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M. Mohamed Essa
Mohammed Akbar
Gilles Guillemin *Editors*

The Benefits of Natural Products for Neurodegenerative Diseases

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M. Mohamed Essa • Mohammed Akbar
Gilles Guillemin
Editors

The Benefits of Natural Products for Neurodegenerative Diseases

Springer

Editors
M. Mohamed Essa
Food Science and Nutrition
College of Agricultural and Marine Sciences
Sultan Qaboos University
Muscat, Oman

Mohammed Akbar
National Institutes of Health
National Institute on Alcohol Abuse
Rockville, MD, USA

Gilles Guillemin
Australian School of Advanced Medicine
Macquarie University
Sydney, NSW, Australia

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Preface

Few centuries B.C., the Greek physician Hippocrates rightly said, "Let food be thy medicine and medicine thy food." But not only in the Greek civilisation but also in many other cultures, natural products have been used for the relief of neurological symptoms and diseases over the centuries. Natural products can be used for prevention and/or treatment to delay progression or limit symptoms and severity of large number of brain diseases. However, for a large number of them, their specific mechanisms of action often still remain unknown.

Recent investigations and new technologies are progressively unveiling the complex molecular mechanisms of a plethora of plant extracts and natural compounds for their neuroprotective and therapeutic abilities.

This book is focusing on the recent contributions of medicinal natural products chemistry to the discovery of new chemical entities useful to the control and prevention of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, frontotemporal dementia, multiple sclerosis, and neurologic traumatic injuries including stroke, spinal cord injury, brain trauma, and cerebral ischemia.

Muscat, Oman — Mohamed Essa
Sydney, NSW, Australia — Gilles Guillemin
Rockville, MD, USA — Mohammed Akbar

Acknowledgments

We are highly thankful to all the contributors of this book for sharing their expertise to provide evidence-based information on foods and natural products beneficial to brain functions.

We are indebted to our families for their understanding and unconditional support allowing us to spend extra time on completing the book. Many thanks are due to our teachers who have inspired us to investigate the potential health and medicinal benefits of the food and natural products.

The support provided from the research grant from The Research Council, Oman (RC/AGR/FOOD/11/01), is highly acknowledged by us.

We have to thank Springer staff for their valuable assistance and constant support during the different phases of this book publication.

Dr. Mohamed Essa
Prof. Gilles Guillemin
Dr. Mohammed Akbar

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Erratum E1

The original version of this book was revised. An erratum to this book can be found at DOI 10.1007/978-3-319-28383-8_24

Editors Short Biography

Mohamed Essa is an Associate Professor of Nutrition at Sultan Qaboos University, Oman, and holding visiting A/Prof position in Neuropharmacology group, ASAM, Macquarie University, Sydney, Australia. He is an editor-in-chief for *International Journal of Nutrition, Pharmacology, Neurological Diseases* published by Wolters and Kluwer, USA, and involved in editor/reviewer board of various well-known journals such as *Frontiers in Neuroscience*, Biochimie, and *PLOS one*.

He is an expert in the field of Nutritional Neuroscience and published 91 papers, 32 book chapters, and 7 books (4 published and 3 in press). He has strong international collaborations with institutes in the USA, Australia, and India.

Recently he founded a new foundation named "Food and Brain Research Foundation" to support research in nutritional neuroscience. He is holding memberships in various international bodies including American Society for Neurochemistry (ASN), International Society for Neurochemistry (ISN), etc.

He has received so many awards from local and international bodies and this year one of his book titled *Food and Brain health* was awarded as best book in the World by GOURMAND Cook Book Awards. This year he got the National Research Award from Oman. The same book was awarded as best in the best in the world of past 20 years of awards by GOURMAND Cook Book Awards. He has received many research grants from local and international agencies.

Email: drmdessa@gmail.com

Gilles Guillemin has been working in the fields of Neuroimmunology and tryptophan metabolism for more than 20 years. He is the Co-Director of the MND and Neurodegenerative Diseases Research Centre at Macquarie University. Prof Guillemin's team is one of the world's leading research groups working on the involvement of the tryptophan catabolism (via the kynurenine pathway) in human neurodegenerative diseases.

He demonstrated this pathway in multiple sclerosis, Alzheimer's disease, amyotrophic lateral sclerosis, etc. He is the President of the International Society for Tryptophan Research and the Editor-in-Chief of the *International Journal for Tryptophan Research*.

He has published an overall 165 peer-reviewed scientific articles. His current h-index is 42.

Email: gilles.guillemin@MQ.edu.au; Ph. +61 9350 2727

Mohammed Akbar is a cell biologist and Program Officer in the Division of Metabolism and Health Effects at National Institute on Alcohol Abuse and Alcoholism (NIAAA). He received his Ph.D. from Gunma University, School of Medicine, Maebashi, Japan, where he investigated the signal transduction mechanisms of purinergic receptors under the supervision of Prof. Yoichi Kondo. He worked for the pharmaceutical company (Chugai Institute of Molecular Medicine, Chugai Pharmaceuticals, Japan) in the drug discovery projects. After moving to NIH, he has been conducting research on the effects of fatty acids and alcohol and their cell signaling in neuronal and non-neuronal tissues, at the intramural division of NIAAA. Before joining the extramural division, he was engaged in studying non-alcoholic and alcohol-induced liver diseases, dietary supplements and natural products, oxidative stress, and metabolic syndromes. His research interests are cell signaling, oxidative stress, and natural products in metabolic syndromes. He has published more than 30 papers in peer-reviewed journals, co-authored 2 books, and given 13 invited presentations in different countries, including USA, Japan, Austria, Oman, and South Korea.

E-mail: akbarm@mail.nih.gov; Phone: 1-301-443-6009

Epigenetic Modifications in Neurological Diseases: Natural Products as Epigenetic Modulators a Treatment Strategy

Omkaram Gangisetty and Sengottuvelan Murugan

Abstract Epigenetic modifications, including DNA methylation, covalent histone modifications, and small noncoding RNAs, play a key role in regulating the gene expression. This regulatory mechanism is important in cellular differentiation and development. Recent advances in the field of epigenetics extended the role of epigenetic mechanisms in controlling key biological processes such as genome imprinting and X-chromosome inactivation. Aberrant epigenetic modifications are associated with the development of many diseases. The role of epigenetic modifications in various neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, Huntington disease, epilepsy, and multiple sclerosis is rapidly emerging. The use of epigenetic modifying drugs to treat these diseases has been the interest in recent years. A number of natural products having diverse mechanism of action are used for drug discovery. For many years, natural compounds have been used to treat various neurodegenerative diseases, but the use of such compounds as epigenetic modulators to reverse or treat neurological diseases are not well studied. In this chapter, we mainly focus on how various epigenetic modifications play a key role in neurodegenerative diseases, their mechanism of action, and how it acts as a potential therapeutic target for epigenetic drugs to treat these diseases will be discussed.

Keywords DNA methylation • Histone deacetylases (HDACs) • Neurological diseases • Epigenetic modulators • Dietary products

Gangisetty and Murugan contributed equally with all other contributors.

O. Gangisetty (✉)
Endocrine Research Facility, Department of Animal Sciences, Rutgers University, 67 Poultry Farm Lane, New Brunswick, NJ 08901, USA
e-mail: og53@rci.rutgers.edu

S. Murugan (✉)
Hematology/Oncology Division, Perelman School of Medicine, University of Pennsylvania, Clinical Research Building, Philadelphia 19104, PA, USA
e-mail: murugan@mail.med.upenn.edu; velan2009@gmail.com

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Introduction

The eukaryotic genome is organized and packed into chromatin, which is a complex structure composed of DNA, histone, and nonhistone proteins. Chromatin remodeling is a dynamic process that modulates gene expression. Chromatin exists either in a condensed, inactive, transcriptionally repressive state called heterochromatin or transcriptionally active state called euchromatin. The term epigenetics is defined as heritable change in gene expression without altering the DNA sequence. Epigenetic modifications regulating gene expression are reversible and have long lasting effects. These epigenetic modifications control the gene expression during cellular development through DNA methylation, histone code modifications, and small noncoding RNAs (Costa 2008; Kouzarides 2007). All the three mechanisms regulate gene expression without altering the DNA sequence. There is a complex interplay between these three processes to regulate the gene expression (Fig. 1). These epigenetic modifications are involved in a number of essential cellular processes such as transcription, cellular differentiation, development, X-chromosome inactivation, gene imprinting, and cellular responses to environmental stimuli (Guibert and Weber 2013; Klose and Bird 2006; Smith and Meissner 2013; Subramaniam et al. 2014). Aberrant epigenetic modifications have been extensively reported in cancer. In recent years, the upcoming interest is developing on studying the role of epigenetic modifications in a number of neuropsychiatric and neurodegenerative diseases including schizophrenia (Grayson et al. 2005; Veldic et al. 2004, 2005) epilepsy, Alzheimer's disease (AD), Huntington's, and Parkinson's diseases (PD). Abnormal epigenetic mechanisms also have been reported in a number of mental disorders such as Rett, ICF, Fragile-X, and ATRX syndrome (Egger et al. 2004).

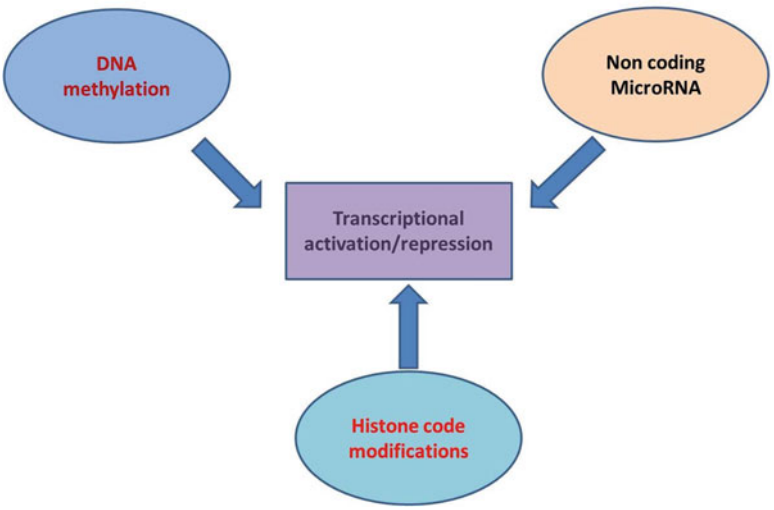


Fig. 1 Epigenetic regulation of gene expression. DNA methylation, histone code modifications, and noncoding microRNAs control gene expression without altering the DNA sequence. There is a complex interplay between the three epigenetic modifications to regulate gene activation or repression

DNA Methylation

DNA methylation is one of the most classically studied epigenetic modifications and involves covalent modification of cytosine residue in the CpG back ground by the addition of a methyl group at the fifth carbon position on its pyrimidine ring. The CpG dinucleotide rich regions are called CpG islands and are found in the promoter regions of the gene. The Promoter CpG island methylation plays an important role in regulating the gene expression by preventing transcription factors binding on to the promoter and thereby recruiting transcriptional repressors on to the promoter regions (Cedar and Bergman 2012). DNA methylation is also involved in silencing of imprinted genes in which only one allele, either paternal or maternal, is expressed (Reik et al. 2001; Edwards and Ferguson-Smith 2007). It is also involved in X chromosome inactivation in women (Jaenisch and Bird 2003). DNA methylation is also associated with maintenance of chromosomal stability and translocation prevention (Bird 2002). In human genome, most of the methylated CpGs occur in repetitive sequences such as long interspersed transposable element 1 (LINE1) and Alu repeats (Edwards and Myers 2008).

DNA Methyl Transferases

DNA methyl transferases catalyze the addition of a methyl group onto the cytosine nucleotide by utilizing *S*-adenosyl methionine (SAM) as the methyl donor. There are about five DNA methyl transferases reported that play a crucial role in establishing and maintaining DNA methylation. They are DNMT1, DNMT3A, DNMT3B, DNMT3L, and DNMT2. They share common structural similarities of having an N terminal regulatory and C terminal catalytic domain. Of all the five members of the DNMT family, DNMT1, 3A, and 3B are required to establish and maintain the genome methylation (Fig. 2).

DNMT1 is most abundant in mammalian cells and first murine DNMT cloned (Bestor 1988). DNMT1 plays an important role in methylating newly synthesized DNA during replication. It has a preference toward hemimethylated DNA and is responsible for copying preexisting methylation patterns to the newly synthesized DNA strand. It is also called maintenance methyl transferase. It is a large protein with around 1620 amino acids in length. It has an N terminal regulatory domain and C terminal catalytic domain. DNMT1 plays an essential role in development. DNMT1 knockout mice results in early embryonic lethality (Li et al. 1992).

DNMT 3A and 3B are other groups of DNMTs that effectively methylate unmethylated DNA de novo. They are considered as de novo DNA methyl transferases. They are encoded by two different genes and have structural homology with N terminal regulatory domain and C terminal catalytic domain. They play an important role in germ cell development and embryogenesis. In addition to redundancy in de novo methylation, these enzymes have different functional roles. DNMT3A is

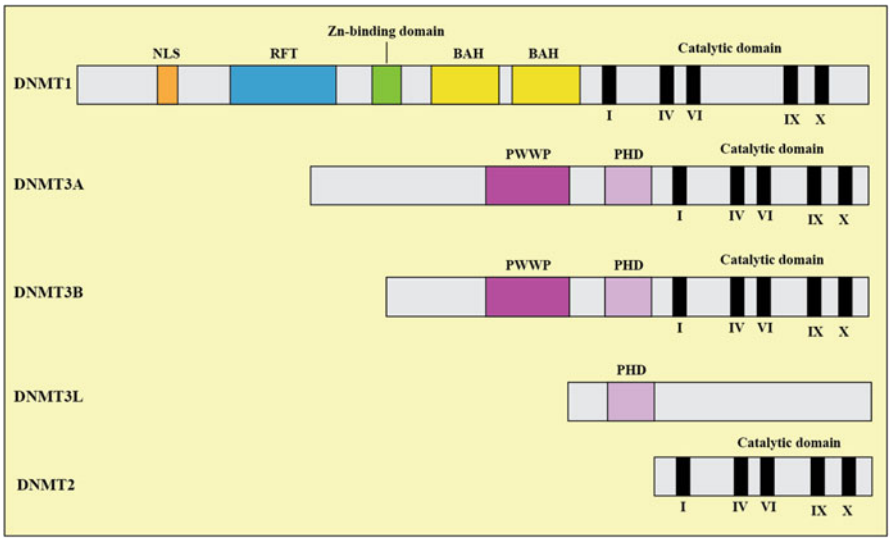


Fig. 2 A schematic representation of DNA methyl transferases (DNMTs). The five member family of DNMTs includes DNMT1, DNMT2, DNMT3A, 3B, and 3L. They all share conserved methyl transferase motifs indicated by *roman numerals* in the catalytic domain at C terminus. The regulatory N terminal domain is represented with NLS (nuclear localization signal), RFT (replication foci targeting domain), BAH (bromo-adjacent homology domain), PWWP (proline–tryptophan–tryptophan–proline) motif, and PHD (plant homeodomain). DNMT3L lacks methyl transferase motifs and is catalytically in active. DNMT2 lacks N terminal regulatory domain

ubiquitously expressed and DNMT3B is expressed at low levels except in testis, thyroid, and bone marrow (Xie et al. 1999). DNMT3B expression is increased in tumor cell lines and focused on methylating CpGs in repetitive sequences of pericentric regions of the chromosome (Hansen et al. 1999; Xu et al. 1999).

DNMT3L is another member of DNA methyl transferase that lacks the methyl transferase activity. It may cooperate with other de novo DNMTs and thereby increase the activity of that enzyme. It plays an important role in genomic imprinting since targeted disruption of DNMT3L resulted in biallelic expression of genes imprinted and expressed from one parental origin (Bourc'his et al. 2001).

DNMT2 was cloned based on its sequence homology with other DNMTs. It is the most conserved and its targeted disruption in ES cells did not detect any effect on global methylation suggesting it is not an essential for DNA methylation (Okano et al. 1998).

DNA Demethylation

DNA methylation has long thought to be a permanent epigenetic mark and is irreversible. However, it is a dynamic process during early mammalian development and alteration of methylation is also important for normal development (Shemer

and Razin 1996). There is considerable evidence supporting genome-wide active demethylation found in zygotes (Mayer et al. 2000; Oswald et al. 2000), primordial germ cells (Morgan et al. 2005; Hajkova et al. 2002), and locus-specific active demethylation observed in somatic cells such as neurons (Ma et al. 2009) and T lymphocytes (Bruniquel and Schwartz 2003). However, the mechanism of active demethylation is not clearly understood. Recent studies showed 5-hydroxymethylcytosine (5hmC) is likely to have an important implication in mammalian genome for active demethylation. The substantial amount of 5hmC has been detected in mouse purkinje neurons (Kriaucionis and Heintz 2009) and in ES cells (Tahiliani et al. 2009) In humans, TET family of proteins, TET1, TET2, and TET3, have been identified to catalyze the conversion of 5mC to 5hmC (Tahiliani et al. 2009).

Histone Code Modifications

In the eukaryotic cell nucleus, DNA is packed with histone octomer composed of two copies each of H2A, H2B, H3, and H4. Chromatin remodeling in the brain is characterized by posttranslational modification of histones. The specific amino acid residues such as lysine, arginine, serine, and threonine at the N terminal tail of histones subjected to potential modifications such as acetylation, methylation, phosphorylation, ubiquitination, and sumoylation. These modifications are associated with transcriptional activation or repression depending on the site of residue and the type of modification thereby forms the histone code. Posttranslational modification of histones is a dynamic and reversible process mediated by two different sets of enzyme complexes that add or remove a particular chemical group in a site-specific manner.

Histone Acetylation and Deacetylation

Histone acetylation is associated with positive transcription. Histone acetylation of lysine residue is one of the well-studied histone modifications. Histone H3 and H4 acetylation increases gene expression by promoting open configuration of chromatin. It is mainly catalyzed by histone acetyl transferases (HATs). They catalyze the transfer of acetyl group from acetyl co-A to lysine residues of histones. These acetyl groups neutralize positive histone charge, thereby opening up the chromatin for transcriptional activation. Some of the HATs include GCN5-related *N* acetyl transferases, MYST HATS, p300/CBP HATS, TATA binding protein-associated factor II (TAF II), RE1 silencing transcription factor (REST), nuclear factor kappa B (NFkB), etc. Acetylation is a transient mark and is vital for precise temporal transcription control. There are a number of acetylation sites on histone residues dynamically regulated by HATs and Histone deacetylases (HDACs). The most important acetylation sites of histone H3 are H3K9, K14, K18, and K56. The histone H4 acetylation sites are H4K5, K8, K12, and K16. Another histone H2B lysine residues are also

acetylated at K7, K16, and K17. All these histone code modifications play a role in transcriptional activation (Strahl and Allis 2000). Histone deacetylation involves removal of acetyl groups of lysine residues in the conserved tails of core histone proteins, thereby altering the negative to the positive charge. This results in the tight interaction of histones with negatively charged DNA, thereby facilitating the closed chromatin structure. It is associated with transcriptional repression catalyzed by HDACs. There are two major family proteins with HDAC activity. Sir2 (silent information regulator-2) or sirtuin (sir2-like protein) family of NAD-dependent HDACs (Class III HDACs) and classical HDAC family protein (De Ruijter et al. 2003; Yang and Seto 2008). The classical HDAC family proteins comprise three different classes such as class I, II, and IV. The class I HDACs includes HDAC1, 2, 3, and 8 which are smaller proteins. The class II HDACs includes HDAC 4, 5, 6, 7, 9, and 10 which are larger proteins (Bjerling et al. 2002; Fischle et al. 2002). The class IV HDAC member includes HDAC11 which has sequence similarity to class I and II HDACs (Gregoretti et al. 2004).

Histone Methylation and Demethylation

Histone methylation is associated with both transcriptional activation and repression depending on the modified amino acid residues. It occurs mainly on lysine and arginine residues either as mono-, di- and, trimethylation. Methylation of H3K4 and H3K36 are associated with transcriptional activation. H3K9, K27, and H4K20 methylations are associated with transcriptional repression (Barski et al. 2007). Histone methyl transferases (HMTs) which catalyze H3K9, K27, and H4K20 include G9a, GLP (Tachibana et al. 2002, 2005), SUV39H1, EZH2 (Cao et al. 2002), and PR-SET7 (Nishioka et al. 2002). However, SET7/9 mediates H3K4-specific methylation (Wang et al. 2003). These enzymes catalyze the transfer of the methyl group from *S*-adenosyl-L-methionine to the lysine residues of histones. Like other histone modifications, histone demethylation also plays an important role in the regulation of gene expression. Lysine-specific demethylase1 (LSD1) is the first reported histone demethylase which act on mono- and dimethylations (Shi et al. 2004). Jumanji domain containing protein is another histone demethylase that acts on trimethylated as well as mono- and dimethylated lysine's (Tsukada et al. 2006; Klose et al. 2006).

The overall epigenetic modification machinery including DNA methylation, demethylation, and various histone code modifications regulate gene expression either positively or negatively constitute the whole epigenome as it is represented in Fig. 3.

Epigenetic Dysregulation in Neurological Diseases

DNA methylation has been implicated in regulation of gene activity in the adult brain. It is linked to activation or repression of genes by synaptic activity. Such mechanisms regulate the expression of specific sets of neuronal genes that are

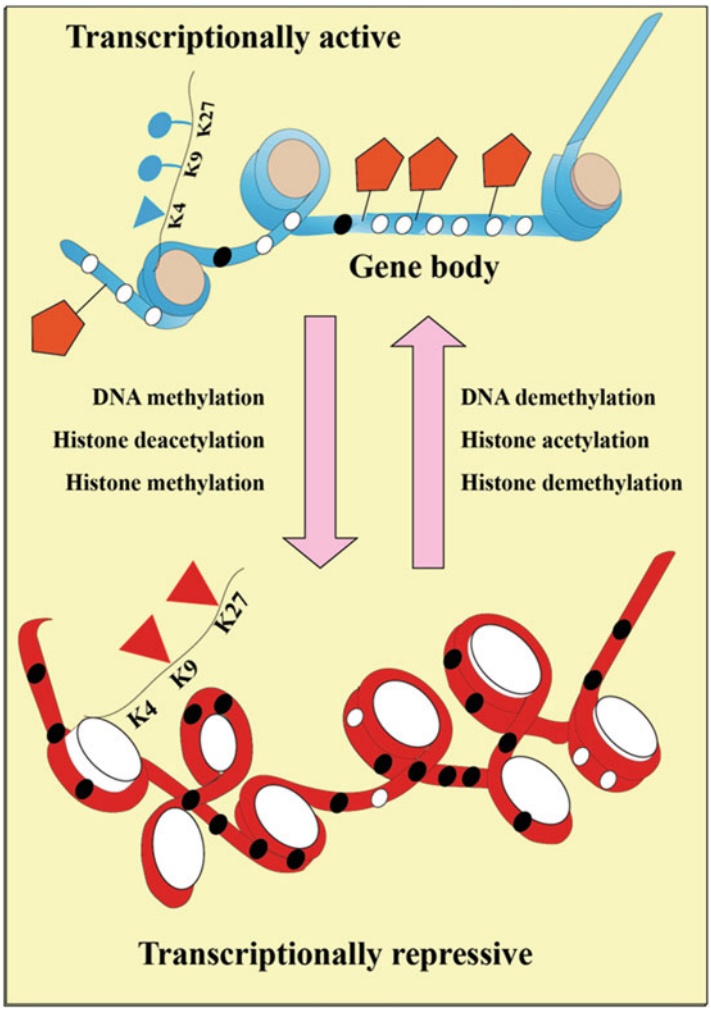


Fig. 3 Euchromatin- and heterochromatin-associated epigenetic modifications. Transcriptionally active euchromatin is represented in *blue* at *top*. DNA wrapped around histones. Unmethyl cytosine residues are represented in *open circles* on DNA. The hydroxyl methyl cytosine was represented as *pentagon structure* on the DNA. The N terminal tail of histone3 (H3) is represented with methyl group (*filled blue inverted triangle*) at K4 and acetyl group (*filled blue circles*) at K9 and K27. Transcriptionally inactive heterochromatin is represented in *red* at *bottom*. DNA wrapped around histones. Methyl cytosine residues are represented in *closed circles* (*filled black circles*). The N terminal tail of H3 is represented with methyl group (*filled red inverted triangle*) at K9 and K27

important for neural activity, survival, and morphology of neurons. DNA methylation patterns were altered in schizophrenia, Alzheimer's, Parkinson's, and other related psychiatric diseases. There is growing evidence that DNA methylation is involved in the pathophysiological mechanism of depression and addiction (Table 1).

Table 1 Epigenetic modifications associated with neurological diseases

Neurological disease	Epigenetic modification	Reference
Schizophrenia		
DNA methylation	Hypermethylation of GADD67 and Reelin in GABAergic neurons.	Veldic et al. (2004), Grayson et al. (2005), Ruzicka et al. (2007)
	Over expression of DNMT1 in GABAergic neurons.	
	Increased plasma homocysteine levels.	Applebaum et al. (2004), Levine et al. (2002), Adler Nevo et al. (2006)
Histone modifications	Increased H3K9 and H3R17 di- and trimethylation at GAD1 promoter.	Akbarian (2010)
	Loss of H3K4 methylation marks and excess of H3K27 methylation at GAD67 promoter.	Huang et al. (2007)
	G9a, GLP, and SETDB1 are increased across genome in lymphocytes.	Wang et al. (2003), Zee et al. (2010)
	HDAC1 expression is increased in prefrontal cortex of schizophrenia patients.	Sharma et al. (2008)
Alzheimer's disease		
DNA methylation	Genome-wide hypomethylation in AD patients.	Mastroeni et al. (2011)
	SAM levels were significantly reduced in AD.	Bottinglieri et al. (1990), Morrison et al. (1996)
	Repetitive Alu elements were hypomethylated with aging but not the repetitive long interspersed transposable elements (LINE-1).	Bollati et al. (2009)
Histone modifications	Accumulation of phospho-H2AX in AD.	Myung et al. (2008)
	Tip60 a HAT acetylates H4 necessary for correct repair of DNA is important in AD.	Stante et al. (2009)
	HDAC2 deficiency results in increased synapse number and memory facilitation supporting the role of histone acetylation and deacetylation in AD.	Guan et al. (2009)
Parkinson's disease		
DNA methylation	Hypomethylation of SCNA gene promoter with increased expression of α-synuclein which aggregates to form Lewy bodies which is hall mark of PD.	Ammal Kaidery et al. (2013) Pieper et al. (2008)
	TNF-α promoter hypomethylation with its increased expression induces dopaminergic neuronal death in substantia nigra in PD	
Histone modifications	α-Synuclein associate with sirt2 a class III HDAC inhibitor inhibit the histone acetylation.	Outeiro et al. (2007)
Epilepsy		

(continued)

Table 1 (continued)

Neurological disease	Epigenetic modification	Reference
DNA methylation	Global hypermethylation in epileptic rats.	Kobow et al. (2013)
	Hypermethylation of calcium calmodulin protein kinase with its reduced expression in epileptic rats.	
Histone modifications	Transient phosphorylation of histone H3 and sustained acetylation of histone H4 were observed in hippocampal neurons in animal model of epilepsy.	Sng et al. (2006)
	Histone H3 and H4 are rapidly deacetylated at promoter of glutamate receptor subtype GluR2 lead to reduced expression after seizure development.	Huang et al. (2002)

Schizophrenia

Schizophrenia is a psychiatric disorder with the positive symptoms such as delusions, hallucinations and disorganized thoughts, social withdrawal, and apathy. There is an evidence that epigenetic mechanisms are involved in pathogenesis of schizophrenia disease. One of the global methylome study identified numerous DNA methylation changes at differentially methylated regions in schizophrenia and bipolar disorder (Xiao et al. 2014). GADD67 and Reelin genes were extensively studied in psychiatric disorders. These genes are downregulated in GABA neurons of the prefrontal cortex of schizophrenia and bipolar disorder patients (Impagnatiello et al. 1998; Guidotti et al. 2000; Fatemi et al. 2000). The downregulation of these gene expressions leads to a decrease in GABAergic transmission, which is an important pathological mechanism that underlies the clinical manifestation of schizophrenia and bipolar disorders (Akbarian et al. 1995; Guidotti et al. 2005; Eastwood and Harrison 2006). Hypermethylation of promoters of GADD67 and Reelin are associated with reduced expression of these genes in GABAergic neurons. There is a characteristic overexpression of DNMT1 in GABAergic neurons responsible for downregulation of GADD67 and Reelin in schizophrenia and bipolar disorder patients (Veldic et al. 2004, 2005; Grayson et al. 2005; Ruzicka et al. 2007). DNMTs utilizes the SAM as a substrate to transfer the methyl group to cysteine there by converting SAM to *S*-adenosyl L-homocysteine (SAH) which is subsequently hydrolyzed to form homocysteine. The plasma homocysteine levels were also reported to be increased in schizophrenia patients (Applebaum et al. 2004; Levine et al. 2002; Adler Nevo et al. 2006). The accumulation of homocysteine has been shown to cause neural damage and cognitive dysfunctions (Krebs et al. 2009).

Histone code modifications are another epigenetic regulators which plays a role in schizophrenia disease. Increased levels of GAD1 promoter H3K9 and H3R17 di- and trimethylation are associated with its reduced expression in

cortical neurons and adjacent nonneuronal cells of post-mortem tissue of schizophrenia patients and are typically associated with neuronal metabolism (Akbarian 2010). GAD67 a GABA synthesis enzyme expression is downregulated in cerebral and cerebellar cortex of schizophrenia, depression, or autism patients and may be contributing to desynchronization of cortical networks and cognitive dysfunction due to defective GABAergic inhibition. The promoter that regulates GAD67 expression is associated with altered histone code modifications, including loss of H3K4 methylation marks and excess of repressive marks such as H3K27 methylation (Huang et al. 2007). The three HMTs G9a, GLP, and SETDB1 that mediate H3K9 di- and trimethylations are increased across the genome in lymphocytes from schizophrenia patients (Wang et al. 2003; Zee et al. 2010). H3K4 methylation levels are reduced at nearly 600 loci, including near multiple NMDA receptor subunits and genes involved in neurodevelopment. HDAC1 expression is increased in the prefrontal cortex of schizophrenia patients (Sharma et al. 2008).

Alzheimer's Disease

Alzheimer's disease (AD) is the age-related most common type of dementia with characteristic features of loss of memory, language, ability to focus, reasoning skills, and visual perception (Blennow et al. 2006). The amyloid precursor protein (APP) which is a membrane protein that is expressed throughout the brain and particularly concentrated in neuronal synapses cleaved to produce β-amyloid plaques is a hallmark of AD. The hyperphosphorylated microtubule-associated protein tau that is expressed in neurons is capable of forming neurofibrillary tangles is another hallmark of AD (Voss and Gamblin 2009). There is a growing evidence suggesting epigenetic mechanisms mediate the risk for AD. Genome-wide hypomethylation has been reported in AD patients (Mastroeni et al. 2011). Global DNA hypomethylation was reported in the entorhinal cortex of AD patients compared to controls (Mastroeni et al. 2010). Studies also reported that folate and SAM levels were significantly reduced in AD (Bottiglieri et al. 1990; Morrison et al. 1996). CpG islands become more methylated with aging, while loci not in CpG islands were hypomethylated (Christensen et al. 2009). Repetitive Alu elements were also hypomethylated with aging, but not the repetitive LINE-1 elements (Bollati et al. 2009). APP and tau genes involved in pathophysiology of AD are affected by epigenetic regulation. In addition to DNA methylation, histone code modification plays an important role in AD. The cleavage of APP generates APP C-terminal peptide (AICD) in addition to Aβ peptide. AICD translocates to the nucleus and acts on specific genes and modify their expression. Over expression of AICD in rat primary cortical neurons associated with increased acetylation of histones H3K14 and H4K5. Fe65 is a binding partner of APP and its interaction with AICD recruits Tip60 to DNA strand breaks. Tip60 an HAT acetylates H4 which is necessary for the correct repair of DNA and

this process could be important in AD (Stante et al. 2009). In AD, an accumulation of phospho-H2AX, an indicator of DNA strand breaks, has been described (Myung et al. 2008). HDAC2 deficiency results in increased synapse number and memory facilitation supporting the role of histone acetylation and deacetylation in human diseases associated with memory impairment such as AD (Guan et al. 2009).

Parkinson's Disease

Parkinson's disease (PD) is another common neurodegenerative disease characterized by progressive loss of substantia nigra dopamine neurons and striatal projections. The typical symptoms include muscle rigidity, tremor, bradykinesia, and postural instability. Genome-wide DNA methylation studies in the brain and blood samples of PD patients were reported to have differential methylation pattern of several genes associated with PD pathology supporting the role of epigenetic dysregulation in PD (Masliah et al. 2013). The presence of Lewy bodies (structures containing aggregates of α-synuclein encoded by gene SNCA) which accumulate at sites where neuronal loss is found is a hallmark of PD. The epigenetic regulation of SNCA gene plays an important role in the pathogenesis of PD. The increased α-synuclein production is associated with PD may result from increased expression of the SNCA gene as a consequence of hypomethylation of this gene (Ammal Kaidery et al. 2013). It has also been reported that α-synuclein sequesters DNMT1 in cytoplasm, leading to global DNA hypomethylation in PD and dementia with characteristic accumulation of Lewy bodies found in post-mortem brains and in transgenic mouse models (Desplats et al. 2011). Another study reported hypomethylation of TNFα promoter and its overexpression induces dopaminergic neuronal cell death in substantia nigra in PD (Pieper et al. 2008).

α-Synuclein can associate with histones and inhibit their acetylation. It is largely associated with Sirt2, a type of NAD-dependent class III HDAC. The inhibition of Sirt2 using siRNA rescued α-synuclein toxicity (Outeiro et al. 2007). Another epigenetic hallmark associated with PD is dopamine depletion observed in this disease is associated with reduction in H3K4me3. Over all epigenetic regulation might have an important role in the pathogenesis of this disease.

Epilepsy

Epilepsy is another common brain disorder affecting millions of people worldwide. In epilepsy, certain brain regions such as the hippocampus is susceptible to electrical discharge that promote some morphological changes such as cell death in the CA1 and mossy fiber sprouting and dispersion of granule cell layer that are thought to be involved in recurrent excitatory circuits that contribute to seizure

susceptibility (Heck et al. 2004). DNA methylation is one of the epigenetic modifications involved in epilepsy (Kobow et al. 2009; Miller-Delaney et al. 2012; Zhu et al. 2012). Kobow et al. 2013 reported global DNA methylation pattern in chronic epileptic rats using methyl seq and showed global hypermethylation of the DNA. They also confirmed hypermethylation of calcium calmodulin-dependent protein kinase with its reduced expression involved in calcium signaling in pilocarpine induced epileptic rat model.

Histone modifications have also been altered in epilepsy induced animal models. In kainic acid induced animal model of epilepsy, transient phosphorylation of histone H3 and sustained acetylation of histone H4 were observed in hippocampal neurons (Sng et al. 2006). Other promoter-specific histone code modifications in epilepsy include hyperacetylation of histone H4 on BDNF promoter which correlate with its increased expression. Histone H3 and H4 are rapidly deacetylated at the promoter of glutamate receptor subtype GluR2 correlated with its reduced expression after seizure development (Huang et al. 2002).

The importance of DNA methylation in association with other neurological disorders such as Rett syndrome and the ICF syndrome has been reported. Rett syndrome is one of the most common mental retardation diseases in females. Mutations of methyl CpG binding protein2 (MeCP2) have been found in 80 % of Rett syndrome patients (Amir et al. 1999). Mutations of DNMT3b have been reported in about 60 % of ICF syndrome patients (Hansen et al. 1999). ICF syndrome patients are characterized with immune defects, chromosomal instability, and neurological defects including mental retardation.

Epigenetic Modulatory Drugs to Treat Neurological Diseases

The most widely studied epigenetic modulatory drugs include DNA methylation inhibitors and HDAC inhibitors. 5-AZA deoxycytidine, zebularine are DNA methylation inhibitors more widely used to treat cancer. These drugs incorporate in to target DNA and bind to DNMT1 during DNA replication to inhibit its activity and therefore require DNA replication to be active (Wu and Santi 1985). However, the use of these drugs for treating brain disorders has limitation since most of the neurons are postmitotic. The mechanism of action of demethylation in postmitotic neurons is not clearly understood. However, the recent study used RG108, a small molecule DNA methylation inhibitor in epilepsy therapeutics suggesting a novel epigenetic modulatory drug to treat neurological diseases (Machnes et al. 2013).

HDAC inhibitors show a promise for cognitive improvement and are being considered for drug development in neurological diseases. Hence, HDAC inhibitors could be used as promising therapeutic agents for diseases associated with dementia and cognitive impairments. The class I HDAC inhibitors such as sodium valproate and sodium butyrate improve memory in AD mouse model (Kilgore et al. 2010). Valproic acid is also used as an anticonvulsant in epileptic patients and as a mood stabilizer in bipolar disorder patients (Phiel et al. 2001). Sodium butyrate and Sirtuin

HDAC siRNA inhibitors are effectively used in a Drosophila model of PD (St Laurent et al. 2013; Outeiro et al. 2007). The use of Valproic acid in the treatment of schizophrenia and bipolar disorders has also been reported (Weaver et al. 2006).

Natural Products as Epigenetic Modulators in Neurological Diseases

Most of the human diseases are significantly influenced by diet in a varying degree. Except the inherited one, some are almost purely influenced by dietary components like the vitamin and mineral deficiency. In the last decade, a new wealth of information has emerged to explain how nutrition and diet affect different diseases to varying degrees. Nutrition and diet have little impact on some diseases, but strongly affects others. Considering the role of epigenetics in human diseases; here, we discuss how the diet affects long-term health by altering the epigenome and how one can prevent degenerative conditions by consuming of diets rich in antioxidants and anti-inflammatory components. This part of the chapter mainly focuses to discuss the promising epigenetic effects of dietary factors (phytochemicals) and their effects in neurodegeneration and neuroprotection.

Phytochemicals

Phytochemicals are considered as nonessential complex chemicals found in plants, particularly in fruits and vegetables. Even though, phytochemicals do not fall under the category of essential nutrients for humans, they are contributing greatly to health and well-being. So far, in nature more than 3000 phytochemicals have been discovered and here we have provided a noncomprehensive list of phytochemicals and examples of how they are broadly subclassified (Liu 2004).

1. *Terpenoids (Isoprenoids)*
   (a) *Carotenoid terpenoids* (lycopene, beta-carotene, alpha-carotene, lutein, zeaxanthin, and astaxanthin)
   (b) *Noncarotenoid terpenoids* (perillyl alcohol, saponins, terpeneol, and terpene limonoids)
2. *Polyphenolics*
   (a) *Flavonoid polyphenolics* (anthocyanins, catechins, isoflavones and hesperetin, naringin, rutin, quercetin, silymarin, tangeretin, and tannins)
   (b) *Phenolic acids* (ellagic acid, chlorogenic acid, *P*-coumaric acid (*para*-coumaric acid), phytic acid, ferulic acid, vanillin, cinnamic acid, and hydroxycinnamic acids)

   (c) *Nonflavonoid polyphenolics* (curcumin, resveratrol, pterostilbene, lignans, and coumestans)

3. *Glucosinolates*

   (a) *Isothiocyanates* (phenethyl isothiocyanate and sulforaphane)
   (b) *Indoles* [Indole-3-Carbinol (I3C)]

4. *Thiosulfonates*
5. *Phytosterols* (beta-sitosterol)
6. *Anthraquinones*

   (a) *Senna*
   (b) *Barbaloin*
   (c) *Hypericin*

7. *Capsaicin*
8. *Piperine*
9. *Chlorophyll* (chlorophyllin)
10. *Betaine*
11. *Pectin*
12. *Oxalic acid*

In recent years, the role of epigenetic modifications in neurodiseases and degeneration has been well established through systematic studies. Many studies have shown that essential nutritional compounds like vitamin B_{12} or folic acid play key role in the modulation of epigenetic changes. Increasing scientific evidence suggests that oxidative stress (cellular and metabolic) has crucial implications for the pathogenesis of many neurodegenerative diseases, including PD, AD, and many others via epigenetic changes (Andersen 2004). Therefore, nonnutritional compounds such as polyphenols have attracted the scientific world as epigenetic modulators mainly through its antioxidant properties. In addition to plant based natural products (compounds), certain secondary metabolites derived from marine and terrestrial micro- and macro-organisms are discovered as drugs that have epigenetic targets such as HDACs. Although, many natural compounds have been shown to possess potential epigenetic modulatory effects in diseases like cancer and atherosclerosis, only fewer natural products inhibitors have been documented in modulating epigenetic pathways in brain-related diseases.

Dietary Factors as Modulators of HDAC Activity

Diet derived bioactive components are able to modulate epigenetic events and their epigenetic targets. For example, genistein, diallyl sulfide, vitamin D3, or all-*trans* retinoic acids have been shown to impact DNA methylation by altering histones and chromatin structure (Bassett and Barnett 2014). Here, we provided a brief

Table 2 Partial list of dietary compounds as HDAC modulators

Inhibitors of HDACs	Inducers of HDACs
• Butyrate	• Theophylline
• Diallyl disulfide	• Resveratrol
• Sulforaphane	
• Isothiocyanates	
• All-*trans* retinoic acid	

introduction of some important bioactive natural compounds and how they target the epigenetic events in disease conditions. Table 2 gives a partial list of dietary compounds that are known to modulate HDAC activity.

