700 scientists from more than 80 countries and discovered more than 6000 potentially novel species [4]. Mostly unexploited sources of potential secondary metabolites have been found from marine sources with promising pharmacological activities and novel pharmacophores due to the diversity of marine habitats and environmental conditions (nutrient availability, sunlight density, and salinity levels) [5]. The saline water from oceans provided several halogenated molecules with promising medicinal activities [5]. More than 20 000 natural products have been isolated so far from different marine sources such as sponges, ascidians, aplysia, algae, corals, bryozoa, worms, sea squirts, sea horses, sea cucumbers, fish species, and microorganisms [6]. The chemical investigations of these marine sources yielded unusual, novel, and different classes of compounds such as alkaloids, terpenoids, steroids, polypeptides, polyethers, macrolides, and polysaccharides [7–9]. A number of drug candidates from marine sources have been approved by the Food and Drug Administration (FDA) to treat several diseases [10–12].

Alzheimer's disease (AD) is a progressive neurodegenerative disease and characterized by a progressive deterioration of memory and higher cortical functions [13]. Approximately, 48 million people worldwide are affected by AD as of 2015 [14]. Originally, this disease was first identified and characterized by German psychiatrist Alois Alzheimer in 1906 [15, 16]. The causes of this disease are not clearly understood. The pathogenesis of AD involved approximately 70% genetics and some other causes such as head injuries, hypertension, and depressions. The characteristic pathology of AD includes extracellular deposition of beta-amyloid (Aβ) in senile plaques [17] and the intracellular formation of neurofibrillary tangles with hyperphosphorylated *tau* proteins [18]. The loss of neuronal

Neuroprotective Natural Products: Clinical Aspects and Mode of Action, First Edition.
Edited by Goutam Brahmachari.

synapses and pyramidal neurons at the presynaptic and postsynaptic terminals of the receptors is also a major hallmark of AD [19, 20]. Some compounds that show interventions with these pathological targets have now been launched in the market to modify the progress or cure to AD [21].

As a consequence of new drug discovery for AD, several natural products have been screened, and many of them (including marine natural products) or their synthetic derivatives are in advance preclinical studies [10–12].

In this book chapter, anti-AD therapeutic potentials of a wide array of marine natural products are described as per their pharmacological targets. Marine natural products under clinical trials are also considered herein along with their mode of actions. In addition, certain plant extracts showing efficacy against AD with specific pharmacological targets are described in this chapter.

7.2 Identification of Potent Anti-Alzheimer's Agents from Marine Sources

AD is a progressive multifarious neurodegenerative disorder of the central nervous system (CNS) and the leading cause of dementia in the late stage of life. Pathologically, it is characterized by the aggregation and abnormal deposition of two proteins, extracellular amyloid β-protein (Aβ) and intracellular neurofibrillary tangles known as *tau protein* and also progressive loss of neurons. Several marine natural products have been found to be effective in the experimental models of AD. The therapeutic potential agents from marine sources are categorized according to their therapeutic targets for AD.

7.2.1 Cholinergic Hypothesis in Treatment of Alzheimer's Disease

The cholinergic hypothesis mainly deals with the neurotransmitter acetylcholine (ACh). It has been proposed that patients with AD are associated with reduced availability of ACh at synapse [22]. Hence, the most and the oldest therapeutics available for AD belong to cholinergic hypothesis [23]. The therapeutics directed toward the cholinergic hypothesis are involved in activating nicotinic acetylcholinesterase (AChE) receptors directly or by inhibition of cholinesterase enzymes.

7.2.1.1 Cholinesterase Inhibitors

ACh is a neurotransmitter released by nerve cells to transmit signals to other cells in order to activate muscles. AChE and butyrylcholinesterase (BuChE) are enzymes present in the CNS to catalyze the hydrolysis of the ACh to choline because continuous stimulation of the muscles, glands, and CNS by ACh may cause fatal convulsions. Patients with AD have shown a reduced availability of ACh. Thus, AChE inhibitors are used as drug to combat some AD-related symptoms and hence improve the cognitive functions through enhanced activation of synapses from very shortage of neurotransmitter [24]. Galantamine and rivastigmine, two naturally derived compounds, are clinically available as AChE inhibitors. In the recent past, extensive research has been carried out in search of identification of other new, selective, and potent AChE inhibitors from different

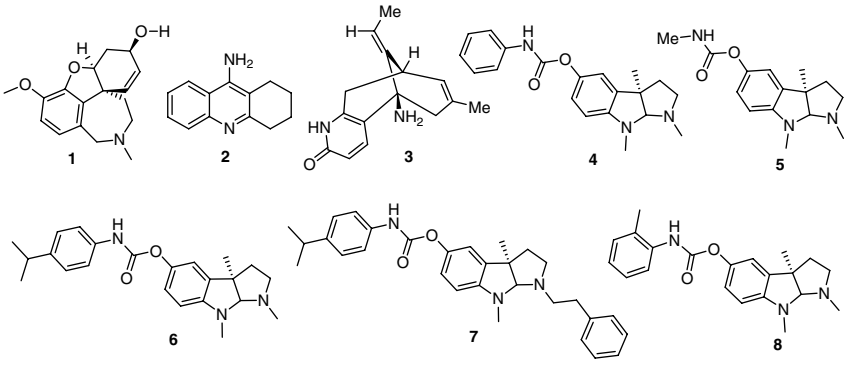


Figure 7.1 Clinically approved anti-Alzheimer's disease agents (**1–8**).

natural sources. Similarly, numerous AChE inhibitors have been isolated from different marine organisms.

Clinically approved drug candidates such as galantamine (**1**), tacrine (**2**), huperzine A (**3**), phenserine (**4**), physostigmine (**5**), cymserine (**6**), *N*-norcymserine (**7**), and tolserine (**8**) (Figure 7.1) belong to alkaloid class of compounds and act as cholinesterase inhibitors. These drug candidates are obtained either directly from natural products or derived from them. This fact has inspired scientists to screen several other new scaffolds in search of new leads for AD.

Two pentacyclic pyridoacridine alkaloids, namely, petrosamine (**9**) and 2-bromoamphimedine (**10**) isolated from a Thai marine sponge, *Petrosia* n. sp., were evaluated by the modified Ellman method for their AChE inhibitory activity with IC_{50} 0.091 and 300 μM, respectively, as compared with clinically approved drug galantamine (IC_{50} 0.590 μM). Compound **9** showed about six times more potency than galantamine, whereas compound **10** possesses very weak activity against AChE enzyme [25]. Two interesting farnesyl acetone derivatives (sesquiterpene), (5*E*, 10*Z*)-6,10,14-trimethylpentadeca-5,10-dien-2,12-dione (**11**) and (5*E*, 9*E*, 13*E*)-6,10,4-trimethyl-pentadeca-5,9,13-trien-2,12-dione (**12**), were isolated from moderate biologically active methanolic extract of the Korean brown alga *Sargassum sagamianum.* Compounds **11** and **12** were also evaluated to have moderate AChE (IC_{50} 65.0 and 48.0 μM, respectively) and BuChE (IC_{50} 34.0 and 23.0 μM, respectively) inhibitory activities [26]. Five new xyloketals, A–E, were isolated from the ethyl acetate extract of the mangrove fungus *Xylaria* sp. from the South China Sea coast [27]. Xyloketals A (**13**), B (**14**), C (**15**), D (**16**), and E (**17**) showed AChE inhibitor activity in dose-dependent manner, and their IC_{50} values were determined as 1.5, 29.9, 137.4, 109.3, and 425.6 μmol l^{-1}, respectively [27, 28]. A new stigmane-type alkaloid, 4-acetoxy-plakinamine B (**18**), isolated from Thai sponge *Corticium* sp., was found to inhibit AChE activity with IC_{50} value of 3.75 μM in a reversible manner [29]. This was the first marine steroid alkaloid as well as non-pregnane alkaloid compound that displayed AChE inhibitor activity. *Ecklonia stolonifera* exhibited potency against both AChE and BuChE enzymes in the screening assay of 27 Korean seaweeds. Furthermore, two sterols (viz., fucosterol (**19**) and 24-hydroperoxy-24-vinylcholesterol (**20**)) and eight phlorotannins (viz., phloroglucinol (**21**), eckstolonol (**22**), eckol (**23**),

phlorofucofuroeckol A (**24**), dieckol (**25**), triphlorethol-A (**26**), 2-phloroeckol (**27**), and 7-phloroeckol (**28**)) were isolated from the biologically active *n*-hexane and ethyl acetate soluble fractions of *E. stolonifera*, respectively. Compounds **22–25**, **27**, and **28** were found to exhibit AChE enzyme inhibitory activity with IC_{50} values of 42.66, 20.56, 4.89, 17.11, 38.13, and 21.11 μM, respectively, whereas both steroids **19** and **20** and two phlorotannins **22** and **24** showed inhibitory activity against BuChE enzyme with IC_{50} values of 421.72, 176.46, 230.27, and 136.71 μM, respectively. Compounds **21** and **26** were found inactive against both AChE and BuChE enzymes. A new pyrroloquinoline alkaloid, marinoquinoline A (**29**), along with two known and related pyrrole derivatives, 3-(2′-aminophenyl) pyrrole (**30**) and 2,2-dimethyl-pyrrolo-1,2-dihydroquinoline (**31**), were isolated from a marine gliding bacterium, *Rapidithrix thailandica*, and assayed for AChE enzyme inhibitory potentials with IC_{50} value of 4.9 μM for compound **29**, whereas compounds **30** and **31** were found to be inactive (Figure 7.2) [30]. The natural product (–)-debromoflustramine B (**32**) found in marine bryozoan *Flustra foliacea* [31] displayed selective BuChE inhibitory activity with an IC_{50} value of 1.37 μM [32]. Pseudozoanthoxanthin (**33**) isolated from yellow pigment of zoanthid crust coral *Parazoanthus axinellae* showed competitive inhibition of AChE with K_i value of 4 μM [33]. Benzenepropanamide or (*E*)-aplysamine-4 (**34**), a bromotyrosine-like amino acid derivative, was isolated from unidentified verongid sponge from the Red Sea. This molecule was found to be noncompetitive reversible inhibitor of AChE with K_i values of 16 and 2 μM determined with different methods such as electric eel and insect recombinant at pH 7.4 [34]. The plastoquinone derivatives sargaquinoic acid (**35**) and sargachromenol (**36**) were isolated from the activity-guided extraction and fractionation of brown algae *S. sagamianum.* Both compounds **35** and **36** were evaluated and found to be moderately active against AChE enzyme with inhibitory concentration in micromole range (IC_{50} 23.2 and 32.7 μM, respectively) [35]. A naphthoquinone derivative, echinochrome A (**37**), was found as a dark-red pigment from sea urchin *Scaphechinus mirabilis*. Compound **37** showed anti-AChE activity in a dose-dependent manner with IC_{50} value of 16.4 μM that is better than those for other related quinone derivatives, sargaquinoic acid (**35**) and sargachromenol (**36**) [36]. Onchidal (**38**), a marine compound, was isolated from the defensive secretion of the mollusc *Onchidella binneyi*, and it showed reversible binding affinity toward AChE enzyme with K_i 300 μM [37]. A rare and new thiophene compound, 8-hydroxy-2-[1-hydroxyethyl]-5,7-dimethoxynaphtho[2,3-*b*]thiophene-4,9-dione (**39**), together with 9 known compounds – namely, anhydrojavanicin (**40**), 8-*O*-methylbostrycoidin (**41**), 8-*O*-methyljavanicin (**42**), botryosphaerone D (**43**), 6-ethyl-5-hydroxy-3,7-dimethoxynaphthoquinone (**44**), 3β,5α-dihydroxy-(22*E*, 24*R*)-ergosta-7,22-dien-6-one (**45**), 3β,5α,14α-trihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**46**), NGA0187 (**47**), and beauvericin (**48**) – was isolated from mangrove endophytic fungus *Aspergillus terreus* collected from South China Sea. **39–48** compounds were evaluated for their anti-AChE activity, but only compounds **40**, **41**, **47**, and **48** showed potentials against AChE enzyme with respective IC_{50} values of 2.01, 6.71, 1.89, and 3.09 μM [38]. Alaternin (**49**), 5,8-dihydroxy-4-methyl-coumarin (**50**), and 3-hydroxy-3,6-dimethyl-2,5-piperazine (**51**) were isolated from the metatrophic fungus *Paecilomyces* sp. (Treel-7),

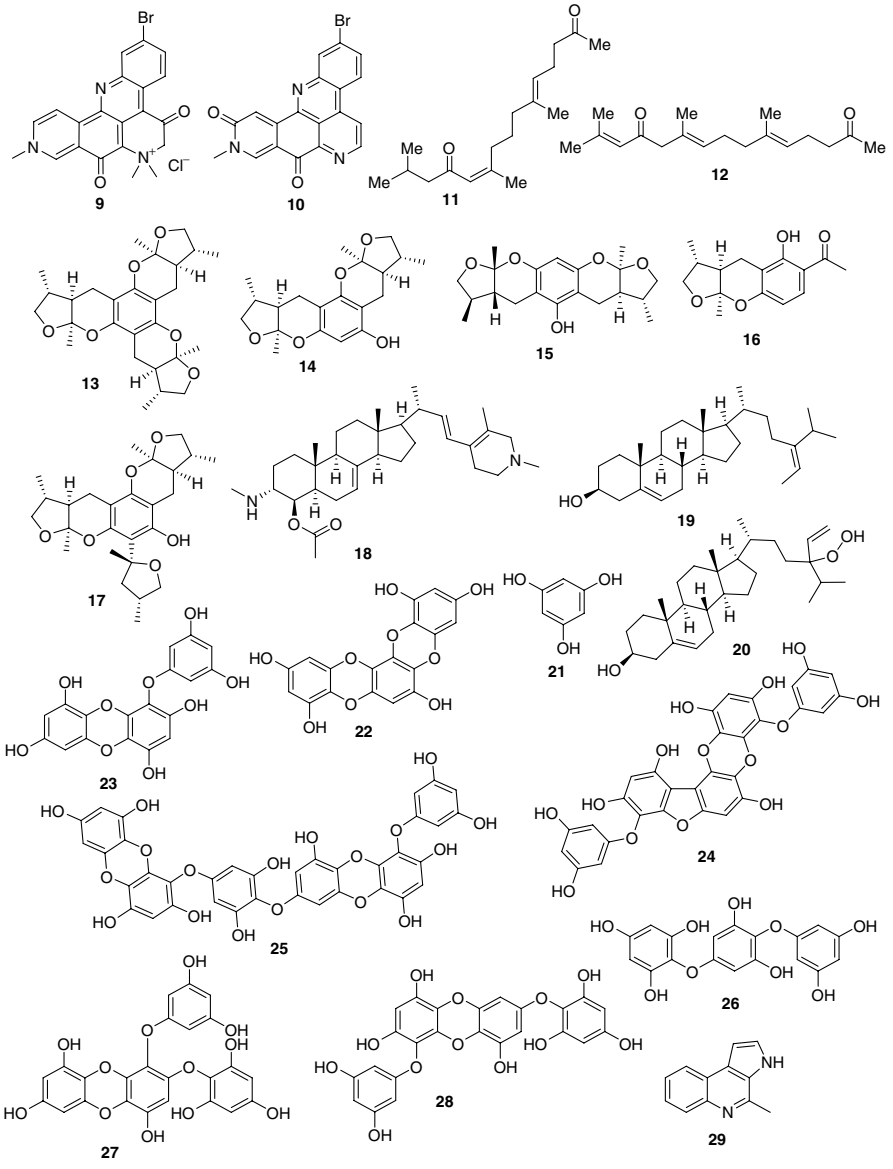


Figure 7.2 Chemical structure of cholinesterase inhibitors (**9–29**).

which was obtained from an estuarine mangrove from the Taiwan Strait (Figure 7.3). Among compounds **49–51**, only alaternin (**49**) exhibited activity against AChE (IC_{50} 0.87 μg ml^{-1}) *in vitro* [39]. In another study, the methanolic extract of mycelium fungus showed the presence of paeciloxanthone (**52**), emodin (**53**), and chrysophanol (**54**), in which only paeciloxanthone (**52**) exhibited inhibitory activity with IC_{50} value of 2.25 μg ml^{-1} [40]. Two novel polymeric 3-alkylpyridinium salts (poly-APS) with 29 (**55**) and 99-carbons (**56**) alkyl side

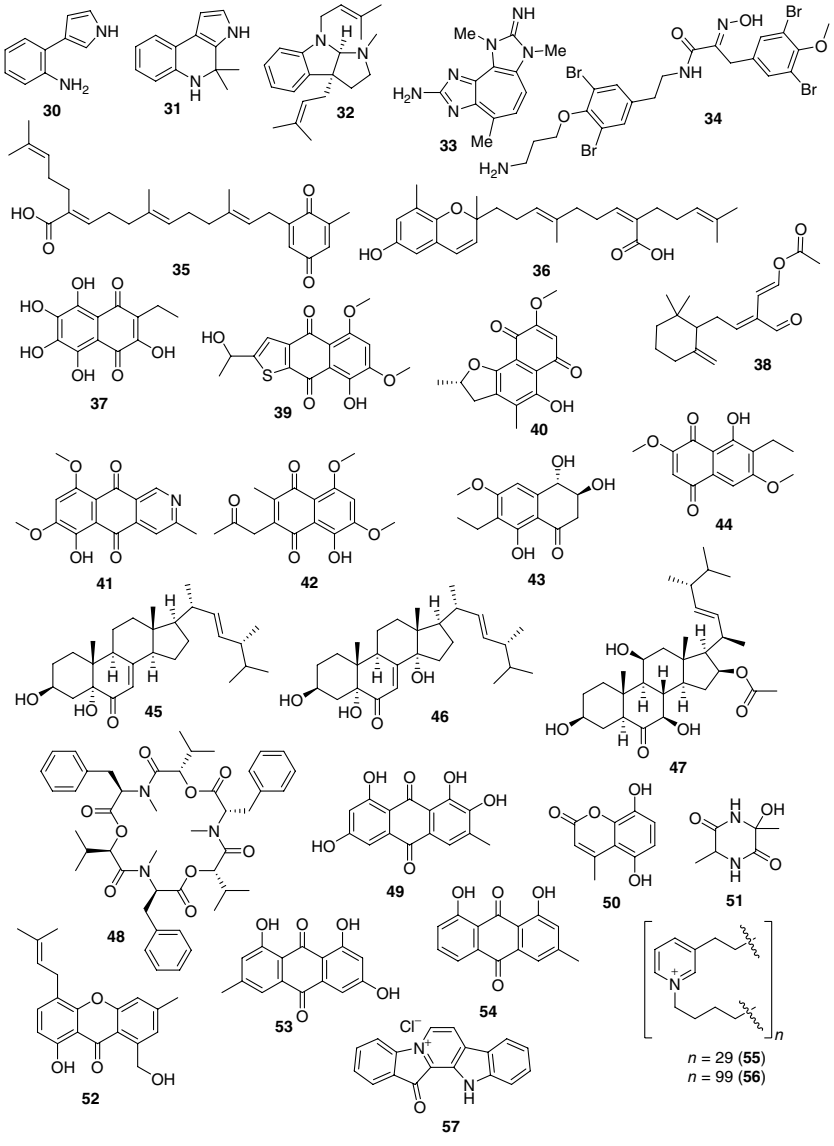


Figure 7.3 Chemical structure (**30–57**) of cholinesterase inhibitors.

chain were isolated from the aqueous extract of marine sponge *Reniera sarai* (Haliclonidae) and found to be effective against AChE enzyme (Figure 7.3).

Poly-APS strongly inhibited the activity of AChE enzymes obtained from different sources such as recombinant insects, electric eel, human erythrocyte, and equine serum BuChE with respective IC_{50} values of 0.06, 0.08, 0.57, and 0.14 μg ml^{-1} [41, 42]. Fascaplysin (**57**), a bis-indole alkaloid isolated from the marine sponge *Fascaplysinopsis* Bergquist sp. [43], exhibited potency against AChE and BuChE with IC_{50} values of 1.49 and 90.47 μM, respectively (Figure 7.3). It showed

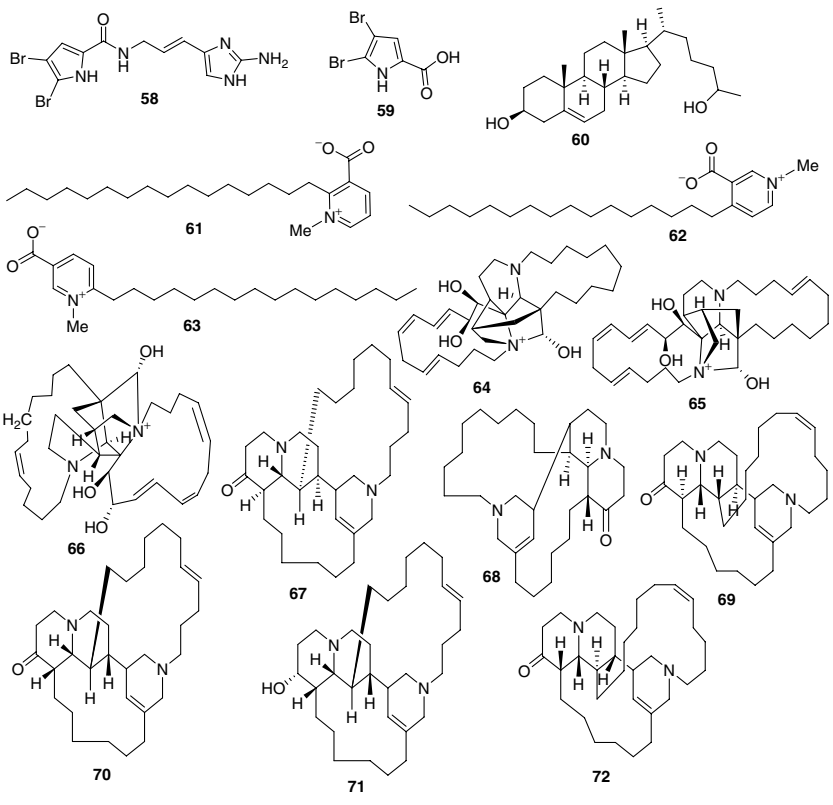


Figure 7.4 Chemical structure (**58–72**) of cholinesterase enzyme inhibitors.

approximately 60-fold more affinity toward AChE than BuChE enzyme [44]. The chemical investigation of *Agelas oroides*, a marine sponge collected from different spots of the Turkish coast of the Mediterranean Sea, revealed the presence of compounds oroidin (**58**), 4,5-dibromopyrrol-2-carboxylic acid (**59**), and 25-hydroxy-24-methylcholesterol (**60**), among which oroidin (**58**) showed 26.24% AChE inhibition at 100 μg ml^{-1} [45]. Three pyridium alkaloids, platisidines A–C (**61–63**), were found in Okinawan marine sponge of the genus *Plakortis* and exhibited AChE inhibitory activity [46]. Structurally complex zwitterion-like diamine alkaloids saraine A–C (**64–66**) together with saraine 1 (**67**), saraine 2 (**68**), saraine 3 (**69**), isosaraine 1 (**70**), isosaraine 2 (**71**), and isosaraine 3 (**72**) were isolated from the sponge *R. sarai* collected from the Bay of Naples (Italy) [47–52]. These compounds were found as potent antibacterial agents [53] as well as potent inhibitors of AChE enzyme in a competitive manner. The inhibition constants (K_i) of the mixture of saraines A–C (**64–66**), saraine C (**66**), saraine 1 (**67**), saraine 3 (**69**), and isosaraine 1 (**70**) were found as 5.7, 8.4, 6.4, 6.4, and 10.7, respectively (Figure 7.4). The inhibition constants (K_i) values were calculated by the electric eel method [54].

Marine microorganisms are the potential source of novel natural products having anti-AD agents. Hence, a number of formulations or extracts, obtained from different marine sources, had been evaluated against AChE enzyme

inhibitor activity. In the same context, 43 marine fungi extracts were collected from sea sediment of the Lianyungang area of China and evaluated against AChE activity. Among the 43 extracts, 15 were found to exhibit AChE inhibitory activity >50% and 3 extracts inhibited the AChE >80% at the concentration of 500 mg ml^{-1}. The three extracts, L1705, S1101, and SH0701, exhibited AChE inhibitory activity in a dose-dependent manner with IC_{50} values 11.3, 72.1, and 7.8 mg ml^{-1}, respectively. The ethyl acetate fraction of the biologically active extract of SH0701 that showed the highest AChE inhibitory activity with an inhibition rate of 71.55% at the concentration of 10 μg ml^{-1} and the fungus SH0701 was identified as *Aspergillus ochraceus* [55]. In another study, 134 extracts (EtOAc, hexane, and BuOH/MeOH) from different species of marine sponges collected from Mauritius waters were screened for AChE inhibitory activity at concentration 0.1 mg ml^{-1} and found that only two extracts displayed potent AChE inhibitory activity with >90% inhibition, whereas the rest of the extracts either possess moderate activity or were found inactive. These two potent extracts were obtained from the ethyl acetate fractions of *Pericharax heteroraphis* and *Amphimedon navalis* sponges having IC_{50} values of 0.018 and 0.016 mg ml^{-1}, respectively. These two extracts from *P. heteroraphis* and *A. navalis* displayed 90% and 96% inhibitions of AChE enzyme, respectively [56].

7.2.1.2 Nicotinic Acetylcholine Receptor Agonists

The nicotinic α_7-receptors are located on both presynaptic and postsynaptic terminals, which suggest the involvement in modulation of synaptic transmission [42, 57]. Therapeutic approaches directed toward the cholinergic system in the brain have focused on the stimulation of postsynaptic muscarinic cholinergic receptors, either directly with muscarinic agonists or indirectly by cholinesterase inhibition [23, 58].

Anabaseine (**73**) is a naturally occurring alkaloid toxin first isolated from marine worm *Paranemertes peregrina* [59] and also found in certain species of *Aphaenogaster* ants [60]. It was found that compound **73** generates current at α_7-subtype receptor equivalent to ACh, which is the indication of agonist property at receptor site [57]. Some synthetic analogs (**74–78**) of anabaseine were found as effective for α_7-nicotinic acetylcholine receptors (nAChRs) with more potency and selectivity than anabaseine (**73**) (Figure 7.5). Analogs such as 3-(2,4-dimethoxybenzylidene)anabaseine (GTS-21, **77**) and 3-(2-methoxy,4-hydroxybenzylidene)anabaseine (4OH-GTS-21, **78**) were shown to possess neuroprotective effects against amyloid toxicity in human and rat cells and also to protect rat neurons from apoptosis and necrosis both *in vitro* and *in vivo* models [61–63].

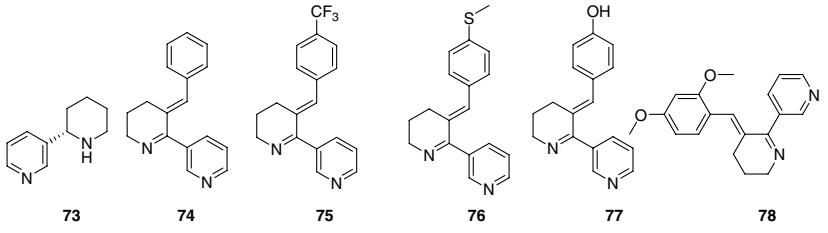


Figure 7.5 Chemical structures (**73–78**) of nicotinic acetylcholine receptor agonists.

7.2.2 Amyloid Cascade Hypothesis in the Treatment of Alzheimer's Disease

The sequential proteolysis of amyloid precursor protein (APP) leads to the generation of a fragment peptide of 36–43 amino acids called as beta-amyloid. The soluble forms of Aβ are commonly found in the brain of vertebrates. Evidences suggest that Aβ peptides play a critical role in the pathogenesis of AD. Initially, Aβ peptides undergo oligomerization and hence deposited extracellularly in the brain or cause neurofibrillogenesis intracellularly. Several marine natural products are known to either inhibit the enzymes involved in Aβ production or reduce the production of Aβ from APP.

7.2.2.1 Secretase Inhibitors

The generation of Aβ peptide from APP has critical role in the pathogenesis of AD. Aβ is a short fragment (peptides of 36–43 amino acids) of APP generated by its sequential cleavage with the action of proteolytic enzymes α-, β-, and γ-secretase (mainly β- and γ-secretase enzymes). The accumulation and aggregation of Aβ peptides lead to the disruption and destruction of nerve cells causing AD. The aggregated form of Aβ is known as *amyloid plaques*. Hence, the molecules having potency to inhibit β-secretase (BACE-1) and γ-secretase may become the drug candidates for treating AD. There are some synthetic molecules that are in clinical trials with β-secretase [64] and γ-secretase inhibitory [65] activity. In search of such inhibitors, several research groups have been involved in developing both synthetic and natural molecules of potential interest.

The bioassay-guided fractionation of active extract from the sponge *Sarcotragus* sp. (Ircinidae) led to the isolation of bioactive prenylated phenyl derivatives as Aβ peptide production inhibitors. A prenylated hydroxybenzoic acid (**79**) was identified in the enzyme-linked immunosorbent assay (ELISA) cell-based assay, which was shown to possess inhibition of Aβ peptide production by 47% at 10 μM concentration. In the mechanistic assay, it was also found to have BACE-1 inhibition 45% at 1 μM concentration. Inspired from this natural product, several potent synthetic analogs were synthesized and evaluated for BACE inhibitory activity [66] (Figure 7.6).

A cytotoxic peptide, tasiamide B (**80**), was isolated from the marine cyanobacterium *Symploca* sp. [67] and was evaluated against BACE-1 activity. Grassystatin A (**81**) and the originally proposed (epimeric) structure of tasiamide B (**82**) were found to be inactive, whereas tasiamide B (**80**) and its synthetic analogs exhibited potent inhibitory activity against BACE-1. Tasiamide B (**80**) and its synthetic precursors and analogs (**83–90**) showed *in vitro* inhibitory activity against BACE-1 enzyme with IC_{50} values of 0.189, 0.073, 0.124, 0.166, 0.0542, 0.211, 0.0488, 0.128, and 0.0572 μM, respectively [68]. Two sulfonic acid derivatives, taurine (**91**) and homotaurine (**92**), are the amino acids found in seaweeds and possess inhibitory activity of Aβ aggregation and reduce amyloid plaque formation. Homotaurine or tramiprosate (**92**) is a 3-amino-1-propanesulfonic acid (Alzhemed™) that was initially launched for clinical trials as nutraceuticals [69], but later, the FDA rejected it as a new dietary ingredient [70]. Homotaurine (**92**) was found to reduce the brain amyloid plaque load up to 30%, and hence, the moderate reduction

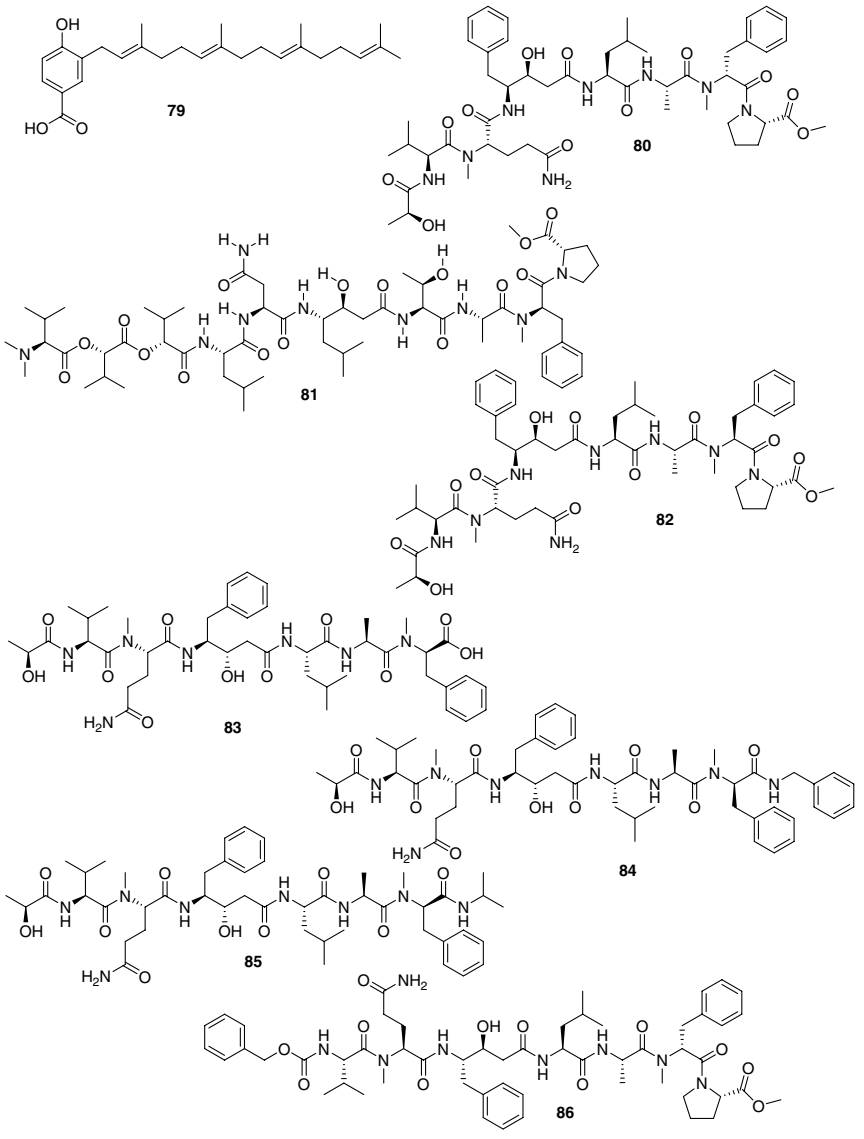


Figure 7.6 Chemical structure of secretase inhibitors (**79–86**).

(~20–30%) was observed in the level of $A\beta_{40}$ and $A\beta_{42}$ in the cerebrum [71]. Similarly, taurine (**91**) also prevented neurotoxicity offered by Aβ [72]. Betaine (**93**), a zwitterionic quaternary ammonium salt found as a catabolite of choline, was isolated from several vegetables and marine sources, and the compound displayed anti-AD activity by altering Aβ formation from APP. Betaine was found as an activator of α-secretase and inhibitor of β-secretase. α-Secretase acts first and cleaves Aβ peptide from APP of different amino acids rather than $A\beta_{40–42}$, which could be more responsible for AD pathogenesis [73]. Five new pyrrolidinone alkaloids, namely, dictyodendrins F–J (**94–98**), were reported from the Australian

marine sponge *Ianthella* sp., and these compounds (**86–90**) were evaluated for their BACE inhibitory activity. The experimental data revealed that compounds **94** and **96–98** inhibited β-secretase enzyme with IC_{50} values of 1.5, 1.0, 2.0, and 2.0 μM, respectively, whereas compound **97** was found to be inactive [74]. The alkaloids ianthellidones A–H (**99–106**) (Figure 7.7) and lamellarins O (**107**), O1 (**108**), O2 (**109**), Q (**110**) together with some known acid derivatives (**111–113**) and a aldehyde derivative (**114**) (Figure 7.8), were found in an Australian marine sponge, *Ianthella* sp. Pyrrolidone and furanone derivatives were isolated as new

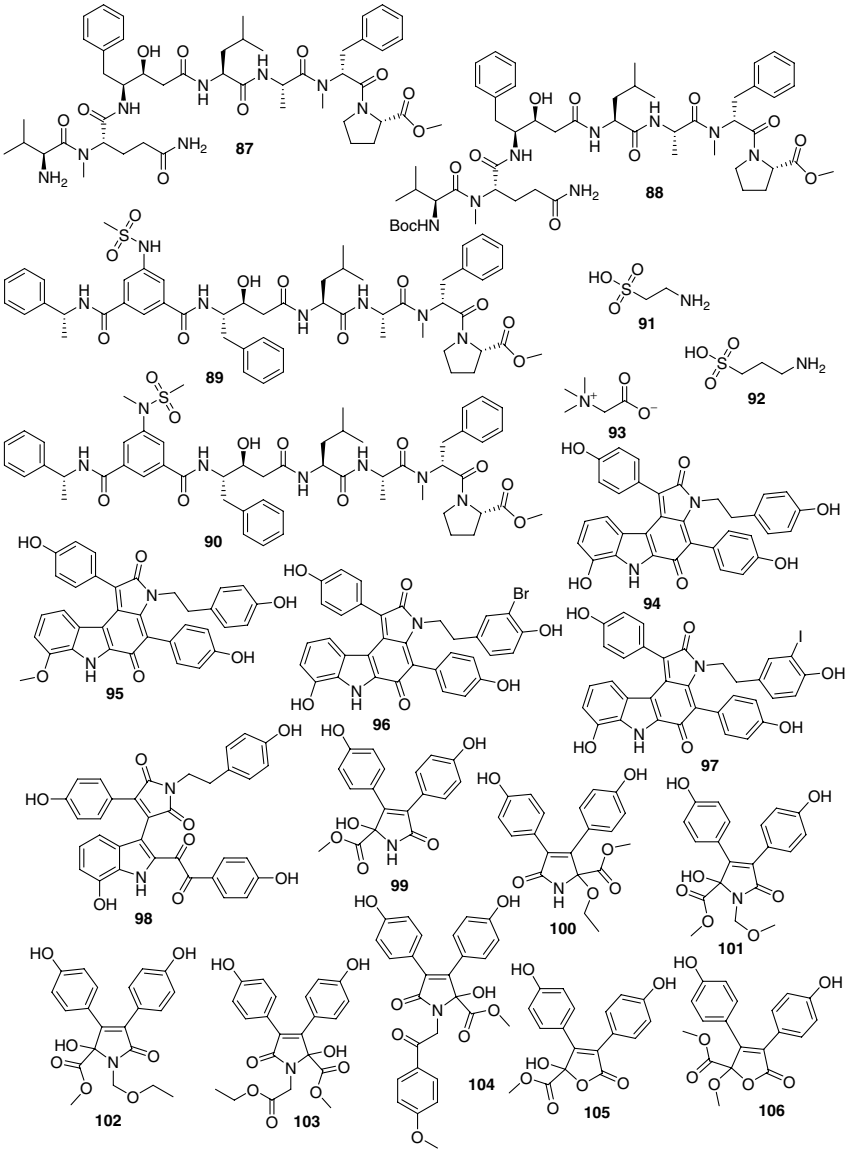


Figure 7.7 Chemical structure of BACE inhibitors (**87–106**).

compounds from this sponge. In an *in vitro* analysis, all 16 compounds (**99–114**) were investigated for their BACE inhibitory activity at 10 μM concentration. Only compounds lamellarin O (**107**), lamellarin O1 (**108**), lamellarin O2 (**109**), and ianthellidone F (**104**) showed inhibition of BACE enzyme activity by 40%, 60%, 40%, and 40%, respectively, at 10 μM concentration, whereas the rest of the compounds were found inactive against the enzyme [75]. Xestosaprol D (**115**), a metabolite of Indo-Pacific marine sponge *Xestospongia* sp., was collected near Turtle Bay, Sangalaki, Indonesia. The predicted log *P* value and low molecular weight of xestosaprol D (**115**) indicated that it has better ability to penetrate the blood–brain barrier (BBB) as compared with peptidic compounds [76]. The penetration of the BBB is a main hurdle to the drug development for the diseases related to the CNS [77, 78]. Compound **115** was found to exhibit BACE inhibitory activity with an IC_{50} value of 30 μM [76]. The other derivatives, xestosaprol F–M (**116–123**), isolated from same sponge exhibited weak β-secretase inhibitory activity as compared with xestosaprol D with IC_{50} values of 135, 155, 82, 163, 90, 93, 98, 104 μM, respectively. Among xestosaprol (F–M), compound **118** was found most active due to its β-orientation of the hydroxyl group at C-3, which is required for activity. Compound **116** was epimeric to compound **118** at C-3 position and found to exhibit weak activity than compound **118** [79]. Omega-3 fatty acids are essential fatty acids generally found in marine organisms. Docosahexaenoic acid (DHA) (**124**) is an essential omega-3 fatty acid found in fishes [80]. DHA is also found in the CNS, cellular membranes, and synaptic junctions; hence, DHA is more related to the function of the CNS, neuroprotection, synaptogenesis, and vision [81–83]. In an *in vivo* model, it was found that DHA reduces Aβ deposition [84, 85]. Another omega-3 fatty acid derivative, neuroprotectin D1 (NPD1) (**125**), was evaluated to possess potency against AD by activating α-secretase and downregulating the activity of β-secretase enzyme. Hence, NPD1 suppresses $A\beta_{42}$ formation from APP [86]. Bryostatin 1 (**126**), a macrolide lactone, was found in bryozoans *Bugula neritina* [87]. Initially, compound **126** was found to be active against protein kinases C, which are promising targets for cancer [88, 89]. Recently, it has been demonstrated that bryostatin 1 possesses considerable potency to reduce the production of $A\beta_{40}$ and $A\beta_{42}$ in double transgenic mouse model [90–92]. Now, bryostatin 1 is under phase II clinical trials for the treatment of AD (https://clinicaltrials.gov/ct2/show/NCT02431468) (Figure 7.8).

Five 24-isopropyl sterol derivatives – namely, topsentinol K (**127**), topsentinol L (**128**), topsentinol K trisulfate (**129**), polasterol B (**130**), and 22-dehydro-24-isopropylcholesterol (**131**) – were isolated from *Topsentia* sp. Among all five compounds, only trisulfated sterol (**129**) was found to exhibit the BACE-1 inhibitory activity with IC_{50} value of 1.2 μM. The structure–activity relationship indicates that the BACE-1 inhibitory activity is mainly due to the presence of sulfate group in the molecule [93] (Figure 7.8).

The peptidic compound HTP-1 (**132**) was isolated from traded species of sea horse, *Hippocampus trimaculatus*, which showed neuroprotective effects against $A\beta_{42}$ on PC12 cells [94]. The naturally occurring compound bastadin 9 (**133**) (Figure 7.9) was isolated from *Ianthella basta* Pallas (Lanthellidae) [95, 96]

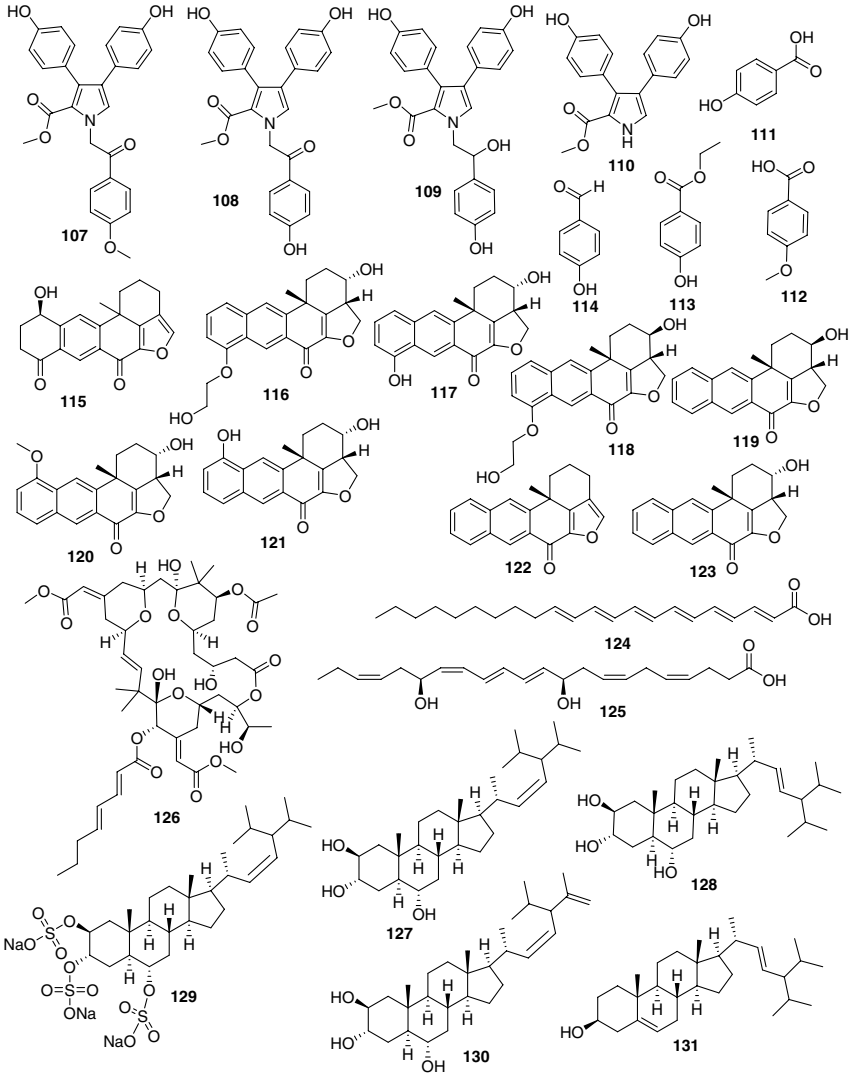


Figure 7.8 Chemical structure of BACE inhibitors (**107–131**).

and *Psammaplysilla purpurea* (Aplysinellidae) [97]. This marine natural product was found to inhibit the activity of β-secretase and hence reduces the level of Aβ peptides from APP in the brain. The cell- and enzyme-based assay revealed the inhibitory effect of bastadin 9 against BACE-1 activity in the cleavage of APP with IC_{50} values of 2.8 and 0.3 μM, respectively. Compound **133** was able to permeate the BBB with nanomolar potency [96]. The series of oxide-based macrocyclic derivatives of bastadin 9 were patented by Bristol-Myers Squibb in 2008 [98]. An unusual cyclobutane ring containing bis-2-amino-imidazolone, dictazole A (**134**), was isolated from *Smenospongia cerebriformis* [99]. The β-secretase

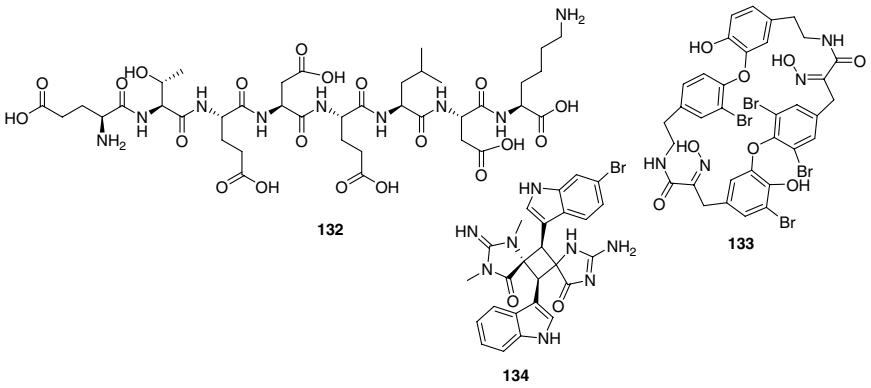


Figure 7.9 Chemical structure of BACE inhibitors (**132–134**).

inhibitory activity of compound **134** was found to be very weak, but due to its unique structural scaffold, it may become a molecule of interest in the drug discovery of AD [100].

7.2.2.2 Anti-aggregation and Clearance Promoters

The physiological role of Aβ is still unknown , but it is commonly found in the brains of all individuals with or without AD. The fatality of the oligomerization of $A\beta_{42}$ has been noted in transgenic mice. Hence, the therapeutic approaches directed toward reduction in Aβ formation, aggregation, and promotion of clearance of soluble and insoluble Aβ peptides may be useful in the development of drugs for AD.

Some marine compounds have been found to possess such kind of potency to protect memory loss. Gymnodimine (**135**), a marine macrocyclic imine compound, was isolated from the extract of dinoflagellates *Karenia selliformis.* The initial study of compound **135** found severe neurotoxic effects due to its antagonistic activity for nicotinic ACh receptors with high affinity for $\alpha_3\beta_2$, $\alpha_4\beta_2$, and chimeric α_7-$5HT_3$ receptor's subtype [101]. Later, Alonso *et al.* studied again and found its beneficial effects against AD. Surprisingly, they found that compound **135** possesses the potency to inhibit the accumulation of intracellular Aβ in cortical neurons and decrease the levels of the hyperphosphorylated isoforms of tau protein. These findings were suggested to be mediated by the increase in the inactive isoform of the glycogen synthase kinase 3 (phospho-GSK-3 Ser9), the decrease in the levels of the active isoform of the ERK1/2 kinase, and the increase in ACh synthesis elicited by long-term exposure of cortical neurons at 50 nM concentration [102]. A naturally occurring spirolide marine compound, 13-desmethyl spirolide C (**136**), was detected in North American and European shellfishes [103, 104]. Initially, compound **136** was evaluated to show great affinity toward both muscarinic and nicotinic ACh receptors with antagonistic property [105], but the ability of 13-desmethyl spirolide C was explored later by Alonso *et al.* Compound **136** was found to display neuroprotective effects by inducing inhibition toward the intracellular Aβ aggregation in an *in vitro* model of 3 × Tg cortical neurons [106]. In an *in vivo* experiment, it was found that

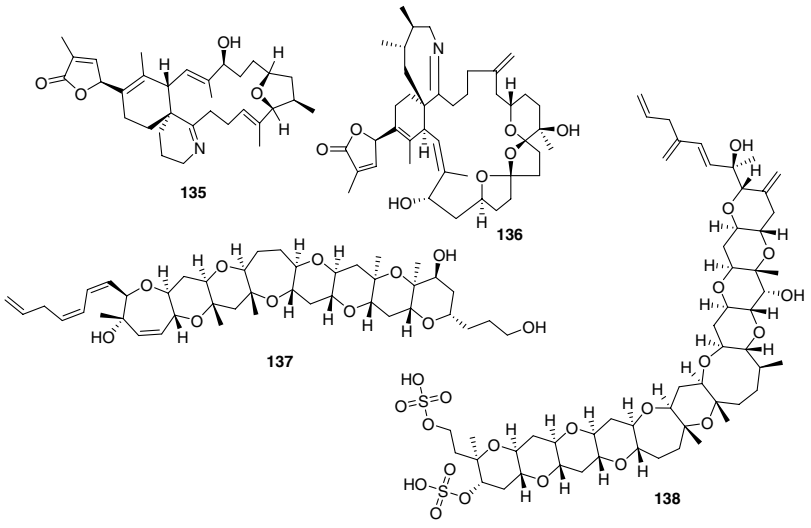


Figure 7.10 Chemical structure of anti-aggregation and clearance promoters (**135–138**).

compound **136** possesses good bioavailability in the brain and a significant reduction in Aβ aggregation in 3 × Tg-AD mice model. The compound was administered via intra-parenteral (i.p.) injection with the dose of 11.9 $\mu g\,kg^{-1}$ [107] (Figure 7.10).

A toxic secondary metabolite, gambierol (**137**), was isolated from the dinoflagellate *Gambierdiscus toxicus* [108]. Gambierol (**137**) is a polycyclic marine ether natural product that displayed potent acute lethal toxicity against mice with LD_{50} 50 $\mu g\,kg^{-1}$ administered via i.p. route. **137** was found to exhibit potency to reduce Aβ extra- and intracellular levels. The reduction in the level of Aβ was achieved by voltage-gated potassium channel (K_v) inhibition in an *in vitro* model of AD [109]. Another phycotoxin, yessotoxin (**138**), was isolated from dinoflagellates of *Protoceratium reticulatum* and *Lingulodinium polyedrum* [110, 111]. This compound was found to reduce the level of Aβ and *tau* hyperphosphorylation by the modulation of protein kinase activity at 1 nM concentration [112]. In addition, small peptide-enriched water-soluble fraction of the extract of salt-tolerant fungus *Phomopsis occulta* collected from the mangroves *Pongamia pinnata* (L.) Pierre was evaluated to exhibit the inhibition of the aggregation of Aβ in *Escherichia coli* model [113].

7.2.3 Kinase Modulators in the Treatment of Alzheimer's Disease (*Tau* Hypothesis)

Glycogen synthase kinase 3 (GSK3) is an enzyme involved in the phosphorylation of *tau* proteins. The phosphorylation of *tau* proteins mediated by GSK3 regulates the binding of *tau* to microtubules, its degradation, and its aggregation. It has been hypothesized that Aβ promotes GSK activation, thereby triggering the hyperphosphorylation of *tau* proteins. The hyperphosphorylation of *tau* proteins is another hallmark of AD [114]. As per the GSK hypothesis of AD, the

hyperactivity of GSK3 results in the impairment of memory, *tau* hyperphosphorylation, and enhancement of the Aβ production as well as the inflammation mediated by microglia [115].

The first report was published in 1996 on the reversible inhibition of GSK3 activity by lithium [116]. Lithium reduces the activity of GSK3 by competing with magnesium ion for binding [117] and inhibiting the phosphorylation of N-terminal serine [118]. It reduces *tau* phosphorylation and, hence, prevents neurotoxicity by decreasing the production of Aβ in transgenic mouse model [119].

In search of potential leads for GSK3 inhibitory activity, several natural products have been evaluated. A marine alkaloidal natural product, manzamine A (**139**), was first isolated by Higa from an Okinawan sponge of the genus *Haliclona* [120]. Manzamine A (**139**) was found to decrease the hyperphosphorylation of *tau* proteins by inhibiting cellular GSK at 10 μM concentration in human neuroblastoma cell lines. The structure–activity relationship of the semisynthetic analogs of manzamine A revealed that the presence of indole and ircinal fragments is necessary for the activity (Figure 7.11).

In the selectivity study of *tau* phosphorylation-promoting enzymes, it was found that compound **139** inhibits GSK3β and CDK5 noncompetitively [121]. The linear furanosesquiterpene palinurin (**140**) was first isolated from Mediterranean sponge *Ircinia variabilis* [122, 123]. Palinurin (**140**) neither competed out by ATP nor peptide substrate to inhibit GSK3β. Compound **140** inhibited GSK3β at an allosteric site with IC_{50} value of 2.6 μM [124]. It was patented by NeuroPharma, a Spanish biotechnology company, for the development of formulation to treat neurodegenerative diseases [125]. Hymenialdisine (**141**), a marine natural product containing bromopyrrole and guanidine fragments, was isolated from the Red Sea sponge *Acanthella* sp. and the Mediterranean sponge *Axinella* sp. in 1982 as well as from *Hymeniacidon* sp. in 1983 and characterized by X-ray structure analysis. Compound **141** was found to be a competitive ATP inhibitor of glycogen synthase kinase 3β (GSK3β) and cyclin-dependent kinase 5/p35 (CDK5/p35) with IC_{50} values of 35 and 28 nM, respectively, in an *in vivo* model [58, 126, 127]. The extract of Australian marine sponge *Callyspongia* sp. displayed casein kinase 1 (CK1), cyclin-dependent kinase 5 (CDK5), and glycogen synthase kinase 3 (GSK3β) inhibitory activities with respective IC_{50} values of 0.03, 0.16, and 0.07 μM. The chemical investigation of this marine sponge yielded bromopyrrole alkaloids, callyspongisines A–D (**142–145**), hymenialdisine (**141**), and 2-bromoaldisine (**146**). A HPLC-DAD-MS analysis revealed the presence of hymenialdisine (**141**) and 2-bromoaldisine (**146**) as major cometabolites [128]. The activity of the extract could be attributed to the major component hymenialdisine (**141**), well known as a kinase inhibitor [126]. Leucettamine B (**147**), isolated from the marine calcareous sponge *Leucetta microraphis* Haeckel [129, 130], was found inactive, whereas its synthetic analog, leucettine L41 (**148**), was found to be GSK3β inhibitory activity with IC_{50} value of 0.38 μM [131]. Variolin B (**149**), a marine alkaloid isolated from Antarctic sponge *Kirkpatrickia variolosa* [132], was evaluated to possess the inhibitory activity against several CDKs and GSK3β. It inhibited the enzymatic activity of CDK1, CDK2, CDK5, and CDK9 and protein kinase GSK3β with IC_{50} values of 60, 80, 90, 26, and 70 nM, respectively [132, 133]. Indirubins were first

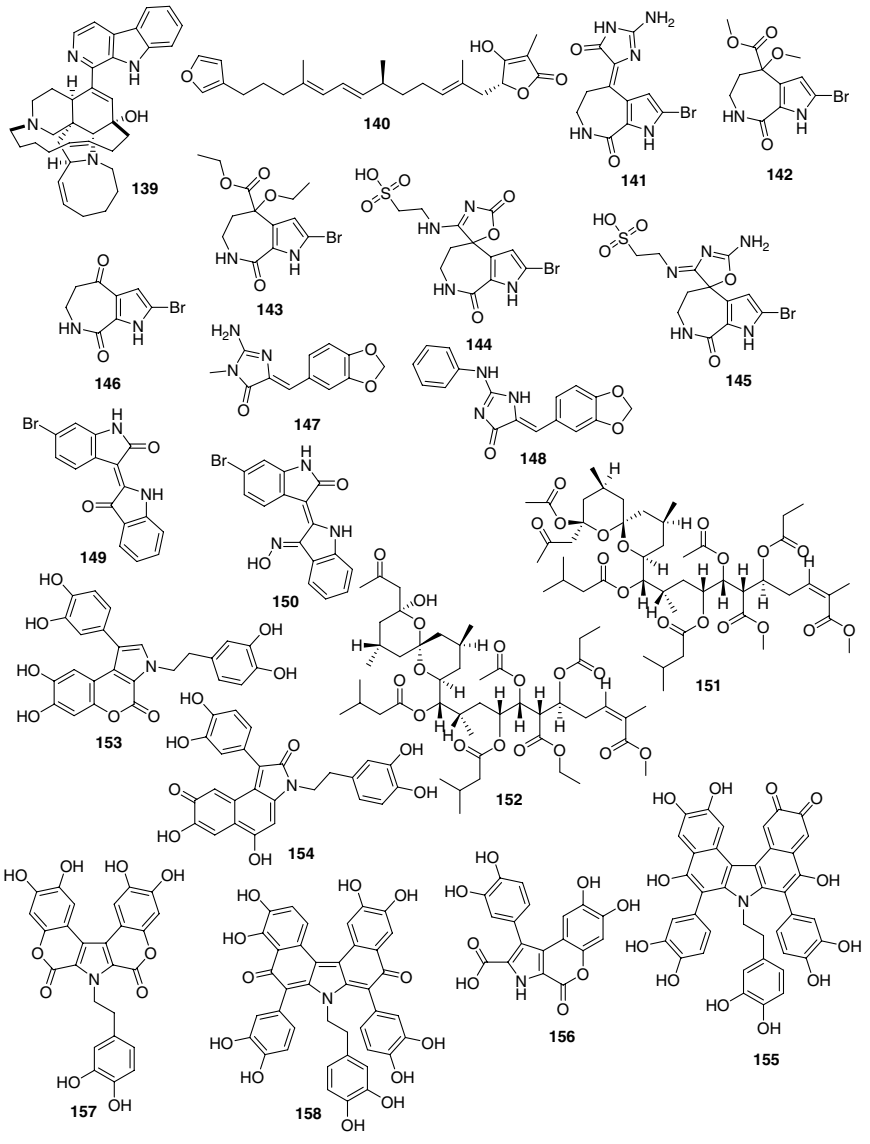


Figure 7.11 Chemical structure of glycogen synthase kinase 3 (GSK3β) inhibitors (**139–158**).

reported for their selective GSK3 inhibitory activity by Meijer *et al.* [134]. 6-Bromoindirubin (**149**) was isolated from the Mediterranean mollusc *Hexaplex trunculus* and displayed selective GSK3β inhibitory activity with IC_{50} value of 45 nM. 6-Bromoindirubin-3′-oxime (**150**), a synthetic analog of compound **149**, inhibited GSK3β by binding with ATP pocket [134]. Didemnaketals D (**151**) and E (**152**), spiroketal-type terpenoids with additional ketal or hemiketal functional group, were isolated from the tunicates of the genus *Didemnum* sp. of the Red Sea. Both compounds were found to exhibit moderate cyclin-dependent kinase and GSK3β inhibitory activity at 10 μg ml^{-1} concentration [135]. Six alkaloids,

ningalins B–G (**153–158**), were found in Australian ascidian of the genus *Didemnum* [136, 137]. Structurally, these compounds have catechol and pentacyclic amines derived from phenylalanine. Ningalins B (**153**), E (**156**), and F (**157**) were found as moderate protein kinase inhibitors against CK1 and GSK3β, whereas ningalins C (**154**), D (**155**), and G (**158**) were found as more potent inhibitors against the three protein kinases, namely, CK1, CDK5, and GSK3β, with IC_{50} values ranging between 0.2–1.4, 0.5–2, and 0.2–0.5 μM, respectively [136] (Figure 7.11).

A known indole alkaloid, meridianin E (**159**), was found in tunicates *Aplidium meridianum* and *Synoicum* sp. [138, 139]. Compound **159** was reported to inhibit the effects of different kinases such as CDK1/B, CDK5/p25, GSK3α, GSK3β, and CK1 with IC_{50} values of 0.18, 0.15, 0.90, 2.5, and 0.40 μM, respectively [140]. The chemical investigation of the sponge *Hemimycale arabica* from the Red Sea yielded a compound, namely, (*Z*)-5-(4-hydroxybenzylidene)-hydantoin (**160**), with the anticancer activity [141, 142]. The *in vitro* analysis of compound **160** found it to be the potent inhibitor of GSK3β with IC_{50} value of 13.7 μM. The synthetic analog (**161**) of compound **160** was found to be more potent against GSK3β with IC_{50} value of 4.2 μM in an *in vitro* study [143]. A chromone derivative (**162**) with lipid side chain was isolated from the marine organisms *Zonaria tournefortii* and *Zonaria spiralis* [144, 145]. Two phloroglucinol-derived lipid metabolites, spiralisones A (**163**) and B (**164**), were found in brown alga *Z. spiralis.* All three compounds (**162–164**) (Figure 7.12) were found as inhibitors of kinases CK1 (all IC_{50} values of 10.0 μM), CDK5/p25 (IC_{50} values of 10.0, 10.0, and 3.0 μM, respectively), and GSK3β (all IC_{50} values of 10.0 μM) [145]. (–)-Agelastatin A (**165**), a novel oroidin alkaloid isolated from axinellid sponge *Agelas dendromorpha*, was evaluated to inhibit the activity of GSK3β enzyme with IC_{50} value of 12 μM [127, 146, 147]. The importance of GSK3 inhibitors from marine sources in search of new lead molecules has been documented.

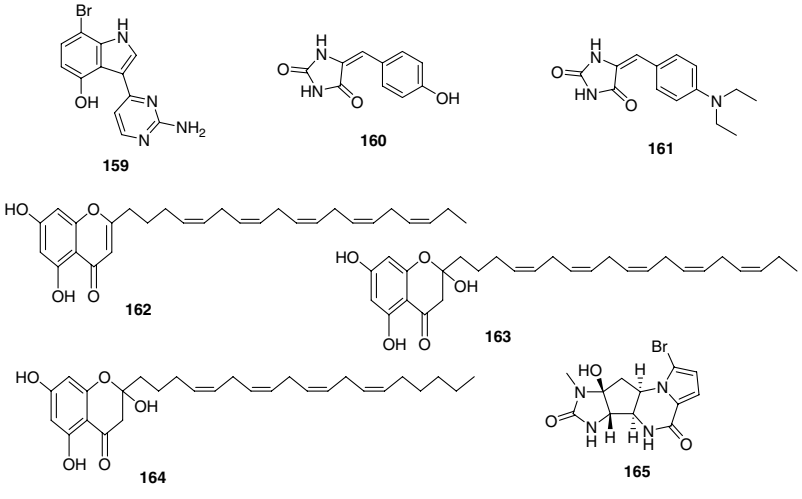


Figure 7.12 Chemical structure of glycogen synthase kinase 3 (GSK3β) inhibitors (**159–165**).

7.2.4 Antioxidant Natural Products

Some marine natural products have been reported for their preventive effects against neuronal oxidative damages. Six diterpenes, namely, gracilin A (**166**), gracilin H (**167**), gracilin K (**168**), gracilin J (**169**), gracilin L (**170**), and tetrahydroaplysulfurin-1 (**171**), were found in marine sponge *Spongionella gracilis.* In *in vitro* oxidative stress model, all these six compounds (**166–171**) showed protective effects on primary cortical neurons from H_2O_2-induced cytotoxicity. Compound **171** was found to be the most potent agent among this series [148] (Figure 7.13).

The eicosapentaenoic acid-enriched phospholipids were extracted from sea cucumber *Cucumaria frondosa* and found to be against hydrogen peroxide (H_2O_2)- and tertiary-butyl hydroperoxide-induced oxidative injury in PC12 cells [149]. Exopolysaccharide, a polysaccharide composed of galactose, glucose, rhamnose, mannose, and glucuronic acid in approximate proportion of 50 : 8 : 1 : 1 : 0.4, was isolated from a marine fungus, *Keissleriella* sp. YS4108, which displayed protective effect against hydrogen peroxide (H_2O_2)-induced oxidative injuries in PC12 cells and also found as free radical scavenger [150, 151].

7.3 Molecules in Clinical Trials for Alzheimer's Disease from Marine Sources

There are several natural products and their derivatives are in advanced phases of drug discovery to treat AD. Homotaurine (tramiprosate, **92**) was qualified up to phase III clinical trials (NCT00314912) for the patients with mild to moderate AD, but later, it was discontinued as the compound did not show enough clinical efficiency [152]. GTS-21 (**77**), a synthetic analog of naturally occurring anabaseine, is in phase II clinical trials (NCT00414622) for its agonist activity against nicotinic ACh receptors. DHA (**124**), a polyunsaturated omega 3-fatty acid, has already qualified for phase III clinical trials (NCT00440050) for slowing the

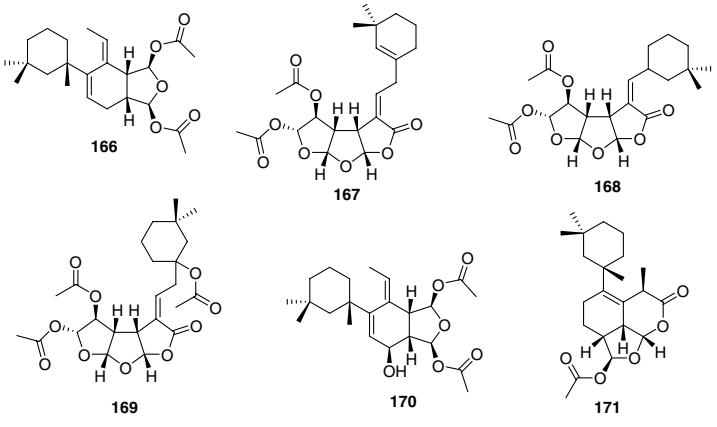


Figure 7.13 Chemical structures of gracilins (**166–171**) as antioxidants.

progression of AD via reducing Aβ formation [153]. Bryostatin 1 (**126**), a clinical trial candidate (NCT00606164, phase II) for AD, exerts its protective effect via the reduction in the Aβ formation and inhibition of GSK3β [154].

7.4 Concluding Remarks

The immense role of marine natural products in drug discovery is supported by huge literature. In search of lead molecules for AD from natural products, majority of compounds and drug candidates have been found from plants, whereas most new leads of clinical trials have been derived from marine sources. These results showed the potential of marine natural products. Hence, the marine natural products could play a very important role in the drug discovery of anti-AD.

A new hope of drug discovery and development from marine sources has been visualized due to its increasing number of molecules with new scaffolds and interesting biological activity. In spite of the large number of marine natural products and related compounds being in clinical and preclinical trials against AD, a large portion of the sea remains unexplored. New approaches are needed to identify the type of organism that produces these secondary metabolites and also to identify gene clusters responsible for biosynthesis of molecule of interest. The biodiversity of ocean is tremendous but not immortal; hence with preserving the beauty and diversity of marine life, we should go further toward the development of drugs from these sources against AD as well as other ailments. The marine natural products need to be explored, so that they could provide new drug targets that are strongly connected with the pathogenesis of AD.

Acknowledgments

Kapil Dev is thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi, for providing senior research fellowship. The CSIR-CDRI communication number for this manuscript is 9268.

Abbreviations

Aβ	amyloid β-protein or beta-amyloid
$Aβ_{40–42}$	beta-amyloid protein (40–42 amino acids)
ACh	acetylcholine
AChE	acetylcholinesterase enzyme
AD	Alzheimer's disease
APP	amyloid precursor protein
ATP	adenosine triphosphate
BACE	beta-secretase enzyme
BBB	blood–brain barrier
BuChE	butyrylcholinesterase

BuOH	*n*-butanol
CNS	central nervous system
CDK	cyclin-dependent kinase
CK1	casein kinase 1
DHA	docosohexaenoic acid
E. coli	*Escherichia coli*
ELISA	enzyme-linked immunosorbent assay
E. stolonifera	*Ecklonia stolonifera*
EtOAc	ethyl acetate
FDA	Food and Drug Administration
GSK3	glycogen synthase kinase 3
GTS-21	3-(2,4-dimethoxybenzylidene)anabaseine
4-OH-GTS-21	3-(2-methoxy-4-hydroxybenzylidene)anabaseine
HPLC-DAD	high-performance liquid chromatography with photodiode array detection
IC_{50}	half maximal inhibitory concentration
i.p.	intra-parenteral
K_i	inhibitor constant
K_v	voltage-gated potassium channels
LD_{50}	lethal dose, 50%
MeOH	methanol
$\mu g\,ml^{-1}$	microgram/milliliter
μM	micromolar
nAChRs	α_7-nicotinic acetylcholine receptor
NPD1	neuroprotectin D1
Poly-APS	poly-3-alkylpyridinium
SH0701	ethyl acetate fraction of *Aspergillus ochraceus*
3 × Tg-AD	the triple transgenic mouse model of *Alzheimer's disease*

References

1 Costello, M.J., Coll, M., Danovaro, R., Halpin, P., Ojaveer, H., and Miloslavich, P. (2010) A census of marine biodiversity knowledge, resources, and future challenges. *PLoS One*, **5** (8), e12110.

2 Fautin, D., Dalton, P., Incze, L.S., Leong, J.-A.C., Pautzke, C., Rosenberg, A., Sandifer, P., Sedberry, G., Tunnell, J.W. Jr., Abbott, I., Brainard, R.E., Brodeur, M., Eldredge, L.G., Feldman, M., Moretzsohn, F., Vroom, P.S., Wainstein, M., and Wolff, N. (2010) An overview of marine biodiversity in United States waters. *PLoS One*, **5** (8), e11914.

3 Miloslavich, P., Díaz, J.M., Klein, E., Alvarado, J.J., Díaz, C., Gobin, J., Escobar-Briones, E., Cruz-Motta, J.J., Weil, E., Cortés, J., Bastidas, A.C., Robertson, R., Zapata, F., Martín, A., Castillo, J., Kazandjian, A., and Ortiz, M. (2010) Marine biodiversity in the Caribbean: regional estimates and distribution patterns. *PLoS One*, **5** (8), e11916.

4 Ausubel, J.H., Crist, D.T., and Waggoner, P.E. (2010) Summary of the First Census of Marine Life, http://www.coml.org (accessed 15 April 2016).

5 Scheuer, P.J. (1990) Some marine ecological phenomena: chemical basis and biomedical potential. *Science*, **248** (4952), 173–177.
6 El Gamal, A.A. (2010) Biological importance of marine algae. *Saudi Pharm. J.*, **18** (1), 1–25.
7 Molinski, T.F., Dalisay, D.S., Lievens, S.L., and Saludes, J.P. (2009) Drug development from marine natural products. *Nat. Rev. Drug Discovery*, **8** (1), 69–85.
8 Thakur, N.L., Thakur, A.N., and Muller, W.E. (2005) Marine natural products in drug discovery. *Nat. Prod. Radiance*, **4** (6), 471–478.
9 Harvey, A.L. (2008) Natural products in drug discovery. *Drug Discovery Today*, **13** (19-20), 894–901.
10 Newman, D.J. and Cragg, G.M. (2004) Marine natural products and related compounds in clinical and advanced preclinical trials. *J. Nat. Prod.*, **67** (8), 1216–1238.
11 Newman, D.J. and Cragg, G.M. (2004) Advanced preclinical and clinical trials of natural products and related compounds from marine sources. *Curr. Med. Chem.*, **11** (13), 1693–1713.
12 Newman, D.J. and Cragg, G.M. (2016) Natural products as sources of new drugs from 1981 to 2014. *J. Nat. Prod.*, **79** (3), 629–661.
13 Leifer, B.P. (2003) Early diagnosis of Alzheimer's disease: clinical and economic benefits. *J. Am. Geriatr. Soc.*, **51** (5 Suppl. Dementia), S281–S288.
14 World Health Organization (2015) WHO Media Centre Updates on Dementia.
15 Alzheimer, A. (1987) About a peculiar disease of the cerebral cortex. By Alois Alzheimer, 1907 (Translated by L. Jarvik and H. Greenson). *Alzheimer Dis. Assoc. Disord.*, **1** (1), 3–8.
16 Tonkonogy, J. and Moak, G.S. (1988) Alois Alzheimer on presenile dementia. *J. Geriatr. Psychiatry Neurol.*, **1** (4), 199–206.
17 Bayer, T.A., Wirths, O., Majtenyi, K., Hartmann, T., Multhaup, G., Beyreuther, K., and Czech, C. (2001) Key factors in Alzheimer's disease: beta-amyloid precursor protein processing, metabolism and intraneuronal transport. *Brain Pathol.*, **11** (1), 1–11.
18 Iqbal, K., Alonso, A.D., Gondal, J.A., Gong, C.X., Haque, N., Khatoon, S., Sengupta, A., Wang, J.Z., and Grundke-Iqbal, I. (2000) Mechanism of neurofibrillary degeneration and pharmacologic therapeutic approach. *J. Neural Transm. Suppl.*, **59**, 213–222.
19 Terry, R.D. (2000) Cell death or synaptic loss in Alzheimer disease. *J. Neuropathol. Exp. Neurol.*, **59** (12), 1118–1119.
20 Lahiri, D.K., Farlow, M.R., Greig, N.H., and Sambamurti, K. (2002) Current drug targets for Alzheimer's disease treatment. *Drug Dev. Res.*, **56** (3), 267–281.
21 Burns, A. and Iliffe, S. (2009) Alzheimer's disease. *Br. Med. J.*, **338**, b158.
22 Parveen, M. and Kumar, S. (2005) *Recent Trends in the Acetylcholinesterase System*, IOS Press.
23 Francis, P.T., Palmer, A.M., Snape, M., and Wilcock, G.K. (1999) The cholinergic hypothesis of Alzheimer's disease: a review of progress. *J. Neurol. Neurosurg. Psychiatry*, **66** (2), 137–147.
24 Ibach, B. and Haen, E. (2004) Acetylcholinesterase inhibition in Alzheimer's disease. *Curr. Pharm. Des.*, **10** (3), 231–251.

25 Nukoolkarn, V.S., Saen-oon, S., Rungrotmongkol, T., Hannongbua, S., Ingkaninan, K., and Suwanborirux, K. (2008) Petrosamine, a potent anticholinesterase pyridoacridine alkaloid from a Thai marine sponge *Petrosia n*. sp. *Bioorg. Med. Chem.*, **16** (13), 6560–6567.

26 Ryu, G., Park, S.H., Kim, E.S., Choi, B.W., Ryu, S.Y., and Lee, B.H. (2003) Cholinesterase inhibitory activity of two farnesylacetone derivatives from the brown alga *Sargassum sagamianum*. *Arch. Pharmacal Res.*, **26** (10), 796–799.

27 Lin, Y., Wu, X., Feng, S., Jiang, G., Luo, J., Zhou, S., Vrijmoed, L.L.P., Jones, E.B.G., Krohn, K., Steingröver, K., and Zsila, F. (2001) Five unique compounds: xyloketals from mangrove fungus *Xylaria* sp. from the South China Sea Coast. *J. Org. Chem.*, **66** (19), 6252–6256.

28 Jinghui, L., Yingbao, Y., Yongcheng, L., Zhiliang, C., and Xiongyu, W. (2004) Effects of metabolites of mangrove fungus *Xylaria* sp. from South China Sea Coast on the activity of acetylcholinesterase *in vitro*. *Zhong Yao Cai*, **27** (4), 261–264.

29 Langjae, R., Bussarawit, S., Yuenyongsawad, S., Ingkaninan, K., and Plubrukarn, A. (2007) Acetylcholinesterase-inhibiting steroidal alkaloid from the sponge *Corticium* sp. *Steroids*, **72** (9-10), 682–685.

30 Sangnoi, Y., Sakulkeo, O., Yuenyongsawad, S., Kanjana-opas, A., Ingkaninan, K., Plubrukarn, A., and Suwanborirux, K. (2008) Acetylcholinesterase-inhibiting activity of pyrrole derivatives from a novel marine gliding bacterium, *Rapidithrix thailandica*. *Mar. Drugs*, **6** (4), 578–586.

31 Holst, P.B., Anthoni, U., Christophersen, C., and Nielsen, P.H. (1994) Marine alkaloids, 15. Two alkaloids, flustramine E and debromoflustramine B, from the arine bryozoan *Flustra foliacea*. *J. Nat. Prod.*, **57** (7), 997–1000.

32 Rivera-Becerril, E., Joseph-Nathan, P., Perez-Alvarez, V.M., and Morales-Rios, M.S. (2008) Synthesis and biological evaluation of (-)- and (+)-debromoflustramine B and its analogues as selective butyrylcholinesterase inhibitors. *J. Med. Chem.*, **51** (17), 5271–5284.

33 Turk, T., Maček, P., and Šuput, D. (1995) Inhibition of acetylcholinesterase by a pseudozoanthoxanthin-like compound isolated from the zoanthid *Parazoanthus axinellae* (O. Schmidt). *Toxicon*, **33** (2), 133–142.

34 Sepcić, K., Mancini, I., Vidic, I., Franssanito, R., Pietra, F., Macek, P., and Turk, T. (2001) Antibacterial and anticholinesterase activities of aplysamine-4, a bromotyrosine-derived metabolite of a Red Sea marine sponge. *J. Nat. Toxins*, **10** (3), 181–191.

35 Choi, B.W., Ryu, G., Park, S.H., Kim, E.S., Shin, J., Roh, S.S., Shin, H.C., and Lee, B.H. (2007) Anticholinesterase activity of plastoquinones from *Sargassum sagamianum*: lead compounds for Alzheimer's disease therapy. *Phytother. Res.*, **21** (5), 423–426.

36 Lee, S.R., Pronto, J.R.D., Sarankhuu, B.-E., Ko, K.S., Rhee, B.D., Kim, N., Mishchenko, N.P., Fedoreyev, S.A., Stonik, V.A., and Han, J. (2014) Acetylcholinesterase inhibitory activity of pigment echinochrome a from sea urchin *Scaphechinus mirabilis*. *Mar. Drugs*, **12** (6), 3560–3573, 3514 pp.

37 Abramson, S.N., Radic, Z., Manker, D., Faulkner, D.J., and Taylor, P. (1989) Onchidal: a naturally occurring irreversible inhibitor of acetylcholinesterase with a novel mechanism of action. *Mol. Pharmacol.*, **36** (3), 349–354.

38 Deng, C.-M., Liu, S.-X., Huang, C.-H., Pang, J.-Y., and Lin, Y.-C. (2013) Secondary metabolites of a mangrove endophytic fungus *Aspergillus terreus* (No. GX7-3B) from the South China Sea. *Mar. Drugs*, **11** (7), 2616–2624, 2619 pp.

39 Wen, L., Du, D., Liu, F., She, Z., Lin, Y., and Zheng, Z. (2007) Secondary metabolites of a marine mangrove fungus *Paecilomyces* sp. Tree 1-7. *Zhongshan Da Xue Xue Bao Zi Ran Ke Xue Ban*, **46** (3), 125–127.

40 Wen, L., Lin, Y.-C., She, Z.-G., Du, D.-S., Chan, W.-L., and Zheng, Z.-H. (2008) Paeciloxanthone, a new cytotoxic xanthone from the marine mangrove fungus *Paecilomyces* sp. (Tree1-7). *J. Asian Nat. Prod. Res.*, **10** (2), 133–137.

41 Turk, T., Frangež, R., and Sepčić, K. (2007) Mechanisms of toxicity of 3-alkylpyridinium polymers from marine sponge *Reniera sarai*. *Mar. Drugs*, **5** (4), 157–167.

42 Alonso, D., Castro, A., and Martinez, A. (2005) Marine compounds for the therapeutic treatment of neurological disorders. *Expert Opin. Ther. Pat.*, **15** (10), 1377–1386.

43 Roll, D.M., Ireland, C.M., Lu, H.S.M., and Clardy, J. (1988) Fascaplysin, an unusual antimicrobial pigment from the marine sponge *Fascaplysinopsis* sp. *J. Org. Chem.*, **53** (14), 3276–3278.

44 Bharate, S.B., Manda, S., Joshi, P., Singh, B., and Vishwakarma, R.A. (2012) Total synthesis and anti-cholinesterase activity of marine-derived bis-indole alkaloid fascaplysin. *MedChemComm*, **3** (9), 1098–1103.

45 Erdogan-Orhan, I., Ozcelik, B., Konuklugil, B., Putz, A., Kaban, U.G., and Proksch, P. (2012) Bioactivity screening of the selected Turkish marine sponges and three compounds from *Agelas oroides*. *Rec. Nat. Prod.*, **6** (4), 356–367.

46 Kubota, T., Ishiguro, Y., Yamamoto, S., Fromont, J., and Kobayashi, J.I. (2010) Plastisidines A-C, N-methylpyridinium alkaloids from an Okinawan marine sponge of *Plakortis species*. *Heterocycles*, **80** (2), 1407–1412.

47 Cimino, G., De Rosa, S., De Stefano, S., and Sodano, G. (1986) Marine natural products: new results from Mediterranean invertebrates. *Pure Appl. Chem.*, **58** (3), 375–386.

48 Cimino, G., De Stefano, S., Scognamiglio, G., Sodano, G., and Trivellone, E. (1986) Sarains. A new class of alkaloids from the marine sponge *Reniera sarai*. *Bull. Soc. Chim. Belg.*, **95** (9-10), 783–800.

49 Cimino, G., Mattia, C.A., Mazzarella, L., Puliti, R., Scognamiglio, G., Spinella, A., and Trivellone, E. (1989) Unprecedented alkaloid skeleton from the Mediterranean sponge *Reniera sarai*: X-ray structure of an acetate derivative of saraine-A. *Tetrahedron*, **45** (12), 3863–3872.

50 Cimino, G., Spinella, A., and Trivellone, E. (1989) Isosarain-1: a new alkaloid from the Mediterranean sponge *Reniera sarai*. *Tetrahedron Lett.*, **30** (1), 133–136.

51 Guo, Y., Madaio, A., Trivelllone, E., Scognamiglio, G., and Cimino, G. (1996) Further studies of alkaloids from *Reniera sarai*: structures of saraine-3 and isosaraine-3; absolute stereochemistry of saraine-1 and saraine-2. *Tetrahedron*, **52** (47), 14961–14974.

52 Guo, Y., Trivellone, E., Scognamiglio, G., and Cimino, G. (1998) Absolute stereochemistry of isosaraine-1 and isosaraine-2. *Tetrahedron Lett.*, **39** (5/6), 463–466.

53 Caprioli, V., Cimino, G., De Giulio, A., Madaio, A., Scognamiglio, G., and Trivellone, E. (1992) Selected biological activities of saraines. *Comp. Biochem. Physiol. B*, **103B** (1), 293–296.

54 Defant, A., Mancini, I., Raspor, L., Guella, G., Turk, T., and Sepcic, K. (2011) New structural insights into saraines A, B, and C, macrocyclic alkaloids from the Mediterranean sponge *Reniera* (Haliclona) *sarai*. *Eur. J. Org. Chem.*, **2011** (20-21), 3761–3767, S3761/3761–S3761/3769.

55 Liu, Y.-W., Shi, D.-H., Chen, A.-J., Zhu, Q., Xu, J.-T., and Zhang, X.-X. (2014) Acetylcholinesterase inhibition effects of marine fungi. *Pharm. Biol.*, **52** (5), 539–543.

56 Beedessee, G., Ramanjooloo, A., Surnam-Boodhun, R., van Soest, R.W.M., and Marie, D.E.P. (2013) Acetylcholinesterase-inhibitory activities of the extracts from sponges collected in Mauritius waters. *Chem. Biodivers.*, **10** (3), 442–451.

57 Kem, W.R., Mahnir, V.M., Papke, R.L., and Lingle, C.J. (1997) Anabaseine is a potent agonist on muscle and neuronal alpha-bungarotoxin-sensitive nicotinic receptors. *J. Pharmacol. Exp. Ther.*, **283** (3), 979–992.

58 Williams, P., Sorribas, A., and Howes, M.-J.R. (2011) Natural products as a source of Alzheimer's drug leads. *Nat. Prod. Rep.*, **28** (1), 48–77.

59 Kem, W.R., Abbott, B.C., and Coates, R.M. (1971) Isolation and structure of a hoplonemertine toxin. *Toxicon*, **9** (1), 15–22.

60 Wheeler, J.W., Olubajo, O., Storm, C.B., and Duffield, R.M. (1981) Anabaseine: venom alkaloid of Aphaenogaster ants. *Science*, **211** (4486), 1051–1052.

61 Martin, E.J., Panickar, K.S., King, M.A., Deyrup, M., Hunter, B.E., Wang, G., and Meyer, E.M. (1994) Cytoprotective actions of 2,4-dimethoxybenzylidene anabaseine in differentiated PC12 cells and septal cholinergic neurons. *Drug Dev. Res.*, **31** (2), 135–141.

62 Meyer, E.M., Kuryatov, A., Gerzanich, V., Lindstrom, J., and Papke, R.L. (1998) Analysis of 3-(4-hydroxy, 2-methoxybenzylidene)anabaseine selectivity and activity at human and rat alpha-7 nicotinic receptors. *J. Pharmacol. Exp. Ther.*, **287** (3), 918–925.

63 Meyer, E.M., King, M.A., and Meyers, C. (1998) Neuroprotective effects of 2,4-dimethoxybenzylidene anabaseine (DMXB) and tetrahydroaminoacridine (THA) in neocortices of nucleus basalis lesioned rats. *Brain Res.*, **786** (1-2), 252–254.

64 Vassar, R. (2014) BACE1 inhibitor drugs in clinical trials for Alzheimer's disease. *Alzheimers Res. Ther.*, **6**, 89.

65 Andersson, E.R. and Lendahl, U. (2014) Therapeutic modulation of Notch signalling – are we there yet? *Nat. Rev. Drug Discovery*, **13** (5), 357–378.

66 Lopez-Ogalla, J., Garcia-Palomero, E., Sanchez-Quesada, J., Rubio, L., Delgado, E., Garcia, P., Medina, M., Castro, A., and Munoz, P. (2014) Bioactive prenylated phenyl derivatives derived from marine natural products: novel scaffolds for the design of BACE inhibitors. *MedChemComm*, **5** (4), 474–488.

67 Williams, P.G., Yoshida, W.Y., Moore, R.E., and Paul, V.J. (2003) The isolation and structure elucidation of tasiamide B, a 4-amino-3-hydroxy-5-phenylpentanoic acid containing peptide from the marine *Cyanobacterium Symploca* sp. *J. Nat. Prod.*, **66** (7), 1006–1009.

68 Liu, Y., Zhang, W., Li, L., Salvador, L.A., Chen, T., Chen, W., Felsenstein, K.M., Ladd, T.B., Price, A.R., Golde, T.E., He, J., Xu, Y., Li, Y., and Luesch, H. (2012) Cyanobacterial peptides as a prototype for the design of potent β-secretase inhibitors and the development of selective chemical probes for other aspartic proteases. *J. Med. Chem.*, **55** (23), 10749–10765.

69 Alzforum http://www.alzforum.org/news/research-news/experts-slam-marketing-tramiprosate-alzhemed-nutraceutical (retrived 12 April 2016).

70 Starling, S. http://www.nutraingredients-usa.com/Regulation/FDA-rejects-homotaurine-NDI-bid-finds-it-is-not-an-aminoacid?utm_source=copyright&utm_medium=OnSite& utm_campaign=copyright (retrived 12 April 2016).

71 Gervais, F., Paquette, J., Morissette, C., Krzywkowski, P., Yu, M., Azzi, M., Lacombe, D., Kong, X., Aman, A., Laurin, J., Szarek, W.A., and Tremblay, P. (2007) Targeting soluble Abeta peptide with tramiprosate for the treatment of brain amyloidosis. *Neurobiol. Aging*, **28** (4), 537–547.

72 Louzada, P.R., Paula Lima, A.C., Mendonca-Silva, D.L., Noel, F., De Mello, F.G., and Ferreira, S.T. (2004) Taurine prevents the neurotoxicity of beta-amyloid and glutamate receptor agonists: activation of GABA receptors and possible implications for Alzheimer's disease and other neurological disorders. *FASEB J.*, **18** (3), 511–518.

73 Liu, X.-P., Qian, X., Xie, Y., Qi, Y., Peng, M.-F., Zhan, B.-C., and Lou, Z.-Q. (2014) Betaine suppressed Aβ generation by altering amyloid precursor protein processing. *Neurol. Sci.*, **35** (7), 1009–1013.

74 Zhang, H., Conte, M.M., Khalil, Z., Huang, X.-C., and Capon, R.J. (2012) New dictyodendrins as BACE inhibitors from a southern Australian marine sponge, *Ianthella* sp. *RSC Adv.*, **2** (10), 4209–4214.

75 Zhang, H., Conte, M.M., Huang, X.C., Khalil, Z., and Capon, R.J. (2012) A search for BACE inhibitors reveals new biosynthetically related pyrrolidones, furanones and pyrroles from a southern Australian marine sponge, *Ianthella* sp. *Org. Biomol. Chem.*, **10** (13), 2656–2663.

76 Millán-Aguiñaga, N., Soria-Mercado, I.E., and Williams, P. (2010) Xestosaprol D and E from the Indonesian marine sponge *Xestospongia* sp. *Tetrahedron Lett.*, **51** (4), 751–753.

77 Pajouhesh, H. and Lenz, G.R. (2005) Medicinal chemical properties of successful central nervous system drugs. *NeuroRx*, **2** (4), 541–553.

78 Hansch, C., Bjorkroth, J.P., and Leo, A. (1987) Hydrophobicity and central nervous system agents: on the principle of minimal hydrophobicity in drug design. *J. Pharm. Sci.*, **76** (9), 663–687.

79 Dai, J., Sorribas, A., Yoshida, W.Y., Kelly, M., and Williams, P.G. (2010) Xestosaprols from the Indonesian marine sponge *Xestospongia* sp. *J. Nat. Prod.*, **73** (6), 1188–1191.

80 Watters, C.A., Edmonds, C.M., Rosner, L.S., Sloss, K.P., and Leung, P. (2012) A cost analysis of EPA and DHA in fish, supplements, and foods. *J. Nutr. Food Sci.*, **2** (8), 159.

81 Bazan, N.G. (2006) Cell survival matters: docosahexaenoic acid signaling, neuroprotection and photoreceptors. *Trends Neurosci.*, **29** (5), 263–271.

82 Lim, G.P., Calon, F., Morihara, T., Yang, F., Teter, B., Ubeda, O., Salem, N. Jr., Frautschy, S.A., and Cole, G.M. (2005) A diet enriched with the omega-3 fatty

acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. *J. Neurosci.*, **25** (12), 3032–3040.

83 Lukiw, W.J., Cui, J.-G., Marcheselli, V.L., Bodker, M., Botkjaer, A., Gotlinger, K., Serhan, C.N., and Bazan, N.G. (2005) A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. *J. Clin. Invest.*, **115** (10), 2774–2783.

84 Calon, F., Lim, G.P., Yang, F., Morihara, T., Teter, B., Ubeda, O., Rostaing, P., Triller, A., Salem, N. Jr., Ashe, K.H., Frautschy, S.A., and Cole, G.M. (2004) Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron*, **43** (5), 633–645.

85 Cole, G.M. and Frautschy, S.A. (2006) Docosahexaenoic acid protects from amyloid and dendritic pathology in an Alzheimer's disease mouse model. *Nutr. Health*, **18** (3), 249–259.

86 Zhao, Y., Calon, F., Julien, C., Winkler, J.W., Petasis, N.A., Lukiw, W.J., and Bazan, N.G. (2011) Docosahexaenoic acid-derived neuroprotectin D1 induces neuronal survival via secretase- and PPARγ-mediated mechanisms in Alzheimer's disease models. *PLoS One*, **6** (1), e15816.

87 Pettit, G.R., Herald, C.L., Doubek, D.L., Herald, D.L., Arnold, E., and Clardy, J. (1982) Isolation and structure of bryostatin 1. *J. Am. Chem. Soc.*, **104** (24), 6846–6848.

88 Kortmansky, J. and Schwartz, G.K. (2003) Bryostatin-1: a novel PKC inhibitor in clinical development. *Cancer Invest.*, **21** (6), 924–936.

89 Ruan, B.F. and Zhu, H.L. (2012) The chemistry and biology of the bryostatins: potential PKC inhibitors in clinical development. *Curr. Med. Chem.*, **19** (16), 2652–2664.

90 Etcheberrigaray, R., Tan, M., Dewachter, I., Kuiperi, C., van der Auwera, I., Wera, S., Qiao, L., Bank, B., Nelson, T.J., Kozikowski, A.P., van Leuven, F., and Alkon, D.L. (2004) Therapeutic effects of PKC activators in Alzheimer's disease transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.*, **101** (30), 11141–11146.

91 Schrott, L.M., Jackson, K., Yi, P., Dietz, F., Johnson, G.S., Basting, T.F., Purdum, G., Tyler, T., Rios, J.D., Castor, T.P., and Alexander, J.S. (2015) Acute oral bryostatin-1 administration improves learning deficits in the APP/PS1 transgenic mouse model of Alzheimer's disease. *Curr. Alzheimer Res.*, **12** (1), 22–31.

92 Mutter, R. and Wills, M. (2000) Chemistry and clinical biology of the bryostatins. *Bioorg. Med. Chem.*, **8** (8), 1841–1860.

93 Dai, J., Sorribas, A., Yoshida, W.Y., Kelly, M., and Williams, P.G. (2010) Topsentinols, 24-isopropyl steroids from the marine sponge *Topsentia sp*. *J. Nat. Prod.*, **73** (9), 1597–1600.

94 Pangestuti, R., Ryu, B., Himaya, S.W.A., and Kim, S.-K. (2013) Optimization of hydrolysis conditions, isolation, and identification of neuroprotective peptides derived from seahorse *Hippocampus trimaculatus*. *Amino Acids*, **45** (2), 369–381.

95 Calcul, L., Inman, W.D., Morris, A.A., Tenney, K., Ratnam, J., McKerrow, J.H., Valeriote, F.A., and Crews, P. (2010) Additional insights on the bastadins: isolation of analogues from the sponge *Ianthella* cf. reticulata and exploration of the oxime configurations. *J. Nat. Prod.*, **73** (3), 365–372.

96 Williams, P., Sorribas, A., and Liang, Z. (2010) New methods to explore marine resources for Alzheimer's therapeutics. *Curr. Alzheimer Res.*, **7** (3), 210–213.

97 Carney, J.R., Scheuer, P.J., and Kelly-Borges, M. (1993) A new bastadin from the sponge *Psammaplysilla purpurea*. *J. Nat. Prod.*, **56** (1), 153–157.

98 Wu, Y.J., Gerritz, S., Shi, S., and Zhu, S. (2010) Oxime-containing macrocyclic acyl guanidines as β-secretase inhibitors. US Patent 7,678,784, dated Mar. 16, 2010.

99 Dai, J., JiméAnez, J.I., Kelly, M., and Williams, P.G. (2010) Dictazoles: potential vinyl cyclobutane biosynthetic precursors to the dictazolines. *J. Org. Chem.*, **75** (7), 2399–2402.

100 Hills, I.D. and Vacca, J.P. (2007) Progress toward a practical BACE-1 inhibitor. *Curr. Opin. Drug Discovery Dev.*, **10** (4), 383–391.

101 Kharrat, R., Servent, D., Girard, E., Ouanounou, G., Amar, M., Marrouchi, R., Benoit, E., and Molgo, J. (2008) The marine phycotoxin gymnodimine targets muscular and neuronal nicotinic acetylcholine receptor subtypes with high affinity. *J. Neurochem.*, **107** (4), 952–963.

102 Alonso, E., Vale, C., Vieytes, M.R., Laferla, F.M., Gimenez-Llort, L., and Botana, L.M. (2011) The cholinergic antagonist gymnodimine improves Aβ and *tau* neuropathology in an *in vitro* model of Alzheimer disease. *Cell. Physiol. Biochem.*, **27** (6), 783–794.

103 Gribble, G.W. (2003) The diversity of naturally produced organohalogens. *Chemosphere*, **52** (2), 289–297.

104 González, A.V., Rodríguez-Velasco, M.L., Ben-Gigirey, B., and Botana, L.M. (2006) First evidence of spirolides in Spanish shellfish. *Toxicon*, **48** (8), 1068–1074.

105 Gill, S., Murphy, M., Clausen, J., Richard, D., Quilliam, M., MacKinnon, S., LaBlanc, P., Mueller, R., and Pulido, O. (2003) Neural injury biomarkers of novel shellfish toxins, spirolides: a pilot study using immunochemical and transcriptional analysis. *Neurotoxicology*, **24** (4-5), 593–604.

106 Alonso, E., Vale, C., Vieytes, M.R., Laferla, F.M., Gimenez-Llort, L., and Botana, L.M. (2011) 13-Desmethyl spirolide-C is neuroprotective and reduces intracellular Aβ and hyperphosphorylated *tau in vitro*. *Neurochem. Int.*, **59** (7), 1056–1065.

107 Alonso, E., Otero, P., Vale, C., Alfonso, A., Antelo, A., Gimenez-Llort, L., Chabaud, L., Guillou, C., and Botana, L.M. (2013) Benefit of 13-desmethyl spirolide C treatment in triple transgenic mouse model of Alzheimer disease: beta-amyloid and neuronal markers improvement. *Curr. Alzheimer Res.*, **10** (3), 279–289.

108 Satake, M., Murata, M., and Yasumoto, T. (1993) Gambierol: a new toxic polyether compound isolated from the marine dinoflagellate *Gambierdiscus toxicus*. *J. Am. Chem. Soc.*, **115** (1), 361–362.

109 Alonso, E., Fuwa, H., Vale, C., Suga, Y., Goto, T., Konno, Y., Sasaki, M., LaFerla, F.M., Vieytes, M.R., Giménez-Llort, L., and Botana, L.M. (2012) Design and synthesis of skeletal analogues of gambierol: attenuation of amyloid-β and *tau* pathology with voltage-gated potassium channel and N-methyl-D-aspartate receptor implications. *J. Am. Chem. Soc.*, **134** (17), 7467–7479.

110 Satake, M., MacKenzie, L., and Yasumoto, T. (1997) Identification of *Protoceratium reticulatum* as the biogenetic origin of yessotoxin. *Nat. Toxins*, **5** (4), 164–167.

111 Paz, B., Riobo, P., Fernandez, M.L., Fraga, S., and Franco, J.M. (2004) Production and release of yessotoxins by the dinoflagellates *Protoceratium reticulatum* and *Lingulodinium polyedrum* in culture. *Toxicon*, **44** (3), 251–258.

112 Alonso, E., Vale, C., Vieytes, M.R., and Botana, L.M. (2013) Translocation of PKC by yessotoxin in an in vitro model of Alzheimer's disease with improvement of *tau* and beta-amyloid pathology. *ACS Chem. Neurosci.*, **4** (7), 1062–1070.

113 Wu, H., Zhang, F., Williamson, N., Jian, J., Zhang, L., Liang, Z., Wang, J., An, L., Tunnacliffe, A., and Zheng, Y. (2014) Effects of secondary metabolite extract from *Phomopsis occulta* on β-amyloid aggregation. *PLoS One*, **9** (10), e109438.

114 Hernandez, F., Gomez de Barreda, E., Fuster-Matanzo, A., Lucas, J.J., and Avila, J. (2010) GSK3: a possible link between beta amyloid peptide and *tau* protein. *Exp. Neurol.*, **223** (2), 322–325.

115 Hooper, C., Killick, R., and Lovestone, S. (2008) The GSK3 hypothesis of Alzheimer's disease. *J. Neurochem.*, **104** (6), 1433–1439.

116 Klein, P.S. and Melton, D.A. (1996) A molecular mechanism for the effect of lithium on development. *Proc. Natl. Acad. Sci. U.S.A.*, **93** (16), 8455–8459.

117 Ryves, W.J. and Harwood, A.J. (2001) Lithium inhibits glycogen synthase kinase-3 by competition for magnesium. *Biochem. Biophys. Res. Commun.*, **280** (3), 720–725.

118 De Sarno, P., Li, X., and Jope, R.S. (2002) Regulation of AKT and glycogen synthase kinase-3 beta phosphorylation by sodium valproate and lithium. *Neuropharmacology*, **43** (7), 1158–1164.

119 Phiel, C.J., Wilson, C.A., Lee, V.M., and Klein, P.S. (2003) GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. *Nature*, **423** (6938), 435–439.

120 Sakai, R., Higa, T., Jefford, C.W., and Bernardinelli, G. (1986) Manzamine A, a novel antitumor alkaloid from a sponge. *J. Am. Chem. Soc.*, **108** (20), 6404–6405.

121 Hamann, M., Alonso, D., Martin-Aparicio, E., Fuertes, A., Perez-Puerto, M.J., Castro, A., Morales, S., Navarro, M.L., Del Monte-Millan, M., Medina, M., Pennaka, H., Balaiah, A., Peng, J., Cook, J., Wahyuono, S., and Martinez, A. (2007) Glycogen synthase kinase-3 (GSK-3) inhibitory activity and structure-activity relationship (SAR) studies of the manzamine alkaloids; potential for Alzheimer's disease. *J. Nat. Prod.*, **70** (9), 1397–1405.

122 Barrow, C.J., Blunt, J.W., Munro, M.H.G., and Perry, N.B. (1988) Oxygenated furanosesterterpene tetronic acids from a sponge of the *Genus Ircinia*. *J. Nat. Prod.*, **51** (6), 1294–1298.

123 Perez Vazquez, M., Gomez Pacios, G., and Fall Diop, Y. (2008) Method for Preparation of a Furanosesterterpene of Marine Origin. PCT/GB2004/050033, December 2, 2004.

124 Bidon-Chanal, A., Fuertes, A., Alonso, D., Pérez, D.I., Martínez, A., Luque, F.J., and Medina, M. (2013) Evidence for a new binding mode to GSK-3: allosteric

regulation by the marine compound palinurin. *Eur. J. Med. Chem.*, **60**, 479–489.

125 Pérez, V.M., Gómez, P.G., and Fall, D.Y. (2008) Method for preparation of a furanosesterpene of marine origin. WO Patent 2008116948A1, dated Jun. 16, 2005.

126 Nguyen, T.N. and Tepe, J.J. (2009) Preparation of hymenialdisine, analogues and their evaluation as kinase inhibitors. *Curr. Med. Chem.*, **16** (24), 3122–3143.

127 Meijer, L., Thunnissen, A.M.W.H., White, A.W., Garnier, M., Nikolic, M., Tsai, L.H., Walter, J., Cleverley, K.E., Salinas, P.C., Wu, Y.Z., Biernat, J., Mandelkow, E.M., Kim, S.H., and Pettit, G.R. (2000) Inhibition of cyclin-dependent kinases, GSK-3β and CK1 by hymenialdisine, a marine sponge constituent. *Chem. Biol.*, **7** (1), 51–63.

128 Plisson, F., Prasad, P., Xiao, X., Piggott, A.M., Huang, X.-c., Khalil, Z., and Capon, R.J. (2014) Callyspongisines A-D: bromopyrrole alkaloids from an Australian marine sponge, *Callyspongia* sp. *Org. Biomol. Chem.*, **12** (10), 1579–1584.

129 Chan, G.W., Mong, S., Hemling, M.E., Freyer, A.J., Offen, P.H., DeBrosse, C.W., Sarau, H.M., and Westley, J.W. (1993) New leukotriene B4 receptor antagonist: leucettamine A and related imidazole alkaloids from the marine sponge *Leucetta microraphis*. *J. Nat. Prod.*, **56** (1), 116–121.

130 Watanabe, K., Tsuda, Y., Iwashima, M., and Iguchi, K. (2000) A new bioactive triene aldehyde from the marine sponge *Leucetta microraphis*. *J. Nat. Prod.*, **63** (2), 258–260.

131 Tahtouh, T., Elkins, J.M., Filippakopoulos, P., Soundararajan, M., Burgy, G., Durieu, E., Cochet, C., Schmid, R.S., Lo, D.C., Delhommel, F., Oberholzer, A.E., Pearl, L.H., Carreaux, F., Bazureau, J.-P., Knapp, S., and Meijer, L. (2012) Selectivity, cocrystal structures, and neuroprotective properties of leucettines, a family of protein kinase inhibitors derived from the marine sponge alkaloid leucettamine B. *J. Med. Chem.*, **55** (21), 9312–9330.

132 Simone, M., Erba, E., Damia, G., Vikhanskaya, F., Di Francesco, A.M., Riccardi, R., Bailly, C., Cuevas, C., Fernandez Sousa-Faro, J.M., and D'Incalci, M. (2005) Variolin B and its derivate deoxy-variolin B: new marine natural compounds with cyclin-dependent kinase inhibitor activity. *Eur. J. Cancer*, **41** (15), 2366–2377.

133 Echalier, A., Bettayeb, K., Ferandin, Y., Lozach, O., Clement, M., Valette, A., Liger, F., Marquet, B., Morris, J.C., Endicott, J.A., Joseph, B., and Meijer, L. (2008) Meriolins (3-(pyrimidin-4-yl)-7-azaindoles): synthesis, kinase inhibitory activity, cellular effects, and structure of a CDK2/cyclin A/meriolin complex. *J. Med. Chem.*, **51** (4), 737–751.

134 Meijer, L., Skaltsounis, A.L., Magiatis, P., Polychronopoulos, P., Knockaert, M., Leost, M., Ryan, X.P., Vonica, C.A., Brivanlou, A., Dajani, R., Crovace, C., Tarricone, C., Musacchio, A., Roe, S.M., Pearl, L., and Greengard, P. (2003) GSK-3-selective inhibitors derived from Tyrian purple indirubins. *Chem. Biol.*, **10** (12), 1255–1266.

135 Mohamed, G.A., Ibrahim, S.R.M., Badr, J.M., and Youssef, D.T.A. (2014) Didemnaketals D and E, bioactive terpenoids from a Red Sea ascidian *Didemnum* species. *Tetrahedron*, **70** (1), 35–40.

136 Plisson, F., Conte, M., Khalil, Z., Huang, X.C., Piggott, A.M., and Capon, R.J. (2012) Kinase inhibitor scaffolds against neurodegenerative diseases from a Southern Australian ascidian, *Didemnum* sp. *ChemMedChem*, **7** (6), 983–990.

137 Kang, H. and Fenical, W. (1997) Ningalins A-D: novel aromatic alkaloids from a western Australian ascidian of the genus *Didemnum*. *J. Org. Chem.*, **62** (10), 3254–3262.

138 Bharate, S.B., Yadav, R.R., Battula, S., and Vishwakarma, R.A. (2012) Meridianins: marine-derived potent kinase inhibitors. *Mini Rev. Med. Chem.*, **12** (7), 618–631.

139 Franco, L.H., Joffé, E.B.d.K., Puricelli, L., Tatian, M., Seldes, A.M., and Palermo, J.A. (1998) Indole alkaloids from the tunicate *Aplidium meridianum*. *J. Nat. Prod.*, **61** (9), 1130–1132.

140 Radwan, M.A.A. and El-Sherbiny, M. (2007) Synthesis and antitumor activity of indolylpyrimidines: marine natural product meridianin D analogues. *Bioorg. Med. Chem.*, **15** (3), 1206–1211.

141 Mudit, M., Khanfar, M., Muralidharan, A., Thomas, S., Shah, G.V., van Soest, R.W.M., and El Sayed, K.A. (2009) Discovery, design, and synthesis of anti-metastatic lead phenylmethylene hydantoins inspired by marine natural products. *Bioorg. Med. Chem.*, **17** (4), 1731–1738.

142 Mudit, M., Khanfar, M., Muralidharan, A., Thomas, S., Shah, G.V., van Soest, R.W.M., and El Sayed, K.A. (2009) Discovery, design, and synthesis of anti-metastatic lead phenylmethylene hydantoins inspired by marine natural products. *Bioorg. Med. Chem.*, **17**, 1731–1738. Erratum in: *Bioorg. Med. Chem.* (2009) **17** (8), 3216–3217.

143 Khanfar, M.A., Abu Asal, B., Mudit, M., Kaddoumi, A., and El Sayed, K.A. (2009) The marine natural-derived inhibitors of glycogen synthase kinase-3β phenylmethylene hydantoins: *in vitro* and *in vivo* activities and pharmacophore modeling. *Bioorg. Med. Chem.*, **17** (16), 6032–6039.

144 Tringali, C. and Piatelli, M. (1982) Two chrome derivatives from the brown alga *Zonaria tournefortii*. *Tetrahedron Lett.*, **23** (14), 1509–1512.

145 Zhang, H., Xiao, X., Conte, M.M., Khalil, Z., and Capon, R.J. (2012) Spiralisones A-D: acylphloroglucinol hemiketals from an Australian marine brown alga, *Zonaria spiralis*. *Org. Biomol. Chem.*, **10** (48), 9671–9676.

146 D'Ambrosio, M.A., Guerriero, C., Debitus, O., Ribes, J., Pusset, S., Leroy, and Pietra, F. (1993) Agelastatin a, a new skeleton cytotoxic alkaloid of the oroidin family. Isolation from the axinellid sponge *Agelas dendromorpha* of the coral sea. *J. Chem. Soc., Chem. Commun.*, (16), 1305–1306.

147 Guerriero, A., D'Ambrosio, M., Chiasera, G., and Pietra, F. (1994) Conformational preferences and absolute configuration of agelastatin A, a cytotoxic alkaloid of the Axinellid Sponge *Agelas dendromorpha* from the Coral Sea, *via* combined molecular modelling, NMR, and exciton splitting for diamide and hydroxyamide derivatives. *Helv. Chim. Acta*, **77** (7), 1895–1902.

148 Leiros, M., Sanchez, J.A., Alonso, E., Rateb, M.E., Houssen, W.E., Ebel, R., Jaspars, M., Alfonso, A., and Botana, L.M. (2014) Spongionella secondary metabolites protect mitochondrial function in cortical neurons against oxidative stress. *Mar. Drugs*, **12** (2), 700–718, 719 pp.

149 Wu, F.-J., Xue, Y., Liu, X.-F., Xue, C.-H., Wang, J.-F., Du, L., Takahashi, K., and Wang, Y.-M. (2014) The protective effect of eicosapentaenoic acid-enriched phospholipids from sea cucumber *Cucumaria frondosa* on oxidative stress in PC12 cells and SAMP8 mice. *Neurochem. Int.*, **64**, 9–17.

150 Sun, C., Shan, C.Y., Gao, X.D., and Tan, R.X. (2005) Protection of PC12 cells from hydrogen peroxide-induced injury by EPS2, an exopolysaccharide from a marine filamentous fungus *Keissleriella sp*. YS4108. *J. Biotechnol.*, **115** (2), 137–144.

151 Sun, C., Wang, J.-W., Fang, L., Gao, X.-D., and Tan, R.-X. (2004) Free radical scavenging and antioxidant activities of EPS2, an exopolysaccharide produced by a marine filamentous fungus *Keissleriella sp*. YS 4108. *Life Sci.*, **75** (9), 1063–1073.

152 Aisen, P.S., Gauthier, S., Ferris, S.H., Saumier, D., Haine, D., Garceau, D., Duong, A., Suhy, J., Oh, J., Lau, W.C., and Sampalis, J. (2011) Tramiprosate in mild-to-moderate Alzheimer's disease – a randomized, double-blind, placebo-controlled, multi-centre study (the Alphase Study). *Arch. Med. Sci.*, **7** (1), 102–111.

153 Quinn, J.F., Raman, R., Thomas, R.G., Yurko-Mauro, K., Nelson, E.B., Van Dyck, C., Galvin, J.E., Emond, J., Jack, C.R. Jr., Weiner, M., Shinto, L., and Aisen, P.S. (2010) Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial. *J. Am. Med. Assoc.*, **304** (17), 1903–1911.

154 Clinical Trials.gov https://clinicaltrials.gov (accessed 21 September 2016).

8

Natural Products against Huntington's Disease (HD): Implications of Neurotoxic Animal Models and Transgenics in Preclinical Studies

Abhijit Dey

Ethnopharmacology and Natural Products Research Laboratory, Department of Life Sciences, Presidency University, 86/1 College Street, Kolkata 700073, India

8.1 Introduction

Huntington's disease (HD) is a chronic progressive and incurable autosomal dominant genetic neurodegenerative fatal disease characterized by abnormal involuntary movements, impaired voluntary movements, and cognitive and psychiatric disturbances associated with neuronal death in corticostriatal circuits [1–4]. Abnormal involuntary movements are associated with chorea and dystonia, whereas impaired voluntary movements involve incoordination and gait balance [1]. Besides, patients exhibit a typical sporadic, rapid, involuntary control of limb movement, limb stiffness, lack of cognition, and serious psychiatric disturbances, leading to death in 15–20 years [5, 6]. HD is caused by a cytosine, adenine, guanine (CAG) repeat mutation in the huntingtin (Htt) gene, encoding the huntingtin protein [1, 7]. The inherited mutation results in the production of an elongated polyglutamine (polyQ) mutant huntingtin protein (mHtt) widely expressed outside the central nervous system (CNS) [1, 2]. Although HD is caused by genetic mutation leading to abnormal CAG expansion within the Htt gene on chromosome 4p16.3, the underlying mechanism of targeting striatal neurons and the process of neuronal cell death are yet to be explored [5]. The cellular functions of the Htt protein have also not yet been fully elucidated [1]. However, expression of mHtt is correlated with transcriptional dysregulation associated with disturbance of histone-modifying complexes and changed interactions with chromatin-related factors [7]. Moreover, altered energy production and dysregulation of neurotransmitter metabolism, receptors, and growth factors are also attributed to the mHtt functionality [1]. Peripheral signs of HD often include weight loss and enhancement in proinflammatory signaling, but their implication in HD pathophysiology is yet to be resolved [2]. Besides, altered innate immune cell function and increased inflammation are observed many years prior to the HD symptom possibly due to the expression of mHtt in innate immune cells themselves [8].

Although the disorder had earlier been reported, Huntington's chorea or HD was first described in detail by George Huntington, an Ohio physician [9]. Initially

Neuroprotective Natural Products: Clinical Aspects and Mode of Action, First Edition.
Edited by Goutam Brahmachari.

HD was described as a chronic encephalitis [10], which was for the first time described as characteristic neuropathologic alteration within the basal ganglia in 1908 [11]. HD is prevalent globally irrespective of the races and ethnic groups [12]. HD is of rare occurrence; worldwide only in 3–7 persons per 100 000 populations are known to suffer from HD, and among 100 000 people, approximately 20 are reported as carriers [13–15]. Moreover, a new mutation rate as high as 1–3% was also observed [16]. In most European populations, a high prevalence (4–8 per 100 000) of HD has been observed with its possible frequent presence in India and Central Asian region. However, limited data is available on its occurrence in Eastern Asian, African, and black American populations. High frequency of HD in Europeans may be attributed to one or very few mutations in the HD gene [17]. In another study, devastating prevalence of HD in Europe, North America, and Australia than in Asia was possibly implicated to the huntingtin gene haplotypes [18]. The prevalence of HD in India was reported to be comparable with that of in Western Europe [19]. Although the average age of onset is 38 years, the pathologic changes manifest in the brain years prior to the appearance of HD symptoms [20].

HD is of genetic origin that still lacks effective treatment strategies due to lack of ample knowledge on its pathogenesis even after almost two decades when the genetics of HD was discovered. Clinical symptoms of HD are mostly treated with conventional therapies due to limitations of treatment to block progressive neuronal loss and behavioral and psychiatric disorders [21]. Non-pharmacological treatments of HD include genetic counseling and therapy and palliative care [22]. To treat the motor, psychiatric, and cognitive decline associated with HD, very few treatments are currently available [23]. Tetrabenazine (TBZ), used to treat chorea, suffers from drug interactions and side effects [24]. Selective serotonin reuptake inhibitors (SSRIs) and mood stabilizers have also been used to manage the early symptoms of HD [23, 25]. Evidence-based treatment approaches are also used besides education and symptomatic relief offered by the medical practitioners to the HD patients, affected families, and caregivers [23, 26, 27]. Incidentally, the high suicidality among HD patients and their caregivers was found to be related to rational suicide attempts [28]. Molecular therapeutic interventions against this incurable disease included silencing of the mutated gene and neutralization of its toxic product [29]. A number of preclinical studies indicated the possible efficacy of RNA interference (RNAi), antisense oligonucleotides (ASOs), ribozymes, DNA enzymes, and genome-editing methods to either silence or restore the mHtt gene [30]. Other advancements in the treatment strategies involve fetal neural transplantation and transglutaminase inhibitors (Tgasei) [5].

8.2 Methodology

By searching PUBMED database using general search strings such as "Huntington's disease," "Huntington's medicinal plants," "Huntington's herb," "3-nitropropionic acid," "huntingtin," "Chinese herbal," "Ayurveda," "neurological disorder, medicinal plants," "Huntington's disease models," and so on, a number of citations were retrieved. Cross-referencing among the retrieved literature produced potential papers and reviews. After initial screening, herbal medicines (extracts or isolated

compounds) are included alphabetically in Tables 8.1 and 8.2, respectively. The major biological effects and possible molecular mode of action of the anti-HD herbal medicines on neurotoxic HD models are summarized. A total of 17 plant species belonging to 16 genera and 16 families, 27 bioactive phytochemicals, and 2 herbal formulations are included in this review. The nomenclature of the plants and the plant families and author's citations were verified from the Missouri Botanical Garden's electronic database (www.tropicos.org). Figure 8.1 represents photographs of few anti-HD botanicals. Figure 8.2 presents the chemical structures of the

Table 8.1 Anti-HD activity of some of the medicinal plants.

Source botanicals	Family	Experimental models	Mechanisms of action	References
Bacopa monnieri (L.) Wettst. leaf powder	Plantaginaceae	3-NP, PP mice	↓Cytotoxicity, antioxidant	[31]
Boerhaavia diffusa L. polyphenol-rich ethanolic extract	Nyctaginaceae	3-NP, rat brain homogenates	Antioxidant	[32]
Centella asiatica (L.) Urb. leaf powder and aqueous extract	Apiaceae	3-NP, PP male mice	Antioxidant	[33]
C. asiatica aqueous extract		3-NP, male mice	Antioxidant, ↓mitochondrial dysfunction, ↑GSH, ↑thiols	[34]
C. asiatica aqueous extract		3-NP, PP mice	Antioxidant, ↑GSH, ↑thiols	[35]
Convolvulus pluricaulis Choisy ethyl acetate fraction of a methanol extract of the whole plant	Convolvulaceae	3-NP, rats	↑Locomotor activity, ↓behavioral damage, ↓body weight deficit, ↑MDA, ↑nitrite, ↑SOD, ↑reduced GSH	[36]
C. pluricaulis standardized hydromethanol extract and its fractions		3-NP, rats	↑Locomotor activity, ↑memory, ↓body weight deficit, ↑oxidative defense	[37]
Gastrodia elata Blume	Orchidaceae	Rats	↑Molecular chaperons	[38]
Luehea divaricata Mart. aqueous extract	Malvaceae	3-NP, rats	↑Locomotor activity, ↓ROS ↓lipid peroxidation, ↑GSH/GSSG ratio, ↑ AChE activity	[39]

(*Continued*)

Table 8.1 (Continued)

Source botanicals	Family	Experimental models	Mechanisms of action	References
Panax ginseng (Korean red ginseng) extract	Araliaceae	3-NP, mice	↓Neurological impairment, lethality, lesion area, neuronal loss, ↓microglial activation, ↓ mitogen-activated protein kinases (MAPKs), ↓NF-κB signal pathway, ↓mRNA expression of TNF-α, IL-1β, IL-6, inducible NO synthase	[40]
P. quinquefolius L. (American ginseng) ground leaves and stems	Araliaceae	3-NP, rodents	↑Behavioral score, ↓striatal lesion volume	[41]
P. quinquefolius L. [Rb extract (with ginsenosides Rb1, Rb3, and Rd)]		3-NP, rodents	↓Motor impairment, ↓striatum cell loss	[41]
Psoralea corylifolia water and 80% ethanol seed extract	Fabaceae	3-NP, PC12 cells	↑Mitochondrial respiration, ↓apoptosis	[42]
Punica granatum L.	Lythraceae	3-NP, PC12 cells	Antioxidant, ↓ROS ↓lipid peroxidation, ↓extracellular NO, ↓lactate/pyruvate ratio, ↓lactase dehydrogenase	[43]
Valeriana officinalis L. ethanolic extract	Caprifoliaceae	3-NP, rat brain homogenates	↓TBARS, antioxidation	[44]

↑Prevented loss of/induced/enhanced/improved/increased/upregulated/elicited/promoted/restored/activated/inhibited depletion/protected. ↓Downregulated/attenuated/ameliorated/reduced/declined/terminated/blocked/prevented/inhibited.

anti-HD phytochemicals drawn by using the ChemDraw software. The characteristics of herbs and herbal constituents, their biological targets, underlying molecular mechanism of action, applicability, and possible clinical trials are also discussed.

8.3 Neurotoxic *In Vitro* and *In Vivo* Anti-HD Models

The following section will elucidate the various neurotoxic *in vitro* and *in vivo* anti-HD models, many of which have been implicated to evaluate anti-HD efficacy of natural products. Figure 8.3 summarizes the anti-HD models in a schematic presentation.

Table 8.2 Anti-HD activity of phytochemicals.

Compound	Source plant	*In vitro*/*in vivo* models	Mode of action	References
Berberine (**1**)	*Berberis* sp. (Berberidaceae) and other plants	Transgenic HD (N171-82Q) mice	↓Motor dysfunction, ↑survival duration, ↑mHtt degradation, ↓autophagy	[45]
Celastrol (**3**)	Root bark of *Tripterygium wilfordii* Hook (Celastraceae)	3-NP, rats	↓Striatal lesion volume	[46]
Curcumin (**4**)	Root of *Curcuma longa* L. (Zingiberaceae)	3-NP, rats	↑Motor and cognitive functions, antioxidant, ↑SDH	[47]
Curcumin (with piperine)		3-NP, rats	↓Behavioral and molecular changes	[48]
(−)-Epigallocatechin-gallate (**5**)	Green tea [*Camellia sinensis* (L.) Kuntze (Theaceae)] leaves	3-NP, rats	NO modulation, antioxidant, ↓mitochondrial dysfunction	[49]
		3-NP, rats	↑Behavioral and biochemical activities, ↑GSH, ↑memory, NO modulation	[50]
Ferulic acid (**6**) (and fish oil)	Plants	3-NP, rats	Antioxidant, ↓MDA, ↓hydroperoxides, ↓NO, ↑AChE, ↑dopamine, ↓mitochondrial dysfunctions	[51]
Genistein (**9**)	Plants	3-NP, ovariectomized rats	↓Memory loss, ↑antioxidant, ↑anti-inflammatory, ↓cholinesterase	[52]
Ginsenosides, Rb1 (**10**), Rb3, or Rd (individually)	*P. quinquefolius* L. (American ginseng) (Araliaceae)	3-NP, rodents	↑Motor function, ↑mortality, ↓striatal lesion volume	[41]
Hesperidin (**11**)	*Citrus* sp. (Rutaceae)	3-NP, rats	↓Behavioral alterations, ↓mitochondrial enzymes complex dysfunction, antioxidant, NO modulation	[53]
L-Theanine (**13**)	Green tea *Camellia sinensis* (L.) Kuntze (Theaceae)	3-NP, rats	↑SOD, ↑GSH, ↑CAT, ↑SDH, antioxidant	[54]

(*Continued*)

Table 8.2 (Continued)

Compound	Source plant	*In vitro/in vivo* models	Mode of action	References
Lycopene (**15**)	*Lycopersicon* sp. (Solanaceae) species and red fruits and vegetables	3-NP, rats	↓Mitochondrial dysfunctions, antioxidant	[55]
Lycopene (with quercetin and poloxamer 188)	Tomatoes	3-NP, rats	↓Anxiety, ↓depression, ↑body weight, ↑locomotor activity	[56]
Melatonin (**16**)	Plants	3-NP, rats	↓Lipid peroxidation, antioxidant	[57]
		3-NP, rats	↑Behavioral improvement, ↓BDNF and GDNF, ↓neuronal loss, antioxidant	[58]
		3-NP, rats	↓Asymmetric rotational behavior, ↑DA, antioxidant	[59]
		3-NP, rats	↑Protection of striatal neuron	[60]
		3-NP, rats	↑Behavioral improvement, ↓dendritic spine damage	[61]
Naringin (**17**)	*Citrus* sp. (Rutaceae)	3-NP, rats	Antioxidant, antiapoptotic, ↓Bad and Bax, ↓cytochrome c release, ↓caspase 3 activation	[62]
		3-NP, rats	↑Nrf2 activation, antioxidant, anti-inflammatory	[63]
		3-NP, rats	↓BBB dysfunction, ↓MMPs 2 and 9, ↑TIMPs 1 and 2, ↓neuroinflammation	[64]
		3-NP, PC12 cells	Antioxidant, ↓lipid peroxidation, ↑mitochondrial membrane potential, ↓apoptosis	[65]
Nicotine (**18**)	*Nicotiana tabacum* L. (Solanaceae)	3-NP, rats	Antioxidant	[66]
Protopanaxatriol	*Panax ginseng* C.A. Mey. (Araliaceae)	3-NP, rats	Antioxidant, ↑Hsp70 expression, ↑body weight, ↑behavior, ↓ROS, ↑Nrf2 entering nucleus	[67]
Puerarin (**19**)	Dried roots of *Pueraria lobata* (Willd.) Ohwi (Fabaceae)	3-NP, rats	↑Weight, ↑locomotor activity, ↓hypothermia, ↓neurotransmitters anomalies, ↓oxidative stress	[68]
		3-NP, rats	↑Antiapoptotic, ↑anti-inflammatory, ↓energy deficit	[69]

Quercetin (**20**)	Plants	3-NP, rats	↓Movement disturbances and anxiety, ↓inflammatory damages	[70]
Resveratrol (**21**)	Red grapes	R6/2 mouse model of HD	↑ERK	[71]
		3-NP, rats	↓Motor and cognitive impairment, antioxidant	[72]
S-Allylcysteine (**22**)	*Allium sativum* L. (Amaryllidaceae)	3-NP, rats	↓Mitochondrial dysfunction, antioxidant	[73]
		3-NP, rats	↓Oxidative damage, ↓energy depletion	[74]
Sesamol (**24**)	*Sesamum indicum* L. (Pedaliaceae)	3-NP, rats	↑Mitochondrial enzymes, ↑body weight, ↑locomotor activity, ↑motor coordination, antioxidant	[75]
Spermidine (**25**)	Plants	3-NP, rats	↑Antioxidant, ↑NMDA receptor antagonistic, ↑anti-inflammatory, ↓alteration in striatal neurotransmitters	[76]
Sulforaphane	*Brassica oleracea* L. (Brassicaceae) (broccoli) or other cruciferous vegetables	HEK293 cells expressing mHtt-94Q	↓mHtt cytotoxicity, ↑mHtt degradation	[77]
		3-NP, mice	↓Striatal toxicity, ↑Keap1–Nrf2–ARE pathway, ↓MAPK and NF-κB pathways	[78]
Trehalose (**26**)	Plants	polyQ, transgenic mice	↓polyQ aggregates in the cerebrum and liver, ↑motor function, ↑life span, ↑polyQ-containing protein stabilization	[79]
Vanillin (**27**) (and agomelatine)	Plants	3-NP, rats	Antioxidant, ↑weight, ↑locomotor activity, ↑learning memory, ↓biochemical impairments	[80]

↑Prevented loss of/induced/enhanced/improved/increased/upregulated/elicited/promoted/restored/activated/inhibited depletion/protected. ↓Downregulated/attenuated/ameliorated/reduced/declined/terminated/blocked/prevented/inhibited.

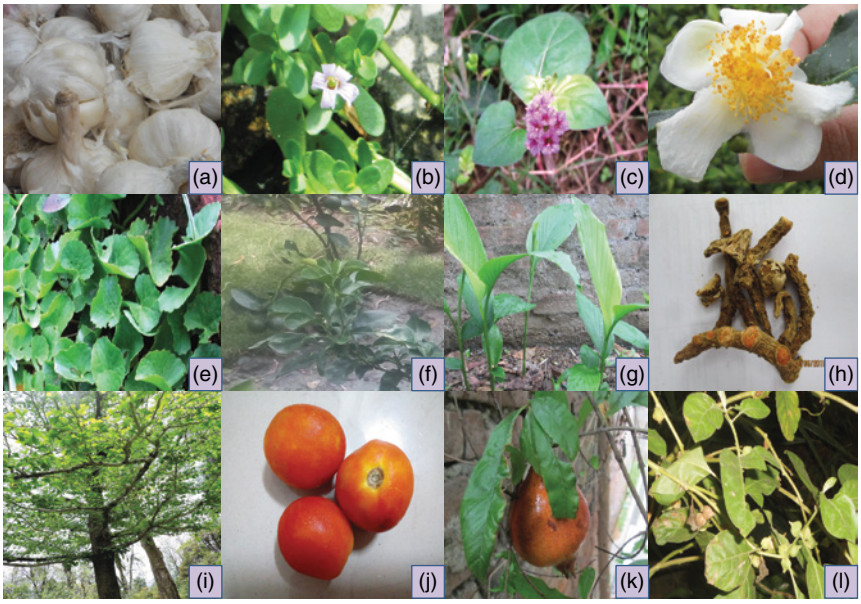


Figure 8.1 Medicinal plants as source of anti-HD botanicals and/or phytochemicals. (a) *Allium sativum* L.; (b) *Bacopa monnieri* (L.) Wettst.; (c) *Boerhaavia diffusa* L.; (d) *Camellia sinensis* (L.) Kuntze; (e) *Centella asiatica* (L.) Urb. (f) *Citrus* sp.; (g,h) *Curcuma longa* L. (habit and roots); (i) *Ginkgo biloba* L.; (j) *Lycopersicon* sp.; (k) *Punica granatum* L.; and (l) *Withania somnifera* L. (Dunal).

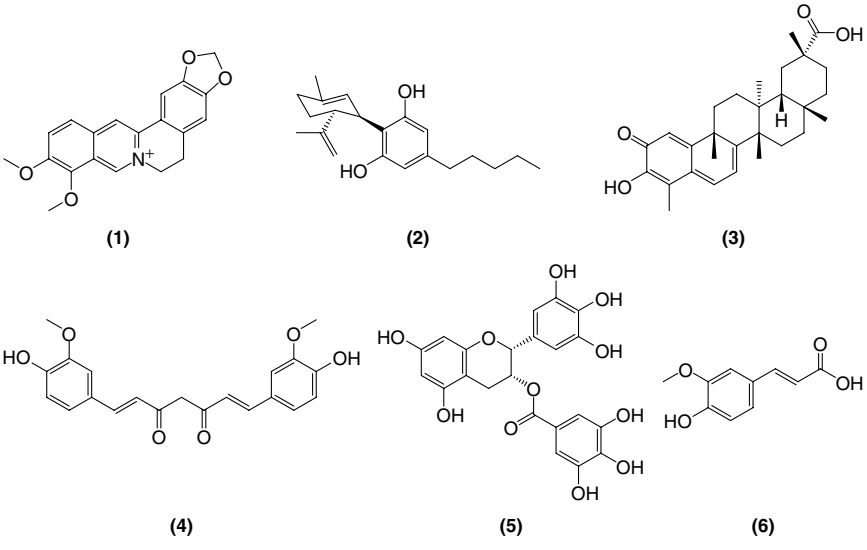


Figure 8.2 Chemical structures of anti-HD phytochemicals **1**, berberine; **2**, cannabidiol; **3**, celastrol; **4**, curcumin; **5**, (–)-epigallocatechin-gallate; **6**, ferulic acid; **7**, fisetin; **8**, galantamine; **9**, genistein; **10**, ginsenoside Rb1; **11**, hesperidin; **12**, kaempferol; **13**, L-theanine; **14**, lutein; **15**, lycopene; **16**, melatonin; **17**, naringin; **18**, nicotine; **19**, puerarin; **20**, quercetin; **21**, resveratrol; **22**, S-allylcysteine; **23**, schisandrin B; **24**, sesamol; **25**, spermidine; **26**, trehalose; and **27**, vanillin.

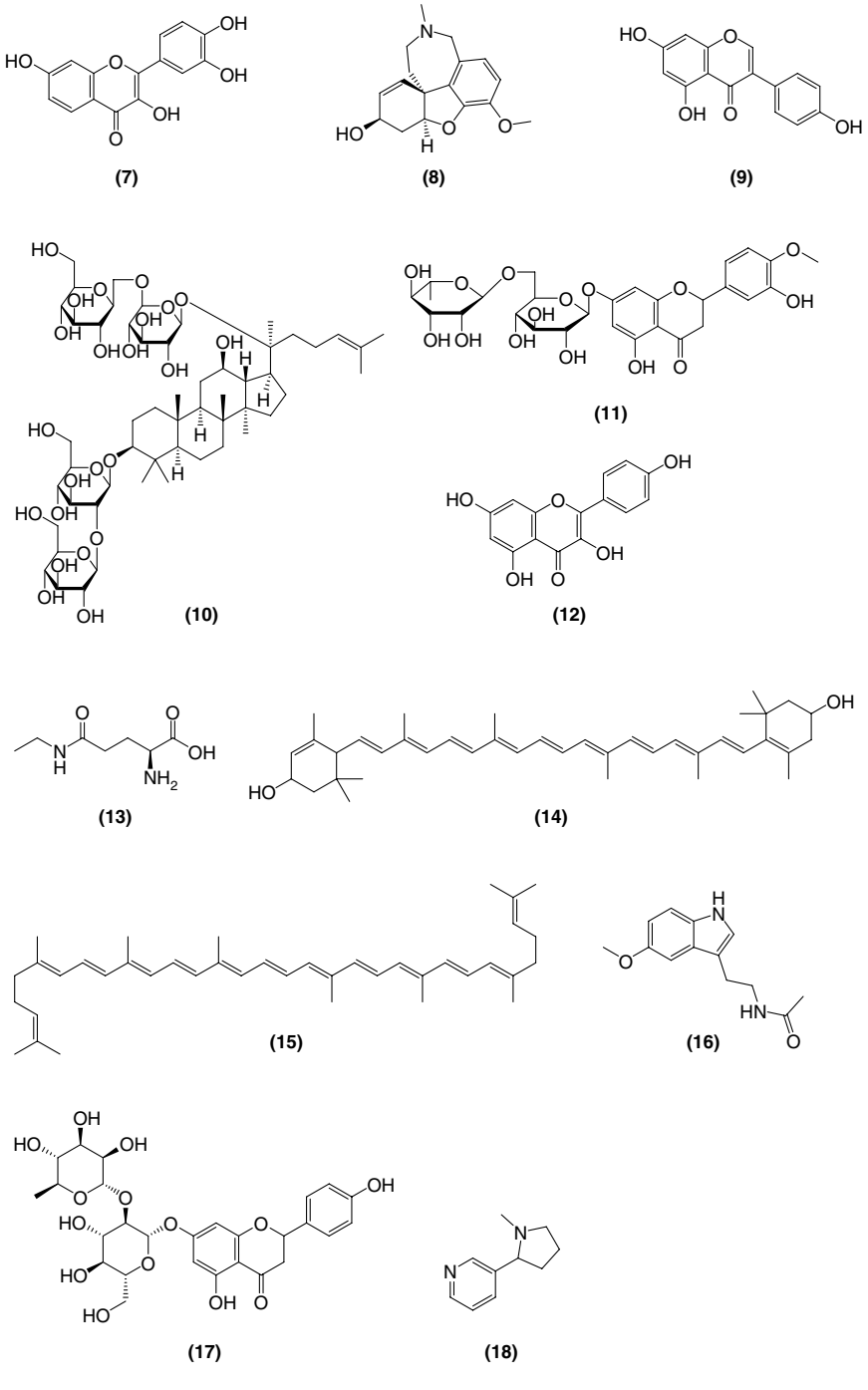


Figure 8.2 (*Continued*)

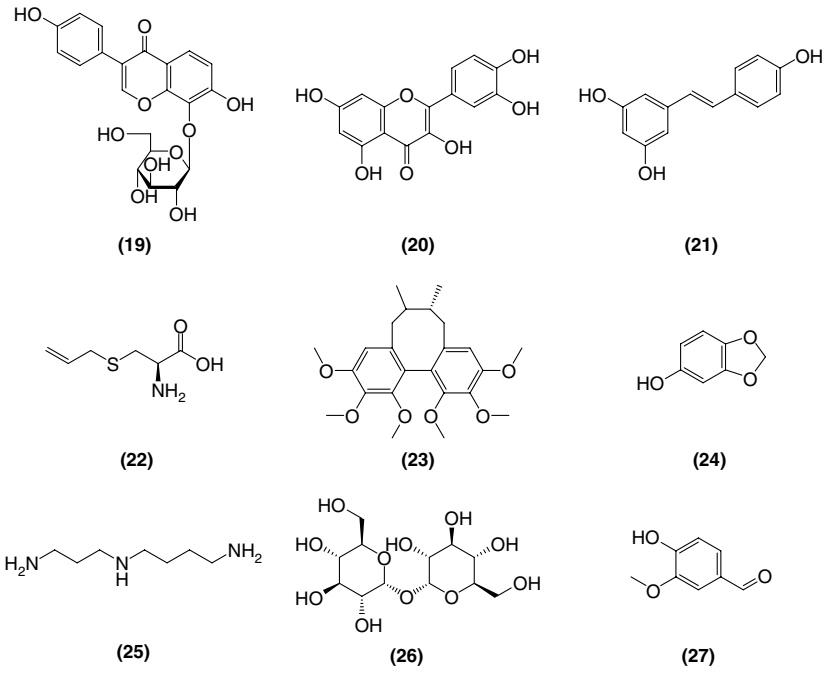


Figure 8.2 (*Continued*)

8.3.1 Excitotoxic Lesion Models

Excitotoxic lesion models mimic some but not all of the behavioral aspects of HD, and they involve apoptosis-mediated destruction of striatal neurons due to possible nuclear factor-κB (NF-κB) activation [81–83]. Kainic acid (KA) and quinolinic acid (QA) are considered as potent excitotoxins producing HD pathophysiology in different HD models.

8.3.1.1 Kainic Acid

KA, an analog of L-glutamic acid and a potent neuroexcitant, is known to cause degeneration of neurons with perikarya within the striatum producing axon-sparing striatal kainate lesion similar to neurochemical and histopathologic changes observed in human HD. Although the neurotoxicity caused by kainate is complex and indirect, specific receptors for kainate were observed via ligand-binding studies [84]. *In vivo* and *in vitro* studies with kainate and its analogs have revealed the effects of these toxins on striatal cholinergic and gamma-aminobutyric acid (GABA)ergic neurons and interaction between injected kainate and synaptically released glutamate [85]. The kainate-injected striatum manifested a total decline in intrinsic neurons, enhanced amount of glial cells, and intact internal capsule fibers [86]. However, as the excitotoxin kills both projection neurons and nicotinamide

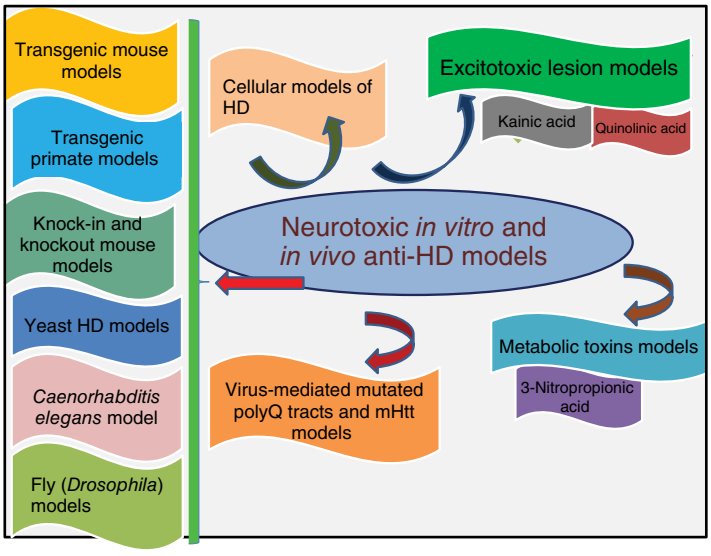


Figure 8.3 A schematic presentation of anti-HD models.

adenine dinucleotide phosphate (NADPH)-positive interneurons, intrastriatal injection of kainate does not mimic the histological hallmarks associated with HD [87, 88].

8.3.1.2 Quinolinic Acid

Chronic administration of the *N*-methyl-D-aspartate (NMDA) receptor agonist and a potent excitotoxin QA, a metabolite of tryptophan along the kynurenine pathway, produced lesions in rats that were very similar to HD. QA also remarkably enhanced the levels of both serotonin/5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (HIAA) and evidenced the role of NMDA receptor-mediated excitotoxicity in HD pathogenesis [89, 90]. QA produced axon-sparing lesions as observed in HD, leading to the depletion of neurotransmitters such as GABA in striatal spiny neurons without affecting dopamine. The lesions produced by KA, ibotenic acid (IA), and *N*-methyl-D-aspartate (MeAsp) were reportedly different from those produced by QA [88]. Derangements in the formation of QA in the brains of HD patients were tentatively indicated [89]. However, in another study, the hypothesis of enhanced levels of QA associated with neuronal degeneration in HD was not evidenced from the postmortem brain tissue from patients [91].

8.3.2 Metabolic Toxin Models

Mitochondrial electron transport chain (ETC) function is selectively inhibited in various tissues, including the brain in patients suffering from neurological disorders [92]. A number of various mitochondrial toxins are known to cause a significant decline in GABAergic neurons and the relative sparing of cholinergic

and NADPH diaphorase-positive interneurons [93]. Mitochondrial toxin-induced neurodegenerative process mimics the pathological hallmarks of HD because neuronal degeneration in HD is known to be affected by a defect in energy metabolism [94, 95].