Genistein

Genistein is mainly a soy-derived compound classified under the category of isoflavones (Fig. 4). Many anticancer studies have shown that genistein affects tumorigenesis/carcinogenesis through epigenetic regulations (Zhang and Chen 2011). Reports suggest that genistein may be involved in inhibiting the DNMTs and can regulate gene expression by erasing DNA methylation at promoter levels. Genistein has been proved to prevent breast cancer risk and promotes DNA demethylation of SF1 promoter in endometrial stromal cells (Khan et al. 2012), a type of cells that is present in the endometrium (the innermost lining) of the uterus. Recently, it has been shown that genistein, inhibits neuroblastoma (NB) growth and tumor microvessel formation in vivo by decreasing hypermethylation levels of tumor suppressor genes (TSGs) such as CHD5 and enhances the expression of CHD5 as well as p53 (Li et al. 2012). In addition, genistein acts as an inhibitor to significantly decrease the expression of DNMT3b in NB model and thereby suggest that genistein could be used as an adjuvant therapeutic agent for NB treatment.

Also, genistein induces chromatin remodeling and DNA methylation, which leads to the activation of TSGs and thereby suppression of the cancer cell survival. Genistein has also been shown to inhibit the DNMT activity, which causes inhibition of DNA methylation and thus may be acting as an anticancer agent (Li and Tollefsbol 2010). Genistein has been observed to enhance the acetylation of histones H3 and H4 in the transcription sites of p21 and p16, thereby, it upregulates the TSGs in prostate cancer cells (Zhang and Chen 2011). Prenatal exposure to genistein possesses estrogenic activity and affects the erythropoiesis in the fetus and alters the gene expression and DNA methylation in hematopoietic cells (Vanhees et al. 2011). Also, genistein causes modulation of the HAT activity and extents the acetylation of histone (Piaz et al. 2011). In breast and prostate cancer cell lines, genistein has been shown to inhibit the proliferation (Moyad 1999) and to compete with estrogen, it prevents the estrogen receptor mediated cell growth (Wang et al. 1996). Another study shows that genistein is positively associated with modulation of DNA methylation at CpG islands of certain genes in prostate of a mouse.

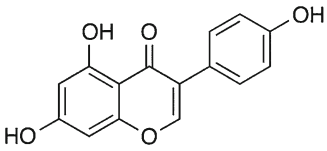


Fig. 4 Structure of genistein

Genistein seems to reduce the hypermethylation status of RARβ, p16, and MGMT genes in vitro and inhibit the activity of DNA methyltransferases dose dependently, showing that genistein can reactivate methylation-silenced genes, via inhibiting DNA methyltransferase (Fang et al. 2005). Supplementation of genistein during the gestation period modulated the site-specific DNA methylation of offspring and changed coat color of heterozygous yellow agouti (Avy/a) pups to black pseudoagouti by reducing the DNA methylation status of the agouti locus (Dolinoy et al. 2006).

Epigallocatechin-3-gallate (EGCG)

Epigallocatechin gallate (EGCG) is a type of catechin (Fig. 5) found highly in green tea and exists in nature as the ester form of epigallocatechin and gallic acid. Trace amounts of (EGCG) are found in plums, onions, hazelnuts, and apple skin. Numerous studies have shown that EGCG has beneficial effects in a broad range of disorders including cancer. Preliminary research shows that EGCG is an inhibitor of various enzymes in epigenetic pathways, such as histone acetyltransferase (Choi et al. 2009) DNA methyltransferase (Choi et al. 2009) or tyrosinase (No et al. 1999).

Decaffeinated green tea and black tea extracts are rich in EGCG and has been shown to inhibit 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced DNA methylation in lung cancer model in A/J mice (Shi et al. 1994). In cancer cells, EGCG reactivated methylation-silenced genes by inhibiting DNMTs and suggest the potential use during carcinogenesis (Fang et al. 2003). In addition, a study demonstrated that EGCG and other tea polyphenols (e.g., catechin and epicatechin) and bioflavonoids (quercetin, fisetin and myricetin) can inhibit Dnmt 1-mediated DNA methylation in a concentration-dependent manner (Lee et al. 2005).

Lycopene

Lycopene is a tetraterpene (carotenoid), possess potent antioxidant property (Fig. 6). Lycopene is mainly present in fruits and vegetables, including tomatoes, watermelon, pink grapefruit, pink guava, and papaya. As an antioxidant, lycopene knows to modulate the expression of many genes that are associated with cell cycle, DNA repair, and apoptosis. Also, it has been shown to alter DNA methylation and upregulate the GSTP1 gene in the breast cancer cell line (King-Batoon et al. 2008). It seems

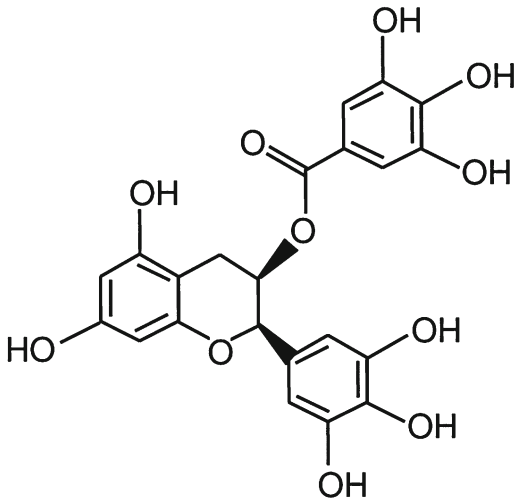


Fig. 5 Structure of epigallocatechin gallate (EGCG)

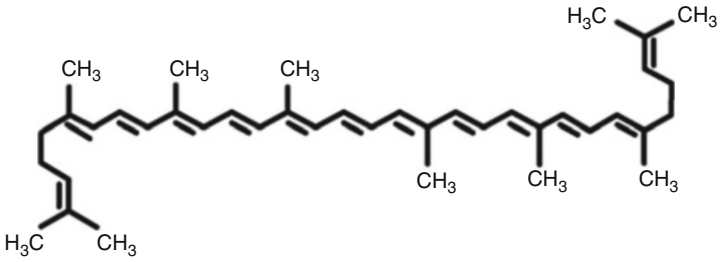


Fig. 6 Structure of lycopene

that lycopene affects gene expression by modifying gene-specific methylation. It has been reported to demethylate the promoter of RARβ2 and HIN-1 genes (King-Batoon et al. 2008).

Resveratrol

Resveratrol is more famous by the name "French Paradox" by preventing many diseases, including neurological disorders such as AD, PD, and stroke. Studies have shown that the potential beneficial effects of resveratrol (Fig. 7) are not only because of its antioxidant and anti-inflammatory action but also due to activation of sirtuin 1 (SIRT1). It is well known that at least in animal models, caloric restriction has been shown to prevent the development of various cancers through sirtuins as a target. Sirtuins are nicotinamide adenine dinucleotide (NAD(+))-dependent HDACs which are involved in aging and reverted significantly in transformed cells. Resveratrol is known to activate the sirtuin 1 (SIRT1), a class III HDAC (Baur 2010) and thereby preventing aging and cancer cell proliferation.

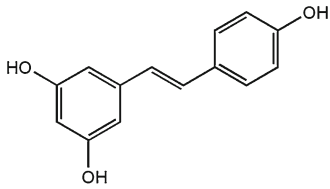


Fig. 7 Structure of resveratrol

In cell lines, resveratrol acts as a weak inhibitor of DNMT activity (Paluszczak et al. 2010) and acts synergistically with adenosine analogues to inhibit methylation of retinoic acid receptor beta 2 gene and thereby increases its expression (Stefanska et al. 2010).

Curcumin

Curcumin is the principal active compound of turmeric and known to have an anti-disease effect in various animal models and in humans (Fig. 8). Effects of curcumin to induce apoptosis in cancer cell lines are well characterized and recently it has been shown to inhibit certain epigenetic enzymes (such as HATs, HDAC1, HDAC3, and HDAC8) in vitro (Reuter et al. 2011; Vahid et al. 2015). The mode of induction of apoptosis by curcumin may vary from cell to cell. For example, in cervical cancer, curcumin inhibits the acetylation of histone and p53 through specific inhibition of p300/CBP (Balasubramanyam et al. 2004b).

In addition, it also induces histone hypoacetylation, activation of poly (ADP) Ribose polymerase- and caspase-3-mediated apoptosis in brain glioma cells (Kang et al. 2006) were observed. In addition, curcumin decreased histone H3 and H4 acetylation and thereby controls the fate of neural stem cells (Kang et al. 2006).

Anacardic Acid

Anacardic acid falls under the category of phenolic lipids and are highly present in the shell of the cashew nuts (Fig. 9). Anacardic acids seem to arrest the growth of cancer cells by inhibiting acetylation, nuclear translocation of p65 and through modulation of NF-kappaB signaling pathway. (Sung et al. 2008). Anacardic acid can inhibit HATs such as p300, PCAF, and Tip60. Also, anacardic acid has been shown to specifically inhibit HAT (Balasubramanyam et al. 2003; Sun et al. 2006).

Garcinol

Garcinol is a highly cytotoxic polyprenylated benzophenone from the fruit G*arcinia indica* (Fig. 10). Garcinol is also a potent inhibitor of different HATs, such as p300 and PCAF (Mai et al. 2006; Chandregowda et al. 2009; Balasubramanyam et al.

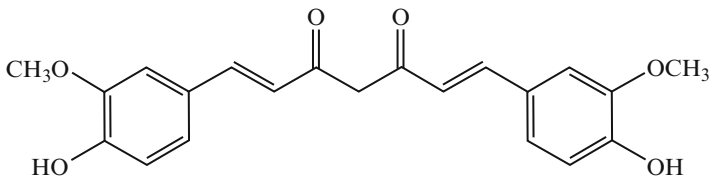
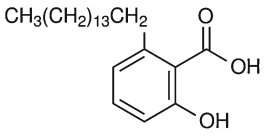


Fig. 8 Structure of curcumin

Fig. 9 Structure of anacardic acid



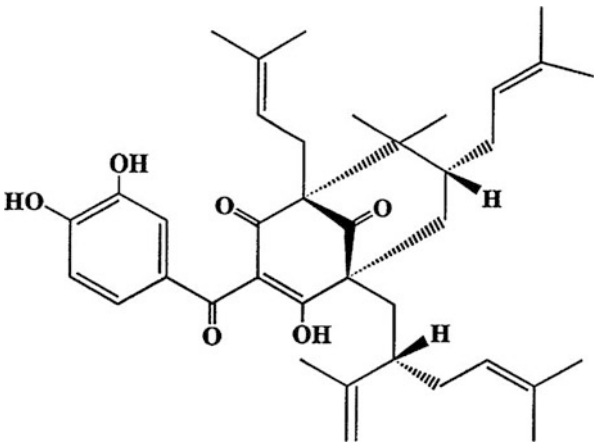


Fig. 10 Structure of garcinol

2004a). Many derivatives of garcinol have been synthesized (1) iso-garcinol (IG), (2) 14-isopropoxy IG (LTK-13), (3) 14-Methoxy IG (LTK-14), and (4) disulfoxy IG (LTK-19). LTK-13, LTK-14, and LTK-19 selectively inhibit p300 and LTK-14 act as a noncompetitive inhibitor of acetyl-coA and histones (Mantelingu et al. 2007).

Plumbagin

Plumbagin is another compound, derived from a root extract of the plant *Plumbago rosea* (Fig. 11), which has been found to potently inhibit HAT activity (Ravindra et al. 2009). Plumbagin and its derivatives possess HAT inhibitory activity and serves as a noncompetitive inhibitor for p300. The single hydroxyl group seems to be crucial for the HAT inhibitory activity.

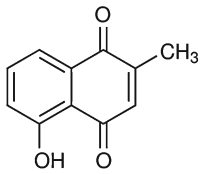


Fig. 11 Structure of plumbagin

Conclusion

A growing body of research suggests that epigenetic defects (epimutations) play a considerable role in human conditions that are strongly influenced by changes in the lifestyle, environment, diet, and pharmacological intervention. Therefore, it is possible that the discovery of novel synthetic and natural dietary compounds or testing the molecules that are already known, may be an effective strategy to treat the epigenetic changes or correct epimutations of various disease states. Although, a variety of compounds have been discovered as epigenetic modulators on various human diseases, such as cancer, obesity, and insulin resistance, only a few compounds (natural or synthetic) are known to target various epigenetic factors in brain disorders which are associated with epigenetic changes. So there is an urgent need in the investigation of phytochemicals as epigenetic modulators in the treatment of neurological diseases.

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Compliance with Ethics Requirements The authors declare that they have no conflicts of interest.

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Effect of Docosahexaenoic Acid (DHA) on Spinal Cord Injury

Sreyashi Samaddar

Abstract Spinal cord injury (SCI) has become one of the most leading concerns in the past decade. Preclinical and research studies are now ongoing trying to understand the molecular mechanisms and develop treatment strategies for this neurodegenerative condition. In the last decade, researchers have deciphered few of the leading players that play a major role in worsening the condition. But till date none of these have been applied to the clinical treatment of patients with SCI. Here in this chapter I discuss about one of the dietary requirements that could ameliorate the condition of these patients.

Keywords Spinal cord injury • DHA • Neurodegeneration • Neurotrauma • Treatment

Introduction

Spinal cord injury (SCI) has become one of the most leading concerns in the past decade. Preclinical and research studies are now ongoing trying to understand the molecular mechanisms and develop treatment strategies for this neurodegenerative condition. In the last decade, researchers have deciphered few of the leading players that play a major role in worsening the condition. But till date none of these have been applied to the clinical treatment of patients with SCI. Here in this chapter I discuss about one of the dietary requirements that could ameliorate the condition of these patients.

S. Samaddar, Ph.D. (✉)
Department of Neuroscience/Biology, Research Foundation CUNY, Staten Island, NY, USA
e-mail: sreyashi.samaddar@gmail.com

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The Spinal Cord

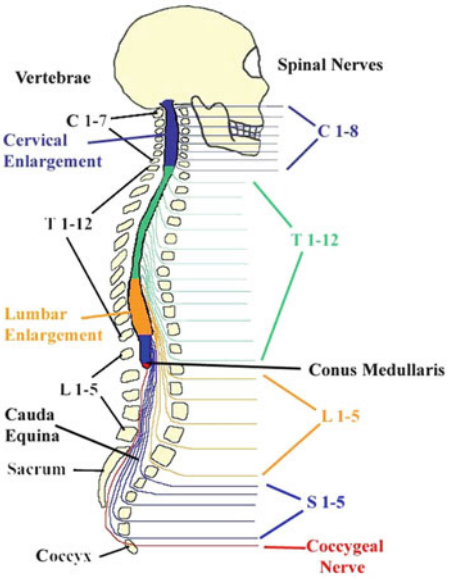
The spinal cord together with the brain forms the central nervous system (CNS). It is the most important component connecting the brain with the rest of the body. It is an extension of the brain stem into the vertebral column through the foramen magnum. The spinal cord is a cylindrical bundle of nerve fibers extending from the medulla oblongata and is well protected inside the vertebral column. The spinal cord can be divided into four different regions: cervical, thoracic, lumbar, and sacral, which are in turn divided into several segments. A pair of nerve exits from each segment. There are 31 pairs of spinal nerves corresponding to the different segments of the spinal cord. Each spinal nerve carries both sensory and motor information. This information controls not just the movement of different parts of the body, but also regulates several of the physiological processes. Many of these commands come directly from the brain, and some are regulated from within the spinal cord. In either way, the proper functioning of the spinal nerves is important in maintaining the essential bodily functions (Fig. 1).

Spinal Cord Injury (SCI)

There are about six million people living with paralysis currently that is approximately 1 in every 50 people. And one of the primary causes of paralysis is spinal cord injury (SCI). SCI currently affects about 300,000 individuals in the USA. It has become an increased cause of concern given that there is no direct cure for SCI. SCI usually results from motor vehicle accidents, falls, athletics and gymnastics, diving into shallow water and to some extent from physical violence. The number of males with spinal injuries is far more than the number of women (Fig. 2).

What Is Spinal Cord Injury (SCI)?

SCI is usually a sudden blow to the spine, that disrupts the vertebral column, and can be accompanied by fracture of bones, tear in the muscles and ligaments, and also damage to the spinal tissue. The spinal cord generally is not completely severed, but the axonal connections, nerve fibers and tracts which carry signals to and from the brain are usually affected. This is among the primary injuries which immediately result from such accidents. However, there are a series of secondary damages at the vascular, biochemical, and neuronal aspects of the functioning of the spinal cord. These secondary injuries include inflammation, hypoxia, ischemia, release of free radicals, increased apoptosis, and glial scarring. Thus, in addition to the immediate damage or disruption of the spinal cord, the following secondary injuries make the process of recovery and regeneration of the damaged neurons even more difficult. The higher the region (in the anatomy of the spinal cord), the greater is the damage. Injuries to the upper part of the spinal cord (in the cervical region) can lead to the most



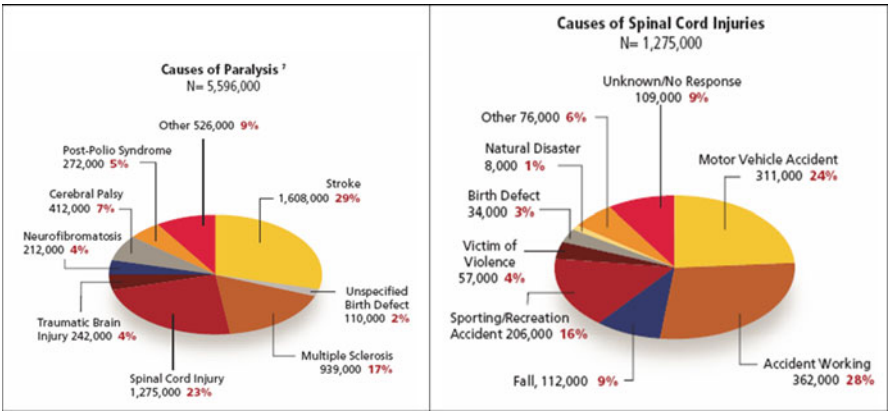


Fig. 1 Illustration of the different parts of the spinal cord

Fig. 2 Pie charts showing the causes of paralysis in general and the different causes of Spinal cord Injury (SCI)

serious injuries, amounting to total loss of movement, paralysis of arms, hands, trunk, and legs (which is also termed as tetraplegia or quadriplegia), complete dependence for everyday life activities, 24 h assistance, impairment of bladder and sexual functions. Injuries to the lower half of the spinal cord (thoracic and lumbar regions) can lead to paralysis of trunk and legs (condition termed paraplegia), partial dependence on aid, movement on a wheelchair depending on the severity of the injury. The injury can be complete or incomplete. In a complete spinal cord injury, the cord in unable to send signals or perform its function below the level of injury, leading to paralysis below the injury level. In the case of incomplete injury, there is still some sensation and function below the injury level for which it does not lead to total paralysis.

A series of damages occur at the molecular level which start after the primary injury and have long-lasting effects on the extent of damage, making it progressively worse and attenuating the recovery process. The processes include inflammation caused by the disruption of the blood–brain barrier followed by an invasion of immune cells in the area of injury, resulting in an inflammatory response killing nearby neurons and oligodendrocytes. This inflammation in turn gives rise to the production of highly reactive form of oxygen molecules called free radicals which are capable of changing the chemical structure of molecules in cells. The very common neurotransmitter in the spinal cord, glutamate is excessively released after injury, overstimulating the nerve cells and destroying them at and beyond the point of injury. Apoptosis is a very common phenomenon that sets in few days after the injury. The still surviving nerve cells which could have helped in regeneration and recovering the severed spinal cord enters the apoptotic pathway. Furthermore, excessive accumulation of astrocytes at the site of injury forms glial scar, which physically obstructs axon growth and disrupts communication with the growth factors. The initial injury which sometimes causes compression of the spinal cord blocks the blood flow, causing swelling and leaking and thus blocking nutrients and oxygen reaching the injury site. All these molecular events slow down the recovery process and sometimes make the injury progressively worse.

Treatments Currently Available

In the case of an SCI, initial medical help is very crucial to avoid further long-term injuries. At any point in time it is very important to keep the head, neck, and the body of the individual aligned till the time medical help arrives. The individual should not move unless it is necessary. Once the individual reaches the emergency room, sometimes they undergo surgery to get rid of extra fluids, fix torn tissues and other physical damages to the body. But there is literally no way to reverse the damage done to the spinal cord. Other treatments include braces or traction to immobilize the spine in order to avoid further injury, also some initial medications like methylprednisolone which if administered within the first 8 h of injury is assumed to decrease the further nerve loss and reduce inflammation. Initial treatments are critical in the sense that they definitely do not change the damage that has already been done, but does help in decreasing any further injuries or help reduce the extent of secondary injuries that will shortly follow after 1–3 days. Medical tests to diagnose the position of the spinal cord and the extent of injury include magnetic resonance imaging (MRI), neurological computerized tomography (CT), and plain X-Rays. These tests help us to know the position of the chest and the skull, exact regions of the injury, inflammation, bone fractures, bleeding, compression of the spinal cord and to know if any injury has occurred to any spinal ligaments (http://www.ninds.nih.gov/disorders/sci/detail_sci.htm).

As a later and long-term therapy comes in the physical therapy and rehabilitation which can help the patient recover slowly from the initial trauma, enabling him/her

to become more and more independent to perform everyday functions and lead a better life. These therapies help in muscle strengthening and mobility, use of assistive devices like leg braces, and wheelchairs, occupational therapy to improve fine motor skills, vocational therapy helping to get back to work, recreational therapy to engage in sports and other social activities, better adjustment of bladder and bowel movements (Tator and Benzel 2000). Controlling diet to control obesity and providing the right nutrient is an essential part of the posttraumatic treatment.

Ongoing Research with SCI

Scientists have been highly involved in studying every aspect of SCI extensively trying to understand the molecular mechanisms which make the recovery process very difficult and target molecules to change events that attenuate the process of regeneration. The challenge lies in the fact that there are multiple molecules and cells involved, each of which have their signaling cascades, making it difficult to treat the disease with just one target. More than one molecule and perhaps mechanisms have to be regulated, at the same time to achieve the goal of combating the condition.

Spinal cord injury research is supported by the National Institute of Neurological Disorders and Stroke (NINDS), a part of the National Institutes of Health (NIH). It is also supported by other NIH components, the Department of Veterans Affairs, other Federal agencies, research institutions, and voluntary health organizations like National Institute of Child Health and Human Development, Hill Foundation for Families Living With Disabilities, and many other similar organizations.

Currently different SCI models are being used to study the molecular mechanisms which mimic the injury type in humans. The two most commonly used models are contusion or an acute injury to the spinal cord with an electromagnetic impactor or weight-drop technique (Cheriyan et al. 2014) and the compression injury model with a specific force and for a particular duration. Other models include distraction (stretching of the cord), dislocation (mechanical displacement of the vertebrae), transection (partial or complete surgical transection), or chemical injury models (using molecules that play a role in causing the secondary injury) (Cheriyan et al. 2014).

Preclinical research for the treatment of SCI mainly revolves around the efforts of trying to modulate the signaling cascades leading to secondary damage. Several groups have been targeting different molecules which lead to excitotoxicity, oxidative stress, inflammation, blockade of regeneration. Riluzole, a sodium channel blocker, has been shown to successfully reduce excitotoxicity following SCI. This compound has been approved for ALS and is currently in the Phase I/II trial for SCI (Mu et al. 2000; Schwartz and Fehlings 2001; McAdoo et al. 2005). Minocycline, which is a synthetic tetracycline derivative clinically used to treat acne, has been shown to be highly neuroprotective in the recent years. It is known to inhibit caspase-1 and caspase-3, inhibit production of NO, decrease glutamate excitotoxicity and many others. Use of minocycline to treat animals with SCI has proved to result

in improved hindlimb function, reduced lesion size, and superior behavioral recovery when compared to commonly used methylprednisolone (Wells et al. 2003). Cethrin, an antagonist of Rho, is being used in the Phase I/II clinical trial to treat patients with complete cervical and thoracic injury. Rho, a small intracellular family of G-proteins, gets activated by glial cells at the lesion site in SCI. Cethrin, a Rho antagonist, is shown to reduce tissue damage, dampen apoptosis and thus enhance the process of regeneration, leading to improved locomotory functions (Dubreuil et al. 2003). A lot of work is also ongoing in the direction of trying mechanisms to remyelinate the demyelinated axons which have been spared during the injury.

Methylprednisolone, a synthetic corticosteroid, is currently widely used in the clinic in the treatment of SCI, after the results that came from the three trials in the National Acute Spinal Cord Injury Studies (NASCIS). However, this compound has been shown to cause various side effects, urging discovery and development of other potential therapeutic compounds for SCI.

Cellular transplants after SCI have been demonstrated to be successful in animal studies. Mouse ES cells transplanted into rats with spinal cord injury have resulted in better functional recovery (McDonald et al. 1999). The next step would be to try transplanting cells, induced pluripotent cells in human clinical trials (Nakamura and Okano 2013). Neuralstem, Inc. is in the process of Phase I clinical testing of human neural stem cells (NS1-566) in the treatment of SCI to reestablish the broken circuitry of the spinal cord, in collaboration with UC San Diego Health System. This trial is intended for four patients with complete thoracic (T2–T12) SCI and who are within 2-year time window post-injury. Success with the same cell line has been already witnessed with ALS patients.

Omega-3 Fatty Acids

It would be difficult to find an individual in the recent scientific world who is not aware of the benefits of Omega-3 fatty acids. This is just a brief recapitulation of what we already know about this particular type of fatty acid.

Biochemically, fatty acids are carboxylic acids with a long aliphatic chain which can be either saturated (with no double bonds between carbon atoms) or unsaturated (with one or more double bonds). Essential fatty acids are a class of unsaturated fatty acids which is essential for humans and other animals that cannot be synthesized and thus have to be ingested in the form of food. The most important essential fatty acid for humans is omega-3 fatty acid and omega-6 fatty acid or linoleic acid (LA). The three types of omega-3 polyunsaturated fatty acids are alpha-linolenic acid (ALA, found in plant oils), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA, fish oil). DHA can either be obtained from mother's milk or can be synthesized from omega-3 fatty acid ALA. The foods rich in omega-3 fatty acids are fish, fish oil, walnuts, flaxseed, flaxseed oil, and leafy vegetables. Since our body lacks the desaturase enzyme needed to transform omega-6 fatty acid to omega-3 fatty acids, including the abovementioned foods in regular diet is extremely essential (Fig. 3).

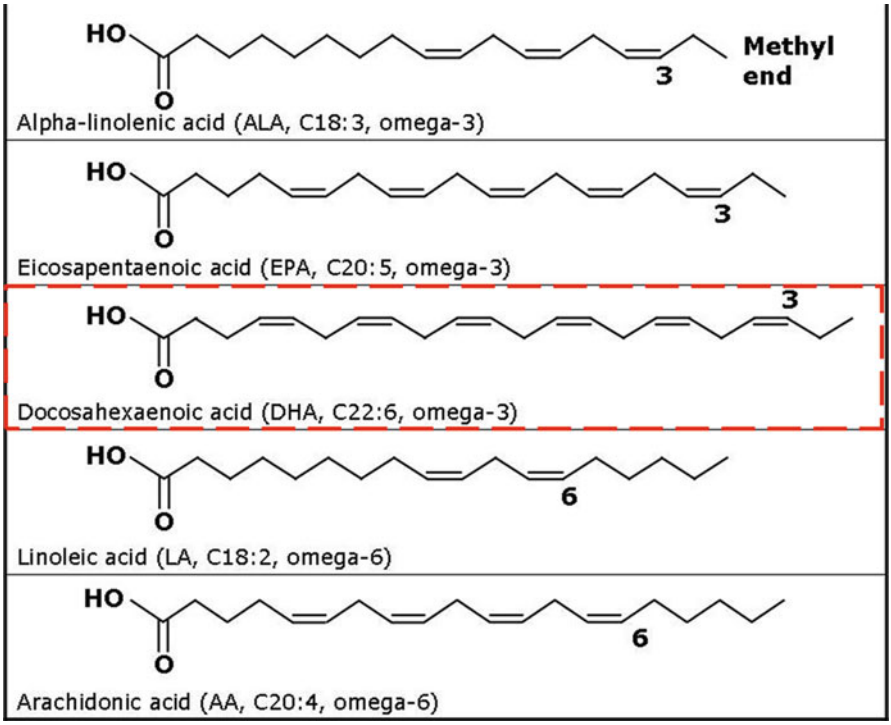


Fig. 3 Different types of essential fatty acids

Why Are Omega-3 Fatty Acids So Important?

At the molecular level omega-3 fatty acids are an integral part of cell membranes, and affect the activity of the cell membrane receptors, binding to their respective substrates, downstream signaling cascade, which will eventually either regulate the expression of various proteases, or regulate gene expression at the nuclear level. DHA is an essential component for brain and central nervous system development in infants. It is also required in adults to maintain proper neurological functioning. DHA is taken up by brain in preference to other fatty acids (Horrocks and Yeo 1999). Deficits in DHA have been associated with numerous physiological disorders like rheumatoid arthritis, diabetes mellitus, and cardiovascular disease; some forms of cancer; neurological disorders like depression, Alzheimer's disease, attention deficit hyperactivity disorder (ADHD), and unipolar disorder. DHA has been shown to help in the growth of nerves by regulating nerve growth factors. In infants, DHA in the brain helps in learning ability, and also helps to maintain neuron functioning in the case of adults (Horrocks and Yeo 1999). The multifaceted function of DHA has been well studied and is known to ameliorate the symptoms of many disorders.

DHA is also known to modulate anti-inflammatory and pro-inflammatory factors. Membrane-bound phospholipase A2 forms free DHA, which ultimately is transformed into docosatriene, a compound that inhibits inflammatory genes and pro-inflammatory factors like NF-κB, TNF-α, which on the one hand decreases expression of pro-apoptotic proteins like Bax and Bik and on the other hand increases expression of anti-apoptotic proteins like Bcl-2. These factors eventually help in neuroprotection and cell survival (Mukherjee et al. 2004; Bazan 2006). DHA is also known to have an antioxidant effect (Ephraim et al. 2002; Satkunendrarajah and Fehlings 2013). Apart from the neuroprotective role of DHA, it seems to regulate the ion channels like sodium, potassium, and calcium (Vreugdenhil et al. 1996). Since one of the crucial secondary effects of SCI is excitotoxicity resulting from excessive release of the excitatory neurotransmitter glutamate mediated by calcium-mediated exocytosis, regulation of ion channels can provide effective means of controlling deleterious effects of over-excitability. In addition to its structural contribution, DHA seems to regulate signal transduction, gene expression, inflammation, and ion channel function (Satkunendrarajah and Fehlings 2013).

Role of DHA, an Omega-3 Fatty Acid in Treating SCI

SCI is perhaps the worst of its kind it terms of trauma, long-lasting injuries, progressively worsening conditions, and the posttraumatic challenges faced by the patient pertaining to regular life activities, emotional uncertainties of also the family members who manage extremely delicate situations in which they have to deal with the growing frustration outbursts of the patient and at the same time provide all kinds of support, emotional and physical.

Presently, with treatments for SCI still very scarce, the multifaceted properties of DHA are being applied with the intention to combat the condition from all aspects. Several groups have studied the effects of DHA on SCI, in mostly rodents, rats and mice. Different injury models have been used mimicking the injuries in humans, from commonly used compression to contusion as well as hemisection, transection, and thoracic cervical spondylotic myelopathy (CSM). Treatments include bolus intravenous injection (250–500 nmol/kg), dietary supplementation (750 mg/kg/day to 1.2 g/100 g), and also the use of transgenic mice. Treating with bolus I.V. injection of DHA resulted in improvement in locomotor function (horizontal ladder and beam walk), increase in neurons and oligodendrocytes (white matter), and reduction in the oxidation, apoptosis, and the lesion size of the injury (King et al. 2006). Pretreatment with bolus along with dietary omega-3 fatty acids on T-10 contusion NYU impactor improves bladder function, locomotor function, axonal conduction measured by transcranial magnetic motor evoked potential, survival of neurons and oligodendrocytes (Figueroa et al. 2012, 2013). Dietary supplementation of DHA

alone has shown to increase spinal cord injury learning, ameliorate expression of pro-restorative signaling molecule mRNA like BDNF, CREB, syntaxin-3 (Joseph et al. 2012; Langston et al. 2012). Bolus injection along with dietary supplementation of T-12 compression studies is shown to attenuate inflammation, oxidative stress, size of the lesion cavity, and lipid and protein peroxidation (Huang et al. 2007; Lim et al. 2012).T-12 spinal compression in rats followed by treatment with bolus DHA or along with dietary supplementation revealed reduced neutrophil infiltration and increased neuronal integrity by MAP-2 in immunohistochemistry (Ward et al. 2010; Hall et al. 2012). Treatment with fenretinide, a synthetic retinoid derivative with antioxidant and anti-inflammatory properties widely used in cancer treatments, resulted in reduced and oxidative stress and inflammation measured by TNF-α, revealing a potential relationship of fenretinide positively regulating the levels of DHA homeostasis (López-Vales et al. 2010). Elevated endogenous omega-3-polyunsaturated fatty acid levels in transgenic *fat-1* mice lead to improved outcome after spinal cord injury associated with increased neuronal and oligodendrocyte survival and reduced loss of neurofilaments, microglia activation, and expression of pro-inflammatory mediators (Lim et al. 2013).

Mechanism of Action of DHA

DHA, the commonly used omega-3-polyunsaturated fatty acid, has been proved to have beneficial outcome in the treatment of spinal cord injury beyond any reasonable doubt. But the precise mechanism by which this molecule accomplishes this task is not yet well known. Several groups have been studying the mechanism, but still it is not well understood.

DHA is known to regulate transmembrane receptors like G-proteins, thus regulating a plethora of signaling cascade resulting in the receptor activation and inactivation. Also loss of DHA leads to reduced levels of phosphatidylserine (PS), indicating a possible regulation of cell signaling via PS (Salem et al. 2001). Fish oil (High DHA/EPA) increased insulin secretion but had no effect on mice deficient for GPR 120, a polyunsaturated fatty acid receptor (Furutani et al. 2015). Application of DHA for amelioration of depression revealed that the antidepressant effect of DHA is due to the translocation of Gsα from lipid raft and enhancing the actions of adenylate cyclase (Zhu et al. 2015). Neuroprotectin D1 (NPD1), derived from DHA, is shown to downregulate the expression of pro-inflammatory enzyme COX-2, and apoptosis in HNG cells and Alzheimer's disease mouse models. It also shifts the cleavage of β-amyloid precursor protein (βAPP) holoenzyme from an amyloidogenic into the non-amyloidogenic pathway, thus rescuing brain cells in the early stages of neurodegeneration (Zhao et al. 2011). All these above studies suggest that the scientific community is trying to delineate the mechanism from all angles, but we are yet to join the dots.

Sources of DHA

As already mentioned, since DHA cannot be synthesized in the human body it has to be consumed as a part of the regular diet. A typical American diet contains more of omega-6 than omega-3 fatty acids, and the former tends to add up to the fat content in the system. There are a bunch of food sources that are rich in omega-3 fatty acids and are good sources of DHA like walnuts, sardines, salmon, brussels sprouts, cauliflower, tofu, green leafy vegetables with flaxseed at the top of the list. Below is a detailed table of the various food sources and their DRI/DV (Dietary Reference Intakes/Daily Value) (Fig. 4).

Commercially Available DHA

There are a dozen different types of dietary supplements of DHA, which are commercially available. These supplements are manufactured by various manufacturers and aimed at different age groups; hence, their strengths are different and also they are sometimes available in combination with other vitamins. DHA supplements are consumed by pregnant women or women who are trying to get pregnant (since the first few weeks in pregnancy are very crucial for brain and spinal cord formation) along with prenatal vitamins. Supplementary DHA is also available for lactating mothers, in the form of postnatal DHA. They are usually consumed by growing toddlers and children, who have higher needs for DHA, in order to maintain a developing brain and visual system, and are available as Kids DHA, in different kid-friendly forms, thus making it likable to kids. Also DHA is required for adults, who need to maintain proper brain functioning, retention capability, memory, and also other physiological conditions like cardiovascular function (Fig. 5).

Conclusion

The above review summarizes the challenges involved in the treatment of spinal cord injury, and discusses the potential benefits of the omega-3-polyunsaturated fatty acid, DHA as an emerging therapeutic for the same purpose.

The situation encountered by a patient suffering from SCI is very different from any other type of injury. Unlike injuries in other parts of the body, leaving out the probability of amputation of a limb, or other devastating situations beyond treatment—which sometime involve multiple fractures, lesions, bruises—injury in the spinal cord is a progressively worsening condition and leads to neurodegeneration. Patients with spinal cord injury face multiple secondary damages, which alter their way of living for the rest of their lives. Depending on the extent of injury they may require 24 h physical aid and extreme levels of assistance which cause emotional and depressing side effects, and they may have an inability to work and may

Food Source	DRI/DV (%)	Healthiest World Ranking
Flaxseeds	133	excellent
Walnut	113	excellent
Sardines	61	Very good
Salmon	55	Very good
Brussels sprouts	11	Very good
Cauliflower	9	Very good
Tofu	28	Good
Broccoli	8	Good
Cod	8	Good
Collard greens	8	Good
Spinach	7	Good
Kale	5	Good
Romaine Lettuce	5	Good
Green beans	5	Good

Fig. 4 Various sources of DHA, ranked according to excellence

Fig. 5 Different types of commercially available DHA, dietary supplements

experience disruption of urinary and sexual functions. The families of the patients also have to undergo a lot of emotional upheavals trying to cope with this traumatic situation. There are several rehabilitation centers which provide not only physiological assistance to these patients but also the emotional support that is required for the patients and their families.

The discovery of an effective treatment for SCI is thus urgent. Since the secondary damage is multidimensional and deteriorates with time, dealing with the condition from one end will not be helpful, or if helpful at all will not be sustainable in a typically hostile condition. Therefore, a treatment which is multifaceted and can simultaneously modulate various functions at the cellular and nuclear level is required. Moreover, given the fragile condition of the patients, a less invasive or maybe noninvasive treatment is the ideal therapy desired. DHA either as injection or in regular diet or as a dietary supplement has huge potential given the improvements reported in rodent studies. Though clinical trials have to be done in human patients and this will require some time, if successful, DHA could prove to be a blessing for thousands of people currently suffering from SCI. Moreover, preclinical studies to lay out the mechanism are underway, to understand the big picture and implement it in clinical treatments.

Compliance with Ethics Requirements The author declares that he/she has no conflicts of interest.

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Dietary Polyphenols as Potential Remedy for Dementia

Abhishek Desai

Abstract With increasing life expectancy as a result of better quality of life and improved health care, the incidence of aging related diseases and disorders is heading toward epidemic proportions. Dementia, a spectrum of neurological diseases associated with aging, is an increasingly prevalent disease. No cure exists yet for dementia; however, there are many potential candidates for treatment of dementia that merit more exploration. Polyphenols, which constitute one such class of compounds, are dietary agents that are globally found in commonly consumed food. Many processes that are associated with the pathophysiology of dementia can be modulated by polyphenols. Polyphenolic compounds can alleviate oxidative stress by acting as direct scavengers of free radicals and clearing superoxide and hydroxyl radicals and by increasing the level of antioxidant enzymes such as glutathione peroxidase. They also chelate metal ions to prevent free radical formation. Polyphenols can also combat inflammation by affecting transcription factors such as NF-κB. Some polyphenols may have the potential to inhibit excitotoxicity by regulating intracellular calcium ion concentration, inhibiting glutamate receptors and increasing glutamate reuptake at the synapse. The cognitive decline in dementia due to decreased availability of acetylcholine can also be countered by polyphenols that inhibit acetyl-cholinesterase activity. Taken together, these findings suggest that increasing the consumption of polyphenol rich food may alleviate the effects of dementia. Moreover, their effects on controlling multiple mechanisms that are associated with dementia may also prevent or slow down the onset and progress of this devastating disease.

Keywords Polyphenols • Dementia • Curcumin • Catechin • Diet

The term dementia covers a broad spectrum of neurological disorders. General cognitive impairment may progressively increase with time to eventually interfere with normal daily activities that depend on thinking, memory, communication, attentiveness, and judgment. The cause of dementia may be traced to genetics (e.g., Huntington's

A. Desai (✉)
National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD, USA
e-mail: abhishrekdesai@gmail.com; Abhishek.Desai@nih.gov

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disease—a progressive, degenerative disease caused by a dominant mutation in a gene on chromosome 4 that leads to cognitive decline and dysregulated mood and motor control), lifestyle choices (e.g., Korsakoff syndrome—a chronic memory disorder that is probably caused by energy depletion in the brain due to severe thiamine (vitamin B-1) deficiency; this effect is often associated with alcohol abuse and malnutrition that leads to the vitamin deficiency), or a combination of the two along with unclear predisposing factors. Alzheimer's disease is the most common cause of dementia, accounting for about two-thirds of all dementia cases. It is often associated with aging as individuals over 65 years are more prone to developing this disease. However, it is no longer considered to be a consequence of aging. Vascular dementia or vascular cognitive impairment (dementia caused by inadequate/interrupted blood flow to the brain; 10 % of all dementia cases), dementia with Lewy bodies (aggregations of α-synuclein), Parkinson's disease, and frontotemporal dementia are regarded as other forms of dementia. Although dementia is thus categorized into many forms, distinguishing between these forms is not always simple, and many cases have pathological signs indicating an overlap or multiple forms of dementia. Such cases are often diagnosed as cases of "mixed dementia". Between the year 2000 and 2010, the proportion of deaths resulting from heart disease, stroke, and prostate cancer decreased by 16 %, 23 %, and 8 %, respectively, whereas the proportion resulting from AD increased by 68 %. By 2050, Alzheimer's disease alone is expected to inflict nearly a million new cases per year with the total estimated prevalence expected to be 13.8 million (Alzheimer's Association 2014). Two factors are responsible for the devastation caused by dementia: (1) *Most dementia cases are progressive in nature*. Dementia often progresses from barely detectable symptoms to total compromise in cognition. (2) *Most dementia cases are irreversible*. There is no cure for dementia. After diagnosis, dementia is often managed by using drugs that regulate the activity of neurotransmitters (e.g., Memantine, an NMDA receptor antagonist; donepezil, a cholinesterase inhibitor) and by making lifestyle changes (e.g., diet changes, regular exercise, and daily mental activities). Dementia has complex and incompletely understood etiology and pathophysiology and diverse symptoms and accompanying effects. Therefore, potential therapeutic agents are likely to be effective if they can simultaneously target multiple processes involved in the disease. Many dietary agents consist of active compounds that have such effects. Of late, there has been renewed interest in many natural compounds as these have been recognized to be commonly present and better tolerated with less side effects. With the growing advocacy about lifestyle changes, closer attention is being focused on improving our diet. Dietary compounds can be attractive candidates, not only because they can affect many facets of the pathology, but also because they are widely believed to have few adverse effects and have historically documented use. One of the classes of such compounds is the polyphenols.

Polyphenols are present in the most commonly consumed food, including fruits, vegetables and grains. According to recent estimates, the intake of polyphenols by adults in the USA is 250–350 mg per day (Bai et al. 2014; Sebastian et al. 2015). Chemically, a polyphenol is a compound that has many hydroxyl groups attached to an aromatic ring. Hundreds of polyphenols that are found in plants are classified on the basis of the number of phenol rings in the compound as well as by the way that

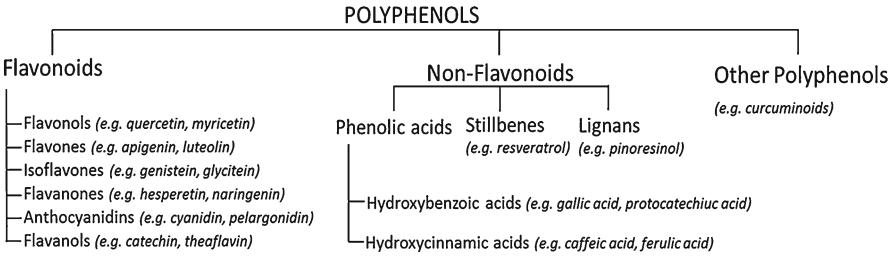

Fig. 1 Types of polyphenols

they are bound together. They are present as monomers (e.g. catechin—present abundantly in green tea) as well as polymers (e.g. condensed tannins—present in black tea, pears, and apples) (Vrhovsek et al. 2004). Polyphenols that have two aromatic rings together with three carbon atoms that form an oxygenated heterocyclic are categorized as flavonoids, which have six subtypes (Fig. 1) (Manach et al. 2004). The nonflavonoid polyphenols are classified as phenolic acids (derivatives of either benzoic or cinnamic acid), lignans (two phenylpropane units), and stilbenes (Manach et al. 2004).

Epidemiological studies have reported that intake of flavonoids reduces the risk of dementia (Commenges et al. 2000; Engelhart et al. 2002). Polyphenols can potentially influence different factors that converge in the onset and progression of dementia (Letenneur et al. 2007). Prominent factors among these are oxidative stress, inflammation, and excitotoxicity. Attenuation of these pathological phenomena by polyphenols can contrite to neuroprotection and preservation of cognitive function (Fig. 2).

Oxidative Stress

Free radicals are species with an unpaired electron, which makes them highly reactive. These species can react with cellular components in a chain reaction that can spread and cause extensive damage through processes like lipid peroxidation and DNA/protein oxidation. Free radicals generated due to metabolic activity are usually efficiently neutralized by the antioxidant system of the body. This antioxidant system includes glutathione, and uric acid that directly neutralizes free radicals along with antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase that catalyze the conversion of superoxide to hydrogen peroxide (superoxide dismutase) and the conversion of hydrogen peroxide to water and oxygen (catalase/glutathione peroxidase). Oxidative stress is a condition caused when the antioxidant system of the body is overwhelmed by excessive free radical production. Certain agents obtained through diet can also act as antioxidants (e.g., Vitamins A, C, and E). Polyphenolic compounds are also scavengers of free radicals.

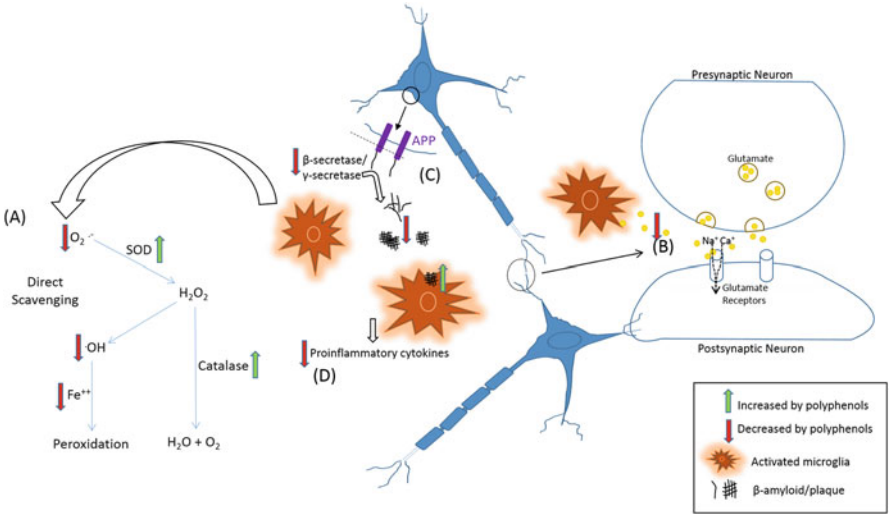

Fig. 2 Polyphenols can potentially alleviate dementia by multiple mechanisms. Polyphenols have different modes of antioxidant action (**a**). They can also decrease intracellular calcium ion concentration to reduce excitotoxicity (**b**), suppress the production of amyloid plaques by inhibiting beta secretase, aggregation of amyloid, and increasing phagocytosis of amyloid by microglia (**c**). They may also limit inflammation by reducing the production of inflammatory cytokines (**d**)

Oxidative stress is associated with both aging and neurodegenerative diseases (Floyd and Hensley 2002; Lin and Beal 2006). Oxidative stress is an important feature in vascular dementia and Alzheimer's disease (Bennett et al. 2008). Amyloid β leads to lipid peroxidation (Butterfield et al. 1994; Avdulov et al. 1997) and accumulation of ceramides and cholesterol in neurons, which are elevated in vulnerable brain regions of patients suffering from Alzheimer's disease (Cutler et al. 2004). Numerous polyphenolic compounds have been shown to have antioxidant properties either as pure compounds or as components of extracts. These antioxidant properties can be ascribed to different mechanisms.

Direct Scavenging

The antioxidant activity of polyphenols may be based on free radical scavenging capacity; polyphenols including gallic acid, tannic acid, caffeic acid, quercetin, rutin, ferulic acid, resveratrol have direct free radical scavenging activity (Sánchez-Moreno et al. 1999). When tested for DPPH (1,1-diphenyl-2-picrylhydrazyl) radical and superoxide radical scavenging activities, polyphenols isolated from apple pomace, which included (−)-epicatechin, its dimer (procyanidin B2), trimer, tetramer and oligomer, quercetin glycosides, chlorogenic acid, phloridzin, and 3-hydroxyphloridzin, showed strong antioxidant activities; their DPPH scavenging activities were 2–3 times and superoxide scavenging activities 10–30 times those of vitamins

C and E (Lu and Foo 2000). Tea polyphenols and tea pigments (isolated from black tea) also scavenge superoxide and hydroxyl radicals (Yaping et al. 2003). Simple and complex polyphenols from red wine also exhibit free radical scavenging activity (Lodovici et al. 2001).

Metal Ion Chelation

Free metal ions can catalyze the formation of free radicals; therefore chelation of these metal ions can also prevent the formation of free radicals. Moreover, copper and iron can mediate aggregation of amyloid in Alzheimer's disease (Finefrock et al. 2003). Most polyphenols are effective metal ion chelators. Metal ions such as Fe^{2+} and Fe^{3+} can coordinate up to three catecholate or gallate groups (Perron and Brumaghim 2009). 10 μmol/l of baicalein and baicalin, the flavonoids in the radix of *Scutellaria baicalensis* Georgi, effectively inhibited lipid peroxidation of rat brain cortex mitochondria induced by Fe^{2+}-ascorbic acid (Gao et al. 1999). Certain endogenous proteins also serve to sequester metal ions. Among polyphenols, quercetin decreased while kaempferol, genistein, and biochanin-A increased the expression of metallothionein, a metal binding protein, in Caco-2 cells (Kuo et al. 1998).

Regulation of Antioxidant Enzymes

Increasing the expression and activity of antioxidant enzymes is an effective way to combat free radicals. Chronic exposure to flavonol rich red wine led to an increase in the activity of catalase and glutathione peroxidase while decreasing lipid peroxidation in the kidney in rats (Rodrigo et al. 2002). Similarly, oral treatment with soy isoflavones increased, reduced glutathione by increasing the enzymes *glutathione S-transferase* and *glutathione reductase* in potassium bromate-induced renal oxidative stress (Khan and Sultana 2004). Quercetin and onion extract can increase glutathione content by upregulating the expression of the γ-*glutamylcysteine synthetase* containing antioxidant-/electrophile-response elements (Myhrstad et al. 2002).

Excitotoxicity

Glutamate is the principal excitatory neurotransmitter in the brain. It acts through ionotropic receptors that are ligand-activated ion channels and metabotropic receptors, which are G protein-coupled receptors. Though glutamate has an essential role in memory processes, overstimulation of glutamatergic signaling can lead to excitotoxicity. Excitotoxicity involves the large influx of calcium ions through the ionotropic glutamate receptors, thereby causing activation of calcium-dependent

lytic enzymes such as phospholipases and calpains leading to cell death. Excitotoxicity is an important component in the pathology of neurodegeneration (Dong et al. 2009). Numerous studies report a reduction in glutamate excitotoxicity by polyphenols in neurons and glia in vitro (Gottlieb et al. 2006; Lee et al. 2003; Lemus-Molina et al. 2009; Ibarretxe et al. 2006; Yazawa et al. 2006; Chen et al. 2008). Pretreatment with curcumin and cotreatment with tannic acid decreases the intracellular calcium load due to glutamate stimulation in rat cortical neurons (Yazawa et al. 2006). Protein kinase C (PKC) is a calcium-regulated kinase that modulates NMDA receptor function. Curcumin inhibits the activation of NMDA receptors through PKC (Yazawa et al. 2006). The polyphenols morin and mangiferin inhibit excitotoxic cell death in cortical neurons induced by AMPA (3-amino-5-hydroxy-4-methylisoxazole propionic acid), an agonist of AMPA ionotropic glutamate receptors (Ibarretxe et al. 2006). Treatment with these polyphenols also attenuates AMPA-induced intracellular calcium overload in cortical neurons. The polyphenols baicalin and baicalein extracted from dried *S. baicalensis* also protect against excitotoxic cell death. Baicalin and baicalein attenuate intracellular calcium increase in primary neuronal culture due to glutamate-NMDA treatment and also decrease neuronal NMDA receptor-mediated excitotoxic cell death (Lee et al. 2003). Threohydroxyaspartate (THA) is an inhibitor of glutamate uptake and causes accumulation of glutamate at synapses leading to excitotoxicity. Pretreatment of Rat lumbar spinal cord explants with epigallocatechin gallate (EGCG) protects motor neurons against THA-induced cell death and reduces accumulation of glutamate in the medium (Yu et al. 2010). A similar effect of epicatechin gallate is reported wherein the polyphenol enhances glutamate uptake in C6 glioma cells (Abib et al. 2008).

Inflammation

Glial cells in the brain act as local mediators of immunity. Activated astrocytes and microglia can produce proinflammatory cytokines, chemokines and cell adhesion molecules. Inflammation is associated with neurodegeneration (Griffin 2006), though whether inflammation per se is the primary causal factor in the onset of dementia and whether blocking all aspects of inflammation is therapeutic is still controversial (Enciu and Popescu 2013). In addition to neuroinflammation originating in the brain, systemic inflammation also leads to production of proinflammatory cytokines by immune system components such as macrophages that can gain access to the brain. Although the brain is protected by a blood–brain barrier, a highly selective permeability barrier made by the endothelial cells, the integrity of this barrier can be compromised by the disease. A recent publication by Takeda et al. (2013) reported that APP transgenic mice, a model of Alzheimer's disease, have increased permeability of the blood–brain barrier after peripheral injection of lipopolysaccharide (LPS), suggesting greater susceptibility to immune challenges in the Alzheimer's disease model. Secretion of the proinflammatory cytokine IL-1β by

microglia can increase acetylcholinesterase activity (Li et al. 2000), which leads to enhanced degradation of acetylcholine, a neurotransmitter vital for cognition. Numerous polyphenolic compounds have anti-inflammatory activity. In LPS-stimulated RAW 264.7 cells, a macrophage cell line, fisetin, kaempferol, and quercetin reduce the DNA binding activity of nuclear factor kappa B (NF-κB), a transcription factor regulating inflammation by promoting the expression of the inflammatory mediators iNOS and Cox-2 (Wang et al. 2006). Quercetin acts through the inhibition of Src- and Syk-mediated PI3K-(p85) tyrosine phosphorylation and subsequent TLR4/MyD88/PI3K complex formation to decrease inflammation (Endale et al. 2013). Luteolin, a polyphenol found in celery, green pepper, and dandelions, limits LPS-induced inflammation in RAW 264.7 cells by inhibiting iNOS-mediated nitric oxide production and prostaglandin E2 synthesis. This inhibition was affected by a decrease in the activation of the transcription factors NF-κB and AP-1 (Park and Song 2013). Similarly, the stilbenoids resveratrol, found abundantly in red wine, and arachidin-1 and piceatannol found in peanuts, also have an anti-inflammatory effect in RAW 264.7 cells. These stilbenoids decrease LPS-induced NF-κB activation in RAW 264.7 cells along with inhibition of PGE2 and nitric oxide without affecting the expression of cox-2 and iNOS (Djoko et al. 2007). The anti-inflammatory effects of polyphenols have also been reported in in vivo studies. Daily feeding of polyphenol-rich grape extract for 3 weeks to rats reduced serum phospholipase A2 levels and reversed hematocrit decline due to LPS injection (Tsao et al. 2012).

Owing to the astounding diversity of polyphenols, a detailed account of each requires an entire volume. Catechins and curcumin will be reviewed here as representatives of flavonoid and nonflavonoid polyphenols. These are chosen as they have been extensively studied and also because unlike many polyphenols, they can cross the blood–brain barrier.

Curcumin

Curcumin or 1,7-bis(4-hydroxy-3-methoxyphenyl)-1*E*,6*E*-heptadiene-3,5-dione is one of the three curcuminoids found in turmeric, a powder from the rhizome of *Curcuma longa*, the other two being demethoxycurcumin and bisdemethoxycurcumin. It has been used for thousands of years in food preparations as a spice and also as a folk medicine in India and Asia. For instance, it has been used for its medicinal properties in the “Siddha medicine” of southern India, the oldest known medicinal system. In India, turmeric also has a religious significance and is used in many auspicious rituals such as weddings. Indeed, the use of turmeric as a treatment for a diverse array of conditions from minor burns and insect bites to wounds and cough and cold signify multiple mechanisms of action of this agent. Modern science now attests that curcumin has antioxidant, -inflammatory, and -microbial activity, which may underlie its beneficial effects. These effects make curcumin a good candidate for the prevention and control of neurodegenerative diseases.

Alzheimer's disease incidences are less in India as compared to the USA (Ganguli et al. 2000) and the extensive use of curcumin in India has been posited as one of the reasons for this difference. Incubation of amyloid β40 (Aβ40) peptide with curcumin prevents the formation of amyloid oligomers; addition of curcumin to preformed Aβ40 aggregates leads to disaggregation (Ono et al. 2004; Yang et al. 2005). 20 μM curcumin blocked copper-induced increases in APP (amyloid precursor protein) and BACE (β-site APP-cleaving enzyme) in PC12 cells (Lin et al. 2008). Curcumin also suppresses Aβ42-induced increases in BACE-1 rat primary neuronal culture possibly acting through antioxidant mechanisms (Shimmyo et al. 2008). Curcumin has been reported to have antioxidant and anti-amyloid actions in the transgenic mouse model APPSw, which carries a human familial AD gene (APP with the "Swedish" double mutation). These mice have age-related deposition of amyloid plaques accompanied by a decrease in cognitive function and increase in oxidative stress and inflammation (Lim et al. 2001). The mice, when fed with low doses of curcumin for 6 months, had lower brain level of the proinflammatory cytokine IL-1β and reduced oxidized protein levels, suggesting an antioxidant effect of the curcumin treatment. Moreover, microglial staining decreased in the cortex and hippocampus except in the vicinity of the plaques, which suggests that curcumin suppresses inflammation while preserving, or even enhancing, site-specific microglial action to clear the plaques. In an elegant experiment, Garcia-Alloza et al. (2007) injected curcumin through the tail vein of mice daily for 7 days and visualized the binding of curcumin to amyloid plaques. In this experiment, transgenic APPswe/PS1dE9 mice were used to model Alzheimer's disease as these mice have distinct deposits of amyloid plaques by 7–8 months of age. Daily intravenous injections of 7.5 mg/kg of curcumin for 7 days resulted in the plaques and the Aβ deposits in the walls of the arteries to show green fluorescence, indicating that curcumin can cross the blood–brain barrier and bind to amyloid plaques. This binding was visualized directly in the cerebral cortex through thin glass cover slips fixed over a small cranial aperture by multiphoton microscopy. Curcumin treatment also decreased the amyloid burden by reducing the plaque size as well as by preventing the formation of plaques. In another study, Aβ40 and Aβ42 peptides were directly infused in the brains of aging female rats to model Alzheimer's disease. Curcumin feeding prevented decline in spatial memory in rats due to amyloid peptide infusions while decreasing the number and size of Aβ deposits and preventing a decline in synaptophysin and PSD-95, a postsynaptic marker. Curcumin also decreased the levels of F2 isoprostanes which are lipid peroxidation products of arachidonic acid, indicating reduced oxidative damage. Curcumin reduced the overall phosphotyrosine labeled microglial immunoreactivity while increasing it in and around the amyloid plaques (Frautschy et al. 2001). This finding, when taken along with that of Lim et al., further strengthens the inference that curcumin likely acts by microglia-mediated clearing amyloid deposits while dampening the nonspecific microglia-mediated inflammation in the surrounding tissue.

A double-blind, placebo-controlled, randomized, 6-month trial employing elderly Chinese having symptoms of progressive cognitive decline was undertaken in Hong Kong to evaluate the effect of 1 or 4 g of curcumin taken orally

with meals (along with daily 120 mg standardized ginkgo leaf extract) (Baum et al. 2008). The trial was not conclusive regarding the effect of the curcumin on cognition mainly because the placebo group did not show a decline in cognition during the 6-month trial period. No side effects of curcumin were reported for the doses used.

Another randomized, double blind, placebo controlled study was recently concluded at the University of California, Los Angeles that included 36 subjects with mild-to-moderate probable Alzheimer's disease (Ringman et al. 2012). The subjects had the placebo, 2 or 4 g of Curcumin C3 Complex (Sabinsa Corporation, Piscataway, NJ, USA) per day in two separate doses for 24 weeks. No clinical or biochemical evidence was observed, proving the efficacy of curcumin administration in Alzheimer's disease. Curcumin intake had no statistically significant effect on cognitive function assessed by the mini mental score exam (MMSE) or ADAS-cog (Alzheimer's disease assessment scale—cognitive subportion) and did not alter the level of Aβ40 and Aβ42 in either the plasma or CSF. However, a case study conducted in Japan reported significant improvement due to curcumin (Hishikawa et al. 2012). Three patients with Alzheimer's disease exhibiting severe cognitive decline accompanied by Behavioral and Psychological Symptoms of Dementia (BPSD) showed substantial improvement in cognition (with improved parameters such as recognition and calculation), reduced BPSD, and less burden to caregivers. A study conducted at Ohio State University found that healthy 40- to 60-year old human subjects consuming Longvida Optimized Curcumin from *Curcuma Longa* root (Verdure Sciences, Noblesville, IN, USA) at 400 mg powder per day containing 80 mg curcumin for 4 weeks had lowered plasma Aβ levels compared to the placebo treated group *(*DiSilvestro et al. 2012).

Catechins

Catechins are flavanols that include catechin, (−)-epicatechin, epigallocatechin, (−)-epicatechin gallate, and epigallocatechin gallate (EGCG). They are the most dominant flavonoids consumed in the USA (Bai et al. 2014). These polyphenolic compounds are found in many commonly consumed foods, such as fruits (apples and grapes, especially black grapes), berries (blackberries, cherries, and raspberries), beans (flava beans), and beverages (tea, cocoa, and red wine). Dark chocolate is also a good source of (−)-epicatechin. Catechins are antioxidants that can act as free radical scavengers (Nanjo et al. 1996), metal ion chelators (Mandel et al. 2004*;* Reznichenko et al. 2006), and affect antioxidant enzyme activity in the brain (Levites et al. 2001). In models of neurotoxicity, catechin treatment not only offers neuroprotection but also results in improvement in behavior. EGCG protected PC12 cells from 6-hydroxydopamine hydrochloride (6-OHDA)-induced apoptosis (Nie et al. 2002) and 1-methyl-4-phenyl-pyridine (MPP+)-induced cell death (Ye et al. 2012) with an increase in antioxidant enzyme expression, possibly through regulation of the SIRT1/PGC-1α signaling pathway.

Catechin can prevent cell death by directly inhibiting the activity of caspase-3 (Yazawa et al. 2006), the enzyme that causes apoptosis. Experiments in animal models have borne out these in vitro results. In an experimental model of Parkinson's disease produced by unilateral 6-OHDA intrastriatal injection in rats, catechin treatment improves working memory, reduced 6-OHDA-induced rotational behavior, partially restored striatal tyrosine hydroxylase expression, and increased glutathione levels in the mesencephalon (Teixeira et al. 2013). Similarly, in a recent publication in a rat dementia model induced by injection of streptozotocin in the brain ventricles, EGCG treatment for 4 weeks preserves spatial memory and restored glutathione peroxidase and acetylcholinesterase activity (Biasibetti et al. 2013). Aqueous extracts of green and black tea inhibited the activities of human acetylcholinesterase and human butyrylcholinesterase in vitro in a dose-dependent manner. Green tea also inhibited β-secretase activity (Okello et al. 2004). However, the active ingredients of the tea extracts were not analyzed by the authors. A recent study has investigated the effects of isolated flavonoids on the APP processing by βγ-secretase using luciferase expression as a reporter for amyloid APP processing and found that (−)-epicatechin (EC50 of 20.5 nM) and epigallocatechin (EC50 of 18.6 nM), but not ECG or EGCG, potently reduced luciferase expression; however, at higher concentration of 10 μm (−)-epicatechin did not affect luciferase expression whereas epigallocatechin potentiated it (Cox et al. 2015). The same study employed neurons from TASTPM mice, which express the APP K595 N/M596 L Swedish double point mutation and the PS1 M146 V familial mutation and have enhanced amyloidogenic processing and cognitive deficits, to confirm the effect of (−)-epicatechin. In vitro treatment with 100 nM (−)-epicatechin for 6 h led to a 30 % decrease in secreted Aβ42 by TASTPM embryonic neurons. In addition, chronic daily oral treatment of 15 mg (−)-epicatechin for 21 days led to reduced levels of Aβ40 and 42 and a decrease in Aβ plaques in the cortex of TASTPM mice. The treatment also reduced neuronal endogenous BACE-1 activity, thereby suggesting that the reduction in amyloid levels may be due to reduced activity of BACE-1. Indeed, substitution of alanine by threonine at 673 position of APP due to a single nucleotide polymorphism confers protection against Alzheimer's disease by making the APP resistant to cleavage by BACE-1 (Jonsson et al. 2012), indicating that BACE-1 inhibition may be effective to prevent Alzheimer's disease.

Catechins are extensively metabolized after absorption and form conjugates such as glucuronides and sulfonated metabolites. The activity of (−)-epicatechin and its phase II metabolite has been elegantly demonstrated by Wang et al. (2012). Chronic daily oral treatment with monomeric polyphenols from grape (with catechin and (−)-epicatechin as constituents) at 80 mg/kg for 5 months improved spatial memory in 12 months old Tg2576 mice with reductions in oligomeric Aβ as well as Aβ40 and 42 in the brain. Glucuronide metabolites of catechin and (−)-epicatechin were also found in plasma and brain. Treatment of hippocampal slices from Tg2576 mice with synthetic 3-*O*-Me-EC-Gluc, a phase II metabolite of (−)-epicatechin that accumulates in the brain, increased long term potentiation (LTP) without any effect on the basal LTP in brain slices from age-matched wild-type mice. This increase in LTP in Tg2576 was due to CaMKII-mediated CREB phosphorylation.

Treatment of elderly individuals with mild cognitive impairment for 12 weeks with 444–621 ml/day (depending on the body weight) of grape juice resulted in improved memory compared to those that were given a placebo, a beverage with similar color, flavor, and carbohydrate composition but without polyphenols (Krikorian et al. 2010). Similar results have been reported recently for cocoa and (−)-epicatechin. Intake of 900 mg cocoa flavanols and 138 mg of (−)-epicatechin per day by elderly subjects for 3 months improves cognitive function by enhancing local or global blood flow (Brickman et al. 2014).

Overall, the consensus regarding the beneficial effects of polyphenols is strong in case of in vitro studies that are confined to assessing specific pathological mechanisms. A less emphatic agreement is noted for the effects in small animal models which all but disappears in case studies with human subjects. Conflicting observations regarding the effects of polyphenols can be due to possible weaknesses in the design of the human studies, such as lack of robustness due to very few participants, difficulty in ensuring treatment compliance, inadequate inclusion/exclusion criteria, and lack of control for confounding factors related to genetic and lifestyle differences. Other factors such as concentration of polyphenols consumed, in vivo metabolism and bioavailability, and local tissue concentrations at the target site merit careful attention.

Caveats and Considerations

An obvious confound in many studies arises due to the use of crude extracts and juices as the agents of intervention. The rationale for using them is the relatively easy availability, their high polyphenolic content and also the fact that these are often normally consumed as food. However, in such cases, the effects of other non-polyphenolic agents, either independently or by interaction with polyphenols, are impossible to rule out.

A dietary compound has to pass through many hurdles before it can access the target organ, more so in case of an immunoprivileged organ such as the brain. The solubility of the polyphenol and its stability and interaction with other compounds in the presence of digestive enzymes and low pH partly dictates its potential bioavailability. For example, the intake of epigallocatechin by Caco-2 cells doubles when the pH is decreased from 7.4 to 5.0 (Vaidyanathan and Walle 2003). Also, the metabolism of (−)-epicatechin in Caco-2 cells partly depends on the presence and type of other polyphenols (Sanchez-Bridge et al. 2015). Polyphenols can diffuse into the enterocytes lining the intestinal lumen and may be taken up via transport through the monocarboxylic acid transporter (Vaidyanathan and Walle 2003). The polyphenols are conjugated in the enterocytes and are either secreted back into the intestinal lumen via ABC transporters or in the mesenteric vein. They are then taken up and metabolized by the liver and are partially excreted in the bile (Crespy et al. 2003). In most cases, the absorption of polyphenols in the small intestine is relatively poor. On reaching the colon, these compounds are metabolized by the colon

microbiota (Duynhoven et al. 2011; Marín et al. 2015). The limited net absorption coupled with extensive metabolism results in very low levels of polyphenol bioavailability. This raises questions for some of the actions ascribed to polyphenols such as antioxidant activity, which may require higher local polyphenol concentration. However, the bioavailability of polyphenols can be dose dependent (Baba et al. 2001) indicating that polyphenols concentrations may be elevated by saturation of enterocyte transporters (Silberberg et al. 2006). Similarly, repeated administration of the grape seed polyphenols also increases bioavailability and accumulation in the brain (Ferruzzi et al. 2009). Although extensive biotransformation of polyphenols can often form metabolites that have less or no activity of the parent compound, some metabolites may retain the original activity or even have independent effects. Metabolites of quercetin have antioxidant and anti-inflammatory effects (Yamamoto et al. 1999; Loke et al. 2008; Moon et al. 2001). Similarly, a metabolite of (−)-epicatechin increases synaptic transmission and long term potentiation in hippocampus slices (Wang et al. 2012).

It is plausible that consumption of food with high polyphenolic content by healthy people can act as a prophylactic measure to prevent or slow the onset of dementia by combating the processes such as chronic inflammation and elevated oxidative stress. Owing to the limits imposed on the bioavailability by absorption and metabolism, high intake of polyphenol rich foods is unlikely to produce toxicity. So despite the lack of conclusive evidence for the benefits of polyphenols in dementia in humans, dietary polyphenols may be beneficial in more ways than one.

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Spices: Potential Therapeutics for Alzheimer's Disease

N. Satheeshkumar, R.S.K. Vijayan, A. Lingesh, S. Santhikumar, and Ch. Vishnuvardhan

Abstract India has traditionally been known to all over the world for spices and medicinal plants. Spices exhibit a wide range of pharmacological activities. In contemporary, Indian spices are used to rustle up delicious delicacies. However, the Indian spices are more than just adjuvant which adds aroma and fragrance to foods. A few spices are very widely used and grown commercially in many countries, contain many important chemical constituents in the form of essential oil, oleoresin, oleogum, and resins, which impart flavor, pungency, and color to the prepared dishes, simultaneously exerts diverse therapeutic benefits. Ayurveda, the traditional systems of medicine in India has many evidences for the utilization of spices to cure various diseases. Some of the activities have been scientifically proven. Among various indications central nervous system disorders are of prime importance and it has been evident in traditional books and published reports that spices in fact protect and cure neuronal ailments. Likewise there are many spices found in India used for culinary purpose and have been found to have reported specific activities against brain disorders. About 400 B.C., Hippocrates rightly said "Let food be thy medicine and medicine thy food." This review focuses on the importance of spices in therapeutics and the till date scientific findings of Indian spices in CNS pharmacology and explores the potential of Indian spices to cure CNS disorders.

Keywords Indian spices • Ayurveda • Toxicity • Neurodegenerative disorders • Alzheimer's disease

N. Satheeshkumar, M. Pharm., Ph.D. (✉) • A. Lingesh • S. Santhikumar • Ch. Vishnuvardhan
Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research, Hyderabad (NIPER-H), Balanagar, Hyderabad 500037, Telangana, India
e-mail: satish.niperhyd@gov.in

R.S.K. Vijayan
Department of Chemistry, Temple University, Philadelphia, PA 19122-1801, USA

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Introduction

Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disorder and is the major cause of dementia particularly in the older population. Based on the available epidemiological data, WHO estimates 35.6 million worldwide have AD, and its incidence is estimated to double by 2020 and triple by 2030, as the population ages. Neuropathological hallmark of AD includes neuritic plaques and neurofibrillary tangles. Neuritic plaques confined to the cerebral cortex contain aggregates of amyloid-β (Aβ) and the neurofibrillary tangles found in the neurons contain filamentous hyperphosphorylated form of tau protein. The earliest clinical features of AD are an inability to acquire new memories and difficulty in recalling recently observed facts followed by a prodromal phase of amnesic problems that eventually progresses to dementia. Current FDA approved drugs for the treatment of AD includes cholinesterase inhibitors (ChEIs) namely donepezil, rivastigmine, galantamine, and a partial *N*-methyl-D-aspartate (NMDA) antagonist memantine.

ChEIs constitute the first line pharmacotherapy for the symptomatic treatment of mild to moderate AD. They work by increasing the cholinergic transmission through the inhibition of enzymes (cholinesterases) that act to break down acetylcholine. This results in an increased concentration of the neurotransmitter acetylcholine at cholinergic synapses. Memantine, the first in a class of NMDA receptor antagonist, is used for treating moderate to severe AD and is believed to exert neuroprotective effects by decreasing excitotoxicity due to the overstimulation of glutamate receptors, especially of the NMDA subtype.

Incidentally, two of the clinically approved cholinesterase inhibitors, galantamine and rivastigmine (a synthetic analog of physostigmine) are based on natural products. Galantamine is a natural product isolated from the bulbous herbaceous plant *Galanthus woronowii* and bulbs of different species belonging to the Amaryllidaceae family. Rivastigmine is a synthetic analog of the cholinesterase inhibitor alkaloid physostigmine extracted from the seeds of *Physostigma venosum*. Unlike physostigmine, its synthetic analog rivastigmine displays longer duration of action and lower side effect profile. To date, there is no cure for AD and current therapy focuses on symptomatic approaches to improve both cognitive and behavioral symptoms. Hence, there is an impassioned case for developing newer therapies to prevent or stop the progression of the disease. In this chapter, we discuss about the therapeutic benefits of some popular Indian spices that holds benefit in treating AD.

Due to the lesser side effects and good tolerability, the natural remedies are the call of the day, especially the food additives have a good role to play in curing some of these problems effectively, which has been reported in ancient literatures of traditional medicines in India and China. The ancient practices lured researchers to find the scientific base regarding the health benefits. And the result as dramatic as some current reviews stated that the Indian spices are having a definite effect in treating AD. There are many scientific experimental reports published in different journals, providing a hand full of data to prove that the spices are actually beneficial in CNS ailments. The phytoconstituents present in the spices is trending in the phytopharmacological research to find out the potential leads to treat deleterious nervous disorders.

Spices are natural food adjuncts that have been in use for culinary purposes from ancient time. Spices embellish foods by imparting its characteristic color, complement it with flavor and aroma, also they mask other flavors, augment the taste and act as preservative in some foods (Parry 1969; Manandhar 1996). Spices are usually aromatic and pungent, it is due to their essential oil content which also imparts them antiseptic and preservative properties (Achinewu et al. 1995). No country in the world produces as many kinds of spices as India. Because of its climatic diversity almost all spices are grown in this country. In almost all of the 29 states and 9 union territories of India, at least one spice is grown in abundance. Different spices dominate in different regions in the country. In the north, cinnamon, nutmeg, bay leaves, cloves, cumin, and coriander are used more, while in the south, mustard seeds, fenugreek seeds, turmeric, peppercorns, chili peppers, and curry leaves feature prominently. The journey of spices faded into the historic antiquity, when humans were learning that wrapping their food in leaves kept the ashes off, retained the juices, and sometimes improved the flavor, or even tenderized tough meat. Profound application of garlic and onion evidenced in Egypt dated back to about 50,000 years in pyramids to preserve human body. Similar way ginger ornamented Chinese medicine chests and pepper in Ayurveda. In those days, spices were as important for medicine, embalming, preserving food, and masking bad odors, as they were for more mundane culinary matters. The story of Indian spices dates back to 7000 years into the past. The art of seasoning is an ancient one in the Indian subcontinent. Particles of cumin, coriander, mustard seeds, and cinnamon have been found in 4500-year-old grinding stones from the prehistoric cities of Mohenjo Daro and Harappa in the Indus Valley. Spices were the exotic treasures that lured explorers to India and brought the drama and romance into history. In spite of all the geopolitical changes that eroded India's wealth, it still remains the bastion of spices, and spices are a proud symbol of Indian culture and heritage. Today, spice use is ubiquitous, but spices are far more important in some cuisines than others. "Japanese dishes are often described as delicate, Indonesian and Szechwan as 'hot,' and middle European and Scandinavian dishes as 'bland' (Sherman and Billing 1999). In India, spices have been traditionally used since ancient times, for the preservation of food products as they have been reported to have antiseptic and disinfectant properties. Spices are an intrinsic part of Indian culture; there are other cultures which have their own spices as well. Ayurveda, the ancient system of therapy in India, quoted ayurvedic herbal formulations with spices as ingredients. The Indian spice market has shown remarkable growth in spice and culinary herb exports. Indian spices manufacturers are making substantial efforts to improve the quality of spices backed up by technological advancement in order to tap the international market. Advanced technologies such as carbon dioxide extraction, cryogrinding, encapsulation of spice oil are being undertaken to ensure high quality of spices and their derivatives. India is also leading in the field of spice derivatives, meeting almost 70 % of the total demand of spice oils and oleoresins. India meets almost 65 % of the global demand for derivatives like spice oils and oleoresins.

In this review, we considered some of the potential Indian spices scientifically proven for their specific efficacy in AD. Spices pharmacology with major pathways through which they are acting is depicted in Fig. 1. Spices, their molecular mechanism, and the ailments they can cure have been listed in Table 1. The following is brief details of the pharmacological profile of each spice.

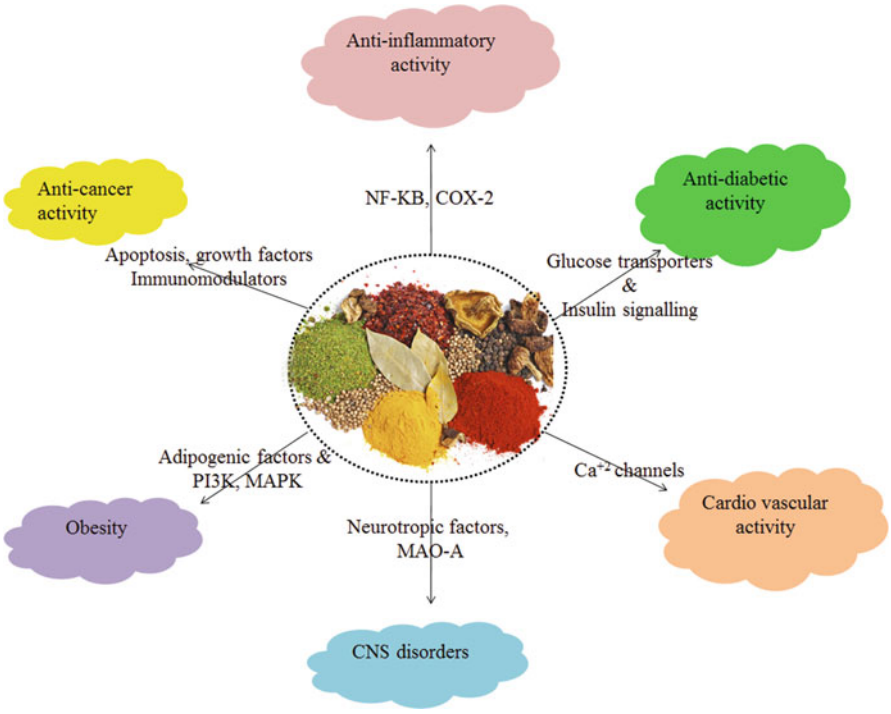

Fig. 1 Spices pharmacology activity with major pathways

Allium sativum L.