8.3.3 3-Nitropropionic Acid

3-Nitropropionic acid (3-NPA), a widely distributed plant and fungal neurotoxin, is reported to damage the basal ganglia, hippocampus, spinal tracts, and peripheral nerves in animals [96]. Before the recent use of 3-NPA as a chemical tool for investigation of various neurodegenerative disorders in humans, 3-NPA intoxication caused substantial problem in domestic livestock following ingestion of plants. Moreover, tragic consequences due to its intoxication in humans consuming moldy sugarcane were also reported from China [97]. 3-NPA intoxication via consumption of mildewed sugarcane possibly caused putaminal necrosis with delayed dystonia in children [96]. 3-NPA, a suicide inhibitor of the Krebs cycle enzyme succinate dehydrogenase (SDH) (an enzyme located in the mitochondrial inner membrane that carries out oxidation of succinate to fumarate), was observed to produce histochemical and pathological similarities to the neuropathologic and neurochemical features of HD in both rodents and primates [94]. However, acute and large doses of 3-NPA were not found to replicate HD pathology. 3-NPA dose-dependently induces rapid neurological decline via incoordination, drowsiness, weakness, limb paralysis without rigidity but with final recumbence, and death. Therefore, NPA intoxication represents energy deficiency model of brain damage, producing morphological brain injury in a highly predictable anatomical pattern, simultaneously with the onset of clinical recumbence [98]. It was further revealed that the initiation of 3-NPA-induced morphologic injury is not implicated to the leakage of protein-rich fluid into the cerebral parenchyma due to blood–brain barrier (BBB) impairment but would possibly play a role in the severity of injury at some later stages, leading to the development of brain lesions [99]. 3-NPA reportedly reduced the cellular adenosine triphosphate (ATP) levels, thus causing neuronal injury by an excitotoxic mechanism [100]. Systemic administration of 3-NPA to both rats and primates produced selective striatal lesions as a result of secondary excitotoxic mechanisms [101, 102], which precisely mimic the motor and neuropathological symptoms found in HD patients [103]. 3-NPA equally affects both enkephalin and substance P striatal neurons, which is absent in adult-onset HD [104]. However, in some experimental animals, 3-NPA-induced metabolic impairment, motor and behavioral abnormalities, and striatal lesions were reported similar or analogous to that occurring in HD patients. However, the progressive behavioral pathology of HD was not characterized by the excitotoxic animal models [93].

Nonhuman primate models are considered as valuable tools for elucidating human disorders and for designing therapeutic interventions. Due to similar physiological, neurological, and genetic features observed in humans and higher primates, monkey models may be used as valuable aid to understand human

physiology and ailments [105]. Moreover, primate models hold the advantage over rodent models because of their prominent differences in repertoire of movements and the organization of the basal ganglia with that of the rats. Hence, in chronic 3-NPA-insulted nonhuman primates, the observed movement abnormalities were reported to resemble those found in HD patients [93]. In two nonhuman primate models (male *Cebus apella* monkeys) of HD, QA- and 3-NPA-produced behavioral and morphological symptoms similar to the juvenile and akinetic–rigid variants of HD with smaller lesions and spontaneous dyskinesia were observed in 3-NPA-insulted animals [106]. In another neuropathological and behavioral model of HD, the glutamate receptor agonist IA produced unilateral caudate–putamen (CP) lesions in the nonhuman primate baboons in which the lesioned CP manifested a neurodegenerative pattern similar to HD [107]. In addition, bilateral selective excitotoxic lesion of the posterior putamen was cited as an improved and reliable behavioral model of HD in QA- and apomorphine-induced rhesus monkeys [108].

8.3.4 Transgenic Mouse Models

Since 1993, when the mutation as an unstable expansion of CAG repeats in the IT15 gene was identified as the cause of HD, a type II trinucleotide repeat disorder, various mouse models emerged to study the pathological features of HD [109, 110]. Transgenic, knock-in, knockout, and virally inserted mutated polyQ tract models are considered as the major genetic mouse HD models used to explore the various early pathological, molecular, and cellular abnormalities associated with the mutation causing HD [93, 109]. In transgenic mouse model, either the part of mHtt or the full-length mHtt is inserted into the mouse genome followed by its expression alongside the endogenous normal Htt. Shorter size of the transgene and longer size of the CAG repeat were correlated with higher level of expression and severe phenotype [110]. The R6 mouse model overexpressing exon 1 of the human Htt gene with long (141–157) CAG repeat expansions was reported as the first transgenic mouse HD model [93]. In 1996, the R6/1 and R6/2 lines were developed as the first transgenic mouse models of HD followed by the emergence of a number of HD transgenic mouse lines [109, 111]. However, R6/1 and R6/2 lines both expressing exon 1 of the human HD gene with around 115 and 150 CAG repeats, respectively, have been considered as the most commonly used models to testify novel therapies for HD [111, 112]. Each R6 model was found to be unique and partially mimicking the HD phenotype found in humans with some expected differences in symptoms due to some major differences in the normal behavioral properties of rodents and humans. However, the best analogous features observed in human and rodents were aggregate formation, cell death, transmitter changes, and alterations in neurogenesis [112].

In addition, full-length human IT15 gene has also been used to generate transgenic mice with full-length Htt [93]. However, in transgenic mice containing the full-length human HD complementary DNA (cDNA) with 44 CAG repeats, no abnormal protein expression and neurodegeneration or behavioral anomalies were noted. The repeat stability in this experiment was possibly attributed to the

genomic sequences [113]. Moreover, mice expressing a full-length IT15 cDNA clone with 48 or 89 CAG repeats manifested progressive motor dysfunction with neuron loss in the striatum [114]. Similarly, yeast artificial chromosome (YAC) transgenic mice expressed a full-length IT15 gene with 72 repeats with a selective loss of medium spiny neurons in the lateral striatum with a slow rate of disease progression corresponding to smaller repeat units and lower level of transgene expression [115]. In addition, unlike the R6/1 mouse model of HD, the YAC128 mouse model represented selective degeneration and nuclear localization of mutant Htt with a pattern of degeneration quite similar to that of human HD [116]. Selective degeneration in YAC mouse models represents an exciting tool that recapitulated the region specific loss occurring in HD brain [117].

8.3.4.1 Knock-In and Knockout Mouse Models

Knock-in mouse HD models have the advantage of carrying the mutation causing the disease in the appropriate murine genomic and protein context under the endogenous Hdh promoter with early behavioral, molecular, cellular, and neuropathological anomalies [118]. Knock-in mouse models also represent early motor phenotype resembling that of early HD besides having a slower progress rate providing more time for in-depth analysis [93, 119]. In contrast, early homozygous knockout mutants in mice were found to be embryonic lethal, indicating their indispensable role in embryonic development [93, 120].

8.3.4.2 Virus-Mediated Mutated polyQ Tracts and mHtt Models

Precise localization of injection and gene expression are the major advantages of viral vector-mediated incorporation of full or partial genes [93]. In a direct viral approach, intrastriatal expression of 97Q-GFP in the adult rat brain led to rapid formation of intraneuronal aggregate [121]. A recombinant adeno-associated viral (AAV) vector delivering small interfering RNA (siRNA) reduced the striatal Htt aggregations, partially corrected the aberrant striatal transcriptional profile, and improved behavioral anomalies in a YAC128 mouse model of HD [122]. Earlier, AAV-mediated RNAi for HD in R6/1- and N171-82Q fragment-based HD mouse models with aggressive and rapidly progressing phenotype were reported for having various limitations [123–126]. In another AAV vector approach in mice, overexpression of mHtt was not correlated with major perturbation in basal levels of autophagy in the animals [127]. Furthermore, lentiviral-mediated vascular endothelial growth factor $(VEGF)_{165}$ expression was reported to be neuroprotective in both human neuroblastoma SH-SY5Y and rat primary striatal cultures [128].

8.3.4.3 Transgenic Primate Models

The recent development of transgenic HD primates represents an exciting option to study human genetic disorders including HD and to develop therapeutic strategies incorporating gene expression and metabolite profiling and noninvasive imaging. Moreover, comparable neuropathology and clinical features (such as rigidity, seizure, dystonia, bradykinesia, and chorea) between human and nonhuman primates are considered added advantages to design novel treatment strategies [129]. Transgenic HD rhesus macaque model expressing polyQ-expanded

Htt produced nuclear inclusions and neuropil aggregates in the brains besides displaying dystonia and chorea and was suggested as alternative and important tool in elucidating the underlying molecular pathogenesis of HD [105]. However, a number of issues such as technical difficulty and financial and ethical costs are associated with the transgenic primate disease models [130].

8.3.5 Fly (Drosophila) Models

Invertebrate models such as *Drosophila* models of a number of neurodegenerative diseases have exhibited remarkable similarities to the human diseases [131]. Moreover, these models have also been implicated to the testing of a number of therapeutic compounds *in vivo* to generate rational treatment strategies [132]. *Drosophila melanogaster* houses an Htt protein that interacts with the endogenous pathways in a conserved manner. Due to their rapid life cycle, cost-effective maintenance, availability of an array of well-established *in vivo* genetic devices, possibility to perform rapid genome-wide screenings for enhancers or suppressors of the mHtt phenotype, tissue-specific and temporally regulated expression of foreign genes and overexpression of human mHtt gene displaying protein aggregation, neurological and behavioral anomalies, and a shortened longevity in engineered transgenic flies, *Drosophila* models are considered as exciting tools for studying HD pathology for their therapeutic interventions [133, 134]. Moreover, since dominant neurodegenerative diseases mostly result from gain-of-function mutations in single genes, engineered *Drosophila* readily expresses phenotypes closely mimicking human disease. In addition, *Drosophila* possesses a fully active nervous system with specialized job distribution such as vision, memory etc. [133]. HD-associated modulations in behavior in *Drosophila* also provide an insight into the earliest stages of HD when treatment strategies might be more efficacious [135]. *Drosophila* model of HD elucidated a number of novel HD therapeutics that may be tested in mammalian HD models [136].

8.3.6 *Caenorhabditis elegans* Model

Caenorhabditis elegans represents another excellent and relatively simple model to study various complex human neurodegenerative diseases and to elucidate their possible therapeutic interventions. Besides having a well-defined and genetically tractable nervous system, the nematode possesses complex biochemical pathways that are mostly conserved like in mammals [137–139]. The role of polyQ expansions in perturbing transcription of cyclic adenosine monophosphate (cAMP) response element–binding protein/CREB-binding protein (CREB/CBP) targets and specific targeting of histone deacetylases (HDACs) in reducing related neurodegeneration were also demonstrated using a *C. elegans* model [140]. The ability of sirtuin-1 (SIRT1) to protect neurons was well-elucidated in mouse as well as in *C. elegans* HD models, and therapeutic interventions via targeting SIRT1 have been cited as a possible treatment strategy against HD [141]. In a *C. elegans* model of polyQ toxicity, the effects of transactive response (TAR) DNA-binding protein 43 (TDP-43) and fused-in-sarcoma (FUS) and the interaction between TDP-43 and the survival factor progranulin (PGRN) in producing neurodegenerative phenotypes were

documented [142]. *C. elegans* has been mentioned as an instructive against polyQ expansion disorders such as HD [143].

8.3.7 Yeast HD Models

Glutamine-encoding trinucleotide expansions were initially used to produce yeast HD models [93]. Htt aggregation in yeast was reportedly dependent on the length of the polyQ expansion and the expression of chaperone proteins [144]. In a yeast model, for the first time, a direct connection between expanded polyQ domain aggregation and its cytotoxicity and the role of Rnq1 in its prion conformation in polyQ aggregation were reported [145]. Prion-dependent polyQ aggregation and its toxicity in a yeast model were elucidated by chaperone proteins [146]. Moreover, in yeast PMS1 homolog 1 (pms1) and MutS protein homolog 2 (msh2) mutants, the mismatch repair system prevented small changes in CAG repeats with its inability to protect from larger changes [147].

8.3.8 Cellular Models of HD

Cell cultures are considered as more simplified models when compared with complex *in vivo* models. Cell lines expressing mHtt represent stable and controlled genetic and transcriptional expressions, minimizing the biological and experimental variability [93]. Mouse clonal striatal cells expressing human wild-type and mHtt cDNAs and mouse neurons fused with mouse teratoma cells transfected with polyQ-containing peptides are considered as available *in vitro* HD models [148, 149]. In a pheochromocytoma (PC12) HD cell model expressing exon 1 of wild-type or mHtt, CB1 receptor via G-protein alpha subtype i/o (G(i/o))-linked, extracellular signal-regulated kinase (ERK)-dependent signal transduction was reported as a therapeutic target in HD [150]. The protective role of activating transcription factor 3 (ATF3) against the toxic effects of the N-terminal fragment of mHtt-N63 was elucidated by a stable PC12 cell line [151]. PC12 cell lines expressing mHtt-N63 also exhibited toxicity and decreased histone acetylation [152]. Clonal striata-derived cells expressing various N-terminal 548-amino acid Htt fragments (with 26, 67, 105, or 118 glutamines) were used to study the gene expression profiles in HD [153]. Cellular models expressing full-length or truncated forms of wild-type and mHtt represent excellent alternatives to study the pathogenic mechanisms and to formulate therapeutic strategies against various neurodegenerative disorders including HD.

8.4 Anti-HD Natural Products and Implications of HD Models

8.4.1 Anti-HD Properties of Medicinal Plants

8.4.1.1 *Bacopa monnieri* (L.) Wettst. (Plantaginaceae)

Bacopa monnieri or water hyssop or "Brahmi" has been used in the Ayurvedic medicine for centuries as a brain tonic to enhance memory, learning, and concentration and also against anxiety or epilepsy [154, 155]. In clinical trials, the

plant extracts were reported efficacious against attention deficit hyperactivity disorder (ADHD) [156] and multitasking stress reactivity and mood [157] and as a pro-neurocognitive agent [158]. Preclinical studies indicated its effectiveness against epilepsy [159], Parkinson's disease (PD) [160], dementia [161], Alzheimer's disease (AD) [31], and an array of other neurological diseases. Dietary intake of *B. monnieri* leaf powder (0.5% and 1% for 4 weeks) by prepubertal (PP) mice was found to modulate cytoplasmic and mitochondrial oxidative markers in the animals and was also found to ameliorate the effects of neurotoxic prooxidants such as 3-NP [162]. In a PP mouse brain, ethanolic extract of the plant attenuated 3-NP-insulted mitochondrial dysfunctions via antioxidation in striatal mitochondria [163]. An alcoholic leaf extract was also reported to display *in vivo* neuroprotective ability mediated by antioxidation following 3-NP insult [164]. Besides the various extracts of *B. monnieri* exhibited *in vitro* anti-HD properties via their antioxidative and cytotoxic abilities and also via retaining mitochondrial functionality [163, 164].

8.4.1.2 *Boerhaavia diffusa* L. (Nyctaginaceae)

Boerhaavia diffusa (commonly known as Punarnava), an Ayurvedic and Unani medicinal plant grown primarily in the wastelands, is known to produce various high-value biopharmaceuticals [165, 166]. Roots of the plants have been reported as potent antioxidant, antistress, and anticonvulsant agents [167]. Root ethanol extracts and the isolated alkaloid punarnavine reportedly exerted antidepressant-like activity in experimental animals with possible modulation of the monoaminergic and GABAergic systems [167, 168]. A polyphenol-rich ethanolic extract protected 3-NP-insulted rat brain homogenates via antioxidative mechanisms [32].

8.4.1.3 *Calendula officinalis* L. (Asteraceae)

Calendula officinalis or "pot marigold," a traditional and homeopathic medicine and a food component and colorant, has been reported for its potent antioxidant, anti-inflammatory, estrogenic, and CNS properties [169–171]. *C. officinalis* exerted its neuroprotective properties via preventing monosodium glutamate (MSG)-induced excitotoxic brain damage [172], subacute effect of exposure to cigarette smoke [173], and learning and memory deficits [174] *in vivo*. *C. officinalis* flower extract protected 3-NP-insulted rats via its antioxidant, anti-inflammatory, and estrogenic responses [171].

8.4.1.4 *Cannabis sativa* L. (Cannabaceae)

Human civilization is known to use cannabis preparations for over 5000 years [175]. Cannabinoids isolated from herbal cannabis interact with the body's endogenous cannabinoid systems, leading to psychomotor disturbances and respiratory and cardiovascular health risks [176]. However, cannabidiol (CBD) (**2**), the main non-psychotropic cannabinoid, exerted its anti-PD effects via enhancing life quality and via improving rapid eye movement (REM) and REM sleep behavior disorder (RBD) in PD patients without causing any psychiatric comorbidity [177, 178]. Cannabinoids also reduced oxidative stress (OS), neuroinflammation, and amyloid plaque formation associated with late-onset Alzheimer's

disease (LOAD) [179]. *Cannabis sativa* and its constituents exhibited anti-HD efficacy in 3-NP-insulted rats via antioxidation [180]. In addition, Sativex®, a commercial formulation made up of cannabis constituents, has been very near to a clinical anti-HD trial [181]. Endocannabinoid system [CB(1) and CB(2) receptors] was reportedly involved in exerting neuroprotection by Sativex®-like combination of phytocannabinoids on a malonate-lesioned inflammatory rat model of HD [182]. In addition, cannabinoid CB2 receptor agonists protected the mouse striatum against malonate-induced death [183], and hence, modulation of endocannabinoid system was suggested as a possible therapeutic strategy to treat neurodegeneration in HD [184].

8.4.1.5 *Centella asiatica* (L.) Urb. (Apiaceae)

Centella asiatica, commonly known as Gotu kola and Thannkuni, is an age-old Ayurvedic medicinal plant used to treat neurological disturbances due to its nootropic and pro-cognitive properties [185, 186]. Several *in vitro* and *in vivo* studies evidenced its therapeutic activity against PD [187], migraine [188], learning and memory deficit [189], neurotoxin-induced cognitive dysfunction [190], and so on. The plant has been reported as a neuroprotector by preventing neurobehavioral and neurochemical changes in middle cerebral artery occlusion (MCAO) in rats [191]. Asiaticoside and madecassoside isolated from the plant were reported to show anti-PD effects in experimental rat [192, 193]. Asiatic acid, a pentacyclic triterpene isolated from the herb, was reported to ameliorate glutamate-induced cognitive deficits in animals [192]. *C. asiatica* leaf powder and aqueous extract protected 3-NP-challenged PP male mice (0.5% and 1% for 4 weeks, dietary intake) via antioxidation. The aqueous extract showed enhanced antioxidant ability and elevated glutathione/glutathione disulfide (GSH/GSSG) and thiols against 3-NP-induced neurotoxicity in male mice [33]. Neuroprotection was also offered by the extract (5 mg kg^{-1} body weight for 10 days) followed by 3-NP administration in the brain of PP mice [35]. Studies also indicated the anti-HD potential of the plant in 3-NP-treated brain mitochondria [34].

8.4.1.6 *Convolvulus pluricaulis* Choisy (Convolvulaceae)

Convolvulus pluricaulis has been implicated in the treatment of various mental disorders such as anxiety, neurosis, epilepsy, and insomnia and as a brain tonic in Ayurveda as "Medhya Rasayana" (nervine tonic), and it also serves as one of the four ingredients in the commercialized botanical sources of "Shankhpushpi" [194–196]. The plant preparations have exerted anticonvulsant, neuroprotective, and cholinergic properties in various animal models [194, 197]. Moreover, coumarins from the plant exhibited memory-enhancing activity on scopolamine-induced amnesia in mice [196]. Pretreatment with an ethyl acetate fraction (20 mg kg^{-1}) of a methanol extract of the whole plant ameliorated the locomotor, behavioral, and body weight deficit in 3-NP-challenged rats besides enhancing the malondialdehyde (MDA) and nitrite levels and restoring superoxide dismutase (SOD) and reduced GSH levels in the striatum and cortex of the treated animals [36]. *C. pluricaulis* standardized hydromethanol extract (200 mg kg^{-1}) and its ethyl acetate (30 mg kg^{-1}) and butanol (50 mg kg^{-1}) fractions

protected against 3-NP (10 mg kg^{-1}, i.p. for 14 days) insult in rats via enhancing locomotor activity, memory, body weight, and oxidative defense [37].

8.4.1.7 *Garcinia kola* Heckel (Clusiaceae)

The genus *Garcinia* serves as an active ingredient in a number of Ayurvedic preparations used against various pathophysiological disorders [198]. *Garcinia kola* is traditionally used against coughs, fever, and malaria in Central Africa [199]. Kolaviron, isolated from *G. kola*, inhibited acetylcholinesterase (AChE) activities and protected against ischemia/reperfusion injury in rat brain [200, 201]. Neuroprotective effect of kolaviron was noticed against vanadium-induced OS in rat brain due to reduction of the thiobarbituric acid reactive substances (TBARS) and increase in SOD in brain regions [202]. Kolaviron also protected the brain of Wistar albino rats exposed to gamma radiation [203]. *G. kola* water extract demonstrated 3-NP-insulted neuronal protection in mice [204].

8.4.1.8 *Gastrodia elata* Blume (Orchidaceae)

Gastrodia elata has long been used in traditional medicine as anticonvulsant, analgesic, and sedative medications [205]. The plant has been reported in the traditional Chinese herbal medicine for the treatment of headaches, dizziness, and epilepsy [206]. In various preclinical studies, *G. elata* and/or its primary active constituent gastrodin exerted *in vivo* neuroprotective properties against AD [207], PD [208], migraine [209], stroke [210], memory deficit [211], and so on. One-year-old rats were administered with *G. elata* (~2.5 g kg^{-1} day^{-1} for 3 months), which was found to be a modulator of brain proteome by upregulating molecular chaperons related to proteins involved in HD [38]. Moreover, *G. elata* exhibited *in vitro* anti-mHtt aggregation properties mediated by upregulation of adenosine A_2A receptor [A(2A)-R] and induction of proteasomal properties [212].

8.4.1.9 *Ginkgo biloba* L. (Ginkgoaceae)

Ginkgo biloba extract obtained from *G. biloba* trees (native to China) is a common complementary and integrative health (CIH) product in the United States. Ginkgo tree has long been used in traditional Chinese and Japanese cooking and medicine against various ailments [213]. *G. biloba*/*G. biloba* extract (EGb) 761® (EGb 761), a standardized *G. biloba* extract consisting of ginkgolides, bilobalide, and flavonoids, is considered as a powerful antioxidant popularly known to exhibit neuroprotective effects [213, 214]. Besides its well-established neuroprotective properties, EGb 761 has been known to increase the brain levels of neurotransmitters such as dopamine, noradrenaline, and acetylcholine [215]. *G. biloba* exerted its neuroprotective abilities against ischemic brain damage (via inhibiting the extrinsic apoptotic pathway) [216], AD [215], depression [217], focal cerebral ischemia [218], tardive dyskinesia [219], and so on. Moreover, *G. biloba* improved cerebral oxygen supply in elderly patients with preexisting cerebral ischemia [220]. EGb 761 was also suggested as an efficacious alternative treatment for the children with ADHD [221]. Various ginkgolides isolated from the plant exhibited their efficacy against severe hemorrhagic stroke [222], focal cerebral ischemia [223], high-altitude cerebral edema [224], migraine aura [225],

AD [226], and many more in different preclinical and clinical studies. EGb 761 protected 3-NP-challenged rats via preventing apoptosis and oxidation [227].

8.4.1.10 *Luehea divaricata* Mart. (Malvaceae)

Luehea divaricata, popularly known as Açoita-cavalo, is a native tree of the Brazilian Cerrado used for skin wounds and pimples, as vaginal washes, and as diuretic and anti-inflammatory agents [228–230]. Aqueous extract (500, 1000 mg kg^{-1}) protected 3-NP-treated rats (20 mg kg^{-1}) via restoring locomotor activity, reducing the levels of reactive oxygen species (ROS) and lipid peroxidation, enhancing the activity of AChE, and restoring the GSH/GSSG ratio in the toxin-insulted animals [39].

8.4.1.11 *Olea europaea* L. (Oleaceae)

Olea europaea, found in the Mediterranean region, is the most popular member and the only food plant of the genus *Olea* [231–233]. Olive leaf extract suppressed lead poisoning-induced brain impairment in mice [234]. Oleanolic acid, an active constituent of *O. europaea*, exerted neuroprotection against cerebral ischemia [235]. Dietary virgin olive oil (VOO) prevented hypoxia–reoxygenation injury in rat brain by reducing BBB permeability [236]. The neuroprotective effect of olive leaf extract was also demonstrated with the improvement of BBB permeability in rats against cerebral ischemia/reperfusion injury [237]. Extra VOO protected 3-NP-intoxicated rats via brain oxidation [238].

8.4.1.12 *Panax ginseng* C.A. Mey. and *Panax quinquefolius* L. (Araliaceae)

For over 2000 years, *Panax ginseng* has been used as a traditional herbal medicine possessing antianxiety, antidepressant, and pro-cognitive properties [239]. In various animal models, *P. ginseng* and its constituents, ginsenosides, exhibited neuroprotective efficacy against cognitive impairment [240], prenatal stress [241], traumatic brain injury (TBI) [242], autoimmune encephalomyelitis [243], and so on *in vivo*. *Panax quinquefolius* (North American ginseng) reportedly houses a distinct ginsenoside profile from *P. ginseng*, and it has exhibited promising pro-cognitive activities in preclinical as well as in clinical studies [244]. Korean red ginseng (*P. ginseng*) saponins protected 3-NP-insulted rats and improved their behavioral deficits [245]. American ginseng (*P. quinquefolius*, ground leaves and stems) improved the behavioral score and declined the striatal lesion volume in 3-NP-exposed experimental animals. A partially purified extract (Rb extract) containing ginsenosides Rb1, Rb3, and Rd significantly improved 3-NP-induced motor impairment and striatal cell loss [41]. Korean red ginseng hot water extract (50, 100, and 250 mg kg^{-1} day^{-1}, per os (p.o.)) reduced neurological impairment and loss and also declined the levels of NF-κB, tumor necrosis factor-α (TNF-α), and interleukin (IL)-1β and IL-6 in the male mice exposed to 3-NP [40].

8.4.1.13 *Psoralea corylifolia* L. (Fabaceae)

Psoralea corylifolia has traditionally been used in Chinese and Indian medicine to improve vitality and to treat various inflammatory diseases [246]. *P. corylifolia* seed-derived psoralidin exhibited antidepressant-like effects in mice [247].

Besides, the plant and its active principles demonstrated antistress [248] and brain monoamine oxidase (MAO) inhibitory [249] activities in experimental animals. Pretreatment with *P. corylifolia* seeds extracted in water and 80% ethanol protected against 3-NP-insulted mitochondrial dysfunction in cultured rat PC12 cells via enhancing mitochondrial respiration and ameliorating 3-NP (25 μM)-induced apoptosis [42].

8.4.1.14 *Punica granatum* L. (Lythraceae)

The pomegranate, *Punica granatum*, is an ancient and mystical fruit borne on a long-living tree cultivated throughout the different parts of the globe [250]. Recent reports indicated *P. granatum* or its active principles displaying bioactivities such as antioxidant, anti-inflammatory, and neuroprotective efficacies [251]. *P. granatum* prevented diabetes mellitus-induced learning and memory impairment [252], brain ischemia, AD [250], depression [253], and so on *in vivo*. Pomegranate seed oil protected PC12 cells against 3-NP (100 mM) insult via increasing the enzymatic and nonenzymatic antioxidant levels to possibly inhibit ROS via upregulation of antioxidant gene and via decreasing lipid peroxidation, extracellular nitric oxide (NO), lactate/pyruvate ratio, and lactate dehydrogenase (LDH) [43].

8.4.1.15 *Valeriana officinalis* L. (Caprifoliaceae)

Valeriana officinalis is used in various traditional herbal medicines as mild sedative, hypnotic, tranquilizer, and antiepileptic agent [254–256]. The plant and derived components exerted anticonvulsant [255], sedative and sleep-enhancing [257], antioxidant, and anti-neurotoxic properties *in vitro* and/or *in vivo* [44]. Ethanolic extract (0–60 $\mu g\,ml^{-1}$) of the plant protected against 3-NP-induced increment in TBARS in rat brain homogenates via its antioxidation abilities [44].

8.4.1.16 *Withania somnifera* L. (Dunal) (Solanaceae)

Withania somnifera ("Ashwagandha") has long been used in Ayurveda to treat various neurological disorders [258]. The plant reportedly prevented ischemic stroke [259], human immunodeficiency virus (HIV)-associated neurocognitive disorders (HAND) [260], hypobaric hypoxia-induced memory impairment [261], AD [262], and so on in various animal models. In addition, a major component of *Withania* root extract withanolide A protected rats against neurodegeneration during hypoxia [263]. Withanolide A was also reported to promote neuritic regeneration and synaptic reconstruction in severely damaged neurons [264]. *W. somnifera* was reported for its GABA-mimetic activity in mouse neurons [265] and helped in dendrite formation [266]. The root extract of the plant protected against the mitochondrial alterations and behavioral damages in 3-NP-treated rats [267].

8.4.2 Anti-HD Activity of Phytochemicals

8.4.2.1 α-Mangostin

α-Mangostin is a natural xanthonoid derived from the edible *Garcinia mangostana* L. (Clusiaceae) fruits [268]. The compound, as a potential antioxidant,

showed modulatory effects on the GSH system in rat brain synaptosomes treated with ferrous sulfate ($FeSO_4$) [269]. It protected 3-NP-treated cerebellar granule neurons (CGNs) via antioxidative mechanisms [270].

8.4.2.2 Astragalan

Astragalan is a natural polysaccharide isolated from *Astragalus membranaceus* Moench (Fabaceae) [271]. As an antiapoptotic agent, it showed neuroprotective effects in rats with ischemic brain injury [272]. In order to prevent polyQ-exposed proteotoxicity in *C. elegans*, the compound was found to modulate the transcription factor abnormal dauer formation-16/forkhead box O (DAF-16/FOXO) [271].

8.4.2.3 Berberine

Berberine (**1**), a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids, is isolated from *Berberis* sp. (Berberidaceae) and other plants. It exerted protective effects on transgenic HD (N171-82Q) mice attenuating motor dysfunction and enhancing the survival duration in the animals by degrading the mHtt and by promoting autophagy [45]. Moreover, it prevented neuronal damage by inhibiting glial-mediated inflammation in TBI *in vitro* and *in vivo* [273]. It also acted as a neuroprotective against via modulation of apoptotic pathways *in vitro* [274].

8.4.2.4 Celastrol

Celastrol (**3**) is a triterpenoid isolated from the Chinese herb *Tripterygium wilfordii* Hook. f. (Celastraceae). It protected against mitochondrial injury and inhibited p38 mitogen-activated protein kinase (p38 MAPK) in neurotoxic PD models [275]. Neuroprotective effect of celastrolin in a *Drosophila* model of PD was attributed to its antioxidant and anti-inflammatory properties [276]. Celastrol reportedly decreased striatal lesion volume in 3-NP-exposed rats [46]. In mutant polyQ protein-expressing cells, celastrol regulated heat shock protein (HSP) gene expression [277].

8.4.2.5 Curcumin

Curcumin (**4**) is a diarylheptanoid and the major curcuminoid of dietary turmeric [*Curcuma longa* L. (Zingiberaceae)] [278]. Curcumin-encapsulated poly(lactic-*co*-glycolic) acid (PLGA) nanoparticles (Cur-PLGA-NPs) induced adult neurogenesis in an AD model via canonical wnt/β-catenin pathway [279]. It (10, 20, and 50 $mg\,kg^{-1}$, p.o. once daily) prevented OS, elevated the level of SDH, and improved motor and cognitive abilities in rats exposed to 3-NP [47]. Curcumin (25 $mg\,kg^{-1}$) with piperine (2.5 $mg\,kg^{-1}$, as a bioavailability enhancer) displayed more pronounced effects on 3-NP (10 $mg\,kg^{-1}$)-treated rats in reducing behavioral and molecular changes induced by the toxin when compared with curcumin (only)-treated animals [48].

8.4.2.6 (–)-Epigallocatechin-gallate

(–)-Epigallocatechin-gallate (EGCG) (**5**) is the polyphenol (a type of catechin) isolated from the tea plant *Camellia sinensis* (L.) Kuntze (Theaceae) [280]. EGCG

(10, 20, and 40 mg kg^{-1} for 14 days) protected 3-NP-induced HD rat models against behavioral changes, OS, mitochondrial dysfunction, and striatal injury possibly via NO modulation [49]. In another related study, EGCG (10, 20, and 40 mg kg^{-1}) improved 3-NP-induced behavioral and biochemical changes and altered GSH level *in vivo* by modulating NO [50].

8.4.2.7 Ferulic Acid

Ferulic acid (**6**) is a hydroxycinnamic acid, a phenolic compound isolated from plants [281]. Ferulic acid (with fish oil) was found to exert pronounced neuroprotective activity when compared to its individual components in protecting rats from 3-NP toxicity via reducing the levels of MDA, hydroperoxides, and NO, restoring AChE and dopamine levels, and preventing mitochondrial dysfunctions [51].

8.4.2.8 Fisetin

Fisetin (**7**) is a dietary bioflavonoid naturally occurring in many fruits and vegetables [282]. It exerted neuroprotection as an anti-inflammatory agent against aluminum chloride ($AlCl_3$)-induced neurotoxicity [283] and lipopolysaccharide (LPS)-induced microglial activation [284]. In PC12 cells and in *Drosophila* expressing mutant Httex1 and in R6/2 mouse model of HD, fisetin attenuated mHtt-mediated damage via upregulation of ERK [71].

8.4.2.9 Galantamine

Galantamine (**8**) is an alkaloid derived from the bulbs and flowers of *Galanthus* sp. and some other genera [285]. In a 3-NP-insulted rat model of HD, it exerted neuroprotection via regulating nicotinic acetylcholine receptor (nAChR) [286].

8.4.2.10 Genistein

Genistein (**9**) is a phytoestrogen and an isoflavone isolated from the plants including *Genista* species [287]. It (5, 10, and 20 mg kg^{-1}) prevented memory loss in 3-NP (20 mg kg^{-1})-induced ovariectomized rats via antioxidant, anti-inflammatory, and cholinesterase inhibitory properties [52].

8.4.2.11 Ginsenosides

Ginsenosides (**10**) are plant saponins produced by ginseng plant *P. quinquefolius* L. (Araliaceae) [288]. In a rodent model of HD, they (Rb1, Rb3, or Rd) improved mortality and motor functionality and reduced toxin-induced striatal lesion volume [41].

8.4.2.12 Hesperidin

Hesperidin (**11**) is a flavanone glycoside isolated from *Citrus* sp. (Rutaceae) [289]. It offered neuroprotection in rats as an antioxidant and an antiapoptotic agent besides elevating the MDA levels and improving locomotor activity in the animals [290]. It also prevented behavioral alterations and mitochondrial dysfunction possibly via modulating NO mechanism [53]. Hesperidin pretreatment protected neuronal hypoxia–ischemic brain injury in neonatal rat via antioxidation and phosphorylated protein kinase B (Akt) activation [291].

8.4.2.13 Kaempferol

Kaempferol (**12**) is a natural flavonol found in many plant foods [292]. It has also been reported to ameliorate neurotoxicity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse PD models [293]. Brain injury and neuroinflammation in rats were ameliorated by kaempferol glycosides isolated from *Carthamus tinctorius* via inhibition of signal transducer and activator of transcription 3 (STAT3) and NF-κB activation [294]. In 3-NP-insulted rats, kaempferol enhanced animal life span and protected against motor impairment and striatal lesions [295].

8.4.2.14 L-Theanine

L-Theanine (**13**) is an amino acid analog found in green tea *C. sinensis* (L.) Kuntze (Theaceae) [296]. It (100 and 200 mg kg^{-1}) protected 3-NP (10 mg kg^{-1})-insulted rats via reducing the OS and also via restoring the levels of SOD, GSH, catalase (CAT), and SDH [54].

8.4.2.15 Lutein

Lutein (**14**), a xanthophyll, is considered as a carotenoid-based nutritional source derived from plant-based dietary sources [297]. It exerted its neuroprotective effects in a rat model of retinal detachment [298] and prevented inflammation in retinal ischemic/hypoxic injury [299]. It protected 3-NP-insulted rats via antioxidative mechanisms [300].

8.4.2.16 Lycopene

Lycopene (**15**), a carotene, is found in *Lycopersicon* sp. (Solanaceae) (tomato) and in many red fruits and vegetables [301]. Lycopene ameliorated OS and neurobehavioral abnormalities in a mouse PD model [302]. In another study, mitochondrial dysfunctions in 3-NP-intoxicated rats were found to be attenuated by lycopene (10 mg kg^{-1}, orally for 15 days) via its antioxidative ability [55]. Lycopene (with quercetin and poloxamer 188) reduced anxiety and depression and enhanced body weight and locomotor activity in 3-NP (10 mg kg^{-1})-treated rats [56].

8.4.2.17 Melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine) (**16**) is present in animals, plants, fungi, and bacteria. It improved asymmetric rotational behavior and restored dopamine level in the affected striatum of the intoxicated animal model [59]. Behavioral damage, modulation of brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and neuronal loss were noted in 3-NP-induced rats, which were substantially ameliorated by melatonin (1 mg kg^{-1} per body weight for 21 days) [58]. Further, 3-NP toxicity in rat striatal neuron was found to be protected by the compound [60]. Carvedilol and melatonin were also found to be effective in the same HD model, making melatonin a suitable candidate for anti-HD therapy [57]. Melatonin caused behavioral improvement and prevented dendritic spine damage in rats exposed to 3-NP [61].

8.4.2.18 Naringin

Naringin (**17**) is a natural dietary flavonoid isolated from *Citrus* sp. (Rutaceae) and other plants [303]. It exerted neuroprotection by upregulating BDNF and VEGF and prevented neuronal apoptosis in rats after spinal cord injury [304]. It was found to reduce the levels of Bad and Bax and inhibit the release of cytochrome c and activation of caspase-3 in the same model [62]. Anti-HD activity of the compound (80 mg kg body weight per day, orally) in the animal system was further attributed to its antioxidant and anti-inflammatory abilities [63]. Naringin protected 3-NP-insulted rats via decreasing BBB dysfunction, downregulating the expressions of matrix metalloproteinases (MMPs) 2 and 9, and upregulating the tissue inhibitors of metalloproteinases (TIMPs) 1 and 2, as well as via reducing the markers for neuroinflammation [64]. Naringin, as an antioxidant, reduced lipid peroxidation, restored mitochondrial membrane potential, and prevented apoptosis via regulating B-cell lymphoma 2 and Bcl-2-associated X protein expressions in 3-NP-insulted PC12 cells [65].

8.4.2.19 Nicotine

Nicotine (**18**) is a stimulant drug and a potent parasympathomimetic alkaloid isolated from the members of the plant family Solanaceae (viz. *Nicotiana tabacum* L.). It was also found to prolong the life span and repair olfactory and motor deficits and reduce levodopa-induced dyskinesias in *Drosophila* and monkey PD models, respectively [305, 306]. 3-NP-induced OS in synaptosomes of Wistar rats was protected by the antioxidative ability of nicotine [66].

8.4.2.20 Onjisaponin B

Onjisaponin B is isolated from Radix Polygalae (*Polygala tenuifolia* Willd., Polygalaceae). In PC12 cell expressing mHtt, it mediated autophagy via 5′-adenosine monophosphate-activated protein kinase–mammalian target of rapamycin (AMPK–mTOR) signaling [307].

8.4.2.21 Protopanaxatriol

Protopanaxatriol is extracted from *P. ginseng* C.A. Mey. (Araliaceae). It (5, 10, and 20 mg kg^{-1}) acted as an antioxidant in 3-NP-intoxicated rats via upregulating Hsp70 expression, increasing body weight, preventing the changes in behavior, reducing ROS levels, and enhancing nuclear factor (erythroid-derived 2)-like 2 [Nrf2] entering the nucleus [67].

8.4.2.22 Puerarin

Puerarin (**19**) is an isoflavonoid isolated from the dried roots of *Pueraria* sp. such as *P. lobata* (Willd.) Ohwi (Fabaceae) and many other plants [68, 69]. It (200 mg kg^{-1}) prevented weight loss, OS, and hypothermia, restored locomotor activity, and blocked neurotransmitter anomalies in 3-NP (20 mg kg^{-1})-insulted rats [68]. Moreover, it (200 mg kg^{-1}) exhibited antiapoptotic and anti-inflammatory response and prevented energy deficit in 3-NP (20 mg kg^{-1})-treated rats [69].

8.4.2.23 Quercetin

Quercetin (**20**) is a flavonol occurring in many plant species. Quercetin with fish oil elevated AChE activity and reduced mitochondrial dysfunctions in 3-NP-treated rat HD models [308]. Quercetin protected against hypobaric hypoxia-induced hippocampal neurodegeneration in rat [309], prevented oxidative damage and neuronal apoptosis in rat hippocampus [310], and protected against diabetic neuropathy [311]. The compound prevented movement disturbances and anxiety and inflammatory damages in 3-NP-treated rats [70].

8.4.2.24 Resveratrol

Resveratrol (**21**) is a stilbenoid and a phytoalexin found in many plants. It (5 and 10 mg kg^{-1}, orally, once daily for 8 days) was found to protect against 3-NP-induced motor and cognitive damage in rats [72]. In R6/2 mouse model of HD, it also offered neuroprotection via ERK activation [71].

8.4.2.25 *S*-Allylcysteine

S-Allylcysteine (**22**) is a garlic [*Allium sativum* L. (Amaryllidaceae)]-derived organic compound and a derivative of the amino acid cysteine [312]. *S*-Allylcysteine attenuated oxidative damage to protect against focal cerebral ischemia [313] and mitigated QA-induced neurotoxicity in rats [314]. 3-NP-induced hyperactivity, oxidation, and mitochondrial dysfunction in rats were ameliorated by the compound [73]. Administration of *S*-Allylcysteine was found to protect against oxidative damage and energy depletion in a rat neurotoxic model [74].

8.4.2.26 (–)Schisandrin B

(–)Schisandrin B (**23**) is an antioxidant lignan from *Schisandra chinensis* (Turcz.) Baill. (Schisandraceae) [315]. The compound showed antiapoptotic and anti-necrotic properties in order to attenuate 3-NP toxicity in PC12 cells [316].

8.4.2.27 Sesamol

Sesamol (**24**) is one of the lignin derivatives isolated from *Sesamum indicum* L. (Pedaliaceae). It reversed the effect of PD in a rat model [317] and suppressed neuroinflammation in rat model of diabetic neuropathy [318]. The compound, at doses of 5, 10, and 20 mg kg^{-1}, showed enhanced locomotor activity, motor coordination, and body weight and prevented oxidative damage caused by 3-NP-induced toxicity [75].

8.4.2.28 Spermidine

Spermidine (**25**) (5 and 10 mg kg^{-1}), a polyamine, enhanced antioxidant and anti-inflammatory properties, promoted NMDA receptor antagonistic activities, and prevented alteration in striatal neurotransmitters in QA-induced rats [319]. It (5 and 10 mg) also attenuated 3-NP-induced striatal toxicity in rats via preventing motor coordination, OS, and striatal neurotransmitter levels [76]. Polyamines (including spermidine) were reported efficacious against age-induced memory impairment (AMI) [320].

8.4.2.29 Sulforaphane

Sulforaphane, an isothiocyanate isolated from *Brassica oleracea* L. (Brassicaceae) (broccoli) or other cruciferous vegetables, prevented mHtt cytotoxicity and facilitated mHtt degradation in HEK293 cells expressing N-terminal Htt containing 94 CAG repeats (mHtt-94Q) via enhanced proteasome and autophagy activities [77]. In addition, it protected mice from 3-NP-induced striatal toxicity via upregulation of Kelch-like ECH-associated protein 1 (Keap1)–Nrf2–ARE pathway and downregulation of mitogen-activated protein kinases (MAPK)s and NF-κB pathways [78]. It also exhibited anticonvulsant properties and improved mitochondrial function in mice [321] and attenuated the loss of cholinergic neurons in the brains of AD-like in order to reduce neurobehavioral deficits in the animals [322].

8.4.2.30 Trehalose

Trehalose (**26**) is a naturally occurring α-linked disaccharide. It protected against spinal cord ischemia in rabbits [323] and increased motor neuron survival and mitigated autophagic flux defect against amyotrophic lateral sclerosis in mice [324]. Trehalose, when administered orally, prevented polyQ aggregates in the cerebrum and liver and enhanced motor function in transgenic mice expressing polyQ [79].

8.4.2.31 Vanillin

Vanillin (**27**) (and agomelatine) prevented weight loss, enhanced locomotor activity and learning memory, and protected against toxin-induced biochemical changes in 3-NP-induced rats [80].

8.4.3 Herbal Formulations with Anti-HD Properties

Besides plant extracts, semi-purified fractions, and isolated compounds investigated for their anti-HD properties, a few traditional polyherbal formulations have also been tested for their anti-HD efficacy in clinical trials [325]. Yi-Gan San (YGS) + Chaihu-Jia-Longgu-Muli Tan (CLMT), two Chinese medicines administered in a crossover manner, reduced HD symptoms in HD patients [326]. YGS exerted neuroprotection in patients of schizophrenia [327], borderline personality disorder (BPD) [328], dementia [329], and tardive dyskinesia [330]. CLMT-derived saponins exhibited neuroprotective ability against depression and stress-induced apoptosis in experimental animals [331–333]. Similarly, a number of popular age-old anti-PD traditional formulations in forms of pills, decoctions, recipe, capsules, tablets, and so on include Bak Foong Pill, Bushen Huoxue, Bushen Yanggan Xifeng, Bushen Yanggan, Chunghyuldan, Chuanxiong Chatiao Pulvis, Da-Bu-Yin-Wan, Dangguijakyak-San, Huanglian, Jiedu, Kangzhen Zhijing, Liuwei Dihuang, Qian-Zheng-San, Qing-Xuan, San-Huang-Xie-Xin-Tang, and so on [334]. Bushen Huoxue Granule plus Western medicine improved motor function in PD patients, whereas Bushen Huoxue Granule alone prevented the side effects of Madopar [335, 336]. Moreover, Banxia Houpo Tang, another Chinese herbal formulation, reduced pneumonia risk in older dementia patients, whereas Kampo kami-shoyo-san (TJ-24) decreased the symptoms of antipsychotic-induced parkinsonism [337, 338]. Huanglian Jiedu Tang improved

the ability to study and memory in AD rats [339]. Another orally administered Chinese traditional medicine, SuHeXiang Wan (SHXW), has been a popular choice against seizures, infantile convulsion, stroke, and so on. A modified SHXW essential oil mixture was found to exert sedative, anticonvulsant, and antioxidative potential upon fragrance inhalation. Another modified SHXW (KSOP1009) prevented apoptosis and ROS production *in vitro* [340]. The oriental medicine Jangwonhwan, composed of a boiled extract of 12 medicinal herbs/mushrooms, has popularly been used against cognitive dysfunction. A modified Jangwonhwan (LMK02-Jangwonhwan) composed of seven medicinal plants/mushrooms has been reported to attenuate AD-like pathology, whereas another LMK03-Jangwonhwan also suppressed AD-like pathology in the brain of Tg-APPswe/PS1dE9 mouse AD model [341]. Tong Luo Jiu Nao (TLJN), composed of ginsenoside Rg1 and geniposide, has been reported to protect against cerebral stroke, vascular dementia, and AD and to improve learning and memory [342, 343]. In addition, Trasina, an Ayurvedic formulation primarily used to improve memory and intellect, has also exerted anti-AD properties *in vivo* [344]. Efficacy of traditional Chinese medicine (TCM) is often attributed to their ability to modulate multiple targets associated with complex disease pathogenesis associated with most neurological disorders [345].

8.5 Synergism: A Novel Approach against Neurological Disorders

Traditional and Western medicine differs significantly in the usage of drugs as traditional medicine mostly prescribes direct use of plant parts, whereas Western medicine emphasizes on isolation of active components derived from botanicals. Hence, the traditional medicine, mostly in their polyherbal preparations, possesses holistic and multi-target curative properties different from the conventional medications [346]. Possible synergistic interaction among phytoconstituents may mutually promote the disease-modifying ability of certain herbal extracts and formulations acting on multiple targets especially against complex syndromes such as HD [325]. Efficacy of certain ethnopharmacological polyherbal formulations is often attributed to probable synergistic interaction among its constituents [347–349]. Polyherbal decoctions and infusions with pronounced therapeutic properties are often ascribed to synergy among the active principles [350]. Moreover, various parts of the same plant mixed in certain formulations have also exhibited synergism [351]. Thus, multifactorial nature of pathogenesis of many diseases can be treated via combined efficacy of the complex nature of the herbal formulations [352, 353]. In addition, additive and antagonistic properties may also contribute toward the efficaciousness of traditional polyherbal preparations [350]. Cotyledon powder of *Mucuna pruriens* demonstrated higher anti-PD properties compared to the isolated compound L-3,4-dihydroxyphenylalanine (l-DOPA) in a 6-hydroxydopamine (6-OHDA)-treated rat PD model [354]. Similarly, EGb 761 but not ginkgolides A and B suppressed mouse brain MAO activity *in vitro* [355]. Moreover, CNS-active natural products derived from multi-target compounds have been reported, and multi-target-directed

ligands (MTDLs) have been designed to achieve single compounds having the ability to modulate multiple targets concurrently [356].

8.6 Discussion

Autophagy is an essential process that targets damaged organelles and toxic or aggregated proteins to the lysosome for degradation. It becomes dysfunctional in many neurodegenerative disorders including HD in which protein aggregation is formed due to expansion of the polyQ tract in the N-terminus of the Htt protein [357]. Targeting defective autophagy–lysosomal function is considered as one of the treatment modalities in HD [358]. Onjisaponin B from Radix Polygalae modulated autophagy via AMPK–mTOR signaling in PC12 cells expressing mHtt [307]. Moreover, sulforaphane prevented mHtt cytotoxicity and facilitated mHtt degradation in HEK293 cells expressing mHtt-94Q via enhanced proteasome and autophagy activities [77]. Disease progression in HD has been attributed to OS and consequent protein oxidation, misfolding and degradation, and energy deficiency [359]. An array of natural antioxidants has been reported in this review exhibiting anti-HD properties by antioxidation via modulating the biochemical and molecular parameters associated with HD pathogenesis. In addition, impaired mitochondrial and metabolic dysfunction has been reported to play major roles in the pathogenesis of HD [360]. mHtt is known to compromise cytosolic and mitochondrial calcium homeostasis, which in turn damages mitochondrial functionality responsible for neuronal loss and death associated with HD [361]. A number of natural compounds reported herein have been known to modulate mitochondrial function in order to exert neuroprotective efficacy against HD. Upregulation and increased level of the proapoptotic proteins have also been implicated in cell death in HD pathogenesis [362]. Caspase-2-mediated selective neuronal cell death in the striatum and cortex is reportedly associated with HD [363]. Compounds such as naringin and puerarin exerted their anti-HD efficacy via antiapoptotic properties. Furthermore, autopsied brain tissue of HD patients displayed lower levels of acetylcholine [364]. A number of AChE inhibitors have been implicated against the cognitive decline and dementia in HD [365]. *L. divaricata* aqueous extract enhanced the activity of AChE and restored the GSH/GSSG ratio in the 3-NP-insulted animals [39].

Most herbal products suffer from quality issues including product substitution, contamination, and use of fillers, which reduce their efficacy and dilute the confidence level of the users [366]. Authentication of herbs, advance chemical and biological standardization methods, and quality control of herbal preparations are of utmost importance, which may be achieved via elucidating the major bioactive subset of phytochemicals via mass spectroscopy, HPLC, and so on and more recently via evaluating bioactivity by studying the expression using mRNA microarray method [367]. In addition, DNA barcoding in combination with high-resolution melting analysis (Bar-HRM) has been applied to authenticate herbal samples for uplifting the quality of the herbal preparations as well as for fostering trust in the customers [368].

Another prime consideration for CNS-active drugs is their ability to cross BBB since it restricts drug permeability via modulating uptake and efflux of drugs [369, 370]. BBB protects the brain from toxic metabolites and xenobiotics via separating blood circulation and CNS to maintain its normal homeostasis [371, 372]. An array of natural products has been documented to ameliorate many CNS diseases via regulating the signal transductions associated with the breakdown of BBB [372]. VOO reportedly protected against hypoxia–reoxygenation-insulted rat brain by reducing BBB permeability [236]. Naringin also protected 3-NP-insulted rats via decreasing BBB dysfunction [64]. Thus natural products are cited as exciting options in the progress in the development of novel therapeutics, preventing BBB breakdown especially in the molecular pathogenesis of neurological disorders [372].

Poor bioavailability of phytochemicals is considered as one of the limitations of herbal therapy, which may be negated by using novel delivery systems modulating the pharmacokinetic properties of existing drugs [373]. Most of the anti-HD compounds reported herewith suffer from bioavailability issues. A modified curcumin known as *Theracurmin* exhibited remarkably enhanced oral bioavailability during repetitive systemic exposure to high concentrations [374]. In addition, piperine improved absorption and bioavailability of curcumin in animals and human volunteers [375]. However, in another study, piperine reportedly nullified antidiabetic and antioxidant potential of curcumin owing to its biotransformation [376]. Absolute bioavailability of α-mangostin, another reported anti-HD phytochemical, was also increased in animals when administered orally as a soft capsule [377]. Similarly, unfavorable physicochemical and pharmacokinetic features of celastrol were negated by nanoencapsulation, which was also correlated with its enhanced bioactive efficacy [378]. A self-microemulsifying drug delivery system (SMEDDS) dispersible tablet has also been reported for the oral administration of poorly water-soluble drug celastrol [379]. Chitosan and aspartic acid-encapsulated nanoparticles remarkably enhanced the effectiveness of EGCG [380]. Piperine reportedly enhanced the bioavailability of EGCG, whereas peracetylation increased the *in vitro* bioactivity and bioavailability of the compound [381, 382]. In another study, complexation of fisetin with novel cyclosophoroase dimer exhibited improved bioactivity via enhancement of the solubility and bioavailability of fisetin [383]. Moreover, bioavailability and bioactivity of fisetin were further improved via liposomal encapsulation and nanoemulsion [384, 385]. Methylated resveratrol showed enhanced bioavailability, whereas tomato paste-derived lycopene demonstrated superior bioavailability when compared with the fresh tomato-derived lycopene [386, 387].

Although no adverse side effects have been noticed, many CNS-active herbal drugs, when applied individually, suffer from inadequacy and inconclusiveness due to methodological constraints, namely, small size of the samples, poor experimental designing and statistical analysis, and also the time span of treatment, offering mostly symptomatic relief within that short period [388]. Since many of these herbal constituents probably act as disease-modifying agents for presymptomatic people and against early signs of neurodegenerative diseases, insufficiency in biomarker selection has been a major constraint to evaluate presymptomatic efficacy of the herbal preparations [389, 390]. In-depth preclinical

and clinical trials are still needed to elucidate the therapeutic efficacy of herbs, herbal products, and phytochemicals against complex syndromes such as neurological disorders including HD. Standardization of herbal preparations to work on multiple targets and their stability, formulations of doses, study of potential side effects, and development of tolerance are needed to be demonstrated through the rigors of scientific trials in order to accept these traditional and alternative preparations against neurological disorders not only for symptomatic relief but also as long-lasting disease-modifying agents [391].

8.7 Concluding Remarks

In this review, we presented a comprehensive account of the anti-HD efficacy of herbs and herbal products with notes on their effectiveness against similar neurodegenerative diseases demonstrated via various *in vitro* and *in vivo* models. Scientifically proven herb-based alternative and complementary therapeutics may serve as efficacious aid to conventional medication, which suffers from potential limitations such as development of drug resistance and unwanted adverse effects. HD is one of the deadliest diseases mostly affecting the older people. The present medication provides only systematic relief without delaying the onset of the disease or curing the disease. Therefore, besides conventional medication, an array of plant-based complementary, alternative, or adjunctive therapies have been reported with their proven efficacy against the biochemical and molecular modulations associated with HD pathophysiology in HD models and patients. However, most of the preclinical studies have not been supported with clinical trials, and only the traditional Chinese formulation YGS and Tan CLMT have been administered in HD patients in a crossover manner. Hence, clinical outcomes of various studies are needed to be evaluated in order to accept the efficacy of herbal medication in mainstream medicine. In addition, possible synergy among multi-target phytoconstituents may modulate the complex underlying mechanisms of pathogenesis of HD more effectively when compared to individual compounds.

Abbreviations

3-NP/3-NPA	3-nitropropionic acid
5-HT	serotonin/5-hydroxytryptamine
6-OHDA	6-hydroxydopamine
A(2A)-R	adenosine A_2A receptor
AAV	adeno-associated viral
AChE	acetylcholinesterase
AD	Alzheimer's disease
ADHD	attention deficit hyperactivity disorder
Akt	protein kinase B
$AlCl_3$	aluminum chloride

AMPK-mTOR	5′-adenosine monophosphate-activated protein kinase–mammalian target of rapamycin
ASOs	antisense oligonucleotides
ATF3	activating transcription factor 3
ATP	adenosine triphosphate
Bar-HRM	DNA barcoding in combination with high-resolution melting analysis
BBB	blood–brain barrier
BDNF	brain-derived neurotrophic factor
CAG	cytosine, adenine, guanine
cAMP	cyclic adenosine monophosphate
CAT	catalase
CBD	cannabidiol
cDNA	complementary DNA
CGNs	cerebellar granule neurons
CIH	complementary and integrative health
CLMT	Chaihu-Jia-Longgu-Muli Tan
CNS	central nervous system
CP	caudate–putamen
CREB/CBP	cAMP response element–binding protein/CREB-binding protein
Cur-PLGA-NPs	curcumin-encapsulated poly(lactic-co-glycolic) acid (PLGA) nanoparticles
DAF-16/FOXO	dauer formation-16/forkhead box O
DOPA	L-3,4-dihydroxyphenylalanine
EGb	Ginkgo biloba extract
EGCG	(–)-epigallocatechin-gallate
ERK	extracellular signal-regulated kinase
ETC	electron transport chain
$FeSO_4$	ferrous sulfate
FUS	fused-in-sarcoma
G(i/o)	G-protein alpha subtype i/o
GABA	gamma-aminobutyric acid
GDNF	glial cell line-derived neurotrophic factor
GSH	glutathione
GSH/GSSG	glutathione/glutathione disulfide
HANDs	human immunodeficiency virus (HIV)-associated neurocognitive disorders
HD	Huntington's disease
HIAA	5-hydroxyindoleacetic acid
HSP	heat shock protein
Htt	huntingtin
IA	ibotenic acid
IL	interleukin
KA	kainic acid
Keap1	Kelch-like ECH-associated protein 1
LDH	lactate dehydrogenase

MAO	monoamine oxidase
MAPKs	mitogen-activated protein kinases
MCAO	middle cerebral artery occlusion
MDA	malondialdehyde
MeAsp	*N*-methyl-D-aspartate
mHtt	mutant huntingtin protein
MMPs	matrix metalloproteinases
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
msh2	MutS protein homolog 2
MTDLs	multi-target-directed ligands
NADPH	nicotinamide adenine dinucleotide phosphate
NF-KB	nuclear factor-κB
NMDA	*N*-methyl-D-aspartate
NO	nitric oxide
Nrf2	nuclear factor (erythroid-derived 2)-like 2
OS	oxidative stress
p38 MAPK	p38 mitogen-activated protein kinase
PC12	pheochromocytoma
PD	Parkinson's disease
PGRN	progranulin
pms1	PMS1 homolog 1
polyQ	polyglutamine
PP	prepubertal
QA	quinolinic acid
RBD	REM sleep behavior disorder
REM	rapid eye movement
RNAi	RNA interference
ROS	reactive oxygen species
SDH	succinate dehydrogenase
SHXW	SuHeXiang Wan
siRNA	small interfering RNA
SIRT1	sirtuin-1
SOD	superoxide dismutase
SSRIs	selective serotonin reuptake inhibitors
STAT3	signal transducer and activator of transcription 3
TAR	transactive response
TBARS	thiobarbituric acid reactive substances
TBI	traumatic brain injury
TBZ	tetrabenazine
TCM	traditional Chinese medicine
TDP-4	TAR DNA-binding protein 43
Tgasei	transglutaminase inhibitors
TIMPs	tissue inhibitors of metalloproteinases
TNF-α	tumor necrosis factor-α
VEGF	vascular endothelial growth factor
YAC	yeast artificial chromosome
YGS	Yi-Gan San

References

1 Squitieri, F. and de Yebenes, J.G. (2015) Profile of pridopidine and its potential in the treatment of Huntington disease: the evidence to date. *Drug Des. Dev. Ther.*, **28** (9), 5827–5833.
2 Carroll, J.B., Bates, G.P., Steffan, J., Saft, C., and Tabrizi, S.J. (2015) Treating the whole body in Huntington's disease. *Lancet Neurol.*, **14** (11), 1135–1142.
3 Young, D. (2016) Gene therapy-based modeling of neurodegenerative disorders: Huntington's disease. *Methods Mol. Biol.*, **1382**, 383–395.
4 McBride, J.L. and Clark, R.L. (2016) Stereotaxic surgical targeting of the nonhuman primate caudate and putamen: gene therapy for Huntington's disease. *Methods Mol. Biol.*, **1382**, 409–428.
5 Kumar, A., Singh, S.K., Kumar, V., Kumar, D., Agarwal, S., and Rana, M.K. (2015) Huntington's disease: an update of therapeutic strategies. *Gene*, **556** (2), 91–97.
6 Merienne, N. and Déglon, N. (2015) Gene silencing approaches for the treatment of Huntington's disease. *Med. Sci. (Paris)*, **31** (2), 159–167.
7 Thomas, E.A. (2015) DNA methylation in Huntington's disease: implications for transgenerational effects. *Neurosci. Lett.*, **625**, 34–39, pii: S0304-3940(15)30223-30228.
8 Andre, R., Carty, L., and Tabrizi, S.J. (2015) Disruption of immune cell function by mutant huntingtin in Huntington's disease pathogenesis. *Curr. Opin. Pharmacol.*, **10** (26), 33–38.
9 Huntington, G. (1872) On chorea. *Med. Surg. Rep.* (Philadelphia), **26**, 317–321.
10 Bruyn, G.W., Bots, G.T.A.M., and Dom, R. (1979) in *Huntington's Disease, Advances in Neurology*, vol. **23** (eds T.N. Chase, N.S. Wexler, and A. Barbeau), Raven Press, New York, pp. 83–93.
11 Jergelsma, G. (1908) Nue anatomische befunde bei paralysis agitans und bei chronischer progessiver chorea. *Neurol. Centralbl.*, **27**, 995–996.
12 Kremer, B., Goldberg, P., Andrew, S.E., Theilmann, J., Telenius, H., Zeisler, J., Squitieri, F., Lin, B., Bassett, A., and Almqvist, E. (1994) A worldwide study of the Huntington's disease mutation. The sensitivity and specificity of measuring CAG repeats. *N. Engl. J. Med.*, **330** (20), 1401–1406.
13 Kent, A. (2004) Huntington's disease. *Nurs. Stand.*, **18** (32), 45–51.
14 Nayak, A., Ansar, R., Verma, S.K., Bonifati, D.M., and Kishore, U. (2011) Huntington's disease: an immune perspective. *Neurol. Res. Int.*, **2011**, 563784.
15 Choudhary, S., Kumar, P., and Malik, J. (2013) Plants and phytochemicals for Huntington's disease. *Pharmacogn. Rev.*, **7** (14), 81–91.
16 Myers, R.H., MacDonald, M.E., Koroshetz, W.J., Duyao, M.P., Ambrose, C.M., Taylor, S.A., Barnes, G., Srinidhi, J., Lin, C.S., and Whaley, W.L. (1993) De novo expansion of a (CAG)n repeat in sporadic Huntington's disease. *Nat. Genet.*, **5** (2), 168–173.
17 Harper, P.S. (1992) The epidemiology of Huntington's disease. *Hum. Genet.*, **89** (4), 365–376.
18 Pringsheim, T., Wiltshire, K., Day, L., Dykeman, J., Steeves, T., and Jette, N. (2012) The incidence and prevalence of Huntington's disease: a systematic review and meta-analysis. *Mov. Disord.*, **27** (9), 1083–1091.

19 Jha, S. and Patel, R. (2004) Some observations on the spectrum of dementia. *Neurol. India*, **52** (2), 213–214.

20 Paulsen, J.S., Zhao, H., Stout, J.C., Brinkman, R.R., Guttman, M., Ross, C.A., Como, P., Manning, C., Hayden, M.R., Shoulson, I., and Huntington Study Group (2001) Clinical markers of early disease in persons near onset of Huntington's disease. *Neurology*, **57** (4), 658–662.

21 Walker, F.O. (2007) Huntington's disease. *Lancet*, **369**, 218–228.

22 Phillips, W., Shannon, K.M., and Barker, R.A. (2008) The current clinical management of Huntington's disease. *Mov. Disord.*, **23**, 1491–1504.

23 Killoran, A. and Biglan, K.M. (2014) Current therapeutic options for Huntington's disease: good clinical practice versus evidence-based approaches? *Mov. Disord.*, **29**, 1404–1413.

24 Setter, S.M., Neumiller, J.J., Dobbins, E.K., Wood, L., Clark, J., DuVall, C.A., and Santiago, A. (2009) Treatment of chorea associated with Huntington's disease: focus on tetrabenazine. *Consult. Pharm.*, **24** (7), 524–537.

25 Labuschagne, I., Jones, R., Callaghan, J., Whitehead, D., Dumas, E.M., Say, M.J., Hart, E.P., Justo, D., Coleman, A., Dar Santos, R.C., Frost, C., Craufurd, D., Tabrizi, S.J., and Stout, J.C. (2013) TRACK-HD Investigators. Emotional face recognition deficits and medication effects in pre-manifest through stage-II Huntington's disease. *Psychiatry Res.*, **207**, 118–126.

26 Frank, S. and Jankovic, J. (2010) Advances in the pharmacological management of Huntington's disease. *Drugs*, **70**, 561–571.

27 Mestre, T.A. and Ferreira, J.J. (2012) An evidence-based approach in the treatment of Huntington's disease. *Parkinsonism Relat. Disord.*, **18**, 316–320.

28 Halpin, M. (2012) Accounts of suicidality in the Huntington disease community. *Omega (Westport)*, **65**, 317–334.

29 Milewski, M., Hoffman-Zacharska, D., and Ball, J. (2015) Molecular therapeutic strategies for Huntington's disease. [Article in Polish]. *Postepy Biochem.*, **61** (1), 18–24.

30 Godinho, B.M., Malhotra, M., O'Driscoll, C.M., and Cryan, J.F. (2015) Delivering a disease-modifying treatment for Huntington's disease. *Drug Discov. Today*, **20** (1), 50–64.

31 Uabundit, N., Wattanathorn, J., Mucimapura, S., and Ingkaninan, K. (2010) Cognitive enhancement and neuroprotective effects of *Bacopa monnieri* in Alzheimer's disease model. *J. Ethnopharmacol.*, **127** (1), 26–31.

32 Ayyappan, P., Palayyan, S.R., and Kozhiparambil Gopalan, R. (2016) Attenuation of oxidative damage by *Boerhaavia diffusa* L. against different neurotoxic agents in rat brain homogenate. *J. Diet. Suppl.*, **13** (3), 300–312.

33 Shinomol, G.K. and Muralidhara (2008) Prophylactic neuroprotective property of *Centella asiatica* against 3-nitropropionic acid induced oxidative stress and mitochondrial dysfunctions in brain regions of prepubertal mice. *Neurotoxicology*, **29**, 948–957.

34 Shinomol, G.K. and Muralidhara (2008) Effect of *Centella asiatica* leaf powder on oxidative markers in brain regions of prepubertal mice in vivo and its in vitro efficacy to ameliorate 3-NPA-induced oxidative stress in mitochondria. *Phytomedicine*, **15**, 971–984.

35 Shinomol, G.K., Ravikumar, H., and Muralidhara (2010) Prophylaxis with *Centella asiatica* confers protection to prepubertal mice against 3-nitropropionic-acid-induced oxidative stress in brain. *Phytother. Res.*, **24**, 885–892.

36 Kaur, M., Prakash, A., and Kalia, A.N. (2015) Neuroprotective potential of antioxidant potent fractions from *Convolvulus pluricaulis* Chois. in 3-nitropropionic acid challenged rats. *Nutr. Neurosci.*, **19** (2), 70–78.

37 Malik, J., Choudhary, S., and Kumar, P. (2015) Protective effect of *Convolvulus pluricaulis* standardized extract and its fractions against 3-nitropropionic acid-induced neurotoxicity in rats. *Pharm. Biol.*, **53** (10), 1448–1457.

38 Manavalan, A., Feng, L., Sze, S.K., Hu, J.M., and Heese, K. (2012) New insights into the brain protein metabolism of *Gastrodia elata*-treated rats by quantitative proteomics. *J. Proteomics*, **75**, 2468–2479.

39 Courtes, A.A., Arantes, L.P., Barcelos, R.P., da Silva, I.K., Boligon, A.A., Athayde, M.L., Puntel, R.L., and Soares, F.A. (2015) Protective effects of aqueous extract of *Luehea divaricata* against behavioral and oxidative changes induced by 3-nitropropionic acid in rats. *Evid Based Complement. Alternat. Med.*, **2015**, 723431.

40 Jang, M., Lee, M.J., Kim, C.S., and Cho, I.H. (2013) Korean Red Ginseng extract attenuates 3-nitropropionicc-induced Huntington's-like symptoms. *Evid Based Complement. Alternat. Med.*, **2013**, 237207.

41 Lian, X.Y., Zhang, Z., and Stringer, J.L. (2005) Protective effects of ginseng components in a rodent model of neurodegeneration. *Ann. Neurol.*, **57**, 642–648.

42 Im, A.R., Chae, S.W., Zhang, G.J., and Lee, M.Y. (2014) Neuroprotective effects of *Psoralea corylifolia* Linn seed extracts on mitochondrial dysfunction induced by 3-nitropropionic acid. *BMC Complement. Altern. Med.*, **14**, 370.

43 Al-Sabahi, B.N., Fatope, M.O., Essa, M.M., Subash, S., Al-Busafi, S.N., Al-Kusaibi, F.S., and Manivasagam, T. (2014) Pomegranate seed oil: effect on 3-nitropropionic acid-induced neurotoxicity in PC12 cells and elucidation of unsaturated fatty acids composition. *Nutr. Neurosci.* doi: http://dx.doi.org/10.1179/1476830514Y.0000000155.

44 Sudati, J.H., Fachinetto, R., Pereira, R.P., Boligon, A.A., Athayde, M.L., Soares, F.A., de Vargas Barbosa, N.B., and Rocha, J.B. (2009) *In vitro* antioxidant activity of *Valeriana officinalis* against different neurotoxic agents. *Neurochem. Res.*, **34** (8), 1372–1379.

45 Jiang, W., Wei, W., Gaertig, M.A., Li, S., and Li, X.J. (2015) Therapeutic effect of berberine on Huntington's disease transgenic mouse model. *PLoS ONE*, **10** (7), e0134142.

46 Cleren, C., Calingasan, N.Y., Chen, J., and Beal, M.F. (2005) Celastrol protects against MPTP- and 3-nitropropionic acid-induced neurotoxicity. *J. Neurochem.*, **94**, 995–1004.

47 Kumar, P., Padi, S.S., Naidu, P.S., and Kumar, A. (2007) Possible neuroprotective mechanisms of curcumin in attenuating 3-nitropropionic acid-induced neurotoxicity. *Methods Find Exp. Clin. Pharmacol.*, **29**, 19–25.

48 Singh, S., Jamwal, S., and Kumar, P. (2015) Piperine enhances the protective effect of curcumin against 3-NP induced neurotoxicity: possible neurotransmitters modulation mechanism. *Neurochem. Res.*, **40** (8), 1758–1766.

49 Kumar, P. and Kumar, A. (2009) Protective effects of epigallocatechin gallate following 3-nitropropionic acid-induced brain damage: possible nitric oxide mechanisms. *Psychopharmacology (Berl.)*, **207**, 257–270.

50 Kumar, P. and Kumar, A. (2009) Effect of lycopene and epigallocatechin-3-gallate against 3-nitropropionic acid induced cognitive dysfunction and glutathione depletion in rat: a novel nitric oxide mechanism. *Food Chem. Toxicol.*, **47**, 2522–2530.

51 Joseph, K.D. (2014) Neuroprotective efficacy of a combination of fish oil and ferulic acid against 3-nitropropionic acid-induced oxidative stress and neurotoxicity in rats: behavioural and biochemical evidence. *Appl. Physiol. Nutr. Metab.*, **39** (4), 487–496.

52 Menze, E.T., Esmat, A., Tadros, M.G., Abdel-Naim, A.B., and Khalifa, A.E. (2015) Genistein improves 3-NPA-induced memory impairment in ovariectomized rats: impact of its antioxidant, anti-inflammatory and acetylcholinesterase modulatory properties. *PLoS ONE*, **10** (2), e0117223.

53 Kumar, P. and Kumar, A. (2010) Protective effect of hesperidin and naringin against 3-nitropropionic acid induced Huntington's like symptoms in rats: possible role of nitric oxide. *Behav. Brain Res.*, **206**, 38–46.

54 Thangarajan, S., Deivasigamani, A., Natarajan, S.S., Krishnan, P., and Mohanan, S.K. (2014) Neuroprotective activity of L-theanine on 3-nitropropionic acid-induced neurotoxicity in rat striatum. *Int. J. Neurosci.*, **124** (9), 673–684.

55 Sandhir, R., Mehrotra, A., and Kamboj, S.S. (2010) Lycopene prevents 3-nitropropionic acid-induced mitochondrial oxidative stress and dysfunctions in nervous system. *Neurochem. Int.*, **57**, 579–587.

56 Jain, D. and Gangshettiwar, A. (2014) Combination of lycopene, quercetin and poloxamer 188 alleviates anxiety and depression in 3-nitropropionic acid-induced Huntington's disease in rats. *J. Intercult. Ethnopharmacol.*, **3** (4), 186–191.

57 Tasset, I., Espínola, C., Medina, F.J., Feijóo, M., Ruiz, C., Moreno, E., Gómez, M.M., Collado, J.A., Muñoz, C., Muntané, J., Montilla, P., and Túnez, I. (2009) Neuroprotective effect of carvedilol and melatonin on 3-nitropropionic acid-induced neurotoxicity in neuroblastoma. *J. Physiol. Biochem.*, **65**, 291–296.

58 Tasset, I., Agüera, E., Olmo-Camacho, R., Escribano, B., Sánchez-López, F., Delgado, M.J., Cruz, A.H., Gascón, F., Luque, E., Peña, J., Jimena, I.M., and Túnez, I. (2011) Melatonin improves 3-nitropropionic acid induced behavioral alterations and neurotrophic factors levels. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **35**, 1944–1949.

59 Nam, E., Lee, S.M., Koh, S.E., Joo, W.S., Maeng, S., Im, H.I., and Kim, Y.S. (2005) Melatonin protects against neuronal damage induced by 3-nitropropionic acid in rat striatum. *Brain Res.*, **1046**, 90–96.

60 Mu, S., OuYang, L., Liu, B., Zhu, Y., Li, K., Zhan, M., Liu, Z., Jia, Y., and Lei, W. (2011) Protective effect of melatonin on 3-NP induced striatal interneuron injury in rats. *Neurochem. Int.*, **59**, 224–234.

61 Chakraborty, J., Nthenge-Ngumbau, D.N., Rajamma, U., and Mohanakumar, K.P. (2014) Melatonin protects against behavioural dysfunctions and dendritic spine damage in 3-nitropropionic acid-induced rat model of Huntington's disease. *Behav. Brain Res.*, **264**, 91–104.

62 Gopinath, K., Prakash, D., and Sudhandiran, G. (2011) Neuroprotective effect of naringin, a dietary flavonoid against 3-nitropropionic acid-induced neuronal apoptosis. *Neurochem. Res.*, **59**, 1066–1073.

63 Gopinath, K. and Sudhandiran, G. (2012) Naringin modulates oxidative stress and inflammation in 3-nitropropionic acid-induced neurodegeneration through the activation of nuclear factor-erythroid 2-related factor-2 signalling pathway. *Neuroscience*, **227**, 134–143.

64 Gopinath, K. and Sudhandiran, G. (2015) Protective effect of naringin on 3-nitropropionic acid-induced neurodegeneration through the modulation of matrix metalloproteinases and glial fibrillary acidic protein. *Can. J. Physiol. Pharmacol.*, **94** (1), 65–71.

65 Kulasekaran, G. and Ganapasam, S. (2015) Neuroprotective efficacy of naringin on 3-nitropropionic acid-induced mitochondrial dysfunction through the modulation of Nrf2 signaling pathway in PC12 cells. *Mol. Cell. Biochem.*, **409** (1-2), 199–211.

66 Túnez, I., Montilla, P., Muñoz, M.C., and Drucker-Colín, R. (2004) Effect of nicotine on 3-nitropropionic acid-induced oxidative stress in synaptosomes. *Eur. J. Pharmacol.*, **504**, 169–175.

67 Gao, Y., Chu, S.F., Li, J.P., Zhang, Z., Yan, J.Q., Wen, Z.L., Xia, C.Y., Mou, Z., Wang, Z.Z., He, W.B., Guo, X.F., Wei, G.N., and Chen, N.H. (2015) Protopanaxtriol protects against 3-nitropropionic acid-induced oxidative stress in a rat model of Huntington's disease. *Acta Pharmacol. Sin.*, **36** (3), 311–322.

68 Mahdy, H.M., Mohamed, M.R., Emam, M.A., Karim, A.M., Abdel-Naim, A.B., and Khalifa, A.E. (2014) Puerarin ameliorates 3-nitropropionic acid-induced neurotoxicity in rats: possible neuromodulation and antioxidant mechanisms. *Neurochem. Res.*, **39** (2), 321–332.

69 Mahdy, H.M., Mohamed, M.R., Emam, M.A., Karim, A.M., Abdel-Naim, A.B., and Khalifa, A.E. (2014) The anti-apoptotic and anti-inflammatory properties of puerarin attenuate 3-nitropropionic-acid induced neurotoxicity in rats. *Can. J. Physiol. Pharmacol.*, **92** (3), 252–258.

70 Chakraborty, J., Singh, R., Dutta, D., Naskar, A., Rajamma, U., and Mohanakumar, K.P. (2014) Quercetin improves behavioral deficiencies, restores astrocytes and microglia, and reduces serotonin metabolism in 3-nitropropionic acid-induced rat model of Huntington's disease. *CNS Neurosci. Ther.*, **20**, 10–19.

71 Maher, P., Dargusch, R., Bodai, L., Gerard, P.E., Purcell, J.M., and Marsh, J.L. (2011) ERK activation by the polyphenols fisetin and resveratrol provides neuroprotection in multiple models of Huntington's disease. *Hum. Mol. Genet.*, **20**, 261–270.

72 Kumar, P., Padi, S.S., Naidu, P.S., and Kumar, A. (2006) Effect of resveratrol on 3-nitropropionic acid-induced biochemical and behavioural changes: possible neuroprotective mechanisms. *Behav. Pharmacol.*, **17**, 485–492.

73 Herrera-Mundo, M.N., Silva-Adaya, D., Maldonado, P.D., Galván-Arzate, S., Andrés-Martínez, L., Pérez-De La Cruz, V., Pedraza-Chaverrí, J., and Santamaría, A. (2006) S-Allylcysteine prevents the rat from 3-nitropropionic acid-induced hyperactivity, early markers of oxidative stress and mitochondrial dysfunction. *Neurosci. Res.*, **56**, 39–44.

74 Elinos-Calderón, D., Robledo-Arratia, Y., Pérez-De La Cruz, V., Maldonado, P.D., Galván-Arzate, S., Pedraza-Chaverrí, J., and Santamaría, A. (2010) Antioxidant strategy to rescue synaptosomes from oxidative damage and energy failure in neurotoxic models in rats: protective role of S-allylcysteine. *J. Neural Transm.*, **117**, 35–44.

75 Kumar, P., Kalonia, H., and Kumar, A. (2009) Sesamol attenuate 3-nitropropionic acid-induced Huntington-like behavioral, biochemical, and cellular alterations in rats. *J. Asian Nat. Prod. Res.*, **11**, 439–450.

76 Jamwal, S. and Kumar, P. (2015) Spermidine ameliorates 3-nitropropionic acid (3-NP)-induced striatal toxicity: possible role of oxidative stress, neuroinflammation, and neurotransmitters. *Physiol. Behav.*, **155**, 180–187.

77 Liu, Y., Hettinger, C.L., Zhang, D., Rezvani, K., Wang, X., and Wang, H. (2014) Sulforaphane enhances proteasomal and autophagic activities in mice and is a potential therapeutic reagent for Huntington's disease. *J. Neurochem.*, **129** (3), 539–547.

78 Jang, M. and Cho, I.H. (2015) Sulforaphane ameliorates 3-nitropropionic acid-induced striatal toxicity by activating the Keap1-Nrf2-ARE pathway and inhibiting the MAPKs and NF-κB pathways. *Mol. Neurobiol.*, **53** (4), 2619–2635.

79 Tanaka, M., Machida, Y., Niu, S., Ikeda, T., Jana, N.R., Doi, H., Kurosawa, M., Nekooki, M., and Nukina, N. (2004) Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. *Nat. Med.*, **10**, 148–154.

80 Gupta, S. and Sharma, B. (2014) Pharmacological benefits of agomelatine and vanillin in experimental model of Huntington's disease. *Pharmacol. Biochem. Behav.*, **122**, 122–135.

81 Sanberg, P.R. and Coyle, J.T. (1984) Scientific approaches to Huntington's disease. *Crit. Rev. Clin. Neurobiol.*, **1**, 1–44.

82 Qin, Z.H., Wang, Y.M., and Chase, T.N. (1996) Stimulation of *N*-methyl-D-aspartate receptors induces apoptosis in rat brain. *Brain Res.*, **725**, 166–176.

83 Qin, Z.H., Wang, Y.M., Nakai, M., and Chase, T.N. (1998) Nuclear factor-kB contributes to excitotoxin-induced apoptosis in rat striatum. *Mol. Pharmacol.*, **53**, 33–42.

84 Coyle, J.T., Ferkany, J.W., and Zaczek, R. (1983) Kainic acid: insights from a neurotoxin into the pathophysiology of Huntington's disease. *Neurobehav. Toxicol. Teratol.*, **5** (6), 617–624.

85 Coyle, J.T. (1979) An animal model for Huntington's disease. *Biol. Psychiatry*, **14** (2), 251–276.

86 Schwarcz, R. and Coyle, J.T. (1977) Striatal lesions with kainic acid: neurochemical characteristics. *Brain Res.*, **127** (2), 235–249.

87 Beal, M.F., Marshall, P.E., Burd, G.D., Landis, D.M., and Martin, J.B. (1985) Excitotoxin lesions do not mimic the alteration of somatostatin in Huntington's disease. *Brain Res.*, **361** (1-2), 135–145.

88 Beal, M.F., Kowall, N.W., Ellison, D.W., Mazurek, M.F., Swartz, K.J., and Martin, J.B. (1986) Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature*, **321** (6066), 168–171.

89 Bruyn, R.P. and Stoof, J.C. (1990) The quinolinic acid hypothesis in Huntington's chorea. *J. Neurol. Sci.*, **95** (1), 29–38.

90 Beal, M.F., Ferrante, R.J., Swartz, K.J., and Kowall, N.W. (1991) Chronic quinolinic acid lesions in rats closely resemble Huntington's disease. *J. Neurosci.*, **11** (6), 1649–1659.

91 Reynolds, G.P., Pearson, S.J., Halket, J., and Sandler, M. (1988) Brain quinolinic acid in Huntington's disease. *J. Neurochem.*, **50** (6), 1959–1960.

92 Smith, T.S. and Bennett, J.P. Jr. (1997) Mitochondrial toxins in models of neurodegenerative diseases. I: *In vivo* brain hydroxyl radical production during systemic MPTP treatment or following microdialysis infusion of methylpyridinium or azide ions. *Brain Res.*, **765** (2), 183–188.

93 Wang, L.H. and Qin, Z.H. (2006) Animal models of Huntington's disease: implications in uncovering pathogenic mechanisms and developing therapies. *Acta Pharmacol. Sin.*, **27** (10), 1287–1302.

94 Beal, M.F. (1994) Neurochemistry and toxin models in Huntington's disease. *Curr. Opin. Neurol.*, **7** (6), 542–547.

95 Koroshetz, W.J., Jenkins, B.G., Rosen, B.R., and Beal, M.F. (1997) Energy metabolism defects in Huntington's disease and effects of coenzyme Q10. *Ann. Neurol.*, **41** (2), 160–165.

96 Ludolph, A.C., He, F., Spencer, P.S., Hammerstad, J., and Sabri, M. (1991) 3-Nitropropionic acid-exogenous animal neurotoxin and possible human striatal toxin. *Can. J. Neurol. Sci.*, **18** (4), 492–498.

97 Hamilton, B.F., Gould, D.H., and Gustine, D.L. (2000) in *Mitochondrial Inhibitors and Neurodegenerative Disorders* (eds P.R. Sanberg, H. Nishino, and C.V. Borlongan), Humana Press, Totowa, NJ, pp. 21–33.

98 Hamilton, B.F. and Gould, D.H. (1987) Nature and distribution of brain lesions in rats intoxicated with 3-nitropropionic acid: a type of hypoxic (energy deficient) brain damage. *Acta Neuropathol.*, **72** (3), 286–297.

99 Hamilton, B.F. and Gould, D.H. (1987) Correlation of morphologic brain lesions with physiologic alterations and blood-brain barrier impairment in 3-nitropropionic acid toxicity in rats. *Acta Neuropathol.*, **74** (1), 67–74.

100 Ludolph, A.C., Seelig, M., and Ludolph, A. (1992) 3-Nitropropionic acid decreases cellular energy levels and causes neuronal degeneration in cortical explants. *Neurodegeneration*, **1**, 151–161.

101 Beal, M.F., Brouillet, E., Jenkins, B.G., Ferrante, R.J., Kowall, N.W., Miller, J.M., Storey, E., Srivastava, R., Rosen, B.R., and Hyman, B.T. (1993) Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. *J. Neurosci.*, **13** (10), 4181–4192.

102 Brouillet, E., Jenkins, B.G., Hyman, B.T., Ferrante, R.J., Kowall, N.W., Srivastava, R., Roy, D.S., Rosen, B.R., and Beal, M.F. (1993) Age-dependent vulnerability of the striatum to the mitochondrial toxin 3-nitropropionic acid. *J. Neurochem.*, **60** (1), 356–359.

103 Ferrante, R.J. (1792) Mouse models of Huntington's disease and methodological considerations for therapeutic trials. *Biochim. Biophys. Acta*, **1792** (6), 506–520.

104 Sun, Z., Xie, J., and Reiner, A. (2002) The differential vulnerability of striatal projection neurons in 3-nitropropionic acid-treated rats does not match that typical of adult-onset Huntington's disease. *Exp. Neurol.*, **176** (1), 55–65.

105 Yang, S.H., Cheng, P.H., Banta, H., Piotrowska-Nitsche, K., Yang, J.J., Cheng, E.C., Snyder, B., Larkin, K., Liu, J., Orkin, J., and Fang, Z.H. (2008) Towards a transgenic model of Huntington's disease in a non-human primate. *Nature*, **453** (7197), 921–924.

106 Roitberg, B.Z., Emborg, M.E., Sramek, J.G., Palfi, S., and Kordower, J.H. (2002) Behavioral and morphological comparison of two nonhuman primate models of Huntington's disease. *Neurosurgery*, **50** (1), 137–145.

107 Hantraye, P., Riche, D., Maziere, M., and Isacson, O. (1990) A primate model of Huntington's disease: behavioral and anatomical studies of unilateral excitotoxic lesions of the caudate-putamen in the baboon. *Exp. Neurol.*, **108** (2), 91–104.

108 Burns, L.H., Pakzaban, P., Deacon, T.W., Brownell, A.L., Tatter, S.B., Jenkins, B.G., and Isacson, O. (1995) Selective putaminal excitotoxic lesions in non-human primates model the movement disorder of Huntington disease. *Neuroscience*, **64** (4), 1007–1017.

109 Menalled, L.B. and Chesselet, M.F. (2002) Mouse models of Huntington's disease. *Trends Pharmacol. Sci.*, **23** (1), 32–39.

110 Hickey, M.A. and Chesselet, M.F. (2003) The use of transgenic and knock-in mice to study Huntington's disease. *Cytogenet. Genome Res.*, **100** (1-4), 276–286.

111 Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., Lawton, M., Trottier, Y., Lehrach, H., Davies, S.W., and Bates, G.P. (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell*, **87** (3), 493–506.

112 Li, J.Y., Popovic, N., and Brundin, P. (2005) The use of the R6 transgenic mouse models of Huntington's disease in attempts to develop novel therapeutic strategies. *NeuroRx*, **2** (3), 447–464.

113 Goldberg, Y.P., Kalchman, M.A., Metzler, M., Nasir, J., Zeisler, J., Graham, R., Koide, H.B., O'Kusky, J., Sharp, A.H., Ross, C.A., Jirik, F., and Hayden, M.R. (1996) Absence of disease phenotype and intergenerational stability of the CAG repeat in transgenic mice expressing the human Huntington disease transcript. *Hum. Mol. Genet.*, **5** (2), 177–185.

114 Reddy, P.H., Williams, M., Charles, V., Garrett, L., Pike-Buchanan, L., Whetsell, W.O. Jr., Miller, G., and Tagle, D.A. (1998) Behavioural abnormalities and selective neuronal loss in HD transgenic mice expressing mutated full-length HD cDNA. *Nat. Genet.*, **20** (2), 198–202.

115 Hodgson, J.G., Agopyan, N., Gutekunst, C.A., Leavitt, B.R., LePiane, F., Singaraja, R., Smith, D.J., Bissada, N., McCutcheon, K., Nasir, J., Jamot, L., Li, X.J., Stevens, M.E., Rosemond, E., Roder, J.C., Phillips, A.G., Rubin, E.M., Hersch, S.M., and Hayden, M.R. (1999) A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. *Neuron*, **23** (1), 181–192.

116 Van Raamsdonk, J.M., Murphy, Z., Slow, E.J., Leavitt, B.R., and Hayden, M.R. (2005) Selective degeneration and nuclear localization of mutant huntingtin in the YAC128 mouse model of Huntington disease. *Hum. Mol. Genet.*, **14** (24), 3823–3835.

117 Van Raamsdonk, J.M., Warby, S.C., and Hayden, M.R. (2007) Selective degeneration in YAC mouse models of Huntington disease. *Brain Res. Bull.*, **72** (2-3), 124–131.

118 Menalled, L.B. (2005) Knock-in mouse models of Huntington's disease. *NeuroRx*, **2** (3), 465–470.

119 Menalled, L.B., Sison, J.D., Wu, Y., Olivieri, M., Li, X.J., Li, H., Zeitlin, S., and Chesselet, M.F. (2002) Early motor dysfunction and striosomal distribution of huntingtin microaggregates in Huntington's disease knock-in mice. *J. Neurosci.*, **22** (18), 8266–8276.

120 Duyao, M.P., Auerbach, A.B., Ryan, A., Persichetti, F., Barnes, G.T., McNeil, S.M., Ge, P., Vonsattel, J.P., Gusella, J.F., and Joyner, A.L. (1995) Inactivation of the mouse Huntington's disease gene homolog Hdh. *Science*, **269** (5222), 407–410.

121 Senut, M.C., Suhr, S.T., Kaspar, B., and Gage, F.H. (2000) Intraneuronal aggregate formation and cell death after viral expression of expanded polyglutamine tracts in the adult rat brain. *J. Neurosci.*, **20** (1), 219–229.

122 Stanek, L.M., Sardi, S.P., Mastis, B., Richards, A.R., Treleaven, C.M., Taksir, T., Misra, K., Cheng, S.H., and Shihabuddin, L.S. (2014) Silencing mutant huntingtin by adeno-associated virus-mediated RNA interference ameliorates disease manifestations in the YAC128 mouse model of Huntington's disease. *Hum. Gene Ther.*, **25** (5), 461–474.

123 Rodriguez-Lebron, E., Denovan-Wright, E.M., Nash, K., Lewin, A.S., and Mandel, R.J. (2005) Intrastriatal rAAV-mediated delivery of anti-huntingtin shRNAs induces partial reversal of disease progression in R6/1 Huntington's disease transgenic mice. *Mol. Ther.*, **12** (4), 618–633.

124 Harper, S.Q., Staber, P.D., He, X., Eliason, S.L., Martins, I.H., Mao, Q., Yang, L., Kotin, R.M., Paulson, H.L., and Davidson, B.L. (2005) RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. *Proc. Natl. Acad. Sci. U.S.A.*, **102** (16), 5820–5825.

125 Machida, Y., Okada, T., Kurosawa, M., Oyama, F., Ozawa, K., and Nukina, N. (2006) rAAV-mediated shRNA ameliorated neuropathology in Huntington disease model mouse. *Biochem. Biophys. Res. Commun.*, **343** (1), 190–197.

126 Boudreau, R.L., McBride, J.L., Martins, I., Shen, S., Xing, Y., Carter, B.J., and Davidson, B.L. (2009) Nonallele-specific silencing of mutant and wild-type huntingtin demonstrates therapeutic efficacy in Huntington's disease mice. *Mol. Ther.*, **17** (6), 1053–1063.

127 Baldo, B., Soylu, R., and Petersén, A. (2013) Maintenance of basal levels of autophagy in Huntington's disease mouse models displaying metabolic dysfunction. *PLoS ONE*, **8** (12), e83050.

128 Ellison, S.M., Trabalza, A., Tisato, V., Pazarentzos, E., Lee, S., Papadaki, V., Goniotaki, D., Morgan, S., Mirzaei, N., and Mazarakis, N.D. (2013) Dose-dependent neuroprotection of $VEGF_{165}$ in Huntington's disease striatum. *Mol. Ther.*, **21** (10), 1862–1875.

129 Yang, S.H. and Chan, A.W. (2011) Transgenic animal models of Huntington's disease. *Curr. Top. Behav. Neurosci.*, **7**, 61–85.

130 Editorial (2008) Transgenic primate models inch forward. *Nat. Neurosci.*, **11**, 729.

131 Sang, T.-K. and Jackson, G.R. (2005) Drosophila models of neurodegenerative disease. *NeuroRx*, **2** (3), 438–446.

132 Lu, B. and Vogel, H. (2009) Drosophila models of neurodegenerative diseases. *Annu. Rev. Pathol.*, **4**, 315–342.

133 Marsh, J.L., Pallos, J., and Thompson, L.M. (2003) Fly models of Huntington's disease. *Hum. Mol. Genet.*, **12** (Suppl. 2), R187–R193.

134 Lewis, E.A. and Smith, G.A. (2015) Using Drosophila models of Huntington's disease as a translatable tool. *J. Neurosci. Methods*, **265**, 89–98, pii: S0165-0270(15)00281-00282.

135 Green, E.W. and Giorgini, F. (2012) Choosing and using Drosophila models to characterize modifiers of Huntington's disease. *Biochem. Soc. Trans.*, **40** (4), 739–745.

136 Krench, M. and Littleton, J.T. (2013) Modeling Huntington disease in Drosophila: insights into axonal transport defects and modifiers of toxicity. *Fly* (Austin), **7** (4), 229–236.

137 Link, C.D. (2006) *C. elegans* models of age-associated neurodegenerative diseases: lessons from transgenic worm models of Alzheimer's disease. *Exp. Gerontol.*, **41** (10), 1007–1013.

138 Calahorro, F. and Ruiz-Rubio, M. (2011) *Caenorhabditis elegans* as an experimental tool for the study of complex neurological diseases: Parkinson's disease, Alzheimer's disease and autism spectrum disorder. *Invert. Neurosci.*, **11** (2), 73–83.

139 Alexander, A.G., Marfil, V., and Li, C. (2014) Use of *Caenorhabditis elegans* as a model to study Alzheimer's disease and other neurodegenerative diseases. *Front. Genet.*, **5**, 279.

140 Bates, E.A., Victor, M., Jones, A.K., Shi, Y., and Hart, A.C. (2006) Differential contributions of *Caenorhabditis elegans* histone deacetylases to huntingtin polyglutamine toxicity. *J. Neurosci.*, **26** (10), 2830–2838.

141 Duan, W. (2013) Targeting sirtuin-1 in Huntington's disease: rationale and current status. *CNS Drugs*, **27** (5), 345–352.

142 Tauffenberger, A., Chitramuthu, B.P., Bateman, A., Bennett, H.P., and Parker, J.A. (2013) Reduction of polyglutamine toxicity by TDP-43, FUS and progranulin in Huntington's disease models. *Hum. Mol. Genet.*, **22** (4), 782–794.

143 Parker, J.A., Holbert, S., Lambert, E., Abderrahmane, S., and Néri, C. (2004) Genetic and pharmacological suppression of polyglutamine-dependent neuronal dysfunction in *Caenorhabditis elegans*. *J. Mol. Neurosci.*, **23** (1-2), 61–68.

144 Krobitsch, S. and Lindquist, S. (2000) Aggregation of huntingtin in yeast varies with the length of the polyglutamine expansion and the expression of chaperone proteins. *Proc. Natl. Acad. Sci. U.S.A.*, **97** (4), 1589–1594.

145 Meriin, A.B., Zhang, X., He, X., Newnam, G.P., Chernoff, Y.O., and Sherman, M.Y. (2002) Huntington toxicity in yeast model depends on polyglutamine

aggregation mediated by a prion-like protein Rnq1. *J. Cell Biol.*, **157** (6), 997–1004.

146 Gokhale, K.C., Newnam, G.P., Sherman, M.Y., and Chernoff, Y.O. (2005) Modulation of prion-dependent polyglutamine aggregation and toxicity by chaperone proteins in the yeast model. *J. Biol. Chem.*, **280** (24), 22809–22818.

147 Schweitzer, J.K. and Livingston, D.M. (1997) Destabilization of CAG trinucleotide repeat tracts by mismatch repair mutations in yeast. *Hum. Mol. Genet.*, **6** (3), 349–355.

148 Kim, M., Lee, H.S., LaForet, G., McIntyre, C., Martin, E.J., Chang, P., Kim, T.W., Williams, M., Reddy, P.H., Tagle, D., Boyce, F.M., Won, L., Heller, A., Aronin, N., and DiFiglia, M. (1999) Mutant huntingtin expression in clonal striatal cells: dissociation of inclusion formation and neuronal survival by caspase inhibition. *J. Neurosci.*, **19** (3), 964–973.

149 Rigamonti, D., Bauer, J.H., De-Fraja, C., Conti, L., Sipione, S., Sciorati, C., Clementi, E., Hackam, A., Hayden, M.R., Li, Y., Cooper, J.K., Ross, C.A., Govoni, S., Vincenz, C., and Cattaneo, E. (2000) Wild-type huntingtin protects from apoptosis upstream of caspase-3. *J. Neurosci.*, **20** (10), 3705–3713.

150 Scotter, E.L., Goodfellow, C.E., Graham, E.S., Dragunow, M., and Glass, M. (2010) Neuroprotective potential of CB_1 receptor agonists in an *in vitro* model of Huntington's disease. *Br. J. Pharmacol.*, **160** (3), 747–761.

151 Liang, Y., Jiang, H., Ratovitski, T., Jie, C., Nakamura, M., Hirschhorn, R.R., Wang, X., Smith, W.W., Hai, T., Poirier, M.A., and Ross, C.A. (2009) ATF3 plays a protective role against toxicity by N-terminal fragment of mutant huntingtin in stable PC12 cell line. *Brain Res.*, **1286**, 221–229.

152 Igarashi, S., Morita, H., Bennett, K.M., Tanaka, Y., Engelender, S., Peters, M.F., Cooper, J.K., Wood, J.D., Sawa, A., and Ross, C.A. (2003) Inducible PC12 cell model of Huntington's disease shows toxicity and decreased histone acetylation. *NeuroReport*, **14** (4), 565–568.

153 Sipione, S., Rigamonti, D., Valenza, M., Zuccato, C., Conti, L., Pritchard, J., Kooperberg, C., Olson, J.M., and Cattaneo, E. (2002) Early transcriptional profiles in huntingtin-inducible striatal cells by microarray analyses. *Hum. Mol. Genet.*, **11** (17), 1953–1965.

154 Anonymous (2004) Bacopa monnieri. Monograph. *Altern. Med. Rev.*, **9** (1), 79–85.

155 Phulara, S.C., Shukla, V., Tiwari, S., and Pandey, R. (2015) *Bacopa monnieri* promotes longevity in *Caenorhabditis elegans* under stress conditions. *Pharmacogn. Mag.*, **11** (42), 410–416.

156 Kean, J.D., Kaufman, J., Lomas, J., Goh, A., White, D., Simpson, D., Scholey, A., Singh, H., Sarris, J., Zangara, A., and Stough, C. (2015) A randomized controlled trial investigating the effects of a special extract of *Bacopa monnieri* (CDRI 08) on hyperactivity and inattention in male children and adolescents: BACHI study protocol (ANZCTRN12612000827831). *Nutrients*, **7** (12), 9931–9945.

157 Benson, S., Downey, L.A., Stough, C., Wetherell, M., Zangara, A., and Scholey, A. (2014) An acute, double-blind, placebo-controlled cross-over study of 320 mg and 640 mg doses of *Bacopa monnieri* (CDRI 08) on multitasking stress reactivity and mood. *Phytother. Res.*, **28** (4), 551–559.

158 Downey, L.A., Kean, J., Nemeh, F., Lau, A., Poll, A., Gregory, R., Murray, M., Rourke, J., Patak, B., Pase, M.P., Zangara, A., Lomas, J., Scholey, A., and Stough, C. (2013) An acute, double-blind, placebo-controlled crossover study of 320 mg and 640 mg doses of a special extract of *Bacopa monnieri* (CDRI 08) on sustained cognitive performance. *Phytother. Res.*, **27** (9), 1407–1413.

159 Mathew, J., Gangadharan, G., Kuruvilla, K.P., and Paulose, C.S. (2011) Behavioral deficit and decreased GABA receptor functional regulation in the hippocampus of epileptic rats: effect of *Bacopa monnieri*. *Neurochem. Res.*, **36** (1), 7–16.

160 Jadiya, P., Khan, A., Sammi, S.R., Kaur, S., Mir, S.S., and Nazir, A. (2011) Anti-Parkinsonian effects of Bacopa monnieri: insights from transgenic and pharmacological *Caenorhabditis elegans* models of Parkinson's disease. *Biochem. Biophys. Res. Commun.*, **413** (4), 605–610.

161 Saini, N., Singh, D., and Sandhir, R. (2012) Neuroprotective effects of *Bacopa monnieri* in experimental model of dementia. *Neurochem. Res.*, **37** (9), 1928–1937.

162 Shinomol, G.K. and Muralidhara (2011) *Bacopa monnieri* modulates endogenous cytoplasmic and mitochondrial oxidative markers in prepubertal mice brain. *Phytomedicine*, **18**, 317–326.

163 Shinomol, G.K., Bharath, M.M.S., and Muralidhara (2012) Pretreatment with *Bacopa monnieri* extract offsets 3-nitropropionic acid induced mitochondrial oxidative stress and dysfunctions in the striatum of prepubertal mouse brain. *Can. J. Physiol. Pharmacol.*, **90**, 595–606.

164 Shinomol, G.K., Bharath, M.M.S., and Muralidhara (2012) Neuromodulatory propensity of *Bacopa monnieri* leaf extract against 3-nitropropionic acid-induced oxidative stress: *in vitro* and *in vivo* evidences. *Neurotox. Res.*, **22**, 102–114.

165 Aher, V., Chattopadhyay, P., Goyary, D., and Veer, V. (2013) Evaluation of the genotoxic and antigenotoxic potential of the alkaloid punarnavine from *Boerhaavia diffusa*. *Planta Med.*, **79** (11), 939–945.

166 Patil, K.S. and Bhalsing, S.R. (2015) Efficient micropropagation and assessment of genetic fidelity of *Boerhaavia diffusa* L- High trade medicinal plant. *Physiol. Mol. Biol. Plants*, **21** (3), 425–432.

167 Dhingra, D. and Valecha, R. (2014) Evidence for involvement of the monoaminergic system in antidepressant-like activity of an ethanol extract of *Boerhaavia diffusa* and its isolated constituent, punarnavine, in mice. *Pharm. Biol.*, **52** (6), 767–774.

168 Dhingra, D. and Valecha, R. (2014) Punarnavine, an alkaloid isolated from ethanolic extract of *Boerhaavia diffusa* Linn. reverses depression-like behaviour in mice subjected to chronic unpredictable mild stress. *Indian J. Exp. Biol.*, **52** (8), 799–807.

169 Cetkovic, G.S., Djilas, S.M., Brunet, C., and Tumbas, V.T. (2004) Antioxidant properties of marigold extracts. *Food Res. Int.*, **37**, 643–650.

170 Duke, J.A. (2008) *Pot Marigold an Herbal Florin for Health*, Mary Ann Liebert Inc, New Rochelle, NY.

171 Shivasharan, B.D., Nagakannan, P., Thippeswamy, B.S., Veerapur, V.P., Bansal, P., and Unnikrishnan, M.K. (2013) Protective effect of *Calendula officinalis*

Linn. flowers against 3-nitropropionic acid induced experimental Huntington's disease in rats. *Drug Chem. Toxicol.*, **36**, 466–473.

172 Shivasharan, B.D., Nagakannan, P., Thippeswamy, B.S., and Veerapur, V.P. (2013) Protective effect of *Calendula officinalis* L. flowers against monosodium glutamate induced oxidative stress and excitotoxic brain damage in rats. *Indian J. Clin. Biochem.*, **28** (3), 292–298.

173 Ozkol, H., Tülüce, Y., and Koyuncu, I. (2012) Subacute effect of cigarette smoke exposure in rats: protection by pot marigold (*Calendula officinalis* L.) extract. *Toxicol. Ind. Health*, **28** (1), 3–9.

174 Moradkhani, S., Salehi, I., Abdolmaleki, S., and Komaki, A. (2015) Effect of Calendula officinalis hydroalcoholic extract on passive avoidance learning and memory in streptozotocin-induced diabetic rats. *Ancient Sci. Life*, **34** (3), 156–161.

175 Turner, C.E., Elsohly, M.A., and Boeren, E.G. (1980) Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. *J. Nat. Prod.*, **43** (2), 169–234.

176 Ashton, C.H. (2001) Pharmacology and effects of cannabis: a brief review. *Br. J. Psychiatry*, **178** (2), 101–106.

177 Chagas, M.H., Zuardi, A.W., Tumas, V., Pena-Pereira, M.A., Sobreira, E.T., Bergamaschi, M.M., dos Santos, A.C., Teixeira, A.L., Hallak, J.E., and Crippa, J.A. (2014) Effects of cannabidiol in the treatment of patients with Parkinson's disease: an exploratory double-blind trial. *J. Psychopharmacol.*, **28** (11), 1088–1098.

178 Chagas, M.H., Eckeli, A.L., Zuardi, A.W., Pena-Pereira, M.A., Sobreira-Neto, M.A., Sobreira, E.T., Camilo, M.R., Bergamaschi, M.M., Schenck, C.H., Hallak, J.E., Tumas, V., and Crippa, J.A. (2014) Cannabidiol can improve complex sleep-related behaviours associated with rapid eye movement sleep behaviour disorder in Parkinson's disease patients: a case series. *J. Clin. Pharm. Ther.*, **39** (5), 564–566.

179 Ahmed, A., van der Marck, M.A., van den Elsen, G., and Olde Rikkert, M. (2015) Cannabinoids in late-onset Alzheimer's disease. *Clin. Pharmacol. Ther.*, **97** (6), 597–606.

180 Sagredo, O., Pazos, M.R., Satta, V., Ramos, J.A., Pertwee, R.G., and Fernández-Ruiz, J. (2011) Neuroprotective effects of phytocannabinoid-based medicines in experimental models of Huntington's disease. *J. Neurosci. Res.*, **89**, 1509–1518.

181 Sagredo, O., Pazos, M.R., Valdeolivas, S., and Fernandez-Ruiz, J. (2012) Cannabinoids: novel medicines for the treatment of Huntington's disease. *Recent Pat. CNS Drug Discov.*, **7**, 41–48.

182 Valdeolivas, S., Satta, V., Pertwee, R.G., Fernández-Ruiz, J., and Sagredo, O. (2012) Sativex-like combination of phytocannabinoids is neuroprotective in malonate-lesioned rats, an inflammatory model of Huntington's disease: role of CB1 and CB2 receptors. *ACS Chem. Neurosci.*, **3** (5), 400–406.

183 Sagredo, O., González, S., Aroyo, I., Pazos, M.R., Benito, C., Lastres-Becker, I., Romero, J.P., Tolón, R.M., Mechoulam, R., Brouillet, E., Romero, J., and Fernández-Ruiz, J. (2009) Cannabinoid CB2 receptor agonists protect the

striatum against malonate toxicity: relevance for Huntington's disease. *Glia*, **57** (11), 1154–1167.

184 Lastres-Becker, I., Bizat, N., Boyer, F., Hantraye, P., Brouillet, E., and Fernández-Ruiz, J. (2003) Effects of cannabinoids in the rat model of Huntington's disease generated by an intrastriatal injection of malonate. *NeuroReport*, **14** (6), 813–816.

185 Shinomol, G.K., Muralidhara, and Bharath, M.M.S. (2011) Exploring the role of "Brahmi" (*Bocopa monnieri* and *Centella asiatica*) in brain function and therapy. *Recent Pat. Endocr. Metab. Immune Drug Discov.*, **5** (1), 33–49.

186 Marques, N.F., Stefanello, S.T., Froeder, A.L., Busanello, A., Boligon, A.A., Athayde, M.L., Soares, F.A., and Fachinetto, R. (2015) *Centella asiatica* and its fractions reduces lipid peroxidation induced by quinolinic acid and sodium nitroprusside in rat brain regions. *Neurochem. Res.*, **40** (6), 1197–1210.

187 Berrocal, R., Vasudevaraju, P., Indi, S.S., Sambasiva, R., Krothapalli, R.S., and Rao, K.S. (2014) *In vitro* evidence that an aqueous extract of *Centella asiatica* modulates α-synuclein aggregation dynamics. *J. Alzheimers Dis.*, **39** (2), 457–465.

188 Bobade, V., Bodhankar, S.L., Aswar, U., Vishwaraman, M., and Thakurdesai, P. (2015) Prophylactic effects of asiaticoside-based standardized extract of *Centella asiatica* (L.) Urban leaves on experimental migraine: involvement of 5HT1A/1B receptors. *Chin. J. Nat. Med.*, **13** (4), 274–282.

189 Doknark, S., Mingmalairak, S., Vattanajun, A., Tantisira, B., and Tantisira, M.H. (2014) Study of ameliorating effects of ethanolic extract of *Centella asiatica* on learning and memory deficit in animal models. *J. Med. Assoc. Thai*, **97** (Suppl. 2), S68–S76.

190 Prakash, A. and Kumar, A. (2013) Mitoprotective effect of *Centella asiatica* against aluminum-induced neurotoxicity in rats: possible relevance to its anti-oxidant and anti-apoptosis mechanism. *Neurol. Sci.*, **34** (8), 1403–1409.

191 Tabassum, R., Vaibhav, K., Shrivastava, P., Khan, A., Ahmed, M.E., Javed, H., Islam, F., Ahmad, S., Siddiqui, M.S., Safhi, M.M., and Islam, F. (2013) *Centella asiatica* attenuates the neurobehavioral, neurochemical and histological changes in transient focal middle cerebral artery occlusion rats. *Neurol. Sci.*, **34**, 925–933.

192 Xu, M.F., Xiong, Y.Y., Liu, J.K., Qian, J.J., Zhu, L., and Gao, J. (2012) Asiatic acid, a pentacyclic triterpene in *Centella asiatica*, attenuates glutamate-induced cognitive deficits in mice and apoptosis in SH-SY5Y cells. *Acta Pharmacol. Sin.*, **33**, 578–587.

193 Xu, C.L., Qu, R., Zhang, J., Li, L.F., and Ma, S.P. (2013) Neuroprotective effects of madecassoside in early stage of Parkinson's disease induced by MPTP in rats. *Fitoterapia*, **90**, 112–118.

194 Verma, S., Sinha, R., Kumar, P., Amin, F., Jain, J., and Tanwar, S. (2012) Study of *Convolvulus pluricaulis* for antioxidant and anticonvulsant activity. *Cent. Nerv. Syst. Agents Med. Chem.*, **12** (1), 55–59.

195 Sethiya, N.K., Raja, M.K., and Mishra, S.H. (2013) Antioxidant markers based TLC-DPPH differentiation on four commercialized botanical sources of Shankhpushpi (A Medhya Rasayana): a preliminary assessment. *J. Adv. Pharm. Technol. Res.*, **4** (1), 25–30.

196 Malik, J., Karan, M., and Vasisht, K. (2015) Attenuating effect of bioactive coumarins from *Convolvulus pluricaulis* on scopolamine-induced amnesia in mice. *Nat. Prod. Res.*, **30** (5), 578–582.

197 Sharma, K., Bhatnagar, M., and Kulkarni, S.K. (2010) Effect of *Convolvulus pluricaulis* Choisy and *Asparagus racemosus* Willd on learning and memory in young and old mice: a comparative evaluation. *Indian J. Exp. Biol.*, **48** (5), 479–485.

198 Hemshekhar, M., Sunitha, K., Santhosh, M.S., Devaraja, S., Kemparaju, K., Vishwanath, B.S., Niranjana, S.R., and Girish, K.S. (2011) An overview on genus G*arcinia*: phytochemical and therapeutical aspects. *Phytochem. Rev.*, **10** (3), 325–351.

199 Konziase, B. (2015) Protective activity of biflavanones from *Garcinia kola* against *Plasmodium* infection. *J. Ethnopharmacol.*, **172**, 214–218.

200 Ijomone, O.M. and Obi, A.U. (2013) Kolaviron, isolated from *Garcinia kola*, inhibits acetylcholinesterase activities in the hippocampus and striatum of wistar rats. *Ann. Neurosci.*, **20** (2), 42–46.

201 Akinmoladun, A.C., Akinrinola, B.L., Olaleye, M.T., and Farombi, E.O. (2015) Kolaviron, a *Garcinia kola* biflavonoid complex, protects against ischemia/reperfusion injury: pertinent mechanistic insights from biochemical and physical evaluations in rat brain. *Neurochem. Res.*, **40** (4), 777–787.

202 Igado, O.O., Olopade, J.O., Adesida, A., Aina, O.O., and Farombi, E.O. (2012) Morphological and biochemical investigation into the possible neuroprotective effects of kolaviron (*Garcinia kola* bioflavonoid) on the brains of rats exposed to vanadium. *Drug Chem. Toxicol.*, **35**, 371–380.

203 Adaramoye, O.A. (2010) Protective effect of kolaviron, a biflavonoid from *Garcinia kola* seeds, in brain of Wistar albino rats exposed to gamma-radiation. *Biol. Pharm. Bull.*, **33**, 260–266.

204 Ajayi, S.A., Ofusori, D.A., Ojo, G.B., Ayoka, O.A., Abayomi, T.A., and Tijani, A.A. (2011) The microstructural effects of aqueous extract of *Garcinia kola* (Linn) on the hippocampus and cerebellum of malnourished mice. *Asian Pac. J. Trop. Biomed.*, **1**, 261–265.

205 Jang, J.H., Son, Y., Kang, S.S., Bae, C.S., Kim, J.C., Kim, S.H., Shin, T., and Moon, C. (2015) Neuropharmacological potential of *Gastrodia elata* blume and its components. *Evid Based Complement. Alternat. Med.*, **2015**, 309261.

206 Tsai, C.F., Huang, C.L., Lin, Y.L., Lee, Y.C., Yang, Y.C., and Huang, N.K. (2011) The neuroprotective effects of an extract of *Gastrodia elata*. *J. Ethnopharmacol.*, **138** (1), 119–125.

207 Hu, Y., Li, C., and Shen, W. (2014) Gastrodin alleviates memory deficits and reduces neuropathology in a mouse model of Alzheimer's disease. *Neuropathology*, **34** (4), 370–377.

208 Doo, A.R., Kim, S.N., Hahm, D.H., Yoo, H.H., Park, J.Y., Lee, H., Jeon, S., Kim, J., Park, S.U., and Park, H.J. (2014) *Gastrodia elata* Blume alleviates L-DOPA-induced dyskinesia by normalizing FosB and ERK activation in a 6-OHDA-lesioned Parkinson's disease mouse model. *BMC Complement. Alternat. Med.*, **14**, 107.

209 Wang, Q., Shen, L., Ma, S., Chen, M., Lin, X., Hong, Y., Liang, S., and Feng, Y. (2015) Effects of Ligusticum chuanxiong and Gastrodia elata on blood-brain barrier permeability in migraine rats. *Pharmazie*, **70** (6), 421–426.

210 Yu, S.J., Kim, J.R., Lee, C.K., Han, J.E., Lee, J.H., Kim, H.S., Hong, J.H., and Kang, S.G. (2005) *Gastrodia elata* blume and an active component, p-hydroxybenzyl alcohol reduce focal ischemic brain injury through antioxidant related gene expressions. *Biol. Pharm. Bull.*, **28** (6), 1016–1020.

211 Wu, L.Y., Chen, W.C., Tsai, F.S., Tsai, C.C., Wu, C.R., and Lin, L.W. (2015) p-hydroxybenzyl alcohol, an active phenolic ingredient of gastrodia elata, reverses the cycloheximide-induced memory deficit by activating the adrenal gland in rats. *Am. J. Chin. Med.*, **43** (08), 1593–1604. doi: 10.1142/s0192415x15500901

212 Huang, C.L., Yang, J.M., Wang, K.C., Lee, Y.C., Lin, Y.L., Yang, Y.C., and Huang, N.K. (2011) *Gastrodia elata* prevents huntingtin aggregations through activation of the adenosine A_2A receptor and ubiquitin proteasome system. *J. Ethnopharmacol.*, **138**, 162–168.

213 Nash, K.M. and Shah, Z.A. (2015) Current perspectives on the beneficial role of *Ginkgo biloba* in neurological and cerebrovascular disorders. *Integr. Med. Insights*, **10**, 1–9.

214 Kwon, K.J., Lee, E.J., Cho, K.S., Cho, D.H., Shin, C.Y., and Han, S.H. (2015) *Ginkgo biloba* extract (Egb761) attenuates zinc-induced tau phosphorylation at Ser262 by regulating GSK3β activity in rat primary cortical neurons. *Food Funct.*, **6** (6), 2058–2067.

215 Stein, C., Hopfeld, J., Lau, H., and Klein, J. (2015) Effects of *Ginkgo biloba* Extract EGb 761, donepezil and their combination on central cholinergic function in aged rats. *J. Pharm. Pharm. Sci.*, **18** (4), 634–646.

216 Tulsulkar, J., Glueck, B., Hinds, T.D. Jr., and Shah, Z.A. (2015) *Ginkgo biloba* extract prevents female mice from ischemic brain damage and the mechanism is independent of the HO1/Wnt pathway. *Transl. Stroke Res.*, **7** (2), 120–131.

217 Yeh, K.Y., Shou, S.S., Lin, Y.X., Chen, C.C., Chiang, C.Y., and Yeh, C.Y. (2015) Effect of *Ginkgo biloba* extract on lipopolysaccharide-induced anhedonic depressive-like behavior in male rats. *Phytother. Res.*, **29** (2), 260–266.

218 Yin, B., Xu, Y., Wei, R., and Luo, B. (2014) *Ginkgo biloba* on focal cerebral ischemia: a systematic review and meta-analysis. *Am. J. Chin. Med.*, **42** (4), 769–783.

219 An, H.M., Tan, Y.L., Shi, J., Wang, Z.R., Li, J., Wang, Y.C., Kosten, T.R., Zhou, D.F., Yang, F.D., and Zhang, X.Y. (2013) Extract of *Ginkgo biloba* is equivalent to vitamin E in attenuating and preventing vacuous chewing movements in a rat model of tardive dyskinesia. *Behav. Pharmacol.*, **24** (7), 610–616.

220 Xu, L., Hu, Z., Shen, J., and McQuillan, P.M. (2015) Effects of *Ginkgo biloba* extract on cerebral oxygen and glucose metabolism in elderly patients with pre-existing cerebral ischemia. *Complement. Ther. Med.*, **23** (2), 220–225.

221 Uebel-von Sandersleben, H., Rothenberger, A., Albrecht, B., Rothenberger, L.G., Klement, S., and Bock, N. (2014) *Ginkgo biloba* extract EGb 761® in children with ADHD. Z. Kinder Jugendpsychiatr. Psychother., **42** (5), 337–347.

222 Chi, C.L., Shen, D.F., Wang, P.J., Li, H.L., and Zhang, L. (2015) Effect of ginkgolide B on brain metabolism and tissue oxygenation in severe haemorrhagic stroke. *Int. J. Clin. Exp. Med.*, **8** (3), 3522–3529.

223 Wang, X., Jiang, C.M., Wan, H.Y., Wu, J.L., Quan, W.Q., Wu, K.Y., and Li, D. (2014) Neuroprotection against permanent focal cerebral ischemia by ginkgolides A and B is associated with obstruction of the mitochondrial apoptotic pathway via inhibition of c-Jun N-terminal kinase in rats. *J. Neurosci. Res.*, **92** (2), 232–242.

224 Botao, Y., Ma, J., Xiao, W., Xiang, Q., Fan, K., Hou, J., Wu, J., and Jing, W. (2013) Protective effect of ginkgolide B on high altitude cerebral edema of rats. *High Alt. Med. Biol.*, **14** (1), 61–64.

225 Allais, G., D'Andrea, G., Maggio, M., and Benedetto, C. (2013) The efficacy of ginkgolide B in the acute treatment of migraine aura: an open preliminary trial. *Neurol. Sci.*, **34** (1), S161–S163.

226 Ahlemeyer, B. and Krieglstein, J. (2003) Pharmacological studies supporting the therapeutic use of *Ginkgo biloba* extract for Alzheimer's disease. *Pharmacopsychiatry*, **36** (1), S8–S14.

227 Mahdy, H.M., Tadros, M.G., Mohamed, M.R., Karim, A.M., and Khalifa, A.E. (2011) The effect of *Ginkgo biloba* extract on 3-nitropropionic acid-induced neurotoxicity in rats. *Neurochem. Int.*, **59**, 770–778.

228 Lorenzi, H. (1998) *Árvores brasileiras: Manual de identificação e cultivo de plantas arbóreas nativas do Brasil*, 2nd edn, vol. **1**, Plantarum, Nova Odessa.

229 Tanaka, J.C.A., Da Silva, C.C., Dias Filho, B.P., Nakamura, C.V., De Carvalho, J.E., and Foglio, M.A. (2005) Chemical constituents of *Luehea divaricata* Mart. (Tiliaceae). *Quim. Nova*, **28** (5), 834–837.

230 Bieski, I.G.C., Rios Santos, F., de Oliveira, R.M., Espinosa, M.M., Macedo, M., Albuquerque, U.P., and de Oliveira Martins, D.T. (2012) Ethnopharmacology of medicinal plants of the pantanal region (mato grosso, Brazil). *Evid Based Complement. Alternat. Med.*, **2012**, 1–36. doi: 10.1155/2012/272749

231 Kaniewski, D., Van Campo, E., Boiy, T., Terral, J.F., Khadari, B., and Besnard, G. (2012) Primary domestication and early uses of the emblematic olive tree: palaeobotanical, historical and molecular evidence from the Middle East. *Biol. Rev. Camb. Philos. Soc.*, **87** (4), 885–899.

232 Zohary, D., Hopf, M., and Weiss, E. (2012) *Domestication of Plants in the Old World: The Origin and Spread of Domesticated Plants in Southwest Asia, Europe, and the Mediterranean Basin*, Oxford University Press, Oxford.

233 Sarwar, M. (2013) The theatrical usefulness of olive *Olea europaea* L.(Oleaceae family) nutrition in human health: a review. *Sky J. Med. Plant Res.*, **2** (1), 1–4.

234 Wang, Y., Wang, S., Cui, W., He, J., Wang, Z., and Yang, X. (2013) Olive leaf extract inhibits lead poisoning-induced brain injury. *Neural Regen. Res.*, **8** (22), 2021–2029.

235 Caltana, L., Nieto, M.L., and Brusco, A. (2015) Oleanolic acid: a promising neuroprotective agent for cerebral ischemia. *Neural. Regen. Res.*, **10** (4), 540–541.

236 Mohagheghi, F., Bigdeli, M.R., Rasoulian, B., Zeinanloo, A.A., and Khoshbaten, A. (2010) Dietary virgin olive oil reduces blood brain barrier permeability,

brain edema, and brain injury in rats subjected to ischemia-reperfusion. *Sci. World J.*, **10**, 1180–1191.

237 Mohagheghi, F., Bigdeli, M.R., Rasoulian, B., Hashemi, P., and Pour, M.R. (2011) The neuroprotective effect of olive leaf extract is related to improved blood-brain barrier permeability and brain edema in rat with experimental focal cerebral ischemia. *Phytomedicine*, **18**, 170–175.

238 Tasset, I., Pontes, A.J., Hinojosa, A.J., de la Torre, R., and Túnez, I. (2011) Olive oil reduces oxidative damage in a 3-nitropropionic acid-induced Huntington's disease-like rat model. *Nutr. Neurosci.*, **14**, 106–111.

239 Ong, W.Y., Farooqui, T., Koh, H.L., Farooqui, A.A., and Ling, E.A. (2015) Protective effects of ginseng on neurological disorders. *Front. Aging Neurosci.*, **7**, 129.

240 Park, C.H., Park, S.K., Seung, T.W., Jin, D.E., Guo, T., and Heo, H.J. (2015) Effect of ginseng (*Panax ginseng*) berry EtOAc fraction on cognitive impairment in C57BL/6 mice under high-fat diet inducement. *Evid Based Complement. Alternat. Med.*, **2015**, 316527.

241 Kim, Y.O., Lee, H.Y., Won, H., Nah, S.S., Lee, H.Y., Kim, H.K., Kwon, J.T., and Kim, H.J. (2015) Influence of *Panax ginseng* on the offspring of adult rats exposed to prenatal stress. *Int. J. Mol. Med.*, **35** (1), 103–109.

242 Hu, B.Y., Liu, X.J., Qiang, R., Jiang, Z.L., Xu, L.H., Wang, G.H., Li, X., and Peng, B. (2014) Treatment with ginseng total saponins improves the neurorestoration of rat after traumatic brain injury. *J. Ethnopharmacol.*, **155** (2), 1243–1255.

243 Zhu, D., Liu, M., Yang, Y., Ma, L., Jiang, Y., Zhou, L., Huang, Q., Pi, R., and Chen, X. (2014) Ginsenoside Rd ameliorates experimental autoimmune encephalomyelitis in C57BL/6 mice. *J. Neurosci. Res.*, **92** (9), 1217–1226.

244 Scholey, A., Ossoukhova, A., Owen, L., Ibarra, A., Pipingas, A., He, K., Roller, M., and Stough, C. (2010) Effects of American ginseng (*Panax quinquefolius*) on neurocognitive function: an acute, randomised, double-blind, placebo-controlled, crossover study. *Psychopharmacology (Berl)*, **212** (3), 345–356.

245 Kim, J.H., Kim, S., Yoon, I.S., Lee, J.H., Jang, B.J., Jeong, S.M., Lee, J.H., Lee, B.H., Han, J.S., Oh, S., Kim, H.C., Park, T.K., Rhim, H., and Nah, S.Y. (2005) Protective effects of ginseng saponins on 3-nitropropionic acid-induced striatal degeneration in rats. *Neuropharmacology*, **48**, 743–756.

246 Tang, S.Y., Gruber, J., Wong, K.P., and Halliwell, B. (2007) *Psoralea corylifolia* L. inhibits mitochondrial complex I and proteasome activities in SH-SY5Y cells. *Ann. N.Y. Acad. Sci.*, **1100**, 486–496.

247 Yi, L.T., Li, Y.C., Pan, Y., Li, J.M., Xu, Q., Mo, S.F., Qiao, C.F., Jiang, F.X., Xu, H.X., Lu, X.B., Kong, L.D., and Kung, H.F. (2007) Antidepressant-like effects of psoralidin isolated from the seeds of Psoralea Corylifolia in the forced swimming test in mice. *Progr. Neuropsychopharmacol. Biol. Psychiatry*, **32** (2), 510–519.

248 Chen, Y., Wang, H.D., Xia, X., Kung, H.F., Pan, Y., and Kong, L.D. (2007) Behavioral and biochemical studies of total furocoumarins from seeds of Psoralea corylifolia in the chronic mild stress model of depression in mice. *Phytomedicine*, **14** (7-8), 523–529.

249 Kong, L.D., Tan, R.X., Woo, A.Y., and Cheng, C.H. (2001) Inhibition of rat brain monoamine oxidase activities by psoralen and isopsoralen: implications for the treatment of affective disorders. *Pharmacol. Toxicol.*, **88** (2), 75–80.

250 Jurenka, J.S. (2008) Therapeutic applications of pomegranate (Punica granatum L.): a review. *Altern. Med. Rev.*, **13** (2), 128–144.

251 Forouzanfar, F., Afkhami Goli, A., Asadpour, E., Ghorbani, A., and Sadeghnia, H.R. (2013) Protective effect of *Punica granatum* L. against serum/glucose deprivation-induced PC12 cells injury. *Evid Based Complement. Alternat. Med.*, **2013**, 716730.

252 Cambay, Z., Baydas, G., Tuzcu, M., and Bal, R. (2011) Pomegranate (*Punica granatum* L.) flower improves learning and memory performances impaired by diabetes mellitus in rats. *Acta Physiol. Hung.*, **98** (4), 409–420.

253 Naveen, S., Siddalingaswamy, M., Singsit, D., and Khanum, F. (2013) Anti-depressive effect of polyphenols and omega-3 fatty acid from pomegranate peel and flax seed in mice exposed to chronic mild stress. *Psychiatry Clin. Neurosci.*, **67** (7), 501–508.

254 Malva, J.O., Santos, S., and Macedo, T. (2004) Neuroprotective properties of *Valeriana officinalis* extracts. *Neurotox. Res.*, **6** (2), 131–140.

255 Rezvani, M.E., Roohbakhsh, A., Allahtavakoli, M., and Shamsizadeh, A. (2010) Anticonvulsant effect of aqueous extract of *Valeriana officinalis* in amygdala-kindled rats: possible involvement of adenosine. *J. Ethnopharmacol.*, **127** (2), 313–318.

256 Nam, S.M., Choi, J.H., Yoo, D.Y., Kim, W., Jung, H.Y., Kim, J.W., Kang, S.Y., Park, J., Kim, D.W., Kim, W.J., Yoon, Y.S., and Hwang, I.K. (2013) *Valeriana officinalis* extract and its main component, valerenic acid, ameliorate D-galactose-induced reductions in memory, cell proliferation, and neuroblast differentiation by reducing corticosterone levels and lipid peroxidation. *Exp. Gerontol.*, **48** (11), 1369–1377.

257 Fernández, S., Wasowski, C., Paladini, A.C., and Marder, M. (2004) Sedative and sleep-enhancing properties of linarin, a flavonoid-isolated from *Valeriana officinalis*. *Pharmacol. Biochem. Behav.*, **77** (2), 399–404.

258 Durg, S., Dhadde, S.B., Vandal, R., Shivakumar, B.S., and Charan, C.S. (2015) *Withania somnifera* (Ashwagandha) in neurobehavioural disorders induced by brain oxidative stress in rodents: a systematic review and meta-analysis. *J. Pharm. Pharmacol.*, **67** (7), 879–899.

259 Raghavan, A. and Shah, Z.A. (2015) *Withania somnifera* improves ischemic stroke outcomes by attenuating PARP1-AIF-mediated caspase-independent apoptosis. *Mol. Neurobiol.*, **52** (3), 1093–1105.

260 Kurapati, K.R., Atluri, V.S., Samikkannu, T., and Nair, M.P. (2013) Ashwagandha (*Withania somnifera*) reverses β-amyloid1-42 induced toxicity in human neuronal cells: implications in HIV-associated neurocognitive disorders (HAND). *PLoS ONE*, **8** (10), e77624.

261 Baitharu, I., Jain, V., Deep, S.N., Hota, K.B., Hota, S.K., Prasad, D., and Ilavazhagan, G. (2013) *Withania somnifera* root extract ameliorates hypobaric hypoxia induced memory impairment in rats. *J. Ethnopharmacol.*, **145** (2), 431–441.

262 Sehgal, N., Gupta, A., Valli, R.K., Joshi, S.D., Mills, J.T., Hamel, E., Khanna, P., Jain, S.C., Thakur, S.S., and Ravindranath, V. (2012) *Withania somnifera* reverses Alzheimer's disease pathology by enhancing low-density lipoprotein receptor-related protein in liver. *Proc. Natl. Acad. Sci. U.S.A.*, **109** (9), 3510–3515.

263 Baitharu, I., Jain, V., Deep, S.N., Shroff, S., Sahu, J.K., Naik, P.K., and Ilavazhagan, G. (2014) Withanolide A prevents neurodegeneration by modulating hippocampal glutathione biosynthesis during hypoxia. *PLoS ONE*, **9** (10), e105311.

264 Kuboyama, T., Tohda, C., and Komatsu, K. (2005) Neuritic regeneration and synaptic reconstruction induced by withanolide A. *Br. J. Pharmacol.*, **144** (7), 961–971.

265 Yin, H., Cho, D.H., Park, S.J., and Han, S.K. (2013) GABA-mimetic actions of *Withania somnifera* on substantia gelatinosa neurons of the trigeminal subnucleus caudalis in mice. *Am. J. Chin. Med.*, **41**, 1043–1051.

266 Singh, N., Bhalla, M., de Jager, P., and Gilca, M. (2011) An overview on ashwagandha: a Rasayana (rejuvenator) of Ayurveda. *Afr. J. Tradit. Complement. Altern. Med.*, **8**, 208–213.

267 Kumar, P. and Kumar, A. (2009) Possible neuroprotective effect of *Withania somnifera* root extract against 3-nitropropionic acid-induced behavioral, biochemical, and mitochondrial dysfunction in an animal model of Huntington's disease. *J. Med. Food*, **12**, 591–600.

268 Pan-In, P., Wongsomboon, A., Kokpol, C., Chaichanawongsaroj, N., and Wanichwecharungruang, S. (2015) Depositing α-mangostin nanoparticles to sebaceous gland area for acne treatment. *J. Pharmacol. Sci.*, **129** (4), 226–232, S1347-8613(15)00227-3.

269 Márquez-Valadez, B., Maldonado, P.D., Galván-Arzate, S., Méndez-Cuesta, L.A., Pérez-De La Cruz, V., Pedraza-Chaverrí, J., Chánez-Cárdenas, M.E., and Santamaría, A. (2012) Alpha-mangostin induces changes in glutathione levels associated with glutathione peroxidase activity in rat brain synaptosomes. *Nutr. Neurosci.*, **15**, 13–19.

270 Pedraza-Chaverrí, J., Reyes-Fermín, L.M., Nolasco-Amaya, E.G., Orozco-Ibarra, M., Medina-Campos, O.N., González-Cuahutencos, O., Rivero-Cruz, I., and Mata, R. (2009) ROS scavenging capacity and neuroprotective effect of alpha-mangostin against 3-nitropropionic acid in cerebellar granule neurons. *Exp. Toxicol. Pathol.*, **61**, 491–501.

271 Zhang, H., Pan, N., Xiong, S., Zou, S., Li, H., Xiao, L., Cao, Z., Tunnacliffe, A., and Huang, Z. (2012) Inhibition of polyglutamine-mediated proteotoxicity by *Astragalus membranaceus* polysaccharide through the DAF-16/FOXO transcription factor in *Caenorhabditis elegans*. *Biochem. J.*, **441**, 417–424.

272 Yan, L. and Zhou, Q.H. (2012) Study on neuroprotective effects of astragalan in rats with ischemic brain injury and its mechanisms. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*, **28**, 373–377.

273 Chen, C.C., Hung, T.H., Lee, C.Y., Wang, L.F., Wu, C.H., Ke, C.H., and Chen, S.F. (2014) Berberine protects against neuronal damage via suppression of glia-mediated inflammation in traumatic brain injury. *PLoS ONE*, **9** (12), e115694.

274 Simões, P.E.N., Frozza, R.L., Hoppe, J.B., de Melo Menezes, B., and Salbego, C.G. (2014) Berberine was neuroprotective against an in vitro model of brain ischemia: survival and apoptosis pathways involved. *Brain Res.*, **1557**, 26–33.

275 Choi, B.S., Kim, H., Lee, H.J., Sapkota, K., Park, S.E., Kim, S., and Kim, S.J. (2014) Celastrol from 'Thunder God Vine' protects SH-SY5Y cells through the

preservation of mitochondrial function and inhibition of p38 MAPK in a rotenone model of Parkinson's disease. *Neurochem. Res.*, **39**, 84–96.

276 Faust, K., Gehrke, S., Yang, Y., Yang, L., Beal, M.F., and Lu, B. (2009) Neuroprotective effects of compounds with antioxidant and anti-inflammatory properties in a *Drosophila* model of Parkinson's disease. *BMC Neurosci.*, **10**, 109.

277 Zhang, Y.Q. and Sarge, K.D. (2007) Celastrol inhibits polyglutamine aggregation and toxicity though induction of the heat shock response. *J. Mol. Med.* (Berl.), **85**, 1421–1428.

278 Naz, R.K., Lough, M.L., and Barthelmess, E.K. (2016) Curcumin: a novel non-steroidal contraceptive with antimicrobial properties. *Front. Biosci.* (Elite Ed.), **8**, 113–128.

279 Tiwari, S.K., Agarwal, S., Seth, B., Yadav, A., Nair, S., Bhatnagar, P., Karmakar, M., Kumari, M., Chauhan, L.K., Patel, D.K., Srivastava, V., Singh, D., Gupta, S.K., Tripathi, A., Chaturvedi, R.K., and Gupta, K.C. (2014) Curcumin-loaded nanoparticles potently induce adult neurogenesis and reverse cognitive deficits in Alzheimer's disease model via canonical Wnt/β-catenin pathway. *ACS Nano*, **8**, 76–103.

280 Cheng-Chung, W.J., Huang, H.C., Chen, W.J., Huang, C.N., Peng, C.H., and Lin, C.L. (2015) Epigallocatechin gallate attenuates amyloid β-induced inflammation and neurotoxicity in EOC 13.31 microglia. *Eur. J. Pharmacol.*, **770**, 16–24.

281 Sajjadi, S.E., Shokoohinia, Y., and Moayedi, N.-S. (2012) Isolation and identification of ferulic acid from aerial parts of *Kelussia odoratissima* Mozaff. *Jundishapur J. Nat. Pharm. Prod.*, **7** (4), 159–162.

282 Zhuo, W., Zhang, L., Zhu, Y., Zhu, B., and Chen, Z. (2015) Fisetin, a dietary bioflavonoid, reverses acquired Cisplatin-resistance of lung adenocarcinoma cells through MAPK/Survivin/Caspase pathway. *Am. J. Transl. Res.*, **7** (10), 2045–2052.

283 Prakash, D., Gopinath, K., and Sudhandiran, G. (2013) Fisetin enhances behavioral performances and attenuates reactive gliosis and inflammation during aluminum chloride-induced neurotoxicity. *Neuromol. Med.*, **15**, 192–208.

284 Zheng, L.T., Ock, J., Kwon, B.M., and Suk, K. (2008) Suppressive effects of flavonoid fisetin on lipopolysaccharide-induced microglial activation and neurotoxicity. *Int. Immunopharmacol.*, **8**, 484–494.

285 Berkov, S., Bastida, J., Sidjimova, B., Viladomat, F., and Codina, C. (2011) Alkaloid diversity in *Galanthus elwesii* and *Galanthus nivalis*. *Chem. Biodivers.*, **8** (1), 115–130.

286 Park, J.E., Lee, S.T., Im, W.S., Chu, K., and Kim, M. (2008) Galantamine reduces striatal degeneration in 3-nitropropionic acid model of Huntington's disease. *Neurosci. Lett.*, **448**, 143–147.

287 Tosun, F., Erdem, C.K., and Eroğlu, Y. (2003) Determination of genistein in the Turkish Genista L. species by LC-MS. *Pharmazie*, **58** (8), 549–550.

288 Ivanov, D.A., Georgakopoulos, J.R., and Bernards, M.A. (2015) The chemoattractant potential of ginsenosides in the ginseng – pythium irregulare pathosystem. *Phytochemistry*, **122**, 56–64, S0031-9422(15)30106-0.

289 Ahmadi, A., Shadboorestan, A., Nabavi, S.F., Setzer, W.N., and Nabavi, S.M. (2015) The role of Hesperidin in cell signal transduction pathway for the prevention or treatment of cancer. *Curr. Med. Chem.*, **22** (30), 3462–3471.

290 Menze, E.T., Tadros, M.G., Abdel-Tawab, A.M., and Khalifa, A.E. (2012) Potential neuroprotective effects of hesperidin on 3-nitropropionic acid-induced neurotoxicity in rats. *Neurotoxicology*, **33**, 1265–1275.

291 Rong, Z., Pan, R., Xu, Y., Zhang, C., Cao, Y., and Liu, D. (2013) Hesperidin pretreatment protects hypoxia-ischemic brain injury in neonatal rat. *Neuroscience*, **255**, 292–299.

292 Alkhalidy, H., Moore, W., Zhang, Y., McMillan, R., Wang, A., Ali, M., Suh, K.S., Zhen, W., Cheng, Z., Jia, Z., Hulver, M., and Liu, D. (2015) Small molecule kaempferol promotes insulin sensitivity and preserved pancreatic β-cell mass in middle-aged obese diabetic mice. *J. Diabetes Res.*, **2015**, 532984.

293 Li, S. and Pu, X.P. (2011) Neuroprotective effect of kaempferol against a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced mouse model of Parkinson's disease. *Biol. Pharm. Bull.*, **34**, 1291–1296.

294 Yu, L., Chen, C., Wang, L.F., Kuang, X., Liu, K., Zhang, H., and Du, J.R. (2013) Neuroprotective effect of kaempferol glycosides against brain injury and neuroinflammation by inhibiting the activation of NF-κB and STAT3 in transient focal stroke. *PLoS ONE*, **8**, e55839.

295 Lagoa, R., Lopez-Sanchez, C., Samhan-Arias, A.K., Ganan, C.M., Garcia-Martinez, V., and Gutierrez-Merino, C. (2009) Kaempferol protects against rat striatal degeneration induced by 3-nitropropionic acid. *J. Neurochem.*, **111**, 473–487.

296 Ben, P., Zhang, Z., Xuan, C., Sun, S., Shen, L., Gao, Y., Cao, X., Zhou, Y., Lan, L., Yin, Z., and Luo, L. (2015) Protective effect of L-theanine on cadmium-induced apoptosis in PC12 cells by inhibiting the mitochondria-mediated pathway. *Neurochem. Res.*, **40** (8), 1661–1670.

297 Bernstein, P.S., Li, B., Vachali, P.P., Gorusupudi, A., Shyam, R., Henriksen, B.S., and Nolan, J.M. (2015) Lutein, zeaxanthin, and meso-zeaxanthin: the basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease. *Prog. Retin Eye Res.*, **50**, 34–66, S1350-9462(15)00086-5.

298 Woo, T.T., Li, S.Y., Lai, W.W., Wong, D., and Lo, A.C. (2013) Neuroprotective effects of lutein in a rat model of retinal detachment. *Graefes Arch. Clin. Exp. Ophthalmol.*, **251**, 41–51.

299 Li, S.Y., Fung, F.K., Fu, Z.J., Wong, D., Chan, H.H., and Lo, A.C. (2012) Anti-inflammatory effects of lutein in retinal ischemic/hypoxic injury: *in vivo* and *in vitro* studies. *Invest. Ophthalmol. Vis. Sci.*, **53**, 5976–5984.