Allium sativum (Lilliaceae) commonly known as garlic, historically known for its hypocholesterolemic effects in relation to cardiovascular functions. Garlic's therapeutic applications include AD, cancer, cardiovascular disease, stress, infections, and dermatological infections. Its therapeutic activities are attributed mainly to the organosulfur compounds present; of these, diallyl sulfide (DAS) is a potent oil soluble component which has shown a protective action against cancer in animal models. DAS is the degradation product of Allicin, which itself is formed by cell decomposition. Aged garlic extract and *S*-allylcysteine has alleviated the oxidative stress, caspase 3 activation, PARP activation, and DNA fragmentation in Aβ-induced neurotoxicity in PC12 cells (Peng et al. 2002). In several other in vitro studies, both of these two showed protective effects against Aβ-induced neuronal toxicity (Borek 2006). The central nervous system activity of this plant has been evaluated by many researchers; in one study, aged garlic extract per oral treatment at 40 mg/kg daily for 4 weeks minimized the biomarkers Aβ 40 and 42 levels and enhanced formation of sAPP alpha levels in Tg2576 mice (Chauhan 2003). Allicin

Table 1 Molecular mechanisms and therapeutic implications of Indian spices

S. no.	Plant name/ phytoconstituent	Disease	Molecular mechanism
1	Cinnamon	Antidiabetic	Enhances tristetraprolin, glucose transporter GLUT4, and inhibits insulin signaling (Heping et al. 2007, 2010)
		Anti-inflammatory	Inflammatory intermediates (Kwon et al. 2011)
		Neurodegenerative disorders	Neurotrophic factors (Arundhati et al. 2013)
2	Piperine	Anti-inflammatory	Inhibits TNF-α-induced adhesion of neutrophils by suppression of NF-κB and IκB kinase activation (Kumar et al. 2007)
		Irritable bowel syndrome and pain	TRPV-1 (Szallasi 2005)
3	Diosgenin	Anticancer and anti-inflammatory	Increased apoptosis, suppression of NF-κB, and COX-2 (Gupta et al. 2010)
4	Garlic	Anticancer	DNA adduct formation, P21WAF1 inhibition, and induction of apoptosis (Raghu et al. 2012)
		Atherosclerosis	Antiplatelet, hypolipidemic activity, and HMG-coA inhibition (Banerjee and Maulik 2002)
5	Ginger	Arthritis and pain	Vanilloid receptor-1 agonist (Rosenbaum and Simon 2007)
		Antihypertensive/cardio protective	Ca^{2+} channel blocker and inhibition of platelet aggregation (Ghayur and Gilani 2005; Vasanthi and Parameswari 2010)
		Anti-inflammatory and anticancer	Inhibitor of NF-KB and AP-1 proteins (Lee et al. 2012) (Ling et al. 2010)
6	Capsaicin	Arthritis and pain	TRPV-1 receptor agonist (Pingle et al. 2007)
7	Rosmarinic acid	Anti-inflammatory and anticancer	IKK-β, ItK, and PLC-γ1 inhibition (Kim et al. 2015)
8	Eugenol/isoeugenol	Neurodegenerative diseases, analgesic, and antidepressant	Antioxidant activity, Ca^{2+} channel blocker, cholinesterase, and MAO inhibition (Rasool et al. 2014; Seo et al. 2013; Dalai et al. 2014; Tao et al. 2005)
		Anticancer and anti-inflammatory	Cox-2 and NF-κB inhibition (Kim et al. 2003; Murakami et al. 2003)
		Antidiabetic and antihypertensive	In vitro α-amylase, lipase, and ACE activity inhibition (Mnafgui et al. 2013)

a molluscicidal component obtained from garlic is capable of uncompetitively inhibiting AChE (Singh and Singh 1996). Another 21 day study found that rats treated with fresh garlic homogenate at 250 mg/kg showed memory enhancement and it was associated with an increase in brain 5-HT levels and its metabolism (Haider et al. 2008). Ethyl acetate fraction of aged garlic extract reduced oxidative stress in PC 12 cells and improved cognitive impairment in Aβ-induced neurotoxicity (Jeong et al. 2013).

Carum carvi L.

Carum carvi (Umbelliferae) commonly known as caraway, meridian fennel, or Persian cumin. The major phytoconstituents reported in *C. carvi* are carvone, epoxy carvones, limonene, α-pinene, carveol, dihydrocarveol, thymol, and myrcene. α-Pinene, carvacrol, and dihydrocarvone were found to have potent inhibitory activity against AChE with an IC_{50} value of 0.28 mg/ml, 0.17 mg/ml, and 0.78 mg/ml, respectively (Yeom et al. 2012). Limonene another active component uncompetitively inhibited acetylcholinesterase enzyme in snail *Lymnaea acuminata* (Kumar et al. 2009).

Crocus sativus L.

The stigma of *Crocus sativus* (Iridaceae), commonly known as saffron, is the world's most expensive spice cultivated in various parts of the world, which was used to treat depression. Interest in search of an alternative to the expensive stigma led to the research on the petal of *C. sativus* and was found to be as effective as the stigma of *C. sativus*. Safranal has also showed protective effect against cerebral ischemia-induced, quinolinic acid-induced oxidative damage in rat hippocampus, as the hippocampus is one of the major parts to be affected in AD it may be useful (Hosseinzadeh and Talebzadeh 2005; Hosseinzadeh and Sadeghnia 2005). In a study, *C. sativus* stigma and its constituent showed good antioxidant properties and inhibited Aβ fibrillogenesis in a concentration and time-dependent manner, its major constituent *trans*-crocin-4 has shown better anti-amyloidogenic activity than nonsugar estered dimethyl crocetin establishing the role of sugar in its activity (Papandreou et al. 2006). Saffron, crocetin, and safranal have reduced the oxidative stress, decreased the caspase-3 activation in neuronal SH-SY5Y cells and enhanced the memory in aged mice by its antioxidant mechanism and anticholinesterase activity (Papandreou et al. 2011). Crocin modified as has improved the hyoscine unpaired acquisition/performance activity but does not alter intact memory (Hosseinzadeh and Ziaei 2006), similarly in passive

avoidance test it possess good activity which support the implication of *C. sativus* stigma extract in learning, memory and can be potentially introduced as new drug to treat cognitive dysfunctions of AD (Pitsikas et al. 2007). In a recent study conducted saffron has shown protective effect against aluminum-induced neurotoxicity, although there was no enhancement in cognition, treatment has enhanced the antioxidant activity, MAO enzyme activity, and reduced the acetyl and butyryl cholinesterase activity in the whole brain and cerebellum (Linardaki et al. 2013).

Curcuma longa L.

Curcuma longa or turmeric (Zingiberaceae) is the most studied medicinal plants throughout the world, curcumin is the active components of turmeric. It was used from ancient times on the Indian subcontinent to treat various illnesses such as skin diseases, microbial diseases, intermittent fevers, hepatic disorders, inflammations, constipation, iron chelating, and neuroprotectant in the treatment of various neurological disorders. Curcumin has the potential to treat Alzheimer's and Parkinson diseases (Goel et al. 2008) through modulation of numerous molecular targets (Pari et al. 2008). A water extract of turmeric has reduced the H_2O_2-induced neurotoxicity in PC 12 cells by significantly elevating cell survival, antioxidant enzyme activities, and decreasing levels of MDA (Koo et al. 2004). It has reduced the oxidized protein levels and inflammatory cytokine IL-1β level, insoluble and soluble Aβ in APPsw mice. Curcumin has also reduced in vitro formation of Aβ 40 and 42 from Aβ 40 and 42, it is also having metal chelating activity against Cu and Fe which are indicated in Aβ aggregation. Curcumin crossed the blood–brain barrier in Tg2576 mice and reduced the amyloid plaque burden after binding to the plaques. Beta amyloid elicits toxicity by producing peroxidase from heme which is inhibited by curcumin, it also increased the phagocytosis of Aβ cells (Aggarwal and Harikumar 2009). The anti-amyloidogenic activity of curcumin is by inhibition Aβ (1–40)-induced MAP kinase and downstream activation of ERK-1/2 and Elk-1 (Kannappan et al. 2011). These neuroprotective effects of cholinesterase inhibitors might partly contribute to the clinical efficacy in AD treatment and may lead to the development of new therapeutic treatments for hypoxic/ischemic brain injury (Ahmed and Gilani 2009). *C.longa*, protects neuronal cells against Aβ-induced toxicity (Park et al. 2008), data indicate that curcumin protected PC12 cells against Aβ-induced neurotoxicity through inhibition of oxidative damage, intracellular calcium influx, and tau hyperphosphorylation. Curcumin administration increased hippocampal neurogenesis in chronically stressed rats, similar to classic antidepressant imipramine treatment (Xu et al. 2007). Prolonged treatment of curcumin has enhanced hippocampal neurogenesis, cognition in spatial, and nonspatial memory in aged rats. This may be by modulating the synaptic plasticity and cell growth (Dong et al. 2012).

Capsicum annum L. var. angulosum

Capsicum annum (Solanaceae) is well known to all Indian kitchens as it is one of the integral part of Indian food to make it delicious, there are two varieties found for the species, one is sweet and another is piquant. Traditionally it has many acclaimed uses in digestive disorders. Discovery of capsaicin receptor TRPV-1 is a breakthrough in pain research, it causes analgesia at lower concentrations but relieves pain after prolonged exposure by desensitization of the TRPV-1 receptor. Recently the phytoconstituents of *Capsicum* mainly capsaicin were found to be potential in treating AD by effectively inhibiting Aβ plaque formation (Szabo et al. 2004). In another study, Capsicum water extract has shown AChE and BChE inhibitory activity with EC_{50} 3.19 ± 0.04 and 3.01 ± 0.03, respectively, and exhibited good antioxidant activities in vitro (Ogunruku et al. 2014). Capsaicin the major constituent of capsicum attenuated the cognitive impairment in cold water stress-induced cognition impairment, treatment increased synapsin 1 and PSD93 memory associated proteins, and reduced tau hyperphosphorylation by activating protein phosphatase 2A (Jiang et al. 2013). In another study, capsaicin-enhanced membrane bound APP levels by 1.7 times without having any effect on BACE and PKC levels in rat brain cortical samples hinting its effect on amyloidogenic processing of APP (Magdolna et al. 2009). Capsaicin also exhibited anticholinesterase activity with 62.7 ± 0.79 and 75.3 ± 0.98 percentage inhibitions of AChE and BChE, respectively (Orhan et al. 2007).

Cinnamomum cassia

Cinnamomum cassia. Blume (Lauraceae), commonly known as cinnamon, has a long history of use as spice and flavoring agent. Major constituents of cinnamon bark are cinnamic acid, cinnamaldehyde, and cinnamic alcohol. A number of pharmacological and clinical effects have been observed with it. It effectively reverses the scopolamine-induced memory impairment in rat model, enhanced choline acetyltransferase activity, and acetylcholine content in frontal cortex (dos Santos-Neto et al. 2006). In a dose-dependent manner its extract significantly protected against glutamate-induced cell death and also inhibited glutamate-induced Ca^{2+} influx. These results demonstrate the neuroprotective effect of *Cinnamomum cassia* bark on glutamate-induced neuronal exitotoxicity like AD (Shimada et al. 2000).

Coriandrum sativum L.

Coriandrum sativum (Umbelliferae), commonly known as Coriander (Dhaniya), has been traditionally used as a part of spices and in medicine for its carminative, diuretic, and anticonvulsant effects. Among medicinal plants, *C. sativum* L. has

been recommended for relief of anxiety and insomnia in Iranian folk medicine (Emamghoreishi et al. 2005). The major constituents of coriander are fatty oils including petrolselenic, linoleic and oleic acids, essential oils including linalool, camphor, geraniol, α-pinene, and γ-terpene. It also contains polyphenolic acids ferulic acid, gallic acid, caffeic acid, salicylic acid, esculin, esculetin, and umbelliferone. Its leaves have efficiently enhanced memory in young and aged mice in a dose-dependent manner, also reversed the scopolamine- and diazepam-induced amnesia when given for 45 days. There was a decrease in serum cholesterol and acetylcholinesterase activity in the brain, which might be the possible mechanism through which it reduced the cognitive impairment (Mani et al. 2011). Coriander fruit extract reversed the scopolamine-induced amnesia in a dose of 100, 200, and 300 mg/kg in a dose-dependent manner and inhibited lipid peroxidation greater extent than ascorbic acid in brain and liver (Sushruta and Choi 2012).

Elettaria cardamomum L.

Elettaria cardamomum (Zingiberceae), commonly known as cardamom, is traditionally used in various gastrointestinal, cardiovascular, and neuronal disorders. The chemical composition of it considerably varies with age, region, and the variety of cardamom. Major components of its volatile oil are 1,8-cineole, α-terpinyl acetate, and limonene, whose composition varies highly with storage conditions. The extracts of *E. cardamomum* used as one of the ingredients of the polyherbal formulation for treating dementia of AD, namely Unmadnashak Ghrita (UG) containing *E. cardamomum* (6 g), *Ferula narthex* (6 g), *Gardenia gummifera* (6 g), *Bacopa monneri* (6 g), and cow's ghee (clarified butter fat) (76 g) was developed for their neuropharmacological activities. UG formulation showed CNS-depressant activity and anticonvulsant activity in mice (Achliya et al. 2004). Brahmi rasayana (BR), another formulation is used as a memory enhancer. It comprises coarse seed powders of *E. cardamomum*, dried leaves of *Bacopa monnieri*, the flower buds of *Eugenia caryophyllus*, bark of the shoots of *Cinnamomum zeylanicum*, and fruits of *Piper longum* and *Piper nigrum* were prepared as per standard ayurvedic procedures (Hanumanthachar and Parle 2006b). The extract is also used in one of the herbal combination useful in the treatment of anxiety and insomnia.

Ferula asafoetida L.

Ferula asafoetida (Apiaceae), asafoetida is the gum from rhizome of several *Ferula* spices. Ferulic acid, umbelliferone are active molluscicidal components that inhibited the activity of alkaline phosphatase and acetylcholinesterase enzymes. Ferulic acid, umbelliferone are competitive inhibitor of alkaline phosphatase and competitive inhibitors of acetylcholinesterase (Kumar et al. 2009). The gum extract has

shown dose-dependent memory enhancing potential in scopolamine-induced amnesia, increased serum thiols, and inhibited brain acetylcholinesterase activity (Vijayalakshmi et al. 2011).

Foeniculum vulgare Mill

Foeniculum vulgare (Umbelliferae), commonly known as Fennel belongs to the family Umbelliferae, is quite a famous recipe of the food. Traditionally it has very rich application for curing the gastric disorders. The essential oil of *F. vulgare* has very high inhibitory activity against both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes (Orhan et al. 2008b).

Murraya koenigii L.

Murraya koenigii (Rutaceae) leaves, commonly known as curry leaf, are added routinely to Indian gravy and vegetarian dishes as a favorite condiment. Ethnomedical knowledge suggests that *M.koenigii* is used as a stimulant, antidysentric, and for the management of diabetes mellitus. The *M. koenigii* leaves produced a dose-dependent memory enhancement and reduced scopolamine, diazepam-induced amnesia in young and aged mice, possibly by increasing brain cholinesterase activity, antioxidant activity, and decreasing total cholesterol levels (Mani and Parle 2009). Mahanimbine a carbazole alkaloid isolated from a petroleum ether extract of leaves inhibited acetylcholinesterase activity in a dose-dependent manner (Kumar et al. 2010). Total alkaloidal extract of curry leaves improved memory in both young and aged mice, ameliorated scopolamine- and diazepam-induced amnesia, reduced brain cholinesterase levels, and inhibited beta secretase enzyme which drives amyloidogenic metabolism of APP with an IC_{50} of 1.7 μg/ml (Mani et al. 2012).

Myristica fragrans Houtt. Nutmeg

Myristica fragrans (Myristicaceae) is an evergreen tree. Hydroalcoholic extract of *M. fragrans* was tested for in vitro acetylcholinesterase inhibitory activity. It showed 50 % inhibition of AChE activity at concentrations of 100–150 μg/ml (Mukherjee et al. 2007). The *n*-hexane extract of *M. fragrans* seeds showed brain AChE inhibition and antidepressant activity (Dhingra and Amandeep 2006; Dhingra et al. 2006). Treatment with *M.fragrans* seed *n*-hexane extract improved memory of both young and aged mice in scopolamine- and diazepam-induced amnesia at 5 mg/kg dose orally (Parle et al. 2004).

Piper nigrum L.

Piper nigrum (Piperaceae), commonly known as black pepper, is indigenous to south India and it is the world's most traded spice. It is a potential functional food for improving brain function. The main constituent of it is piperine an alkaloid which is used as traditional medicine in Asian countries. Piperine is of great clinical significance as it enhances bioavailability of drugs and other compounds by inhibiting drug metabolizing enzymes. Piperine treatment alleviated neurotoxicity in hippocampus induced by intracerebroventricular administration of ethylcholine aziridinium ion and improved cognition impairment with lipid peroxidation inhibition and decreased acetylcholinesterase activity. In another study, it enhanced water retention time and decreased escape latency which was comparable with donepezil in diazepam treated rats (Chonpathompikunlert et al. 2010; Wattanathorn et al. 2008).

Pimpinella anisum L.

Pimpinella anisum (Apiaceae) is a flowering plant in the family Apiaceae native to the eastern Mediterranean region and southwest Asia it is known for its flavor that resembles licorice, fennel, and tarragon. In India, it grows to a small extent as a culinary herb or as a garden plant. The major products are anis oil and oleoresin anis. Chinese star anise showed anticholinesterase activity with more specificity toward acetylcholinesterase than butyrylcholinesterase and the major constituent responsible for its activity is Anethole (Bhadra et al. 2011).

Rosmarinus officinalis L.

It is a perennial herb, commonly known as Rosemary, is a member of the mint family Lamiaceae. A major constituent of rosemary is rosmarinic acid, *p*-cymene, linalool, and thymol. Further different extracts, rosmarinic acid, and essential oil of *Rosmarinus officinalis* L. were tested for its AChE and BChE inhibitory activity, of these rosmarinic acid showed 85.8 % AChE inhibition at 1 mg/ml (Orhan et al. 2008a).

Salvia officinalis L.

Salvia officinalis (Lamiaceae) leaves are traditionally used for various pharmacological activities. Ethanolic extract of sage leaves increased memory of passive avoidance learning in rats (Eidi et al. 2006). Standardized extract of *S. officinalis* on acute cognitive performance in older adults has significantly improved secondary memory performance, which is consistent with in vitro acetylcholinesterase

inhibitory activity (Scholey et al. 2008). Ethanolic extract of dried sage leaf has shown anxiolytic activity in lower doses and increased alertness at higher doses on a psychological stress battery in healthy young volunteers (Kennedy et al. 2005).

Syzygium aromaticum L.

Syzygium aromaticum (Myrtaceae), cloves are the aromatic flower buds of *S. aromaticum*. Cloves are ingredients of not only Ayurveda but also Chinese medicine and western herbalism. Its essential oil is used in dentistry, as neuronal stimulant and cognition enhancers in Ayurveda and Iranian traditional medicine. Eugenol is the major constituent of the clove essential oil, it has shown to possess better cholinesterase inhibitory activity than clove extract and its oil has a mixed type of inhibition of AChE and noncompetitive inhibition for BChE. Eugenol also ameliorated the memory impairment and improved learning in mouse models, improved neuronal and vascular functions in the diabetic mouse brain. Eugenol showed neuroprotective activity against Aβ (1–40) in PC-12 cells in a dose-dependent manner, possibly by its calcium channel blocking activity (Irie 2006).

Trigonella foenum graecum L.

Trigonella foenum graecum (Leguminacae), commonly called fenugreek, is an annual herb extensively used as a spice in India and the Mediterranean region and is known to possess a number of medicinal properties. The dried seeds of fenugreek are used as a spice while the leaves are used as a vegetable in the Indian culinary arts. Fenugreek seeds are rich source of protein, fiber, and omega 3 fatty acids while the leaves are sources of β-carotene, iron, calcium, magnesium, potassium, and vitamin C. Therapeutically active constituents in fenugreek are majorly diosgenin and 4-hydroxyisoleucine. Steroidal sapogenins and mucilaginous fibers present in the seed and leaves of this legume plant contribute to antidiabetic, hypocholesterolemic activities which are demonstrated in animal studies and several human trials (Srinivasan 2006). Total alkaloidal fraction and ethyl acetate fraction of the alcohol extract showed potent acetyl cholinesterase inhibitory activity (Satheeshkumar et al. 2010).

Zingiber officinale R.

Zingiber officinale (Zingiberaceae), Ginger rhizome has been used for centuries to treat dementia in South Asia. It is a well-known ayurvedic rasayana (rejuvenator) drug, extracts significantly reversed the amnesia induced by diazepam and

scopolamine, also reversed aging induced amnesia due to natural aging of mice and increased brain acetyl cholinesterase inhibition activity (Hanumanthachar and Parle 2006a). In another study, a hydromethanolic extract of dried ginger showed a unique combination of muscarinic, Ca^{2+} channel antagonist, and BuChE inhibitory activities, which may be beneficial in exitotoxic conditions such as AD. 6-Gingerol also showed a specific anti-BuChE effect (Ghayur et al. 2008). It protects PC-12 neuronal cells from direct Aβ (1–42) insult and may be potentially important resources to discover drug candidates against the onset of AD (Kim Darrick et al. 2007). The plant also possess good antioxidant properties, thus can be used in AD (Topic et al. 2002). Ginger extract (GE) prevents the activation of THP-1 cells by lipopolysaccharide, proinflammatory cytokines, and fibrillar amyloid peptide Aβ (1–42), a major component of neuritic plaques, a neuropathological hallmark of AD. This may be useful in delaying the onset and the progression of neurodegenerative disorders involving chronically activated microglial cells in the central nervous system (Grzanna et al. 2004). Hexane fraction of ginger inhibits proinflammatory mediators TNF-α, IL-1β, NO, and PGE_2 through suppression of MAPKinase NF-κB pathway from activated microglia and prevents neurodegenerative diseases (Jung et al. 2009).

Toxicity of Indian Spices

Most of the studies have reported low level or lack of toxicity of most spices, but there are reports of toxicity with spices at higher doses like curcumin showed hepatotoxicity, nuclear, genome, and mitochondrial damage in human hepatoma G2 cells and carcinogenicity in rodents (Burgos-Moron et al. 2010). Piperine at 10 and 20 mg/kg showed anti-implantation activity and reduced mating performance and fertility (Daware et al. 2000). Significant reduction in hemoglobin content, increase in sperm count, and motility was reported with *Cinnamon zeylanicum* and *Piper longum* (Abdulkarim 2010). Nutmeg is well-known psychotomimetic substance substitute does not produce neurotoxicity at low doses, but produces hallucinations, convulsions, and palpitations at higher doses. It also has anticholinergic like effects, which are attributed to its major constituent Myristicin which is also a mono amine oxidase inhibitor. There are even fatal myristicin poisoning cases which have been reported (Demetriades et al. 2005). Ginger at higher doses may cause heart burns, acts as gastric irritant and also showed increases in early embryonic loss (Ali et al. 2008). Capsicum is considered as safe as food by USFDA, but toxicity reported with its major constituent Capsaicin, which inhibited the growth of multipotent neural progenitor cells in a dose-dependent manner and was cytotoxic at higher doses but did not alter learning and memory in mice (Kong et al. 2010). Anaphylaxis was observed in celery sensitive patients after consuming coriander and aniseed, which developed into angioedema, urticaria, bronchospasm, and rhinoconjunctivitis with the development of IgE antibodies (Stager et al. 1991). Toxicity studies reported cerebellar lesions in rats treated with peppermint oil, pulegone, and menthol at

higher doses. Pulegone is hepatotoxic compound and its concentration should not exceed 1 % in all mentha preparations for pharmaceutical or cosmetic usage (Nair 2000). Estragole one of the constituent in various spices *Pimpinella anisum* (anise oil), *Piper betel* (betel nut), *Foeniculum vulgare* (fennel), *Hyssopus officinalis* (hyssop), *Illicium verum* (Chinese star anise), *Illicium anisatum* (Japanese star anise) metabolically produces 1-hydroxyestragole, and 1-sulphoxyestragole which binds to DNA and are highly carcinogenic especially for liver (Bristol 2011).

Modern Medicines from Spices and Scope of Lead Finding in AD

Indian spices which were a major part of Ayurveda, the holistic Indian medical system has attracted the attention of many researchers and pharmaceutical industries. Their researches lead to identification, separation, and characterization of specific constituents which are responsible for their pharmacological efficacy, the major constituents with crucial pharmacological profile is depicted in Fig. 2. Their mechanism of action established and the pathways through which they act were elucidated. Many clinical trials were conducted to extrapolate their in vivo efficacy to human therapeutic usage, but with inconclusive/ambiguous outcomes. Curcumin the most studied spice constituent underwent several clinical trials for ailments ranging from Irritable bowel syndrome to cancer. It showed decrease in macrophage uptake in Aβ cells and was safe and increased vitamin E concentrations in Alzheimer's patients. It also exhibited efficacy and safety in Dejerine–Sottas disease, currently there are 4–5 trials running for its activity in AD. Piperine another spices constituent is currently used as an excipient to increase the bioavailability of drugs. Clinical trials conducted on the Indian spices for various ailments are depicted in Table 2 and the evolution of spices from culinary ingredients to drug targets is depicted in Fig. 3.

Conclusion

Most of the Indian spices can combat oxidative stress in various brain regions. Although there are vast scientific data supporting spices as a cure for AD, there are very few positive clinical outcomes. The major reason behind their failure is their sparingly soluble nature and lower bioavailability; further more compounds should cross the blood–brain barrier to elicit pharmacological activity. It is because of their lower bioavailability the dose that needs to be taken is high to elicit therapeutically significant action; although they are safer at higher doses with minimal toxicity they are not palatable. Currently there is extensive research going on to improve their bioavailability through pharmaceutical manipulations like nanoformulation

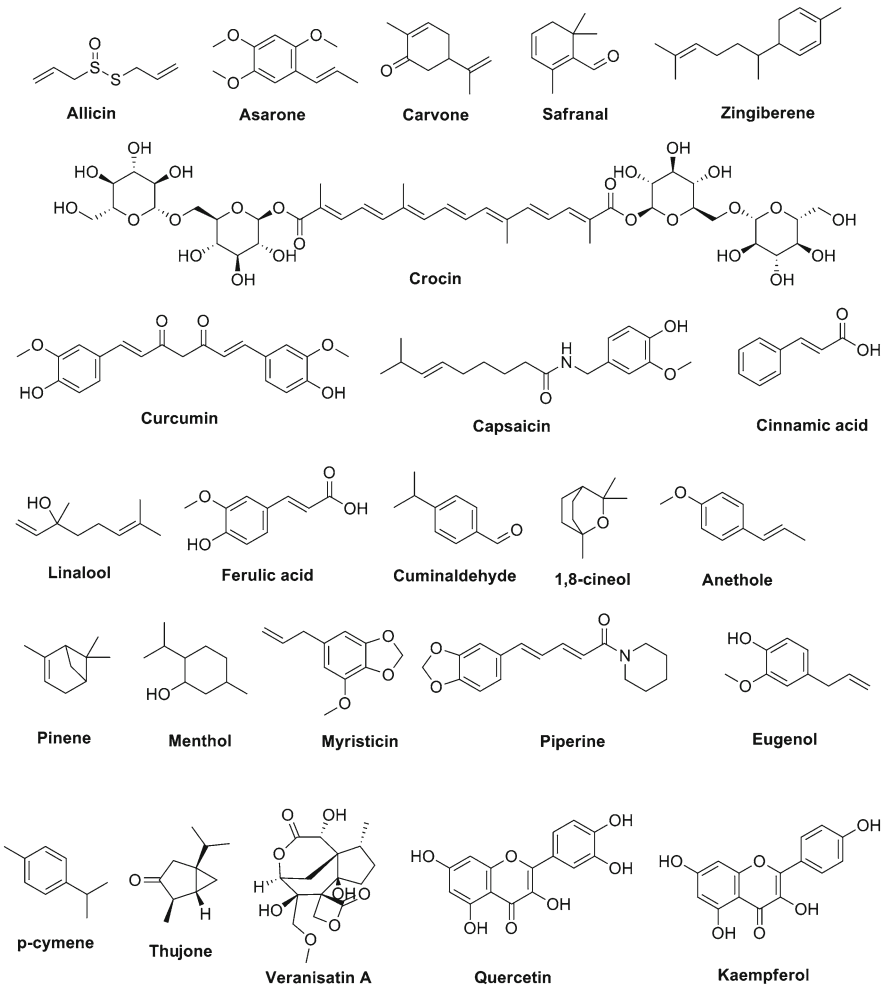

Fig. 2 Major phytoconstituents of Indian spices

preparations of spice ingredients, formulating them along with bioavailability enhancers. There is still need for extensive clinical trials on spices to evaluate their ingredients as disease curatives, as adjuncts to drug therapy, which can reduce the burden of adverse effects due to drugs and as a preventive measure to disease. Tremendous effort was put into the research of CNS drug discovery with meager outcomes; spices which are used as adjuvant in culinary purposes have therapeutic significance with minimal adverse effects in CNS disorders. Many of spices mechanisms need to be established and further scientific developments have to be done in their physiochemical perspective to make them successful clinical outcomes from mere lead compounds.

Table 2 Clinical trials conducted and their outcomes of Indian spices

S. no.	Plant/phyto-constituent name	Clinical trial conducted	Outcome
1	Garlic	Hypocholesterolemic, antiatherogenic, and antihypertensive activity	Most of the studies showed reduction in cholesterol, platelet aggregation, and mixed results obtained in oxidative stress and blood pressure reduction (Mikaili et al. 2013)
2	*Acorus calamus*	Antidepressant activity	Reduction in degree of severity of depression (Hashmat et al. 2013)
		Anti-ischemic activity	Improvement of chest pain, ECG, and decrease in serum cholesterol, LDL, and increasing HDL (Hashmat et al. 2013)
3	*Carum carvi*	Herbal bioenhancer	Improved pharmacokinetic profile of antitubercular drugs rifampicin, isoniazid, and pyrazinamide (Choudhary et al. 2014)
		Anti-obesity activity	Significant reduction in body weight, BMI, body fat percentage, and body size (Kazemipoor et al. 2013)
4	*Crocus sativus*	Antidepressant activity	Improved symptoms of major depressive disorder (Hausenblas et al. 2013)
5	Curcumin	Anticancer activity	Showed efficacy against colorectal, pancreatic, breast, prostate, lung, head and neck cancers, and multiple myeloma (Gupta et al. 2013)
		Anti-inflammatory activity	Improved symptoms in Crohn disease, ulcerative proctitis, ulcerative colitis, inflammatory bowel disease, irritable bowl syndrome, rheumatoid arthritis, osteoarthritis, chronic anterior uvetis, recurrent anterior uvetis, and postoperative inflammation (Gupta et al. 2013)
		Antiulcer activity	Reduced formation of peptic, gastric ulcers, and *Helecobacter pylori* infection (Gupta et al. 2013)
	Curcumin	Antipsoriatic activity	Well-tolerated antipsoriatic activity but with low response rate (Gupta et al. 2013)
		Cardio protective activity	Reduced serum lipid peroxides, total serum cholesterol and increased HDL cholesterol, and triglycerides (Gupta et al. 2013)
		Antidiabetic activity	Reduced fasting blood glucose levels, improved endothelial functioning, and improved symptoms of diabetic nephropathy and microangiopathy (Gupta et al. 2013)
		Dejerine–Sottas disease	Showed efficacy (Gupta et al. 2013)
		Recurrent respiratory tract infections	Immunomodulatory effect with reduced infection (Gupta et al. 2013)
6	Capsaicin	Neuropathic pain	Significant pain reduction (Anand and Bley 2011)
7	Cinnamon	Antidiabetic activity	Most of the studies showed reduction in fasting blood glucose and variability found in their effect on serum cholesterol (Rafehi et al. 2012)
8	*Salvia officinalis*	AD	Significantly improved cognitive function in mild to moderate AD (Akhondzadeh et al. 2003)

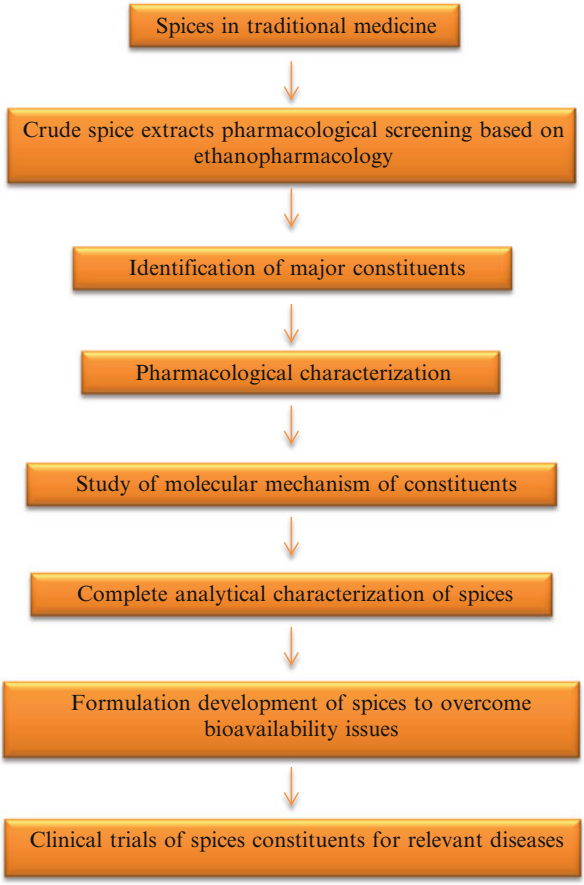

Fig. 3 Evolution of spices from culinary ingredients to drug targets

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Application of Bioactive Compounds from *Scutellaria* in Neurologic Disorders

Farhan Hussain, Sandeep Mittal, Nirmal Joshee, and Prahlad Parajuli

Abstract Inflammation of the brain is one of the most highly researched yet mysterious areas in modern day neurology. The process of inflammation is a normal mechanism of wound healing that can result from acute injuries such as traumas or can be caused by genetic/environmental factors. After the initial insult, the immune system defenses, specifically microglial cells, are activated in order to combat the infection or injury. However, prolonged or chronic inflammation is often deleterious due mainly to accumulation of free reactive oxygen species (ROS) and other pro-inflammatory cytokines in the brain FADDIN EN.CITE. Plant-derived natural compounds have the potential to ameliorate the causes and symptoms of neuroinflammation, due to their various anti-oxidant and anti-inflammatory activities, without completely muting the immune defenses. *Scutellaria* is a perennial plant in the mint family that has been used to treat diseases in Asia and Eastern Europe throughout history. This chapter reviews the active components of various *Scutellaria* species and their mechanisms of action to prevent chronic neurologic disorders involving neuroinflammation and neurodegeneration.

Keywords *Scutellaria* • Flavonoids • Neurodegeneration • Neuroinflammation • Neuroprotection

F. Hussain • P. Parajuli, Ph.D. (✉)
Department of Neurosurgery, Wayne State University School of Medicine, and Karmanos Cancer Institute, Lande Research Building, #460, 540 E. Canfield St., Detroit, MI 48201, USA
e-mail: pparajuli@med.wayne.edu

S. Mittal
Departments of Neurosurgery and Oncology, Wayne State University School of Medicine, and Karmanos Cancer Institute, 930 Harper POB, 4100 John R. St, Detroit, MI 48201, USA

N. Joshee
Graduate Program in Biotechnology, Agricultural Research Station, Fort Valley State University, Fort Valley, GA, USA

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Introduction

Inflammation of the brain is one of the most highly researched yet mysterious areas in modern day neurology. The process of inflammation is a normal mechanism that can result from acute injuries such as traumas or can be caused by genetic/environmental factors (Blasko et al. 2004; Lopez-Lluch et al. 2015). These pathologic states cause an increase in oxidative stress by encouraging production of free reactive oxygen species (ROS) and other proinflammatory factors in the immediate vicinity (Blasko et al. 2004; Mittal et al. 2014). After the initial insult, the immune system defenses, specifically microglial cells, are activated in order to combat the infection or injury (Blasko et al. 2004; Hsieh and Yang 2013). Microglia are the resident immune cells located in the central nervous system (CNS) and are the first responders to damaging stimuli. They produce inflammatory cytokines such as tumor necrosis factor (TNF) or interleukins that feed into a loop that promotes even more production of these compounds that can spread to other regions (Mittal et al. 2014; Hsieh and Yang 2013). As the tissue becomes inflamed due to immune cell invasion, the resulting chronic inflammation can lead to neuronal cell death and neurodegenerative diseases. For example, long-term neural inflammation has been linked to a higher incidence of Alzheimer's disease as well as Parkinson's disease (Blasko et al. 2004; Mittal et al. 2014). As dangerous as chronic inflammation has proven to be, experimental studies suggest that short-term inflammation can also be quite damaging. Investigations involving rats that were subjected to pro-inflammatory factors exhibited decreased cognitive abilities, suggesting a link to clinical depression (Elmore et al. 2014; Yang et al. 2014). When it comes to treating the causes and symptoms of neuroinflammation, the problem lies in the ability of the therapy to effectively cross the blood–brain barrier (BBB) and to not completely mute immune defenses. In this chapter, we will discuss natural compounds that can accomplish these goals while also preventing the deleterious effects of inflammation. Specifically, the focus will be on *Scutellaria*, a perennial plant in the mint family that has been used to treat diseases in Asia and Eastern Europe throughout history (Hsieh and Yang 2013; Yang et al. 2014). For centuries, these regions have used *Scutellaria* extracts to treat diseases such as hepatitis and rabies with beneficial results (Zhou et al. 2014; Joshee et al. 2013). These beneficial activities have been attributed to a group of phenolic compounds called flavonoids present in the plant, which exhibit antioxidant and anti-inflammatory properties. We will review the active components of various *Scutellaria* species and their mechanisms of action to prevent chronic neurologic disorders involving neuroinflammation and neurodegeneration (Fig. 1).

Fig. 1 *Scutellaria lateriflora*, known commonly as American skullcap, is a hardy perennial herb of the mint (Lamiaceae) family native to North America. Photographed by NJ in the greenhouse at FVSU

Mechanism of Oxidative Stress-Induced Inflammation

Oxidative stress is a term used to describe the negative inflammatory effects of excess ROS on tissues including those of the brain. Inflammation is a normal defense mechanism of the body used against pathogens and microbes. However, chronic inflammation caused by ROS buildup leads to deleterious effects and even cell death (Lopez-Lluch et al. 2015; Mittal et al. 2014; Hayashi and Cortopassi 2015). Increased levels of ROS have been associated with many neurodegenerative diseases such as Parkinson's and Alzheimer's as well as acute brain injuries (Hayashi and Cortopassi 2015; Popa-Wagner et al. 2013). In normal circumstances, ROS such as superoxide are produced by mitochondrial oxidative metabolism or nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and exhibit a regulatory role. Superoxide dismutase (SOD) located within the mitochondria rapidly reacts with the superoxide to produce hydrogen peroxide (H_2O_2). This H_2O_2 has been demonstrated to play a crucial role in cell signaling for growth and proliferation (Lopez-Lluch et al. 2015; Fischer and Maier 2015). However, H_2O_2 can also react with ferrous iron to form the hydroxyl radical, which is a more toxic and aggressive ROS (Mittal et al. 2014; Starkov 2010). In addition, reactive nitrogen species (RNS) can be formed by the interaction of nitric oxide (NO) produced by nitric oxide synthase (NOS) and superoxide (O_2^-) from the electron transport chain. ROS and RNS contribute to inflammation due to their high reactivity and redox potentials. As a result, these species can damage cells by causing the oxidation of DNA, protein, and lipids. Specifically, the mitochondrial DNA is most susceptible to mutation causing mitochondrial

dysfunction and enhanced ROS production (Mittal et al. 2014; Fischer and Maier 2015). In addition, ROS species also induce the release of pro-inflammatory cytokines from immune cells, resulting in an exacerbation of the already active inflammation (Mittal et al. 2014; Hsieh and Yang 2013; Hayashi and Cortopassi 2015). As stated above, ROS can play a regulatory role in a healthy system, however, once a pathogenic state is induced, these species can accumulate and cause many harmful changes.

Inflammation and Neurodegeneration

The brain is especially susceptible to the negative effects of oxidative stress because of its high energy needs and heavy oxygen consumption, while having minimal protection due to low levels of antioxidants (Popa-Wagner et al. 2013). Injured neuronal cells are also not easily rescued often leading to permanent cell loss (Keddy et al. 2012). ROS are produced more rapidly in the CNS and are transformed into toxic products faster because of the abundance of ferrous iron in CNS mitochondria (Hayashi and Cortopassi 2015; Popa-Wagner et al. 2013). As a result, the mitochondria rapidly lose function and are not able to sustain neuronal energy needs. Damaged mitochondria begin to release cytochrome *C* intracellularly, initiating the caspase pathway. This signals the cell for apoptosis thereby leading to eventual cell death (Hsieh and Yang 2013). In addition, the abundance of ROS and RNS leads to the activation of astrocytes and microglial cells. These glial cells release multiple pro-inflammatory cytokines such as TNF, NO, histamines, thrombins, and other cytotoxic compounds. Accumulation of these products leads to chronic inflammation and neuronal degradation (Hsieh and Yang 2013; Herrera et al. 2015) (Fig. 2).

Microglial activation is a complicated process that occurs after these immune cells interact with the previously mentioned pro-inflammatory substances. Once activated, microglia begin to proliferate, release more inflammatory compounds, and migrate toward the site of insult (Hsieh and Yang 2013; Herrera et al. 2015; Gehrmann et al. 1995). Initially, the first responders are activated into nonphagocytic microglia. If the injury persists and causes neuronal death, the previously activated cells can transform into their phagocytic counterparts (Gehrmann et al. 1995; Bradley 2008). Microglia are also capable of producing a significant amount of glutamate and aspartate when activated, leading to increased cytotoxicity (Gehrmann et al. 1995; Keilhoff et al. 2015). This function along with their phagocytic abilities and the production of pro-inflammatory cytokines cause microglia to produce chronic inflammation and cell death.