300 Binawade, Y. and Jagtap, A. (2013) Neuroprotective effect of lutein against 3-nitropropionic acid-induced Huntington's disease-like symptoms: possible behavioral, biochemical, and cellular alterations. *J. Med. Food*, **16**, 934–943.

301 Cooperstone, J.L., Ralston, R.A., Riedl, K.M., Haufe, T.C., Schweiggert, R.M., King, S.A., Timmers, C.D., Francis, D.M., Lesinski, G.B., Clinton, S.K., and Schwartz, S.J. (2015) Enhanced bioavailability of lycopene when consumed as cis-isomers from tangerine compared to red tomato juice, a randomized, cross-over clinical trial. *Mol. Nutr. Food Res.*, **59** (4), 658–669.

302 Liu, C.B., Wang, R., Pan, H.B., Ding, Q.F., and Lu, F.B. (2013) Effect of lycopene on oxidative stress and behavioral deficits in rotenone induced model of Parkinson's disease. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*, **29**, 380–384.

303 Zhang, Y.S., Li, Y., Wang, Y., Sun, S.Y., Jiang, T., Li, C., Cui, S.X., and Qu, X.J. (2015) Naringin, a natural dietary compound, prevents intestinal tumorigenesis in Apc Min/+ mouse model. *J. Cancer Res. Clin. Oncol.*, **142** (5), 913–925.

304 Rong, W., Wang, J., Liu, X., Jiang, L., Wei, F., Hu, X., Han, X., and Liu, Z. (2012) Naringin treatment improves functional recovery by increasing BDNF and VEGF expression, inhibiting neuronal apoptosis after spinal cord injury. *Neurochem. Res.*, **37**, 1615–1623.

305 Chambers, R.P., Call, G.B., Meyer, D., Smith, J., Techau, J.A., Pearman, K., and Buhlman, L.M. (2013) Nicotine increases lifespan and rescues olfactory and motor deficits in a Drosophila model of Parkinson's disease. *Behav. Brain Res.*, **253**, 95–102.

306 Quik, M., Mallela, A., Ly, J., and Zhang, D. (2013) Nicotine reduces established levodopa-induced dyskinesias in a monkey model of Parkinson's disease. *Mov. Disord.*, **28**, 1398–1406.

307 Wu, A.G., Wong, V.K., Xu, S.W., Chan, W.K., Ng, C.I., Liu, L., and Law, B.Y. (2013) Onjisaponin B derived from Radix Polygalae enhances autophagy and accelerates the degradation of mutant α-synuclein and huntingtin in PC-12 cells. *Int. J. Mol. Sci.*, **14**, 22618–22641.

308 Denny, J.K.M. and Muralidhara (2013) Enhanced neuroprotective effect of fish oil in combination with quercetin against 3-nitropropionic acid induced oxidative stress in rat brain. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **40**, 83–92.

309 Prasad, J., Baitharu, I., Sharma, A.K., Dutta, R., Prasad, D., and Singh, S.B. (2013) Quercetin reverses hypobaric hypoxia-induced hippocampal neurodegeneration and improves memory function in the rat. *High Alt. Med. Biol.*, **14**, 383–394.

310 Kanter, M., Unsal, C., Aktas, C., and Erboga, M. (2013) Neuroprotective effect of quercetin against oxidative damage and neuronal apoptosis caused by cadmium in hippocampus. *Toxicol. Ind. Health*, **32** (3), 541–550. doi: 10.1177/0748233713504810

311 Ferreira, P.E., Lopes, C.R., Alves, A.M., Alves, É.P., Linden, D.R., Zanoni, J.N., and Buttow, N.C. (2013) Diabetic neuropathy: an evaluation of the use of quercetin in the cecum of rats. *World J. Gastroenterol.*, **19**, 6416–6426.

312 Gómez-Sierra, T., Molina-Jijón, E., Tapia, E., Hernández-Pando, R., García-Niño, W.R., Maldonado, P.D., Reyes, J.L., Barrera-Oviedo, D., Torres, I., and Pedraza-Chaverri, J. (2014) S-allylcysteine prevents cisplatin-induced nephrotoxicity and oxidative stress. *J. Pharm. Pharmacol.*, **66** (9), 1271–1281.

313 Ashafaq, M., Khan, M.M., Shadab Raza, S., Ahmad, A., Khuwaja, G., Javed, H., Khan, A., Islam, F., Siddiqui, M.S., Safhi, M.M., and Islam, F. (2012) S-allyl cysteine mitigates oxidative damage and improves neurologic deficit in a rat model of focal cerebral ischemia. *Nutr. Res.*, **32**, 133–143.

314 Pérez-Severiano, F., Rodríguez-Pérez, M., Pedraza-Chaverrí, J., Maldonado, P.D., Medina-Campos, O.N., Ortíz-Plata, A., Sánchez-García, A., Villeda-Hernández, J., Galván-Arzate, S., Aguilera, P., and Santamaría, A. (2004) S-Allylcysteine,

a garlic-derived antioxidant, ameliorates quinolinic acid-induced neurotoxicity and oxidative damage in rats. *Neurochem. Int.*, **45**, 1175–1183.

315 Giridharan, V.V., Thandavarayan, R.A., Arumugam, S., Mizuno, M., Nawa, H., Suzuki, K., Ko, K.M., Krishnamurthy, P., Watanabe, K., and Konishi, T. (2015) Schisandrin B ameliorates ICV-infused amyloid β induced oxidative stress and neuronal dysfunction through inhibiting RAGE/NF-κB/MAPK and up-regulating HSP/Beclin expression. *PLoS ONE*, **10** (11), e0142483.

316 Lam, P.Y. and Ko, K.M. (2012) Beneficial effect of (-)schisandrin B against 3-nitropropionic acid-induced cell death in PC12 cells. *Biofactors*, **38**, 219–225.

317 Sonia, A.M., Sarkar, A., Anand, K., Ambasta, R.K., and Kumar, P. (2013) Sesamol and naringenin reverse the effect of rotenone-induced PD rat model. *Neuroscience*, **254**, 379–394.

318 Chopra, K., Tiwari, V., Arora, V., and Kuhad, A. (2010) Sesamol suppresses neuro-inflammatory cascade in experimental model of diabetic neuropathy. *J. Pain*, **11**, 950–957.

319 Jamwal, S., Singh, S., Kaur, N., and Kumar, P. (2015) Protective effect of spermidine against excitotoxic neuronal death induced by quinolinic acid in rats: possible neurotransmitters and neuroinflammatory mechanism. *Neurotox. Res.*, **28** (2), 171–184.

320 Sigrist, S.J., Carmona-Gutierrez, D., Gupta, V.K., Bhukel, A., Mertel, S., Eisenberg, T., and Madeo, F. (2014) Spermidine-triggered autophagy ameliorates memory during aging. *Autophagy*, **10**, 178–179.

321 Carrasco-Pozo, C., Tan, K.N., and Borges, K. (2015) Sulforaphane is anticonvulsant and improves mitochondrial function. *J. Neurochem.*, **135** (5), 932–942.

322 Zhang, R., Zhang, J., Fang, L., Li, X., Zhao, Y., Shi, W., and An, L. (2014) Neuroprotective effects of sulforaphane on cholinergic neurons in mice with Alzheimer's disease-like lesions. *Int. J. Mol. Sci.*, **15** (8), 14396–14410.

323 Takahashi, S., Isaka, M., Hamaishi, M., Imai, K., Orihashi, K., and Sueda, T. (2013) Trehalose protects against spinal cord ischemia in rabbits. *J. Vasc. Surg.*, **60**, 490–496.

324 Zhang, X., Chen, S., Song, L., Tang, Y., Shen, Y., Jia, L., and Le, W. (2014) MTOR-independent, autophagic enhancer trehalose prolongs motor neuron survival and ameliorates the autophagic flux defect in a mouse model of amyotrophic lateral sclerosis. *Autophagy*, **10**, 588–602.

325 Dey, A. and De, J.N. (2015) Neuroprotective therapeutics from botanicals and phytochemicals against Huntington's disease and related neurodegenerative disorders. *J. Herb. Med.*, **5**, 1–19.

326 Satoh, T., Takahashi, T., Iwasaki, K., Tago, H., Seki, T., Yaegashi, N., Tobita, M., and Arai, H. (2009) Traditional Chinese medicine on four patients with Huntington's disease. *Mov. Discord.*, **24**, 453–455.

327 Miyaoka, T., Furuya, M., Yasuda, H., Hayashida, M., Nishida, A., Inagaki, T., and Horiguchi, J. (2009) Yi-gan san as adjunctive therapy for treatment-resistant schizophrenia: an open-label study. *Clin. Neuropharmacol.*, **32**, 6–9.

328 Miyaoka, T., Furuya, M., Yasuda, H., Hayashia, M., Inagaki, T., and Horiguchi, J. (2008) Yi-gan san for the treatment of borderline personality disorder: an open-label study. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **32**, 150–154.

329 Iwasaki, K., Satoh-Nakagawa, T., Maruyama, M., Monma, Y., Nemoto, M., Tomita, N., Tanji, H., Fujiwara, H., Seki, T., Fujii, M., Arai, H., and Sasaki, H. (2005) A randomized, observer-blind, controlled trial of the traditional Chinese medicine Yi-Gan San for improvement of behavioral and psychological symptoms and activities of daily living in dementia patients. *J. Clin. Psychiatry*, **66**, 248–252.

330 Miyaoka, T., Furuya, M., Yasuda, H., Hayashida, M., Nishida, A., Inagaki, T., and Horiguchi, J. (2008) Yi-gan san for the treatment of neuroleptic-induced tardive dyskinesia: an open-label study. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **32**, 761–774.

331 Zhu, W., Ma, S., Qu, R., and Kang, D. (2006) Antidepressant-like effect of saponins extracted from Chaihu-jia-longgu-muli-tang and its possible mechanism. *Life Sci.*, **79**, 749–756.

332 Liu, Y., Ma, S., and Qu, R. (2010) SCLM, total saponins extracted from Chaihu-jia-longgu-muli-tang, reduces chronic mild stress-induced apoptosis in the hippocampus in mice. *Pharm. Biol.*, **48**, 840–848.

333 Li, L.F., Lu, J., Li, X.M., Xu, C.L., Yang, J., Qu, R., and Ma, S.P. (2012) Antidepressant-like effects of the saponins extracted from Chaihu-jia-longgu-muli-tang in a rat unpredictable chronic mild stress model. *Fitoterapia*, **83**, 93–103.

334 Dey, A. and De, J.N. (2015) in *Studies in Natural Product Chemistry*, vol. **44** (ed. Atta-ur-Rahman), Elsevier Science, Amsterdam, pp. 447–520.

335 Yang, M.H., Li, M., Dou, Y.Q., Liu, Y., Luo, X.D., Chen, J.Z., and Shi, H.J. (2010) Effects of Bushen Huoxue Granule on motor function in patients with Parkinson's disease: a multicenter, randomized, double-blind and placebo-controlled trial. *Zhong Xi Yi Jie He Xue Bao*, **8**, 231–237.

336 Li, S.D., Yang, M.H., and Liu, Y. (2011) Effect of bushen huoxue granule on dopamine neurotransmitter and dopamine transporter in the brain of patients with Parkinson's disease. *Zhongguo Zhong Xi Yi Jie He Za Zhi*, **31**, 622–625.

337 Ishikawa, T., Funahashi, T., and Kudo, J. (2000) Effectiveness of the Kampo kami-shoyo-san (TJ-24) for tremor of antipsychotic-induced parkinsonism. *Psychiatry Clin. Neurosci.*, **54**, 579–582.

338 Iwasaki, K., Kato, S., Monma, Y., Niu, K., Ohrui, T., Okitsu, R., Higuchi, S., Ozaki, S., Kaneko, N., Seki, T., Nakayama, K., Furukawa, K., Fujii, M., and Arai, H. (2007) A pilot study of banxia houpu tang, a traditional Chinese medicine, for reducing pneumonia risk in older adults with dementia. *J. Am. Geriatr. Soc.*, **55**, 2035–2040.

339 Fang, Q., Zhan, X.P., Mo, J.L., and Sun, M. (2004) The effect of huanglian jiedu tang on Alzheimer's disease and its influence on cytokines [Article in Chinese]. *Zhongguo Zhong Yao Za Zhi*, **29** (6), 575–578.

340 Jeon, S., Bose, S., Hur, J., Jun, K., Kim, Y.K., Cho, K.S., and Koo, B.S. (2011) A modified formulation of Chinese traditional medicine improves memory impairment and reduces Aβ level in the Tg-APPswe/PS1dE9 mouse model of Alzheimer's disease. *J. Ethnopharmacol.*, **137** (1), 783–789.

341 Seo, J.S., Jung, E.Y., Kim, J.H., Lyu, Y.S., Han, P.L., and Kang, H.W. (2010) A modified preparation (LMK03) of the oriental medicine Jangwonhwan reduces Abeta(1-42) level in the brain of Tg-APPswe/PS1dE9 mouse model of Alzheimer disease. *J. Ethnopharmacol.*, **130** (3), 578–585.

342 Liu, Y., Hua, Q., Lei, H., and Li, P. (2011) Effect of Tong Luo Jiu Nao on Aβ-degrading enzymes in AD rat brains. *J. Ethnopharmacol.*, **137** (2), 1035–1046.

343 He, P., Li, P., Hua, Q., Liu, Y., Staufenbiel, M., Li, R., and Shen, Y. (2013) Chronic administration of anti-stroke herbal medicine TongLuoJiuNao reduces amyloidogenic processing of amyloid precursor protein in a mouse model of Alzheimer's disease. *PLoS ONE*, **8** (3), e58181.

344 Bhattacharya, S.K. and Kumar, A. (1997) Effect of Trasina, an ayurvedic herbal formulation, on experimental models of Alzheimer's disease and central cholinergic markers in rats. *J. Altern. Complement. Med.*, **3** (4), 327–336.

345 Liu, P., Kong, M., Yuan, S., Liu, J., and Wang, P. (2014) History and experience: a survey of traditional Chinese medicine treatment for Alzheimer's disease. *Evid. Based Complement. Alternat. Med.*, **2014**, 642128.

346 Wang, Y., Zheng, C., Huang, C., Li, Y., Chen, X., Wu, Z., Wang, Z., Xiao, W. and Zhang, B. (2015) Systems pharmacology dissecting holistic medicine for treatment of complex diseases: an example using cardiocerebrovascular diseases treated by TCM. *Evid. Based Complement. and Alternat. Med.*, 2015, 980190. doi: 10.1155/2015/980190.

347 Dey, A. and De, J.N. (2012) Anti snake venom botanicals used by the ethnic groups of Purulia District, West Bengal, India. *J. Herbs Spices Med. Plants*, **8**, 152–165.

348 Dey, A. and De, J.N. (2012) Ethnomedicinal plants used by the tribals of Purulia district, West Bengal, India against gastrointestinal disorders. *J. Ethnopharmacol.*, **143** (1), 68–80.

349 Modak, B.K., Gorai, P., Dhan, R., Mukherjee, A., and Dey, A. (2015) Tradition in treating taboo: Folkloric medicinal wisdom of the aboriginals of Purulia district, West Bengal, India against sexual, gynaecological and related disorders. *J. Ethnopharmacol.*, **169**, 370–386.

350 Guimarães, R., Barros, L., Carvalho, A.M., and Ferreira, I.C. (2011) Infusions and decoctions of mixed herbs used in folk medicine: synergism in antioxidant potential. *Phytother. Res.*, **25**, 1209–1214.

351 van Vuuren, S.F. and Viljoen, A.M. (2008) *In vitro* evidence of phyto-synergy for plant part combinations of *Croton gratissimus* (Euphorbiaceae) used in African traditional healing. *J. Ethnopharmacol.*, **119**, 700–704.

352 Wink, M. (2008) Evolutionary advantage and molecular modes of action of multicomponent mixtures used in phytomedicine. *Curr. Drug Metabol.*, **9**, 996–1009.

353 Efferth, T. and Koch, E. (2011) Complex interactions between phytochemicals. The multitarget therapeutic concept of phytotherapy. *Curr. Drug Targets*, **12**, 122–132.

354 Manyam, B.V., Dhanasekaran, M., and Hare, T.A. (2004) Neuroprotective effects of the antiparkinson drug *Mucuna pruriens*. *Phytother. Res.*, **2004** (18), 706–712.

355 Wu, W.R. and Zhu, X.Z. (1999) Involvement of monoamine oxidase inhibition in neuroprotective and neurorestorative effects of *Ginkgo biloba* extract against MPTP-induced nigrostriatal dopaminergic toxicity in C57 mice. *Life Sci.*, **65**, 157–164.

356 Chen, X. and Decker, M. (2013) Multi-target compounds acting in the central nervous system designed from natural products. *Curr. Med. Chem.*, **20** (13), 1673–1685.

357 Martin, D.D., Ladha, S., Ehrnhoefer, D.E., and Hayden, M.R. (2015) Autophagy in Huntington disease and huntingtin in autophagy. *Trends Neurosci.*, **38** (1), 26–35.

358 Cortes, C.J. and La Spada, A.R. (2014) The many faces of autophagy dysfunction in Huntington's disease: from mechanism to therapy. *Drug Discov. Today*, **19** (7), 963–971.

359 Sorolla, M.A., Rodríguez-Colman, M.J., Vall-llaura, N., Tamarit, J., Ros, J., and Cabiscol, E. (2012) Protein oxidation in Huntington disease. *Biofactors*, **38** (3), 173–185.

360 Chen, C.M. (2011) Mitochondrial dysfunction, metabolic deficits, and increased oxidative stress in Huntington's disease. *Chang Gung Med. J.*, **34** (2), 135–152.

361 Quintanilla, R.A. and Johnson, G.V. (2009) Role of mitochondrial dysfunction in the pathogenesis of Huntington's disease. *Brain Res. Bull.*, **80** (4-5), 242–247.

362 Hickey, M.A. and Chesselet, M.F. (2003) Apoptosis in Huntington's disease. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **27** (2), 255–265.

363 Hermel, E., Gafni, J., Propp, S.S., Leavitt, B.R., Wellington, C.L., Young, J.E., Hackam, A.S., Logvinova, A.V., Peel, A.L., Chen, S.F., Hook, V., Singaraja, R., Krajewski, S., Goldsmith, P.C., Ellerby, H.M., Hayden, M.R., Bredesen, D.E., and Ellerby, L.M. (2004) Specific caspase interactions and amplification are involved in selective neuronal vulnerability in Huntington's disease. *Cell Death Differ.*, **11** (4), 424–438.

364 Manyam, B.V., Giacobini, E., and Colliver, J.A. (1990) Cerebrospinal fluid acetylcholinesterase and choline measurements in Huntington's disease. *J. Neurol.*, **237** (5), 281–284.

365 Vattakatuchery, J.J. and Kurien, R. (2013) Acetylcholinesterase inhibitors in cognitive impairment in Huntington's disease: a brief review. *World J. Psychiatry.*, **3** (3), 62–64.

366 Newmaster, S.G., Grguric, M., Shanmughanandhan, D., Ramalingam, S., and Ragupathy, S. (2013) DNA barcoding detects contamination and substitution in North American herbal products. *BMC Med.*, **11**, 222.

367 Sinha, G. (2005) Medicine of the masses. *Nat. Med.*, **11**, 9–10.

368 Osathanunkul, M., Madesis, P., and de Boer, H. (2015) Bar-HRM for authentication of plant-based medicines: evaluation of three medicinal products derived from acanthaceae species. *PLoS ONE*, **10** (5), e0128476.

369 Ohtsuki, S. (2004) Physiological function of blood-brain barrier transporters as the CNS supporting and protecting system. *Yakugaku Zasshi*, **124** (11), 791–802.

370 Urquhart, B.L. and Kim, R.B. (2009) Blood-brain barrier transporters and response to CNS-active drugs. *Eur. J. Clin. Pharmacol.*, **65** (11), 1063–1070.

371 Fricker, G. (2008) Drug interactions with natural products at the blood brain barrier. *Curr. Drug Metabol.*, **9** (10), 1019–1026.

372 Kam, A., Li, K.M., Razmovski-Naumovski, V., Nammi, S., Chan, K., Li, Y., and Li, G.Q. (2012) The protective effects of natural products on blood-brain barrier breakdown. *Curr. Med. Chem.*, **19** (12), 1830–1845.

373 Aqil, F., Munagala, R., Jeyabalan, J., and Vadhanam, M.V. (2013) Bioavailability of phytochemicals and its enhancement by drug delivery systems. *Cancer Lett.*, **334** (1), 133–141.

374 Kanai, M., Otsuka, Y., Otsuka, K., Sato, M., Nishimura, T., Mori, Y., Kawaguchi, M., Hatano, E., Kodama, Y., Matsumoto, S., Murakami, Y., Imaizumi, A., Chiba, T., Nishihira, J., and Shibata, H. (2013) A phase I study investigating the safety and pharmacokinetics of highly bioavailable curcumin (Theracurmin®) in cancer patients. *Cancer Chemother. Pharmacol.*, **71** (6), 1521–1530.

375 Shoba, G., Joy, D., Joseph, T., Majeed, M., Rajendran, R., and Srinivas, P.S. (1998) Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med.*, **64**, 353–356.

376 Arcaro, C.A., Gutierres, V.O., Assis, R.P., Moreira, T.F., Costa, P.I., Baviera, A.M., and Brunetti, I.L. (2014) Piperine, a natural bioenhancer, nullifies the antidiabetic and antioxidant activities of curcumin in streptozotocin-diabetic rats. *PLoS ONE*, **9** (12), e113993.

377 Zhao, Y., Tang, G., Tang, Q., Zhang, J., Hou, Y., Cai, E., Liu, S., Lei, D., Zhang, L., and Wang, S. (2015) A method of effectively improved α-mangostin bioavailability. *Eur. J. Drug Metabol. Pharmacokinet.*, **41** (5), 605–613.

378 Sanna, V., Chamcheu, J.C., Pala, N., Mukhtar, H., Sechi, M., and Siddiqui, I.A. (2015) Nanoencapsulation of natural triterpenoid celastrol for prostate cancer treatment. *Int. J. Nanomed.*, **10**, 6835–6846.

379 Qi, X., Qin, J., Ma, N., Chou, X., and Wu, Z. (2014) Solid self-microemulsifying dispersible tablets of celastrol: formulation development, characterization and bioavailability evaluation. *Int. J. Pharm.*, **472** (1-2), 40–47.

380 Hong, Z., Xu, Y., Yin, J.F., Jin, J., Jiang, Y., and Du, Q. (2014) Improving the effectiveness of (-)-epigallocatechin gallate (EGCG) against rabbit atherosclerosis by EGCG-loaded nanoparticles prepared from chitosan and polyaspartic acid. *J. Agric. Food. Chem.*, **62** (52), 12603–12609.

381 Lambert, J.D., Hong, J., Kim, D.H., Mishin, V.M., and Yang, C.S. (2004) Piperine enhances the bioavailability of the tea polyphenol (−)-epigallocatechin-3-gallate in mice. *J. Nutr.*, **134** (8), 1948–1952.

382 Lambert, J.D., Sang, S., Hong, J., Kwon, S.J., Lee, M.J., Ho, C.T., and Yang, C.S. (2006) Peracetylation as a means of enhancing in vitro bioactivity and bioavailability of epigallocatechin-3-gallate. *Drug Metab. Dispos.*, **34** (12), 2111–2116.

383 Jeong, D., Choi, J.M., Choi, Y., Jeong, K., Cho, E., and Jung, S. (2013) Complexation of fisetin with novel cyclosophoroase dimer to improve solubility and bioavailability. *Carbohydr. Polym.*, **97** (1), 196–202.

384 Seguin, J., Brullé, L., Boyer, R., Lu, Y.M., Romano, M.R., Touil, Y.S., Daniel Scherman, D., Bessodes, M., Mignet, N., and Chabot, G.G. (2013) Liposomal encapsulation of the natural flavonoid fisetin improves bioavailability and antitumor efficacy. *Int. J. Pharm.*, **444** (1), 146–154.

385 Ragelle, H., Crauste-Manciet, S., Seguin, J., Brossard, D., Scherman, D., Arnaud, P., and Chabot, G.G. (2012) Nanoemulsion formulation of fisetin improves bioavailability and antitumour activity in mice. *Int. J. Pharm.*, **427** (2), 452–459.

386 Walle, T. (2011) Bioavailability of resveratrol. *Ann. N.Y. Acad. Sci.*, **1215** (1), 9–15.

387 Gärtner, C., Stahl, W., and Sies, H. (1997) Lycopene is more bioavailable from tomato paste than from fresh tomatoes. *Am. J. Clin. Nutr.*, **66** (1), 116–122.

388 Tian, J., Shi, J., Zhang, X., and Wang, Y. (2010) Herbal therapy: a new pathway for the treatment of Alzheimer's disease. *Alzheimers Res. Ther.*, **2** (5), 30.

389 Mani, R.B. (2004) The evaluation of disease modifying therapies in Alzheimer's disease: a regulatory viewpoint. *Stat. Med.*, **23** (2), 305–314.

390 Ho, Y.S., So, K.F., and Chang, R.C.C. (2010) Anti-aging herbal medicine—How and why can they be used in aging-associated neurodegenerative diseases? *Ageing Res. Rev.*, **9** (3), 354–362.

391 Li, X.Z., Zhang, S.N., Liu, S.M., and Lu, F. (2013) Recent advances in herbal medicines treating Parkinson's disease. *Fitoterapia*, **84**, 273–285.

9

Possible Role of Neuroprotectants and Natural Products in Epilepsy: Clinical Aspects and Mode of Action

Anil Kumar, Manveen Bhardwaj, and Harshpreet Kaur

Panjab University, Department of Pharmacology, UGC Centre of Advanced Study, Pharmacology Division, University Institute of Pharmaceutical Sciences, Chandigarh 160014, India

9.1 Introduction

Epilepsy is a chronic neurological condition of recurring seizures/syndromes with unique clinical manifestations [1]. Chronic epileptic condition produces complex neurobehavioral alterations due to the excitability of epileptic neurons in various brain regions [2]. Epileptogenesis causes long-term changes in the cellular and molecular pathomechanisms in the brain. First-generation antiepileptic drugs (AEDs) such as phenytoin, carbamazepine, valproate, ethosuximide, phenobarbital, and primidone that were introduced between 1910 and 1970 are still in clinical use despite their toxicity as a limiting factor. Drugs such as vigabatrin, gabapentin, felbamate, lamotrigine, oxcarbazepine, tiagabine, and topiramate, introduced around 20 years later, were termed as the *new drugs* or *second-generation drugs*. Third-generation drugs such as brivaracetam, carabersat, carisbamate, DP-valproic acid, eslicarbazepine, fluorofelbamate, fosphenytoin, ganaxolone, lacosamide, losigamone, pregabalin, remacemide, retigabine, rufinamide, safinamide, seletracetam, soretolide, stiripentol, talampanel, and valrocemid are few of the recent promising anticonvulsants that are either in preclinical or in various clinical phases of development [3]. AEDs are generally preventive in nature and have no influence on the course of the disease as well as no detrimental effects, which occur after long time. Therefore, approximately one third of people with epilepsy (PWE) have drug-resistant seizures. Surgery is highly effective and generally considered safe for selected patients, but it is still underused because many PWE have a single site of origin, so surgery is not required in that case [4]. Besides beneficial effects of newer AEDs, many undesired central nervous system (CNS) effects such as impaired cognitive performance and psychiatric complications have also been frequently observed in patients. Therefore, to control the epileptogenesis effectively, there is immediate need either to develop or to adopt an alternative approach for its better management. There are few Ayurvedic preparations that are in the market and have claimed to treat epilepsy but do not have sound scientific supports. These market

Neuroprotective Natural Products: Clinical Aspects and Mode of Action, First Edition.
Edited by Goutam Brahmachari.

preparations are like APSA from IMIS, NED Forte from Charak, tantu pashan from Sagar, and Zandopa from Zandu, which are in clinical use to treat epilepsy. Beyond seizure inhibition, these days' research has been focused on the therapeutic modifications in the molecular and cellular response with new opportunities to target epilepsy [5]. Aromatic plants are used medicinally because of their volatile oils and chemical component, and many of them have CNS properties including their antiepileptic action. They are also used as folk medicine and traditional drug [6]. Recently, studies have been conducted on the potential role of essential oils and their components for the development of new therapy that might be having the better effect than the current therapeutics.

9.2 Global Prevalence of Natural Products in Epilepsy

Approximately 67 million people worldwide suffer from the problem of epilepsy or related complications [7]. The lifetime prevalence of a seizure (excluding febrile seizures) is 2–5%. Literature and media demonstrate increasing citations of the benefits of herbals and/or natural products (dietary supplements) against epilepsy. However, concern has been raised about efficacy and safety of these natural products due to their side effects or drug interaction [8]. The use of dietary supplements has also increased among the aged population due to their claimed therapeutic potentials and benefits, and such prevalence was 14.2% in 1998. In 2002, 18.8% of American adults used herbals or other natural products as dietary supplements against epilepsy and related problems. The recently increased trends for herbals and natural products have again been noticed worldwide. According to the American National Health Interview Survey (2007), 23 393 adults (38.3%) and 9417 children (11.8%) used complementary and alternative medicine (CAM) therapies for various reasons [9]. These therapies were used more frequently by people with higher level of education, women, and the Native American population. Around 17.7% adults use natural products as CAM. Concordantly, the annual out-of-pocket cost in the United States for purchase of these natural products for 2007 was estimated to be $14.8 billion, which is one third of the out-of-pocket amount spent on prescription drugs [10]. The frequency of use of specific natural products in developed countries is parallel to the available evidence supporting their use.

9.3 Pathophysiology of Epilepsy

Clinical signs of epilepsy arise due to excessively synchronized activity of selected group(s) of neurons. One of the essential dominant pathomechanisms of epilepsy includes glutamate-induced excitotoxicity that leads to neuronal cell death. It has been demonstrated that an increased glutamate binding to postsynaptic receptors causes the opening of sodium and calcium channels that is responsible for triggering neuronal cell death. There is an increase in depolarization of neurons as the conductance increases during excitability. Besides, the concentration of various amino acids such as norepinephrine, acetylcholine (ACh), and γ-aminobutyric acid (GABA) also fluctuates. GABA (major inhibitory neurotransmitter) level decreases during epilepsy due to the action of the enzyme GABA transaminase,

responsible for degradation of the GABA and reducing its concentration in the brain. Furthermore, an increase in the glutamine synthetase activity is also responsible for decrease in GABA activity.

Both apoptosis and necrosis are cellular events involved in the pathogenesis of epilepsy [11]. Cytoskeleton degeneration in which nitric oxide (NO) synthase interferes with oxidative metabolism causes the accumulation of free radicals [12, 13]. Several intracellular proteins are also involved, which cause calcium overload. Energy deprivation and failure of neuronal homeostasis occur due to excessive entry of calcium into the mitochondria [11], after which apoptotic cell destruction starts with the activation of pro-caspases [13]. Additional factors contributing to neuronal cell damage include growth factor withdrawal and cytokine, toxin, or protein accumulation. These cellular cascade mechanisms contribute to neuronal cell death in a very complex manner, which is yet to be understood to comprehend the exact pathophysiology of epilepsy [14].

9.3.1 Oxidative Stress

The brain utilizes the highest amount of oxygen in comparison to other parts of the body. Polyunsaturated fatty acids are more prone to lipid peroxidation, which are present in high concentration in the brain. Oxidation of the biomolecules such as proteins, lipids, and nucleotides causes cell disruption and cell damage [15]. Deactivation of the various enzymes occurs by the oxidation of these proteins. Membrane fluidity and permeability are also affected by the peroxidation of lipids [16]. Oxidative stress is significantly involved in the pathogenesis of various neurological conditions and neurodegenerative disorders [17]. At the cellular level, seizures precipitate due to calcium influx, voltage-gated, and *N*-methyl-D-aspartate (NMDA)-dependent ion channel. High level of intracellular calcium can induce reactive oxygen species (ROS) [18]. At the physiological level, enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and nonenzymatic (vitamin C, vitamin E, and reduced form of glutathione) molecules scavenge ROS. During oxidative stress, the antioxidant defense system is weakened, and oxidants such as ROS increase [19].

9.3.2 Mitochondrial Electron Transport Chain

Phosphorylation of the electron transport chain (ETC) is critical for the regulation of membrane permeability, neurotransmitter biosynthesis, and exocytosis. Epilepsy, particularly temporal lobe epilepsy (TLE), appears when ETC enzymes do not function properly. ROS also inactivate mitochondrial ETC. Mitochondrial ETC consists of complex I, which is a major producer of $O^{2\bullet-}$ and is the main cause behind mitochondrial dysfunction [20]. Impaired mitochondrial ETC function, as a result of ROS, may lead to Ca^{2+}-dependent depolarization of the mitochondrial membrane potential that reduced the production of ATP, incomplete oxygen consumption, and overproduction of ROS [21].

9.3.3 Brain Inflammatory Pathways

Proconvulsants or brain injury activates Toll-like receptors (TLRs) in astrocytes and microglia cells. Overexpression of TLR4 in these cell leads to the production

of proinflammatory mediators (e.g., chemokines, cytokines, complement factors, cell adhesion molecules, and prostaglandins) and gliotransmitters. TLR4 causes Src kinase-dependent phosphorylation of the NMDA receptors, thereby resulting in NMDA-dependent Ca^{2+} influx and altering neuronal excitability. IL-1β and tumor necrosis factor) (TNF)-α decrease the expression of GABA-mediated Cl^- ion channels [22]. Activation of plasminogen and complement system affects blood–brain barrier (BBB) permeability and causes serum albumin accumulation, which induces long-lasting hyperexcitability. These changes exert direct effect on neuronal excitability and decrease seizure threshold. Once seizure develops, it activates the transcription of inflammatory genes and changes in cytokine production. Treatment of epilepsy with COX-2 inhibitors, antibodies against endothelial cell adhesion molecules [23], and immunosuppressant drugs [24] and mammalian target of rapamycin (*m*TOR) inhibitors suggests the role of neuroinflammation in epileptogenesis. Studies have demonstrated that quercetin [25, 26], luteolin [27], and rutin [28] attenuate seizures by reducing neuroinflammation.

9.3.4 Apoptosis

Seizures significantly cause the loss of neurons of hippocampal CA1 and CA3 regions. Apoptosis includes two major pathways – extrinsic (activation of death receptors of TNF) and intrinsic (mitochondrial). In extrinsic pathways, activation of cell-surface-expressed death receptors of the TNF superfamily leads to the formation of caspases [29]. Caspases belong to the aspartate-specific cysteine protease family. Caspase cascade cleaves the intracellular structural and functional proteins and DNA. Apoptotic DNA fragmentation has also been noticed after prolonged seizures [30, 31]. Caspase-8 is cleaved after seizure induction. In intrinsic pathway, high Ca^+ level, ROS/RNS (Reactive Nitrogen Species), and dimerization interactions of Bcl-2 family proteins cause alteration in intracellular homeostasis or DNA damage [32], which leads to mitochondrial dysfunction. Vitamin C, SOD mimetics, and melatonin are some of the antioxidants that prevent seizure-induced neuronal death (Figure 9.1) [33, 34].

9.4 Role of Neurotransmitters in Neuronal Excitation

Different neurotransmitters or neuromodulators are known to play important roles in neuronal excitation. Some of these neurotransmitters with diverse chemical nature coexist in the same nerve terminal and act on more than one receptor. Neurotransmitters such as GABA, glutamate, and ACh play key role in proper functioning of the limbic areas. Fine-tuning of these neurotransmitters is essential for maintenance of homeostasis, human emotions, behavior, and cognitive functions.

9.4.1 γ-Aminobutyric Acid (GABA)

GABA is the principal inhibitory neurotransmitter in the central cortex [35]. GABA-based positive modulators are antiepileptic in nature [36]. At GABAergic

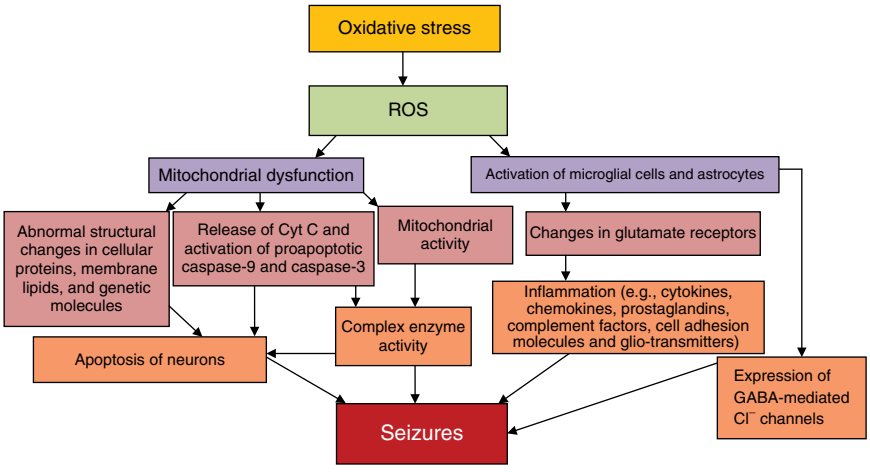
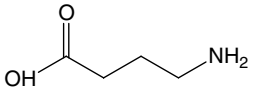


Figure 9.1 Pathophysiology of epilepsy.

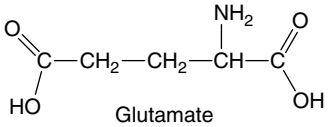
axon terminals, GABA is formed, where glutamic acid decarboxylase (GAD) decarboxylates the glutamic acid to GABA. GABAA receptors (ligand-gated ion channels) increase inward chloride conductance, thus hyperpolarizing the neurons and showing inhibitory effect on neurons. GABAB receptors are G-protein-linked receptors that act when potassium conductance is increased and thus hyperpolarize the neurons. GABA transporters are also involved in the epileptogenesis [37].



GABA

9.4.2 Glutamate

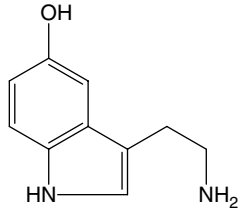
The level of GABA is reduced in epilepsy [38]. There is a change in the number and sensitivity of different glutamate receptor subtypes [39]. Two glutamate receptors, NMDA [40] and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) [41], play important role in epileptogenesis. NMDA receptors are composed of subunits NR1 and NR2A and NR2B, which differ in functional properties. Any change in these subunits also plays a role in synaptic plasticity [42, 43]. Long-lasting excitatory synaptic neurotransmission is dependent upon the extracellular concentration of glutamate. Glutamate receptors are permeable to Na^+ and K^+, thus responsible for membrane depolarization. NMDA receptors also possess Ca^+ channels that contribute to hyperexcitability of neurons [44].



Glutamate

9.4.3 Serotonin

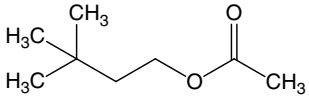
Studies revealed that reduction of brain serotonin concentration significantly increases seizure susceptibility [45, 46]. 5-HT1A receptor activation increases potassium conductance and hyperpolarizes the neurons [47, 48] and demonstrates anticonvulsant effect in experimental models of seizure [49]. Emotional changes in kindling model are due to the hypoactivity of serotonin in the hippocampus and prefrontal cortex [50].



Serotonin

9.4.4 Acetylcholine (ACh)

The concentration of ACh rises during seizures [51]. There is evidence that on electrical stimulation, there occurs a significant rise of ACh in the nucleus amygdaloideum lateralis [52, 53]. Isoniazid and pentylenetetrazol (PTZ) have different mechanisms to reduce GABAA receptor function and induce tonic–clonic convulsions [54, 55]. Both the drugs also increase hippocampal ACh release in a dose-dependent manner. Activation of muscarinic receptors causes the initiation of the seizures [56].



Acetylcholine

Considering the aforementioned parameters as an important factor in the pathophysiology of epilepsy, a neuroprotectant that can target these pathways is depicted in Figure 9.2.

9.5 Role of Neuroprotectants in Seizures

Neuroprotectants protect against injury or loss of neurons. Neuronal loss occurs in different disease conditions involving oxidative stress, excitotoxicity, mitochondrial dysfunction, and so on. Pharmacological neuroprotection against seizures can be categorized as primary and secondary. If AEDs and compounds act on voltage-sensitive Na^+ and Ca^{2+} channels or on glutamate receptors, then it is a primary neuroprotection, and when they act on the cascade leading to necrosis or apoptosis, it is called as the *secondary neuroprotection* [15]. Other possibilities may diminish the long-term morphological and functional consequences of seizures. Given in the following are various categories of neuroprotectants.

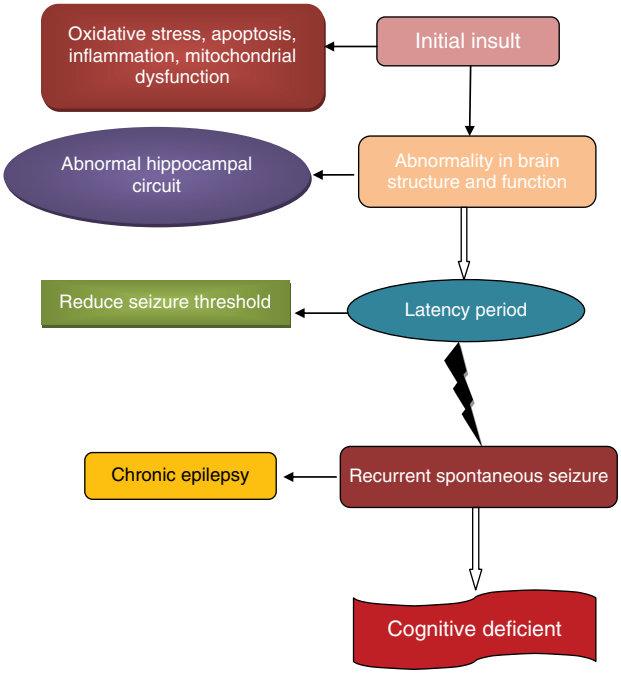


Figure 9.2 Diagram showing the cascade of seizure induction, which causes cognitive deficiency.

9.5.1 Alkaloids

Alkaloids such as aconitum alkaloids interact with voltage-dependent Na^+ channel that has an exceptional relationship with neuronal excitability. Most of the tested aconitum alkaloids inhibit epileptiform activity. Apart from aconitum alkaloids, there are many other kinds of alkaloids: isoquinoline alkaloids such as berberine [57], montanine [58], and tetrahydropalmatine [59]; indole alkaloids such as ibogaine [60]; piperidine alkaloids such as piperine [61]; amide alkaloids such as piplartine [62]; tetracyclic oxindole alkaloids such as rhynchophylline and isorhynchophylline [63]; aporphine alkaloids such as nantenine [64]; erythrine by-products such as erysothrine [65]; (+)-erythravine and (+)-11-α-hydroxyerythravine [66]; and raubasine [67], which possess significant anticonvulsant activity. Anticonvulsant activities of the aforementioned alkaloids were well investigated in various experimental models including PTZ, maximal electroshock (MES), and kainic acid- (KA), bicuculline-, pilocarpine-, or NMDA-induced convulsions. Both montanine and berberine exhibited significant anticonvulsant activity by modulating neurotransmitter systems; montanine protected PTZ-provoked convulsion as well. Erysothrine reduced all the convulsions [65], whereas (+)-erythravine and (+)-11-α-hydroxyerythravine were found inactive in NMDA- and PTZ-induced seizures, respectively. Piplartine in the PTZ-induced convulsion model decreased the latency to death in mice [62]. At lower doses, nantenine reduced PTZ- and MES-induced

seizures, probably attributed to nantenine's stimulation and the resultant decrease in Ca^{2+} influx into the cell [64]. Piperine delayed time latency of convulsion as well as mortality. Further, piperine increases percentage of survival by acting on the GABAergic system against pilocarpine-induced convulsion [61]. Raubasine attenuated PTZ- and bicuculline-induced convulsions due to its benzodiazepine agonist-like activity [67]. Furthermore, ibogaine produced anticonvulsant activity using MES- and NMDA-induced models. The results showed that ibogaine has NMDA receptor blocking activity [60].

9.5.2 Flavonoids

Flavonoids are widely distributed in plants with diverse pharmacological properties including antiseizure properties. Many flavonoids prevent tonic–clonic seizures. Antiseizure properties of flavonoids including bioflavonoids have already been thoroughly discussed in Chapter 10 of this book.

9.5.3 Terpenoids

α-Terpineol, a relatively common monoterpene present in many medicinal plants, showed protective effects against PTZ- and MES-induced convulsive seizures in mice. Anticonvulsant activities of linalool enantiomers were found in PTZ, PTX (Picrotoxin), and MES models; (*R*)-(−)-linalool was reported to be potent than its enantiomeric form [68, 69].

Isopulegol, a product formed by cyclization of citronellol, exhibited anticonvulsive effects against PTZ-induced model [70]. Safranal, an active monoterpene aldehyde, interacts with GABAA and shows beneficial effect against PTZ-induced convulsions in mice by interacting with GABA–BZD receptor complex [71, 72]. Carvacrol and borneol, two monoterpenes present in the essential oils of numerous medicinal plants, were reported to be effective in preventing clonic seizures induced by PTZ and tonic convulsions induced by MES, and the effects of borneol are through the modulation of GABAergic system [73]. Eugenol, a phenolic monoterpene extracted from cloves, is also useful in various medicines including in ameliorating epileptic seizures because of its modulating effect on neuronal excitability. Anticonvulsant effect of eugenol is associated primarily with its specific effect on ionic currents, which increase voltage-gated Na^{+} currents (INa) inactivation and suppress the noninactivating INa [Ina]. Some diterpenes and their derivatives also demonstrated good anticonvulsive effects in preclinical experimental models. Phytol can reduce pilocarpine-induced seizures by modulating neurotransmitters rather than GABAergic system [74]. Abietic acid has a potential anticonvulsant property. Moreover, three isomeric cannabinoids (a group of 21-carbon-containing terpenophenolic compounds) derived from *Cannabis sativa* – namely, Δ^8-tetrahydrocannabinol (Δ^8-THC), Δ^9-tetrahydrocannabinol (Δ9-THC), and cannabidiol (CBD) – were reported to possess anticonvulsant activities [75]. Both Δ^8-THC and Δ^9-THC dose-dependently protected tonic convulsion against MES in rats [76]. CB1 receptors in cannabinoid have anticonvulsant effects [77]. Ursolic acid, a pentacyclic triterpenoid, is widely distributed in plants and possesses anticonvulsant activity as observed in reducing the number and mortality against PTZ-induced seizures in

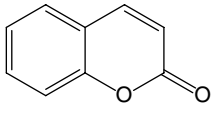
mice [78]. Ursolic acid stearoyl glucoside, a terpenoid isolated from *Lantana camara* L., exhibited anticonvulsive effect [79]. This effect is mediated by facilitation of GABA transmission, and it has been evaluated in MES- and isoniazid-induced seizures in rats.

9.5.4 Saponins

Saponins are potential bioactive agents in treating epilepsy. The saponin fraction obtained from *Astragalus mongholicus* showed significant anticonvulsant effect against acute PTZ-induced seizures [80]. Saponin-rich fraction (SFG) of *Ficus platyphylla* stem bark also demonstrated anticonvulsive effects against both PTZ- and strychnine-induced seizures *in vivo* but failed to protect mice against MES test [81, 82].

9.5.5 Coumarins

Coumarins are plant-derived polyphenolic compounds, having fused benzene and α-pyrone rings. These compounds possess a wide range of pharmacologic and biochemical applications [83]. *Esculetin* (6,7-dihydroxycoumarin) decreases seizure response induced probably through the GABAergic mechanism. The protective anticonvulsant effects of imperatorin and osthole (two natural coumarin derivatives) were shown to have activity against MES-induced convulsions in mice [84]. The anticonvulsant effects of four linear furanocoumarins revealed that the compounds contained substitutions at the C-8 position of the psoralen ring (imperatorin and xanthotoxin). Furanocoumarins, from the fruits of *Heracleum crenatifolium*, such as isopimpinellin and byak-angelicol both show much stronger anticonvulsant activities. Coumarin derivatives such as *heraclenin* and *oxypeucedanin* for GABA-induced chloride current enhancement bear an epoxylated oxyprenyl residue at the C-8 position and C-5 position, respectively; and heraclenin showed a >10-fold loss of activity (31% vs. 547%). However, the fact that the effect of the tested coumarins occurred at very high concentrations (100 lm) implies that coumarins exert their anticonvulsant effects by interacting with other receptors while not exclusively via the GABAA receptor [85].



Coumarin

9.5.6 Antioxidants

Oxidative stress by the generation of free radicals contributes to the initiation and progression of epilepsy after brain injury. Therefore, antioxidant therapies aimed at reducing oxidative stress have received considerable attention in the treatment of epilepsy. Cells contain natural defense system composed of enzymes that neutralize free radicals such as SOD, CAT, and peroxidase and antioxidants such as vitamins C and E, glutathione, ferritin, and uric acid. These systems help

the cell to maintain its homeostasis by neutralizing the oxidative effects of oxygen and its reactive metabolites. Antioxidants therapies involve either the administration of antioxidants, which may react with free radicals, or the strengthening of the endogenous antioxidant defenses by enhancing the activity of SOD, CAT, and GPx. Antioxidants can give protection against excitotoxic cell death in various *in vitro* systems, including selective neuronal loss induced by burst discharges, which can be ameliorated by vitamin E [142]. Vitamin E and glutathione reduce neurodegeneration induced by seizures.

9.6 Natural Plants against Epilepsy

Various natural plants that are examined in epilepsy are listed as follows.

9.6.1 *Nardostachys jatamansi* (Jatamansi)

Nardostachys jatamansi DC. (Valerianaceae) is used to treat epilepsy, hysteria, syncope, and mental weakness. The ethanol extract of *N. jatamansi* increased the seizure threshold in the experimental model of generalized tonic–clonic seizures with very low neurotoxic effect [86]. The combination study underscores the significance of the synergistic effect of *N. jatamansi* in combination with phenytoin.

9.6.2 *Cotyledon orbiculata* (Seredile, Plakkie, Imphewula)

Cotyledon orbiculata L. (Crassulaceae) juice is used to treat epilepsy. The leaves of *C. orbiculata* contain saponins that are of triterpenoid type, and the triterpene steroids present in *C. orbiculata* contribute to the anticonvulsant activity of the plant [87]. NMDLA (N-methyl-D,L-aspartic acid) is a specific agonist at NMDLA receptors that are implicated in the pathogenesis of epilepsy. It produces effects similar to glutamic acid at NMDLA receptors and exerts its convulsant effect by activating the receptors to enhance glutaminergic neurotransmission. *C. orbiculata* delays the onset of NMDLA convulsion.

9.6.3 *Laurus nobilis*

Leaves of the *Laurus nobilis* Linn. (Lauraceae) are used to treat epilepsy, neuralgia, and parkinsonism [88].

9.6.4 *Bacopa monnieri* (Brahmi)

Bacopa monnieri, an Indian herbal drug, is a reputed nootropic plant and is commonly used to treat asthma, epilepsy, insanity, and hoarseness. It is a major constituent of Medhya Rasayana formulations [89]. *B. monnieri* treatment ($300\,mg\,kg^{-1}$ (oral) body weight per day for 15 days) to epileptic rat prevents the occurrence of seizures, thereby reducing the impairment on the peripheral nervous system [90]. Treatment with *B. monnieri* extract shows a therapeutic effect, and it has immense clinical significance in the therapeutic management of epilepsy. *B. monnieri* treatment significantly reverses the downregulated mGluR8

gene expression. The glutamatergic system, predominantly the NMDA receptor, has important functions in the neonatal period in neurodevelopment.

9.6.5 *Rhizoma pinelliae*

It is a tuber of *Rhizoma ternate* (Thumb, Family: Araceae). Ethanol fraction from *Rhizoma pinelliae* Praeparatum (EFRP) was reported to reduce the rate of nikethamide (NKTM)-induced convulsion death and to prolong the latency; however, the extract had no effect on the convulsion latency, thereby suggesting that EFRP possesses the potential to modify the course of convulsive episodes via interfering with seizure threshold and blocking seizure propagation. It provides pharmacological support for the use of *R. pinelliae* Praeparatum in the treatment of insomnia and central nervous disorders [91].

9.6.6 *Taxus wallichiana* (Himalayan Yew)

Taxus wallichiana Zucc. (Himalayan yew) is often used in epilepsy. The genus *Taxus* (Taxaceae) is well known for its famous anticancer agent. Leaves of the plant are used to make herbal tea for indigestion and epilepsy [92].

9.6.7 *Sutherlandia frutescens* (Umwele, Cancerbush)

The aerial parts of *Sutherlandia frutescens* R. BR. (Fabaceae) are extensively used in childhood convulsions and epilepsy. *S. frutescens*'s shoot aqueous extract (50–400 mg kg^{-1} intraperitoneally i.p.) significantly delayed the onset of, and antagonized, PTZ-induced seizures. The plant's shoot aqueous extract (50–400 mg kg^{-1} i.p.) also profoundly antagonized picrotoxin (PCT)-induced seizures [93]. *S. frutescens* contains several bioactive chemical compounds, including the nonprotein amino acid L-canavanine; pinitol; GABA; asparagine; methyl- and propyl-parabens; small amount of saponins, especially the novel triterpenoid glucoside tagged as "SU1"; sigma-4-en-3-one; and gamma-sitosterol [9, 27, 35, 38]. L-Canavanine, a natural L-arginine analog, and its metabolite canaline possess antitumor properties [12, 36] and are likely to contribute to the *in vitro* antiproliferative and apoptotic effects of *S. frutescens* extract.

9.6.8 *Ficus platyphylla* (Dell-Holl)

Ficus platyphylla (Moraceae) is a Nigerian traditional medicine used to treat psychosis, depression, epilepsy, pain, and inflammation for many years; the crude methanol extract of *F. platyphylla* stem bark contains sedative principles with potential neuroleptic, analgesic, and anti-inflammatory properties [94].

9.6.9 *Scutellaria baicalensis* (Skullcaps)

Scutellaria baicalensis (Lamiaceae) is one of the most important medicinal herbs in traditional Korean medicine. Flavonoids from *S. baicalensis* may exert pharmacologically and clinically important profiles including anxiolysis, anticonvulsion, myorelaxation, and sedation because they have high affinity for the benzodiazepine binding site of GABAA receptors [69]. The total

extract from *S. baicalensis* partially blocked suppression of locomotion as well as behavioral changes induced by electroshock stress [95].

9.6.10 *Harpagophytum procumbens* (Devil's Claw)

Harpagophytum procumbens is widely used in South African traditional medicine. Aqueous root extract of *H. procumbens* possesses anticonvulsant activity. The effectiveness of the plant's extract in the experimental convulsion suggests that the herb can be used in both petit and grand mal types of epilepsy. The plant's extract appears to be relatively more effective in PTZ- and PCT-induced convulsions [96]. *H. procumbens* significantly delayed the onset of seizures induced by PTZ and also significantly antagonized PCT-induced seizures. *H. procumbens* secondary root aqueous extract probably produces its anticonvulsant activity by enhancing GABAergic neurotransmission because its ability to depress the CNS by one or more of the known mechanisms of anticonvulsant action [11], which may include altered Na^+/K^+-ATPase expression [9], phosphate metabolism [3], and inhibition of expression of inducible NO.

9.6.11 *Delphinium denudatum* (Jadwar)

Delphinium denudatum is an indigenous medicinal herb popularly known as Jadwar by the traditional healers. It is used for the treatment of epilepsy. Aqueous fraction (AF) exhibited dose-dependent activity against hind limb tonic extension (HLTE) phase of MES and comparatively stronger anticonvulsant activity against seizures induced by PTZ. These anticonvulsant compounds from AF possibly interact with GABA(A) receptor to produce blockade of epileptiform activity.

9.6.12 *Withania somnifera* (Ashwagandha)

The root extract of *Withania somnifera along with* lithium pilocarpine treatment for 7 days reduced mortality up to 60% without affecting latency of forelimb clonus. Furthermore, *W. somnifera* when combined with the standard AEDs is able to reduce significantly the effective dose of diazepam and clonazepam to offer full protection with no mortality. Ashwagandharishta and flaxseed oil exhibit antiepileptic activity. They also have excellent anti-postictal depression effect. The observed activity is because of the inhibition of voltage-dependent Na^+ channels and blocking of glutaminergic excitation mediated by the NMDA receptor. The safety and efficacy of this plant has been thoroughly presented in Chapter 12 of this book.

9.6.13 *Magnolia grandiflora* (Him-Champa)

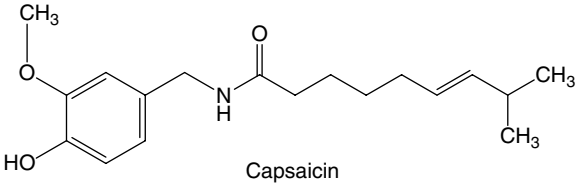
The ethyl ether (EE) and hydroalcoholic extract (HE) of *Magnolia grandiflora* L. (Magnoliaceae) seeds, when orally administered in a single dose of 250 and 200 mg kg^{-1}, exhibited abolition of the extensor reflex of maximal electrical stimulus-induced seizure test in 50% and 40% of the experimental animals, respectively. They significantly prolonged the sleeping time induced by pentobarbital [96].

9.7 Natural Plants Examined in Epilepsy

Various natural compounds that are examined in epilepsy are listed as follows.

9.7.1 Capsaicin

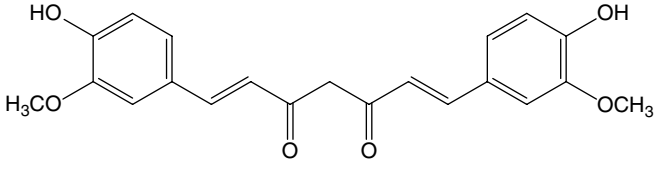
It is the foremost ingredient present in hot pepper. It belongs to the genus *Capsicum.* Along with food, it is extensively utilized as a traditional medicine worldwide for the treatment of various disorders. Most of the AEDs target the ion channel activity such as glutamate and transient receptor potential (TRP) channels that induce epileptic seizures and peripheral pain. With the activation of the TRPV1 channels, there is increased entry of Na^+ and Ca^{2+}, which results in increase in neuronal excitability. Recent studies have reported that TRPV1 channels may be a novel antiepileptic potential target [97]. In fact, it was reported that expression of TRPV1 has been increased in the dentate gyrus of mice with TLE. Capsaicin acts on these TRPV1 channels and decreases epileptic seizures. It can replace the depleted antioxidant molecules and antioxidant enzymes in the erythrocytes and in the liver and decrease the elevated lipid peroxide content [98].



Capsaicin

9.7.2 Curcumin

Turmeric is the active component that is obtained from the rhizomes of *Curcuma longa.* It is nontoxic and has a number of pharmacological effects such as antioxidant, anti-inflammatory, antiamyloid, and anticancer. Due to its property of crossing the BBB, it has a number of neurological effects. Due to its antioxidant effect, it plays role in the prevention of seizure-induced pathology. Curcumin pretreatment significantly caused reduction in astrocytic activation, neuronal cell death, and oxidative stress in the hippocampus [99]. Because of its antiapoptotic, anti-inflammatory, and antioxidant properties, curcumin therapy may be useful for preventing chronic epilepsy [100].

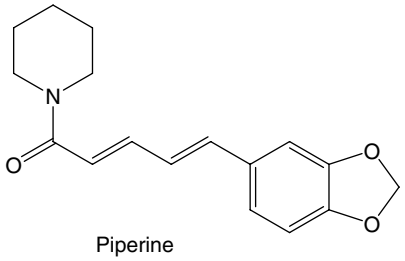


Curcumin

9.7.3 Piperine

This alkaloid shows the antidepressant and anxiolytic effect via the serotonergic system. Serotonergic system modulates the function of GABA. *Piperine* causes

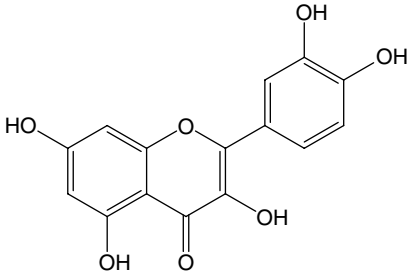
the activation of serotonergic receptors that are located on the interneurons, which result in the enhancement of the release of GABA. In the case of PTZ- and PCT-induced seizures, the inhibitory effect of piperine has been observed [101]. *Piperin*e can block convulsions induced by intracerebroventricular injection of threshold doses of kainate and have only slight effects on convulsions induced by L-glutamate, NMDA, or guanidinosuccinate.



Piperine

9.7.4 Quercetin

Quercetin, found in a variety of fruits and vegetables, shows anticonvulsant effect. It modulates GABAA receptor and acts as NMDA receptor antagonist. Previous reports also suggest that it shows proconvulsant activity [102] and, depending upon the timing and dose, protective and toxic effect. Due to its narrow therapeutic dose range, the risk of neurotoxicity is not negligible. Moreover, a previous research suggested that quercetin prevents tissue damage through various mechanisms, for example, by inhibiting neuronal apoptosis in the brain [103], but it is not clear whether quercetin treatment can reduce SE-induced neuronal injury in the hippocampus and which molecular mechanisms can mediate this effect [104].



Quercetine

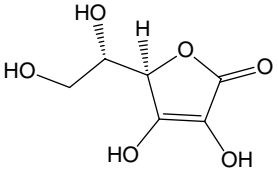
9.7.5 Cod Liver Oil

It is well known for preventing rickets and osteomalacia in children and adults, respectively. It contains n–3 polyunsaturated fatty acids, which modulate immune response. Omega-3 fatty acids have neuroprotective activity against epilepsy-induced hippocampal damage [105].

9.7.6 Vitamin C

Vitamins play a great role in controlling the seizures and even preventing the adverse effects of AEDs. Out of all the vitamins, vitamin C has the strong

persuasion on the brain tissue especially during seizures. In the CNS, the hippocampus is more vulnerable to oxidative stress. During seizures, the demand of oxygen is increased, and it causes reduction in ATP and inhibits mitochondrial respiratory enzymes while damaging the DNA [143]. Vitamin C consolidates the damages by decreasing lipid peroxidation and supports the SOD and CAT activity. It also inhibits the production of free radicals that are responsible for neurodegeneration [106].



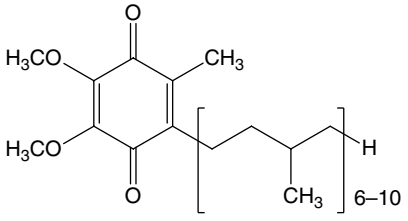
Vitamin C

9.7.7 Lycopene

Lycopene is a carotenoid antioxidant. It is mainly present in tomatoes. In recent years, it has gained scientific interest because of its neuroprotective properties against various central nervous disorders. Recent reports show that oxidative stress and mitochondrial dysfunction are some of the precipitating factors of epilepsy. Lycopene has been shown to produce beneficial effect against PTZ-induced seizures by inhibiting both factors [107].

9.7.8 Coenzyme Q10

It is a ubiquinone with antioxidant activity. It mainly inhibits mitochondrial dysfunctions. It shows beneficial effects in the case of neurodegenerative disorders such as Alzheimer's disease [108]. It mainly inhibits the microglia and shows protective effects in the case of neurodegeneration. Previous studies showed that CoQ10 has protective effect against seizure as well [109].

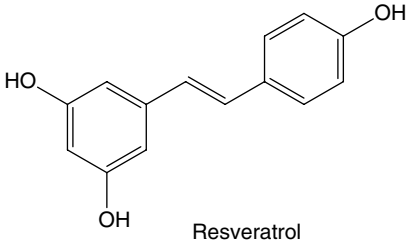


CoQ10

9.7.9 Resveratrol

Resveratrol (3,5,40-trihydroxystilbene), a naturally occurring phytoalexin present in high concentrations in the skin of red grapes, belongs to the polyphenol group of plant compounds. *Resveratrol* exists in *cis* and *trans* isomeric forms; however, the *trans* isomer is the major form that contributes to its biological activity. Resveratrol mediates a wide range of biological activities with multisystem benefits. It can protect the heart from ischemia by inhibiting peroxidation of

low-density lipoproteins (LDL) via free radical scavenging mechanisms. It can also act as an anti-inflammatory agent. It is a potent neuroprotective compound that mediates its effects mainly via inhibition of oxidative stress. A study on chronic administration of resveratrol suggests that resveratrol pretreatment partially protects rat's hippocampal neurons against KA-induced damage *in vivo*. Acute resveratrol pretreatment is also associated with reductions in the severity of KA-induced SE.



Resveratrol

9.8 German Herbs in Epilepsy

Before the modern anticonvulsant drugs, there was use of herbal remedies in Central Europe to treat epilepsy (Figure 9.3).

Tabernaemontanus recommended that drinking a schnapps (an alcoholic distillate) prepared from the roots of *Angelica archangelica* can be used to treat epilepsy. *A. archangelica* is a potent antagonist of calcium uptake. Coriander oil activates GABAA receptor responsible for potentiating barbiturate effects. Anise oil in wine is advisable because oil from the fruits contains eugenol, anethole, methyl chavicol, anisaldehyde, and estragole and shows anticonvulsive effects [110]. Anise oil suppressed MES- and PTZ-induced seizures and also increased seizure threshold. Rubbing thyme (*Thymus vulgaris*) under the nose can treat epilepsy. There are two main types of thyme chemotype, namely, the geraniol and linalool chemotypes. Linalool has anticonvulsant activity [111].

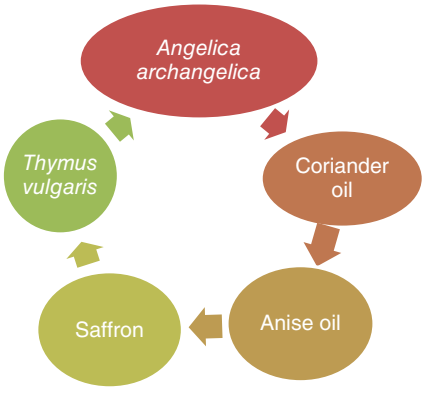


Figure 9.3 A list of German herbs.

9.9 Complement and Alternative Medicine

Herbal therapies are among the most commonly used forms of CAM therapies for patients, according to the National Institutes of Health National Center of Complementary and Alternative Medicine. Herbal traditions include traditional Chinese medicine, Ayurveda, and other culturally specific practices in which plant materials, processed or not, are ingested by persons with the intention of reducing symptoms or curing diseases (Figure 9.4).

9.9.1 Psychological Therapies and Mind–Body Techniques

Psychological therapies and mind–body techniques cover a variety of methods that include behavioral inhibition of symptoms at seizure onset [112], cognitive behavioral therapy [113], relaxation techniques such as yoga and related meditative techniques [114, 115], and neuron feedback [116, 117], which are useful for children [118] and adults. It is not viable to evaluate these treatments in double-blinded, randomized, controlled trials. Till date no benefits have been found in psychological therapies [119] and yoga [120] in patients with epilepsy. Nevertheless, investigators continue to conduct open trials to show therapeutic effects without side effects [121].

9.9.2 Homeopathy

Samuel Hahnemann was the father of modern-day homeopathy who proposed that "like should be cured with like," meaning that homeopathic preparation includes plants or animal products and, when given to healthy volunteers, shows similar symptoms [122, 123]. In excess of a placebo effect, homeopathic medicines have no benefits [124]. However, homeopathic preparations such as belladonna can reduce tonic–clonic seizures [125].

9.9.3 Acupuncture

Major component of TCM (Traditional Chinese Medicine) is acupuncture, which is used for centuries for PWE. It is used in conjugation with herbs [126]. In a variety of animal models, acupuncture appears to have anticonvulsant effects [127, 128].

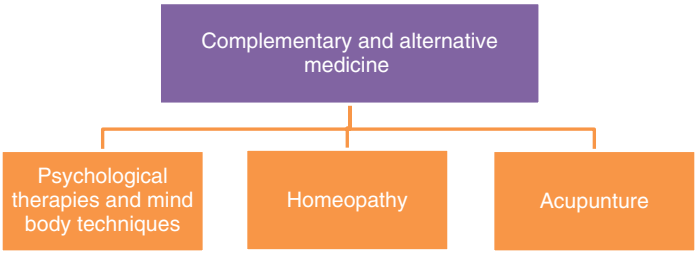


Figure 9.4 Divisions of the complementary and alternative medicines.

9.10 Marketed Formulation of Natural Products in India

There are number of marketed Ayurvedic formulations that are available in the Indian market. List of the formulations is available in Table 9.1.

9.11 Herbs That Induce Seizures

There are number of herbs that induce seizures [129–131, 144]. These drugs are listed in Table 9.2.

9.12 Interaction of Natural Products with Antiepileptic Drugs (AEDs)

Herbal medicines for epilepsy are increasing over the counter. But now it has been realized that it interacts with AEDs. A cohort study of 400 patients with epilepsy reports that CAM for general health purposes are used by 34% of people. They all believed that these medicines had little or no effect on epilepsy. Neurotoxin compounds are present in a large number of herbs [132].

Table 9.1 List of marketed formulations.

S. No.	Product name	Manufacturer
1	Ashwagandharishta (20–40 ml)	Zandu Pharmaceutical Works Ltd
2	Bali tail	Sandu Pharmaceuticals Ltd
3	Brahmi ghrita (12 mg)	Shree Baidyanath Ayurved Pvt Ltd
4	Chandanadi tail	Arya Vaidya Pharmacy
5	Chaturmukha rasa (125–250 mg)	Dabur India Ltd
6	Haratala bhasma (1/4–1/2 ratti)	Divya Pharmacy
7	Kalyanaka ghrita (125 mg)	Nagarjuna Ayurvedic Group
8	Kumaryasava (125 mg)	Dabur India Ltd
9	Mahakalyanaka ghrita (125 mg)	Nagarjuna Ayurvedic Group
10	Mahamrityunjaya rasa (125–250 mg)	Uma Ayurvedics Pvt Ltd
11	Rajata bhasma (1/2 ratti)	Divya Pharmacy
12	Saraswatarishta (20–40 ml)	Uma Ayurvedics Pvt Ltd
13	Sarpagandha vati (125 mg)	Dabur India Ltd
14	Swarna bhasma (1/8–1/4 ratti)	Shree Dhootapapeshwar Ltd
15	Svarnamakshika bhasma (102 ratti)	Divya Pharmacy
16	Vatkulantak rasa (125–250 mg)	Dabur India Ltd
17	Yogendra rasa (125 mg)	Dabur India Ltd

Table 9.2 List of herbal drugs that show epileptic behavior.