The most important and well studied of these pro-inflammatory cytokines is TNF-α. This is a pleiotropic substance that regulates multiple functions such as the activation of microglial cells, firing of glutamatergic neurons, and immune responses (Bradley 2008; Qin et al. 2015). Interestingly, TNF-α has both proliferative and apoptotic effects on cells depending on the receptor that it binds to (Bradley 2008;

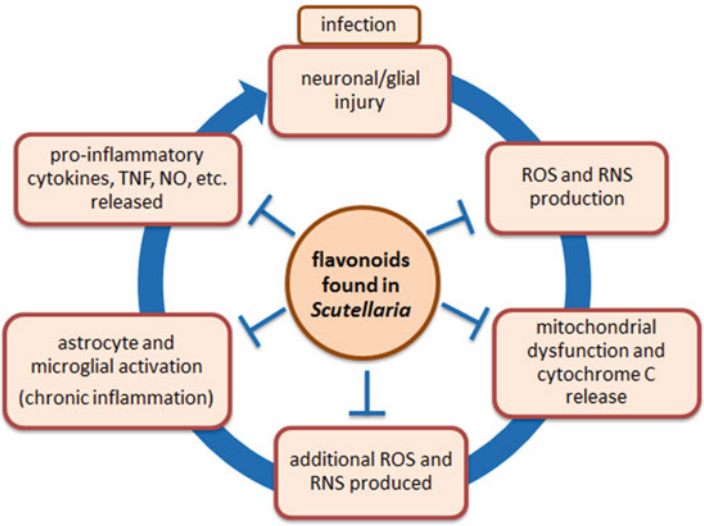

Fig. 2 Schematic representation of the vicious cycle of chronic inflammation. Pathologic or physiologic insult of the neurons or glial cells result in initial inflammatory signals inviting microglia to the site of injury in the normal wound healing process. Prolonged injury or infection, however, can lead to the release of ROS, RNS, and TNF-α, which leads to further activation of microglia via NF-κB activation and tissue damage due to mitochondrial dysfunction and caspase-mediated apoptosis. *Scutellaria* flavonoids have been shown to inhibit the process at various stages, as described in the text

Belarbi et al. 2012). As a result, a balanced level of this cytokine is produced within the body to maintain homeostasis. The more well-known, negative effects occur when TNF-α is produced in excessive amounts during pathological states. In fact, the TNF-α concentration correlates directly with the severity of disease (Bradley 2008; Belarbi et al. 2012). The mechanisms by which TNF-α promotes inflammation are not completely understood; but the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway, which will be discussed later in the chapter, is one of the best documented.

Lipopolysaccharides (LPS) also play a prominent role in causing of chronic inflammation in the brain. LPS is a class of endotoxin produced by gram-negative bacteria created by the bonding of a lipid and a polysaccharide (Qin et al. 2007; Tang et al. 2015). High levels of LPS can lead to microglial activation and TNF-α production, thereby producing an inflammatory response (Qin et al. 2007). Even if the LPS are localized outside of the brain, a small amount may cross the BBB. Due to the feedback nature of neuronal inflammation, the local activation of microglia may lead to the paracrine activation of other microglial cells (Qin et al. 2007; Tang et al. 2015). As a result, even a small neuronal insult due to LPS may yield a severe inflammatory response.

Traditional Medicines for Inflammation

One of the methods used to fight against the accumulation of ROS is to treat patients with phytochemicals that are naturally found in plants, herbs, etc. (Keddy et al. 2012; Hugel 2015). This broad category of natural chemicals contains many compounds that have antioxidant and anti-inflammatory properties. An example of this is the phenolics founds in the oil palm extract. The phenolic antioxidants found in this plant act as neuroprotective agents due to their high redox potential, allowing them to act as reducing agents and ROS scavengers (Keddy et al. 2012; Hugel 2015). The class of phytochemicals that is the focus of this chapter consists of a large group of polyphenols called flavonoids. These compounds are naturally found in all plants and are known to be produced under stressful living conditions or microbial infection (Keddy et al. 2012; Hugel 2015). Almost every group of flavonoid exhibits antioxidant activity due to the ROS scavenging function of their active hydroxyl groups. Additionally, many flavonoids exhibit anti-inflammatory activity. This is achieved by the competitive inhibition of enzymes, which are involved in the production of pro-inflammatory compounds such as NO, leukotrienes, and cytokines (Hugel 2015; Kumar and Pandey 2013).

Background of Scutellaria

Flavonoids as a whole can be separated into smaller subcategories by their configurations, different functional groups, and the number of hydroxyl groups (Kumar and Pandey 2013; Shi et al. 2014). We will specifically examine the flavonoids found in the *Scutellaria* plant and their neuroprotective effects. *Scutellaria* is a perennial herb in the Lamiaceae (formerly known as Labiatae) family that has been used for medicinal purposes across Asia and Eastern Europe for centuries (Yang et al. 2014; Shi et al. 2014; Guo et al. 2011). There are over 350 different species under this genus each carrying a unique assortment of active components (Nikbin et al. 2014; Paton 1990). Traditionally, *Scutellaria* has been used to treat inflammatory illnesses ranging from hepatitis to diarrhea (Shi et al. 2014; Guo et al. 2011). In more recent studies, *S. lateriflora*, also known as American scullcap (Fig. 1) was found to be an effective treatment for anxiety disorders (Guo et al. 2011; Brock et al. 2014). This plant was traditionally used by Native Americans to support a woman during menstruation and also used as an aid in the removal of the placenta after childbirth due to its sedative properties (Kumar and Pandey 2013; Brock et al. 2012). Although *S. lateriflora* was disregarded for some time due to its perceived ineffectiveness, a double-blind study conducted by Brock et al. proved that administration of the plant extract lowered general anxiety levels (Brock et al. 2014). This study is particularly intriguing because patients who experienced reduced anxiety after taking the extract did not experience the fatigue or loss of cognition associated with other antianxiety medications (Brock et al. 2012, 2014). However, there is a large variation in the effects seen across the different species of *Scutellaria* and in the individual species

themselves. For example, the flavonoid composition in the root may be very different from that found in the leaves or stem of the plant. Moreover, the antioxidant capacity of various *Scutellaria* species has been seen to vary dramatically between the fresh and air dried tissues (Vaidya et al. 2014).

The most important aspect to determine the effectiveness of the medicinal use of *Scutellaria* is the environment that the plant was grown in. There has been evidence showing that flavonoids are produced in larger quantities during times environmentally stressful rather than stable growing periods (Guo et al. 2011; Brock et al. 2014). Differing variables such as soil composition, humidity, temperature, and sunlight exposure can all have an impact on what specific flavonoids are produced (Guo et al. 2011; Brock et al. 2014). To offset these variations, in vitro transformed hairy root culture technique has proven beneficial, as bioactive metabolite production can be achieved in controlled conditions (Marsh et al. 2014).

The bioactive compounds are usually extracted from either the leaf or the root of the plant and studied for their medicinal properties. Some of the most common neuroprotective flavonoids found in the plant include wogonin, wogonoside, baicalin, baicalein, scutellarin, and oroxylin (Yang et al. 2014). Each of these has a glycosylated and an aglycosylated form. It has been reported that the latter is a more active antioxidant and has a greater anti-inflammatory effect. As a result, research on various flavonoids of *Scutellaria* centers more on the aglycosylated form because of its added therapeutic effects (Brock et al. 2012; Li et al. 2012).

When *Scutellaria* is administered orally, there are two main mechanisms through which the flavonoids are absorbed. First, some active components can enter the circulatory system in the upper small intestine through active transport (Patel et al. 2013; Zhang et al. 2015). However, the majority of absorption occurs through a process requiring the bacterial flora in the lower small intestine. These flora use their beta-glucuronide activity of the glycosylated flavonoids, making the resultant aglycosylated compounds readily available for absorption (Patel et al. 2013). For example, when baicalin enters into the intestine, it is metabolized to oroxylin A and baicalein (the aglycosylated form of baicalin) (Patel et al. 2013; Yu et al. 2013). These flavonoids have specific individual effects but can also interact with each other when taken together to produce new responses.

Therapeutic Benefits of Crude Scutellaria *Extracts*

Some benefits of *Scutellaria* are seen most prominently when using the crude extract containing all flavonoids. For example, the combined antioxidative effects of the plant have shown to improve learning and decrease memory impairment (Wang et al. 2013; Zhang et al. 2013). This applies specifically to Alzheimer's disease, which is characterized by impairments of the previously mentioned points. Alzheimer's disease is caused by accumulation of β-amyloid protein (Wang et al. 2013; Zhang et al. 2013; Heo et al. 2004). Intracellular buildup of this protein leads to cell apoptosis through generations of endoplasmic reticulum stress and oxidative stress. The cell death process is accomplished through several proteins that regulate

apoptosis and hippocampal neuronal loss. Several well-characterized genes such as caspase-3, Bax, and cytochrome C have shown to be pro-apoptotic when transcribed in large quantities (Wang et al. 2013; Heo et al. 2004). Specifically, activation of caspase-3 can lead to cytochrome C release from mitochondria which triggers apoptosis. On the other hand, the *BCL2* gene is an antiapoptotic gene preventing cell death (Wang et al. 2013; Zhang et al. 2013). *BAX* and *BCL2* are related genes that act antagonistically to each other. *BAX* increases the permeability of the outer mitochondrial membrane allowing cytochrome C to escape while the *BCL2* gene has the opposite effect (Wang et al. 2013; Heo et al. 2004). β-Amyloid protein has a function similar to the BAX protein (also known as bcl-2-like protein 4) in that it also increases permeability of the outer mitochondrial membrane. Released cytochrome *C* activates pathways that cause the release of calcium intracellularly (Wang et al. 2013; Kana et al. 2015). This increased calcium concentration, in turn stimulates the release of cytochrome *C* further promoting apoptosis. The crude extract of the *Scutellaria* plant has shown the ability to downregulate caspase-3 expression, reduce the calcium release, and has an antioxidant effect that inhibits pro-apoptotic pathways (Wang et al. 2013; Kana et al. 2015). Additionally, treatment with plant extract also caused the inhibition of β-secretase, which is a key enzyme in the production of β-amyloid (Wang et al. 2013). As a result, the combination of all active flavonoids within a crude extract of *Scutellaria* can have significant antioxidant activity that can promote neuronal health and prevent degeneration.

Wogonin

Wogonin, 5,7-dihydroxy-8-methoxyflavone, is one of the most studied flavonoids in *Scutellaria* due to its strong anti-inflammatory/antioxidant effects. It is the aglycosylated form of wogonoside and like other flavonoids, wogonin exhibits ROS scavenging activity in preventing the accumulation of toxic oxygen or nitrogen radicals (Yu et al. 2013; Wang et al. 2015). The mechanism by which wogonin achieves its anti-inflammatory function is by competitively inhibiting the enzymes used in the production of TNF-α, cytokines, IL-1β, and NOS (Wang et al. 2015; Lee et al. 2003). Studies have shown that wogonin directly contributes to lower levels of NO and inflammatory cytokines (Yu et al. 2013; Lee and Park 2015). Lower activity of these compounds prevents the inflammatory activation of microglial cells, thereby preventing chronic inflammation.

Moreover, the wogonin has been shown to inhibit the action of NF-κB pathway in microglia. NF-κB is a well-documented transcription factor that becomes activated by ROS accumulation and serves to worsen inflammation by promoting the release of pro-inflammatory factors such as TNF. Originally, NF-κB is located in the cytoplasm and is kept inactive by IκB (Zhou et al. 2014; Sun et al. 2015). When a cell is exposed to negative external stimuli such as ROS or LPS, the IκB is cleaved allowing the NF-κB to enter the nucleus. Here, it upregulates TNF gene expression and promotes inflammation (Zhou et al. 2014; Lee et al. 2003; Sun et al. 2015). Increased levels of TNF feedback and further activate NF-κB. Wogonin is able to inhibit this

pathway through its antioxidant activity because the pathway requires ROS to become active (Lee et al. 2003; Sun et al. 2015). Wogonin has been shown to enhance the recovery of neurologic function via modulation of toll-like receptor 4 (TLR4)-mediated NF-κB activity, as early as 24 h after treatment and up to several days post-treatment in a mouse model of traumatic brain injury (TBI) (Chen et al. 2012). The TLRs are expressed on innate immune cells, including microglia and astrocytes, and cause their activation upon binding with pathogen-associated molecular patterns (PAMPs), such as LPS as well as cellular damage-associated molecular patterns (DAMPs), such as heat shock proteins (HSP) and hypoxia-inducible factors (HIF).

Finally, wogonin has been shown to inhibit the release of intracellular calcium from the endoplasmic reticulum via the calcium-signal transducer and activator of transcription (STAT) pathway (Lee and Park 2015). This inhibition limits the activity of calcium-dependent kinases that activate multiple pro-inflammatory pathways (Lee et al. 2003; Lee and Park 2015). The importance of the anti-inflammatory/antioxidant effects of wogonin have been proven through multiple studies and may contribute to future medicines and therapies.

Baicalin

Baicalin and its aglycone baicalein are both found in high quantities in the *Scutellaria baicalensis* species. The people of East Asia have historically used this plant to treat inflammatory diseases, hypercholesterolemia, and hypertension (Lee et al. 2014; Chen et al. 2015). Additionally, *S. baicalensis* has been used to treat bacterial as well as viral infections (Lee et al. 2014; Chen et al. 2015). Baicalin has been shown to inhibit macrophage activation and reduces the amount of inflammatory cytokines by inhibiting the activation of T-helper 17 (Th17) and T-helper 1 cells. Th17 cell release the cytokine IL-17 which serves to further progress the inflammatory state through the following methods (Yang et al. 2013; Zou et al. 2014). IL-17 promotes leukocyte infiltration into damaged areas, stimulates tissue death, and creates a feedback loop for more inflammatory cytokines to be produced (Yang et al. 2013). Because of the inhibition of Th17 by baicalin, the inflammatory feedback loop cannot be completed and lower levels of IL-17 are produced. Another therapeutic effect of baicalin treatment is the reduction of matrix metalloproteinases (MMPs). These compounds have the ability to increase the permeability of the BBB by destroying the microvascular extracellular matrix (Zhou et al. 2014; Zou et al. 2014). Increased levels of MMPs have been associated with brain edema and neurodegeneration. Baicalin was shown to decrease both the expression of the MMP protein as well as the MMP mRNA (Zhou et al. 2014; Yang et al. 2013). In addition, like wogonin, baicalin exhibits antioxidant activity as well as inhibition of the NF-κB pathway. Baicalin has also been shown to protect microglia from activation-induced cell death by attenuating NO production via inhibition of NF-κB signaling (Suk et al. 2003).

Baicalin exhibits further anti-inflammatory effect by the downregulation of the toll-like receptor 2 (TLR2) signaling. The TLR2 signaling can be turned on by DAMPs released upon oxidative stress or glucose depletion (Li et al. 2012; Luo

et al. 2012). Activation of this pathway causes microglial activation, increased production of TNF, and promotion of pro-inflammatory factors. Baicalin inhibits the expression of TLR2 thereby reducing the inflammatory reaction to pathological or physiological events (Li et al. 2012; Luo et al. 2012).

Scutellarin

TLR4 is a receptor associated with the low-grade inflammation in the brain caused by hypertension (Eissler et al. 2011). Like TLR2, TLR4 causes microglial activation and produces inflammation in the presence of high blood pressure (Luo et al. 2012; Chen et al. 2013). This molecule functions by activating the NF-κB pro-inflammatory signaling pathway and also by inducing vascular constriction thereby exacerbating the hypertensive event (Eissler et al. 2011; Chen et al. 2013). Scutellarin, a flavonoid found in *Scutellaria*, has been shown to inhibit the transcription of TLR4 and lower blood pressure (Luo et al. 2012; Eissler et al. 2011; Chen et al. 2013). The suppression of this gene prevents the activation of microglial cells, resulting in decreased inflammation. Therefore, scutellarin is especially important when it comes to attenuating the long-term damage associated with strokes and hemorrhages while also harboring the potential to treat hypertension (Luo et al. 2012; Eissler et al. 2011; Chen et al. 2013).

Oroxylin A

Yet another flavonoid found in the *Scutellaria* species has been oroxylin A. Similar to the previously mentioned components of *Scutellaria*, this compound also exhibits antioxidant and anti-inflammatory characteristics. Oroxylin A is specifically known to target LPS-induced inflammation (Liu et al. 2012; Huang et al. 2015). LPS has the ability to activate microglia within the brain resulting in release of pro-inflammatory cytokines (IL-1β and IL-6) and NO (Liu et al. 2012). Unlike the other flavonoids mentioned, oroxylin A does not mainly function through the inhibition of the NF-κB transcription factor. Rather, its anti-inflammatory effects are more heavily produced through the inhibition of the Janus Kinase (JAK)/STAT pathway (Liu et al. 2012; Huang et al. 2015; Rawlings et al. 2004). This heavily researched pathway is important in cell signaling involved with the homeostasis of mammals. Initially, various molecules such as interferons, interleukins, or growth factors activate this signaling system by binding to JAK (Huang et al. 2015; Rawlings et al. 2004). Once activated, the JAK auto-phosphorylates causing the STAT protein to bind to it. After one STAT1 protein has bound to JAK, another STAT1 protein binds forming a dimer that is translocated to the nucleus. Here, the JAK/STAT upregulates various genes involved with inflammation and apoptosis (Rawlings et al. 2004). Oroxylin A functions to prevent the release of IL-1β and IL-6 by inhibiting the phosphorylation of STAT1. As a result, the JAK/STAT1 pathway cannot complete its translocation process and upregulation of pro-inflammatory genes are not accomplished (Liu et al. 2012; Rawlings et al. 2004).

Oroxylin has also been shown to increase memory retention in mouse models via enhanced production of brain-derived neurotrophic factor (BDNF) in the hippocampus (Kim et al. 2014). The neuroprotective mechanism used by oroxylin A involves adenosine A2 receptor stimulation (Jeon et al. 2012) and blocking of γ-aminobutyric acid (GABA) receptor (Huen et al. 2003). Oroxylin A and its synthetic analog (5,7-dihydroxy-6-methoxy-4′-phenoxyflavone) showed remarkably improved attention-deficit/hyperactivity disorder (ADHD)-like behaviors in spontaneously hypertensive rats via inhibition of dopamine uptake (dela Pena et al. 2013; Yoon et al. 2013).

Neuroprotection (Cell Death Prevention) Versus Anticancer (Cell Death Induction) Activities of *Scutellaria* Extracts

Several groups, including ours, have extensively reported on the anticancer activities of crude *Scutellaria* extracts and isolated phytochemicals in several cancer models in vitro as well as in vivo (Dandawate et al. 2012; Parajuli et al. 2009, 2011) (reviewed in Patel et al. 2013). As discussed above in relation to neuroprotective activity, the anticancer activity of *Scutellaria* is also often associated with inhibition of NF-κB signaling. However, the outcome of NF-κB inhibition in these two systems can be completely different depending on the context. While in cancer cells, activation of NF-κB promotes cell survival and inhibition thereof would induce apoptosis. On the other hand, in microglia and astrocytes, activation of NF-κB leads to enhanced production of ROS and RNS, leading to apoptosis; inhibition of NF-κB in this scenario results in the protection of these immune cells from apoptosis (Fig. 3). Moreover, induction of apoptosis in cancer cells by *Scutellaria* has been associated with mitochondrial dysfunction and activation of caspases (Ge et al. 2015; Shu et al. 2014; Lin et al. 2011); and inhibition of lipoxygenase-12 (Leung et al. 2007). Interestingly, baicalin-mediated antiapoptotic activity in microglia via inhibition of NO was NF-κB specific and did not involve caspase or lipoxygenase activity (Suk et al. 2003).

Through previous studies completed by our group, we have demonstrated the antiproliferative activity of crude *Scutellaria* extracts on cancer cells. Although specific components of the plant have shown this ability when administered individually, the anticancer activity appears to be much more potent in crude extracts containing an assortment of flavonoids (Guo et al. 2011). The exact mechanism by which this occurs is not clear, but one theory states that *Scutellaria* acts through inhibition of the Akt pathway (Guo et al. 2011; Patel et al. 2013). In order to properly understand this process, it must be noted that cancer cells rely heavily on glycolysis for their energy needs. Healthy cells, on the other hand, get the majority of their energy needs met through respiration while using glycolysis minimally (Patel et al. 2013; Marchiq and Pouyssegur 2015). In the previously mentioned pathway, Akt is a protein kinase that acts to phosphorylate glycogen synthase kinase-3-β (GSK3-β) (Patel et al. 2013; Parajuli et al. 2011; Jain et al. 2015). The

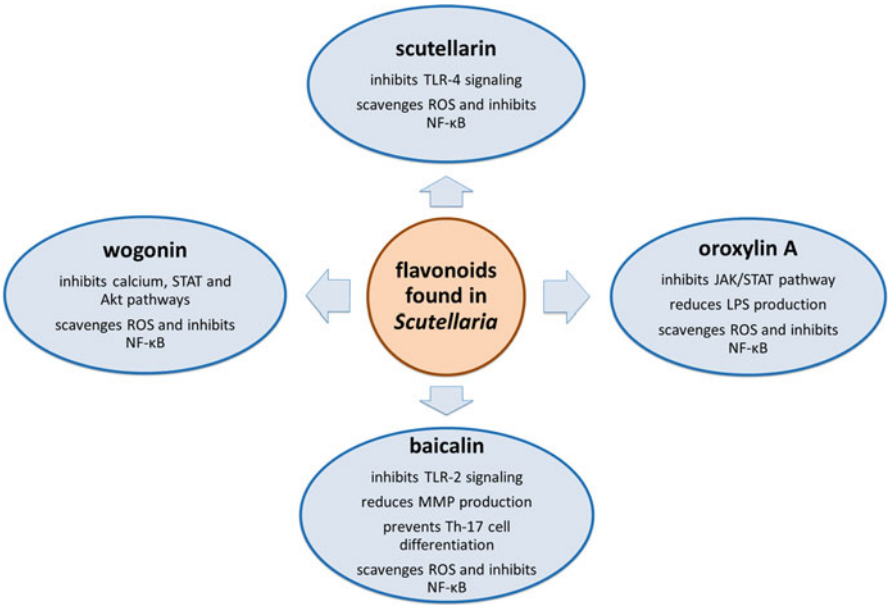

Fig. 3 Schematic representation of the underlying molecular mechanisms of neuroprotection by select *Scutellaria* flavonoids. Scavenging ROS appears to be one of the common anti-inflammatory mechanisms of all the flavonoids. Although all flavonoids eventually resulted in the inhibition of NF-κB, the upstream mechanisms leading to this event were different for many flavonoids. While baicalin and scutellarein inhibited the expression of TLR2 and TLR4, respectively; wogonin inhibited the Akt signaling. Wogonin and oroxylin A have also been reported to inhibit the inflammatory JAK/STAT signaling pathway

function of GSK3-β is to phosphorylate glycogen synthase (GS) thereby inactivating it. However, when GSK3-β is phosphorylated by Akt, it becomes inactivated and is not able to stop the GS activity (Patel et al. 2013). In cancer cells, Akt is hyperactivated leading to the unhindered activity of GS (Parajuli et al. 2011). Crude extract of *Scutellaria* has shown the ability to downregulate Akt by preventing its kinase activity allowing GSK3-β to phosphorylate and inactivate GS (Patel et al. 2013; Parajuli et al. 2011). The result of this inactivation would prevent cancer cells from fulfilling their energy needs causing cell death. Additionally, because normal cells rely minimally on glycolysis, they are not as heavily effected by Akt deactivation (Parajuli et al. 2011; Marchiq and Pouyssegur 2015). This is just one example of how *Scutellaria* is showing promise by causing selective cell death while not exhibiting the neurotoxicity prevalent among other treatments.

Scutellaria flavonoids, while on one hand, inhibit the production of ROS in injured microglia; they have in fact been shown to enhance ROS production in gliomas, leading to endoplasmic reticulum stress-related protein activation and caspase-mediated apoptosis (Parajuli et al. 2009). On the other hand, ROS production in

normal astrocytes remains unaffected by flavonoids (Patel et al. 2013; Tsai et al. 2012). Overall, it seems that using the active components of *Scutellaria* in concert with modern medicines may increase the efficacy of treatment.

Conclusion

Traditional medicines have long been overlooked in western culture, but are now making a resurgence due to recent experimental findings. Active ingredients in plants such as Scutellaria have shown promise in treating chronic inflammatory diseases and cancer. Each flavonoid found within *Scutellaria* has unique mechanisms of action, meaning that inflammation and diseases can be attacked on multiple fronts. What makes this plant so special is its ability to produce therapeutic benefits in patients while avoiding many of the serious side effects of other common medicines. The only setback for studying *Scutellaria* lies in the variation of active components that occurs due to the wide array of species and contrasting growing conditions. As a result, many studies are conflicted as to what the benefits of *Scutellaria* are due to the inconsistency of results. However, once more research is completed into correlating the growing environment of the plant with its active components, there will be a much greater consensus on *Scutellaria's* true anti-inflammatory benefits. Overall, the potential of this plant species to treat many neuroinflammatory and neurodegenerative diseases is astounding and deserves a closer look in the scientific community.

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Compliance with Ethics Requirements The authors declare that they have no conflicts of interest.

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Alzheimer's Disease and Medicinal Plants: An Overview

S. Manoharan, M. Mohamed Essa, A. Vinoth, R. Kowsalya, A. Manimaran, and R. Selvasundaram

Abstract Alzheimer's disease (AD) is progressive neurodegenerative disorder and identified as a major health concern globally. Individuals with AD and their care givers are affected in personal, emotional, financial, and social levels. Due to its significant impact and heavy burden on the individual, the patients' families, and society, it is highly needed to search for cost effective, long-time retention therapeutic targets. In recent decades, there are lots of research conducted the possible benefit of natural products and their active components on AD and other neurodegenerative disease, which are discussed here.

Keywords Alzheimer's Disease • Natural products • Acetyl choline esterase

Introduction

Alzheimer's disease is one of the most pathologic complex, heterogeneous disorders and is characterized by the presence of senile plaques (spherical accumulations of β-amyloid proteins), neurofibrillary tangles (composed of paired helical filaments and other proteins), and impairment of memory and cognitive ability. This progressive neurodegenerative disorder is identified as a major health concern in almost all countries. While dementia is recognized as the first clinical features, retrieval of distant memories is preserved relatively well in the course of the disease. Once dementia is progressing, additional impairment in cognitive ability such as the ability to calculate and to use common objects and tools is most common. The etiology of Alzheimer's disease is multifactorial and thus several mechanisms contribute to its pathogenesis, especially nerve cell death. Alzheimer's disease is usually diagnosed with standard mental

S. Manoharan (✉) • A. Vinoth • R. Kowsalya • A. Manimaran • R. Selvasundaram
Department of Biochemistry and Biotechnology, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India
e-mail: sakshiman@rediffmail.com

M.M. Essa
Department of Food Science and Nutrition, Sultan Qaboos University, Muscat, Oman

Ageing and Dementia Research Group, Sultan Qaboos University, Muscat, Oman

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function tests. Examining the patients for signs of intellectual impairment, including ability to recall about self, place, and time. Other diagnostic modalities include attention span, working memory, speed of information processing, and mood and personality. Brain imaging and blood tests are also performed to rule out other medical causes. Interactions between environmental and genetic risk factors could play a major role in the pathogenesis of Alzheimer's disease. Inhibition of acetyl choline hydrolysis using natural or synthetic products could improve the cholinergic function in Alzheimer's disease. (Irvine et al. 2008; Zilkova and Koson 2006; Shao and Xiao 2013).

Epidemiology of Alzheimer's Disease

Worldwide around 15–20 million people are found to suffer from Alzheimer's disease. It has been estimated that the incidence of Alzheimer's disease rises as the population grows and the average life span lengthens. The incidence of Alzheimer's disease will double worldwide by the year 2050 (Hebert et al. 2013). Alzheimer's disease and other forms of dementia are still challenging health problem worldwide, especially among the elderly in developing countries. It has been pointed out that the age-specific prevalence of Alzheimer's disease doubles every 5 years in older people after age 65 (Florent-Bechard et al. 2007).

Epidemiological studies have reported that the incidence of Alzheimer's disease in developing countries was 3–4 %. The Alzheimer's disease incidence in persons aged 65+ years in urban areas of China was between 3.5 and 4.8 % (Kalaria et al. 2008). Wimo and Prince (2010) pointed out that the prevalence of dementia will increase to 65.7 million by 2030 and to 115.4 million by the year 2050. Dementia incidence increases from the current figure of 25 million to 30 million by the year 2020 (Rodríguez et al. 2008). It has also been estimated that over 15 million Americans will be affected by dementia by the year 2050 (Qiu et al. 2009). Population-based studies in Europe suggested that the age-standardized prevalence in people 65+ years old is 6.4 % of dementia and 4.4 % of Alzheimer's disease (Scalmana et al. 2013). In the USA, the study of a national representative sample of people aged >70 years yielded a prevalence of Alzheimer's disease by 9.7 %. The incidence of dementia in India and rural Latin America was approximately a quarter of the incidence rates in European countries (Qiu et al. 2009). It has been reported that dementia affected 2.7 % of the Indian populations between 1996 and 2006, Alzheimer's disease being the most common cause (1.3 %) (Aprahamian et al. 2013).

Risk Factors of Alzheimer's Disease

The exact etiology of Alzheimer's disease is the unknown. However, accumulating evidence clearly pointed out that soluble amyloid β-peptide is the proximate cause of synaptic injuries and neuronal death early in the disease. Brain inflammation

plays prominent role in the pathogenesis of Alzheimer's disease (Schott and Revesz 2013). Excessive generation of ROS accompanied with depleted antioxidants in the brain could play a role in Alzheimer's disease pathogenesis (Zhao and Zhao 2013). Aluminum, a neurotoxic agent, mediates Alzheimer's disease through excessive generation of free radicals in the brain, which in turn causes neuropathological alterations (Querfurth and LaFerla 2010). Extensive studies also indicated that Alzheimer's disease begins with neuronal apoptosis (Bertoni-Freddari et al. 2009).

Apolipoprotein E polymorphism is documented as a major genetic risk factor for the development of Alzheimer's disease (Liu et al. 2014). High intake of cholesterol, PUFA deficiency, and diabetes mellitus are associated with increased risk of Alzheimer's disease (Fonteh et al. 2014). Decosahexanoic acid (DHA) deficiency was correlated with impairment in brain function (Kuratko et al. 2013). Studies reported that moderate fish consumption was associated with reduced risk of impaired cognitive functions (Hu et al. 2013). Though various risk factors such as diet, culture, lifestyle, socioeconomic status, and head injury were proposed, the degree of Alzheimer's disease pathogenesis of each factor is controversial. However, chronic inflammatory reactions in the brain and old age are the two major risk factors for the development of Alzheimer's disease (Borenstein et al. 2006). Higher levels of iPF2α-VI in CSF, plasma, and urine were reported in Alzheimer's disease patients (Schneider et al. 2009). Alzheimer's disease also develops due to brain tumors, stroke, epilepsy, Parkinson's disease, and Huntington's disease (Mattson and Meffert 2006).

Metabolic ailments, including hypothyroidism, hypoglycemia, and vitamin deficiencies were proposed as possible risk factors of Alzheimer's disease development. The other risk factors include alcohol and drug abuse, chronic sleep deprivation, emotional trauma, psychosis, and infections such as meningitis, encephalitis, and syphilis (Sperlinga et al. 2011). The deleterious effect of heavy alcohol intake emerges from a study suggesting that heavier drinkers at middle age had a more than threefold increased risk of dementia and Alzheimer's disease in their later life (Qiu 2011). Regular physical exercise was reported to be associated with a delay in the onset of dementia and Alzheimer's disease among cognitively healthy elderly (Barnes and Yaffe 2011).

Symptoms of Alzheimer's Disease

In Alzheimer's disease, the symptoms develop slowly and gradually worsen over time. The major symptoms of Alzheimer's disease include:

1. Difficulty remembering recent events
2. Defect in behavior and thinking abilities
3. Confusion, irritability, aggression, mood swings, trouble with language, and long-term memory loss
4. Impairment in learning and recall of recently learned information
5. Difficulties in word-finding
6. Object agnosia, impaired face recognition, and alexia
7. Impaired reasoning, judgment, and problem solving

Clinical Features of Alzheimer's Disease

The Alzheimer's disease brain shows multiple pathological features, including amyloid plaques, neurofibrillary tangles, elevated levels of advanced glycation end products and their receptor, oxidative damage, and inflammation. Alzheimer's disease brains is predominantly characterized by the presence of senile plaques and neurofibrillary tangles, which are thought to play a central role in the inflammatory cascade.

Staging of Alzheimer's Disease

Alzheimer's disease develops or proceeds in a stepwise manner. The seven stage model of Alzheimer's disease is represented as follows:

Stage 1	No impairment
Stage 2	Minimal impairment/normal forgetfulness
Stage 3	Early confusional/mild cognitive impairment
Stage 4	Late confusional/mild Alzheimer's disease
Stage 5	Early dementia/moderate Alzheimer's disease
Stage 6	Middle dementia/moderately severe Alzheimer's disease
Stage 7	Late or severe dementia and failure to thrive

Treatment

No effective treatment is yet available to halt the progression or prevent the development of Alzheimer's disease. This could be mainly attributed to the complex pathophysiology of Alzheimer's disease, which is poorly understood. Acetylcholine esterase inhibitors are the only agents approved by the Food and Drug Administration for the treatment of Alzheimer's disease. Inhibition of acetylcholine esterase serves as a strategy for the treatment of Alzheimer's disease, senile dementia, ataxia, myasthenia Gravis, and Parkinson's disease (Anonymous 2000; Brenner 2000; Rahman and Choudhary 2001). The first acetylcholine esterase inhibitors specifically approved for the treatment of Alzheimer's disease was 1,2,3,4-tetrahydro-9-aminoacridine (tacrine) (Whitehouse 1993). Currently, several acetylcholine esterase inhibitors, such as donepezil (Kelly et al. 1997), galantamine (Scott and Goa 2000), and rivastigmine (Gottwald and Rozanski 1999), are available for the treatment of patients with Alzheimer's disease.

Medicinal Plants and Alzheimer's Disease

Medicinal plants play a pivotal role in the treatment of several disorders including Alzheimer's disease. The pharmacology and therapeutic potential of medicinal plants could be attributed to their proactive chemical constituents. Traditional herbal medicines are still recommended throughout the world as a memory enhancers or to treat dementia-related disorders. It has been reported that several medicinal plants showed acetylcholine esterase inhibitory activity and so may be relevant to the treatment of neurodegenerative disorders such as Alzheimer's disease. *Bacopa monniera and Ginkgo biloba* are well-known cognitive enhancers in Indian and Chinese traditional medicine systems. *Ginkgo biloba* leaf and *Lycium barbarum* fruit extracts are used as memory enhancers and also have strong antioxidant and anti-inflammatory effects (Kim and Oh 2012).

Currais et al. (2014) identified for the first time, the alkaloid voacamine as a major compound in *Voacanga africana* with potent biological activity in several cell-based assays relevant to Alzheimer's disease. Oh et al. (2004) reported that *Epimedium koreanum* and *Acorus calamus* improved cognitive function for anticholinesterase activity. *Panax ginseng* had cognitive enhancing effects and huperzine A from *Huperzia serrata* has been claimed to be effective in the treatment of Alzheimer's disease (Ma and Yu 1990; Huang 1999). Some alkyl pyridinium polymers from *Reniera sarai*, dehydroevodiamine from *Evodia rutaecarpa*, and protopine from *Corydalis ternata* have been reported to be acetylcholine esterase inhibitors (Sepeic et al. 1998; Park et al. 1996; Kim et al. 1998).

Ozarowski et al. (2013) reported that *Rosmarinus officinalis* leaf extract (200 mg/kg bw, p.o.) improved the memory improvement in rat brain. They suggested that inhibition of acetylcholine esterase in the brain by the *Rosmarinus officinalis* leaf extract could be the possible mechanism. Sun et al. (2014) demonstrated that diterpenes from the twigs of *Croton yanhuii* could be used as antineurodegenerative agents for Alzheimer's disease and other biological disorders. Lin et al. (2008) have tested the antiacetyl choline esterase activities of 26 traditional Chinese medicinal herbs. They suggested that, of 26 herbs tested, the aqueous and ethanolic extracts of *Radix paeoniaerubra*, *Radix paeoniaelaba*, *Radix salviaemitiorrhizae*, *Radix etrhizomerhei*, *Radix polygoni multiflori*, *Caulis spatholobi*, and the ethanol extracts of *Radix salviaemiltiorrhizae*, *Radix paeoniaelaba*, *Radix etrhizomerhei*, and *Caulis spatholobi* strongly inhibited the acetylcholine esterase at 50 μg/ml.

Siqueira et al. (2003) demonstrated the dose- and time-dependent anticholine esterase activity of *Ptychopetalum olacoides* roots—in rat frontal cortex, hippocampus, and striatum. Ingkaninan et al. (2003) reported the antiacetyl choline esterase activity of methanolic extract of *Abutilon indicum*. Lopez et al. (2002) demonstrated the antiacetylcholine esterase activity of Assoanine, a sterol alkaloids from *Narcissus assoanus*. Pereira et al. (2009) reported that the aqueous extract of *C. roseus* significantly inhibited the activity of acetylcholine esterase. Chonpathompikunlert et al. (2010) pointed out that piperine protected neurodegeneration and cognitive impairment in ethylcholine aziridinium-induced Alzheimer's disease in male Wistar rats. They concluded that the antilipid peroxidative and antiacetyl choline esterase activity of piperine might have played a role in the protection of neurodegeneration and improvement in memory impairment.

Fujiwara et al. (2011) explored the memory improving potential of Yokukansan, a traditional Japanese medicine. They suggested that Yokukansan significantly inhibited Amyloid β aggregation in a dose-dependent manner. They concluded that Yokukansan significantly prevented the accumulation of Aβ fibrils in vitro and in vivo. Uabundit et al. (2010) focused *Bacopa monnieri* as a potent cognitive enhancer and neuroprotectant against Alzheimer's disease. They reported that *B. monnieri* mitigated the reduction of neurons and cholinergic neuron densities in AF64A-induced Alzheimer's disease in Wistar rats. Zandi et al. (2004) reported that dietary antioxidants vitamin E and C can able to reduce the risk of Alzheimer's disease.

Profound studies demonstrated that compounds possessing antioxidant activity attenuated the oxidative stress induced by amyloid protein (A), inhibited the formation and extension of amyloid fibrils, and decreased the plaque burden (Ono et al. 2003, 2006; Choi et al. 2011). Seo et al. (2010) pointed out that Jangwonhwan, an oriental medicine, ameliorated Alzheimer's disease like pathology in the brain of Tg-APPswe/PS 1 dE9 by reducing Aβ (1–42) level and β-amyloid deposition. Hage et al. (2010) reported that aqueous extract of *Pterocarpus erinaceus* bark, showed a promising effect against Alzheimer's disease. They suggested that the extract decreased β-amyloid peptide production in CHO cells. Figueiró et al. (2011) suggested that the Amazonian herbal Marapuama attenuated cognitive impairment and neuroglial degeneration in a mouse Alzheimer model. Shivakumar et al. (2011) evaluated the memory enhancing activity of SR-105 in experimental animals. Yassin et al. (2013) they suggested that the aqueous infusions of *Boswellia serrata* significantly ameliorated the neurodegenerative characteristics of Alzheimer's disease in rats. The medicinal plants and active constituents that are tested against Alzheimer's disease are given in Figs. 1, 2, 3, and 4.