S. no.	Name (botanical name)	Seizure potential
1	Asafoetida (*Ferula assafoetida*)	Seizures possible in susceptible individuals
2	Bearberry (*Arctostaphylos uva-ursi*)	May cause seizures
3	Betel nuts (*Areca catechu*)	Seizure has been reported with high doses
4	Black cohosh (*Actaea racemosa*)	One seizure case reported. Should be used cautiously in patients with history of seizure disorder
5	Black tea (*Camellia sinensis*)	Seizure has been reported with caffeine overdose
6	Clove (*Syzygium aromaticum*)	When taken orally in large doses, in undiluted oil form, or clove cigarettes, side effects may include seizures
7	Coffee (*Coffea*)	Caution is advised with large quantities of caffeine/coffee for persons with higher disposition to seizures
8	Damiana (*Turnera diffusa*)	Lowers seizure threshold. Report of tetanus-like seizures
9	Ephedra (*Ephedra vulgaris*)	May cause or worsen seizures
10	Ergot (*Claviceps*)	Overdose can lead to seizures
11	Eucalyptus (*Eucalyptus globulus*)	Seizures are possible when oil is taken orally
12	Evening primrose oil (*Oenothera*)	May lower seizure threshold. Has the potential to manifest temporal lobe epilepsy
13	Ginkgo biloba (*Ginkgo*)	May reduce seizure threshold. Seizures and coma have occurred after ingestion of ginkgo seeds
14	Ginseng (*Panax*)	May lower seizure threshold
15	Goldenseal/berberine (*Hydrastis canadensis*)	May lower seizure threshold. Overdose may lead to seizures
16	Green tea (*C. sinensis*)	May lower seizure threshold if more than three cups per day are consumed. Seizures have been reported with overdose
17	Guarana (*Paullinia cupana*)	May lower seizure threshold
18	Jaborandi/pilocarpine (*Pilocarpus pennatifolius*)	Poisoning from pilocarpine eye drops can cause seizures
19	Juniper oil (*Juniperus communis*)	Reported to induce seizures
20	Kava-kava (*Piper methysticum*)	Generalized tonic–clonic seizures from toxicity and acute withdrawal
21	Ma huang (*J. communis*)	Reported to cause seizures
22	Monkshood (*Aconitum*)	May cause seizures
23	Oleander (*Nerium oleander*)	Eating the leaves, flower, or bark of common oleander may cause seizures

(Continued)

Table 9.2 (Continued)

S. no.	Name (botanical name)	Seizure potential
24	Pennyroyal oil (*Hedeoma pulegioides*)	Toxicity may cause seizures
25	Sage (*Salvia officinalis*)	Extended intake or overdose can cause seizures
26	Starflower/borage (*Borago officinalis*)	May lower seizure threshold
27	St. John's wort (*Hypericum perforatum*)	Seizures reported
28	Thuja oil (*Thuja occidentalis*)	Causes seizures and in high doses leads to tonic–clonic seizures
29	Uva-ursi (*A. uva-ursi*)	Reported to cause seizures
30	Water hemlock (*Cicuta*)	One case of seizure reported
31	Wormwood (*Artemisia absinthium*)	May lower seizure threshold
32	Yohimbe (*Pausinystalia johimbe*)	Reported to cause seizures. May change seizure threshold

Evening primrose oil (EPO) is a popular herb used in the treatment of premenstrual syndrome, diabetic neuropathy, Sjögren's syndrome, and attention deficit hyperactivity disorder. It shows the healing effect of the omega-6 fatty acid γ-linoleic acid (GLA) but lowers the seizure threshold at the same time. Ginseng (reported by 17%), ginkgo biloba (16%), and St. John's wort (13%) are the three products that are used because these extracts are generally used for amelioration of symptoms of anxiety, depression, and memory deficits, which are commonly encountered comorbidities of epilepsy [133]. While these herbs show beneficial effects on seizures, there are also reports that show that they aggravate seizures as well. There are evidences that gingko biloba may be epileptogenic (the seeds), while other parts (the leaves and the stem) may protect against seizure activity [134]. In contrast, the effect of St. John's wort on seizures may depend on the extraction method [135]. Clinically, gingko biloba and St. John's wort [136] have interactions with hepatically metabolized AEDs. The most frequently used products in this population of PWE are echinacea (11%), garlic (10%), cranberry (9%), and soy (8%). These are commonly used in the general population as immune enhancers (echinacea, garlic) and for prevention and ameliorations of symptoms of urinary tract infections (cranberry) and menopause (soy) [137]. These products neither have beneficial effects nor have detrimental effects on seizures. However, their presumed effects on the P450 system potentially interact with AEDs that are metabolized by the liver. Although anticonvulsant effect of melatonin [138], kava-kava [139], and valerian [140] is reported, melatonin [141] and kava-kava are also associated with aggravation of epilepsy. Interaction of herbals drugs with AEDs are shown in Table 9.3.

Table 9.3 Interaction of herbal drugs with antiepileptic drugs (AEDs).

1	Diazepam + Chinese herb *Saiboku-to* ⟶ anxiolytic-like effect
2	Diazepam + *Ginkgo biloba* ⟶ inhibit GABA synthesis
3	Phenytoin + *Centella asiatica* ⟶ decrease in the effective dose of AEDs
4	Valproate + *Centella asiatica* ⟶ decrease in the effective dose of AEDs
5	Gabapentin + *Centella asiatica* ⟶ decrease in the effective dose of AEDs
6	Carbamazepine + *Sho-seiryuto* and *Sho-saiko-to* ⟶ affect pharmacokinetics
7	Carbamazepine + *Cassia auriculata* ⟶ increase its blood concentrations
8	Carbamazepine + *Paeoniae Radix* ⟶ decrease the carbamazepine T_{max}
9	Carbamazepine + *Mentat* ⟶ decrease its metabolism
10	Phenytoin + *Mentat* ⟶ decrease its metabolism
11	Phenytoin + *Paeoniae Radix* ⟶ increase phenytoin T_{max}
12	Phenytoin + *Shankhapushpi* ⟶ reduction in plasma concentration
13	Diazepam + *Angelica dahurica* ⟶ increase first-pass metabolism
14	Carbamazepine + *Hypericum perforatum* ⟶ increase metabolism
15	Phenytoin + *Piper longum* ⟶ increase the mean phenytoin serum concentration

9.13 Concluding Remarks

Although epilepsy is a well-known neurological problem, its pathophysiology is still unclear in terms of cellular and molecular cascades that would have to be explored for suitable drug targets. Besides, presently available AEDs also suffer from significant side effects and drug interactions. Different neuroprotectants of diverse nature are being tried with limited success. In search of new and more efficacious drugs, researchers have been engaged to explore therapeutic potentials of plant-based bioactive molecules, particularly belonging to the alkaloid, flavonoid, terpenoid, saponin, and coumarin families, which have been found responsible for their anticonvulsant properties. These plant-based lead compounds are also associated with anti-inflammatory, neuroprotective, and cognition-enhancing activities in addition to their antiepileptic actions. Such natural molecules have an added advantage of possessing pleiotropic mode of action. These plant-based lead molecules are believed to be safer in comparison with conventional modern medicine. Current market trend of using herbal/dietary supplements is impressive. Presently various market preparations based on natural products with different claims are available in the market. Importantly, these plant-based drugs should be investigated on par with modern medicine in the light of modern tools and technologies. There is no doubt that these plant-based drugs have tremendous clinical therapeutic potential to treat several neurological problems including epilepsy. Hence, detailed investigations are warranted to evaluate their scientific status/claims in terms of their safety, efficacy, and quality as medicines.

References

1 Diniz, T.C., Silva, J.C., Ribeiro, F.P., Freitas, A.G., Quintans, L.J., and Almeida, J.R. (2015) The role of flavonoids on oxidative stress in epilepsy. *Oxid. Med. Cell. Longevity*, **1** (7), 1756–1760.

2 Iannotti, F.A., Hill, C.L., Leo, A., Alhusaini, A., Soubrane, C., Mazzarella, E., Russo, E., Whalley, B.J., Marzo, V., and Stephens, G.J. (2014) Nonpsychotropic plant cannabinoids, cannabidivarin (CBDV) and cannabidiol (CBD), activate and desensitize transient receptor potential vanilloid 1 (TRPV1) channels *in vitro*: potential for the treatment of neuronal hyperexcitability. *ACS Chem. Neurosci.*, **5** (11), 1131–1141.

3 Luszczk, J.J. (2009) Third-generation antiepileptic drugs: mechanisms of action, pharmacokinetics and interactions. *Pharmacol. Rep.*, **61**, 197–216.

4 Engel, J., Wiebe, S., French, J., Sperling, M., Williamson, P., Spencer, D., Gumnit, R., Zahn, C., Westbrook, E., Enos, B., Quality Standards Subcommittee of the American Academy of Neurology, American Epilepsy Society, and American Association of Neurological Surgeons (2003) Practice parameter: temporal lobe and localized neocortical resections for epilepsy: report of the Quality Standards Subcommittee of the American Academy of Neurology, in association with the American Epilepsy Society and the American Association of Neurological Surgeons. *J. Neurol.*, **60**, 538–547.

5 Kannappan, R., Gupta, S.C., Kim, J.H., Reuter, S., and Aggarwal, B.B. (1998) Neuroprotection by spice-derived nutraceuticals: you are what you eat!. *J. Pharmacol. Exp. Ther.*, **284**, 2474–2494.

6 Sucher, N.J. (2006) Insights from molecular investigations of traditional Chinese herbal stroke medicines: implications for neuroprotective epilepsy therapy. *J. Epilepsy Behav.*, **8**, 350–362.

7 Hauser, W.A. and Kurland, L.T. (1975) The epidemiology of epilepsy in Rochester, Minnesota, 1935 through 1967. *Epilepsia*, **16**, 1–66.

8 Sander, J.W., Hart, Y.M., Johnson, A.L., and Shorvon, S.D. (1990) National General Practice Study of Epilepsy: newly diagnosed epileptic seizures in a general population. *Lancet*, **336**, 1267–1271.

9 Langler, A., Spix, C., Edelhauser, F., Kameda, G., Kaatsch, P., and Seifert, G. (2011) Use of homeopathy in pediatric oncology in Germany. *Evid. Based Complement. Alternat. Med.*, **867**, 151–157.

10 Doering, J.H. (2013) Pattern and predictors of complementary and alternative medicine (CAM) use among pediatric patients with epilepsy. *Epilepsy Behav.*, **29** (1), 41–46.

11 Vajda, F.J. (2002) Neuroprotection and neurodegenerative disease. *J. Clin. Neurosci.*, **9**, 4–8.

12 Bittigau, P. and Ikonomidou, C. (1997) Glutamate in neurologic diseases. *J. Child Neurol.*, **12**, 471–485.

13 Moshe, S.L. and Decker, J.F. (2004) Susceptibility of immature and adult brains to seizure effects. *Lancet*, **3** (10), 608–617.

14 Micha, K., Trojnar, R.M., and Chrooeciñska, M. (2002) Neuroprotective effects of antiepileptic drugs. *Pol. J. Pharmacol.*, **54**, 557–566.

15 Fujikawa, D.G., Itabashi, H.H., Wu, A., and Shinmei, S.S. (2002) Status epilepticus-induced neuronal loss in human without systemic complications or epilepsy. *Epilepsia*, **41**, 981–991.
16 Winyard, P.G., Mody, C.J., and Hacob, C. (2005) Oxidative activation of antioxidant defense. *Trends Biochem. Sci.*, **30**, 453–461.
17 Brown, G.C. and Borutaite, V. (2001) Nitric oxide, mitochondria, and cell death. *IUBMB Life*, **52**, 189–195.
18 Patel, M. (2004) Mitochondrial dysfunction and oxidative stress: cause and consequence of epileptic seizures. *Free Radical Biol. Med.*, **37**, 1951–1962.
19 Taylor, R.W., Schaefer, A.M., McDonnell, M.T., Petty, R.K., Thomas, A.M., Blakely, E.L., Hayes, C.M., McFarland, R., and Turnbull, D.M. (2004) Catastrophic presentation of mitochondrial disease due to a mutation in the tRNA (His) gene. *Neurology*, **66**, 1420–1423.
20 Waldbaum, S. and Patel, M. (2010) Mitochondria, oxidative stress, and temporal lobe epilepsy. *Epilepsy Res.*, **88** (1), 23–45.
21 Maaloufa, M., Sullivanb, P.G., Davisb, L., Kima, D.Y., and Rhoa, J.M. (2007) Ketones inhibit mitochondrial production of reactive oxygen species production following glutamate excitotoxicity by increasing NADH oxidation. *Neuroscience*, **145** (1), 256–264.
22 Fabene, P.F., Mora, G.N., Martinelslo, M., Rossi, B., Merigo, F., Ottoboni, L., Bach, S., Angiari, S., Benati, D., Chakir, A., Zanetti, L., Schio, F., Osculati, A., Marzola, P., Nicolato, E., Homeister, J.W., Xia, L., Lowe, J.B., McEver, R.P., Osculati, F., Sbarbati, A., Butcher, E.C., and Constantin, G. (2008) A role for leukocyte endothelial adhesion mechanisms in epilepsy. *Nat. Med.*, **14**, 1377–1383.
23 Zeng, L.H., Rensing, N.R., and Wong, M. (2009) The mammalian target of rapamycin signalling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. *J. Neurosci.*, **29**, 6964–6972.
24 Ravizza, T., Balosso, S., and Vezzani, A. (2011) Inflammation and prevention of epileptogenesis. *Neurosci. Lett.*, **497**, 223–230.
25 Kempuraj, D., Madhappan, B., Christodoulou, S., Boucher, W., Cao, J., Papadopoulou, N., Cetrulo, C.L., and Theo-harides, T.C. (2005) Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. *Br. J. Pharmacol.*, **145**, 934–944.
26 Joshi, D., Naidu, P.S., Singh, A., and Kulkarni, S.K. (2005) Protective effect of quercetin on alcohol abstinence-induced anxiety and convulsions. *J. Med. Food*, **8**, 392–396.
27 Shaikh, M.F., Tan, K.N., and Borges, K. (2013) Anticonvulsant screening of luteolin in four mouse seizure models. *Neurosci. Lett.*, **550**, 195–199.
28 Nassiri-Asl, M., Shariati-Rad, S., and Zamansoltani, F. (2008) Anticonvulsive effects of intracerebroventricular administration of rutin in rats. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, **32**, 989–993.
29 Henshall, D.C. and Simon, R.P. (2005) Epilepsy and apoptosis pathways. *J. Cereb. Blood Flow Metab.*, **25**, 1557–1572.
30 Bengzon, J., Kokaia, Z., Elmer, E., Nanobashvili, A., Kokaia, M., and Lindvall, O. (1997) Apoptosis and proliferation of dentate gyrus neurons after single

and intermittent limbic seizures. *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 10432–10437.

31 Zhang, L.X., Smith, M.A., Li, X.L., Weiss, S.R., and Post, R.M. (1988) Apoptosis of hippocampal neurons after amygdale kindled seizures. *Mol. Brain Res.*, **55**, 198–208.

32 Henshall, D.C., Bonislawski, D.P., Skradski, S.L., Meller, R., Lan, J.Q., and Simon, R.P. (2001) Cleavage of bid may amplify caspase-8-induced neuronal death following focally evoked limbic seizures. *Neurobiol. Dis.*, **8**, 568–580.

33 Mohanan, P.V. and Yamamoto, H.A. (2002) Preventive effect of melatonin against brain mitochondria DNA damage, lipid peroxidation and seizures induced by kainic acid. *Toxicol. Lett.*, **129**, 99–105.

34 Barros, D.O., Xavier, S.M., Barbosa, C.O., Silva, R.F., Freitas, R.L., Maia, F.D., Oliveira, A.A., Freitas, R.M., and Taka-hashi, R.N. (2007) Effects of the vitamin E in catalase activities in hippocampus after status epilepticus induced by pilocarpine in Wistar rats. *Neurosci. Lett.*, **416**, 227–230.

35 Schwartz, R.D. (1988) The GABA-A receptor-gated ion channel: biochemical and pharmacological studies of structure and function. *Biochem. Pharmacol.*, **37**, 3369–3375.

36 Treiman, D.M. (2001) GABAergic mechanisms in epilepsy. *Epilepsia*, **42** (3), 8–12.

37 Doi, T., Ueda, Y., Nagatomo, K., and Willmore, L.J. (2009) Role of glutamate and GABA transporters in development of pentylenetetrazol-kindling. *Neurochem. Res.*, **34** (7), 1324–1331.

38 Corda, M.G., Orlandi, M., Lecca, D., Carboni, G., Frau, V., and Giorgi, O. (1991) Pentylenetetrazole-induced kindling in rats: effect of GABA function inhibitors. *Pharmacol. Biochem. Behav.*, **40**, 329–333.

39 Schroeder, H., Becker, A., and Hoellt, V. (1998) Sensitivity and density of glutamate receptor subtypes in the hippocampal formation are altered in pentylenetetrazole-kindled rats. *Exp. Brain Res.*, **120** (4), 527–530.

40 Zhu, L.J., Chen, Z., Zhang, L.S., Xu, S.J., Xu, A., and Luo, J.H. (2004) Spatiotemporal changes of the *N*-methyl-d-aspartate receptor subunit levels in rats with pentylenetetrazole-induced seizures. *Neurosci. Lett.*, **356** (1), 53–56.

41 Ekonomou, A., Smith, A.L., and Angelatou, F. (2001) Changes in AMPA receptor binding and subunit messenger RNA expression in hippocampus and cortex in the pentylenetetrazole-induced 'kindling' model of epilepsy. *Mol. Brain Res.*, **95**, 27–35.

42 Barria, A. and Malinow, R. (2002) Subunit-specific NMDA receptor trafficking to synapses. *Neuron*, **35**, 345–353.

43 Chen, N., Luo, T., and Raymond, L.A. (1999) Subtype-dependence of NMDA receptor channel open probability. *J. Neurosci.*, **19**, 6844–6854.

44 Mody, I. and Heinemann, U. (1987) NMDA receptors of dentate gyrus granule cells participate in synaptic transmission following kindling. *Nature*, **326**, 701–704.

45 Wenger, G.R., Stitzel, R.E., and Craig, C.R. (1973) The role of biogenic amines in the reserpine-induced alteration of minimal electroshock seizure thresholds in the mouse. *Neuropharmacology*, **12**, 693–703.

46 Lazarova, M., Bendotti, C., and Samanin, R. (1983) Studies on the role of serotonin in different regions of the rat central nervous system on pentylenetetrazol-induced seizures and the effect of Di-n-propylacetate. *Arch. Pharmacal Res.*, **322**, 147–152.

47 Beck, S.G. and Choi, K.C. (1991) 5-Hydroxytryptamine hyperpolarizes CA3 hippocampal pyramidal cells through an increase in potassium conductance. *Neurosci. Lett.*, **133**, 93–96.

48 Okuhara, D.Y. and Beck, S.G. (1994) 5-HT1A receptor linked to inward rectifying potassium current in hippocampal CA3 pyramidal cells. *J. Neurophysiol.*, **71**, 2161–2167.

49 Statnick, M.A., Dailey, J.W., Jobe, P.C., and Browning, R.A. (1996) Abnormalities in 5-HT1A and 5-HT1B receptor binding in severe-seizure genetically epilepsy-prone rats (GEPR-9s). *Neuropharmacology*, **35**, 111–118.

50 Szyndlera, J., Rok, P., Maciejak, P., Walkowiak, J., Agnieszka, I., Conkowskaa, C., Jaroszc, H.S., Clowskaa, A.W., Zienowicz, M.C., Lehner, M.C., Bidzinski, A., Kostowskia, W., and Plaznik, A. (2002) Effects of pentylenetetrazol-induced kindling of seizures on rat emotional behavior and brain monoaminergic systems. *Pharmacol. Biochem. Behav.*, **73**, 851–861.

51 Jope, R.S., Simonato, M., and Lally, K. (1987) Acetylcholine content in rat brain is elevated by status epilepticus induced by lithium and pilocarpine. *J. Neurochem.*, **49**, 944–951.

52 Baptista, T., Weiss, S.R.B., Zocchi, A., Sitcoske, M., and Post, L. (1994) Electrical kindling is associated with increases in amygdale acetylcholine levels: an *in vivo* microdialysis study. *J. Neurosci.*, **167**, 133–136.

53 Burchfiel, J.L., Duchowny, M.S., and Duffy, F.H. (1979) Neuronal supersensitivity to acetylcholine induced by kindling in the rat hippocampus. *Science*, **204**, 1096–1098.

54 Horton, W.R., Chapman, A.G., and Meldrum, B.S. (1979) Isoniazid as a glutamic acid decarboxylase inhibitor. *J. Neurochem.*, **33**, 745–748.

55 Squires, S.F., Saederup, E., Crawley, J.N., Skolnick, P., and Paul, S.M. (1984) Convulsant potencies of tetrazole are highly correlated with action on GABA/picrotoxin receptor complexes in brain. *Life Sci.*, **35**, 1439–1444.

56 Serra, M., Dazzi, L., Cagetti, E., Chessa, M.F., Pisu, M.G., Sanna, A., and Biggio, G. (1997) Effect of pentylenetetrazole-induced kindling on acetylcholine release in the hippocampus of freely moving rats. *J. Neurochem.*, **68** (1), 313–318.

57 Bhutada, P., Mundhada, Y., and Bansod, K. (2010) Anticonvulsant activity of berberine, an isoquinoline alkaloid in mice. *Epilepsy Behav.*, **18**, 207–210.

58 da Silva, A.F.S., de Andrade, J.P., and Bevilaqua, L.R.M. (2006) Anxiolytic-, antidepressant- and anticonvulsant-like effects of the alkaloid montanine isolated from Hippeastrum vittatum. *Pharmacol. Biochem. Behav.*, **85**, 148–154.

59 Lin, M.T., Wang, J.J., and Young, M.S. (2002) The protective effect of dl-tetrahydropalmatine against the development of amygdala kindling seizures in rats. *Neurosci. Lett.*, **320**, 113–116.

60 Chen, K., Kokate, T.G., and Donevan, S.D. (1996) Ibogaine block of the NMDA receptor: *in vitro* and *in vivo* studies. *Neuropharmacology*, **35**, 423–431.

61 da Cruz, G.M., Felipe, C.F., and Scorza, F.A. (2013) Piperine decreases pilocarpine-induced convulsions by GABAergic mechanisms. *Pharmacol. Biochem. Behav.*, **104**, 144–153.

62 Felipe, F.C.B., Sousa, J.T., and Souza, L.E.D.O. (2007) Piplartine, an amide alkaloid from Piper tuberculatum, presents anxiolytic and antidepressant effects in mice. *Phytomedicine*, **14**, 605–612.

63 Kang, T.H., Murakami, Y., and Matsumoto, K. (2002) Rhynchophylline and isorhynchophylline inhibit NMDA receptors expressed in Xenopus oocytes. *Eur. J. Pharmacol.*, **455**, 27–34.

64 Ribeiro, R.A. and Leite, J.R. (2003) Nantenine alkaloid presents anticonvulsant effect on two classical animal models. *Phytomedicine*, **10**, 5063–5568.

65 Rosa, D.S., Faggion, S.A., and Gavin, A.S. (2012) Erysothrine, an alkaloid extracted from flowers of Erythrina mulungu Mart. ex Benth: evaluating its anticonvulsant and anxiolytic potential. *Epilepsy Behav.*, **23**, 205–212.

66 Faggion, S.A., Cunha, A.O., and Fachim, H.A. (2011) Anticonvulsant profile of the alkaloids (+)-erythravine and (+)-11-alpha-hydroxyerythravine isolated from the flowers of Erythrina mulungu Mart. ex Benth (Leguminosae-Papilionaceae). *Epilepsy Behav.*, **20**, 441–446.

67 Charveron, M., Assie, M.B., and Stenger, A. (1984) Benzodiazepine agonist type activity of raubasine, a Rauwolfia serpentina alkaloid. *Eur. J. Pharmacol.*, **106**, 313–317.

68 Avallone, R., Zanoli, P., and Puia, G. (2000) Pharmacological profile of apigenin, a flavonoid isolated from Matricaria chamomilla. *Biochem. Pharmacol.*, **59**, 1387–1394.

69 Park, H.G., Yoon, S.Y., Choi, J.Y. *et al.* (2007) Anticonvulsant effect of wogonin isolated from *Scutellaria baicalensis*. *Eur. J. Pharmacol.*, **574**, 112–119.

70 Silva, M.I., Silva, M.A., and de Aquino Neto, M.R. (2009) Effects of isopulegol on pentylenetetrazol-induced convulsions in mice: possible involvement of GABAergic system and antioxidant activity. *Fitoterapia*, **80**, 506–513.

71 Hosseinzadeh, H. and Sadeghnia, H.R. (2007) Protective effect of safranal on pentylenetetrazol-induced seizures in the rat: involvement of GABAergic and opioids systems. *Phytomedicine*, **14**, 256–262.

72 Hosseinzadeh, H. and Talebzadeh, F. (2005) Anticonvulsant evaluation of safranal and crocin from Crocus sativus in mice. *Fitoterapia*, **76**, 722–724.

73 Quintans-Junior, L.J., Guimaraes, A.G., and Araujo, B.E.S. (2010) Carvacrol, (−)-borneol and citral reduce convulsant activity in rodents. *Afr. J. Biotechnol.*, **9**, 6566–6572.

74 Costa, J.P., Ferreira, P.B., and De Sousa, D.P. (2012) Anticonvulsant effect of phytol in a pilocarpine model in mice. *Neurosci. Lett.*, **523**, 115–118.

75 Jones, N.A., Hill, A.J., Smith, I., Bevan, S.A., Willians, C.M., and Whalley, B.J. (2010) Cannabidiol displays antiepileptic form an antiseizure properties in-vitro and in-vivo. *J. Pharmacol. Exp. Ther.*, **332** (2), 567–577.

76 Zhu, H.L., Wan, J.B., Wang, Y.T., Li, B.C., Xiang, C., He, J., and Li, P. (2014) Medicinal compounds with antiepileptic/anticonvulsant activities. *Epilepsia*, **55** (1), 3–16.

77 Wallace, M.J., Wiley, J.L., and Martin, B.R. (2001) Assessment of the role of CB1 receptors in cannabinoid anticonvulsant effects. *Eur. J. Pharmacol.*, **428**, 51–57.

78 Taviano, M.F., Miceli, N., and Monforte, M.T. (2007) Ursolic acid plays a role in Nepeta sibthorpii bentham CNS depressing effects. *Phytother. Res.*, **21**, 382–385.

79 Kazmi, I., Gupta, G., and Afzal, M. (2012) Anticonvulsant and depressant-like activity of ursolic acid stearoyl glucoside isolated from *Lantana camara* L. (verbenaceae). *Asian Pac. J. Cancer Prev.*, **2**, S453–S456.

80 Lian, X.Y., Zhang, T.Z., and Stringer, J.L. (2005) Anticonvulsant activity of ginseng on seizures induced by chemical convulsants. *Epilepsia*, **46**, 15–22.

81 Mu, Q.Z., Lu, J.R., and Zhou, Q.L. (1986) Two new antiepilepsy compounds otophyllosides A and B. *Sci. Sin., Ser. B*, **29**, 295–301.

82 Zhou, J. (1991) Bioactive glycosides from Chinese medicines. *Mem. Inst. Oswaldo Cruz*, **86**, 231–234.

83 Jalsrai, A., Grecksch, G., and Becker, A. (2010) Evaluation of the effects of *Astragalus mongholicus* Bunge saponin extract on central nervous system functions. *J. Ethnopharmacol.*, **131**, 544–549.

84 Woo, T.S., Yoon, S.Y., and de la Pena, I.C. (2011) Anticonvulsant effect of Artemisia capillaris Herba in mice. *Biomol. Ther.*, **19**, 342–347.

85 Luszczki, J.J., Glowniak, K., and Czuczwar, S.J. (2007) Time-course and dose response relationships of imperatorin in the mouse maximal electroshock seizure threshold model. *Neurosci. Res.*, **59**, 18–22.

86 Luszczki, J.J., Andres-Mach, M., and Cisowski, W. (2009) Osthole suppresses seizures in the mouse maximal electroshock seizure model. *Eur. J. Pharmacol.*, **607**, 107–109.

87 Rao, V.S., Rao, A., and Karanth, S.K. (2005) Anticonvulsant and neurotoxicity profile of *Nardostachys jatamansi* in rats. *J. Ethnopharmacol.*, **102**, 351–356.

88 Amabeoku, G.J., Green, I., and Kabatendu, J. (2007) Anticonvulsant activity of *Cotyledon orbiculata* L. (Crassulaceae) leaf extract in mice. *J. Ethnopharmacol.*, **112**, 101–107.

89 Sayyah, M., Valizadeh, J., and Kamalinejad, M. (2002) Anticonvulsant activity of the leaf essential oil of *Laurus nobilis* against pentylenetetrazole and maximal electroshock induced seizures. *Phytomedicine*, **9**, 212–216.

90 Vohara, D., Pal, S.N., and Pillai, K.K. (2002) Protection from Phenytoin induced cognitive deficit by Bacopa monnieri, a reputed Nootropic plant. *J. Ethnopharmacol.*, **71**, 383–390.

91 Mathew, J., Paul, J., Nandhu, M.S., and Paulose, C.S. (2010) Increased excitability and metabolism in pilocarpine induced epileptic rats: effect of Bacopa monnieri. *Fitoterapia*, **81**, 546–551.

92 Wu, X.Y., Zhao, J.L., Zhang, M., Li, F., Zhao, T., and Yang, L.Q. (2011) Sedative, hypnotic and anticonvulsant activities of ethanol fraction from rhizoma Pinelliae praeparatum. *J. Ethnopharmacol.*, **135**, 325–329.

93 Nisar, M., Khan, I., Simjee, S.U., Gilani, A.H., Obaidullah, and Perveen, H. (2008) Anticonvulsant, analgesic and antipyretic activities of Taxus wallichiana Zucc. *J. Ethnopharmacol.*, **116**, 490–494.

94 Ojewole, J.A. (2008) Anticonvulsant property of *Sutherlandia frutescens* R. BR. (variety Incana E. MEY.) [Fabaceae] shoot aqueous extract. *Brain Res. Bull.*, **75**, 126–132.

95 Chindo, B.A., Anuka, A.J., McNeil, L., Yaro, A.H., Adamu, S.S., and Amos, S. (2009) Anticonvulsant property of saponin from Ficus platyphylla stem bark. *Brain Res. Bull.*, **78**, 276–282.

96 Mahomedi, I.M. and Ojewole, J.A. (2006) Anticonvulsant activity of *Harpagophytum procumbens* DC [Pedaliaceae] secondary root aqueous extract in mice. *Brain Res. Bull.*, **69**, 57–62.

97 Nazirog, M. (2015) Involvement of apoptosis and calcium accumulation through Trpv1 channels in neurobiology of epilepsy. *Neuroscience*, **293**, 55–66.

98 Lee, T.H., Lee, J.G., Yon, J.M., Ohb, K.W., Baek, I.J., Nahmd, S.S., Lee, B.J., Yun, Y.W., and Nama, S.Y. (2011) Capsaicin prevents kainic acid-induced epileptogenesis in mice. *Neurochem. Int.*, **58**, 634–640.

99 Jiang, Z., Guo, M., Shi, C., Wang, H., Yao, L., Liu, L., Xie, C., Pu, S., Lachaud, G., Shen, J., Zhu, M., and Mu, L. (2015) Protection against cognitive impairment and modification of epileptogenesis with curcumin in a post-status epilepticus model of temporal lobe epilepsy. *Neuroscience*, **310**, 362–371.

100 Iang, Z., Guo, M., Shi, C., Wang, H., Yao, L., Liu, C., Xie, C., Pu, S., Lachaud, G., Shen, J., Zhu, M., Mu, L., and Park, H. (2015) Protection against cognitive impairment and modification of epileptogenesis with curcumin in a post-status epilepticus model of temporal lobe epilepsy. *J. Neurosci.*, **310**, 362–371.

101 Pal, A., Nayak, S., Sahu, P.K., and Swain, T. (2011) Piperine protects epilepsy associated depression: a study on role of monoamines. *Eur. Rev. Med. Pharmacol. Sci.*, **15**, 1288–1295.

102 Sefil, F., Kahraman, I., Dokuyucu, R., Gokce, H., Ozturk, A., Tutuk, O., Aydin, M., Ozkan, U., and Pinar, N. (2014) Ameliorating effect of quercetin on acute pentylenetetrazole induced seizures in rats. *Int. J. Clin. Exp. Med.*, **7** (9), 2471–2477.

103 Hu, K., Li, S.U., Xiao, B., Bi, F., Lu, X.Q., and Wu, X.M. (2011) Protective effects of quercetin against status epilepticus induced hippocampal neuronal injury in rats: involvement of X-linked inhibitor of apoptosis protein. *Acta Neurol. Belg.*, **111**, 205–212.

104 Nassir, M., Sahar, M., Abbasi, E., Yonesi, F., Haghighi, M.R., Lotfizadeh, M., and Bazahang, P. (2014) Chronic administration of quercetin prevent spatial learning and memory deficits provoked by chronic stress in rats. *Behav. Brain Res.*, **15** (270), 196–205.

105 Mannaaa, F., El-Shamy, K.A., El-Shaikh, K.A., and El-Kassaby, M. (2011) Efficacy of fish liver oil and propolis as neuroprotective agents in pilocarpine epileptic rats treated with valproate. *Pathophysiology*, **18**, 287–294.

106 Glazer, E. and Czuczwar, S. (2014) Vitamin C: a new auxiliary treatment of epilepsy. *Pharmacol. Rep.*, **66**, 529–533.

107 Bhardwaj, M. and Kumar, A. (2016) Neuroprotective effect of lycopene against PTZ-induced kindling seizures in mice: possible behavioural, biochemical and mitochondrial dysfunction. *Phytother. Res.*, **30** (2), 306–313.

108 Singh, A. and Kumar, A. (2015) Minocycline modulates the neuroprotective effect of coenzyme q10 against amyloid beta 1-42 induced cognitive

dysfunction in rats: behavioral and biochemical evidence. *Front. Pharmacol.*, **11** (7), 268.

109 Berbel-Garcia, A., Barbera-Farre, J.R., Etessam, J.P., Salio, A.M., Cabello, A., Gutierrez-Rivas, E., and Campos, Y. (2004) Coenzyme Q10 improves lactic acidosis, stroke like episodes, and epilepsy in a patient with MELAS mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke like episodes. *Clin. Neuropharmacol.*, **27** (4), 187–191.

110 Adams, M., Schneider, S., Kluge, M., Kessler, M., and Hamburger, M. (2012) Epilepsy in the renaissance: a survey of remedies from 16th and17th century German herbals. *J. Ethnopharmacol.*, **143**, 1–13.

111 Sakurada, T., Kuwahata, H., Katsuyama, S., Komatsu, T., Morrone, L.A., Corasaniti, M.T., Bagetta, G., and Sakurada, S. (2009) Intraplantar injection of Bergamot essential oil into the mouse hind paw: effects on capsaicin-induced nociceptive behaviors. *Int. Rev. Neurobiol.*, **85**, 237–248.

112 Andrews, D.J., Reiter, J.M., Devinsky, O., Schachter, S., and Pacia, S. (2005) *Complementary and Alternative Therapies for Epilepsy* (eds Orrin Devinsky, Steven Sahachter, and Steven Pacia), Demos Medical Publishing, New York, pp. 33–42.

113 Dahl, J.C. and Lundgren, T.L. (2008) in *Behavioral Aspects of Epilepsy: Principles & Practice* (eds S.C. Schachter, G.L. Holmes, and D.G.A.K. Trenite), Demos Medical Publishing, New York, pp. 245–252.

114 Yardi, N. (2001) Yoga for control of epilepsy. *Seizure*, **10**, 7–12.

115 Lansky, E.P. (2006) Transcendental meditation: a double-edged sword in epilepsy? *Epilepsy Behav.*, **9**, 394–400.

116 Sterman, M.B. and Egner, T. (2006) Foundation and practice of neurofeedback for the treatment of epilepsy. *Appl. Psychophysiol. Biofeedback*, **31**, 21–35.

117 Egner, T. and Sterman, M.B. (2006) Neurofeedback treatment of epilepsy: from basic rationale to practical application. *Expert Rev. Neurother.*, **6**, 247–257.

118 Kneen, R. and Appleton, R.E. (2006) Alternative approaches to conventional antiepileptic drugs in the management of paediatric epilepsy. *Arch. Dis. Child.*, **91**, 936–941.

119 Ramaratnam, S., Baker, G., Goldstein, L., and Ramaratnam, S. (2005) Psychological treatments for epilepsy. *Cochrane Database Syst. Rev.*, 3. doi: https://dx.doi.org/10.1002/14651858.CD002029.pub3.

120 Ramaratnam, S. and Sridharan, K. (2000) Yoga for epilepsy. *Cochrane Database Syst. Rev.*, 3. doi: https://dx.doi.org/10.1002/14651858.CD001524.

121 Rajesh, B., Jayachandran, D., Mohandas, G., and Radhakrishnan, K. (2006) A pilot study of a yoga meditation protocol for patients with medically refractory epilepsy. *J. Altern. Complement. Med.*, **12**, 367–371.

122 Vickers, A. and Zollman, C. (1999) ABC of complementary medicine: homoeopathy. *Br. Med. J.*, **319**, 1115–1118.

123 Taffler, S. (2005) in *Complementary and Alternative Therapies for Epilepsy* (eds O. Devinsky, S. Schachter, and S. Pacia), Demos Medical Publishing, New York, pp. 157–164.

124 Shang, A., Huwiler-Muntener, K., and Nartey, L. (2005) Are the clinical effects of homoeopathy placebo effects? Comparative study of placebo-controlled trials of homoeopathy and allopathy. *Lancet*, **366**, 726–732.

125 Varshney, J.P. (2007) Clinical management of idiopathic epilepsy in dogs with homeopathic. Belladonna 200C: a case series. *Homeopathy*, **96**, 46–48.

126 Lai, C.W. and Lai, Y.H. (1991) History of epilepsy in Chinese traditional medicine. *Epilepsia*, **32**, 299–302.

127 Jin, H.B., Li, B., and Gu, J. (2005) Electro-acupuncture improves epileptic seizures induced by kainic acid in taurine-depletion rats. *Acupunct. Electrother. Res.*, **30**, 207–217.

128 Shu, J., Liu, R.Y., and Huang, X.F. (2005) Efficacy of ear-point stimulation on experimentally induced seizure. *Acupunct. Electrother. Res.*, **30**, 43–52.

129 Tyagi, A. and Delanty, N. (2003) Herbal remedies, dietary supplements, and seizures. *Epilepsia*, **44** (2), 228–235.

130 MedlinePlus www.nlm.nih.gov/medlineplus/druginformation.html (accessed 12 July 2016).

131 National Center for Complementary and Alternative Medicine www.nccam.nih.gov/health/alerts/silver/silver.pdf (accessed 12 July 2016).

132 Vaddadi, K.S. (1981) The use of gamma-linoleic acid and linoleic acid to differentiate between temporal lobe epilepsy and schizophrenia. *Prostaglandins Med.*, **6**, 375–379.

133 LaFrance, W.C., Kanner, J., and Hermann, B. (2008) Psychiatric comorbidities in epilepsy. *Int. Rev. Neurobiol.*, **83**, 347–383.

134 Harms, S.L., Eberly, L.E., Garrard, J.M., Hardie, N.A., Bland, P.C., and Leppik, I.E. (2005) Prevalence of appropriate and problematic antiepileptic combination therapy in older people in the nursing home. *J. Am. Geriatr. Soc.*, **53**, 1023–1028.

135 Ivetic, V., Popovic, M., Dukic, M., Barak, N., and Pilija, O. (2002) St. John's wort (*Hypericum perforatum* L.) and kindling epilepsy in rabbit. *Phytomedicine*, **9**, 496–499.

136 Borrelli, F. and Izzo, A.A. (2009) Herb-drug interactions with St John's wort (Hypericum perforatum): an update on clinical observations. *Am. Assoc. Pharm. Sci. J.*, **11**, 710–727.

137 Hinnell, C., Williams, J., Metcalfe, A., Patte, S.B., Parker, R., Wiebe, S., and Jette, N. (2010) Health status and health-related behaviors in epilepsy compared to other chronic conditions-A national population-based study. *Epilepsia*, **51** (5), 853–861.

138 Peled, N., Shorer, Z., Peled, E., and Pillar, G. (2001) Melatonin effect on seizures in children with severe neurologic deficit disorders. *Epilepsia*, **42**, 1208–1210.

139 Spinella, M. (2001) Herbal medicines and epilepsy: the potential for benefit and adverse effects. *Epilepsy Behav.*, **2**, 524–532.

140 Eadie, M.J. (2004) Could valerian have been the first anticonvulsant? *Epilepsia*, **45**, 1338–1343.

141 Sheldon, S.H. (1988) Pro-convulsant effects of oral melatonin in neurologically disabled children. *Lancet*, **351**, 1254–1255.

142 Kovacsova, M., Barta, A., Parohova, J., Vrankova, S., and Pechanova, O. (2010) Neuroprotective mechanisms of natural polyphenolic compounds. *Act. Nerv. Super. Rediviva*, **52** (3), 181–186.

143 Xavier, S.M., Barbosa, C.O., Barros, D.O., Silva, R.F., Oliveira, A.A., and Freitas, R.M. (2007) Vitamin C antioxidant effects in hippocampus of adult Wistar rats after seizures and status epilepticus induced by pilocarpine. *Neurosci. Lett.*, **420**, 76–79.

144 Burkhard, P.R., Burkhardt, K., Haenggeli, C.A., and Landis, T. (1999) Plant induced seizures: reappearance of an old problem. *J. Neurol.*, **246**, 667–670.

10

Neuroprotective Effects of Flavonoids in Epilepsy

Hossein Hosseinzadeh[1] *and Marjan Nassiri-Asl*[2]

[1] *Mashhad University of Medical Sciences, Pharmaceutical Research Center, School of Pharmacy, Department of Pharmacodynamics and Toxicology, Vakilabad Blvd., Mashhad 1365-91775, Iran*
[2] *Qazvin University of Medical Sciences, Cellular and Molecular Research Centre, School of Medicine, Department of Pharmacology, Bahonar Blvd., Qazvin 341197-5981, Iran*

10.1 Introduction

Epilepsy is one of the most common neurological disorders, affecting approximately 1% of the general population [1]. Antiepileptic drugs (AEDs) are of potential use for the treatment of epilepsy with approximately 70% of children achieving good control with medications alone [1]. Despite the therapeutic arsenal of old and new AEDs, approximately 30% of patients with epilepsy still suffer from seizures. Thus, there remains a substantial need for the development of more efficacious AEDs for patients with refractory seizures. The search for new therapies with better efficacy and tolerability remains an important goal [2].

Gamma-aminobutyric acid (GABA) (γ-aminobutyric acid) is an inhibitory neurotransmitter in the central nervous system (CNS) [3]. The benzodiazepines have been found to enhance inhibitory neurotransmission by allosterically modulating GABA receptor-mediated chloride ion currents [2] and inducing anticonvulsant, sedative, or anxiolytic effects [4]. GABA receptors are heteropentamers that are made up of 19 subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π and ρ1–3) [5]. As the decision of Nomenclature Committee of the International Union of Pharmacology (IUPHAR), the $GABA_A$ ρ receptors belong to the $GABA_A$-R family. It is recommended that the name of GABAc receptor should not be used as the sole name for the ρ receptors [6]. Most fast synaptic inhibition in the mature brain is mediated by $GABA_A$ receptors (GABARs), whereas slow inhibition is mediated by $GABA_B$ receptors [7]. Six benzodiazepines, including diazepam, lorazepam, clonazepam, nitrazepam, clorazepate, and clobazam, play important roles in the treatment of epilepsy. It seems that they have antiseizure effects that are shown to be of different degrees by these six compounds. One possible mechanism is that they may interact with the GABA–benzodiazepine allosteric receptor sites [3].

Flavonoids are polyphenolic compounds that have 15 carbons, with two aromatic rings connected by a three-carbon bridge (C6–C3–C6). Dietary flavonoids are classified as flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones, or

Neuroprotective Natural Products: Clinical Aspects and Mode of Action, First Edition.
Edited by Goutam Brahmachari.

isoflavones, while those that are comparatively minor components of the diet are dihydroflavonols, flavan-3,4-diols, coumarins, chalcones, dihydrochalcones, and aurones [8]. Major dietary sources of flavonoids include fruits, vegetables, cereals, tea, and fruit juices [9]. The chemical structures of some of these are shown in Figure 10.1. Basic science has provided strong evidence for research and development of novel AEDs from medicinal plants [10]. Anticonvulsant effects of several

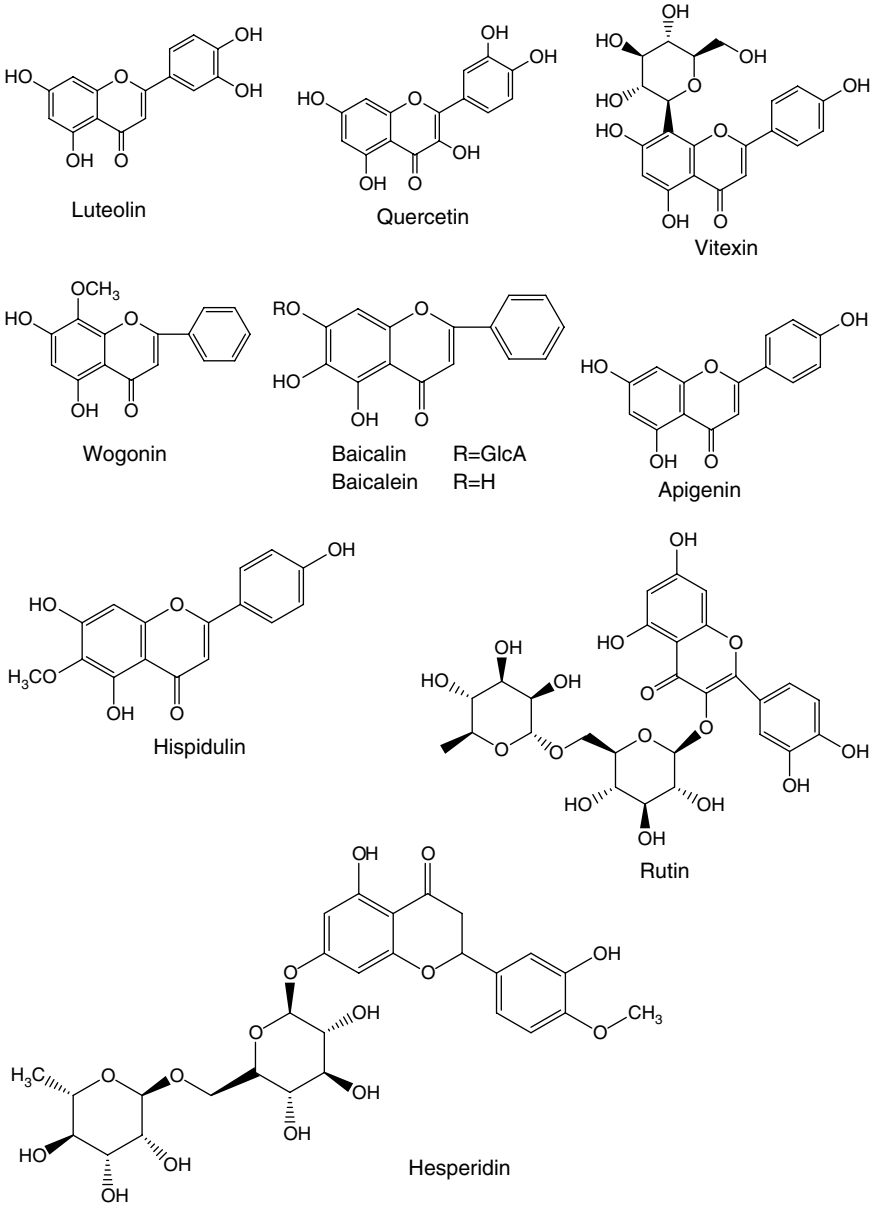


Figure 10.1 Chemical structure of some important natural flavonoids.

herbal medicines have been studied and established [11–17]. It seems that clinical studies with selected standardized botanical extracts and plant-derived compounds are necessary [18].

10.2 Natural Flavonoids with Antiepileptic Potential

It was shown that flavonoids interact with the benzodiazepine site of the GABA receptor and various voltage-gated ion channels [18]. Electrophysiological studies have shown two binding sites for flavonoids: the first is a high-affinity flumazenil-sensitive site and the second, a low-affinity flumazenil-insensitive site [4]. Up to 1983, there was no scientific literature regarding the role of flavonoids in the CNS [19]. However, in recent years, there have been several studies that have focused on the neuroprotective effects of flavonoids in *in vitro* and *in vivo* models. We have selected some that have been shown to be effective in animal epilepsy models. In this chapter, we will describe some of anticonvulsant effects and possible mechanisms. For more details, see Table 10.1.

10.2.1 Rutin

Rutin (3,3′,4′,5,7-pentahydrohyflavone-3-rhamnoglucoside) is a flavonoid that is an important dietary constituent of fruits and vegetables [38, 39]. It has anticonvulsant effects in different models [20–22] (Table 10.1).

10.2.2 Quercetin

Quercetin is a flavonoid (3,3′,4′,5,7-pentahydroxyflavone) found in a variety of fruits and vegetables [23]. It has anticonvulsant activity [23–25] (Table 10.1). It seems that the anticonvulsant effects of quercetin are mostly dependent on the model of seizure and its specific dose [23]. Flavonoids have modulatory effects on $GABA_A$ and $GABA_A$ ρ receptors. Quercetin at 30 μM inhibited α1β1γ2 $GABA_A$ and ρ1 $GABA_C$ ($GABA_A$ ρ) receptors in *Xenopus laevis* oocytes. It seems that flavonols such as apigenin and chrysin have similar effects. However, flavone, or α-naphthoflavone, has shown a different profile [40]. $GABA_A$ρ receptors are insensitive to the competitive antagonist of GABA and have low affinity to allosteric modulators of GABA such as benzodiazepine and barbiturates. Moreover, it has been shown that quercetin dose-dependently antagonized $GABA_A\rho_1$ receptors through a redox-independent allosteric mechanism and that ascorbic acid could inhibit this effect [41].

10.2.3 Vitexin

Vitexin (5,7,4-trihydroxyflavone-8-glucoside) is a C-glycosylated flavone found in numerous plants such as *Passiflora* sp., bamboo leaves, pigeon pea leaves, and mung beans [26]. Flumazenil reversed the anticonvulsant effects of rutin and vitexin. The suppression of the anticonvulsant effects of rutin and vitexin by flumazenil suggests that there is an interaction among rutin, vitexin, and the benzodiazepine receptor [20, 26] (Table 10.1).

Table 10.1 Anticonvulsant effects of some flavonoids in animals.

Flavonoids	Classification	Dose	Model	Animal	Effects
Rutin	Flavonol	150 nM, i.c.v.	PTZ (90 mg kg^{-1}, i.p.)	Rats	Increased onset of and GTCS [20]
		100 mg kg^{-1}, i.p.	Kindling (PTZ, 35 mg kg^{-1}, i.p.)	Rats	Attenuated seizure severity [21]
		100, 200 mg kg^{-1}, i.p.	KA (10 mg kg^{-1}, i.p.)	Mice	Decreased seizure scores and lipid peroxidation in the hippocampus [22]
Quercetin	Flavonol	50 mg kg^{-1}, i.p.	Kindling (PTZ, 35 mg kg^{-1})	Rats	Attenuated seizure severity [23]
		100 mg kg^{-1}, i.p.	On the test day after kindling (PTZ, 35 mg kg^{-1}, i,p.)	Rats	Increased onset of GTCS and its reduced duration [24]
		10, 20 mg kg^{-1}, i.p.	PTZ (45 mg kg^{-1}, i.p.)	Rats	Prevented seizure [25]
Vitexin	Flavone	100, 200 μM, i.c.v.	PTZ (90 mg kg^{-1}, i.p.)	Rats	Increased onset of MCS and GTCS [26]
Hesperidin	Flavanone	200 mg kg^{-1}, i.p.	Kindling (PTZ, 40 mg kg^{-1}, i.p.)	Mice	Attenuated behavioral, biochemical, and mitochondrial alterations induced by PTZ [27]
		200 mg kg^{-1}, p.o.	PTZ (80 mg kg^{-1}, i.p.)	Mice	Enhanced onset of clonic and tonic phases of convulsion [28]
Apigenin	Flavonol	25, 50 mg kg^{-1}, i.p.	KA (40 mg kg^{-1}, i.p.)	Mice	Decreased onset and scores of seizure [29]
Wogonin	Flavanone	5, 10 mg kg^{-1}, i.p.	PTZ (70 mg kg^{-1}, i.p.)	Rats	Decreased seizure responses [30]
		10 mg kg^{-1}	PTZ (100 mg kg^{-1}, i.p.)		
		5, 10 mg kg^{-1}, i.p.	Electroshock (convulsive current 50)		

Baicalein	Flavone	5 mg kg^{-1}	PTZ (70 mg kg^{-1}, i.p.)	Mice	Showed significant anticonvulsant activity [31]
		5, 10 mg kg^{-1}, i.p.	Electroshock (convulsive current 50)		Decreased electrogenic response score [31]
		100 mg kg^{-1}, i.p.	Pilocarpine (400 mg kg^{-1}, i.p.)	Rats	Pretreatment significantly delayed the onset of the first limbic seizures and SE and inhibited the increment in lipid peroxidation and nitrite content and the decrement in glutathione activity in the hippocampus [32]
Hispidulin	Flavone	10 mg kg^{-1}	Standardized handling procedure	Seizure-prone Mongolian gerbil	Reduced the number of suffering animals from seizure [33]
		10, 50 mg kg^{-1}, i.p.	KA (15 mg kg^{-1}, i.p.)	Rats	Attenuated neuronal cell death via suppression of inflammatory responses and MAPK activation [34]
Naringin	Flavanone	40, 80 mg kg^{-1}, i.p.	KA (10 mg kg^{-1}, i.p.)	Rats	Suppressed seizure in SE [35]
		80 mg kg^{-1}, i.p.	KA (0.2 μg/4 μl, intrahippocampal injection)	Mice	Increased the onset of seizure and decreased the occurrence of chronic SRS [36]
		80 mg kg^{-1}, i.p	PTZ (60 mg kg^{-1})	Rats	Prolonged the induction of myoclonic jerks [37]

Kainic acid (KA), minimal clonic seizure (MCS), generalized tonic–clonic seizure (GTCS), mitogen-activated protein kinases (MAPK), pentylenetetrazole (PTZ), oral (p.o.), status epilepticus (SE), and spontaneous recurrent seizure (SRS).

10.2.4 Hesperidin

Hesperidin is a bioflavonoid that is found in oranges and lemons. It enhanced the effect of diazepam. However, it was not a ligand for benzodiazepine binding site [42]. Hesperidin could potentiate the protective effect of *N*-nitro-L-arginine methyl ester (L-NAME) on kindling induced by pentylenetetrazole (PTZ) [27]. Its oral administration suppressed the convulsant effects of PTZ [28].

10.2.5 Apigenin

Apigenin (4′,5,7-trihydroxyflavone) is a flavonoid abundant in many fruits and vegetables [9]. It acts as a GABA antagonist at flumazenil-insensitive $\alpha_1\beta_2$ GABA receptors, and it also has an enhancing effect on the modulatory activity of diazepam of GABA receptors [43]. Apigenin, as an active component of *Matricaria chamomilla* extract, did not show anticonvulsant activity against picrotoxin, and it was suggested that apigenin could decrease GABA-activated chloride ion channel [44]. However, in contrast to these studies, it was found that apigenin has anticonvulsant activity [29] and could inhibit glutamate release from hippocampal nerve terminals, possibly via reducing Ca^{2+} entry, which is mediated by the Cav2.2 (N-type) and Cav2.1 (P/Q-type) channels, in rats [45] (Table 10.1).

10.2.6 Oroxylin A

Oroxylin A (5,7-dihydroxy-6-methoxyflavone), a flavonoid constituent of *Scutellaria baicalensis*, is an orally active benzodiazepine binding site ligand with some antagonistic properties that selectively abolished the anxiolytic, myorelaxant, and motor incoordination provided by diazepam ($1\,mg\,kg^{-1}$) in animals. It seems that these antagonistic effects may be mediated by antagonistic action on $\alpha_{2,3,5}$ subunits of $GABA_A$ receptor. But oroxylin A ($3.67–60\,mg\,kg^{-1}$) was not effective in picrotoxin-induced seizure alone, and pretreatment with it did not reverse the anticonvulsant effects of diazepam [46]. In one study by Zhang *et al.* (2009), the anticonvulsant activity of *Scutellaria lateriflora* was studied with total extract and fraction A. Results showed that fraction A of the extract, which contained 10 flavonoids and 2 phenylethanoid glycosides, has significant anticonvulsant activity compared with the total extract in PTZ-induced seizure in rats [47]. The structures of several active flavonoids of *S. lateriflora*, including baicalin, baicalein, wogonin, and oroxylin A, are shown in Figure 10.1.

10.2.7 Wogonin

Wogonin, a flavonoid constituent of *S. baicalensis*, could enhance Cl^- influx from IMR-32 cells (neuroblastoma cells) when the current was inhibited by flumazenil. More studies are needed to determine the relationship between this flavonoid and $GABA_A$ subunits [30] (Table 10.1).

10.2.8 Baicalein

Baicalein (5,6,7-trihydroxyflavone), but not baicalin and oroxylin A, has shown anticonvulsant effects that were mediated by the benzodiazepine binding site of

$GABA_A$ receptor and increased Cl^- influx from IMR-32 cells. Flumazenil suppressed its anticonvulsant effect. In the study about the structure of *S. baicalensis* flavones, it was concluded that the 6-hydroxy group of baicalein and 8-methoxy group of wogonin increase the agonistic properties, while the 6-methoxy group of oroxylin A induces the antagonistic effect on $GABA_A$ receptors [31] (Table 10.1). Moreover, baicalein has anticonvulsant effects in the pilocarpine model of epilepsy in that it seems that its anticonvulsant effects are not directly dependent on $GABA_A$ receptors [32] (Table 10.1). However, intracerebroventricular administration of baicalein (0.02–2 pmol) did not have an anticonvulsant effect, and its anxiolytic and sedative effects were dependent on $GABA_A$ non-benzodiazepine sites in mice [48].

10.2.9 Luteolin

Luteolin (3′,4′,5,7-tetrahydroxyflavone) is found in vegetables and medicinal plants. In recent years, its anticonvulsant effects have been studied. Acute luteolin administration had no effects in the 6 Hz PTZ and maximal electroshock tests (MEST). Furthermore, chronic luteolin administration had no effect in the 6 Hz model and pilocarpine model with second-hit PTZ seizure model [49]. In contrast, it was reported that pretreatment with luteolin ($10\,mg\,kg^{-1}$, 2 w) could reduce the frequency of seizure using the PTZ model in mice [50].

10.2.10 Hispidulin

Hispidulin (4′,5,7-trihydroxy-6-methoxyflavone) is a potent benzodiazepine receptor ligand with anticonvulsant effects [33, 34] and acts as a positive modulator of $GABA_A$ receptors [33] (Table 10.1). It has been found in many herbal medicines such as in Asteraceae and Lamiaceae families [51]. It has several pharmacological properties such as neuroprotective effect and antioxidant, anticancer, and anti-osteoporotic activities [52–55].

10.2.11 Naringin

Naringin (4′,5,7-trihydroxyflavanone 7-rhamnoglucoside) is a flavanone glycoside that has anticonvulsant effects in kainic acid and PTZ models [35–37] (Table 10.1). Flumazenil suppressed the antiepileptic effect of naringin. Thus, it seems that the anticonvulsant effects of naringin occur via the modulation of the benzodiazepine site of $GABA_A$ receptor [37].

10.3 Discovery and Development of Newer Agents

It has been shown that substitution of a hydroxyl at the 2′ carbon of a flavonoid molecule enhanced binding affinities for the benzodiazepine receptor on the GABA receptor. The substitution of a hydroxyl group for a hydrogen at the 2′ carbon resulted in sevenfold or higher affinity for the benzodiazepine receptor [56]. 6-Methylflavanone and 6-methylflavone were allosteric positive modulators at human recombinant $\alpha_1\beta_2\gamma_{2L}$, $\alpha_2\beta_2\gamma_{2L}$, and $\alpha_1\beta_2$ $GABA_A$ receptors expressed in *X. laevis* oocytes [57].

The efficacy of flavone 6-substitutions, including 6-fluoroflavone, 6-chloroflavone, 6-bromoflavone, and 2′-hydroxyflavone, on $GABA_A$ receptors was compared. It was found that increasing the width of the single atom on the substituent at the 6-position could increase binding affinity, but the overall volume of the substituent at this position determined the efficacy of the aforementioned compounds [58]. 6-Bromoflavone has been shown to be a positive modulator of $GABA_A$ receptors and acts through the flumazenil-sensitive high-affinity benzodiazepine site [58]. 6-Bromoflavone (10 μM) is a selective benzodiazepine receptor ligand and has an anxiolytic effect that is 10 times more potent than diazepam [59]. But 6-fluoro- or 6-chloroflavones are neutralizing modulators [58]. Flavan-3-ol compounds of *Vitis labrusca* extract could prevent PTZ-induced oxidative damage in rats. It seems that this extract may be used to develop new therapeutic agent against seizure disorders [60].

Applying the linear quantitative structure–activity relationships (QSAR) has estimated the binding affinities for some newly synthesized flavonoids by displaying 2-,7-substitutions in the benzopyrone backbone. The presence of the β-naphthyl group substituting the benzopyrone nucleus has highly improved the binding affinities for the benzodiazepine binding site of the $GABA_A$ receptor complex [61]. Flavone (2-phenylchromone) has shown potential for application in a diversity of pharmacological targets [62]. There are some studies that have tried to find synthetic flavone derivatives with higher affinities for the $GABA_A$ receptor [62–64]. Recently, some benzyl-substituted flavone derivatives have been synthesized, and the regioselectivity of benzyl ether compounds was reported [65].

10.4 Concluding Remarks

Epilepsy is one of the neurodegenerative disorders that need more efficient and safer treatments. In recent years, natural flavonoids have shown good neuroprotective effects in the treatment of epilepsy in *in vitro* and *in vivo* studies so that there is a case to start clinical studies. Several semisynthetic and synthetic derivatives of flavonoids with greater efficacy that bind the GABA receptors via either the benzodiazepine binding site or an alternative site have been studied. Thus, further extensive studies that focus on the anticonvulsant effects of these compounds in basic and clinical studies including their improved pharmacodynamics and pharmacokinetics are needed.

Abbreviations

AEDs	antiepileptic drugs
GABA	gamma-aminobutyric acid
IUPHAR	Nomenclature Committee of the International Union of Pharmacology
L-NAME	*N*-nitro-L-arginine methyl ester
MEST	maximal electroshock tests

PTZ	pentylenetetrazole
QSAR	quantitative structure–activity relationships

References

1 Sankaraneni, R. and Lachhwani, D. (2015) Antiepileptic drugs-a review. *Pediatr. Ann.*, **44** (2), e36–e42.
2 Bialer, M. and White, H.S. (2010) Key factors in the discovery and development of new antiepileptic drugs. *Nat. Rev. Drug Discovery*, **9** (1), 68–82.
3 Porter, R.G. and Meldrum, B.S. (2012) in *Basic and Clinical Pharmacology*, 12th edn (eds B.G. Katzung and S.B. Masters), McGraw-Hill, New York, pp. 379–381.
4 Jäger, A.K. and Saaby, L. (2011) Flavonoids and the CNS. *Molecules*, **16** (2), 1471–1485.
5 Rudolph, U. and Knoflach, F. (2011) Beyond classical benzodiazepines: novel therapeutic potential of $GABA_A$ receptor subtypes. *Nat. Rev. Drug Discovery*, **10** (9), 685–697.
6 Olsen, R.W. and Sieghart, W. (2008) International Union of Pharmacology. LXX. Subtypes of γ-aminobutyric acid A receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol. Rev.*, **60** (3), 243–260.
7 Brooks-Kayal, A.R., Raol, Y.H., and Russek, S.J. (2009) Alteration of epileptogenesis genes. *Neurotherapeutics*, **6** (2), 312–318.
8 Crozier, A., Jaganath, I.B., and Clifford, M.N. (2009) Dietary phenolics: chemistry, bioavailability and effects on health. *Nat. Prod. Rep.*, **26** (8), 1001–1043.
9 Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L. (2004) Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.*, **79** (5), 727–747.
10 Zhu, H.L., Wan, J.B., Wang, Y.T., Li, B.C., Xiang, C., He, J., and Li, P. (2014) Medicinal compounds with antiepileptic/anticonvulsant activities. *Epilepsia*, **55** (1), 3–16.
11 Hosseinzadeh, H., Ramezani, M., Shafaei, H., and Taghiabadi, E. (2013) Anticonvulsant effect of *Berberis integerrima* L. root extracts in mice. *J. Acupunct. Meridian Stud.*, **6** (1), 12–17.
12 Hosseinzadeh, H., Sayadi, S.K.M., Taghiabadi, E., and Razavi, B.M. (2009) Anticonvulsant effect of *Citrus bigaradia Duh*. leaves extracts in mice. *Pharmacologyonline*, **3**, 412–418.
13 Sadeghnia, H.R., Cortez, M.A., Liu, D., Hosseinzadeh, H., and Snead, O.C. III (2008) Antiabsence effects of safranal in acute experimental seizure models: EEG and autoradiography. *J. Pharm. Pharm. Sci.*, **11** (3), 1–14.
14 Hosseinzadeh, H. and Sadeghnia, H.R. (2007) Protective effect of safranal on pentylenetetrazol-induced seizures in the rat: involvement of GABAergic and opioids systems. *Phytomedicine*, **14** (4), 256–262.
15 Hosseinzadeh, H. and Talebzadeh, F. (2005) Anticonvulsant evaluation of safranal and crocin from *Crocus sativus* in mice. *Fitoterapia*, **76** (7–8), 722–724.
16 Hosseinzadeh, H., Karimi, G.R., and Rakhshanizadeh, M. (2005) Anticonvulsant effect of *Hypericum perforatum*: role of nitric oxide. *J. Ethnopharmacol.*, **98** (1–2), 207–208.

17 Hosseinzadeh, H., Parvardeh, S., Nassiri-Asl, M., and Mansouri, M.T. (2005) Intracerebroventricular administration of thymoquinone, the major constituent of Nigella sativa seeds, suppresses epileptic seizures in rats. *Med. Sci. Monit.*, **11** (4), BR106–BR110.

18 Sucher, N.J. and Carles, M.C. (2015) A pharmacological basis of herbal medicines for epilepsy. *Epilepsy Behav.*, **52** (Pt. B), 308–318.

19 Marder, M. and Paladini, A.C. (2002) GABA(A)-receptor ligands of flavonoid structure. *Curr. Top. Med. Chem.*, **2** (8), 853–867.

20 Nassiri-Asl, M., Shariati-Rad, S., and Zamansoltani, F. (2008) Anticonvulsive effects of intracerebroventricular administration of rutin in rats. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, **32**, 989–993.

21 Nassiri-Asl, M., Mortazavi, S.R., Samiee-Rad, F., Zangivand, A.A., Safdari, F., Saroukhani, S., and Abbasi, E. (2010) The effects of rutin on the development of pentylenetetrazole kindling and memory retrieval in rats. *Epilepsy Behav.*, **18**, 50–53.

22 Nassiri-Asl, M., Naserpour Farivar, T., Abbasi, E., Sadeghnia, H.R., Sheikhi, M., Lotfizadeh, M., and Bazahang, P. (2013) Effects of rutin on oxidative stress in kainic acid-induced seizure in mice. *J. Integr. Med.*, **11** (5), 337–342.

23 Nassiri-Asl, M., Moghbelinejad, S., Haghighi, M.R., Lotfizadeh, M., Abbasi, E., Yonesi, F., and Bazahang, P. (2013) Effects of quercetin on oxidative stress and memory retrieval in kindled rats. *Epilepsy Behav.*, **28** (2), 151–155.

24 Nassiri-Asl, M., Hajiali, F., Taghiloo, M., Abbasi, E., Mohseni, F., and Yousefi, F. (2016) Comparison between the effects of quercetin on seizure threshold in acute and chronic seizure models. *Toxicol. Ind. Health*, **32** (5), 936–944.

25 Sefil, F., Kahraman, I., Dokuyucu, R., Gokce, H., Ozturk, A., Tutuk, O., Aydin, M., Ozkan, U., and Pinar, N. (2014) Ameliorating effect of quercetin on acute pentylenetetrazole induced seizures in rats. *Int. J. Clin. Exp. Med.*, **7** (9), 2471–2477.

26 Abbasi, E., Nassiri-Asl, M., Shafeei, M., and Sheikhi, M. (2012) Neuroprotective effects of vitexin, a flavonoid, on pentylenetetrazole-induced seizure in rats. *Chem. Biol. Drug Des.*, **80** (2), 274–278.

27 Kumar, A., Lalitha, S., and Mishra, J. (2013) Possible nitric oxide mechanism in the protective effect of hesperidin against pentylenetetrazole (PTZ)-induced kindling and associated cognitive dysfunction in mice. *Epilepsy Behav.*, **29** (1), 103–111.

28 Kumar, A., Lalitha, S., and Mishra, J. (2014) Hesperidin potentiates the neuroprotective effects of diazepam and gabapentin against pentylenetetrazole-induced convulsions in mice: possible behavioral, biochemical and mitochondrial alterations. *Indian J. Pharmacol.*, **46** (3), 309–315.

29 Han, J.Y., Ahn, S.Y., Kim, C.S., Yoo, S.K., Kim, S.K., Kim, H.C., Hong, J.T., and Oh, K.W. (2012) Protection of apigenin against kainate-induced excitotoxicity by anti-oxidative effects. *Biol. Pharm. Bull.*, **35** (9), 1440–1446.

30 Park, H.G., Yoon, S.Y., Choi, J.Y., Lee, G.S., Choi, J.H., Shin, C.Y., Son, K.H., Lee, Y.S., Kim, W.K., Ryu, J.H., Ko, K.H., and Cheong, J.H. (2007) Anticonvulsant effect of wogonin isolated from *Scutellaria baicalensis*. *Eur. J. Pharmacol.*, **574** (2–3), 112–119.

31 Yoon, S.Y., dela Peña, I.C., Shin, C.Y., Son, K.H., Lee, Y.S., Ryu, J.H., Cheong, J.H., and Ko, K.H. (2011) Convulsion-related activities of *Scutellaria* flavones are related to the 5,7-dihydroxyl structures. *Eur. J. Pharmacol.*, **659** (2–3), 155–160.

32 Liu, Y.F., Gao, F., Li, X.W., Jia, R.H., Meng, X.D., Zhao, R., Jing, Y.Y., Wang, Y., and Jiang, W. (2012) The anticonvulsant and neuroprotective effects of baicalin on pilocarpine-induced epileptic model in rats. *Neurochem. Res.*, **37** (8), 1670–1680.

33 Kavvadias, D., Sand, P., Youdim, K.A., Qaiser, M.Z., Rice-Evans, C., Baur, R., Sigel, E., Rausch, W.D., Riederer, P., and Schreier, P. (2004) The flavone hispidulin, a benzodiazepine receptor ligand with positive allosteric properties, traverses the blood-brain barrier and exhibits anticonvulsive effects. *Br. J. Pharmacol.*, **142** (5), 811–820.

34 Lin, T.Y., Lu, C.W., Wang, S.J., and Huang, S.K. (2015) Protective effect of hispidulin on kainic acid-induced seizures and neurotoxicity in rats. *Eur. J. Pharmacol.*, **755**, 6–15.

35 Golechha, M., Chaudhry, U., Bhatia, J., Saluja, D., and Arya, D.S. (2011) Naringin protects against kainic acid-induced status epilepticus in rats: evidence for an antioxidant, anti-inflammatory and neuroprotective intervention. *Biol. Pharm. Bull.*, **34** (3), 360–365.

36 Jeong, K.H., Jung, U.J., and Kim, S.R. (2015) Naringin attenuates autophagic stress and neuroinflammation in kainic acid-treated hippocampus *in vivo*. *Evid. Based Complement. Alternat. Med.*, **2015**, 354326.

37 Golechha, M., Sarangal, V., Bhatia, J., Chaudhry, U., Saluja, D., and Arya, D.S. (2014) Naringin ameliorates pentylenetetrazol-induced seizures and associated oxidative stress, inflammation, and cognitive impairment in rats: possible mechanisms of neuroprotection. *Epilepsy Behav.*, **41**, 98–102.

38 Kuntic, V., Pejic, N., Ivkovic, B., Vujic, Z., Ilic, K., Micic, S., and Vukojević, V. (2007) Isocratic RP-HPLC method for rutin determination in solid oral dosage forms. *J. Pharm. Biomed. Anal.*, **43**, 718–721.

39 Huang, J., Zhu, M., Tao, Y., Wang, S., Chen, J., Sun, W., and Li, S. (2012) Therapeutic properties of quercetin on monosodium urate crystal-induced inflammation in rat. *J. Pharm. Pharmacol.*, **64** (8), 1119–1127.

40 Goutman, J.D., Waxemberg, M.D., Doñate-Oliver, F., Pomata, P.E., and Calvo, D.J. (2003) Flavonoid modulation of ionic currents mediated by GABA(A) and GABA(C) receptors. *Eur. J. Pharmacol.*, **461** (2–3), 79–87.

41 Calero, C.I., Beltrán González, A.N., Gasulla, J., Alvarez, S., Evelson, P., and Calvo, D.J. (2013) Quercetin antagonism of $GABA_{A\rho_1}$ receptors is prevented by ascorbic acid through a redox-independent mechanism. *Eur. J. Pharmacol.*, **714** (1–3), 274–280.

42 Fernández, S.P., Wasowski, C., Paladini, A.C., and Marder, M. (2005) Synergistic interaction between hesperidin, a natural flavonoid, and diazepam. *Eur. J. Pharmacol.*, **512** (2–3), 189–198.

43 Campbell, E.L., Chebib, M., and Johnston, G.A. (2004) The dietary flavonoids apigenin and (–)-epigallocatechin gallate enhance the positive modulation by diazepam of the activation by GABA of recombinant GABA(A) receptors. *Biochem. Pharmacol.*, **68** (8), 1631–1638.

44 Avallone, R., Zanoli, P., Puia, G., Kleinschnitz, M., Schreier, P., and Baraldi, M. (2000) Pharmacological profile of apigenin, a flavonoid isolated from *Matricaria chamomilla*. *Biochem. Pharmacol.*, **59** (11), 1387–1394.

45 Chang, C.Y., Lin, T.Y., Lu, C.W., Wang, C.C., Wang, Y.C., Chou, S.S., and Wang, S.J. (2015) Apigenin, a natural flavonoid, inhibits glutamate release in the rat hippocampus. *Eur. J. Pharmacol.*, **762**, 72–81.

46 Huen, M.S., Leung, J.W., Ng, W., Lui, W.S., Chan, M.N., Wong, J.T., and Xue, H. (2003) 5,7-Dihydroxy-6-methoxyflavone, a benzodiazepine site ligand isolated from *Scutellaria baicalensis* Georgi, with selective antagonistic properties. *Biochem. Pharmacol.*, **66** (1), 125–132.

47 Zhang, Z., Lian, X.Y., Li, S., and Stringer, J.L. (2009) Characterization of chemical ingredients and anticonvulsant activity of American skullcap (*Scutellaria lateriflora*). *Phytomedicine*, **16** (5), 485–493.

48 de Carvalho, R.S., Duarte, F.S., and de Lima, T.C. (2011) Involvement of GABAergic non-benzodiazepine sites in the anxiolytic-like and sedative effects of the flavonoid baicalein in mice. *Behav. Brain Res.*, **221** (1), 75–82.

49 Shaikh, M.F., Tan, K.N., and Borges, K. (2013) Anticonvulsant screening of luteolin in four mouse seizure models. *Neurosci. Lett.*, **550**, 195–199.

50 Birman, H., Dar, K.A., Kapucu, A., Acar, S., and Uzüm, G. (2012) Effects of luteolin on liver, kidney and brain in pentylentetrazol-induced seizures: involvement of metalloproteinases and NOS activities. *Balkan Med. J.*, **29** (2), 188–196.

51 Atif, M., Ali, I., Hussain, A., Hyder, S.V., Niaz, B., Khan, F.A., Maalik, A., and Farooq, U. (2015) Pharmacological assessment of hispidulin-A natural bioactive flavone. *Acta Pol. Pharm.*, **72** (5), 829–842.

52 Lin, T.Y., Lu, C.W., Wang, C.C., Lu, J.F., and Wang, S.J. (2012) Hispidulin inhibits the release of glutamate in rat cerebrocortical nerve terminals. *Toxicol. Appl. Pharmacol.*, **263** (2), 233–243.

53 Dabaghi-Barbosa, P., Mariante Rocha, A., Franco da Cruz Lima, A., Heleno de Oliveira, B., Benigna Martinelli de Oliveira, M., Gunilla Skare Carnieri, E., Cadena, S.M., and Eliane Merlin Rocha, M. (2005) Hispidulin: antioxidant properties and effect on mitochondrial energy metabolism. *Free Radical Res.*, **39** (12), 1305–1315.

54 Gao, H., Xie, J., Peng, J., Han, Y., Jiang, Q., Han, M., and Wang, C. (2015) Hispidulin inhibits proliferation and enhances chemosensitivity of gallbladder cancer cells by targeting HIF-1α. *Exp. Cell. Res.*, **332** (2), 236–246.

55 Zhou, R., Wang, Z., and Ma, C. (2014) Hispidulin exerts anti-osteoporotic activity in ovariectomized mice via activating AMPK signaling pathway. *Cell Biochem. Biophys.*, **69** (2), 311–317.

56 Huen, M.S., Hui, K.M., Leung, J.W., Sigel, E., Baur, R., Wong, J.T., and Xue, H. (2003) Naturally occurring 2′-hydroxyl-substituted flavonoids as high-affinity benzodiazepine site ligands. *Biochem. Pharmacol.*, **66** (12), 2397–2407.

57 Hall, B.J., Chebib, M., Hanrahan, J.R., and Johnston, G.A. (2005) 6-Methylflavanone, a more efficacious positive allosteric modulator of gamma-aminobutyric acid (GABA) action at human recombinant alpha$_2$ beta$_2$ gamma$_{2L}$ than at alpha$_1$beta$_2$gamma$_{2L}$ and alpha$_1$beta$_2$ GABA(A) receptors expressed in Xenopus oocytes. *Eur. J. Pharmacol.*, **512** (2–3), 97–104.

58 Ren, L., Chan, W.M., Wang, F., Xu, Z., Zhao, C., Mat, W.K., Chai, Y., Wong, J.T., Tsang, S.Y., and Xue, H. (2011) Effects of flavone 6-substitutions on $GABA_A$ receptors efficacy. *Eur. J. Pharmacol.*, **670** (1), 121–129.

59 Viola, H., Marder, M., Wolfman, C., Wasowski, C., Medina, J.H., and Paladini, A.C. (1997) 6-Bromo-3′-nitroflavone, a new high affinity benzodiazepine receptor agonist recognizes two populations of central cortical binding sites. *Bioorg. Med. Chem. Lett.*, **7**, 373–378.

60 Scola, G., Scheffel, T., Gambato, G., Freitas, S., Dani, C., Funchal, C., Gomez, R., Coitinho, A., and Salvador, M. (2013) Flavan-3-ol compounds prevent pentylenetetrazol-induced oxidative damage in rats without producing mutations and genotoxicity. *Neurosci. Lett.*, **534**, 145–149.

61 Duchowicz, P.R., Vitale, M.G., Castro, E.A., Autino, J.C., Romanelli, G.P., and Bennardi, D.O. (2008) QSAR modeling of the interaction of flavonoids with GABA(A) receptor. *Eur. J. Med. Chem.*, **43** (8), 1593–1602.

62 Deeb, O., Shaik, B., and Agrawal, V.K. (2014) Exploring QSARs of the interaction of flavonoids with GABA (A) receptor using MLR, ANN and SVM techniques. *J. Enzyme Inhib. Med. Chem.*, **29** (5), 670–676.

63 Huang, X., Liu, T., Gu, J., Luo, X., Ji, R., Cao, Y., Xue, H., Wong, J.T., Wong, B.L., Pei, G., Jiang, H., and Chen, K. (2001) 3D-QSAR model of flavonoids binding at benzodiazepine site in $GABA_A$ receptors. *J. Med. Chem.*, **44**, 1883–1891.

64 Hong, X. and Hopfinger, A.J. (2003) 3D-pharmacophores of flavonoid binding at the benzodiazepine GABA(A) receptor site using 4D-QSAR analysis. *J. Chem. Inf. Comput. Sci.*, **43** (1), 324–336.

65 Wang, C.Q., Chen, X., Jiang, J.H., Tang, H., Zhu, K.K., Zhou, Y.J., Zheng, C.H., and Zhu, J. (2015) Acidic rearrangement of benzyl group in flavone benzyl ethers and its regioselectivity. *Chin. Chem. Lett.*, **26**, 793–796.

11

The Role of Noncompetitive Antagonists of the *N*-Methyl-D-aspartate (NMDA) Receptors in Treatment-Resistant Depression

Gianluca Serafini[1], Shawn Hayley[2], Mehdi Ghasemi[3], and Mario Amore[1]

[1] *University of Genoa, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, Section of Psychiatry, IRCCS San Martino, Largo Rosanna Benzi 10 16132, Genoa, Italy*
[2] *Carleton University, Department of Neuroscience, 1125 Colonel By Drive, Ottawa, K1S 5B6 ON, Canada*
[3] *University of Massachusetts Medical Center, Department of Neurology, 55 Lake Avenue North, Worcester, MA 01655, USA*

11.1 Introduction

Major depressive disorder (MDD) is commonly associated with frequent relapses, reducing quality of life and promoting severe psychosocial impairment [1–3]. Importantly, MDD may also be accompanied by increased suicidal behaviors [4–6]. Despite the fact that many compounds are available for the treatment of MDD [7], a sizeable proportion of patients do not achieve complete recovery and showed treatment resistance [8, 9].

Refractory depression is a disabling illness that is associated with a significant functional impairment and poor outcome [10–12] and may be found in nearly 30% of all depressed patients [13]. Many criteria have been proposed to define treatment-resistant depression (TRD) [13, 14]. In general, this condition is defined as a failure to respond to at least two different types of antidepressants for a period longer than 4 weeks at the maximum recommended dose.

Unfortunately, the pathophysiology of TRD is still poorly understood and does not fit well within the confines of the long-standing monoamine hypothesis for depression, which essentially postulates the emergence of the disorder as a result of a specific deficit in the synaptic availability of monoamine neurotransmitters (e.g., norepinephrine, dopamine, or serotonin) [15]. Considering the fact that the therapeutic effect of the most common antidepressant medications emerges only after 4–12 weeks of treatment [16, 17], it is critical to identify novel targets for the development of faster-acting drugs for suicidal cases, as well as those with TRD [5–7, 18].

The excitatory amino acid neurotransmitter glutamate has emerged as a potential target for depression beyond monoamines. Indeed, it has been implicated in major affective and anxiety disorders, schizophrenia, and substance abuse/dependence. Glutamate is well positioned to modulate depressive symptomology, as it has neurodevelopmental and neurotrophic effects, along with a modulatory role on other neurotransmitter systems, which underlie its role in learning and memory and the general processing of environmental stimuli [7].

Neuroprotective Natural Products: Clinical Aspects and Mode of Action, First Edition.
Edited by Goutam Brahmachari.

This excitatory neurotransmitter is mainly released by neurons, binds to specific receptors, and is later removed by specific reuptake transporters. Glutamate receptor systems may be classified as "ionotropic" or "metabotropic." Ionotropic glutamate receptors include (i) *N*-methyl-D-aspartate (NMDA), (ii) alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), or (iii) kainate receptors with each group having various subtypes. Most pharmaceutical research has focused on the modulation of NMDA receptors, but recently attention has also been devoted to drugs that act on AMPA and metabotropic receptors.

The first observation that glutamate-modulating medications may be beneficial in treating mood disorders was reported by Crane [19, 20] in the late 1950s. In his study, he found that when tuberculosis patients were treated with the antibiotic D-cycloserine, they showed improvement in their mood and depressive behavior as well. Thus, it soon became clear that when used as antibiotic, D-cycloserine had significant antidepressant properties. Interestingly, when administrated at lower doses, D-cycloserine can be considered a partial NMDA receptor agonist stimulating glutamate release, but when administered at higher doses, it acts as an N-methyl-D-aspartate receptor (NMDAR) antagonist blocking the release of glutamate. The antidepressant effects of D-cycloserine could be related to its capacity to reduce NMDAR function. In a recent study in which high doses of D-cycloserine were used to treatment-resistant MDD patients in addition to their antidepressant medication, D-cycloserine reduced depressive symptoms by 50% in a significant number of these patients [21].

Many studies have focused on the antidepressant potential of another NMDA antagonist, namely, ketamine, which is a high-affinity NMDA receptor antagonist. Indeed, accumulating data indicate marked antidepressant properties of ketamine, even in the treatment of resistant patients. To date, the antidepressant effects of ketamine have been demonstrated in both animal models and human studies of depression. There is consistent evidence (e.g., case reports/series, prospective open-label, double-blind placebo- or active-controlled studies) showing the rapid and sustained antidepressant activity of this compound.

In this chapter, we mainly aimed to review the current literature focusing on the role of noncompetitive antagonists of the NMDA receptors, in particular ketamine, in TRD.

11.2 Noncompetitive Antagonists of the NMDA Receptors: Ketamine and Its Mechanism of Action

Given the potential of noncompetitive antagonists of the NMDA receptors to induce rapid reduction of treatment-resistant depressive symptoms in MDD patients, there is a growing interest concerning the application of this novel medication [22–26]. Indeed, it has been clearly shown that ketamine may induce a rapid and significant clinical response (e.g., within hours) in TRD patients who were refractory to other conventional treatment strategies.

One of the most important effects of ketamine has also been the reduction of suicide risk [27–31]; however, whether these antisuicidal properties may be independent of antidepressive and anxiolytic effects is still elusive [32]. According to the existing evidence, it seems that ketamine may address some critical unmet needs in

the treatment of both MDD and TRD. This is a very important issue as, with the exception of lithium and clozapine, there are currently no further treatments that may possess specific antisuicidal properties [33]. Therefore, there is an increasing interest in translating ketamine research evidence to clinical practice [34].

Ketamine has been reported to induce a blockade of the NMDA receptors at the phencyclidine binding site. However, it is also able to exert activity on intracellular pathways linked to the inhibition of these receptors with multiple consequences. Several recent authors [35–37] have suggested that ketamine is able to regulate dopamine transmission, and this might contribute to some of its behavioral effects; however, its well-established neuroplastic effects are also likely involved.

Finally, ketamine has also been reported to bind opioid μ and sigma receptors and modulate dopamine neurotransmission [38]; however, whether its antidepressant activity may be linked to this opioid and dopamine signaling is quite controversial.

11.2.1 The Antidepressant Efficacy of Ketamine

Several meta-analytic studies [39–47] using randomized short-term trials have clearly demonstrated the rapid antidepressant properties of ketamine when compared with placebo- or active-controlled conditions.

When examining the pooled data analyses, the efficacy of ketamine compared with saline or midazolam placebo has been reported within a few hours with higher antidepressant effects after 24 h that can persist for 7 days. Conversely, moderate antidepressant effects have been reported after 7 days following the initial dose. When combining findings derived from controlled and noncontrolled studies, a higher variability of cumulative rates of antidepressant effects was apparent with short-term treatment with beneficial responses ranging from approximately 30% to 90% [15, 34].

Large effects reported for the short-term antidepressant response (pooled response rate (RR) 50% vs. 13%, RR 2.6; pooled response rate 31% vs. 7%, RR 3.4) and remission (pooled remission rate 28% vs. 3%, RR 5.2; pooled remission rates 24% vs. 6%, RR 2.6) have been observed when restricting the analysis of findings from randomized crossover trials in the meta-analytic study by Xu and colleagues [39].

The long-term benefits reported for ketamine have been controversial, with the antidepressant effects being inconsistent after 14 days from the initial administration. Indeed, significant antidepressant effects of ketamine after 12–14 days from the initial ketamine administration have been found by Coyle and Laws [44], but decidedly weaker (not significant) effects after 14 days of treatment have been reported by Romeo *et al.* [43]. Similarly, Newport and colleagues [40] showed very low pooled rates of treatment response to ketamine when compared with controls after 14 days of ketamine administration (11% vs. 0%). In this study, only a statistical trend was found after 14 days of treatment (OR 4.4) [40].

Other data from placebo-controlled studies found that significant depressive relapse rates have been reported after 7 days of treatment among individuals who positively responded to the initial ketamine infusions, with almost all patients relapsing after 2 weeks [27, 29, 48, 49]. Thus, the long-term antidepressant effects of ketamine warrant further investigations through more well-designed prospective studies.

In their meta-analytic study, Coyle and Laws [44] explored the effects of either single or serial infusions of ketamine on depressive symptoms. Based on the 21 studies that were selected, more pronounced reductions of depressive symptoms were reported in patients who were treated with serial ketamine infusions over 12–14 days (vs. controls) relative to those who were treated with only a single ketamine infusion. Further, response rates of 71–89% after the first infusion have been found in patients with TRD who were administered up to six infusions of ketamine at 0.5 mg kg^{-1} [50, 51].

When compared with single-infusion findings, response rates with serial infusions of ketamine result in greater clinical responses that were maintained throughout the infusion period and with up to 18–19 day mean/median times to relapse [50, 51]. However, it is noteworthy that case reports or case series studies have indicated more sustained effects for ketamine (e.g., one patient who remained without depressive symptoms for a period of 3 months after the last ketamine administration) [50], but these studies can obviously not be easily generalized.

The potential for ketamine to increase overall remission, when administered twice weekly over 2 weeks, has been demonstrated by two open-label studies [52]. In a study by Rasmussen and colleagues [52] that involved TRD patients, 50% of patients showed remission; moreover, cumulative remission rates correlated with the number of infusions with rates of 10%, 40%, and 50% after one, two, and four ketamine infusions, respectively. In fact, a positive treatment response was found in 92% of individuals with TRD and 67% actually displayed full remission. Unfortunately, 60% of patients who remitted when treated by serial ketamine infusions eventually relapsed over the subsequent 4 weeks, even if they were also taking oral antidepressant medications.

Finally, the potential to reduce suicidal ideation within some hours of the first ketamine administration has been reported in patients with both major and bipolar depression [27, 28, 30, 49]. According to a pooled analysis of seven trials, Xu and colleagues [39] found a significant effect of ketamine on suicidality after both 24 h and 3 days of infusions. Similarly, Murrough *et al.* [31] found a significant reduction of suicidal ideation in patients who were treated with ketamine relative to midazolam after 48 h but not 72 h or 7 days following treatment. In addition, based on two uncontrolled studies [28, 52], higher reductions of suicidal ideation after 12–14 days of treatment in patients who have been treated with repeated ketamine infusions over a 2-week period were observed. To the best of our knowledge, there are no studies that investigated the antidepressant potential of ketamine in subjects with prior suicide attempts or those who eventually died by suicide.

11.2.2 Safety and Tolerability of Ketamine

While the short-term safety and tolerability of ketamine have been widely demonstrated by several studies, there are only case reports or case series describing the successful long-term effects of repeated infusions of ketamine [53–56]. Indeed, there are no controlled or noncontrolled studies investigating the long-term potential of repeated ketamine infusions after the initial antidepressant response to this compound.

The abuse potential of this compound is currently the most commonly stressed long-term safety issue regarding its utilization in clinical practice. The abuse is believed to be mainly due to its dissociative properties, a well-known adverse effect related to the prolonged ketamine administration [57, 58]. There could be specific subgroups of patients who are more likely than others to develop this type of abuse/dependence (e.g., those who have a prior history of abuse/dependence with other psychoactive substances). Disturbingly, some authors [59, 60] reported the emergence of ketamine dependence with the subsequent need of its rapid escalation of use in individuals that showed an initial positive response to this compound. Kalsi *et al*. [61] also reported the existence of specific risk factors for ketamine dependence in subgroups of patients such as young adults and individuals with prior use of other illicit substances.

Some preclinical evidence raises the possibility that adverse effects, such as neurocognitive dysfunction, urinary cystitis, and adverse brain structural changes, may occur in frequent ketamine users [62–65]. However, other studies proposed that ketamine may actually enhance neurocognitive performance, although this improvement is a matter of debate. Significant improvements in visual and working memory were repeated after open-label ketamine infusions over 2 weeks in 15 TRD patients [66]. However, the improvements in depressive symptoms may mostly explain the improvements in cognition.

Nuechterlein and colleagues [67] by performing a secondary analysis of findings related to a randomized short-term antidepressant efficacy trial [68] reported neurocognitive performance using the MATRICS Consensus Cognitive Battery at baseline and at 24, 48, 72 h, and 7 days of treatment with ketamine [68]. Unfortunately, they found no differential effect of ketamine on neurocognitive performance. In addition, Murrough and colleagues [69] found that a single ketamine administration was associated with a small but significant delayed recall shortly after the initial infusion, but this effect dissipated with time. Given the mixed findings, it seems that ketamine has complex effects on neurocognitive performance, but long-term disturbances were not obvious in patients with TRD.

11.3 Other Noncompetitive NMDA Antagonists: Selective GluN2B Subunit NMDA Antagonists

Preclinical studies and clinical trials have begun to uncover the potential for both GluN2B subunit NMDA antagonists as possible novel antidepressants. Indeed, the antidepressant activity of CP-101,606, a selective GluN2B subunit antagonist, has been demonstrated by a randomized, placebo-controlled, double-blind study in patients with TRD [70]. Importantly, only mild adverse effects were in association with drug administration and resolved within 6 h. Interestingly, MK-0657a, another NMDA GluN2B selective antagonist, showed similar antidepressant efficacy after oral administration, with clinical effect emerging after 5 days for a treatment period of 12 days [71]. Furthermore, Lima-Ojeda and colleagues [72] reported that the GluN2B antagonist Ro 25-6981 prevented the neurotoxicity induced by the NMDA antagonist MK-801.

We recently published a comprehensive review [7] concerning the efficacy of glutamatergic agents in both clinical and preclinical studies. Based on this paper and the present one, it is clear that when considering the potential of selective GluN2B subunit NMDA receptor antagonists, some shortcomings/limitations should be considered. First, the small sample size that did not allow the generalization of the main results requires further additional studies with larger samples. Moreover, the long-lasting and more sustained antidepressant effects of medications such as ketamine, which shows a robust but only rapid onset of action, need to be confirmed. In addition, whether there may exist effective ketamine substitutes devoid of adverse effects remains to be determined.

11.4 Other Noncompetitive NMDA Antagonists: Glycine Binding Site Modulators

Historically, there have been suggestions of clinically meaningful psychotropic effects of D-cycloserine in MDD. Trullas and Skolnick [73], more than 20 years ago, first hypothesized the antidepressant potential of the partial agonists of the glycine site of the NMDA receptors complex using 1-aminocyclopropanecarboxylic acid. Later, antidepressant effects of higher doses of D-cycloserine in individuals with TRD were reported in a double-blind trial without any significant adverse effects [21].

In addition, because of their interaction with the NMDA receptor glycine binding site, 7-chlorokynurenic acid (7-CTKA) and tetrapeptide GLYX-13A have been proposed as medications with possible antidepressant properties. Chen *et al.* [74] also suggested the antidepressant potential of 7-CTKA, a glycine binding site NMDA antagonist having well-known neuroprotective properties. The antidepressant activity of 1-week treatment with 7-CTKA was demonstrated in a forced swimming test, along with its ability to reverse chronic stress-induced anhedonia [75].

Similarly to ketamine's effects, 7-CTKA also activated the mechanistic target of rapamycin mTOR pathway and enhanced phosphorylation of glycogen synthase kinase-3β (GSK-3β) in the medial prefrontal cortex. Furthermore, GSK-3β activation using the agonist LY294002 reduced the antidepressant potential of 7-CTKA. Bradley and colleagues [76] further suggested that the GSK-3β pathway was an important mechanism in the antidepressant activity of 7-CTKA, and importantly, in contrast to ketamine, 7-CTKA was not associated with rewarding effects in the conditioned place preference test [74].

As suggested by some researchers [76–79], there are other glycine-acting molecules such as the partial agonist GLYX-13 associated with antidepressant properties. Importantly, GLYX-13 showed modest benefits based on some phase II clinical trials and was not associated with the emergence of adverse effects. Moskal and colleagues [79] likewise reported that a single dose of GLYX-13 is able to significantly improve depressive symptoms for at least 3–7 days without psychotomimetic effects in individuals that were resistant to at least one antidepressant trial.

Despite these encouraging reports, many limitations are apparent including small sample size that did not allow the generalization of findings, the mixed

nature of the selected study populations, as well as the different criteria used worldwide to define and group treatment-resistant depressed patients [21].

11.5 AMPA Receptor Activation: A Possible Adjunctive Antidepressant Role?

Another possible strategy to circumvent the adverse effects of ketamine may involve the administration of a reduced ketamine dosage with another add-on medication having antidepressant properties or alternatively a pharmacological compound with highly selective ketamine-like mechanisms of action.

Several authors [7, 80, 81] suggested the importance of AMPA receptor activation in order to achieve the antidepressant-like effects of NMDA receptor antagonism. As repeatedly documented [82–85], the activation of AMPA receptors may be considered a critical synaptic event for the antidepressant-like activity of ketamine and other antidepressant medications, such as fluoxetine, imipramine, or tianeptine. Moreover, the AMPA/kainate receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline-2,3-dione suppressed the antidepressant-like effects evoked by the aforementioned glycine partial agonist GLYX-13 [77, 85, 86]. Yet, the antidepressant-like activity of CGP 37849, an NMDA receptors antagonist in the forced swimming test, was not reversed by the pretreatment with the AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*] quinoxaline-2,3-dione [87].

Akinfiresoye and Tizabi [88] reported the successful combined antidepressant effects of AMPA modulators after chronic treatment with subclinical ketamine doses. This was associated with significantly elevated Brain-derived neurotrophic factor BDNF and synapsin I levels, along with mTOR activation in the hippocampus [88]. Accordingly, the administration of LY392098, an AMPA receptor-positive allosteric modulator, reversed the reduced weight loss and immobility in the tail suspension test in mice. Conversely, Farley and colleagues [89] found that the antidepressant effects associated with chronic fluoxetine treatment may be attenuated with AMPA receptor antagonists. Wolak and coworkers [90] also highlighted the link between AMPA receptor-positive allosteric modulation and antidepressant-like activity of currently available medications such as imipramine and reboxetine in the forced swimming test. Unfortunately, studies with Org 26775, an AMPA receptor-positive allosteric modulator, did not demonstrate significant differences between the tested drug and placebo in depressed patients. As suggested by some other investigators [91, 92], a possible bias may be related to the main targets of these studies, which were performed predominantly based on dose-dependent safety and tolerability and not primarily the antidepressant efficacy.

Another means of facilitating AMPA receptor-induced antidepressant actions might be to modify the surface expression of GluA1 and GluA2 AMPA receptor subunits, as is evident with anticonvulsant medications such as lamotrigine and riluzole. Indeed, various animal studies confirmed the antidepressant-like activities of both lamotrigine and riluzole. For instance, riluzole administration was found to reduce the hyperemotional response in olfactory bulbectomized rats and attenuate the anhedonia induced by chronic unpredictable stress [93, 94].

Li *et al*. [95, 96] additionally demonstrated the antidepressant-like activity of lamotrigine in the chronic unpredictable stress paradigm together with increased BDNF after its chronic administration. Du and colleagues [97] suggested that the anticonvulsant effects of these drugs stem from the inhibition of the voltage-gated sodium channels but that their antidepressant effects may be aligned with astroglial glutamate uptake and expression of the glutamate excitatory amino acid transporter [98].

These preclinical results have been confirmed by many clinical studies that demonstrated the antimanic and antidepressant effects of lamotrigine in bipolar disorder [99–101]. Some open-label and placebo-controlled studies [98, 102] demonstrated a significant reduction of depressive symptoms in patients with TRD, as did various randomized controlled trials [103, 104]. Some reports [105, 106] focused on the efficacy of riluzole to reduce depressive symptoms in patients with major depression and bipolar disorder. According to a recent double-blind, placebo-controlled study of Ibrahim and colleagues [107], patients with TRD who received a single dose of ketamine and riluzole 6 h after ketamine administration for a period of 4 weeks showed a significant reduction of depressive symptoms, despite the difference between riluzole and placebo treatment being not significant [107].

Overall, AMPA receptor activation may be presumably identified as a very promising route to develop new and alternative antidepressant strategies for both major depression and TRD; however, additional longitudinal studies will be required in order to better characterize and translate preliminary results into clinical practice.

11.6 Discussion and Future Directions

TRD is a very disabling condition and its pathogenesis is still, to date, largely unclear. The resistance to most commonly available medications, the delayed onset of action even in the best of outcomes, and the emergence of adverse effects represent the most crucial barriers of modern pharmacotherapy of depression. In recent years, a possible role for glutamate in the pathophysiology of depression has been suggested and presents a novel shift in treatment strategies. Indeed, glutamatergic-acting drugs may be the next generation of novel and specific pharmacological strategy for TRD. Hence, the antidepressant activity of highly promising glutamatergic agents such as ketamine and CP-101,606 has been proposed [7].

The administration of ketamine in patients with TRD may provide several advantages in clinical practice [108]. First, according to the most relevant preclinical and clinical evidence, a single dose of ketamine is able to exert a rapid antidepressant activity (within hours). The effects of this innovative compound have been also described in individuals who were commonly refractory to conventional antidepressant medications. Furthermore, ketamine is a valid treatment option for subjects with TRD not only according to its advantages in terms of rapid onset of action (e.g., in most patients after a single infusion) but also for its effects on core depressive symptoms such as hopelessness [109], which is widely recognized as a relevant risk factor for suicidal behavior.

Unfortunately, ketamine administration has several shortcomings. First, transient psychoactive/hemodynamic adverse effects such as dissociation, blurred vision, dizziness, headache, anxiety, and irritability have been reported after ketamine infusion. Cardiorespiratory monitoring needs to be considered a critical component of risk management given the possible emergence of hemodynamic changes related to ketamine administration. Importantly, abuse liability and dependence have been also described [110, 111].

Another relevant caveat associated with ketamine utilization in the clinical practice is represented by the fact that the main molecular mechanisms underlying its antidepressant activity are only partially clarified. The most plausible and commonly shared hypothesis indicates the involvement of multiple intracellular pathways induced by ketamine administration with the final result of enhanced synaptogenesis. Indeed, ketamine is able to rapidly reverse behavioral and neuronal associated changes induced by chronic stress maybe due to its ability to enhance BDNF levels [112]. Ketamine is also a rapid neuroplastic modulator agent due to its potential to enhance the mTOR-dependent synapse formation [85]. In addition, ketamine usually consists of two enantiomers, (*R*)- and (*S*)-, and it is predominantly metabolized in norketamine, which is an active ketamine metabolite. Based on some existing evidence [113, 114], (*S*)-ketamine is nearly four times more active due to its better pharmacokinetic properties and enhanced tolerability when compared with (*R*)-enantiomer. Importantly, less psychotomimetic side effects have been reported with (*S*)-ketamine administration.

According to existing evidence, further noncompetitive antagonists of the NMDA receptors displayed antidepressant-like activity in both animal and human models of depression. There are both preclinical evidence and clinical studies suggesting the antidepressant potential of specific NMDA receptor antagonists such as MK-0657 or CP-101,606, Glyx-13, and lanicemine. These novel agents may offer a new strategic opportunity; however, unlike ketamine, the antidepressant potential associated with the use of these medications emerges only after few days from its administration.

Other possible strategies may involve the co-administration of reduced ketamine doses with other add-on medications having antidepressant-like properties (for review see [115]). For instance, the co-administration of ketamine with AMPA receptor modulators may be considered. In fact, we know that the antagonism at specific NMDA receptor subunits may be associated with antidepressant effects via AMPA receptors [116]. The ideal glutamatergic compound associated with antidepressant activity might be achieved through both the inhibition of NMDA receptors and the enhancement of AMPA receptors; thus, a dynamic interplay between these two types of receptors may maximize benefits.

11.7 Concluding Remarks

Overall, the application of NMDA receptor antagonists for treating refractory major depression will require further studies aimed at refining how these agents alone and when co-administered with complementary drugs might increase clinical efficacy

while reducing the likelihood of adverse effects. Given the numerous mechanisms that underlie the multitude of symptoms evident with severe depression, it is likely that the most efficacious strategies will involve the co-administration of highly specialized agents that target parallel brain processes. For instance, the co-application of immune regulatory proteins that have trophic effects could be useful, particularly for depressive cases with marked fatigue and general malaise [117]. It is even probable that such drugs might act on non-neuronal targets, most notably glia and immune cells that have been implicated in several aspects of depression. Whatever the case, more personalized approaches will be the way of the future.

Abbreviations

AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
7-CTKA	7-chlorokynurenic acid
GSK-3β	glycogen synthase kinase-3β
MDD	major depressive disorder
NMDA	*N*-methyl-D-aspartate
TRD	treatment-resistant depression

References

1 Soleimani, L., Lapidus, K.A., and Iosifescu, D.V. (2011) Diagnosis and treatment of major depressive disorder. *Neurol. Clin.*, **29**, 177–179.

2 Langlieb, A.M. and Guico-Pabia, C.J. (2010) Beyond symptomatic improvement: assessing real-world outcomes in patients with major depressive disorder. *Prim. Care Companion J. Clin. Psychiatry*, **12**, 1–12.

3 Greer, T.L., Kurian, B.T., and Trivedi, M.H. (2010) Defining and measuring functional recovery from depression. *CNS Drugs*, **24**, 267–284.

4 Pompili, M., Iliceto, P., Luciano, D., Innamorati, M., Serafini, G., Del Casale, A., Tatarelli, R., Girardi, P., and Lester, D. (2011) Higher hopelessness and suicide risk predict lower self-deception among psychiatric patients and non-clinical individuals. *Riv. Psichiatr.*, **46**, 24–30.

5 Serafini, G., Pompili, M., Innamorati, M., Giordano, G., Montebovi, F., Sher, L., Dwivedi, Y., and Girardi, P. (2012) The role of microRNAs in synaptic plasticity, major affective disorders and suicidal behavior. *Neurosci. Res.*, **73**, 179–190.

6 Serafini, G., Pompili, M., Innamorati, M., Fusar-Poli, P., Akiskal, H.S., Rihmer, Z., Lester, D., Romano, A., de Oliveira, I.R., Strusi, L., Ferracuti, S., Girardi, P., and Tatarelli, R. (2011) Affective temperamental profiles are associated with white matter hyperintensity and suicidal risk in patients with mood disorders. *J. Affect. Disord.*, **129**, 47–55.

7 Serafini, G., Pompili, M., Innamorati, M., Dwivedi, Y., Brahmachari, G., and Girardi, P. (2013) Pharmacological properties of glutamatergic drugs targeting NMDA receptors and their application in major depression. *Curr. Pharm. Des.*, **19**, 1898–1922.

8 Fava, M., Rush, A.J., Wisniewski, S.R., Nierenberg, A.A., Alpert, J.E., McGrath, P.J., Thase, M.E., Warden, D., Biggs, M., Luther, J.F., Niederehe, G., Ritz, L., and Trivedi, M.H. (2006) A comparison of mirtazapine and nortriptyline following two consecutive failed medication treatments for depressed outpatients: a STAR*D report. *Am. J. Psychiatry*, **163**, 1161–1172.

9 Petersen, T., Gordon, J.A., Kant, A., Fava, M., Rosenbaum, J.F., and Nierenberg, A.A. (2001) Treatment resistant depression and axis I comorbidity. *Psychol. Med.*, **31**, 1223–1239.

10 Holtzheimer, P.E. and Mayberg, H.S. (2011) Stuck in a rut: rethinking depression and its treatment. *Trends Neurosci.*, **34**, 1–9.

11 Warden, D., Rush, A.J., Trivedi, M.H., Fava, M., and Wisniewski, S.R. (2007) STAR*D project results: comprehensive review of findings. *Curr. Psychiatry Rep.*, **9**, 449–459.

12 Crown, W.H., Finkelstein, S., Berndt, E.R., Ling, D., Poret, A.W., Rush, A.J., and Russell, J.M. (2002) The impact of treatment resistant depression on health care utilization and costs. *J. Clin. Psychiatry*, **63**, 963–971.

13 Rush, A.J., Trivedi, M.H., Wisniewski, S.R., Nierenberg, A.A., Stewart, J.W., Warden, D., Niederehe, G., Thase, M.E., Lavori, P.W., Lebowitz, B.D., McGrath, P.J., Rosenbaum, J.F., Sackeim, H.A., Kupfer, D.J., Luther, J., and Fava, M. (2006) Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am. J. Psychiatry*, **163**, 1905–1917.

14 Trivedi, M.H., Fava, M., Wisniewski, S.R., Thase, M.E., Quitkin, F., Warden, D., Ritz, L., Nierenberg, A.A., Lebowitz, B.D., Biggs, M.M., Luther, J.F., Shores-Wilson, K., and Rush, A.J. (2006) STAR*D Study Team: medication augmentation after the failure of SSRIs for depression. *N. Engl. J. Med.*, **354**, 1243–1252.

15 Murrough, J.W. and Charney, D.S. (2012) Is there anything really novel on the antidepressant horizon? *Curr. Psychiatry Rep.*, **14**, 643–649.

16 Belmaker, R.H. (2008) The future of depression psychopharmacology. *CNS Spectr.*, **13**, 682–687.

17 Rush, A.J., Trivedi, M.H., Wisniewski, S.R., Stewart, J.W., Nierenberg, A.A., Thase, M.E., Ritz, L., Biggs, M.M., Warden, D., Luther, J.F., Shores-Wilson, K., Niederehe, G., Fava, M., and STAR*D Study Team (2006) Bupropion-SR, sertraline, or venlafaxine- XR after failure of SSRIs for depression. *N. Engl. J. Med.*, **354**, 1231–1242.

18 Lopez, A.D., Mathers, C.D., Ezzati, M., Jamison, D.T., and Murray, C.J. (2006) Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *Lancet*, **367**, 1747–1757.

19 Crane, G.E. (1959) Cycloserine as an antidepressant agent. *Am. J. Psychiatry*, **115**, 1025–1056.

20 Crane, G.E. (1961) The psychotropic effects of cycloserine: a new use for an antibiotic. *Compr. Psychiatry*, **2**, 51–59.

21 Heresco-Levy, U., Gelfin, G., Bloch, B., Levin, R., Edelman, S., Javitt, D.C., and Kremer, I. (2013) A randomized add-on trial of high dose D-cycloserine for treatment-resistant depression. *Int. J. Neuropsychopharmacol.*, **16** (3), 501–506.

22 Zarate, C.A. Jr. and Niciu, M.J. (2015) Ketamine for depression: evidence, challenges and promise. *World Psychiatry*, **14**, 358–360.

23 Ketamine Advocacy Network http://www.ketamine advocacynetwork.org/ (accessed 29 December 2015).

24 Hamilton, J. (2015) Club Drug Ketamine Gains Traction as a Treatment for Depression. National Public Radio. http://www.npr.org/sections/health-shots/2015/09/28/443203592/clubdrug-ketamine-gains-traction-as-a-treatment-for-depression (accessed 29 December 2015).

25 Pollack, A. and Special, K. (2014) A Hallucinogen, Raises Hopes and Concerns as a Treatment for Depression. The New York Times. http://www.nytimes.com/2014/12/10/business/special-k-ahallucinogen- raises-hopes-and-concerns-as-a-treatment-fordepression. html?_r = 0 (accessed 29 December 2015).

26 Ghasemi, M., Kazemi, M.H., Yoosefi, A., Ghasemi, A., Paragomi, P., Amini, H., and Afzali, M.H. (2014) Rapid antidepressant effects of repeated doses of ketamine compared with electroconvulsive therapy in hospitalized patients with major depressive disorder. *Psychiatry Res.*, **215** (2), 355–361.

27 DiazGranados, N., Ibrahim, L.A., Brutsche, N.E., Ameli, R., Henter, I.D., Luckenbaugh, D.A., Machado-Vieira, R., and Zarate, C.A. Jr. (2010) Rapid resolution of suicidal ideation after a single infusion of an NMDA antagonist in patients with treatment-resistant major depressive disorder. *J. Clin. Psychiatry*, **71**, 1605–1611.

28 Price, R.B., Nock, M., Charney, D.S., and Matthew, S.J. (2009) Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. *Biol. Psychiatry*, **66**, 522–526.

29 Zarate, C.A. Jr., Brutsche, N.E., Ibrahim, L., Franco-Chaves, J., Diazgranados, N., Cravchik, A., Selter, J., Marquardt, C.A., Liberty, V., and Luckenbaugh, D.A. (2012) Replication of ketamine's antidepressant efficacy in bipolar depression: a randomized controlled add-on trial. *Biol. Psychiatry*, **71**, 939–946.

30 Ballard, E.D., Ionescu, D.F., Vande Voort, J.L., Niciu, M.J., Richards, E.M., Luckenbaugh, D.A., Brutsché, N.E., Ameli, R., Furey, M.L., and Zarate, C.A. Jr. (2014) Improvement in suicidal ideation after ketamine infusion: relationship to reductions in depression and anxiety. *J. Psychiatr. Res.*, **58**, 161–166.

31 Murrough, J.W., Soleimani, L., DeWilde, K.E., Collins, K.A., Lapidus, K.A., Iacoviello, B.M., Lener, M., Kautz, M., Kim, J., Stern, J.B., Price, R.B., Perez, A.M., Brallier, J.W., Rodriguez, G.J., Goodman, W.K., Iosifescu, D.V., and Charney, D.S. (2015) Ketamine for rapid reduction of suicidal ideation: a randomized controlled trial. *Psychol. Med.*, **45**, 3571–3580.

32 Reinstatler, L. and Youssef, N.A. (2015) Ketamine as a potential treatment for suicidal ideation: a systematic review of the literature. *Drugs R D*, **15**, 37–43.

33 Griffiths, J.J., Zarate, C.A., and Rasimas, J.J. (2015) Existing and novel biological therapeutics in suicide prevention. *Am. J. Prev. Med.*, **47** (3, Suppl. 2), S195–S203.

34 Diamond, P.R., Farmery, A.D., Atkinson, S., Haldar, J., Williams, N., Cowen, P.J., Geddes, J.R., and McShane, R. (2014) Ketamine infusions for treatment resistant depression: a series of 28 patients treated weekly or twice weekly in an ECT clinic. *J. Psychopharmacol.*, **28**, 536–544.

35 Krystal, J.H., Perry, E.B. Jr., Gueorguieva, R., Belger, A., Madonick, S.H., Abi-Dargham, A., Cooper, T.B., Macdougall, L., Abi-Saab, W., and D'Souza, D.C. (2005) Comparative and interactive human psychopharmacologic effects of

ketamine and amphetamine: implications for glutamatergic and dopaminergic model psychoses and cognitive function. *Arch. Gen. Psychiatry*, **62**, 985–994.

36 Smith, G.S., Schloesser, R., Brodie, J.D., Dewey, S.L., Logan, J., Vitkun, S.A., Simkowitz, P., Hurley, A., Cooper, T., Volkow, N.D., and Cancro, R. (1998) Glutamate modulation of dopamine measured in vivo with positron emission tomography (PET) and 11C-raclopride in normal human subjects. *Neuropsychopharmacology*, **18**, 18–25.

37 Vollenweider, F.X., Vontobel, P., Oye, I., Hell, D., and Leenders, K.L. (2000) Effects of (S)-ketamine on striatal dopamine: a [11C] raclopride PET study of a model psychosis in humans. *J. Psychiatr. Res.*, **34**, 35–43.

38 Howland, R.H. (2013) Ketamine for the treatment of depression. *J. Psychosoc. Nurs. Ment. Health Serv.*, **51**, 11–14.

39 Xu, Y., Hackett, M., Carter, G., Loo, C., Gálvez, V., Glozier, N., Glue, P., Lapidus, K., McGirr, A., Somogyi, A.A., Mitchell, P.B., and Rodgers, A. (2015) Effects of low-dose and very low-dose ketamine among patients with major depression: a systematic review and meta-analysis. *Int. J. Neuropsychopharmacol.*, **19** (4).

40 Newport, D.J., Carpenter, L.L., McDonald, W.M., Potash, J.B., Tohen, M., Nemeroff, C.B., and APA Council of Research Task Force on Novel Biomarkers and Treatments (2015) Ketamine and other NMDA antagonists: early clinical trials and possible mechanisms in depression. *Am. J. Psychiatry*, **172**, 950–966.

41 McGirr, A., Berlim, M.T., Bond, D.J., Fleck, M.P., Yatham, L.N., and Lam, R.W. (2015) A systematic review and meta-analysis of randomized, double-blind, placebo-controlled trials of ketamine in the rapid treatment of major depressive episodes. *Psychol. Med.*, **45**, 693–704.

42 Lee, E.E., Della Selva, M.P., Liu, A., and Himelhoch, S. (2015) Ketamine as a novel treatment for major depressive disorder and bipolar depression: a systematic review and quantitative meta-analysis. *Gen. Hosp. Psychiatry*, **37**, 178–184.

43 Romeo, B., Choucha, W., Fossati, P., and Rotge, J.Y. (2015) Meta-analysis of short- and mid-term efficacy of ketamine in unipolar and bipolar depression. *Psychiatry Res.*, **230**, 682–688.

44 Coyle, C.M. and Laws, K.R. (2015) The use of ketamine as an antidepressant: a systematic review and meta-analysis. *Hum. Psychopharmacol.*, **30**, 152–163.

45 Parsaik, A.K., Singh, B., Khosh-Chashm, D., and Mascarenhas, S.S. (2015) Efficacy of ketamine in bipolar depression: systematic review and meta-analysis. *J. Psychiatr. Pract.*, **21**, 427–435.

46 Fond, G., Loundou, A., Rabu, C., Macgregor, A., Lançon, C., Brittner, M., Micoulaud-Franchi, J.A., Richieri, R., Courtet, P., Abbar, M., Roger, M., Leboyer, M., and Boyer, L. (2014) Ketamine administration in depressive disorders: a systematic review and meta-analysis. *Psychopharmacology* (Berl), **231**, 3663–3676.

47 Caddy, C., Giaroli, G., White, T.P., Shergill, S.S., and Tracy, D.K. (2014) Ketamine as the prototype glutamatergic antidepressant: pharmacodynamics actions, and a systematic review and meta-analysis of efficacy. *Ther. Adv. Psychopharmacol.*, **4**, 75–99.

48 Berman, R.M., Cappiello, A., Anand, A., Oren, D.A., Heninger, G.R., Charney, D.S., and Krystal, J.H. (2000) Antidepressant effects of ketamine in depressed patients. *Biol. Psychiatry*, **47**, 351–354.

49 Zarate, C.A. Jr., Singh, J.B., Carlson, P.J., Brutsche, N.E., Ameli, R., Luckenbaugh, D.A., Charney, D.S., and Manji, H.K. (2006) A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch. Gen. Psychiatry*, **63**, 856–864.

50 aan het Rot, M., Collins, K.A., Murrough, J.W., Perez, A.M., Reich, D.L., Charney, D.S., and Mathew, S.J. (2010) Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. *Biol. Psychiatry*, **67**, 139–145.

51 Murrough, J.W., Perez, A.M., Pillemer, S., Stern, J., Parides, M.K., aan het Rot, M., Collins, K.A., Mathew, S.J., Charney, D.S., and Iosifescu, D.V. (2012) Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment resistant major depression. *Biol. Psychiatry*, **74**, 250–256.

52 Rasmussen, K.G., Lineberry, T.W., Galardy, C.W., Kung, S., Lapid, M.I., Palmer, B.A., Ritter, M.J., Schak, K.M., Sola, C.L., Hanson, A.J., and Frye, M.A. (2013) Serial infusions of low-dose ketamine for major depression. *J. Psychopharmacol.*, **27**, 444–450.

53 Cusin, C., Hilton, G.Q., Nierenberg, A.A., and Fava, M. (2012) Long-term maintenance with intramuscular ketamine for treatment-resistant bipolar II depression. *Am. J. Psychiatry*, **169**, 868–869.

54 Messer, M. and Haller, I.V. (2010) Maintenance ketamine treatment produces long-term recovery from depression. *Prim. Psychiatry*, **17**, 48–50.

55 Segmiller, F., Rüther, T., Linhardt, A., Padberg, F., Berger, M., Pogarell, O., Möller, H.J., Kohler, C., and Schüle, C. (2013) Repeated S-ketamine infusions in therapy resistant depression: a case series. *J. Clin. Pharmacol.*, **53**, 996–998.

56 Szymkowicz, S.M., Finnegan, N., and Dale, R.M. (2013) A 12-month naturalistic observation of three patients receiving repeat intravenous ketamine infusions for their treatment-resistant depression. *J. Affect. Disord.*, **147**, 416–420.

57 Lodge, D. and Mercier, M.S. (2015) Ketamine and phencyclidine: the good, the bad and the unexpected. *Br. J. Pharmacol.*, **172**, 4254–4276.

58 Bobo, W.V. and Miller, S.C. (2002) Ketamine as a preferred substance of abuse. *Am. J. Addict.*, **11**, 332–334.

59 Schak, K.M., Vande Voort, J.L., Johnson, E.K., Kung, S., Leung, J.G., Rasmussen, K.G., Palmer, B.A., and Frye, M.A. (2016) Potential risks of poorly monitored ketamine use in depression treatment. *Am. J. Psychiatry*, **173**, 215–218.

60 Liu, J.X., Zerbo, E., and Ross, S. (2015) Intensive ketamine use for multiple years: a case report. *Am. J. Addict.*, **24**, 7–9.

61 Kalsi, S.S., Wood, D.M., and Dargan, P.I. (2011) The epidemiology and patterns of acute and chronic toxicity associated with recreational ketamine use. *Emerg. Health Threats J.*, **4**, 7107.

62 Morgan, C.J., Muetzelfeldt, L., and Curran, H.V. (2010) Consequences of chronic ketamine self-administration upon neurocognitive function and psychological wellbeing: a 1-year longitudinal study. *Addiction*, **105**, 121–133.

63 Wood, D. (2013) Ketamine and damage to the urinary tract. *Addiction*, **108**, 1515–1516.

64 Wang, C., Zheng, D., Xu, J., Lam, W., and Yew, D.T. (2013) Brain damages in ketamine addicts as revealed by magnetic resonance imaging. *Front. Neuroanat.*, **7**, 23.

65 Liao, Y., Tang, J., Fornito, A., Liu, T., Chen, X., Chen, H., Xiang, X., Wang, X., and Hao, W. (2012) Alterations in regional homogeneity of resting-state brain activity in ketamine addicts. *Neurosci. Lett.*, **522**, 36–40.

66 Shiroma, P.R., Albott, C.S., Johns, B., Thuras, P., Wels, J., and Lim, K.O. (2014) Neurocognitive performance and serial intravenous subanesthetic ketamine in treatment resistant depression. *Int. J. Neuropsychopharmacol.*, **17**, 1805–1813.

67 Nuechterlein, K.H., Green, M.F., Kern, R.S., Baade, L.E., Barch, D.M., Cohen, J.D., Essock, S., Fenton, W.S., Frese, F.J. III, Gold, J.M., Goldberg, T., Heaton, R.K., Keefe, R.S., Kraemer, H., Mesholam-Gately, R., Seidman, L.J., Stover, E., Weinberger, D.R., Young, A.S., Zalcman, S., and Marder, S.R. (2008) The MATRICS consensus cognitive battery, part 1: test selection, reliability, and validity. *Am. J. Psychiatry*, **165**, 203–213.

68 Murrough, J.W., Burdick, K.E., Levitch, C.F., Perez, A.M., Brallier, J.W., Chang, L.C., Foulkes, A., Charney, D.S., Mathew, S.J., and Iosifescu, D.V. (2015) Neurocognitive effects of ketamine and association with antidepressant response in individuals with treatment-resistant depression: a randomized controlled trial. *Neuorpsychopharmacology*, **40**, 1084–1090.

69 Murrough, J.W., Wan, L.B., Iacoviello, B., Collins, K.A., Solon, C., Glicksberg, B., Perez, A.M., Mathew, S.J., Charney, D.S., Iosifescu, D.V., and Burdick, K.E. (2013) Neurocognitive effects of ketamine in treatment-resistant major depression: association with antidepressant response. *Psychopharmacology* (Berl) (in press).

70 Preskorn, S.H., Baker, B., Kolluri, S., Menniti, F.S., Krams, M., and Landen, J.W. (2008) An Innovative design to establish proof of concept of the antidepressant effects of the NR2B subunit selective n-methyl-d-aspartate antagonist, CP-101,606, in patients with treatment-refractory major depressive disorder. *J. Clin. Psychopharmacol.*, **28** (6), 631–637.

71 Ibrahim, L., Diaz Granados, N., Jolkovsky, L., Brutsche, N., Luckenbaugh, D.A., Herring, W.J., Potter, W.Z., and Zarate, C.A. Jr. (2012) A randomized, placebo-controlled, crossover pilot trial of the oral selective NR2B antagonist MK-0657 in patients with treatment-resistant major depressive disorder. *J. Clin. Psychopharmacol.*, **32** (4), 551–557.

72 Lima-Ojeda, J.M., Vogt, M.A., Pfeiffer, N., Dormann, C., Köhr, G., Sprengel, R., Gass, P., and Inta, D. (2013) Pharmacological blockade of GluN2B-containing NMDA receptors induces antidepressant-like effects lacking psychotomimetic action and neurotoxicity in the perinatal and adult rodent brain. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **45**, 28–33.

73 Trullas, R. and Skolnick, P. (1990) Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *Eur. J. Pharmacol.*, **185**, 1–10.

74 Chen, J., Graham, S., Moroni, F., and Simon, R. (1993) A study of the dose dependency of a glycine receptor antagonist in focal ischemia. *J. Pharmacol. Exp. Ther.*, **267**, 937–941.

75 Zhu, W.L., Wang, S.J., Liu, M.M., Shi, H.S., Zhang, R.X., Liu, J.F., Ding, Z.B., and Lu, L. (2013) Glycine site N-methyl-D-aspartate receptor antagonist 7-CTKA produces rapid antidepressant-like effects in male rats. *J. Psychiatry Neurosci.*, **38**, 306–316.

76 Bradley, C.A., Peineau, S., Taghibiglou, C., Nicolas, C.S., Whitcomb, D.J., Bortolotto, Z.A., Kaang, B.K., Cho, K., Wang, Y.T., and Collingridge, G.L. (2012) A pivotal role of GSK-3 in synaptic plasticity. *Front. Mol. Neurosci.*, **5**, 13.

77 Burgdorf, J., Zhang, X.L., Nicholson, K.L., Balster, R.L., Leander, J.D., Stanton, P.K., Gross, A.L., Kroes, R.A., and Moskal, J.R. (2013) GLYX-13, a NMDA receptor glycine-site functional partial agonist, induces antidepressant-like effects without ketamine-like side effects. *Neuropsychopharmacology*, **38**, 729–742.

78 Burgdorf, J., Zhang, X.L., Weiss, C., Matthews, E., Disterhoft, J.F., Stanton, P.K., and Moskal, J.R. (2011) The N-methyl-D-aspartate receptor modulator GLYX-13 enhances learning and memory, in young adult and learning impaired aging rats. *Neurobiol. Aging*, **32** (4), 698–706.

79 Moskal, J.R., Burch, R., Burgdorf, J.S., Kroes, R.A., Stanton, P.K., Disterhoft, J.F., and Leander, J.D. (2014) GLYX-13, an NMDA receptor glycine site functional partial agonist enhances cognition and produces antidepressant effects without the psychotomimetic side effects of NMDA receptor antagonists. *Expert Opin. Investig. Drugs*, **23** (2), 243–254.

80 Sanderson, T.M., Collingridge, G.L., and Fitzjohn, S.M. (2011) Differential trafficking of AMPA receptors following activation of NMDA receptors and mGluRs. *Mol. Brain*, **4**, 30.

81 Unoki, T., Matsuda, S., Kakegawa, W., Van, N.T., Kohda, K., Suzuki, A., Funakoshi, Y., Hasegawa, H., Yuzaki, M., and Kanaho, Y. (2012) NMDA receptor-mediated PIP5K activation to produce PI(4,5)P2 is essential for AMPA receptor endocytosis during LTD. *Neuron*, **73**, 135–148.

82 Svenningsson, P., Tzavara, E.T., Witkin, J.M., Fienberg, A.A., Nomikos, G.G., and Greengard, P. (2002) Involvement of striatal and extrastriatal DARPP-32 in biochemical and behavioral effects of fluoxetine (Prozac). *Proc. Natl. Acad. Sci. U.S.A.*, **99**, 3182–3187.

83 Du, J., Creson, T.K., Wu, L.J., Ren, M., Gray, N.A., Falke, C., Wei, Y., Wang, Y., Blumenthal, R., Machado-Vieira, R., Yuan, P., Chen, G., Zhuo, M., and Manji, H.K. (2008) The role of hippocampal GluR1 and GluR2 receptors in manic-like behavior. *J. Neurosci.*, **28**, 68–77.

84 Du, J., Gray, N.A., Falke, C.A., Chen, W., Yuan, P., Szabo, S.T., Einat, H., and Manji, H.K. (2004) Modulation of synaptic plasticity by antimanic agents: the role of AMPA glutamate receptor subunit 1 synaptic expression. *J. Neurosci.*, **24**, 6578–6589.

85 Li, N., Lee, B., Liu, R.J., Banasr, M., Dwyer, J.M., Iwata, M., Li, X.Y., Aghajanian, G., and Duman, R.S. (2010) mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science*, **329**, 959–964.

86 Maeng, S., Zarate, C.A. Jr., Du, J., Schloesser, R.J., McCammon, J., Chen, G., and Manji, H.K. (2008) Cellular mechanisms underlying the antidepressant effects of ketamine: role of a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol. Psychiatry*, **63**, 349–352.

87 Dybała, M., Siwek, A., Poleszak, E., Pilc, A., and Nowak, G. (2008) Lack of NMDA-AMPA interaction in antidepressant-like effect of CGP 37849, an antagonist of NMDA receptor, in the forced swim test. *J. Neural Transm.* (Vienna), **115**, 1519–1520.

88 Akinfiresoye, L. and Tizabi, Y. (2013) Antidepressant effects of AMPA and ketamine combination: role of hippocampal BDNF, synapsin, and mTOR. *Psychopharmacology* (Berl), **230**, 291–298.
89 Farley, S., Apazoglou, K., Witkin, J.M., Giros, B., and Tzavara, E.T. (2010) Antidepressant-like effects of an AMPA receptor potentiator under a chronic mild stress paradigm. *Int. J. Neuropsychopharmacol.*, **13**, 1207–1218.
90 Wolak, M., Siwek, A., Szewczyk, B., Poleszak, E., Pilc, A., Popik, P., and Nowak, G. (2013) Involvement of NMDA and AMPA receptors in the antidepressant like activity of antidepressant drugs in the forced swim test. *Pharmacol. Rep.*, **65**, 991–997.
91 Nations, K.R., Bursi, R., Dogterom, P., Ereshefsky, L., Gertsik, L., Mant, T., and Schipper, J. (2012) Maximum tolerated dose evaluation of the AMPA modulator org 26576 in healthy volunteers and depressed patients: a summary and method analysis of bridging research in support of phase II dose selection. *Drugs R D*, **12**, 127–139.
92 Nations, K.R., Dogterom, P., Bursi, R., Schipper, J., Greenwald, S., Zraket, D., Gertsik, L., Johnstone, J., Lee, A., Pande, Y., Ruigt, G., and Ereshefsky, L. (2012) Examination of Org 26576, an AMPA receptor positive allosteric modulator, in patients diagnosed with major depressive disorder: an exploratory, randomized, double-blind, placebo controlled trial. *J. Psychopharmacol.*, **26**, 1525–1539.
93 Takahashi, K., Murasawa, H., Yamaguchi, K., Yamada, M., Nakatani, A., Yoshida, M., Iwai, T., Inagaki, M., Yamada, M., and Saitoh, A. (2011) Riluzole rapidly attenuates hyperemotional responses in olfactory bulbectomized rats, an animal model of depression. *Behav. Brain Res.*, **216**, 46–52.
94 Banasr, M., Chowdhury, G.M., Terwilliger, R., Newton, S.S., Duman, R.S., Behar, K.L., and Sanacora, G. (2010) Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate modulating drug riluzole. *Mol. Psychiatry*, **15**, 501–511.
95 Li, N., He, X., Zhang, Y., Qi, X., Li, H., Zhu, X., and He, S. (2011) Brain-derived neurotrophic factor signalling mediates antidepressant effects of lamotrigine. *Int. J. Neuropsychopharmacol.*, **14**, 1091–1098.
96 Li, N., Liu, R.J., Dwyer, J.M., Banasr, M., Lee, B., Son, H., Li, X.Y., Aghajanian, G., and Duman, R.S. (2011) Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biol. Psychiatry*, **69**, 754–761.
97 Du, J., Suzuki, K., Wei, Y., Wang, Y., Blumenthal, R., Chen, Z., Falke, C., Zarate, C.A. Jr., and Manji, H.K. (2007) The anticonvulsants lamotrigine, riluzole, and valproate differentially regulate AMPA receptor membrane localization: relationship to clinical effects in mood disorders. *Neuropsychopharmacology*, **32**, 793–802.
98 Dall'Igna, O.P., Bobermin, L.D., Souza, D.O., and Quincozes-Santos, A. (2013) Riluzole increases glutamate uptake by cultured C6 astroglial cells. *Int. J. Dev. Neurosci.*, **31**, 482–486.
99 Gabriel, A. (2006) Lamotrigine adjunctive treatment in resistant unipolar depression: an open, descriptive study. *Depress. Anxiety*, **23**, 485–488.
100 Calabrese, J.R., Suppes, T., Bowden, C.L., Sachs, G.S., Swann, A.C., McElroy, S.L., Kusumakar, V., Ascher, J.A., Earl, N.L., Greene, P.L., and Monaghan, E.T.

(2000) A double-blind, placebo-controlled, prophylaxis study of lamotrigine in rapid-cycling bipolar disorder. *J. Clin. Psychiatry*, **61**, 841–850.

101 Goodwin, G.M., Bowden, C.L., Calabrese, J.R., Grunze, H., Kasper, S., White, R., and Greene, P. (2004) Leadbetter R. A pooled analysis of 2 placebo-controlled 18-month trials of lamotrigine and lithium maintenance in bipolar I disorder. *J. Clin. Psychiatry*, **65**, 432–441.

102 Frye, M.A., Ketter, T.A., Kimbrell, T.A., Dunn, R.T., Speer, A.M., Osuch, E.A., Luckenbaugh, D.A., Cora-Ocatelli, G., and Leverich, G.S. (2000) Post RM.A placebo-controlled study of lamotrigine and gabapentin monotherapy in refractory mood disorders. *J. Clin. Psychopharmacol.*, **20**, 607–614.

103 Normann, C., Hummel, B., Schärer, L.O., Hörn, M., Grunze, H., and Walden, J. (2002) Lamotrigine as adjunct to paroxetine in acute depression: a placebo controlled, double-blind study. *J. Clin. Psychiatry*, **63**, 337–344.

104 Barbosa, L., Berk, M., and Vorster, M. (2003) A double-blind, randomized, placebo-controlled trial of augmentation with lamotrigine or placebo in patients concomitantly treated with fluoxetine for resistant major depressive episodes. *J. Clin. Psychiatry*, **64**, 403–407.

105 Zarate, C.A. Jr., Payne, J.L., Quiroz, J., Sporn, J., Denicoff, K.K., Luckenbaugh, D., Charney, D.S., and Manji, H.K. (2004) An open-label trial of riluzole in patients with treatment-resistant major depression. *Am. J. Psychiatry*, **161**, 171–174.

106 Brennan, B.P., Hudson, J.I., Jensen, J.E., McCarthy, J., Roberts, J.L., Prescot, A.P., Cohen, B.M., Pope, H.G. Jr., Renshaw, P.F., and Ongür, D. (2010) Rapid enhancement of glutamatergic neurotransmission in bipolar depression following treatment with riluzole. *Neuropsychopharmacology*, **35**, 834–846.

107 Ibrahim, L., Diazgranados, N., Franco-Chaves, J., Brutsche, N., Henter, I.D., Kronstein, P., Moaddel, R., Wainer, I., Luckenbaugh, D.A., Manji, H.K., and Zarate, C.A. Jr. (2012) Course of improvement in depressive symptoms to a single intravenous infusion of ketamine vs Add-on riluzole: results from a 4-week, double-blind, placebo-controlled study. *Neuropsychopharmacology*, **37**, 1526–1533.

108 Serafini, G., Howland, H.H., Rovedi, F., Girardi, P., and Amore, M. (2014) The role of ketamine in treatment-resistant depression: a systematic review. *Curr. Neuropharmacol.*, **12**, 444–461.

109 Dowben, J.S., Grant, J.S., and Keltner, N.L. (2013) Ketamine as an alternative treatment for treatment-resistant depression. *Perspect. Psychiatr. Care*, **49**, 2–4.

110 White, J.M. and Ryan, C.F. (1996) Pharmacological properties of ketamine. *Drug Alcohol Rev.*, **15**, 145–155.

111 Morgan, C.J., Muetzelfeldt, L., and Curran, H.V. (2009) Ketamine use, cognition and psychological wellbeing: a comparison of frequent, infrequent and ex-users with polydrug and non-using controls. *Addiction*, **104**, 77–87.

112 Duman, R.S. and Li, N. (2012) A neurotrophic hypothesis of depression: role of synaptogenesis in the actions of NMDA receptor antagonists. *Philos. Trans. R. Soc. London, Ser. B*, **367**, 2475–2484.

113 Paul, R., Schaaff, N., Padberg, F., Möller, H.J., and Frodl, T. (2009) Comparison of racemic ketamine and S-ketamine in treatment resistant major depression: report of two cases. *World J. Biol. Psychiatry*, **10**, 241–244.

114 Mion, G. and Villevieille, T. (2013) Ketamine pharmacology: an update (pharmacodynamics and molecular aspects, recent findings). *CNS Neurosci. Ther.*, **19**, 370–380.

115 Ghasemi, M., Phillips, C., Trillo, L., De Miguel, Z., Das, D., and Salehi, A. (2014) The role of NMDA receptors in the pathophysiology and treatment of mood disorders. *Neurosci. Biobehav. Rev.*, **47**, 336–358.

116 Farley, S., Dumas, S., El Mestikawy, S., and Giros, B. (2012) Increased expression of the Vesicular Glutamate Transporter-1 (VGLUT1) in the prefrontal cortex correlates with differential vulnerability to chronic stress in various mouse strains: effects of fluoxetine and MK-801. *Neuropharmacology*, **62**, 503–517.

117 Osborn, M., Rustom, N., Clarke, M., Litteljohn, D., Rudyk, C., Anisman, H., and Hayley, S. (2013) Antidepressant-like effects of erythropoietin: a focus on behavioural and hippocampal processes. *PLoS One*, **8**, e72813.

12

Safety and Efficacy of Ashwagandha (*Withania somnifera*)

Shri K. Mishra[1], *Bharathi A. Venkatachalapathy*[2], *and Hadi M. Khanli*[3]

[1] *Keck School of Medicine, University of Southern California, 1100 North State Street, Clinic Tower, Los Angeles, CA 90033, USA*
[2] *Medical Ayurveda Rejuvenation Center, Newport Beach, CA 92660, USA*
[3] *Olive View UCLA Medical Center, Department of Neurology, 14445 Olive View Drive, Sylmar, CA 91342, USA*

12.1 Introduction

Ayurveda is the oldest continuously practiced medicine. Ayurvedic medicine has gained a huge popularity in modern society, mainly for its healing modalities both preventive and curative and use in chronic ailments. A vast majority of the population in India still depend on this age-old system of medicine for cure as an alternative to allopathic medicine. Ayurvedic texts cover about 2000 species of plants with their medicinal uses as described by ancient Indian medical scholars. Proper documentation is one of the unique features of the ancient Indian classical medicine system [1]. The primeval classics of India laid emphasis on comprehension of plant taxonomy, classification of soil, relevant practices of cultivation such as selection of soil, plant propagation techniques (through seeds, roots, cuttings, apical portions, etc.), plant nourishment, plant diseases, and their management [2]. In general, the science of Ayurveda advocates *Sharad Ritu* (i.e., October and November) as the best season for harvesting of herbs for therapeutic purpose.

However, limited scientific evidence is available to testify the safety and efficacy of Ayurvedic products. The root of the efficacy data is arrived from the long-term clinical experience transmitted and documented among the practitioners [3]. Hence it is difficult to evaluate polyherbal medicines using the conventional array of pharmacological and toxicological methods. Thus, the proponents theorize on holistic use of plant parts or extracts. The fact to be borne in mind is that these materials consist of hundreds of active ingredients. Many Ayurvedic herbs in use today are based on the principle of single-chemical isolation from plants or large-scale synthesis. But in many instances, these single-chemical entities elicit adverse effects when used alone. Therefore, practitioners feel that the active constituents in a plant are rightly balanced within the plant and any possible untoward or toxic effects of one component would be neutralized by the presence of complementary constituents. There are several publications on the potential toxicity of the phyto products.

Neuroprotective Natural Products: Clinical Aspects and Mode of Action, First Edition.
Edited by Goutam Brahmachari.

Contamination of the herbal plants by pesticides, herbicides, naturally occurring toxins, or microbes or adulteration by means of synthetic substitutes is a huge cause for concern. Toxicity manifestations include hepatotoxicity (most prominent – mild elevations of liver enzymes to fulminant liver failure), nephrotoxicity, neurotoxicity, and hematological, mutagenic, and cardiovascular toxicities. Hence, there is a need for a fundamentally different approach for toxicological studies that need to be adopted for Ayurvedic products. In light of the previously stated facts, an integrated approach for safety assessment focusing on the hazard identification is imperative.

Various Ayurvedic herbs are now being used as over-the-counter supplements for their high adaptogenic property and nutritional values. Among them, Ashwagandha is being used in energy enhancement supplements. Hence it is important to discuss the safety and toxicity of Ashwagandha to ensure that the herb is taken under proper guidelines.

12.2 Ashwagandha

12.2.1 The Plant

Taxonomically, Ashwagandha is known as *Withania somnifera* and belongs to the family Solanaceae. This is an Ayurvedic herb used independently as a single herb or as an ingredient in many formulations for various ailments. Ashwagandha means "horse's smell" (ashwa = horse, gandha = smell) in Sanskrit, which is thought to be due to two main reasons: the root itself smells like a horse, and the root is supposed to imbibe you with the strength and virility of a horse [4]. Ayurvedic medicine considers it as a rejuvenating panacea and sex stimulant. It also acts as a tranquilizer and sedative, hence the "somnifera" in its scientific name, from Latin for "sleep." As a Rasayana agent, it is used as a general tonic to increase energy, improve overall health, as well as prevent and cure diseases of the elderly working on various tissues in the body.

Ashwagandha is a well-known Ayurvedic Rasayana and belongs to a subgroup of Rasayanas known as *Medhya rasayanas*. Medhya typically refers to the mind and mental/intellectual capacity. Thus, Medhya Rasayana such as Ashwagandha is used to promote intellect and memory. The cognition-promoting effect of Medhya Rasayanas is best seen in children with memory deficits, or when memory is compromised following head injury, or a prolonged illness and in old age [1]. Ashwagandha appears to significantly reduce the symptoms of stress and its comorbidities (fatigue, temporary cognitive impairment, etc.) as well as biomarkers such as the cortisol. Secondary to its adaptogenic effects, Ashwagandha is able to reduce the perceptions of fatigue with prolonged daily usage [5].