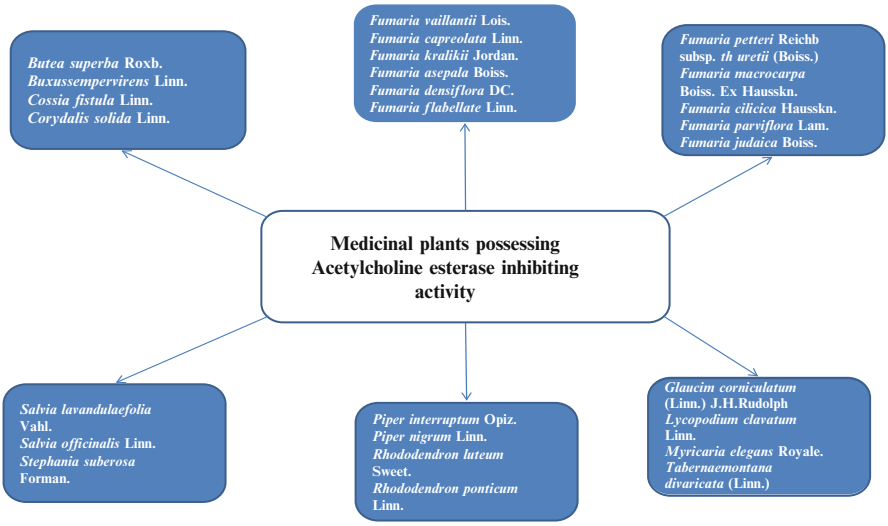

Fig. 1 Medicinal plants that inhibited the activity of acetylcholine esterase. Adapted and modified from Mukherjee et al. (2007)

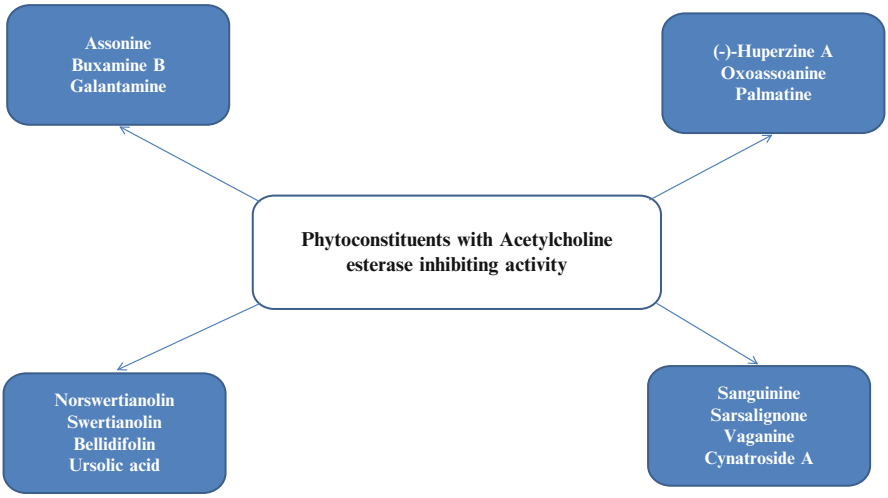

Fig. 2 Bioactive constituents from medicinal plants possessing acetylcholine esterase inhibitory activity. Adapted and modified from Mukherjee et al. (2007)

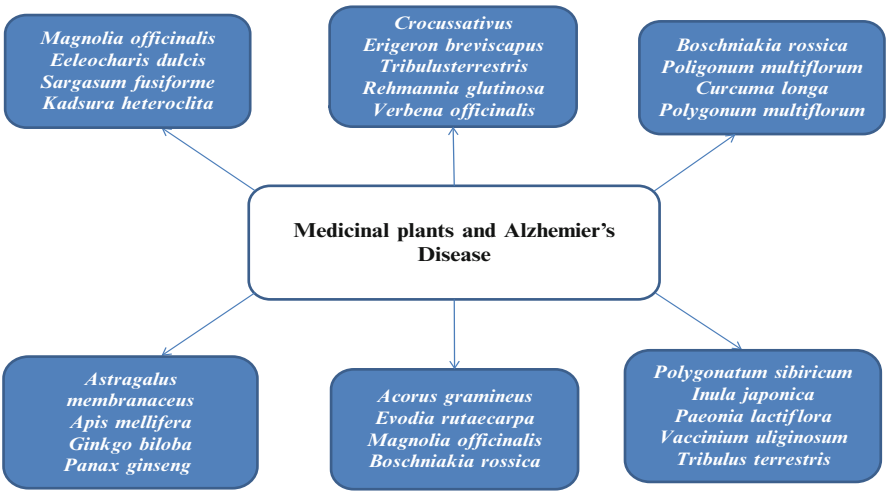

Fig. 3 Medicinal plants that showed beneficial effects against Alzheimer's disease. Adapted and modified from Su et al. (2014)

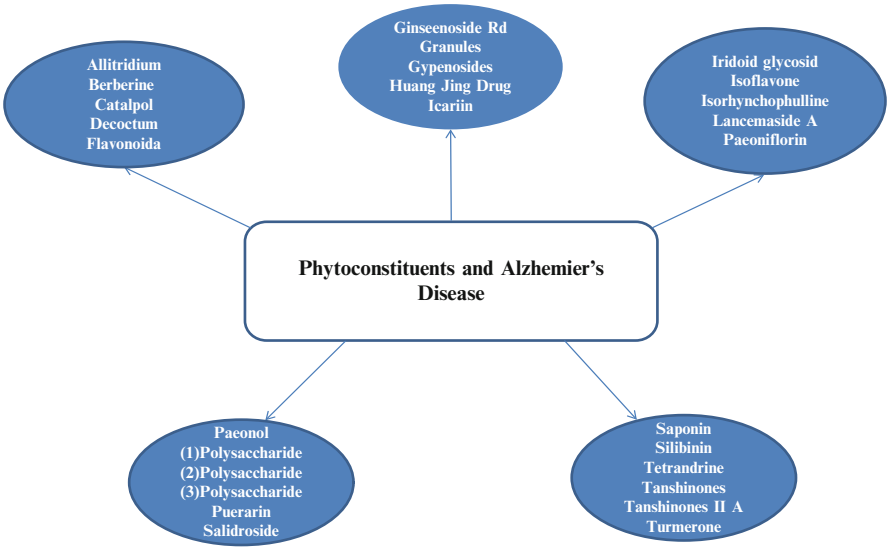

Fig. 4 Phytoconstituents that are tested against Alzheimer's disease targets. Adapted and modified from Su et al. (2014)

Conclusion

Individuals with Alzheimer's disease and their families and friends are affected at personal, emotional, financial, and social levels. Due to its significant impact and heavy burden on the individual, the patients' families, and society, it is most urgent to search for new therapeutic bioactive agents from medicinal plants with fewer side effects for the treatment of Alzheimer's disease.

Compliance with Ethics Requirements The authors declare that they have no conflicts of interest.

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Efficacy of Natural Compounds in Neurodegenerative Disorders

Pranay Srivastava and Rajesh Singh Yadav

Abstract Neurodegenerative disorders represent clusters of serious diseases that results in progressive deterioration of normal structure and physiology of central nervous system. Pathophysiology of Alzheimer's, Parkinson's or other neurodegenerative disorders involves multifaceted permutation of genetic and environmental factors. Combinations of lifestyle modification linked with environmental factor jointly or alone represent the largest share of cases of these disorders. Etiology of such neuronal degeneration involves manifestation of toxic reaction in the form of functional anomalies leading to dysfunction of the ubiquitin–proteasome system, activated inflammatory cascade, compromised neuronal survival pathway, mitochondrial dysfunction and finally neuronal apoptosis/necrosis and cell death. Furthermore, evidences from various studies exhibited role of oxidative stress and compromised anti-oxidant defense system as one of the prime factors associated with activation of various signal transduction pathways that would ultimately lead to the formation of amyloid beta or alpha synuclein in the brain. Keeping in view of complex etiology and pathophysiology along with a miniscule of available treatment options associated with these neurodegenerative disorders, the role of natural agents and herbal extracts as therapeutic alternatives alone or in combination with synthetic drugs could not be ruled out. In the same context the present chapter has been aimed to investigate the role of selected natural plants like Withania somnifera, Bacopa monnieri, Curcuma longa, Centella asiatica, Ocimum sanctum, Nardostachys jatamansi and Emblica officinalis in various neurodegenerative disorders and explore their targets to ameliorate neurotoxicity in various experimental models. The rationale for selection of these plants was based on their strong anti-inflammatory and anti-oxidant potential and large body of evidence that suggest their efficacy in preclinical as well as in clinical studies. Active constituents if these herbals might play an important role in preserving the integrity of various neurotransmitters and their receptor in the brain influencing its functions at the molecular level.

P. Srivastava
Developmental Toxicology Division, Indian Institute of Toxicology Research,
Post Box 80, MG Marg, Lucknow 226 001, UP, India

R.S. Yadav (✉)
Department of Criminology and Forensic Science, School of Applied Sciences,
Dr. Harisingh Gour Central University, Sagar 470 003, MP, India
e-mail: razitrc@gmail.com

Keywords Herbal agents • Neurotoxicity • Alzheimer's disease • Parkinson's disease • Neurodegeneration

Introduction

The term neurodegenerative disorders has been collectively used to describe a variety of conditions whose inception result from progressive deterioration of normal structure and function of neurons predominantly in the central nervous system (CNS). Alzheimer's disease (AD), Parkinson's disease (PD), and ischemia/stroke are the main disease conditions associated with neurodegeneration. Apart from this amyotrophic lateral sclerosis, Huntington's disease, spongiform encephalopathies, prion diseases, head and brain malformations, hydrocephalus, etc. also fall under the category of neurodegenerative disorders (Rubinsztein 2006; Luo and Le 2010). The manifestation of symptoms arising from neurodegeneration depends on disease progression and neuronal network being tricked under foul play. Such a type of progressive breakdown of nervous tissues has been found to be apparently irreversible in nature, and hence, treatment strategies have been aimed to bring symptomatic relief in the deliberating condition. The etiology of neurodegenerative disorders such as AD and PD could be hereditary or/and sporadic grounds (Bigford and Rossi 2014). Chronic exposure to various environmental toxicants, food habits, lifestyles, and brain injury might result in various neurodegenerative disorders. The magnitude of damage inflicted on the nervous system depends on dose, duration, and nature of neurotoxin, exposed to the nervous system. Exposure to wide variety of chemicals such as pesticides, heavy metals, solvents, and food additives has been reported to cause neurotoxicity, which could further develop into a neurodegenerative disorder (Parrón et al. 2011; Baltazar et al. 2014). In addition to AD and PD, stroke, which is also known as brain attack, occurs when blood flow to an area of the brain is impeded, resulting in neuronal death due to oxygen deprivation. It is the fifth leading cause of death and a leading cause of adult disability in the USA.

Leads obtained from various studies have been explored in an attempt to understand the pathophysiology of neurodegenerative disorders, but it still remains elusive. Postmortem studies for AD, PD, and stroke have clearly pointed out various pathological findings such as altered neurotransmitter levels in different parts of the brain, evidence of beta amyloid plaques, tau protein, severe inflammation, etc. Such leads could be utilized for development of suitable therapeutic interventions which could bring clinical relief or abate the symptoms of the diseases. Various pharmacological agents have been utilized for such approach; however, few shortcomings have always been associated with them. A wide variety of natural agents have been explored to fulfill and/or assist the prevalent pharmacological agents in the treatment of neurodegenerative disorders. This chapter provides a general idea about various natural agents that could be utilized for the cure and treatment of various neurodegenerative disorders.

Herbals in Brain Health

Utilization of phytopharmaceuticals has its roots across the globe since ancient times. Indian traditional medicine has played a pivotal role in this regard due to prevalent natural treasure in the form of diverse flora and fauna. A vivid knowledge of ancient as well as scientific Indian literature in the form of sacred texts such as Rigveda, Yajurveda, Samaveda, and Atharvanaveda has served as references to herbal medicines for the treatment of various nervous system disorders. Due to increased load of nervous system disorders and side effects imposed by synthetic drugs, the functional utility of herbs in the treatment of neurological disorders has aroused great interest. Although synthetic drugs are specific in nature, subsequent measures to lower their side effects still remains a challenge. Besides herbals have an unexplored potential in terms of their efficacy and functionality and are preferred due to less side effects. Realizing the potential of herbals in the management of various disorders a number of studies have been carried out to isolate the active molecules which impart the neuroprotective effects (Sun et al. 2015; Ghosh et al. 2015; Fu et al. 2015; Chan et al. 2016).

This review deals with a spectrum of activities that herbs possess against various neurodegenerative disorders. Recent advances have led to the development of novel molecular targets developed through molecular, cellular, and preclinical studies that could be utilized for treatment of neurological ailments through herbal approaches. Botanicals that belong to different families may contain active chemical constituents of the same as well as different classes, yet eliciting its effect on same or different receptors. This type of consolidated literature could pave way for future therapies for prophylaxis as molecular conceptual receptorome approaches by studying the mechanism of action of the chemical constituents at genetic level. In this context various medicinal plants, viz., *Withania somnifera*, *Bacopa monnieri*, *Curcuma longa Centella asiatica*, *Ocimum sanctum*, *Nardostachys jatamansi*, and *Emblica officinalis* have been delved into to check their protective implications on neurodegenerative disorders. Herbals mentioned in Indian traditional medicine and their isolated active constituents so far have demonstrated prophylactic as well as in post disease states their adaptogenic ability.

Withania somnifera (WS) also known as Ashwagandha (Grandhi et al. 1994) belonging to the family Solanaceae has been mentioned in Indian traditional medicine for long. The plant got its name Ashwagandha (Kulkarni and Dhir 2008) in Sanskrit in India as the plant possesses an odor of a sweaty horse. In addition *somnifera* is a Latin word which means sleep inducer, highlighting the sedative properties of the plant. Alkaloids and steroidal lactones constitute the major chemical constituents of the plant along with some flavonoids, tannins, etc. (Rahman et al. 1991, 1993; Choudary et al. 1996). More than 12 alkaloids have been isolated including somniferine, somnine, somniferinine, withananine, pseudo-withanine, etc. In addition steroidal lactones commonly referred to as withanolides were also isolated along with their 40 subtypes (Mirjalili et al. 2009; Bhattarai and Han 2014; Puri et al. 2014).

Bacopa monnieri (BM) is a perennial creeping plant belonging to the family Scrophulariaceae found in wet, damp, and marshy areas (Aguiar and Borowski 2013) throughout the Indian subcontinent. *Bacopa monnieri* is also known as Brahmi, a name derived from Brahma, the creator god of the Hindu pantheon of deities. BM is also mentioned in the Ayurvedic literature for its utilization in various mental conditions like anxiety, poor cognition, and lack of concentration. In addition, studies have highlighted its potential in the treatment of mental illness and epilepsy. Major active compounds of BM include various classes of chemical moieties containing alkaloids like brahmine, herpestine, and nicotine; saponins such as D-mannitol and hersaponin, acid A, and monnierin (Le et al. 2015; Aguiar and Borowski 2013). Bacoside A was responsible for enhancement of the body's antioxidant defense system (Anbarsi, et al. 2006) and memory enhancing activity occurs with its optical isomer bacoside B. Bacogenins A1, A2, and A3 were a mixture of aglycone moiety found on acid hydrolysis together with sapogenins, jujubogenin, and pseudojujubogenin (Rastogi et al. 1994). BM also contains significant amounts of betulic acid, stigmastarol, beta-sitosterol, bacopaside II, bacopaside I, bacopaside X, bacopasaponin C, bacopaside N2, and the minor components were bacopasaponin F, bacopasaponin E, bacopaside N1, bacopaside III, bacopaside IV, and bacopaside V (Murthy et al. 2006; Chakravarty et al. 2001, 2003; Hou et al. 2002). *Curcuma longa* (CL) also known as "Turmeric" is routinely used as spice, food preservative and coloring material in different parts of the world. The active ingredient of turmeric is curcumin which is found to have multiple pharmacological properties including anti-inflammatory, anti-carcinogenic, anti-ischemic and hypotensive (Strimpakos and Sharma, 2008; Aggarwal and Sung, 2009; Esatbeyoglu et al. 2012). Curcumin has also been found to be effective in the treatment of Alzheimer's dementia, neuroleptic-induced tardive dyskinesia and chemical-induced neurotoxicity resulting from lead and cadmium (Garcia-Alloza et al. 2007; Bishnoi et al. 2008). We have also reported that simultaneous treatment with arsenic and curcumin could significantly protect arsenic-induced cholinergic and dopaminergic alterations and oxidative stress in rat brain (Yadav et al. 2009; Srivastava et al. 2014). *Centella asiatica* (CA) is a perennial herb that has been used for centuries in Ayurvedic medicine to treat several disorders, such as insanity, asthma, leprosy, ulcers, and eczema, and for wound healing (Handa et al. 1988; Veerendra Kumar and Gupta 2002). CA exerts a significant neuroprotective effect (Subathra et al. 2005), cardioprotective effect and antigastric ulcer activity (Cheng et al. 2004). The major chemical constituents of the plant include pentacyclic triterpenic acids and their respective glycosides, belonging to ursane or oleanane type, including asiatic acid, asiaticoside, madecassic acid, madecassoside, brahmoside, brahmic acid, brahminoside, thankuniside, isothankuniside, centelloside, madasiatic acid, centic acid, cenellic acid, betulinic acid, and indocentic acid (Zheng and Qin 2007).

Ocimum sanctum (OS) known as "Tulsi" or "Tulasi" in Hindi or Holy Basil in English is considered a sacred plant in the Hindu culture and is a small erect herb

belonging to the family Labiatae (Lamiaceae) usually cultivated in temples and gardens. This herbal plant is known for its medicinal value in various traditional systems of medicine in India, particularly Ayurveda and Unani. The major chemical constituents of OS include oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, and β-caryophyllene. It also contains phenyl 45–73 % and aldehydes 15–25 %. Seeds contain stable oil 17.8 %. Besides this the plant contains alkaloid, glycosides and tannins. The leaves possess ascorbic acid and carotene (Hakkim et al. 2007).

Nardostachys jatamansi (NJ) is a perennial herb which is also known as musk root in English (Valerianaceae) is classified as hypno-sedative in Ayurveda and indigenous to the Himalayan regions of India. The roots of the plant contain essential oil, rich in sesquiterpenes and coumarins. The major chemical constituents reported are jatamansone or valeranone which is the major sesquiterpene; jatamansic acid, nardostachone, dihydrojatamansin, jatamansinol, lignans and neolignans are also present in this plant (Chatterji et al. 1997) while rhizomes contain a terpenoid ester, nardostachysin I (Chatterjee et al. 2000).

Emblica officinalis (EO) syn. *Phyllanthus emblica* belongs to the family Euphorbiaceae and is also known as Amla or Amlaj (Kritikar and Basu 1991). EO is used in Ayurveda, as potent rasayanas, a class of plant-derived drugs reputed to promote health and longevity by increasing defense against disease, arresting the aging process, and revitalizing the body in debilitated conditions (Udupa and Singh 1995). It also contains tannins, phyllembin, rutin, curcuminodes, emblicol, and phenolic compounds (Zhang et al 2000; Singh et al. 2015). Low molecular weight (<1000) hydrolyzable gallotannins (EOT) comprising emblicanin A, emblicanin B, punigluconin, and pedunculagin, isolated from the fresh juice or solvent extracts of Emblica fruits (Ghosal et al. 1996) were shown to have significant antioxidant effects in vitro (Justin Thenmozhi et al. 2016; Tupe et al. 2015).

Role in Alzheimer's Disease

Effect on Various Diagnostic Features of Alzheimer's Disease

AD is the most common cause of dementia in the elderly, characterized by senile plaques, neurofibrillary tangles, and amyloid angiopathy. Amyloid beta is one of the major diagnostic markers for AD, and it has been already demonstrated that amyloid β protein induces neuritic atrophy (Tohda et al. 2004), neuronal cell death, as well as inhibit neurogenesis (Li and Zuo 2005) and memory impairment (Tohda et al. 2003, 2004). Furthermore, NADPH oxidase has been regarded an important source of ROS that mediate the inflammatory response in astrocytes and microglial cells. In fact Aβ induced ROS from NADPH oxidase in astrocytes

is the key factor in mediating neuronal death (Abramov et al. 2004). However, as for other neurodegenerative diseases, a local inflammatory reaction is sustained by activated microglia and reactive astrocytes, as indicated by the presence of antigens associated with microglia/macrophage activation and inflammatory mediators, such as elements of the complement system, cytokines, and free radicals (Perry et al. 2003). There are evidences that suggest that at least part of oxidative mechanism is contributed by the Aβ peptides (Selkoe 2001). In addition, studies have shown that Aβ can induce ROS production in neurons through an NMDA receptor dependent process (De Felice et al. 2007).

Neurochemical cascade that lead to formation of new memory involves neurotransmitter receptor along with activation of CREB and PKC pathways to bring about structural changes for inclusion of receptor and proteins to form long term memory (Lanni et al. 2008; Tully et al. 2003). In addition, amnesia develops due to various neurological problems such as Alzheimer's disease, aging, chronic drug abuse, or head injury and on the contrary, it may be induced by various competitive NMDA receptor antagonists such as AP5, NPC and non-competitive NMDA receptor antagonists such as phencyclidine, ketamine, and MK801 that block long term potentiation induction (Bliss and Collingridge 1993; Gruart et al. 2006; Harney et al. 2006).

The role of phytopharmaceuticals in ameliorating deleterious effect imposed by either Aβ protein or reactive oxygen species could not be ruled out. Herbals such as *B. monnieri* have also been tested to prove their efficacy as nootropics. These herbals act through various mechanistic approaches to improve memory and learning. In this prospect acetylcholinesterase inhibitors (either natural or synthetic) are used to bring symptomatic relief to the patients of Alzheimer's following dementia in the early stages.

Implication on Amyloid Beta (Aβ) Induced Neurological Changes

Withanolide IV is the steroidal lactone, while its active aglycone constituent sominone demonstrated attenuation of Aβ (25–35)-induced axonal, dendritic, and synaptic losses and memory deficits in mice (Kuboyama et al. 2006). These encouraging results have been mediated through the promotion of synaptogenesis; however, the exact mechanism of this action is unclear. Crude alcoholic extract of WS has been found to inhibit acetylcholinesterase, butyrylcholinesterase, and lipoxygenase enzymes in vitro (Khattak et al. 2005; Vinutha et al. 2007). Further, withanolides 1–3 and 4–5 isolated from *A. bracteosa* and *W. somnifera* respectively inhibited acetylcholinesterase and butyrylcholinesterase enzymes in a concentration-dependent manner (Choudhary et al. 2004).

B. monnieri is a traditional Ayurvedic medicine, used for centuries as a nootropic as well as for its utilization in various memory related neurological disorders (Russo and Borrelli 2005; Limpeanchob et al. 2008). Preclinical studies have shown improved learning ability and the memory enhancing effect due to its major active constituent bacosides A and B, present in the ethanol extract (Singh and Dhawan 1992). *B. monnieri* may act through different mechanisms and signaling pathways in adjunct with its different active chemical entities for the improvement of memory. Singh et al. (1990) reported that bacopasides induce membrane dephosphorylation, together with an increase in protein and RNA turnover in specific brain areas. Enhanced protein kinase activity in the hippocampus may be responsible for its memory enhancing activity.

The dose-dependent antiamnesic effect of *B. monnieri* on diazepam-induced anterograde amnesia has been described by Prabhakar et al. (2008) but the probable mechanism of its action still remains elusive as BM is believed to exert its antiamnesic effects by a combination of its antioxidant and anticholinesterase activities (Bhattacharya et al. 2000a, b; Das et al. 2002; Tripathi et al. 1996). Another study by the same group has shown an antiamnesic effect of BM in the scopolamine induced model through Kinase-CREB pathway (Saraf et al. 2010).

Anand et al. (2010) have revealed that L-NNA (a nitric oxide synthase inhibitor)-induced anterograde amnesia was significantly reversed by pretreatment of BM through significant increase of calmodulin (CaM) and pCREB/CREB levels in whole brain lysates. Hence, NO pathway involving calmodulin could be another molecular target for BM to impose its potentiating effect. In addition, BM has also inhibited the amnesic effects of electroshock and immobilization stress (Dhawan et al. 1996) probably through an antioxidant mechanism.

Curcumin treatment has been reported to attenuate cognitive deficits, neuroinflammation and plaque pathology in experimental models of Alzheimer's disease (Garcia-Alloza et al. 2007). Low dose of curcumin (160 ppm) was found to reduce the astrocyte marker glial fibrillary acidic protein and decrease the insoluble beta amyloid (Aβ), soluble Aβ and plaque burden in animal model of Alzheimer's disease (Lim et al. 2001). Curcumin was found to inhibit the formation of Aβ oligomers and fibrils in vitro (Ono et al. 2004, Yang et al. 2005). Recently, Zheng et al. (2016) reported that curcumin administration dramatically reduced Aβ production by downregulating BACE1 expression, preventing synaptic degradation, and improving spatial learning and memory impairment of 5×FAD mice. Further, Ghosh et al. (2016) reported that the curcumin exerts its beneficial effects by modulating different signalling molecules including transcription factors, chemokines, cytokines, tumour suppressor genes, adhesion molecules, microRNAs, etc. It could be a potential agent for multifunctional therapeutic application and recent progress in clinical biology. In a study carried out on transgenic rats it has been demonstrated that CA selectively decreased amyloid β level in the hippocampus region, hydrogen peroxide-induced lipid peroxidation, and DNA cleavage. The results demonstrate that lipid peroxidation is blocked by very low doses of CA, suggesting that

the inhibition of hydroxyl radical induced membrane damage may be one of the primary mechanisms of action of this extract. In addition, the CA inhibited the damage of the supercoiled form of the plasmid DNA due to exposure to hydrogen peroxide and UV light, leading to the formation of single-strand breaks in DNA and finally apoptosis (Dhanasekaran et al. 2009).

Findings regarding the cognitive enhancing effect assessed through animal behavioral models and involvement of an antioxidant mechanism have been demonstrated, indicating that the aqueous extract of CA has a potential to promote learning and memory (Veerendra Kumar and Gupta 2002).

Treatment of the animals with ethanol extract of OS leaves for a week prevented the noise induced changes in cholinergic parameters in the brain (Sembulingam et al. 2005). Ursolic acid separated from OS extract has been reported to reduce free radicals (Balanehru and Nagarajan 1992). Based on the studies the author hypothesized that noise-induced oxidative damage could be attenuated through the free radical scavenging property of the OS.

In behavior models of learning and memory ethanolic extract of NJ has shown significant improved results against diazepam- and scopolamine-induced amnesia highlighting involvement of acetylcholinesterase and cholinergic neurotransmission in NJ to improve memory (Joshi and Parle 2006). In vitro acetylcholinesterase activity of methanolic extract of NJ in traditional medicine supported the fact (Vinutha 2007). Additionally, an antioxidant process may also be support for memory enhancing activity.

Preparation from EO called as Anwala churna has demonstrated in exteroceptive behavioral models comprising plus maze and passive avoidance paradigm (Vasudevan and Parle 2007) memory enhancing effect against diazepam- and scopolamine-induced memory deficits. This action is elicited through cholesterol-lowering and brain anticholinesterase activity (Anila and Vijayalakshmi 2002).

Role in Parkinson's Disease

PD is characterized by reduced activity of dopamine-secreting cells due to their death in the pars compacta region of the substantia nigra (Obeso et al. 2008), giving rise to motor-associated symptoms such as tremor, rigidity, postural instability, and slow movement of the body. Animal models of PD have been used to investigate protective and therapeutic efficacy of botanicals. In this context 6-Hydroxydopamine (6-OHDA), a neurotoxin, is administered to study parkinsonism in rodents. Previous studies have demonstrated that it manifests its toxic effect by acting as an prooxidant (Ahmad et al. 2005) and targeting the antioxidant system of the brain, viz., glutathione-*S*-transferase, glutathione reductase, glutathione peroxidase, superoxide dismutase, and catalase, as shown by their lower activities. In addition, effect on dopamine receptor and reduction in its binding sites together with lowered tyrosine hydroxylase and reduced contents of catecholamine have also been reported. MPTP

is another neurotoxin utilized in the study of PD. In the brain it is converted into MPP^+ which interferes with electron transport chain and leads to accumulation of free radicals (Langston 2002). In a study carried out by Rajasankar et al. (2009) showed mouse treated with MPTP had reduced levels of DA, DOPAC, HVA, GSH, and GPx and induced thiobarbituric acid reactive substance (TBARS) level.

The potential of the WS extracts to quench free radicals has been demonstrated by ameliorating the toxic effect on brain antioxidant system in 6-OHDA induced PD model in a dose-dependent manner (Ahmad et al. 2005) and in MPTP-induced PD model in mice (Sankar et al. 2007). Treatment with WS extract improved motor function and reversed the neurotoxic effect (Rajasankar et al. 2009a, 2009b). The radical scavenging capacity of the active moiety has been accounted for a mechanism through which it acts. The neuroprotective effect of B. monnieri in Parkinson's disease models of animal has been demonstrated by various investigators (Hosamani and Muralidhara 2009, 2010; Singh et al. 2012). It has been showed to diminish alpha synuclein aggregation, dopaminergic neurodegeneration and restore the lipid content in Caenorhabditis elegans (*C. elegans*) to exhibit its protective effect against parkinsonian symptoms (Jadiya et al. 2011). B. monnieri has been found to be effective against paraquat and 1-methyl-4-phenyl-pyridinium iodide (MPP+) induced neurotoxicity in rats and could be used as therapeutic agent in neurodegenerative diseases including Parkinson's disease (Singh et al. 2012)

Protective efficacy of curcumin against 1-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine (MPTP) induced oxidative stress in the brain of mice has been demonstrated (Rajeswari 2006). Treatment with curcumin in 6-OHDA lesioned rats showed significant protection in the number of TH-positive cells in the substantia nigra and dopamine levels in the striatum (Zbarsky et al. 2005). Song et al. (2016) in their studies showed that curcumin protected against 6-OHDA-induced neural impairments in the substantia nigra through improvement in memory, levels of superoxide dismutase, glutathione peroxidase and reduced concentration of malonaldehyde and suggested that curcumin exerts neuroprotection via ameliorating neurofunctions of PD rats (Song et al. 2015). Further, it was also reported that curcumin significantly ameliorate rotenone induced dopaminergic neuronal oxidative damage in the substantia nigra pars compacta (SNpc) of rats via activation of the Akt/Nrf2 signaling pathway (Cui et al. 2016) and anti-apoptotic action. Treatment with CA extract exhibited its neuroprotective effect on MPTP-induced parkinsonism by decreasing the levels of lipid peroxides, protein carbonyl, xanthine oxidase, and the modulating the level of total antioxidants, including SOD, glutathione peroxides, and catalase, towards normal (Haleagrahara and Ponnusamy 2010).

Restoration of activities of glutathione-dependent enzymes, catalase, and superoxide dismutase in a parkinsonism model of 6-OHDA lesioning by ethanolic NJ root extract has been demonstrated. An increase in the number of dopamine receptors in stratum followed by a decrease in dopamine level and its metabolite was also reversed by NJ. It does so by increasing the density of tyrosine hydroxylase immunoreactive (TH-IR) fibers in the ipsilateral striatum (Ahmad et al. 2006).

Role in Ischemia/Stroke

Stroke accompanies rapidly developing loss of brain function(s) due to disturbance in the blood supply to the brain. This can be due to ischemia (lack of blood flow) caused by blockage (thrombosis, arterial embolism), or a hemorrhage (leakage of blood) (Sims and Muyderman 2009). Stroke is a leading cause of death and long-term disability worldwide, yet no satisfactory treatment is available.

In the past, many animal models were developed, including both occlusion of common carotid arteries (CCA) and occlusion of the middle cerebral artery (MCA) (Wang et al. 2005; Chan et al. 1990) in which blood flow is focally or globally, permanently or transiently, and completely or incompletely interrupted. Further, this is accompanied by rapid metabolic changes including decrease in ATP production, neuronal membrane depolarization, activation of glial cells and release of excitatory neurotransmitters, etc. Evidences suggest involvement of oxidative damage accompanying free radical production as the underlying cause of brain damage and neuronal dysfunction. In addition, involvement of NMDA receptor-dependent release of Ca^{2+} ions and mitochondrial dysfunction with neuronal apoptosis may constitute the etiology of brain damage (Choi 1992). Because herbal drugs have a relatively higher therapeutic window, fewer side effects, and are economical, they have gained a lot of acceptance in recent years and are potential candidates for prophylactic treatment of stroke (Siesjo 1992). Chronic pretreatment with hydroalcoholic extract of WS prevented oxidative stress parameters depicted by decreased levels of MDA. In addition, grip strength and rota rod performance were found to be improved as compared to middle cerebral artery occlusion group (Chaudhary et al. 2003). A study involving withanolides has demonstrated to increase the expression of antioxidant enzymes if administered chronically (Bhattacharya et al. 2002).

B. monnieri attenuated the reduced transfer latency in ischemic rats in a step-through test and showed a protective effect on ischemia-induced memory impairment in the plus maze task and reduced infarct size in the ischemic brain. The mechanism for this action was through free radical scavenging evident from decreased nitrite, nitrate, lipid peroxidation, and significantly improved catalase activity (Siesjo 1992).

Treatment with curcumin in rats reduced ischemia induced lipid peroxidation both in the ipsilateral and contralateral hemispheres of brain (Thiyagarajan and Sharma, 2004). Pretreatment with curcumin for 5 days prior to MCAO for 2 hours and for another 3 days after MCAO in rats resulted in significant improvement in grid walking and rota-rod performance as compared to ischemic rats (Shukla et al., 2008). Treatment with curcumin decreased lipid peroxidation, mitochondrial dysfunction, apoptotic indices and glial activation in gerbils subjected to transient global ischemia for 5 minutes as compared to sham (Wang et al., 2005). Rathore et al. (2008) found that treatment with curcuma oil in cerebral ischemia in rats could protect neuronal death due to its anti-oxidant effects and anti-apoptotic property via caspase-dependent pathway. Treatment with curcumin prevent the MCAO injury

induced reduction in γ-enolase expression and exerts its neuroprotective effects in rats function in focal cerebral ischemia by regulating the expression of γ-enolase (Gim et al., 2015). Liu et al. (2014) demonstrated the curcumin may exert neuroprotective effects by increasing mitochondrial biogenesis in cerebral ischemia reperfusion injury in rats. The pentacyclic triterpene from CA, asiatic acid has shown that it is effective in the treatment of focal cerebral ischemia by significantly reducing infarct volume mediated through decreased blood–brain barrier permeability and reduction in mitochondrial injury (Krishnamurthy et al. 2009). In addition, neuroprotection of the BBB has been mediated through its free radical-scavenging property (Jew et al. 2000). On the other hand, attenuation of mitochondrial injury is brought about by markedly reducing cytochrome c released from isolated brain mitochondria preparations exposed to elevated calcium levels, H_2O_2, or nitric oxide. An aqueous extract of CA ameliorated oxidative damage and enhanced acetylcholinesterase activity caused by intra-cerebroventricular injection of colchicine and 3-nitropropionic acid in different studies (Kumar et al. 2009; Shinomol et al. 2010) demonstrating improvement of the antioxidant status of the brain resulted in above effects.

NJ elicits its neuroprotective effect by restoring and ameliorating thiobarbituric acid reactive substance (TBARS), reduced glutathione (GSH), tail group, catalase, and sodium–potassium ATPase activities in middle cerebral artery (MCA) occlusion model of acute cerebral ischemia in rats. In addition, neurobehavioral activities (spontaneous motor activity and motor coordination) also showed the same result. The mechanism of action of NJ in focal ischemia is hypothesized by virtue of its antioxidant property (Salim et al. 2003).

Chronic treatment of OS significantly prevented hypoperfusion-induced functional and structural disturbances including elevated lipid peroxidation and upregulation of superoxide dismutase (SOD) activity accompanied by a fall in tissue total sulfhydryl groups (TSH) in rat forebrains.

Conclusions and Future Directions

Various lines of evidence have demonstrated that neurodegenerative disorders will require several therapeutic interventions to address the varied clinical aspects of the diseases. As drugs and active chemical compounds listed in the present review are antioxidants/metal complexing, bioenergetic, and anti-inflammatory agents, they are potential therapeutic agents for neurodegenerative disorders. In addition, this multidrug therapy provides an upper hand over monotherapies and paves the way to design specific neuroprotective therapies. However, one of the major aspects of such natural compounds is their limited bioavailability, which limits their use as an active pharmacological agent in neurodegenerative therapies. And hence pharmaceutical companies nowadays are quite interested in developing active ingredients with high bioavailability.

Compliance with Ethics Requirements The authors declare that they have no conflicts of interest.

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Impact of Non-Enzymatic Glycation in Neurodegenerative Diseases: Role of Natural Products in Prevention

Saheem Ahmad and Mohammed Farhan

Abstract Non-enzymatic protein glycosylation is the addition of free carbonyls to the free amino groups of proteins, amino acids, lipoproteins and nucleic acids resulting in the formation of early glycation products. The early glycation products are also known as Maillard reaction which undergoes dehydration, cyclization and rearrangement to form advanced glycation end-products (AGEs). By and large the researchers in the past have also established that glycation and the AGEs are responsible for most type of metabolic disorders, including diabetes mellitus, cancer, neurological disorders and aging. The amassing of AGEs in the tissues of neurodegenerative diseases shows its involvement in diseases. Therefore, it is likely that inhibition of glycation reaction may extend the lifespan of an individual. The hunt for inhibitors of glycation, mainly using in vitro models, has identified natural compounds able to prevent glycation, especially polyphenols and other natural antioxidants. Extrapolation of results of in vitro studies on the in vivo situation is not straightforward due to differences in the conditions and mechanism of glycation, and bioavailability problems. Nevertheless, existing data allow postulating that enrichment of diet in natural anti-glycating agents may attenuate glycation and, in consequence may halt the aging and neurological problems.

Keywords Glycation • Advanced glycation end-products (AGEs) • Diabetes mellitus • Neurological disorders • Polyphenols

Introduction

Glycosylation is the reaction between carbohydrates and the other functional groups of another molecule biological molecule (Ashraf et al. 2014). In biological terms, glycosylation is the process in which glycans are attached to the protein, lipid or

S. Ahmad (✉) • M. Farhan
Laboratory of Glycation Biology and Metabolic Disorder, Integral Research Centre-I, Department of Bio-sciences, Integral University, Lucknow, UP, India
e-mail: saheem@iul.ac.in; ahmadsaheem@gmail.com

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other organic molecule in the presence of enzymes. It is one of the forms of post-transnational modification. According to their binding nature, glycans are divided into five classes.

1. *N*-linked glycans specific to nitrogen of the asparagine and arginine side chains.
2. *O*-linked glycans specific to the hydroxyl oxygen of the serine, tyrosine, threonine, and hydroxyproline or hydroxylysine side chains.
3. Phosphoglycans attach through the phosphate of phosphor serine.
4. *C*-glycans. In this very rare type of glycosylation, sugar is added to a carbon on a tryptophan side chain.
5. Glypiation, the addition of a glycosylphosphatidylinositol (GPI) anchor that attaches protein to lipids with the help of glycans linkage.

This glycosylation process is essential and serves many functions such as some of the protein being misfolded without going under the glycosylation process (Drickamer and Taylor 2006).

Glycation (Non-Enzymatic Glycosylation)

The glycosylation process occurs in the absence of an enzyme reaction, and then this phenomenon is known as glycation or non-enzymatic glycosylation. The glycation process occurs both inside (endogenous) and outside the body (exogenous). It is completed through a series of complex reactions starting from the Amadori reaction, Schiff base reactions, and the Maillard reaction, which ultimately produces advanced glycation end products (AGEs; Fig. 1 (Munch et al. 1997; Ahmad et al. 2011).

Food with added sugar cooked at a high temperature (approximately 120 °C) accelerates the exogenous glycation reaction. However, slow cooking for a long time also promotes AGEs formation. Some studies have shown that glycation also contributes to the formation of acrylamide carcinogen during cooking (Stadler et al. 2002). For the last 50 years, food manufacturers have added AGEs to food as flavor enhancers to improve food quality and colorants to improve to make them appealing (Melpomeni et al. 2003).

Glycation Complications in Diseases

Researchers discovered that AGEs are formed after a non-enzymatic reaction to sugar and the amino group freely present on the protein or deoxyribonucleic acid (DNA) (Akhter et al. 2013; Raheem et al. 2014) and this process is involved in ageing. DNA glycation in the nucleus and the cytoplasm alters the protein product, ultimately changing the function of protein (Akhter et al. 2014; Mustafa et al. 2011). Sometimes it generates free radicals, causing structural alteration of the biomolecule. In diabetic patients DNA glycation causes the formation of neo-antigenic epitopes

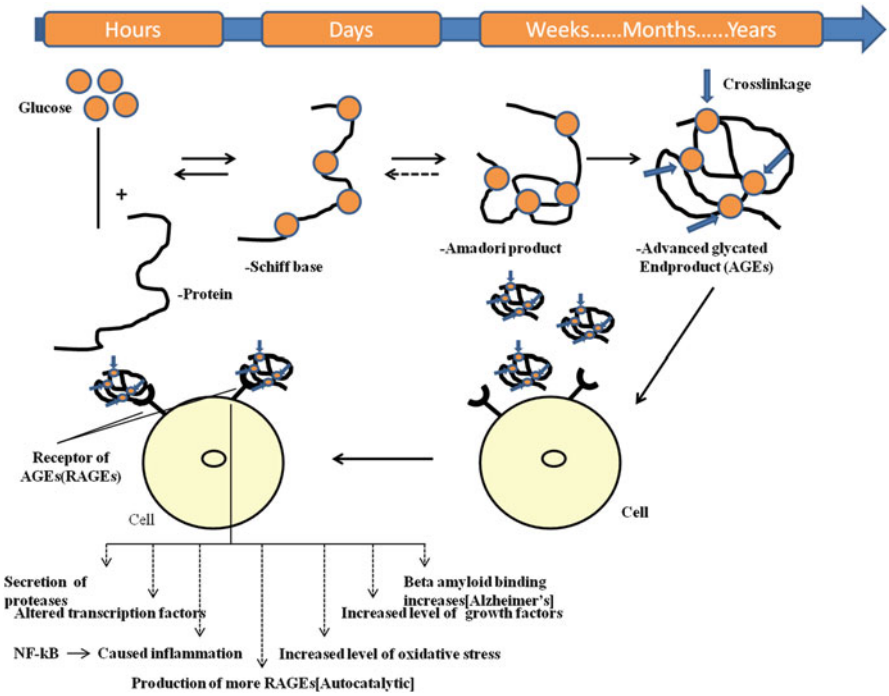

Fig. 1 Pictorial representation of steps involved in the glycation process and its implication in AGE-RAGE signaling

and increases problems in diabetes mellitus (Mustafa et al. 2011; Ahmad et al. 2014; Shahab et al. 2014). Fructose is a prompt glycating agent and a necessary component of the daily diet. In the body, it can modify DNA and generate antibody that can be the cause of the destruction of β-cells of the islets of Langerhans and other problems of diabetes (Takeuchi et al. 2010). Much research shows that hyperglycemia is the first step that causes tissue damage in diabetes because of repeated changes in the metabolism of glucose, or because of the accumulation of AGEs and glycated molecules. Glycation also affects proteins such as serum albumin, lipoprotein, hemoglobin, and insulin, and alters their function (Ahmad et al. 2013a, b, 2014). AGEs cross-link with extracellular matrix protein and activates their respective receptors (RAGEs), resulting in oxidative stress and proinflammatory signaling, which causes microvascular complication, arterial stiffening, and endothelial dysfunction (Negre-Salvayre et al. 2009).