Alongside improvements in all seminal parameters, Ashwagandha is able to increase seminal motility as well; both are thought to underlie pro-fertility effects. Testosterone may be increased in infertile men (who have a reduction in testosterone) and men undergoing strength training, but there is currently no evidence to suggest an inherent testosterone boosting effect in otherwise normal men. According to Ayurvedic pharmacopeia, *W. somnifera* has a bitter and

astringent taste. Its potency is warm, and the end product at the tissue level is sweet, which allows it to have anabolic action. The region of action is in Vata disorders, including muscle wasting. One of its multipurpose effects includes Rasayana or revitalization. As a Rasayana agent, it is used as a general tonic to increase energy, improve overall health, as well as prevent and cure diseases of the elderly working on various tissues in the body. *W. somnifera* could be an effective anti-sarcopenic compound as it helps in tissue buildup through proper tissue metabolism and due to its anabolic properties. The action is attributed to some of its alkaloids and glycosides that act as adaptogens.

The chemical constituents of *W. somnifera* that are biologically active include alkaloids (isopelletierine, anaferine, cuscohygrine, anahygrine, etc.), steroidal lactones (withanolides, withaferins), and saponins [6]. Active principles of Ashwagandha, for instance, the sitoindosides VII–X and withaferin A, have been shown to have significant antistress activity against acute models of experimental stress. Many of its constituents support immunomodulatory actions. The first steroidal lactone that was extracted from *W. somnifera*, named withaferin A, can antagonize vimentin. Besides the inhibition of angiogenesis, it decreases the activity of both NF-κB and Sp1 transcription factors. Withaferin A also downregulates VEGF gene expression and can affect calcium signaling. In addition, the chemical entity can inhibit protein kinase C (PKC), which induces apoptosis [7–9]. Due to the presence of such withanolides, the herb is supposed to exhibit beneficial pharmaceutical effects against a variety of disease manifestations including dementia and muscular discrepancies [10]. Pertinent to the study, the herb has shown to have powerful profibrinolytic properties as well [11].

12.2.2 Safety and Toxicological Evaluation of *Withania somnifera*

The first vital step before medical use of any unknown medicinal herb is the evaluation of its potential toxicity on different systems in the body. Unfortunately, there is no systematic human study to investigate the potential acute or chronic toxicity of *W. somnifera* as whole plant or its different extractions so far [12].

Large doses of *W. somnifera* can be abortifacient. It is rated likely unsafe during pregnancy. There is some evidence that Ashwagandha might cause miscarriages. Not enough is known about the use of Ashwagandha during breast-feeding. It also should be used with caution in patients suffering from hypertension. Ashwagandha might decrease blood pressure. This could cause blood pressure to become too low in people with low blood pressure or interfere with medications used to treat high blood pressure. A small decrease in blood pressure (1.6% systolic and 5.6% diastolic) has been noted to occur alongside a reduction in pulse rate. Ashwagandha might lower blood sugar levels. This could interfere with medications used for diabetes and cause hypoglycemia. Ashwagandha might increase thyroid hormone levels and hence should be avoided in people with thyroid condition or with those on thyroid medication [4].

Medications that decrease the immune system (immunosuppressants) interact with Ashwagandha. Ashwagandha seems to increase immune system response. Some medications that decrease the immune system include azathioprine (Imuran), basiliximab (Simulect), cyclosporine (Neoral, Sandimmune), daclizumab

(Zenapax), muromonab-CD3 (OKT3, Orthoclone OKT3), mycophenolate (CellCept), tacrolimus (FK506, Prograf), sirolimus (Rapamune), prednisone (Deltasone, Orasone), corticosteroids (glucocorticoids), and others. In addition, *W. somnifera* should not be taken with other sedative and antianxiety drugs on a personal basis [13]. Ashwagandha might cause excessive drowsiness in patients taking sedative medications such as benzodiazepines [4].

In one study on rats, *W. somnifera* root (WSR) extract showed no acute toxicity in first 2 weeks after administration of 2000 mg kg^{-1}, once orally. In the subacute study, WSR extract also revealed no toxic signs with oral administration of 500, 1000, and 2000 mg kg^{-1} per day in studied rats. In addition, there were no significant changes in the body weight, organ weight, and biochemical parameters in any of these dosages. No treatment-related gross/histopathological lesions were observed. However, underutilization of glycogen stores due to reduction in metabolic rate can lead to its accumulation in the liver by *W. somnifera* overuse. Structural similarity of some of *W. somnifera* extracts to adrenocortical steroids may lower the ACTH secretion and consequently decrease production of endogenous steroid. Eventually this study demonstrated no observed adverse effects in rats in the dosage of 2000 mg kg^{-1} body weight per day of hydroalcoholic extract of *W. somnifera* and hence may be considered as a safe compound [14]. On the other hand, other studies have mentioned increased catecholamine level in the heart and aortic tissues and decreased activity of adrenal glands as unfavorable side effects of high doses of *W. somnifera* [5].

12.2.3 Effects of Ashwagandha on Muscle Sarcopenia

A recent double-blinded clinical trial of *W. somnifera* on 35 individuals within the age group of 55 and 75 years revealed that *W. somnifera* may have a role in the management of age-related muscle symptoms. The subjects were divided into three groups based on the dosage that each group was receiving including group A (low dosage group), 500 mg of the whole root extract twice daily; group B (high dosage group), 750 mg twice daily; and finally group C (control group), placebo capsules. Muscle strength (MMT), muscle functional performance, routine blood tests, and serum creatine kinase were measured for all subjects prior to and after the trial. The Medical Research Council (MRC) scale was used for scoring the muscle strength, and the standardized functional performance test was used for muscle function tests. The subjects had been followed up every 2 weeks for 2 years.

Decrease in muscle strength is one of the earliest findings of muscle sarcopenia, and the percentage of subjects with an MRC score less than 5 (minimal muscle weakness) increases with age in general for all the muscles tested. None of the subjects who had completed the trial reported any side effect of this herbal medicine. This trial concluded that *W. somnifera* could improve the strength and functioning of the muscle according to the nonstatistically significant result due to the limited sample size, but the trend is promising. There is also a decline in blood creatine kinase levels in the 3-month trial in treatment group, though the difference is not statistically significant (limited sample size with large SD). This finding suggests a possible increase in muscle metabolism or a possible decline in muscle catabolism with *W. somnifera* [9].

In another study designed by Raut *et al.* on 18 healthy volunteers in ages between 18 and 30 years, *W. somnifera* showed a potential effect in reduction of total and LDL cholesterol and an increase in muscle strength especially regarding to hand grip force, quadriceps force, and back extensor force. The increase in quadriceps force and back extensor force was statistically significant, gradually increasing in the follow-ups in day 10, 20, and 30 after starting treatment [10].

12.3 Discussion

Ashwagandha (*W. somnifera* [L.] Dunal), also known as Indian winter cherry, is extensively used in Ayurveda, the traditional health-care system in India. In Ayurveda, certain herbal formulae known as Rasayana are considered to be rejuvenating and are taken as a remedy for general weakness, exhaustion, and stress [15, 16]. Ashwagandha is popularly called Indian ginseng due to its rejuvenating effects. Ashwagandha is shown in the literature to be an anxiolytic, antidepressant, and antistress adaptogen. The withanolides, steroidal lactones, are said to be the important phytochemicals of Ashwagandha and among the active constituents responsible for the therapeutic efficacies of the plant Ashwagandha (*W. somnifera*). Ashwagandha has been shown to decrease cortisol levels in persons under chronic stress, restore healthy adrenal function, and normalize the sympathetic nervous system [16]. Its role in sarcopenia is well defined; however it requires further large sample and long-term trial and proper evaluation.

There are two clinical trials on human subjects that conclude the possible efficacy of *W. somnifera* on improving the strength and functioning of the muscles in sarcopenia. Although the results are not statically significant, because of the small size of these studies, we suggest an important role of herbal medicine particularly *W. somnifera* in the therapeutic management of sarcopenia.

However, further research should be focused in this area in the future to analyze the detailed molecular structures of any herbal compound and evaluate the efficacy of these components on muscle sarcopenia in large sample size clinical trials. We believe that in the near future, the natural therapies can play a significant role in prevention and treatment of the complications of the muscle aging. In general, the type, nature, and extent of effect obtained during toxicity studies can help in adequately classifying herbal medicines as nontoxic, moderately toxic, or severely toxic on selected biological systems.

12.4 Concluding Remarks

Ashwagandha is used as an Ayurvedic household remedy in India as the best tonic for old people and children and as aphrodisiac by young people. The available scientific data support the conclusion that Ashwagandha is a potent regenerative tonic (Rasayana of Ayurveda) due to its multiple pharmacological actions such as antistress, neuroprotective, antitumor, antiarthritic, analgesic, anti-inflammatory, and many more. It is useful in different types of diseases such as

Parkinson, dementia, memory loss, stress-induced diseases, malignoma, and others. However, drug interactions need to be considered when taking any herbs especially adaptogens such as Ashwagandha.

Several scientific studies have been conducted to assess the influence of several factors such as time, season, place of collection, harvesting, and so on, in relation to phytochemistry and clinical efficacy. It is important to develop appropriate methodology of cultivation and harvesting of medicinal plants by integrating the knowledge of traditional and contemporary sciences, which consecutively aid in sustainable deliverance of quality-assured plant drugs, as well as their conservation. With this renaissance of utility of Indian systems of medicine, there is an increasing need to refer to not just the profile of the ingredients but to satisfy the consumers that safety and efficacy of the drugs has indeed been established [2]. There is no large systematic study investigating the safety of *W. somnifera* on humans. However, small studies and several animal studies have showed no significant side effects.

Abbreviations

ACTH	adrenocorticotropic hormone
LDL	low-density lipid
MMT	muscle mass testing
MRC	medical research council
PKC	protein kinase C
W. somnifera	*Withania somnifera*
WSR	*Withania somnifera* root

References

1 Singh, N., Bhalla, M., de Jager, P., and Gilca, M. (2011) An overview on ashwagandha: a rasayana (rejuvenator) of ayurveda. *Afr. J. Tradit. Complement. Altern. Med.*, **8** (5), 208–213.

2 Srikanth, N., Tewari, D., and Mangal, A. (2016) The science of plant life (vriksha ayurveda) in archaic literature: an insight on botanical, agricultural and horticultural aspects of ancient India. *World J. Pharm. Pharmacol. Sci.*, **4** (6), 388–404.

3 Chandramouli, R., Thirunarayanan, T., Mukeshbabu, K., and Sriram, R. (2010) Designing toxicological evaluation of ayurveda and siddha products to cater to global compliance – current practical and regulatory perspectives. *J. Pharm. Sci. Res.*, **2** (12), 867–877.

4 Chandrasekhar, K., Kapoor, J., and Anishetty, S. (2012) A prospective, randomized double-blind, placebo-controlled study of safety and efficacy of a high-concentration full-spectrum extract of ashwagandha root in reducing stress and anxiety in adults. *Indian J. Psychol. Med.*, **34** (3), 255–262.

5 Dhuley, J.N. (2000) Adaptogenic and cardioprotective action of ashwagandha in rats and frogs. *J. Ethnopharmacol.*, **70** (1), 57–63.

6 Rastogi, R.P. and Mehrotra, B.N. (1998) *Compendium of Indian Medicinal Plants*, vol. **6**, Central Drug Research Institute, Lucknow and National Institute of Science Communication.

7 Vanden Berghe, W., Sabbe, L., Kaileh, M., Haegeman, G., and Heyninck, K. (2012) Molecular insight in the multifunctional activities of Withaferin A. *Biochem. Pharmacol.*, **84** (10), 1282–1291.

8 Sen, N., Banerjee, B., Das, B.B., Ganguly, A., Sen, T., Pramanik, S., Mukhopadhyay, S., and Majumder, H.K. (2007) Apoptosis is induced in leishmanial cells by a novel protein kinase inhibitor withaferin A and is facilitated by apoptotic topoisomerase I–DNA complex. *Cell Death Differ.*, **14** (2), 358–367.

9 Mishra, S.K. and Trikamji, B. (2013) A clinical trial with *Withania somnifera* (Solanaceae) extract in the management of sarcopenia (muscle aging). *Signpost Open Access J. Org. Biomol. Chem.*, **1**, 187–194.

10 Raut, A.A., Rege, N.N., Tadvi, F.M., Solanki, P.V., Kene, K.R., Shirolkar, S.G., Pandey, S.N., Vaidya, R.A., and Vaidya, A.B. (2012) Exploratory study to evaluate tolerability, safety, and activity of Ashwagandha (*Withania somnifera*) in healthy volunteers. *J. Ayurveda Integr. Med.*, **3** (3), 111–114.

11 Ku, S.K. and Bae, J.S. (2014) Antiplatelet, anticoagulant, and profibrinolytic activities of withaferin *A*. *Vascul. Pharmacol.*, **60**, 120–126.

12 Mishra, L.C., Singh, B.B., and Dagenais, S. (2000) Scientific basis for the therapeutic use of *Withania somnifera* (*Ashwagandha*): a review. *Altern. Med. Rev.*, **5**, 334–346.

13 Kulkarni, S.K. and Dhir, A. (2008) *Withania somnifera*: an Indian ginseng. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **32** (5), 1093–1105.

14 Prabu, P.C., Panchapakesan, S., and Raj, C.D. (2013) Acute and sub-acute oral toxicity assessment of the hydroalcoholic extract of *Withania somnifera* roots in Wistar rats. *Phytother. Res.*, **27** (8), 1169–1178.

15 Choudhary, B., Shetty, A., and Langade, D.G. (2015) Efficacy of *Ashwagandha* (*Withania somnifera* [L.] Dunal) in improving cardiorespiratory endurance in healthy athletic adults. *AYU*, **36**, 63–68.

16 Dongre, S., Langade, D., and Bhattacharyya, S. (2015) Efficacy and safety of Ashwagandha (*Withania somnifera*) root extract in improving sexual function in women. *Biomed. Res. Int.*, **2015**, 9. doi: 10.1155/2015/284154, Article ID 284154.

13

Cannabinoids: A Group of Promising Neuroprotective Agents

Laura R. Caltana and Alicia Brusco

Universidad de Buenos Aires, Facultad de Medicina. Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Biología Celular y Neurociencia (IBCN), Paraguay 2155, 3rd floor, Ciudad Autónoma de Buenos Aires, Buenos Aires, 1114, Argentina

13.1 Introduction

Cannabis constitutes the third most widely used drug after alcohol and tobacco; on the other hand, abundant evidences in experimental assays and clinical trials show that cannabis has a growing potential to treat different pathologies, including studies proposing cannabis to be the aspirin of the twenty-first century [1].

The endocannabinoid (eCB) system is a neuromodulatory system with important homeostatic roles controlling neuronal function, neurogenesis, inflammation, excitotoxicity, oxidative stress, and synaptic plasticity.

In this context, this chapter summarizes eCB system biology and the potential therapeutic effects of natural cannabinoid compounds on the most representative neuropathologies. To this end, the following sections focus on the cannabinoid system in the central nervous system.

13.1.1 The History of Marijuana

Marijuana is the common name assigned to the dried leaves, flowers, and seeds from the hemp plant *Cannabis sativa.* Natural cannabinoids found in *C. sativa* have been used for medical and recreational purposes for centuries. The first written evidence on the use of marijuana dates back to 2737 BC – in China, marijuana was employed as medicine for the treatment of rheumatism, gout, malaria, and attention deficits. Toxic properties have been also known, but the therapeutic value was considered more relevant. Marijuana was also used in India and by Muslims, who also used it recreationally because alcohol was forbidden by the Koran and introduced the drug in ancient Persia (now Iran) and North Africa in the twelfth century.

Viking and medieval Germans used cannabis for pain relief during labor and toothaches. Marijuana migrated to Africa and Europe and arrived in South America in 1525 with the Spanish conquest and in North America in the hands of the English [2].

Neuroprotective Natural Products: Clinical Aspects and Mode of Action, First Edition.
Edited by Goutam Brahmachari.

13.1.2 Current Context

Several subspecies of the cannabis have been identified so far. *C. sativa*, commonly known as marijuana, contains compounds with and without psychoactive properties. *Cannabis indica*, also known as hemp, is a non-psychoactive form and is used in the oil industry as fuel. *Cannabis ruderalis*, in addition, has also been identified as a psychoactive species [3].

Hemp contains a very low amount of the psychoactive constituent Δ^9-tetrahydrocannabinol (THC) and higher quantities of cannabidiol (CBD), the latter offering medicinal benefits without the cognitive effects and abuse potential associated with THC.

THC is the active compound of *C. sativa* [4]. Cannabinoid receptor type 1 (CB1R) was discovered in 1988 and named after its cannabimimetic properties [5]. THC acting on CB1R present in neurons exerts its psychotropic effects and renders a state of euphoria and sense of well-being. Acute effects of marijuana include memory and learning deficits, changes in perception, difficulty in solving problems, loss of coordination, tachycardia, anxiety, panic attacks, and psychosis-like symptoms [6].

A recent study conducted in the United Kingdom found marijuana smoking to produce the same adverse effects of tobacco smoking, including bronchitis, emphysema, and asthma [7]. Studies on laboratory animals have shown that the chronic stimulation of CB1Rs causes changes in the nervous tissue cells and alters the neuron cytoarchitecture and projections [8, 9]. Magnetic resonance imaging studies have shown that the long-term exposure to cannabis in humans produces anatomical alterations in the brain, such as reduced gray matter volume, and that these alterations are related to the frequency of cannabis use [10–12]. Cannabinoid exposure during prenatal development has also been reported to induce cerebral cortex anatomical alterations in humans [13] and rats [14, 15], showing a reduction in cortical thickness with abnormal layer organization.

Despite the deleterious effects of chronic cannabis consumption, promising studies on animals and humans currently resume the potential effects of cannabinoids for the treatment of diverse pathologies including cancer, ischemia, Parkinson's disease (PD), Alzheimer's disease (AD), glaucoma, pain, and multiple sclerosis (MS).

13.2 The Cannabinoid System

The eCB system is made up of eCBs (their endogenous ligands) and receptors and involves the synthesis and degradation of specific enzyme systems [16]. Different types of agonists include eCBs, synthesized by the endogenous cells, *N*-arachidonoylethanolamide, known as anandamide (*N*-arachidonoylethanolamine or AEA [17] and 2-arachidonoylglycerol (2-AG) [18]), with higher content in the brain than AEA. Other eCBs have been described as 2-arachidonyl glyceryl ether or noladin ether (2-AGE) [19] and *O*-arachidonoyl ethanolamine or virodhamine (O-AEA) [20].

eCBs are synthesized on demand from phospholipids of the plasma membrane through a calcium-dependent process. *N*-Acyltransferase and *N*-arachidonoylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) are involved in AEA biosynthesis, and fatty acid amide hydrolase (FAAH) is the enzyme catalyzing EAA degradation [21]. 2-AG is synthesized from postsynaptic

membrane phospholipids through the combined actions of phospholipase C (PLC) and diacylglycerol lipase (DGL) and is hydrolyzed into arachidonic acid and glycerol, primarily by monoacylglycerol lipase (MGL).

Exogenous cannabinoids are natural derivatives of *C. sativa* (also named phytocannabinoids) (Δ^9-THC, Δ^8-THC, 11-hydroxy-Δ^8-THC-dimethylheptyl (HU-210)) or synthetic (ACEA, *O*-1812 SR141716A, *R*-(+)-WIN55212, JWH-015), among others [22]. The best known exogenous cannabinoid antagonist, AM281 (rimonabant), is used in clinical treatment to block the effects of endogenous agonists.

Cannabinoid agonists activate different cannabinoid receptors (CBRs): CB1Rs are highly expressed in many regions of the brain, including the cerebral cortex, cerebellum, hippocampus, striatum, amygdala, and limbic system, where they play important roles in physiology and neuromodulation as retrograde synaptic messengers; cannabinoid receptor type 2 (CB2R) is expressed primarily in the cells of the immune system, although experimental reports show them to be present in various regions of the adult rodent brain, including the olfactory tubercle, cerebral cortex, striatum, thalamic nuclei, hippocampus, amygdala, substantia nigra, periaqueductal gray matter, paratrochlear nucleus, paralemniscal nucleus, red nucleus, pontine nuclei, inferior colliculus, and the parvocellular portion of the medial vestibular nucleus. CB2Rs are present in both neurons and astrocytes with a postsynaptic localization [23, 24].

Numerous studies have shown the presence of CB1R and CB2R in the retina of several animal species. In human retina, CB1Rs are detected in the outer segments of photoreceptor cells, inner plexiform layer, outer plexiform layer (two synaptic layers of the retina), inner nuclear layer, ganglion cell layer, and retinal pigment epithelium cells. On the other hand, CB2Rs are expressed in human retinal pigment epithelium cells [25]. Evidence also points out that vanilloid receptor type I (TRPV1), which is a potential transient ion channel involved in nociceptive processes, may be a target of eCB anandamide [26].

CB1Rs are located in the presynaptic membrane, and their activity is related to the regulation of many brain functions such as motor coordination, anxiety, memory, and appetite. CB1R and CB2R are G-protein-coupled receptors (GPCR)-type Gi/o with a seven-transmembrane domain structure. CB1Rs inhibit the activation of cAMP synthesis and regulate ion channels by inhibiting voltage-dependent L-, N-, and P/Q-type calcium channel and regulating rectifier potassium channels (Kir) [22, 27]

The eCB system has recently been proposed as a neuromodulator regulating the development of neural cells in embryonic and adult neurogenesis. It has been established that CB1R mRNA is expressed in many developing regions of the brain [28] and that the eCB system promotes cell proliferation of neural progenitors [29, 30].

CBRs are also expressed in embryonic neuroblasts and neural precursors in the adult brain, where they regulate cell proliferation and differentiation. Neurogenesis is impaired in CB1R-deficient mice, which implies that endogenous signaling through this receptor promotes basal levels of neurogenesis *in vivo* [31]. Thus, the eCB system may be involved in controlling structural brain plasticity by modulating cell proliferation, although the roles of CB1R and CB2R in neuronal cell differentiation and specification remain elusive [32, 33]. eCBs and their receptors

also act in the migration of neural cells, particularly immature neurons, and may thus play an instructive and permissive role, presumably by modifying adhesive interactions between neurons and their extracellular substrates [34].

CB1Rs are also expressed in oligodendrocytes during brain development by participating in myelin formation [35, 36] and in neural precursors in the adult brain, thereby regulating astroglial differentiation [29].

The physiological significance of eCBs lies in their function as neuromodulators of synaptic activity, acting as retrograde messengers at the presynaptic level. Since 2001, eCBs have been identified as triggers of short- and long-term plasticity at synapses throughout the brain according to the ubiquitous expression of CB1Rs [37]. eCBs are generally regarded as retrograde signaling, although examples have been found of autocrine action. They are released postsynaptically, cross the synaptic cleft in the reverse direction of normal synaptic transmission, and activate CB1R into presynaptic nerve terminals and postsynaptic CB2R. The eCB system in the brain regulates synaptic communication and affects biological functions such as appetite, anxiety, learning and memory, and growth and development [38]. For example, eCBs are involved in the regulation of energy homeostasis, increasing caloric intake and resulting in consequent weight gain. CB1R blocking causes a reduction in body weight by altering eating habits [39].

Glial cells express CB1R and respond to CB1R agonists, releasing glutamate and thus influencing synaptic transmission. eCBs could have opposite neuromodulatory effects on synaptic transmission acting directly on CB1R presynaptically or enhancing or depressing synaptic transmission [40]. CB1Rs are also present in serotonergic neurons and modulate anxiety/depression behavior in mice [41]. Furthermore, several signaling pathways have proven to be regulated by CBRs. For example, CB1Rs regulate the different members of the family of mitogen-activated protein kinases such as extracellular signal-regulated kinase (ERK), *c*-Jun N-terminal kinase, and p38 [42, 43] and can also activate the signaling pathway phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) [44]. The ability of cannabinoids to activate the PI3K/AKT pathway favoring survival has been reported in *in vitro* studies and can explain their protective role. AKT activation phosphorylates intracellular substrates and promotes neuronal survival by inhibiting apoptosis through phosphorylation and inactivation of glycogen synthase kinase 3 (GSK3) in its two isoforms, GSK3a and GSK3b. However, events mediated by CB1R stimulation *in vivo* remain poorly understood.

Until recently, CB2Rs were thought to be only present in the peripheral and immune tissue, where they have been observed in the marginal zone of the spleen, tonsils, and immune system cells (B lymphocytes, T cells, and monocytes) [45–47]. However, they are known to be also present in the cells of the nervous system, such as microglia [48] and neurons [23] in several brain regions including the anterior olfactory nucleus, cerebral cortex, cerebellum, striatum, brain stem, and hippocampus, where their location is mainly postsynaptic [49].

CB2Rs have been cloned from promyelocytic leukemia cells (HL-60). These receptors share the standardized structure of CB1R with seven transmembrane domains and are also coupled to a G protein. The clone has 68% homology with the amino acid sequence in the transmembrane domains of CB1R and only 44% homology in the total protein [45].

CB2Rs are, similarly to CB1Rs, metabotropic receptors activated by AEA and 2-AG. CB2R plays an important role in pain and inflammation and dramatically increases its expression in inflammatory processes [50], regulating pain by decreasing the release of nociceptive agents such as substance P and histamine. As CB2R activation does not generate the psychotropic effects associated with CB1R, it has become a research target for therapeutic cannabinoid applications with analgesic, anti-inflammatory, and anticancer purposes.

13.3 Cannabinoids and Neuroprotection

The discovery of the eCB signaling system with its ligand network, membrane receptors, and regulatory proteins has revealed possible new therapeutic targets in the cases of obesity, drug abuse, pain, hypertension, and stroke. Although neuroprotection is a relatively new area for cannabinoid agonists, research seems to have reached an advanced stage.

CB1R agonists have shown neuroprotective properties in different animal models during central nervous system injury [51–54] and are also useful for the treatment of pain, spasticity, glaucoma, and other disorders [55]. WIN55,212-2, a nonselective CB1R/CB2R agonist, has exhibited protective effects in models of damaged blood–brain barrier and in models of psychiatric disorders [56, 57]. Furthermore, selective CB2R agonists such as JWH015 have exerted anti-inflammatory effects and pain relief [58, 59].

CBD has agonist activity at CB2R, hence showing anti-inflammatory properties by acting on pain without the impairments of THC and proving efficient in the treatment of neonatal ischemia, PD, and Huntington's disease [60]. The anxiolytic effects of CBD may be attributed to its agonist effect at 5-HT1A receptor [61].

13.3.1 Cannabinoids in Hypoxia/Ischemia

Cerebral ischemia, also called stroke, is the result of a temporary or permanent reduction of cerebral blood flow and is limited to the territory of the cerebral artery. The reduction in flow is, in most cases, caused by artery occlusion or by local thrombosis [62]. According to the World Health Organization, cerebral ischemia affects 15 million people every year and is the third cause of mortality in the adult population. Stroke is also the main cause of long-term motor disability, aphasia, and cognitive impairment [63]. Effective treatment options for functional recovery after stroke are scarce. The administration of tissue plasminogen activator appears as the only treatment approved for the recanalization of the occluded blood vessel, although it is only suitable for a very small number of patients [64].

Recent reports show that the consequences of ischemic injury in the brain, heart, and liver can be ameliorated by cannabinoids [65], which exhibit protective effects against ischemic stroke through molecular mechanisms not fully elucidated yet. In particular, synthetic cannabinoid agonist WIN55,212-2, a psychoactive analog of marijuana components, protects the brain tissue against ischemic damage, an effect apparently mediated by CB1R and blocked by selective CB1R antagonist SR141716A. The neuroprotective action of WIN55,212-2

may involve the inhibition of ion flux through calcium channels and a reduction in glutamate release. Furthermore, the excitotoxicity-induced activation of NMDA glutamatergic receptors in CB1R knockout mice suggests that endogenous cannabinoid signaling can regulate excitotoxicity produced by glutamate release [66].

In addition, treatment with selective CB1R agonist ACEA after occlusion of the middle cerebral artery protects the brain regions affected by the ischemic event, reducing the number of degenerating neurons and the size of the tissue affected and rendering motor recovery [67]. These findings show long-term protective effects, and their physiological basis could be related to anti-inflammatory actions, free radical scavenging, inhibition of glutamate release, and excitotoxicity [68]. Treatment with ACEA also reduces neuronal death, loss of dendrites, and microglial reaction [67], and these parameters have also been observed in other models of hypoxia/ischemia and subsequent treatment with cannabinoid agonists [69–71].

13.3.2 Cannabinoids in Parkinson's Disease

PD affects 1% of the elderly population and is the second most common neurodegenerative disorder. It is associated with loss of 50–70% dopaminergic neurons in the substantia nigra pars compacta [72], producing the depletion of dopamine in the striatum. Levodopa is the primary treatment for PD, although its continuous use produces motor complications. As inflammation is one of the most important pathogenic factors in PD, extensive research focuses on reducing inflammation to prevent dopaminergic neuron death.

CB1R expression is increased in basal ganglia in PD patients, animal models, and *in vitro* assays. Human cell culture models of PD have demonstrated that THC protects neurons through mechanisms mediated by peroxisome proliferator-activated receptor gamma (PPARγ) activation [73]. CB1R activation also protects neurons by reducing S100b protein density, as S100b and CB1R regulate the production of cAMP in opposite ways; CB1R stimulation decreases cAMP and Ca^{2+} entry into cells via voltage-activated Ca^{2+} channels in neurons, reducing S100b neurotoxic signaling [74].

THC and CBD also reduce dopaminergic cell death and lead to a significant recovery in the impairment of dopaminergic transmission in a rat model of hemi Parkinsonism [75]. These effects may be due to the antioxidant properties of these compounds, inhibiting COX-2 activity and modulating glial function [76]. In turn, treatment with WIN55,212-2 (WIN) and selective CB2R agonist JWH015 reduces MPTP-induced microglial activation and motor deficits in a PD model in mice [77, 78].

CB2Rs are also present in neurons located in the brain stem, cerebellum (Purkinje cells and granular neurons), and basal ganglia. The presence of CB2R has also been demonstrated in the substantia nigra in dopaminergic neurons in humans, but its expression is lower in PD as compared with healthy patients [79]. In contrast, CB2Rs expressed in microglial cells are increased in the postmortem tissue of PD patients [80]. Clinical evidence also shows possible effects of CBD in improving life quality [81] and reducing sleep behavior disorders [82].

In summary, cannabinoid signaling and its stimulation seem to be a potential target to reduce dopaminergic neuron degeneration in PD and improve deterioration of control movements, although controlled clinical trials are still necessary to render conclusive results.

13.3.3 Cannabinoids in Alzheimer's Disease

The most common form of dementia, AD, is characterized by the deposition of β-amyloid peptide (βA) in the senile plaque, with activated microglial clusters showing inflammatory processes. The senile plaques express both CB1R and CB2R, as well as markers of microglial activation.

Aso and Ferrer [83] conducted experimental studies demonstrating the multifaceted properties of cannabinoids for the treatment of AD due to their capacity to decrease neuroinflammation, excitotoxicity, mitochondrial dysfunction, and oxidative stress. In an animal model involving βA administration to rats, cannabinoid administration was observed to prevent microglial activation and to attenuate the loss of neuronal markers induced by βA, combining both anti-inflammatory and neuroprotective actions [84].

The analysis of human postmortem samples revealed alterations in eCB composition and signaling in the AD brain. While no correlation has been found between CB1R levels and any AD molecular marker or cognitive status [85], a significant increase in CB2R levels in the AD brain has been well established, mainly corresponding to receptors expressed in the microglia surrounding senile plaques [84].

Several findings indicate that the activation of both CB1R and CB2R by natural or synthetic agonists, in non-psychoactive doses, has beneficial effects in AD experimental models by reducing the harmful βA action and tau phosphorylation, as well as by promoting the brain's intrinsic repair mechanisms [86].

13.3.4 Cannabinoids in Epilepsy

Epilepsy affects about 65 million people worldwide [87] and is a chronic disease classified as a spectrum of disorders due to the variation of its clinical presentation. Epilepsy is characterized by the generation of partial – focalized cortical areas – or generalized seizures, including both brain hemispheres, depending on the magnitude of the brain areas involved. Primary epilepsy is idiopathic, while secondary epilepsy is generated from brain injury such as neurotoxicity, hypoxia, tumors, encephalitis, and trauma, among others [88].

Epileptic seizures are produced by abnormal neuronal electrical activity and a misbalance between excitatory and inhibitory neurotransmitter release. As more than 30% of patients including children present refractory epilepsy or treatment-resistant epilepsy that does not respond to the use of antiepileptic medication [89], the cannabinoid system and its regulation with exogenous compounds may prove to be effective therapies for this pathology.

The pharmacological basis of cannabinoid use in epilepsy is supported by its neuromodulatory effects and its ability to inhibit hyperexcitability, although the underlying mechanisms remain uncertain. While abundant preclinical experimental data show the effectiveness of cannabinoids in the treatment of epilepsy, clinical trial results remain controversial.

CBD has displayed relatively potent anticonvulsant actions with no psychotropic effects in *in vitro* and *in vivo* studies [90–92]. Placebo-controlled trials have demonstrated that CBD has no side effects on the central nervous system or vital signs and that it is well tolerated [93]. In turn, oral cannabis extracts, administered to children and adolescents, have shown beneficial effects, reducing seizures and improving behavior, language, and motor skills, although adverse effects have also been reported [94, 95]. To sum up, controversial clinical data suggest that controlled and blind studies are necessary to evaluate the efficacy and safety of cannabinoid compounds in epilepsy.

13.3.5 Cannabinoids in Multiple Sclerosis

MS is a progressive and chronic autoimmune disease characterized by demyelination and variable axonal loss. Predominantly diagnosed in young adults, MS affects the central nervous system, resulting in physical motor disabilities and deteriorated quality of life.

The administration of THC-CBD (an oromucosal spray containing approximately THC-CBD 1:1 fixed ratio) reduces the spasticity in phase III clinical trials [96–98]. Sativex®, a cannabinoid compound, proves effective in improving MS-related neuropathic pain, which could be related with its actions on cortical pathways modulating glutamate and gamma-aminobutyric acid (GABA) neurotransmission [99]. Nevertheless, the safety and long-term effects of these drugs on cognitive function should be evaluated.

Also, experimental data have shown cannabinoids to exert anti-inflammatory effects, although this aspect has not been explored in patients yet.

13.3.6 Cannabinoids in Psychiatric Disorders

Growing interest focuses on the role of the eCB system in mood disorders, as this neuromodulator system contributes to the maintenance of homeostasis in various physiological states including mood and emotion [100, 101]. In fact, CB1R pharmacological blockade and CB1R genetic ablation induce behavioral alterations in animal models of depression [102]. CB1R-deficient mice show depressive symptoms such as reduced response to stimuli of reward [103, 104], altered autonomic functions [105], deficits in extinction of aversive memories [106], and higher levels of anxiety and stress sensitivity [107, 108].

The relationship between the eCB system and depression is also supported by clinical evidence, as CB1R antagonist rimonabant, prescribed as a drug for weight loss, has been shown to increase the incidence of depression [109]. CB1R is expressed in all primary brain structures involving stress-related behavior, such as the hypothalamus, amygdala, limbic system, cortex, and hippocampus [110], and participates in the control of the hypothalamic–pituitary–adrenal axis [111–113]. Moreover, the lack of CB1R induces alterations in neuronal plasticity and loss [114] and increases susceptibility to neurotoxic insults [115]. The participation of CB1R in neurobiological mechanisms involved in mood and stress disorders is supported by previous findings reporting an increased sensitivity of $CB1R^{-/-}$ mice to soft post-stress anhedonic behavior [107] and increased vulnerability to behavioral

inhibition after repeated stress or acute exposure [116]. In addition, CBD has shown antipsychotic actions due to its capacity to inhibit the degradation of anandamide with no psychotropic effects [117]. Anxiolytic effects of CBD may also be attributed to its agonist effect on the 5-HT1A receptor [118].

In summary, most psychiatric disorders such as schizophrenia, depression, and anxiety are consequences of a high level of inflammatory markers in the brain and cerebrospinal fluid related with microglial and immune cells. CBRs are expressed in microglial cells and increase their expression in inflammatory conditions. Therefore, cannabinoids attenuate the inflammatory profile present in these disorders and could then constitute a therapeutic treatment to be considered [119].

13.3.7 Cannabinoids in Retinal Diseases

The retina expresses both CB1R [120] and CB2R [121]. A recent review has argued that the worldwide presence of cannabis makes it critical to explore its possible therapeutic uses in the treatment and prevention of retinal diseases in humans [122].

In an animal model for autosomal dominant retinitis pigmentosa in rats, the administration of CB1R agonist HU210 has been shown to ameliorate vision loss [123]. Also, animal models of diabetic retinopathy, a pathology associated with oxidative stress and proinflammatory cytokines, have proven CBD treatment to reduce neurotoxicity, inflammation, and blood–retinal barrier breakdown [124]. Regarding glaucoma, a group of optic neuropathies characterized by the cupping of the optic nerve head and selective retinal ganglion cell loss, cannabinoids' ability to modulate intraocular pressure through hypotensive effects acting at CB1R allows them to decrease pressure and reduce retinal ganglion cells loss, possibly by independent mechanisms [125]. Then, CB1R agonists may constitute ideal drugs for topic glaucoma therapy [126].

13.4 Concluding Remarks

The eCB system is activated in inflammatory events, during brain injuries that conduce to neuronal damage and in degenerative brain diseases, to mitigate the deleterious effects on the brain and its consequences.

The cannabinoid system is well characterized, and abundant research supports their role in ameliorating neuropathologies such as ischemia, AD, PD, MS, retinal diseases, and psychiatric disorders. Therefore, the manipulation of the eCB system, using phytocannabinoids or synthetic cannabinoids, could lighten the processing in the treatment and evolution of cerebral diseases. Nowadays, clinical trials have also demonstrated auspicious results, although certain aspects of cannabinoid adverse effects still remain to be elucidated.

It is important that health professionals take into account the possible impact and adverse effects of the use of cannabinoids in the treatment of these pathologies when prescribing as well.

Abbreviations

2-AG	2-arachidonoylglycerol
2-AGE	2-arachidonyl glyceryl ether or noladin ether
5-HT1A	serotonin receptor type 1A
AD	Alzheimer's disease
AEA	anandamide or *N*-arachidonoylethanolamine
βA	β-amyloid peptide
$CB1^{-/-}$	CB1R-deficient mice
CB1R	cannabinoid receptor type 1
CB2R	cannabinoid receptor type 2
CBD	cannabidiol
CBRs	cannabinoid receptors
DGL	diacylglycerol lipase
eCBs	endocannabinoids
ERK	extracellular signal-regulated kinase
FAAH	fatty acid amide hydrolase
GABA	gamma-aminobutyric acid
GPCRs	G-protein-coupled receptors
GSK3	glycogen synthase kinase 3
MGL	monoacylglycerol lipase
MS	multiple sclerosis
NAPE-PLD	*N*-arachidonoylphosphatidylethanolamine-hydrolyzing phospholipase D
O-AEA	*O*-arachidonoyl ethanolamine or virodhamine
PD	Parkinson's disease
PI3K/AKT	phosphatidylinositol 3-kinase/protein kinase B
PLC	phospholipase C
PPARγ	peroxisome proliferator-activated receptor gamma
THC	Δ^9-tetrahydrocannabinol
TRPV1	vanilloid receptor type I

References

1 Baker, D., Pryce, G., Giovannoni, G., and Thompson, A.J. (2003) The therapeutic potential of cannabis. *Lancet Neurol.*, **2** (5), 291–298.

2 Warf, B. (2014) High points: an historical geography of cannabis. *Geogr. Rev.*, **104**, 414–438.

3 Gloos, D. (2015) An overview of products and bias in research. *Neurotherapeutics*, **12** (4), 731–734.

4 Gaoni, Y. and Mechoulam, R. (1964) Isolation, structure and partial synthesis of the active constituent of hashish. *J. Am. Chem. Soc.*, **86**, 1646–1647.

5 Devane, W.A., Dysarz, F.A. III, Johnson, M.R., Melvin, L.S., and Howlett, A.C. (1988) Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharmacol.*, **34**, 605–613.

6 Barkus, E. and Lewis, S. (2008) Schizotypy and psychosis-like experiences from recreational cannabis in a nonclinical sample. *Psychol. Med.*, **38**, 1267–1276.

7 Macleod, J., Robertson, R., Copeland, L., McKenzie, J., Elton, R., and Reid, P. (2015) Cannabis, tobacco smoking, and lung function: a cross-sectional observational study in a general practice population. *Br. J. Gen. Pract.*, **65** (631), e89–e95.

8 Tagliaferro, P., Ramos, A.J., Onaivi, E.S., Evrard, S.G., Lujilde, J., and Brusco, A. (2006) Neuronal cytoskeleton and synaptic densities are altered after a chronic treatment with the cannabinoid receptor agonist WIN 55,212-2. *Brain Res.*, **1085** (1), 163–176.

9 Renard, J., Vitalis, T., Rame, M., Krebs, M.O., Lenkei, Z., Le Pen, G., and Jay, T.M. (2016) Chronic cannabinoid exposure during adolescence leads to long-term structural and functional changes in the prefrontal cortex. *Eur. Neuropsychopharmacol.*, **26** (1), 55–64.

10 Battistella, G., Fornari, E., Annoni, J.M., Chtioui, H., Dao, K., Fabritius, M., Favrat, B., Mall, J., and Giroud, C. (2014) Long-term effects of cannabis on brain structure. *Neuropsychopharmacology*, **39** (9), 2041–2048.

11 Lorenzetti, V., Solowij, N., and Yücel, M. (2015) The role of cannabinoids in neuroanatomic alterations in cannabis users. *Biol. Psychiatry*, **79** (7), e17–31.

12 Yücel, M., Lorenzetti, V., Suo, C., Zalesky, A., Fornito, A., Takagi, M.J., Lubman, D.I., and Solowij, N. (2016) Hippocampal harms, protection and recovery following regular cannabis use. *Transl. Psychiatry*, **6**, e710.

13 El Marroun, H., Tiemeier, H., Franken, I.H., Jaddoe, V.W., van der Lugt, A., Verhulst, F.C., Lahey, B.B., and White, T. (2015) Prenatal cannabis and tobacco exposure in relation to brain morphology: a prospective neuroimaging study in young children. *Biol. Psychiatry*, pii: S0006-3223(15)00692-7.

14 Saez, T.M., Aronne, M.P., Caltana, L., and Brusco, A.H. (2014) Prenatal exposure to the CB1 and CB2 cannabinoid receptor agonist WIN 55,212-2 alters migration of early-born glutamatergic neurons and GABAergic interneurons in the rat cerebral cortex. *J. Neurochem.*, **129** (4), 637–648.

15 de Salas-Quiroga, A., Díaz-Alonso, J., Garcia Rincon, D., Remmers, F., Vega, D., Gómez-Cañas, M., Lutz, B., Guzmán, M., and Galve-Roperh, I. (2015) Prenatal exposure to cannabinoids evokes long-lasting functional alterations by targeting CB1 receptors on developing cortical neurons. *Proc. Natl. Acad. Sci. U.S.A.*, **112** (44), 13693–13698.

16 Lambert, M.D. and Fowler, C.J. (2005) The endocannabinoid system: drug targets, lead compounds, and potential therapeutic applications. *J. Med. Chem.*, **48**, 5059–5087.

17 Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., and Mechoulam, R. (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, **258**, 1946–1949.

18 Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., Compton, D.R., Pertwee, R.G., Griffine, G., Bayewitch, M., Barg, J., and Vogel, Z. (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.*, **50**, 83–90.

19 Hanus, L., Abu-Lafi, S., Fride, E., Breuer, A., Vogel, Z., Shalev, D., Kustanovich, I., and Mechoulam, R. (2001) 2-arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **98** (7), 3662–3665.

20 Porter, A.C., Sauer, J.M., Knierman, M.D., Becker, G.W., Berna, M.J., Bao, J., Nomikos, G.G., Carter, P., Bymaster, F.P., Leese, A.B., and Felder, C.C. (2002) Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J. Pharmacol. Exp. Ther.*, **301** (3), 1020–1024.

21 Okamoto, Y., Wang, J., Morishita, J., and Ueda, N. (2007) Biosynthetic pathways of the endocannabinoid anandamide. *Chem. Biodivers.*, **4**, 1842–1857.

22 Howlett, A.C., Barth, F., Bonner, T.I., Cabral, G., Casellas, P., Devane, W.A., Felder, C.C., Herkenham, M., Mackie, K., Martin, B.R., Mechoulam, R., and Pertwee, R.G. (2002) International union of pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol. Rev.*, **54** (2), 161–202.

23 Gong, J.P., Onaivi, E.S., Ishiguro, H., Liu, Q.R., Tagliaferro, P.A., Brusco, A., and Uhl, G.R. (2006) Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Res.*, **1071** (1), 10–23.

24 Onaivi, E.S., Ishiguro, H., Gong, J.P., Patel, S., Meozzi, P.A., Myers, L., Perchuk, A., Mora, Z., Tagliaferro, P.A., Gardner, E., Brusco, A., Akinshola, B.E., Hope, B., Lujilde, J., Inada, T., Iwasaki, S., Macharia, D., Teasenfitz, L., Arinami, T., and Uhl, G.R. (2008) Brain neuronal CB2 cannabinoid receptors in drug abuse and depression: from mice to human subjects. *PLoS ONE*, **3** (2), e1640.

25 Wei, Y., Wang, X., and Wang, L. (2009) Presence and regulation of cannabinoid receptors in human retinal pigment epithelial cells. *Mol. Vision*, **15**, 1243–1251.

26 Zygmunt, P.M., Petersson, J., Andersson, D.A., Chuang, H., Sorgard, M., Di Marzo, V., Julius, D., and Högestätt, E.D. (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature*, **400**, 452–457.

27 Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., and Bonner, T.I. (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*, **346**, 561–564.

28 Buckley, N.E., Hansson, S., Harta, G., and Mezey, E. (1998) Expression of the CB1 and CB2 receptor messenger RNAs during embryonic development in the rat. *Neuroscience*, **82**, 1131–1149.

29 Aguado, T., Palazuelos, J., Monory, K., Stella, N., Cravat, B., Lutz, B., Marsicano, G., Kokaia, Z., Guzmán, M., and Galve-Roperh, I. (2006) The endocannabinoid system promotes astroglial differentiation by acting on neural progenitors cells. *J. Neurosci.*, **26**, 1551–1561.

30 Berghuis, P., Rajnicek, A.M., Morozov, Y.M., Ross, R., and Mulder, J. (2007) Hardwiring the brain: endocannabinoids shape neuronal connectivity. *Science*, **316**, 1212–1216.

31 Jin, K., Xie, L., Kim, S.H., Parmentier-Batteur, S., Sun, Y., Mao, X.O., Childs, J., and Greenberg, D.A. (2004) Defective adult neurogenesis in CB1 cannabinoid receptor knockout mice. *Mol. Pharmacol.*, **66** (2), 204–208.

32 Galve-Roperh, I., Aguado, T., Palazuelos, J., and Guzmán, M. (2008) Mechanisms of control of neuron survival by the endocannabinoid system. *Curr. Pharm. Des.*, **14** (23), 2279–2288.

33 Galve-Roperh, I., Palazuelos, J., Aguado, T., and Guzmán, M. (2009) The endocannabinoid system and the regulation of neural development: potential

implications in psychiatric disorders. *Eur. Arch. Psychiatry Clin. Neurosci.*, **259** (7), 371–382.

34 Gómez, M., Hernández, M., and Fernández-Ruiz, J. (2008) Cannabinoid signaling system: does it play a function in cell proliferation and migration, neuritic elongation and guidance and synaptogenesis during brain ontogenesis? *Cell Adhes. Migr.*, **2** (4), 246–248.

35 Molina-Holgado, E., Arévalo-Martín, A., Vela, J.M., Almazán, G., Molina-Holgado, F., Borrell, J., and Guaza, C. (2002) Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phos-phatidylinositol-3 kinase/Akt signalling. *J. Neurosci.*, **22**, 9742–9753.

36 Gomez, O., Sanchez-Rodriguez, A., Le, M., Sanchez-Caro, C., Molina-Holgado, F., and Molina-Holgado, E. (2011) Cannabinoid receptor agonists modulate oligodendrocyte differentiation by activating PI3K/Akt and the mammalian target of rapamycin (mTOR) pathways. *Br. J. Pharmacol.*, **163** (7), 1520–1532.

37 Heifets, B. and Castillo, P. (2009) Endocannabinoid signaling and long-term synaptic plasticity. *Annu. Rev. Physiol.*, **71**, 283–306.

38 Alger, B.E. and Kim, J. (2011) Supply and demand for endocannabinoids. *Trends Neurosci.*, **34** (6), 304–315.

39 Rivera, P., Luque-Rojas, M., Pastor, A., Blanco, E., Pavon, F., Serrano, A., Crespillo, A., Vida, M., Grondona, J., Cifuentes, M., Bermudez-Silva, F., de la Torre, R., Rodriguez de Fonseca, F., and Suarez, J. (2013) Diet-dependent modulation of hippocampal expression of endocannabinoid signaling-related proteins in cannabinoid antagonist- treated obese rats. *Eur. J. Neurosci.*, **37** (1), 105–117.

40 Navarrete, M. and Araque, A. (2010) Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. *Neuron*, **68**, 113–126.

41 Häring, M., Enk, V., Rey, A.A., Loch, S., de Azua, I.R., Weber, T., Bartsch, D., Monory, K., and Lutz, B. (2015) Cannabinoid type-1 receptor signaling in central serotonergic neurons regulates anxiety-like behavior and sociability. *Front. Behav. Neurosci.*, **9**, 235.

42 Rueda, D., Galve-Roperh, I., Haro, A., and Guzmán, M. (2000) The CB1 cannabinoid receptor is coupled to the activation of c-Jun N-terminal kinase. *Mol. Pharmacol.*, **58** (4), 814–820.

43 Galve-Roperh, I., Sánchez, C., Cortés, M.L., Gómez del Pulgar, T., Izquierdo, M., and Guzmán, M. (2000) Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. *Nat. Med.*, **6** (3), 313–319.

44 Ozaita, A., Puighermanal, E., and Maldonado, R. (2007) Regulation of PI3K/Akt/GSK3 pathway by cannabinoids in the brain. *J. Neurochem.*, **102** (4), 1105–1114.

45 Munro, S., Thomas, K.L., and Abu-Shaar, M. (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, **365**, 61–65.

46 Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carriere, D., Carayon, P., Bouaboula, M., Shire, D., Le Fur, G., and Casellas, P. (1995) Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur. J. Biochem.*, **232** (1), 54–61.

47 Schatz, A.R., Lee, M., Condie, R.B., Pulaski, J.T., and Kaminski, N.E. (1997) Cannabinoid receptors CB1 and CB2: a characterization of expression and

adenylate cyclase modulation within the immune system. *Toxicol. Appl. Pharmacol.*, **142**, 278–287.

48 Nephi, S. (2010) Cannabinoid and Cannabinoid-Like receptors in microglia, astrocytes and astrocytomas. *Glia*, **58**, 1017–1030.

49 Brusco, A., Tagliaferro, P., Saez, T., and Onaivi, E.S. (2008) Postsynaptic localization of CB2 cannabinoid receptors in the rat hippocampus. *Synapse*, **62**, 944–949.

50 Prather, P.L. (2008) in *Cannabinoids and the Brain* (ed. A. Köfalvi), Springer, New York, pp. 75–90.

51 Docagne, F., Muñetón, V., Clemente, D., Ali, C., Loría, F., Correa, F., Hernangómez, M., Mestre, L., Vivien, D., and Guaza, C. (2007) Excitotoxicity in a chronic model of multiple sclerosis: neuroprotective effects of cannabinoids through CB1 and CB2 receptor activation. *Mol. Cell. Neurosci.*, **34**, 551–561.

52 García-Arencibia, M., González, S., de Lago, E., Ramos, J.A., Mechoulam, R., and Fernández-Ruiz, J. (2007) Evaluation of the neuroprotective effect of cannabinoids in a rat model of Parkinson's disease: importance of antioxidant and cannabinoid receptor independent properties. *Brain Res.*, **1134** (1), 162–170.

53 Zani, A., Braida, D., Capurro, V., and Sala, M. (2007) D-tretrahydrocannabinol (THC) and AM 404 protect against cerebral ischaemia in gerbils through a mechanism involving cannabinoid and opioid receptors. *Br. J. Pharmacol.*, **152**, 1301–1311.

54 Bahremand, A., Nasrabady, S.E., Shafaroodi, H., Ghasemi, M., and Dehpour, A.R. (2009) Involvement of nitrergic system in the anticonvulsant effect of the cannabinoid CB(1) agonist ACEA in the pentylenetetrazole-induced seizure in mice. *Epilepsy Res.*, **84** (23), 110–119.

55 Watson, S., Benson, J., and Joy, J. (2000) Marijuana and medicine: assessing the science base: a summary of the 1999 Institute of Medicine report. *Arch. Gen. Psychiatry*, **57**, 547–552.

56 Chi, O.Z., Barsoum, S., Grayson, J., Hunter, C., Liu, X., and Weiss, H.R. (2012) Effects of cannabinoid receptor agonist WIN 55,212-2 on blood–brain barrier disruption in focal cerebral ischemia in rats. *Pharmacology*, **89** (5-6), 333–338.

57 Spano, M.S., Fattore, L., Cadeddu, F., Fratta, W., and Fadda, P. (2012) Chronic cannabinoid exposure reduces phencyclidine-induced schizophrenia-like positive symptoms in adult rats. *Psychopharmacology (Berl.)*, **225** (3), 531–542.

58 Romero-Sandoval, A., Nutile-McMenemy, N., and DeLeo, J.A. (2008) Spinal microglial and perivascular cell cannabinoid receptor type 2 activation reduces behavioral hypersensitivity without tolerance after peripheral nerve injury. *Anesthesiology*, **108** (4), 722–734.

59 Romero-Sandoval, A., Horvath, R., Landry, R.P., and DeLeo, J.A. (2009) Cannabinoid receptor type 2 activation induces a microglial anti-inflammatory phenotype and reduces migration via MKP induction and ERK dephosphorylation. *Mol. Pain*, **5**, 25.

60 Fernandez-Ruiz, J., Sagredo, O., Pazos, M.R., García, C., Pertwee, R., Mechoulam, R., and Martínez-Olgado, J. (2012) Cannabidiol for neurodegenerative disorders: important new clinical application for this phytocannabinoid? *Br. J. Clin. Pharmacol.*, **75** (2), 323–333.

61 Blessing, E.M., Steenkamp, M.M., Manzanares, J., and Marmar, C.R. (2015) Cannabidiol as a potential treatment for anxiety disorders. *Neurotherapeutics*, **12** (4), 825–836.

62 Dirnagl, U., Simon, R.P., and Hallenbeck, J.M. (2003) Ischemic tolerance and endogenous neuroprotection. *Trends Neurosci.*, **26**, 248–254.

63 Go, A.S., Mozaffarian, D., Roger, V.L., Benjamin, E.J., Berry, J.D., Blaha, M.J., Dai, S., Ford, E.S., Fox, C.S., Franco, S., Fullerton, H.J., Gillespie, C., Hailpern, S.M., Heit, J.A., Howard, V.J., Huffman, M.D., Judd, S.E., Kissela, B.M., Kittner, S.J., Lackland, D.T. *et al.* (2014) Heart disease and stroke statistics--2014 update: a report from the American Heart Association. *Circulation*, **129**, e28–e292.

64 Doyle, K., Simon, R., and Stenzel-Poore, M. (2008) Mechanisms of ischemic brain damage. *Neuropharmacology*, **55** (3), 310–318.

65 Pacher, P. and Hasko, G. (2008) Endocannabinoids and cannabinoid receptors in ischaemia-reperfusion injury and preconditioning. *Br. J. Pharmacol.*, **153**, 252–262.

66 Parmentier-Batteur, S., Jin, K., Mao, X., Xie, L., and Greenberg, D. (2002) Increased severity of stroke in CB1 cannabinoid receptor knock-out mice. *J. Neurosci.*, **22** (22), 9771–9775.

67 Caltana, L., Saez, T.M., Aronne, M.P., and Brusco, A. (2015) Cannabinoid receptor type 1 agonist ACEA improves motor recovery and protects neurons in ischemic stroke in mice. *J. Neurochem.*, **135** (3), 616–629.

68 Van der Stelt, M. and Di Marzo, V. (2005) Cannabinoid receptors and their role in neuroprotection. *Neuromol. Med.*, **7** (1–2), 37–50.

69 Louw, D.F., Yangm, F.W., and Sutherland, G. (2000) The effect of delta-9-tetrahydrocannabinol on forebrain ischemia in rat. *Brain Res.*, **857**, 183–187.

70 Mauler, F., Hinz, V., Augstein, K.H., Fassbender, M., and Horvath, E. (2003) Neuroprotective and brain edema reducing efficacy of the novel cannabinoid receptor agonist BAY 38-7271. *Brain Res.*, **989**, 99–111.

71 Youssef, F.F., Hormuzdi, S.G., Irving, A.J., and Frenguelli, B.G. (2007) Cannabinoid modulation of neuronal function after oxygen/glucose deprivation in area CA1 of the rat hippocampus. *Neuropharmacology*, **52**, 1327–1335.

72 Connolly, B.S. and Lang, A.E. (2014) Pharmacological treatment of Parkinson disease: a review. *J. Am. Med. Assoc.*, **311**, 1670–1683.

73 Carroll, C.B., Zeissler, M.L., Hanemann, C.O., and Zajicek, J.P. (2012) Δ^9-tetrahydrocannabinol (Δ^9-THC) exerts a direct neuroprotective effect in a human cell culture model of Parkinson's disease. *Neuropathol. Appl. Neurobiol.*, **38** (6), 535–547.

74 Iuvone, T., Esposito, G., De Filippis, D., Bisogno, T., Petrosino, S., Scuderi, C., Di Marzo, V., Steardo, L., and Endocannabinoid Research Group (2007) Cannabinoid CB1 receptor stimulation affords neuroprotection in MPTP-induced neurotoxicity by attenuating S100B up-regulation in vitro. *J. Mol. Med.*, **85** (12), 1379–1392.

75 Song, L., Yang, X., Ma, Y., Wu, N., and Liu, Z. (2014) The CB1 cannabinoid receptor agonist reduces L-DOPA-induced motor fluctuation and ERK1/2 phosphorylation in 6-OHDA-lesioned rats. *Drug Des. Dev. Ther.*, **8**, 2173–2179.

76 Lastres-Becker, I., Molina-Holgado, F., Ramos, J.A., Mechoulam, R., and Fernández-Ruiz, J. (2005) Cannabinoids provide neuroprotection against

6-hydroxydopamine toxicity *in vivo* and *in vitro*: relevance to Parkinson's disease. *Neurobiol. Dis.*, **19**, 96–107.

77 Price, D.A., Owens, W.A., Gould, G.G., Frazer, A., Roberts, J.L., Daws, L.C., and Giuffrida, A. (2007) CB1-independent inhibition of dopamine transporter activity by cannabinoids in mouse dorsal striatum. *J. Neurochem.*, **101** (2), 389–396.

78 Price, D.A., Martinez, A.A., Seillier, A., Koek, W., Acosta, Y., Fernandez, E., Strong, R., Lutz, B., Marsicano, G., Roberts, J.L., and Giuffrida, A. (2009) WIN55,212-2, a cannabinoid receptor agonist, protects against nigrostriatal cell loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Eur. J. Neurosci.*, **29** (11), 2177–2186.

79 García, M.C., Cinquina, V., Palomo-Garo, C., Rábano, A., and Fernández-Ruiz, J. (2015) Identification of CB_2 receptors in human nigral neurons that degenerate in Parkinson's disease. *Neurosci. Lett.*, **587**, 1–4.

80 Gómez-Gálvez, Y., Palomo-Garo, C., Fernández-Ruiz, J., and García, C. (2016) Potential of the cannabinoid CB(2) receptor as a pharmacological target against inflammation in Parkinson's disease. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, **64**, 200–208.

81 Chagas, M.H., Zuardi, A.W., Tumas, V., Pena-Pereira, M.A., Sobreira, E.T., Bergamaschi, M.M., dos Santos, A.C., Teixeira, A.L., Hallak, J.E., and Crippa, J.A. (2014) Effects of cannabidiol in the treatment of patients with Parkinson's disease: an exploratory double-blind trial. *J. Psychopharmacol.*, **28** (11), 1088–1098.

82 Chagas, M.H., Eckeli, A.L., Zuardi, A.W., Pena-Pereira, M.A., Sobreira-Neto, M.A., Sobreira, E.T., Camilo, M.R., Bergamaschi, M.M., Schenck, C.H., Hallak, J.E., Tumas, V., and Crippa, J.A. (2014) Cannabidiol can improve complex sleep-related behaviours associated with rapid eye movement sleep behaviour disorder in Parkinson's disease patients: a case series. *J. Clin. Pharm. Ther.*, **39** (5), 564–566.

83 Aso, E. and Ferrer, I. (2014) Cannabinoids for treatment of Alzheimer's disease: moving toward the clinic. *Front. Pharmacol.*, **5**, 37.

84 Ramírez, B.G., Blázquez, C., Gómez del Pulgar, T., Guzmán, M., and de Ceballos, M.L. (2005) Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J. Neurosci.*, **25**, 1904–1913.

85 Solas, M., Francis, P.T., Franco, R., and Ramirez, M.J. (2013) CB2 receptor and amyloid pathology in frontal cortex of Alzheimer's disease patients. *Neurobiol. Aging*, **34** (3), 805–808.

86 Casarejos, M.J., Perucho, J., Gomez, A., Muñoz, M.P., Fernandez-Estevez, M., Sagredo, O., Fernandez Ruiz, J., Guzman, M., de Yebenes, J.G., and Mena, M.A. (2013) Natural cannabinoids improve dopamine neurotransmission and tau and amyloid pathology in a mouse model of tauopathy. *J. Alzheimers Dis.*, **35** (3), 525–539.

87 Rektor, I., Schachter, S.C., Arya, R., Arzy, S., Braakman, H., Brodie, M.J., Brugger, P., Chang, B.S., Guekht, A., Hermann, B., Hesdorffer, D.C., Jones-Gotman, M., Kanner, A.M., Garcia-Larrea, L., Mareš, P., Mula, M., Neufeld, M., Risse, G.L., Ryvlin, P., Seeck, M., Tomson, T., and Korczyn, A.D. (2015) Third international congress on epilepsy, brain and mind: part 2. *Epilepsy Behav.*, **50**, 138–159.

88 Reddy, D.S. (2014) Clinical pharmacology of current antiepileptic drugs. *Int. J. Pharm. Sci. Nanotechnol.*, **7** (1), 2305–2319.

89 French, J.A. (2006) Refractory epilepsy: one size does not fit all. *Epilepsy Curr.*, **6** (6), 177–180.

90 Jones, N.A., Hill, A.J., Smith, I., Bevan, S.A., Williams, C.M., Whalley, B.J., and Stephens, G.J. (2010) Cannabidiol display antiepileptiform and antiseizure properties *in vitro* and *in vivo*. *J. Pharmacol. Exp. Ther.*, **332**, 569–577.

91 Jones, N.A., Glyn, S.E., Akiyama, S., Hill, T.D., Hill, A.J., Weston, S.E., Burnett, M.D., Yamasaki, Y., Stephens, G.J., Whalley, B.J., and Williams, C.M. (2012) Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures. *Seizure*, **21**, 344–352.

92 Shirazi-zand, Z., Ahmad-Molaei, L., Motamedi, F., and Naderi, N. (2013) The role of potassium BK channels in anticonvulsant effect of cannabidiol in pentylenetetrazole and maximal electroshock models of seizure in mice. *Epilepsy Behav.*, **28**, 1–7.

93 Bergamaschi, M.M., Queiroz, R.H., Zuardi, A.W., and Crippa, J.A. (2011) Safety and side effects of cannabidiol, a *Cannabis sativa* constituent. *Curr. Drug Saf.*, **6**, 237–249.

94 Press, C.A., Knupp, K.G., and Chapman, K.E. (2015) Parental reporting of response to oral cannabis extracts for treatment of refractory epilepsy. *Epilepsy Behav.*, **45**, 49–52.

95 Saade, D. and Joshi, C. (2015) Pure cannabidiol in the treatment of malignant migrating partial seizures in infancy: a case report. *Pediatr. Neurol.*, **52** (5), 544–547.

96 Collin, C., Davies, P., Mutiboko, I., and Ratcliffe, S. (2007) Randomized controlled trial of cannabis-based medicine in spasticity caused by multiple sclerosis. *Eur. J. Neurol.*, **14**, 290–296.

97 Novotna, A., Mares, J., Ratcliffe, S., Novakova, I., Vachova, M., Zapletalova, O. *et al.* (2011) A randomized, double-blind, placebo-controlled, parallel-group, enriched-design study of nabiximols* (Sativex®) as add-on therapy, in subjects with refractory spasticity caused by multiple sclerosis. *Eur. J. Neurol.*, **18**, 1122–1131.

98 Zettl, U.K., Rommer, P., Hipp, P., and Patejdl, R. (2016) Evidence for the efficacy and effectiveness of THC-CBD oromucosal spray in symptom management of patients with spasticity due to multiple sclerosis. *Ther. Adv. Neurol. Disord.*, **9** (1), 9–30.

99 Russo, M., Naro, A., Leo, A., Sessa, E., D'Aleo, G., Bramanti, P., and Calabro, R.S. (2016) Evaluating sativex® in neuropathic pain management: a clinical and neurophysiological assessment in multiple sclerosis. *Pain Med.*, **17** (6), 1145–1154.

100 Valverde, O. (2005) Participation of the cannabinoid system in the regulation of emotional-like behavior. *Curr. Pharm. Des.*, **11**, 3421–3429.

101 Moreira, F.A. and Lutz, B. (2008) The endocannabinoid system: emotion, learning and addiction. *Addict. Biol.*, **13**, 196–212.

102 Hill, M.N. and Gorzalka, B.B. (2005) Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? *Behav. Pharmacol.*, **16**, 333–352.

103 Sanchís-Segura, C., Cline, B.H., Marsicano, G., Lutz, B., and Spanagel, R. (2004) Reduced sensitivity to reward in CB1 knockout mice. *Psychopharmacology*, **176**, 223–232.

104 Maldonado, R., Valverde, O., and Berrendero, F. (2006) Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci.*, **29**, 225–232.

105 Cota, D., Marsicano, G., Tschöp, M., Grübler, Y., Flachskamm, C., Schubert, M., Auer, D., Yassouridis, A., Thöne-Reineke, C., Ortmann, S., Tomassoni, F., Cervino, C., Nisoli, E., Linthorst, A.C., Pasquali, R., Lutz, B., Stalla, G.K., and Pagotto, U. (2003) The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J. Clin. Invest.*, **112**, 423–431.

106 Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., Hermann, H., Tang, J., Hofmann, C., Zieglgänsberger, W., Di Marzo, V., and Lutz, B. (2002) The endogenous cannabinoid system controls extinction of aversive memories. *Nature*, **418**, 530–534.

107 Martín, M., Ledent, C., Parmentier, M., Maldonado, R., and Valverde, O. (2002) Involvement of CB1 cannabinoid receptors in emotional behavior. *Psychopharmacology*, **159**, 379–387.

108 Aso, E., Ozaita, A., Valdizán, E.M., Ledent, C., Pazos, A., Maldonado, R., and Valverde, O. (2008) BDNF impairment in the hippocampus is related to enhanced despair behaviour in CB1 knockout mice. *J. Neurochem.*, **105**, 565–572.

109 Christensen, R., Kristensen, P.K., Bartels, E.M., Bliddal, H., and Astrup, A. (2007) Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. *Lancet*, **370**, 1706–1713.

110 Onaivi, E.S., Leonard, C.M., Ishiguro, H., Zhang, P.W., Lin, Z., Akinshola, B.E., and Uhl, G.R. (2002) Endocannabinoids and cannabinoid receptor genetics. *Prog. Neurobiol.*, **66** (5), 307–344.

111 Barna, I., Zelena, D., Arszovszki, A.C., and Ledent, C. (2004) The role of endogenous cannabinoids in the hypothalamo-pituitary–adrenal axis regulation: *in vivo* and *in vitro* studies in CB1 receptor knockout mice. *Life Sci.*, **75**, 2959–2970.

112 Patel, S., Roelke, C.T., Rademacher, D.J., Cullinan, W.E., and Hillard, C.J. (2004) Endocannabinoid signaling negatively modulates stress-induced activation of the hypothalamic-pituitary–adrenal axis. *Endocrinology*, **145**, 5431–5438.

113 Cota, D., Steiner, M.A., Marsicano, G., Cervino, C., Herman, J.P., Grübler, Y., Stalla, J., Pasquali, R., Lutz, B., Stalla, G.K., and Pagotto, U. (2007) Requirement of cannabinoid receptor type 1 for the basal modulation of hypothalamic-pituitary–adrenal axis function. *Endocrinology*, **148**, 1574–1581.

114 Bilkei-Gorzo, A., Racz, I., Valverde, O., Otto, M., Michel, K., Sastre, M., and Zimmer, A. (2005) Early age-related cognitive impairment in mice lacking cannabinoid CB1 receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **102**, 15670–15675.

115 Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., Azad, S.C., Cascio, M.G., Gutiérrez, S.O., van der Stelt, M., López-Rodriguez, M.L., Casanova, E., Schütz, G., Zieglgänsberger, W., Di Marzo, V., Behl, C., and Lutz, B. (2003) CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science*, **302**, 84–88.

116 Fride, E., Suris, R., Weidenfeld, J., and Mechoulam, R. (2005) Differential response to acute and repeated stress in cannabinoid CB1 receptor knockout newborn and adult mice. *Behav. Pharmacol.*, **16**, 431–440.

117 Leweke, F., Piomelli, D., Pahlisch, F., Muhl, D., Gerth, C.W., Hoyer, C., Klosterkötter, J., Hellmich, M., and Koethe, D. (2012) Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Transl. Psychiatry*, **2**, e94.

118 Campos, A.C. and Guimarães, F.S. (2008) Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. *Psychopharmacology*, **199** (2), 223–230.

119 Lisboa, S.F., Gomes, F.V., Guimaraes, F.S., and Campos, A.C. (2016) Microglial cells as a link between cannabinoids and the immune hypothesis of psychiatric disorders. *Front. Neurol.*, **7**, 5.

120 Yazulla, S., Studholme, K.M., McIntosh, H.H., and Deutsh, D.G. (1999) Immonocytochemical localization of cannabinoid CB1 receptor and fatty acid amide hydrolase in rat retina. *J. Comp. Neurol.*, **415**, 80–90.

121 Lopez, E.M., Tagliaferro, A.P., Onaivi, E.S., and López-Costa, J.J. (2011) Distribution of CB2 receptors in adult rat retina. *Synapse*, **65** (5), 388–392.

122 Schwitzer, T., Schwan, R., Angioi-Duprez, K., Giersch, A., and Laprevote, V. (2016) The endocannabinoid system in the retina: from physiology to practical and therapeutic application. *Neural Plast.*, **2016**, 2916732.

123 Lax, P., Esquiva, G., Altavilla, C., and Cuenca, N. (2014) Neuroprotective effects of the cannabinoid agonist HU210 on retinal degeneration. *Exp. Eye Res.*, **120**, 175–185.

124 El-Remessy, A.A.B., Al-Shaabrawey, M., Khalifa, Y., and Tsai, N.-T. (2006) Neuroprotective and blood-retinal barrier- preserving effects of cannabidiol in experimental diabetes. *Am. J. Pathol.*, **168**, 235–244.

125 Cairns, E.A., Baldridge, W.H., and Kelly, M.E.M. (2016) The endocannabinoid system as a therapeutic target in glaucoma. *Neural Plast.*, **2016**, 9364091.

126 Pinar-Sueiro, S., Rodríguez-Puertas, R., and Vecino, E. (2011) Aplicaciones de los cannabinoides en glaucoma. *Arch. Soc. Esp. Oftalmol.*, **86** (1), 16–23.

Index

Neuroprotective Natural Products: Clinical Aspects and Mode of Action, First Edition.
Edited by Goutam Brahmachari.