Human serum albumin glycation is also detected in many diseases. It is ten times more reactive with glucose than with HbA1C protein. Human serum protein is present in plasma and it is the most abundant protein of plasma. Binding with the reducing sugars of plasma, it undergoes structural and functional changes and this property makes it sensitive for glycation. Because of its in vivo glycation process with plasma glucose it can be used as a new disease marker in place of HbA1C for diabetes

(Arasteh et al. 2014). The rapidness of glycation also depends on the nature of the carbohydrate, which binds in the process. For example, when we compare glucose and ribose together, ribose induces faster glycation than the glucose and gives rise to a product similar to amyloid products (Wei et al. 2009). In vivo research shows that in a healthy person glycated albumin is present in between 1 and 10 % (Peters 1996). But in the case of a diabetic patient, it increases two- to three-fold compared with a control (Bourdon et al. 1999). Glycated albumin is also implicated in other diseases, such as retinopathy, neuropathy, nephropathy, and coronary artery diseases (Brownlee 1995).

Glycation and Diabetic Problems

Glycation Complications in Diabetic Retinopathy

Retinopathy is the most common complication of diabetes among individuals between the ages of 30 and 70 years (Frank 2004; Chen et al. 2013). The last stage of retinopathy causes blindness. In brief, the processes involved in retinopathy increase blood vessel density, angiogenesis, the permeability of retinal capillaries and the thickness of the capillary basement membrane (Frank 2004). AGEs play a vital role in the succession of diabetic retinopathy and causes dysfunction and the death of retinal cells. The components of the AGE–RAGE complex may be hopeful targets for the treatment of diabetic retinopathy (Zong et al. 2011). Studies suggest that rats have an accumulation of AGEs, and lose the function of Muller glial cells during diabetic retinopathy (Curtis et al. 2011). Recent findings had revealed that the AGE *N*-epsilon-(carboxymethyl)lysine (N-(ε)-CML) is a modulator in the developmental stages of nonproliferative retinopathy in type 2 diabetic individuals (Choudhuri et al. 2013). Some reports show that AGEs are the major contributors to increasing the permeability of retinal endothelial cells. AGEs cross-linked with protein cause vascular stiffness and ECM protein modification, leading to decreased pericyte adherence (Vasan et al. 2003). AGEs also induce pericyte death and the signaling pathway involved is through the generation of oxidative stress, leading to the inhibition of protein kinase B/Akt phosphorylation (Stitt et al. 2004).

Glycation in Diabetic Cataract

Loss of transparency of the eye lens is the primary step of cataract development and AGEs play a key role in this process (Hashim and Zarina 2011). Reports revealed that in the diabetic individual the progression of cataracts is swift (Harding et al. 1993). In a diabetic individual's cataract the protein of the lens becomes glycated, which ultimately leads to blindness (Luthra and Balasubramanian 1993). Some research reports suggested that AGEs cause vision impairment by accumulating in the eye lens and inducing severe changes in structural protein. Finally, they lead to protein aggregation in the lens and these high molecular weight aggregates scatter light and hamper vision

(Nagaraj et al. 2012). A few reports revealed that AGEs, by changing the surface charge of the protein, affect protein–water and protein–protein interactions and finally decrease the transparent properties of the eye lens (Beswick and Harding 1987; Kumar et al. 2004). In diabetes, a patient's glucose level in aqueous humor increased and induced protein glycation. This process results in the generation of superoxide radicals and production of AGEs. In another piece of research, the binding of AGEs with the RAGE present on the epithelium of the lens elevated O^{2-} and H_2O_2 levels (Gul et al. 2008). All these reports demonstrated that protein glycation altered the protein structure, leading to changes in the amino acid involved in cataract development.

Glycation in Diabetic Neuropathy

Diabetic neuropathy affects both peripheral and autonomic nerves. It also causes diarrhea, constipation, and urinary incontinence. Studies have suggested that AGE–RAGE might play a key role in the pathogenesis of diabetic neuropathy (El-Mesallamy et al. 2011). It has already been shown in previous research that glycolaldehyde (one type of precursor of AGEs) at a physiological concentration slows down the viability of rat Schwann cells. This plays a significant role in diabetic neuropathy. The presence of AGEs has also been examined in the peripheral nerves of diabetic patients. CML is present in the basement cell and in Schwann cells (Sugimoto et al. 1997). Fiber loss, which occurs in human diabetic peripheral nerves, is due to the accumulation of AGEs. AGEs may also interfere with axonal transport and this leads to the development of atrophy and nerve fiber degradation. P0 protein, which is present in nerves modified by AGEs causes demyelination of the nerve fibers (Vlassara et al. 1981). Previous research revealed that the interaction between AGEs and RAGE triggered the transcription factors NF-αB and activator protein-1 (AP-1) and interleukin-6 (Schmidt et al. 1995).

Advanced Glycation in Atherosclerosis

Owing to the invasion and accumulation of white blood cells, the artery wall becomes thicker and atherosclerosis occurs. The accumulation also contains active white blood cells, which are producing inflammation and dead cells, including cholesterol and triglycerides. Atherosclerosis is further promoted by low-density lipoproteins without proper removal of cholesterol and fats from macrophages with the help of functional high-density lipoprotein (HDL). Much research revealed that advanced glycated product plays an important role in the modification of LDL that ultimately promotes atherosclerosis. Basically, AGEs start oxidative reactions that induce the formation of oxidized LDL. A recent study by Naila Rabbani et al. (Rabbani et al. 2011) shows that the glycation of LDL by methylglyoxal (MG) increases arterial atherogenicity. MG attached to the arginine residue of LDL results in the final product hydroimidazolone, also known as the MG-H1 complex. The study revealed that after the modification of

LDL, it becomes smaller and also has an effect on functional changes such as increasing aggregation, binding with proteoglycan, and increasing the accumulation of MG-H1 in the arteries. This peptide mapping and informatics study discloses that MG modified apoB-100 of LDL at the R18 target site. Another study by Basta et al. (Basta et al. 2009) suggests that AGE accumulation might amend vessel wall homeostasis in a pro-atherogenic fashion via multiple mechanisms: extracellular matrix permeability alteration, inflammatory cytokines and growth factor secretion, antithrombotic properties, endothelial alterations, and by the elevated level of adhesion molecules and chemokines on the surface of the vascular cells.

In their 2009 study, Basta et al. examined the plasma level of sRAGE, AGE, and carboxymethyl (lysine) (CML) adduct using an enzyme-linked immunosorbent assay and tissue levels of AGEs and RAGEs were detected by immunohistochemistry. The study was based on 29 patients with carotid atherosclerosis. In those patients, 10 patients had no symptoms of disease and 19 had symptoms. This study revealed that plasma levels of sRAGE were higher in symptomatic patients compared with asymptomatic patients. The researchers concluded that sRAGE in the plasma of symptomatic carotid atherosclerosis is higher than in asymptomatic carotid patients (Basta et al. 2009).

Previous studies showed that AGEs cross-link protein, which is helpful in altering the flexibility and digestibility of the collagen matrix in the vascular cell wall and in the skin. Since the receptor characterization of AGEs, it has been clear that AGEs initiate their biological effects via receptor-coupled signaling pathway. AGEs may also interact with the RAGEs of endothelial cells to activate cellular events such as the regulation of transcription factor NF-kB (Bierhaus et al. 1997), and the activation of p38 MAP kinase, NAD (P) H-oxidase (Wautier et al. 2001), and ERK1/2 MAP kinase cascades. In recent studies, S100/calgranulins have been reported to accumulate at the site of chronic inflammation. HMGB1 (amphoterin) was also identified as an activator of the RAGE–NF-kB axis. In the nucleus, NF-kB increases the transcription of RAGE and other genes relevant to atherogenesis such as VCAM-1, endothelin-1, intracellular adhesion molecule-1, and the pro-inflammatory cytokines interleukin-6, interleukin-1b, and tumor necrosis factor (Fig. 2). In the report by Basta et al. (2005) it is stated that exposure to CML–albumin increased the expression of VCAM-1 in endothelial cells and also RAGES-dependent reactive oxygen species (ROS) formation. These effects were inhibited by NAD (P) H-oxidase inhibition and by precise anti-RAGE antibody. The activation of RAGE genes and the RAGE gene-mediated signaling cascade triggered a vicious cycle. The production of AGE, cytokines, and ROS involved in atherogenesis may be interlinked and may connect, as shown in the schematic Fig. 2.

Role of Glycation in Ageing

Many studies suggested that glycation might play an important role in the process of ageing. The final products of glycation were seen and their accumulations were reported in several previous studies (Peppa et al. 2008; Nowotny et al. 2014). In

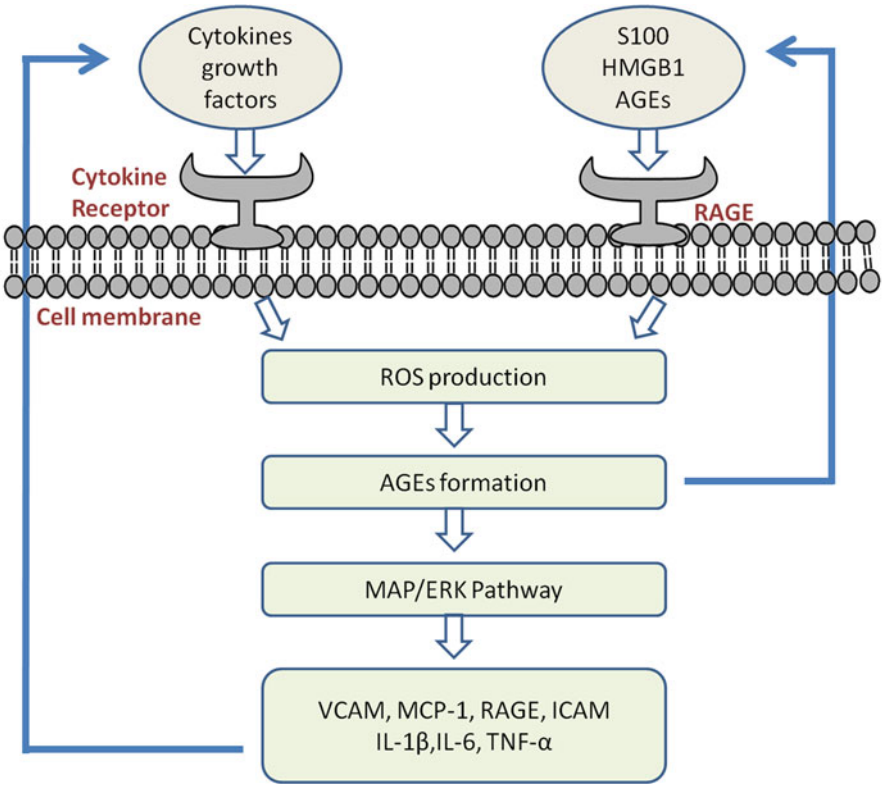

Fig. 2 Effect of ROS and AGEs formation in vascular cells and its involvement in MAP/ERK pathway which leads to the different cytokines secretion

1980, when AGEs were found to accumulate in the tissue of a living organism as it aged, this process engendered the theory of non-enzymatic glycosylation (Monnier et al. 1981). Many problematic age-related changes are actually caused by protein degradation, such as the accumulation of molecular waste, post-translational modification, functional disorders of the tricarboxylic acid cycle, deterioration of functional proteins, and activation of inflammatory pathways controlled by intracellular signaling. These changes are symptomatic of "glycation stress" (Ichihashi et al. 2011). Sometimes, proteases and other oxidizing degradation enzymes are metabolized within the proteasome and the waste product excreted. There are so many other enzymes that can modify AGEs and intermediate compounds. For example, glyoxalase 1 converts the highly reactive α-oxo-aldehydes into the α-hydroxy acids using L-glutathione as a cofactor. However, the activity of proteolytic enzymes decreases with age (Nowotny et al. 2014; Lee and Chang 2014). A decrease in oxidative stress with the application of α-lipoic acid also decreases the accumulation of AGEs, and this was also associated with a reduction in cell cycle reentry and a more euploid neuronal genome (Kuhla et al. 2015). Galactose is more

effective than glucose as a glycating agent. The amount of aldehyde present in the galactose is many times higher than the amount of the glucose (Dworkin and Miller 2000). A high amount of galactose in the diet has been shown to cause premature ageing and in this process cytochrome *c*, which is released from mitochondria, plays an important role by causing apoptosis (Lu et al. 2010). This effect was attenuated by salidroside, known as the inhibitor of RAGE (Mao et al. 2010). Metformin is an inhibitor that is used for the inhibition of AGEs formed by the monosaccharides, also known as geroprotectors (Severin et al. 2013). For the cellular level, aminoguanidine inhibitor was used and reported studies show an increase in the replicative lifespan of the human lung fibroblast from 54 up to 75 population doublings (at 4 mM aminoguanidine) and a decrease in the rate of telomere shortening by about 50%. Several mechanisms are known that can also contribute to the inhibition of glycation (Wang et al. 2007). Thus, it has been postulated that the aggregation of AGEs is the basis of the "biological clock" leading to ageing (Severin et al. 2013). Ageing is related to the chronic low-grade inflammatory status that also causes chronic disease such as kidney disease, age-related muscle wasting, and diabetes mellitus. AGEs are proinflammatory in nature. Studies on people with a low-AGEs diet show decreased inflammation, whereas in subjects with a high-AGE diet had increased inflammation (Van Puyvelde et al. 2014). Half of all the observational studies show the relationship between the inflammatory process and AGEs in food. The level of circulating AGEs and inflammation status are directly related to the dietary intake of AGEs. Restricting AGE intake may lower the level of inflammation and also decrease chronic disease-related inflammation. A previous study on mice (Van Puyvelde et al. 2014) shows that lowering the AGE content and the normal diet significantly curtails AGE accumulation. Short-term human trials revealed that a low AGE diet decreases inflammatory markers and the oxidant burden (Peppa et al. 2008). AGE accumulation plays an important role in skin ageing. The aggregation of AGEs has been noticed in various tissues during diabetes and ageing, including glomerular basement membranes, skeletal and smooth vascular muscles, and articular collagen (Verzijl et al. 2000a; Haus et al. 2007; Sell et al. 1993). Accordingly, the deposition of AGEs in these tissues has been involved in various age-associated pathological conditions such as diabetic angiopathy, nuclear degradation in diabetes, and osteoarthritis (Vlassara et al. 2002; Glenn et al. 2007; Stitt 2001; DeGroot et al. 2001).

The skin is the largest organ of the body. Its easy accessibility is an excellent prospect for the minimally invasive or non-invasive analysis of glycation, taking advantage of the typical autofluorescent properties of AGEs. Therefore, AGEs in skin have been studied thoroughly and detected in diabetes and also during chronological ageing (Jeanmaire et al. 2001; Sell et al. 1996; Schleicher et al. 1997). It is a general view today that AGE aggregation is dependent on the protein turnover value; thus, long-lived proteins are mainly modified by glycation (Verzijl et al. 2000b). Types I and IV collagen, showing a slow turnover value of about 10 years and one dermal protein fibronectin, mainly suffer from glycation at the time of intrinsic chronological ageing (Jeanmaire et al. 2001; Dyer et al. 1993). The first appearance of glycated collagen is observed at the age of 20. The yearly rate of

accumulation is about 3.7% and has increased 30–50% by 80 years of age (Jeanmaire et al. 2001). CML was histochemically detected in the human epidermis from healthy donors (Stitt 2001). The authors identified cytokeratin 10 (CK10) proteins, which are a target protein for CML modification. The total amount of CML in younger donors is less than in older ones. This study shows AGE involvement in the epidermal physiology and the probable involvement of short-lived proteins in glycation chemistry. Additionally, in an in vitro organ skin model, the epidermis and dermis functions were modified by glycation.

The effect of UV irradiation has been mainly recognized by proinflammatory changes, oxidative damage, apoptosis, mutagenesis, and matrix metalloproteinase induction (Nowotny et al. 2014). However, it has been revealed that sun-exposed skin has a higher accumulation of AGEs compared with sun-protected skin (Jeanmaire et al. 2001; Dunn et al. 1991). Accumulation of AGEs was higher in the sun-exposed skin, showing UV irradiation, which may also play a key role in the formation of AGEs in vivo (Mizutari et al. 1997). All those results show that, like smoking, another factor that ages the skin, AGEs accumulation is also involved in the various structural and functional modifications at the time of photoaging. Diet is also an environmental factor for ageing. Dietary AGEs are directly compared with inflammatory markers and serum levels of AGEs in healthy human subjects (Uribarri et al. 2007). It has been commonly accepted that AGEs, once formed, can be removed only when the degradation of the modified proteins occurs. However, it has now become noticeable that in the organism different enzymatic systems seem to be implicated in the degradation or elimination of AGEs. As shown above, GLOI is an enzyme that is also responsible for the elimination of reactive α-dicarbonyl compounds (Ramasamy et al. 2005). AGE existence in biological molecules modifies their functional and biomechanical properties. Lipids, nucleic acids, and proteins can be the main targets of advanced glycation, modifying protein–DNA interactions, enzyme–substrate interactions, protein–protein interactions, epigenetic modulation, and DNA regulation, thus interfering with several physiological functions of the organism. Although, AGEs are themselves reactive molecules, they interact with their receptors and activate various molecular pathways in vivo, thus becoming involved in inflammation, cell proliferation, immune response, and gene expression.

Glycation in Neurodegenerative Diseases

Parkinson's Disease

The blood–brain barrier (BBB) plays an important role in supplying nutrients to brain tissue and also in filtering harmful compounds from the brain back to the circulating blood. Disturbance of the BBB is linked to some neurodegenerative disorders, including Parkinson's disease (PD). Iron deposition, oxidative stress, and mitochondrial impaired function are risk factors for the degradation of the central nervous system (Schipper 2004). Additionally, it has been assumed that

inflammation in the BBB is related to the risk of the increased formation of cytokines, over-activation of the microglia, and the release of ROS (Whitton 2007).

Parkinson's disease is currently the most common neurodegenerative disorder; the current demographic drift indicates a life-time danger approaching 4 % (Schapira 2013). The average age at onset is 70 years, but most of the patients develop early-onset PD, before the age of 50 (Schrag and Schott 2006). The symptoms of this disease are tremors, slowness of movements, rigidity, and postural imbalance. The main pathological abnormalities are the pigment loss in the pigmented cells of the pars compacta in the substantia nigra, and the dopaminergic neurons, which decrease the dopamine level (Lu'o'ng and Nguyen 2012). The pathogenesis of neurodegeneration in PD is that patients have high levels of oxidized lipid and low levels of glutathione (Zeevalk et al. 2008; Obeso et al. 2010; Schapira 2012). The concentration of polyunsaturated free acids and phospholipids, which are highly susceptible to oxidants, is decreased in these patients, although malondialdehyde, a lipid oxidation marker, is increased (Dexter et al. 1989; Zhou et al. 2008).

In the age-related neurodegenerative disorders, such as Alzheimer's, Parkinson's, and Huntington's diseases, tissues have an abnormal accumulation or aggregation of proteins. These proteins come under glycation and form AGEs within the tissue, and AGE accumulation aggravates these diseases. Research by Shaikh et al. published in 2008 revealed that AGE accumulation promotes in vitro cross-linking in alpha-synuclein and accelerates the formation of alpha-synuclein-positive inclusion bodies (Shaikh and Nicholson 2008). As it is already known that alpha-synuclein is a protein that contains 140 amino acids, and is encoded by a single gene of seven exons situated in chromosome 4 (Chen et al. 1995). This protein is present in the nucleus and presynaptic nerve terminals and was also known as a synuclein. Recently, research by Guerrero et al. showed that α-synuclein present in neurons is glycated and glycosylated within the neurons and in vitro, which ultimately affects DNA binding properties. Glycated α-synuclein causes increased genome damage by its direct interaction with DNA and via the increased generation of ROS as a glycation byproduct (Guerrero et al. 2013).

α-Synuclein Aggregation and Cell Death

The aggregation and neurotoxicity of α-synuclein can be grouped into three major classes—mechanical, cellular disruption, toxic loss of function, and toxic gain of function. Common examples are the permeation of cellular membranes by amyloid aggregation. α-Synuclein oligomers bind to the lipid of membranes and break membrane bilayers (Auluck et al. 2010; Van Rooijen et al. 2010). Some α-synuclein has the properties to penetrate the membranes and form pore-like structure (Giehm et al. 2011; Volles and Lansbury 2003). It has also been proposed that amyloid oligomers cause membrane permeation without forming pores (Kayed et al. 2003). It is assumed that this is one of the key mechanisms of protein aggregate toxicity.

Additionally, α-synuclein degradation via proteasome inhibition by the copper-dependent generation of ROS has been proposed for the neurotoxicity of α-synuclein neurotoxicity (Brown 2010; Bennett 2005).

First, glycation reported in the locus ceruleus and substantia nigra shows an increase in immunoreactivity at the periphery of the Lew bodies (LBs) of PD patients (Castellani et al. 1996). Additionally, the colocalization of AGEs with α-synuclein is observed at a very early stage; in this case, α-synuclein is also present at the periphery of LBs (Munch et al. 2000). All the results taken together suggest that glycation might play an important role in the proteolytic resistance of the protein deposits and in the chemical cross-linking. Although glycation was also detected in the amygdala, cerebral cortex, and substantia nigra of older control patients, the level and the number of glycated proteins were substantially higher in PD patients (Dalfo et al. 2005). RAGEs were also highly expressed in PD individuals compared with controls, signifying the role of AGEs in PD.

In PD patients the cellular level of reduced glutathione (GSH) decreases in the early stages of the disease, which ultimately results in a decrease in the activity of the glyoxalase system. Glyoxalase is the main catabolic pathway of important glycation agents such as MG (Thornalley 1998). Carbonyl stress increases the concentration of AGEs, which elevates the oxidative stress that finally induces AGE formation. This harmful cycle may contribute to cell damage previously reported in dopaminergic neurons. Moreover, dopamine degradation and its autoxidation also contribute to increases in the level of oxidative stress (Lee et al. 2009).

Alzheimer's Disease

In a recent survey published in 2014, data showed that between 21 and 35 million individuals worldwide suffer from Alzheimer's disease (Querfurth and LaFerla 2010), most of the cases being found in people over 65 years of age. In 2010, about 486,000 people were dying of dementia (Lozano et al. 2012). This disease was first described by German pathologist and psychiatrist Alois Alzheimer in 1906 (Berchtold and Cotman 1998). The treatment for Alzheimer's is becoming increasingly expensive and is a major problem for developing countries. It belongs to a type of chronic neurodegenerative disease that progresses slowly and becomes worse over time (Burns and Iliffe 2009). The common symptoms of this disease are short-term memory loss. When the disease is advanced, symptoms include disorientation, loss of motivation, and problems with language. The cause of AD is not well understood; various hypotheses have been proposed to explain AD development. Sixty to seventy percent of dementia leads to AD. The term dementia describes symptoms including memory loss and problem-solving, language, and thinking difficulties.

Neurons are injured and dead in the hippocampal region of the AD patient, which is involved with memory and learning, but this degeneration affects the whole brain (Shaffer et al. 2013; Swardfager et al. 2010). Amyloid beta (Aβ) is a type of peptide

that is an abnormal proteolytic byproduct of the transmembrane protein amyloid precursor protein (APP). The function of this protein is unclear, but it may be involved in neuronal development. Aβ is a monomer in nature and contains short regions of beta sheets at high concentrations. After dramatic conformational changes it is a beta sheet-rich tertiary structure that aggregates to form amyloid fibrils. Fibrils make a dense formation outside the neurons. This layer is called neuritic plaque. In Alzheimer's disease, one new protein aggregation was observed in neurons. It is a tau microtubule protein that is highly expressed in neurons. Tau protein works as a microtubule stabilizer in the cell cytoskeleton. Like other microtubule-associated proteins, tau is also regulated by the phosphorylation process. Hyperphosphorylated tau (P-tau) accumulates and forms paired helical filaments, which finally aggregate into masses inside the nerve cells in AD patients. These phenomena are called neurofibrillary tangles (NFTs) and dystrophic neurites linked to amyloid plaques (Goedert et al. 2006).

Recent evidence suggests changes in the cerebrospinal fluid (CSF) levels of P-tau, tau, and Aβ, and that the level of CSF might not be static over the course of the disease. The mechanism behind the senile plaque and NFTs is still unknown at present. Senile plaques and NFTs prompt the injury and death of neurons, and as a consequence memory loss and symptomatic behavioral changes. Inflammation within the brain and increased reactivity of the microglia toward amyloid deposition have been involved in the pathogenesis (Revett et al. 2013).

AGEs in Alzheimer's Disease

Amyloid beta peptide deposition starts early in the course of AD and increases markedly during progression of the disease. The advanced step of Alzheimer's leads to the generation of NFTs, which cause neuronal death (Selkoe 1994). Some studies revealed that the presence of AGEs in the senile plaque and NFTs were identified by the immunohistochemical analysis of samples obtained from the AD patient (Smith et al. 1994; Sasaki et al. 1998). Aβ glycation markedly increases its aggregation in vitro (Vitek et al. 1994). Glycation of tau protein and its hyperphosphorylation enhance the formation of paired helical filaments (Ledesma et al. 1994; Yan et al. 1994). Taking all data together, there is the implication that AGEs may be an important factor involved in the progression of neurodegenerative disorders. The deposition, aggregation and modification of the protein are the well-known part of many pathological processes and play a direct role in tissue damage. From recent studies, it has become clear that AGEs also play a role in neurodegenerative diseases such as AD (Sasaki et al. 2001), amyotrophic lateral sclerosis (ALS) (Kikuchi et al. 2002; Chou et al. 1998), PD (Castellani et al. 1996), and Creutzfeldt–Jakob disease (Sasaki et al. 2002).

Very little is known about the relationship between AD and glucose tolerance, and the higher occurrence of AD among diabetic patients is still controversial. Recently revealed links between AD and type 2 diabetes consist of the detection of

AGEs and increased AGE receptor in the brain tissue of patients with AD (Munch et al. 1998; Yan et al. 1996). Research suggests that patients with diabetes mellitus are at almost double the risk of AD and dementia. AGEs are implicated in diabetic complications, but the degradation of AGEs and AGE-modified protein is not clear at the time of renal dialysis in diabetic patients (Takeuchi et al. 1999; Makita et al. 1994). AGEs of low molecular weight have been shown to be chemically active and also contribute to the damage and further modification of tissue protein (Makita et al. 1994). It is an interesting point to determine that AGE formation is implicated in abnormal tau protein processing and Aβ deposition, which has been detected in the brain of patients at the time of renal dialysis (Harrington et al. 1994). Riviere et al. (1998) measured plasma protein glycation basically derived from glucose in AD patients (Riviere et al. 1998). In plasma, protein glycation evaluated by plasma furosine, was approximately two times greater in AD subjects than in controls. Recently, Shuvaev et al. (2001) quantified the level of an Amadori product, an early glycation product in CSF in late-onset AD and in aging. The amount of an Amadori product in CSF correlated with the glucose concentration of CSF, but did not change as age increased. In brief, the level of CSF Amadori product was found to be 1.7 times higher than in an age-matched control group (Shuvaev et al. 2001).

Role of Natural Products in the Prevention of Neurodegenerative Diseases

It is well established that 80 % of drug molecules are natural products or inspired by natural compounds. Discoveries of new drugs from natural products have been made since the Vedic period. Historically, plants and other natural product-derived drugs were used in the treatment of many major afflictions such as cancer, cardiovascular diseases and neurological conditions and also have their future utilization. The traditional Indian System of Medicine has a very long history of usage in a number of diseases and disorders, but there is a lack of recorded safety and efficacy data. Ayurvedic Indian and Chinese systems are great traditional systems that have relatively organized databases, and more exhaustive descriptions of botanical materials that are available and that can be tested using modern scientific methods. Both systems of medicine thus have an important role to play in the bioprospecting of new medicines. The investigation of natural products as a source of new human therapeutics reached its peak in the western pharmaceutical industry during the period 1970–1980, which resulted in a pharmaceutical landscape extremely influenced by nonsynthetic molecules. Recently, it has been suggested that drug discovery should not always be limited to discovery of a single molecule, and the current belief is that rationally designed polyherbal formulation could also be investigated as an alternative in multitarget 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.

Many inhibitors have been discovered, both natural and synthetic, against the formation of AGEs. Synthetic inhibitors are divided into three classes:(1) Carbonyl-capturing agents, which attenuate carbonyl stress (2) Cross-link breakers(3) Metal ion chelators (Reddy and Beyaz 2006)However, all types of synthetic agents were withdrawn from the clinical trials because of their low efficacy, unsatisfactory safety, and poor pharmacokinetics (Kawanishi et al. 2003; Manzanaro et al. 2006). Aminoguanidine is also a nucleophilic hydrazine synthetic compound that blocks the formation of AGEs withdrawn from the third phase of clinical trials owing to the lack of efficacy and to safety concerns (Thornalley 2003). Alternatively, natural products have been proven to be safe for human consumption and many plant extracts have been tested for their antiglycation activity (Lee et al. 2006). Additionally, numbers of plant-derived agents have been shown to possess hypolipidemic, hypoglycemic, and antioxidant properties (Akhter et al. 2013; Vasu et al. 2005; Iqbal et al. 2014; Hashim et al. 2014). Phenolics (Choudhary et al. 2010), carotenoids (Sun et al. 2011), unsaturated fatty acid (Sun et al. 2010), polysaccharides (Meng et al. 2011), and many others have been shown to have antiglycating properties. Consequently, the consumption of dietary components on a daily basis from the plant source is potentially beneficial for the prevention of diabetes and its complications (Yazdanparast et al. 2007). Some of the best examples are the ethanol fraction of *Melissa officinalis*, L (Lemon balm), which were reported to possess high inhibitory properties on AGEs formation in late glycation (Miroliaei et al. 2011). Green tea significantly decreases the level and the accumulation of AGEs, and in diabetes, it reduced the cross-linking of tail tendon collagen (Babu et al. 2008). Caffeoylquinic, *p*-coumaroylquinic, feruloylquinic, and dicaffeoylquinic acids present in coffee inhibit protein glycation and the formation of dicarbonyl compounds (Verzelloni et al. 2011). Taurine amino acid was shown to decrease acrylamide production in a potato model, implicating its use in food processing to reduce acrylamide formation (Shin et al. 2010).

Polyphenols

The anti-glycation activity of several medicinal herbs and dietary plants was similar to or even stronger than that of aminoguanidine (Ma et al. 2011). Some studies have confirmed that the anti-glycation activity compared significantly with the phenolic content of the experimental plant extracts (Peng et al. 2008). Polyphenols are the most profuse dietary antioxidants, commonly present in the cereals, seeds, fruits, vegetables, nuts, chocolates, and beverages, such as tea, coffee, and wine. They have some health benefits such as the prevention of cancer (Landis-Piwowar et al. 2007), cardiovascular disease (Vinson et al. 2006), and neurodegenerative disease. Polyphenols as groups of natural products contain several sub-groups of phenolic compounds. They are classified by the biological function, source of origin, and chemical structure.

Also, bulk amounts of polyphenols exist in plants as glycosides, with many sugar units and with sugars at various positions of the polyphenol skeletons (Tsao 2010).

On the basis of the chemical structure of the aglycones, polyphenols are divided into the following groups:

1. Phenolic acids

Phenolic acids are the most important antioxidant phytochemicals naturally present in vegetables and fruits. They are non-vitamin in nature. The biological activity of phenolic acid depends on the lipophilicity and presence of ring-substituted hydroxyl groups. Phenolic acid is present in the nonflavonoid polyphenolic compound category, which can be divided into two parts cinnamic acid and benzoic acid derivatives, based on C3–C6 and C1–C6 backbones (Fig. 3) (Tsao 2010).

Cinnamic acid

Caffeic acid (Fig. 4) is a natural cinnamic acid found in herbs and vegetables, e.g., basil, coffee, pear, oregano, and apple (Clifford 1999). Gugliucci et al. in 2009 demonstrated that caffeic acid present in *Ilex paraguariensis* extracts inhibits the formation of fluorescent AGEs in in vivo experiments (Gugliucci et al. 2009). Additionally, extracts from species *Chrysanthemum* (*C. indicum* L. and *C. morifolium* R.) confirmed the marked inhibition of the generation of AGEs and CML in in vitro experiments. Other species of *Chrysanthemum* (*C. morifolium* R.) contain flavonoid glucoside varieties, chlorogenic acid, and apigenin (Tsuji-Naito et al. 2009). Certain *trans* cinnamic acid and quinic acid combined and formed chlorogenic acid, which is present in pineapple, strawberries, and sunflower. 5-caffeoylquinic acid (5-CQA) is a commercially available chlorogenic acid and has been widely studied because of its antioxidant activity. Chlorogenic acids are metal scavengers and free radicals; they may also hinder glucose absorption and have been shown to change the gene expression of antioxidant enzymes (Fiuza

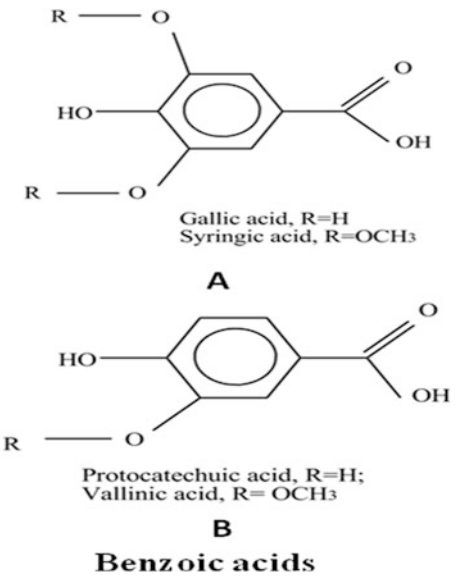

Fig. 3 Different structure(s)(A&B) of benzoic acid

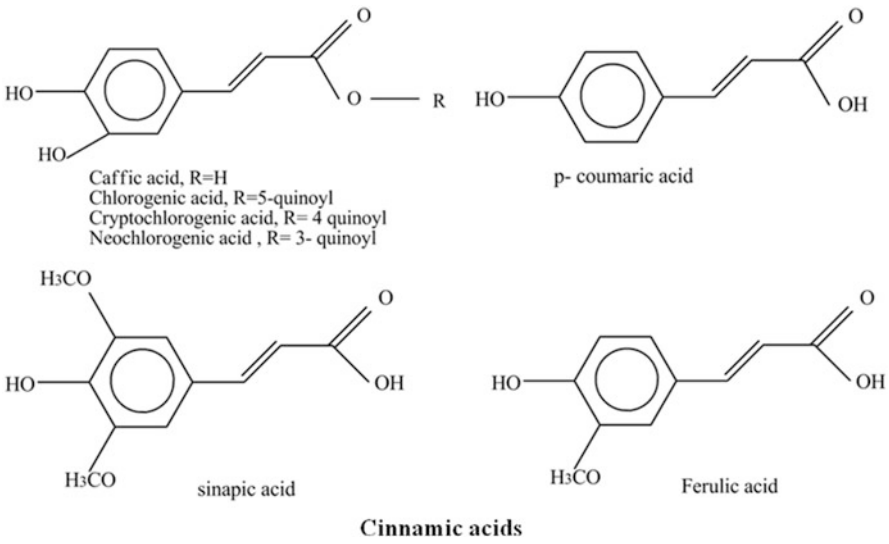

Fig. 4 Phenolic acid- different structure of cinnamic acid

et al. 2004). The chlorogenic acid fraction present in coffee has been shown to inhibit the generation of CML in a concentration-dependent manner. Moreover, polyphenols such as caffeoylquinic, dicaffeoylquinic, p-coumaroylquinic, and feruloylquinic acids contributed to more than 70% of the antioxidant property of the coffee fractions (Verzelloni et al. 2011). In particular, *Ilex paraguariensis*, like coffee, contains a high amount of caffeic acid, generally esterified as chlorogenic acid (Gugliucci et al. 2009). High amounts of chlorogenic acid were also found in *Chrysanthemum morifolium* R. (Tsuji-Naito et al. 2009). Jang et al. isolated three quinic acids derived from the leaves and stem of *Erigeron annuus.* the structure identified is 3-caffroylquinic acid, 3,5-di-*O*-caffrolyl-epi-quinic acid, and 3,5-di-*O*-caffeoylquinic acid methyl ester. The 3,5-di-*O*-caffeoyl-epi-quinic acid compound exhibited most effective activity against AGE generation and in stopping the opacification of rat lenses, whereas 3-caffeoylquinic acid was less effective. Sucrose ester and caffeoyl erigerosides were also more efficient AGE inhibitors than aminoguanidine (Jang et al. 2010).

Ferulic acid

Ferulic acid (FA) is one of the natural cinnamic acids available in food and drinks, e.g., fruits, oats, and vegetables (Wang et al. 2009). It has free radical scavenging properties of oxidized low-density lipoprotein and hydroxyl radicals (Kikuzaki et al. 2002). A few reports have shown that FA reacts with HSA and finally forms a complex structure (Kang et al. 2004). These structures have a decreased HSA α-helix structure, which has led to many other structural changes within the protein. In 2011, Miroliaei et al. published research data that demonstrated that the

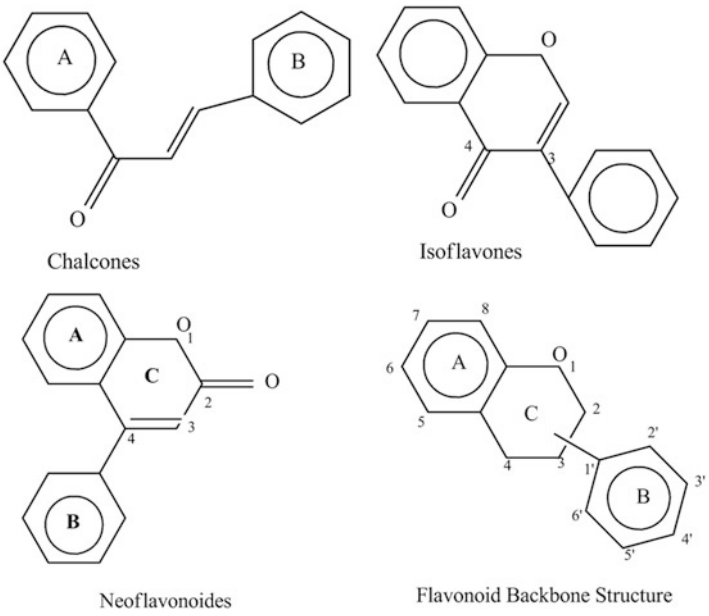

Fig. 5 Structure of various flavonoids

treatment of BSA with the herb *Melissa officinalis* L. prevents structural changes occurring because of D-glucose. The extract contains rosmarinic acid, which has the potential to prevent changes in the glycation site and after that produces a barrier for cross β-structure formation. Additionally, a research paper published by Ma et al. in 2011 showed that the isolation of rosmarinic acid from *Salvia miltiorrhiza Bge* has more inhibitory potential against AGE formation (Ma et al. 2011).

2. Flavonoids

The general structural backbone of flavonoids consists of C6–C3–C6. The two C6 units are phenolic in nature (ring A and ring B; Fig. 5). With the hydroxylation pattern and chromane ring (ring C) variation, flavonoids can be further divided into different sub-groups such as flavanones, flavonols, flavan-3-ols, flavones, and anthocyanins. In the majority of cases of flavonoids, ring B is attached to the C2 position of ring C. In some cases, ring B is attached to the C3 and C4 positions of ring C, known as isoflavones and neoflavonoids respectively. Various types of vegetables, tea, and red wine are rich in flavonoids (Terao et al. 2008). Several flavonoids are antioxidant in nature, effective in trapping free radicals and participating in balancing the overall plant cell redox homeostasis (Hernández et al. 2009). In various lipid system flavonoids show their antioxidant properties, which are helpful in preventing atherosclerosis (Terao et al. 2008). *Cuminum cyminum* (CC), commonly known as jeera, have 51.87% w/w flavonoids, which are

responsible for its antiglycation property. Researchers have revealed that streptozotocin-diabetic rats, when treated with CC, had reduced oxidative stress in the renal region and decreased AGE accumulation by enhancing the antioxidant defense and also reducing lipid peroxidation induced by free radicals. Moreover, experiments suggested that the antihyperglycemic properties of CC may be caused by the protection of surviving pancreatic β cells (Jagtar and Patil 2010).

Owing to the lack of the heterocyclic ring C, chalcones are still present in the flavonoid family (Tsao 2010). The chalcone butein, obtained from an ethyl acetate fraction of *Rhus verniciflua*, is the effective inhibitor of human ALR2 at a concentration of 0.7 μM (IC50 value). Butein also potently inhibits AGE accumulation in vitro. Some research reports say that the methylation or glycosylation of the 3′- or 4′-hydroxyl group decreases this activity, whereas the hydroxyl groups at the 3′-, 4′-, 5-, and 7-positions of flavones raise their AGE-inhibitory activities (Matsuda et al. 2003).

Dihydrochalcones are the major sub-family of flavonoids and is present in *Malus domestica* (apple trees). It is found in large amounts in immature fruits and in leaves (Pontias et al. 2008). Phloridzin and its aglycone phloretin are the simplest forms of dihydrochalcones (Williams 1964). The study by Bernonville et al. (2010) suggests the presence of the combination of phloridzin with two additional dihydrochalcones, identified alone as trilobatin and sieboldin. Phloridzin in the intestine inhibits glucose absorption and renal resorption, which ultimately results in the normalization of glucose in the blood (Herenkranz et al. 2005). On the basis of antioxidant assay results, sieboldin was much more efficient than phloridzin in the inhibition of AGE formation (Dugé de Bernonville et al. 2010).

Isoflavones

In isoflavones, ring B is attached to the C3 (Fig. 5) position of ring C. They are richly present in the leguminous family of plants (Tsao 2010). Soybeans and soy products are particularly important sources of isoflavones, which have both phytoesterogenic and antioxidant activities that may contribute to their potential cardioprotective and anticarcinogenic effects (Rimbach et al. 2008). Daidzein and genistein are two main isoflavones present in soy along with formononetin, glycetein, and biochanin (Mazur et al. 1998). Hsiesh et al. reported in 2009 that soy isoflavone administration significantly attenuates oxidative damage and improves parameters linked to aging and Alzheimer's disease (Hsieh et al. 2009).

Puerarin (daidzein-8-C-glucoside) is another isoflavone glycoside collected from the root of *Pueraria lobata* and it has a range of pharmacological effects, including anti-allergic and anti-hyperglycemic properties (Hsu et al. 2003). Moreover, puerarin has been reported to successfully inhibit AGE formation, which is a common risk factor in diabetic individuals and in neurodegenerative diseases (Kim et al. 2006). In 2010, Kim et al. demonstrated that puerarin treatment administered to mouse mesangial cells increased heme oxygenase-1 (HO-1) protein levels with

increases in dosage (Kim et al. 2010). This enzyme is also helpful in the conversion of heme to biliverdin, which is quickly metabolized to bilirubin (Alam and Cook 2003). Additionally, puerarin treatment increases the phosphorylation of the protein kinase C δ-subunit, which basically regulates the expression of HO-1, inhibiting AGE-induced inflammation in the mesangial cells of mice.

Conclusion

Overall, the research concluded that the process of glycation and AGEs were found in all types of diseases: diabetes, arthritis, and most neurodegenerative disorders. The accumulation of these AGEs present in the tissue of neurodegenerative diseases shows its involvement in these conditions. However, more research is needed to confirm this involvement. In particular, in the case of AD, only a few reports show the modification and accumulation of AGEs in senile plaque, NFTs, and cerebral amyloid angiopathy in these patients. Studies also revealed that Aβ itself glycosylated in vitro, which ultimately promotes the aggregation of Aβ. These studies raise an important question of whether AGE modification of amyloid plaque is a primary event or whether it is a secondary consequence of Aβ aggregation. However, this issue is controversial and requires more study. AGE modification is an important event that occurs in these neurodegenerative diseases. Thus, it could be involved in the early and late prognosis of AD. AGEs are believed to act as good pathogenic propagators in different diseases, particularly in diabetes and some neurodegenerative diseases. It is of great curiosity to identify antiglycative substances and their mode of action. In this book chapter, we endow examples of the anti-glycation ability of plant-derived products (Fig. 6), which directly targets the stages of glycation through different types of action, such as hypoglycemic action, the inhibition of Amadori product formation, the inhibition of AGE precursors, and the reduction of AGE cross-linking. The phenolic content of plant extract has good anti-glycation activity, although there are non-phenolic compounds, such as carotenoids, terpenes, polyunsaturated fatty acid, and melanoidins, that exhibit the great potential to reduce protein glycation. Flavonoids and isoflavones may also have the potential to inhibit glycation in neurodegenerative disease.

As discussed above, the accumulation of AGEs occurs in different neurodegenerative tissue, such as that in Alzheimer's and Parkinson's diseases, and their inhibition may be helpful in the fast treatment of neurodegenerative diseases. However, there are very few studies on the use of natural products for the cure of AD. These plant-derived compounds are attractive candidates for the evolution of a new generation of therapeutics for the treatment of different age-related consequences such as neurodegenerative disease.

Compliance with Ethics Requirements The authors declare that they have no conflicts of interest.

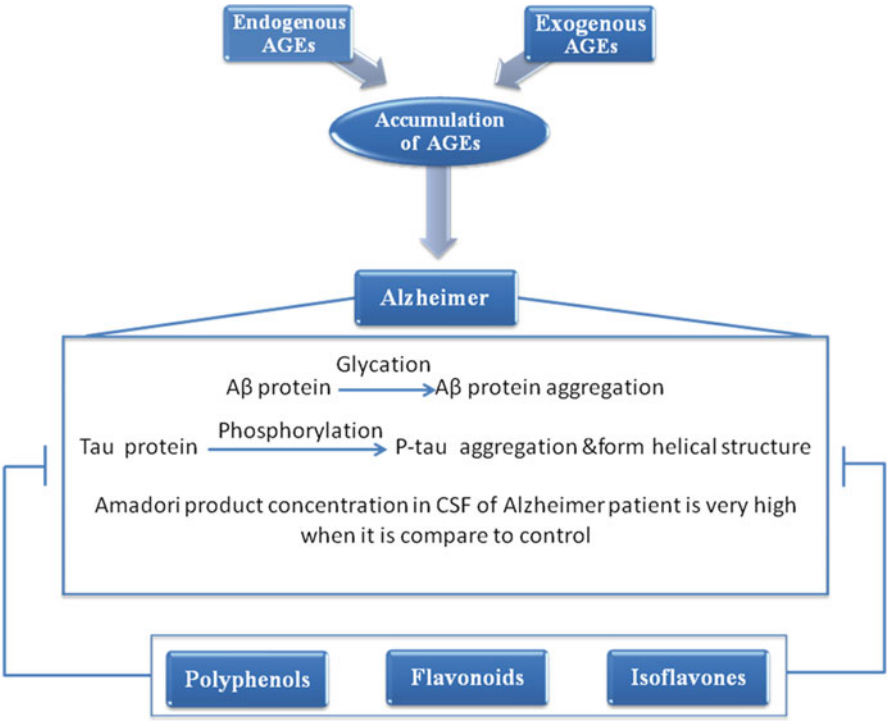

Fig. 6 In Alzheimer Aβ protein aggregates and phosphorylated P-tau protein aggregates and form helical structure. Amadori product concentration in CSF of Alzheimer patient is also high with respective to control. All these process aggravate AD, prevention of all these processes may slow down disease. Polyphenols, Flavonoids and Isoflavones have potential to stop these processes and all these plant derived product may also use for the treatment of Alzheimer disease

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Role of Plant Polyphenols in Alzheimer's Disease

Arokiasamy Justin Thenmozhi, Thamilarasan Manivasagam, and Musthafa Mohamed Essa

Abstract Alzheimer's disease (AD) is the most common neurodegenerative disease, characterized by notable memory loss, cognitive impairment, and personality disorders accompanied with structural abnormalities in the brain of aged population. Currently approved drugs for AD offer symptomatic relief without preventing the progression of the disease and having limited efficacy. Many experiments and clinical trials have shown that the traditional herbal medicine, which has multiple targets, could provide effective treatment of AD. Increasing evidence suggests that the plant derived polyphenols plays a key role in improving cognitive functions and preventing/delaying the onset of certain neurodegenerative diseases including AD. Although several biological effects based on experimental studies could be scientifically explained, the way to bring natural polyphenols into routine clinical application against neurodegeneration seems to be long, because of its low average daily intake, poor availability and few adverse effects. So the better knowledge about intestinal absorption, excretion, intestinal and hepatic metabolism, plasma kinetics, the nature of circulating metabolites, transport, cellular uptake, intracellular metabolism, and accumulation in tissues including brain will facilitate current scientific understanding and offer great hope for the prevention of AD.

Keywords Alzheimer's disease • Polyphenols • Neuroprotection • Pharmacokinetics

A.J. Thenmozhi (✉) • T. Manivasagam
Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar, Chidambaram 608002, Tamil Nadu, India
e-mail: justinthenmozhi@rediffmail.com

M.M. Essa
Department of Food Science and Nutrition, CAMS, Sultan Qaboos University, Muscat, Oman

Ageing and Dementia Research Group, Sultan Qaboos University, Muscat, Oman

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Neurodegenerative Diseases

According to the World Health Organization, the three main causes of death in developing countries are cardiovascular disease, cancer, and neurodegenerative diseases (NDDs). NDDs are defined by the progressive loss of specific neuronal cell populations and are associated with protein aggregates. These abnormal protein depositions are known to occur in more than 25 human NDDs, including Parkinson's, Huntington's, and Alzheimer's diseases (AD), as well as prion diseases, Down syndrome, vascular dementia, multiple system atrophy, amyotrophic lateral sclerosis, and epilepsy. The increasing incidence of NDDs could be partially attributed to the substantial increase in the mean lifespan in developed countries. In individuals aged 55–75 years, the number of NDD cases doubles every 5 years. It has been estimated that AD and PD are the two neurodegenerative diseases with the greatest incidence in the world and that AD accounts for 60–80 % of all NDD diagnosed (Rowinska-Zyrek et al. 2015).

Alzheimer's Disease

In 1906, Alois Alzheimer, a German psychiatrist, described a new neurodegenerative disease that has an insidious onset and a progressive course characterized by memory dysfunction along with other cognitive disturbances, including problems recalling familiar names and objects, changed behavior, and/or impaired semantic abilities. Although AD develops differently for every individual, there are many common symptoms and early symptoms are often mistakenly thought to be "age-related" concerns or manifestations of stress.

The disease course is divided into four stages, with progressive patterns of cognitive and functional impairments

1. Pre-dementia: The early sign of AD is the memory problems, sometimes followed by other thinking problems, such as trouble in finding the right words or poor judgment.
2. Mild AD: As the disease progresses, memory loss worsens, and changes in other cognitive abilities are obvious. This includes getting lost, trouble in handling money and paying bills, repeating questions, taking longer to complete normal daily tasks, poor judgment, losing things or misplacing them in odd places, and mood and personality changes and AD is often diagnosed at this stage.
3. Moderate AD: In this stage, damage occurs in areas of the brain that control language, reasoning, sensory processing, and conscious thought. Symptoms may include increased memory loss and confusion, problems in recognizing family and friends, inability to learn new things, difficulty in carrying out tasks that involve multiple steps, problems in coping with new situations, hallucinations, delusions, and paranoia impulsive behavior.

4. Severe AD: People with severe AD cannot communicate and are completely dependent on others for their care. The person may be in bed most or all of the time as the body shuts down. Their symptoms often include inability to communicate, weight loss, seizures, skin infections, difficulty in swallowing, groaning, moaning, or grunting, increased sleeping, and lack of control of bowel and bladder.

In addition to the cognitive, sensory, and motor deficits caused by the progression of AD, there are a number of behavioral and psychological symptoms related to dementia that include agitation and aggression, wandering, disturbances in the sleep cycle, depression, anxiety, delusions, and hallucinations (WHO 2006).

Epidemiology

According to the 2010 World Alzheimer Report, it is estimated that there are presently 35.6 million people living with AD and related disorders and this number is expected to increase to 115 million by 2050 due to an increasingly aged population. The incidence of the rate of new AD cases is well established at 1 % of those aged 65–70, rising to 6–8 % in those 85 years or more. The duration of illness varies between 2 and 10 years and is reflected in the overall prevalence rates in the 85 years or more, between 10 and 30 %. AD reduces the life expectancy of those affected, with an average survival time after diagnosis of 5–7 years. Patients suffering from AD usually die from secondary infections, e.g., pneumonia or urinary tract infections, 5–15 years after disease onset (Nisbet et al. 2015).

Pathology

On a cellular level, AD is associated with the development of beta-amyloid (Aβ) plaques and neurofibrillary tangles (NFT) within and surrounding neurons of the central nervous system (Kumar et al. 2015). The exact biochemical mechanisms of AD remain unknown, but AD is risen mainly due to diminished synthesis of a vital neurotransmitter acetylcholine (necessary for cognition and memory). The central cholinergic system plays a crucial role in controlling several different functions that comprise cerebral blood flow, cortical activity, sleep-wake cycle, as well as cortical plasticity and the modulating role on cognitive performances and learning memory processes. Most of the studies concerning the role of the cholinergic system in learning and memory have focused on cortex and the hippocampus, because the most rostral parts of the cholinergic cell groups in the basal forebrain innervate the hippocampus (CA2, CA3, and CA4 regions of the hippocampal proper, in the inner part of the molecular layer of the dentate gyrus, and in the subiculum) and cerebral cortex receives cholinergic innervations from the largest group of cholinergic cells in the basal forebrain.

In humans, amyloid precursor protein (APP) found on chromosome 21 undergoes cleavage by three enzymatic complexes: α-secretase, β-secretase, and γ-secretase. Sequential cleavage of APP by α-secretase and γ-secretase leads to the production of a small, nontoxic, and soluble peptide, referred to as p3. The sequential cleavage of APP by β-secretase and γ-secretase leads to the production of the insoluble beta-amyloid protein that deposits into plaques. Plaques deposition was started in the entorhinal cortex and sequentially spread to the hippocampus, the rest of the temporal lobe, the association areas of the prefrontal and parietal cortices, and eventually reach all neocortical areas. According to the amyloid-cascade hypothesis, abnormal generation (or insufficient clearance of Aβ) leads to several secondary events including hyperphosphorylation of the protein tau and the generation of neurofibrillary tangles, inflammation, oxidation, and excitotoxicity. These events lead in turn to the activation of the apoptotic cascade, neuronal cell death, and neurotransmitter deficits. It is the deficit in acetylcholine, and to a lesser extent in norepinephrine and serotonin, that is thought to be responsible for the clinical manifestations of the disease (Chow et al. 2010).

The main alternate hypothesis to the amyloid cascade is the tau hypothesis in which hyperphosphorylation of the tau protein occurs inside the neurons. The tau protein normally binds and stabilizes microtubules, the main component of the cellular cytoskeleton. In AD, tau is hyperphosphorylated leading to the formation of paired helical filaments (PHF) and ultimately forms NFTs. This leads to disintegration of microtubules, the collapse of the intracellular transport system, disruption of biochemical intracellular signaling, and finally causes neuronal death (Nisbet et al. 2015).

Models of AD

Many different animal models, ranging from unicellular organisms (bacteria, yeast) to invertebrates (the roundworm *Caenorhabditis elegans* or the fruit fly *Drosophila melanogaster*) and vertebrates (fish and mammals), are currently used for research on aging and neurodegenerative disorders. They are all of interest and importance, but they also show limitations and drawbacks. Most studies on neurodegenerative diseases have been done in transgenic animals, particularly in mice. Indeed, as the production and handling of transgenic mice is currently quite easy, they have played and continue to play a very important role in biomedical research. In particular, rodent models of AD have been used in the last 20 years to study the pathogenic mechanisms, the progression of the disease, and the efficacy of new drugs in preclinical studies. To date, animal models used in preclinical studies can be distinguished in: (1) Tg models of AD, consisting in single or multi-Tg animals overexpressing APP, PS, and/or Tau mutations; (2) non-Tg models obtained by toxin injection in the brain, including direct injection of Aβ or tau, and models of aging (Gama Sosa et al. 2012).

Genetics

Early Onset of Familial Alzheimer's Disease (FAD)

FAD is a quite rare form, accounting for less than 1% of all cases of AD, because only have a small number of affected families. The genes involved in the pathogenesis of FAD are presenilin 1 (PS1), presenilin 2 (PS2), and APP, located on chromosomes 14, 1, and 21, respectively. APP gene mutations are thought to be key ones in Aβ aggregation, while mutations of the PS1 and PS2 genes result in the enhanced proteolytic processing of APP and lead to the most aggressive form of FAD. It has been established that mutations of the APP gene, located on chromosome 21, are responsible for overexpression of APP, which is inherited in an autosomal-dominant pattern. Most cases of FAD exhibit mutations of the presenilin PS1 and PS2 genes. Over 50 point mutations of the presenilin genes have been described and some of them are shown to be responsible for a selective increase in cleavage and aggregation of soluble Aβ (1–40) and Aβ (1–42) proteins (Maltsev et al. 2011).

Late-Onset alzheimer's Disease

The major risk factor for developing AD, apart from increasing age, is the inheritance of the ε4 allele of the apolipoprotein E (ApoE) gene. The ε4 allele approximately triples the risk of developing AD and reduces the age of onset. Brain ApoE, primarily synthesized by astrocytes, is a component of lipoprotein micelles and has a role in mediating transport and redistribution of cholesterol. ApoE binds to Aβ and likely functions as a chaperone molecule to influence Aβ brain metabolism, deposition, and clearance. The pathogenic mechanism of ApoE in AD is not fully understood, but the ε4 allele is suggested to result in decreased receptor-mediated clearance of Aβ across the blood–brain barrier (BBB). Other studies link the ε4 allele to increased Aβ fibrillogenesis.

A large number of other disease-related loci and candidate genes have been proposed, but associations have not been verified, indicating that each unknown gene only modestly impacts on disease pathogenesis. In most cases, AD is referred to as idiopathic or sporadic, since no direct cause can be found. Numerous environmental factors are suggested to increase the risk for sporadic AD. Head trauma, low education or low cognitive reserve capacity, female gender, hypertension, cardiovascular disease, and a high-cholesterol diet are likely risk factors. Dietary intake of unsaturated fatty acids and antioxidants as well as moderate alcohol consumption could reduce the risk, but findings are somewhat inconsistent (Maltsev et al. 2011).

Treatment

Five drugs are currently approved for the treatment of AD by US Food and Drug Administration (FDA), which includes acetylcholine esterase inhibitors (rivastigmine, galantamine, tacrine, and donepezil) and NMDA receptor antagonist (memantine) that target symptoms at its best. Each drug acts in a different way to delay the breakdown of acetylcholine, a chemical in the brain important for memory. AD is associated with inadequate levels of this important neurotransmitter. Tacrine is rarely prescribed due to its serious side effects (liver damage). In general, rivastigmine, galantamine, and donepezil are most effective when treatment is begun in the early stages. Memantine (Namenda) is the only drug shown to be effective in the later stages of the disease. They have all been shown to modestly slow the progression of cognitive symptoms and reduce problematic behaviors in some people, but at least half of the people who take these drugs do not respond to them (Massoud and Gauthier 2010).

During aging, in addition to Aβ with tau deregulation, genes, chronic inflammation, mitochondrial, metabolic dysfunctions, impaired insulin signaling, oxidative stress, aberrant cell cycle reentry, cholesterol dysmetabolism, as well as metal ion dyshomeostasis synergistically work to promote AD pathological manifestation. Numerous synthetic and natural compounds have been shown to offer neuroprotection against AD, e.g., by preventing the accumulation of Aβ and hyperphosphorylation of tau protein, inhibiting the activity of acetylcholinesterase (AChE), reducing the damage caused by reactive oxygen, and suppressing the inflammatory reaction and neuronal apoptosis. Unfortunately, to date, no pharmacological interventions that effectively prevent or stall AD have been developed. While a single-target therapeutic strategy seems to produce only suboptimal results a broader neuroprotective approach, at least theoretically, appears more appealing. Recently, many experiments and clinical trials have shown that traditional herbal medicine, which has multiple targets, can provide effective treatment of neurological diseases including AD.

Polyphenols

Plants produce a wide variety of secondary metabolites such as alkaloids, steroids, polyphenols, and saponins for different purposes like growth regulation and defense against infections and predations. Polyphenols are a group of antioxidants characterized by the presence of several hydroxyl groups on an aromatic ring. With over 8000 variations having been identified and being so ubiquitous, polyphenols play an integral role in human and animal diets (Ramassamy 2006).

Classifications and Dietary Sources of Polyphenols

Polyphenols are grouped into two main categories: flavonoids and nonflavonoid compounds (Table 1). Flavonoid compounds are classified into two groups: anthoxanthins (flavonol, flavanol, isoflavonoid, flavone, and flavanone) and anthocyanins, while nonflavonoid compounds include phenolic acids, stilbenes, curcuminoids, lignans, and tannins.

Natural polyphenols have been found in many plants and foods such as fruits, vegetables, tea, cereals, medical plants, microalgae, and edible and wild flowers. Some edible and wild fruits have been evaluated, and found that grape, olive, blueberry, sweetsop, mango, and citrus fruits contained high contents of polyphenols. Vegetables are important sources of daily diets and rich in polyphenols. The highest phenolic contents were found in Chinese toon bud, loosestrife, penile leaf, cowpea, caraway, lotus root, sweet potato leaf, soy bean (green), pepper leaf, ginseng leaf, chives, and broccoli (Ross and Kasum 2002).

Polyphenols and Neurodegenerative Diseases

Increasing evidence suggests that plant-derived polyphenols play an important role in improving cognitive functions and preventing/delaying the onset of certain neurodegenerative diseases including AD, Parkinson's disease, and Huntington's disease. The association between polyphenol consumption and lower AD risk is based on several epidemiological studies. A prospective study published in 1997 in the Bordeaux area in France concluded that moderate wine consumption (3–4 glasses per day) negatively correlated to AD risk. Many prospective studies subsequently confirmed a negative relationship between flavonoids intake and incidence of dementia or AD. Although there is still significant debate on the potential benefits of polyphenol with regard to age-related diseases, evidence appears to be growing for these plant-derived compounds as promising dietary/supplemental neuroprotective agents.

Factors Known to Involve in Etiology, Progression, and Pathology of AD

There are eight competing hypotheses which exist to explain the etiology and pathogenesis of AD; they include (1) acceleration of aging; (2) degeneration of cholinergic anatomical pathways, amyloidopathies, and NMDA neurotoxicity; (3) environmental factors such as exposure to metals including aluminum, head

Table 1 The classes and sources of polyphenols

Classes	Groups	Subgroups	Examples	Sources
Flavonoids	Anthoxanthins	Flavonol	3-Hydroxyflavone, azaleatin, fisetin, galangin, gossypetin, kaempferide, kaempferol, isorhamnetin, morin, myricetin, natsudaidain, pachypodol, quercetin, rhamnazin, rhamnetin, etc.	Apples, beans, and onions
		Flavanol	Monomers, e.g., catechin, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), epiafzelechin, fisetinidol, guibourtinidol, mesquitol, robinetinidol, etc.	Berries, cocoa, tea, and onions
			Oligomers and polymers, e.g., theaflavins, thearubigins, condensed tannins, proanthocyanidins etc.	
		Isoflavonoid	Isoflavones—e.g., genistein, daidzein, lonchocarpane, laxiflorane, etc.	Soybeans and legumes
			Isoflavones—e.g., equol, etc.	
		Flavone	Apigenin, luteolin, tangeretin, chrysin, 6-hydroxyflavone, baicalein, scutellarein, wogonin, diosmin, flavoxate, etc.	Citrus fruits, celery, and parsley
		Flavanone	Butin, eriodictyol, hesperetin, hesperidin, homoeriodictyol, isosakuranetin, naringenin, naringin, pinocembrin, poncirin, sakuranetin, sakuranin, sterubin, etc.	Citrus fruits
	Anthocyanins		Aurantinidin, cyanidin, delphinidin, europinidin, luteolinidin, malvidin, pelargonidin, peonidin, petunidin, rosinidin, etc.	Cherries and grapes
Nonflavonoids	Phenolic acids	Derivatives of cinnamic acid	E.g., p-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, sinapic acid, etc.	Red fruits, black radish, and onions
		Derivatives of benzoic acid	E.g., gallic acid, gentisic acid, vanillic acid, protocatechuic acid, syringic acid, etc.	Berries, cherries, kiwis, and apples
	Stilbenes		Resveratrol, pinosylvin, and piceatannol	Red grapes
	Curcuminoids		Curcumin and their derivatives	Curcuma longa
	Lignans		7-Hydroxymatairesinol and enterolactone	Sesame and pumpkin seeds
	Tannins		Hydrolyzable tannins, gallotannins, ellagitannins	Chestnuts, oak

injury, and malnutrition; (4) genetic factors including mutations of amyloid precursor protein (APP) and presenilin (PSEN) genes, and allelic variation in apolipoprotein E (Apo E); (5) a metabolic disorder resulting from mitochondrial dysfunction, oxidative stress, and inflammation; (6) vascular factors such as a compromised blood–brain barrier; (7) immune system dysfunction; and (8) infectious agents (Armstrong 2013). In the present chapter, we specifically focused on the neuroprotective effect of polyphenols on these pathological processes.

Aging

AD may be an accelerated form of natural aging is based on the observation that the many pathological changes in AD are similar to those present in normal aging apart from their severity. Hence, in cognitively normal brain, there is an age-related reduction in brain volume and weight, enlargement of the ventricles, and loss of synapses and dendrites in selected areas. Accompanying these changes are the characteristic pathological features of AD, including SP and NFT. Aging is the required paramount condition on which, in addition to Aβ together with tau deregulation, genes, chronic inflammation, mitochondrial, metabolic dysfunctions, impaired insulin signaling, oxidative stress, aberrant cell cycle reentry, cholesterol dysmetabolism, as well as metal ion dyshomeostasis must synergistically work to promote AD pathological manifestation (Armstrong 2013).

Sirtuin 1 (SIRT1) is known to deacetylate histones and nonhistone proteins including transcription factors, thereby regulating metabolism, stress resistance, cellular survival, cellular senescence/aging, inflammation-immune function, and endothelial functions, and circadian rhythms. Naturally occurring dietary polyphenols, such as resveratrol, curcumin, quercetin, and catechins, have antioxidant and anti-inflammatory properties via modulating different pathways, such as NF-κB- and mitogen activated protein kinase-dependent signaling pathways. In addition, these polyphenols have also been shown to activate SIRT1 directly or indirectly in a variety of models. Therefore, activation of SIRT1 by polyphenols is beneficial for the regulation of calorie restriction, oxidative stress, inflammation, cellular senescence, autophagy/apoptosis, autoimmunity, metabolism, adipogenesis, circadian rhythm, skeletal muscle function, mitochondria biogenesis, and endothelial dysfunction (Chung et al. 2010).

Cholinergic Hypothesis

A specific degeneration of the cholinergic neurotransmitter system was one of the earliest theories as to the cause of AD. Loss of cholinergic activity, atrophy of the nucleus basalis of Meynert as the major source of acetylcholine, and loss

of cortically projecting cholinergic neurons, along with increasing cognitive deficits, are some of notable findings in different neurodegenerative diseases, like Alzheimer's and Parkinson's diseases. Cholinergic dysfunction in neurodegenerative diseases can be the result of reduction in Ach synthesis due to the reduced choline acetyltransferase (ChAT) or choline uptake, cholinergic neuronal and axonal abnormalities, and degeneration of cholinergic neurons (Armstrong 2013).

Several natural polyphenols have shown a cholinesterase inhibitory effect. In most in vivo studies, the anticholinergic activity of polyphenol was accompanied by improvement of cognitive functions, like learning and memory. However, the exact mechanism of interaction of polyphenols with the cholinergic system is still not clear. EGCG and Huperzine A have shown strong anti-acetylcholinesterase activity and resveratrol has shown in a study to block acetylcholine release from adrenal chromaffin cells. Huperzine A has the highest AChE inhibitory activity after donepezil, while tacrine, physostigmine, galantamine, and rivastigmine were less potent (Qian and Ke 2014). Combinatorial regimens of huperzine A with other selective AChE inhibitors have shown even more promising results. Some polyphenols such as huperzine A, quercetin, kuwanon U, E, and C, kaempferol, tri and tetrahydroxy flavone, etc. have shown anti-butyrylcholinesterase effects in addition to their anticholinesterase activity. Not all polyphenols have an anticholinesterase activity. Some of them have a reverse effect. For example, caffeic acid increases AchE activity and expression (Ebrahimi and Schluesener 2012).

Polyphenols and Protective Effects Against NMDA Neurotoxicity

The role of NMDA neurotoxicity and glutamate excitotoxicity in neurodegenerative diseases like Huntington's disease (HD), AD, and even in cognitive impairment associated with aging has been confirmed many years ago. Excessive activation of NMDA receptors induces the production of damaging free radicals (e.g., NO and ROS) and other enzymatic processes that contribute to neuronal damage and cell death. Therefore, blocking the NMDA pathway has been a therapeutic strategy for cognitive impairment not only in neurodegenerative diseases but also in psychiatric disorders with cognitive dysfunction. There is strong evidence of protective effects of several natural polyphenols against NMDA neurotoxicity. Polyphenols act at different locations within the NMDA pathway. In most of these studies, a protective effect against NMDA excitotoxicity has been reported. However, the mechanism of such protection has not been clearly addressed. A few in vitro studies reported a reduction in frequency and amplitude of AMPA/NMDA receptor-mediated spontaneous excitatory postsynaptic currents (sEPSCs) in pyramidal neurons. But it remains unclear whether this is the result of polyphenols' antioxidant activity or their direct NMDA receptor blocking effect (Ebrahimi and Schluesener 2012).

Polyphenols and Amyloidopathies

Tau hyperphosphorylation and beta-amyloid accumulation are believed to be the core pathologies of tauopathies and amyloidopathies. The excessive Aβ accumulation can be the result of either increased production or decreased clearance, which in both situations is toxic to the cells. Aβ aggregation leads to the formation of senile plaques (SP) and stimulates a series of biological signaling pathways which leads to an impairment of neuronal synapses and dendrites through oxidative stress and inflammatory responses. Beside SP and Aβ aggregation, neurofibrillary tangles (NFTs), consisting of abnormally hyperphosphorylated tau protein, are another pathologic hallmark of AD. For a long time, these two pathological features have been the major therapeutic targets for drug development in tau-amyloidopathies. Polyphenols exert their effect through modulation of α-, β-, and γ-secretases, inhibition of Aβ oligomer formation, inhibition of Aβ-induced neurotoxicity, and inhibition of Aβ-induced neuroinflammation. Several natural polyphenols have effectively reduced Aβ deposition and Aβ protein concentrations in the brain and serum, which has shown the most promising anti-amyloidogenic effects. Many studies have shown a direct binding of polyphenols to beta sheet structures. Polyphenol induces α-secretase cleavage activity and inhibits β- and γ-secretases. Several other polyphenols reduce Aβ levels through direct or indirect modulatory effects on α-, β-, and γ-secretases. EGCG, myricetin, quercetin, kaempferol, morin, and apigenin directly inhibit β-secretase activity in a concentration-dependent manner (Shimmyo et al. 2008). Some of polyphenols like dihydroguaiaretic acid, GSE, tannic acid, and wine-related polyphenols affect beta aggregates and destabilize preformed Aβ, while some others affect Aβ (1–40) and Aβ (1–42) and inhibit polymerization and fAβ formation (Ebrahimi and Schluesener 2012).

Theories Based on Environmental Factors

Many environmental factors have been linked to AD, but most studies relate to three such variables, viz., exposure to aluminum (Al), effect of head trauma, and the influence of diet and malnutrition.

Aluminum

Aluminum is one of the most abundant, nonessential metals existing in our environment and is a constituent of cooking utensil, medicine, and drinking water that gain easy access into the body via air, food, and water. Aluminum (Al) is the third most abundant metal in the earth's crust and its role in the etiology and pathogenesis of AD is well-documented in animal experiments and clinical studies.

Aluminum enters into the brain via the specific high affinity receptors for transferrin (TfR) expressed in the blood–brain barrier (Roskams and Connor, 1990) and accumulated in all the regions of rat brain, the maximum being in the hippocampus, which is the site of memory and learning. It is reported that Al is known to accelerate the extracellular Aβ generation and aggregation. Al acts as a cholinotoxin and causes alterations on the cholinergic activity, a key event in the neurochemistry of AD. It causes apoptotic neuronal loss in hippocampus and degeneration of cholinergic terminals in the cortical areas (Armstrong 2013).

Successful chelation therapy for metal poisoning lies in the mobilization of the metal and its excretion from the body by the chelating agents used. This reduces the body burden of the metal and reduces the metal's toxic effects. It has been indicated that dietary phenols, such as catecholates, salicylates, curcumin, and epigallocatechin, can chelate with $Al3^{+}$; therefore, be capable to mobilize Al and to reduce its body burden. Flavonoids are effective metal ion chelators and form stable products with beryllium, aluminum, iron, and zinc ions. For example, in vitro studies confirmed the coordination of hesperidin with Al, forms hesperidin-Al complex (Justin Thenmozhi et al. 2015).

Head Injury

Head trauma results in a primary injury which frequently spreads via inflammatory cytokines to initially unaffected regions, thus amplifying the original injury due to the activation of microglia and central nervous system immune cells. Several observations suggest a link between head injury and AD. In survivors of head injury, APP is observed in neuronal perikarya and in DN surrounding Aβ deposits, as in AD. The formation of Aβ from APP occurs within the synaptic terminal fold of axons, the presence of glia not being necessary for this conversion. Hence, the production of APP may be a component of the brain's response to neuronal injury. Subsequently, it was shown that specific neurons in the medial temporal lobe secreted large quantities of APP and that there were more APP-immunoreactive neurons in these areas in head injury patients. Hence, an increased expression of APP in head trauma cases may be an acute phase response to neuronal injury, the overexpression of APP leading to the deposition of Aβ. Several acute phase proteins are localized within Aβ deposits in AD, including amyloid-P, complement factors, and α-antichymotrypsin. Furthermore, APP maintains cell function by supporting the neuronal growth and survival. The possible neurotrophic action of APP is supported by the observation that it shares structural features with the precursor for the epidermal growth factor. NFT may also be a part of the neuron's response to injury. These studies suggest that the formation of pathological proteins as a result of brain injury is one method by which AD pathology develops and is then propagated within the brain by cell to cell transfer (Armstrong 2013).

Evidence strongly suggests that oxidative stress is a cornerstone event leading to and propagating secondary injury mechanisms such as excitotoxicity, mitochondrial dysfunction, apoptosis, autophagy, brain edema, and inflammation that finally result in brain injury. Resveratrol and other flavonoids such as pycnogenol, epigallocatechin-3-gallate, and wogonin reduced mitochondrial dysfunction, BBB permeability, oxidative stress, and inflammation in traumatic brain injury rat model.

Diet and Malnutrition

This hypothesis is based on clinical observation of AD patients who often exhibit emaciation and cachexia, urinary tract infections, terminal bronchopneumonia, and low triceps skinfold. Low serum albumin, iron, folate, tryptophan, vitamin B 12, and low cerebral metabolism of glucose and oxygen may also be present. These symptoms suggest a protein calorie malnutrition syndrome in AD, which could result in the development of NFT due to chronic nutritional deficiencies of calcium and magnesium. A problem with this type of hypothesis, however, is in determining cause and effect, as malnutrition could be a consequence of the disease resulting from the mental state of the patient. A more direct demonstration of a link between diet and AD involves deposition of Aβ was induced in rabbits fed with high levels of dietary cholesterol. In a human family carrying a mutation of the APP gene (APP 717, Val-glycine), that individuals with AD had a greater vitamin B 12 deficiency, compared with unaffected members. It was concluded that this link was unlikely to be secondary and to be a consequence of impaired dietary intake. A B 12 deficiency could then result in a reduction of monoamine transmitters and in cholinergic activity.

Impact of polyphenols on the serum albumin, iron, folate, tryptophan, vitamin B 12, and low cerebral metabolism of glucose and oxygen in AD models are not investigated so far.

Theories Based on Mitochondrial Dysfunction

Mitochondria play an essential role in the cell well-being. The organelles critically control cellular energy and metabolism as well as intracellular signaling. On the dark side, mitochondria are also key players in modulating cellular death through the release of apoptotic factors, blockade of energy supply, and generation and release of ROS. Apart from ROS enzymatically produced by NADPH oxidases, cytochrome P450-dependent oxygenases and xanthine dehydrogenases, mitochondria are regarded as the primary site of ROS production within cells. The Electron transport systems constantly generate ROS, which are usually kept in

balance by various defense mechanisms, i.e., antioxidative molecules (e.g., glutathione (GSH) or vitamin E) and antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase), as long as ROS levels are in the physiological range. Excess mitochondrial electron leakage, followed by ROS production, occurs in neurodegenerative conditions, paving the way to lipid peroxidation, nucleic acid damage, protein oxidation, and eventually, neuronal death.

In AD, a "two hit" hypothesis has been postulated in which either oxidative stress or alterations of mitotic signaling serve as initiators and are also crucial to propagate disease pathogenesis. Oxidative injury may play a role in Aβ deposition and the complex relationships between this event, excitotoxicity, calcium dysregulation, and ROS generation in AD. Oxidizing conditions cause protein cross-linking and aggregation of Aβ peptides and also contribute to aggregation of tau and other cytoskeletal proteins. Aβ aggregation and its interaction with the neuronal cell membrane induce a sequence of events that leads to the intracellular accumulation of ROS. In addition to the direct induction of oxidative stress, Aβ can also indirectly generate an oxidative microenvironment mediated by the local immune response; indeed, cellular and soluble mediators of inflammation are found in postmortem AD tissue. Aβ accumulation is also considered as a protective consequence with many physiological roles, some of which include redox-active metal sequestration and superoxide dismutase (antioxidant)-like activity. Several markers of oxidative damage to DNA, lipids, and proteins have been widely studied in AD. A significant increase of 8-hydroxy-2 0-deoxyguanosine (8-OHdG) in nuclear DNA (nDNA) and mitochondrial DNA (mtDMA) was found in the parietal cortex of AD patients. These levels were much higher in mtDNA than in nDNA, showing an elevated susceptibility of mitochondria to oxidative stress; elevated levels of 8-OHdG were also detected in lymphocyte DNA from AD donors. In AD brains, lipid peroxidation has been quantitatively assessed by measuring thiobarbituric acid-reactive substances (TBARS), 4-hydroxy-2-nonenal (HNE), malondialdehyde (MDA), lipid hydroperoxides, and isoprostanes. TBARS were found to be increased in AD frontal and temporal cortices and several reports showed an increase in free HNE in multiple AD brain regions including the cerebellum, amygdala, and hippocampus. Regarding protein oxidation, Smith and colleagues found that brain carbonyl levels increase with age, but no difference was observed between aged and AD brains. On the other hand, it has also been demonstrated that, consistent with the regional pattern of AD histopathology, protein carbonyls are significantly increased in both hippocampus and inferior parietal lobule.

Polyphenols are the well-known reducing molecules that are capable of slowing or preventing the oxidation of other molecules. Polyphenols present in food can help limit the oxidative damage by acting directly on reactive oxygen species or by stimulating endogenous defense systems. The phenolic groups in polyphenols can accept an electron to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components. The efficiency of polyphenols as antioxidant compounds greatly depends on their chemical structure. Phenol itself

is inactive as an antioxidant, but ortho- and para-diphenolics have antioxidant capacity, which increases with the substitution of hydrogen atoms by ethyl or *n*-butyl groups. Flavonoids are among the most potent plant antioxidants because they possess one or more of the following structural elements involved in the antiradical activity: (1) an o-diphenolic group (in ring B), (2) a 2–3 double bond conjugated with the 4-oxo function, and (3) hydroxyl groups in positions 3 and 5. Quercetin, a flavonol that combines all of these characteristics, is one of the most potent of natural antioxidants. Also, the antioxidant efficiency of flavonoids is directly correlated with their degree of hydroxylation and decreases with the presence of a sugar moiety (glycosides are not antioxidants, whereas their corresponding aglycones are antioxidant) (Ebrahimi and Schluesener 2012).

Theories Based on Genetics

In 1990s, strong evidence emerged of the connection between familial AD and specific genetic factors. Hence, small numbers of cases were linked to APP mutations and a larger subgroup to PSEN1/2 mutations, while others genes are currently unidentified. In addition, allelic variation in the Apo E locus on chromosome 19 was identified as a significant risk factor, especially in late-onset AD.

APP

A variety of Aβ peptides are formed as a result of secretase cleavage of APP. The most common of these peptides is Aβ 42, found largely in discrete Aβ deposits, whereas the more soluble Aβ40 is also found in association with blood vessels and may develop later in the disease. In addition, mutations of APP within the Aβ coding region may result in the deposition of Aβ 38 in vessel walls, especially in those cases with extensive cerebral amyloid angiopathy. Transgenic mice expressing high levels of APP result in Aβ deposition, synaptic loss, and gliosis. FAD caused by APP 717 (Valine–Isoleucine) mutation has significant numbers of NFT, thus supporting a link between APP and the cytoskeleton. AD cases linked to PSEN1 have greater numbers of SP and NFT compared with cases of sporadic AD (SAD) suggesting that PSEN1 may also increase tau deposition.

PSEN Genes

Full-length PSEN is composed of nine transmembrane domains located on the endoplasmic reticulum membrane. Endoproteolytic cleavage of PSEN and assembly into γ-secretase complex is followed by transport to the cell surface, thus potentially

influencing APP processing. Hence, mutant PSEN1 could enhance 42-specific-γ-secretase cleavage of normal APP resulting in increased deposition of amyloid-forming species. PSEN may also act through loss of function by a reduction in γ-secretase activity.

Apo E

An allelic variation in Apo E has been identified as a major risk factor in late-onset AD, individuals with AD having 2–3 times the frequency of allele ε4 compared with cognitively normal individuals. In addition, allele ε4 may accelerate the development of AD pathology within the aging brain and, hence, is often associated with an earlier disease onset. The relationship between the deposition of Aβ and Apo E genotype has been controversial. The majority of studies, however, report increased amyloid deposition in individuals expressing allele ε4. In addition, the clustering pattern of NP may reflect the degeneration of specific cortical and cortico-hippocampal pathways. Cellular NFTs often occur in regularly distributed clusters along the cortex and NP may develop on the dendrites and axon terminals of NFT containing cells. Individuals expressing allele ε4 are associated with the development of the NP in smaller and denser clusters compared with the other Apo E genotypes, which may reflect a more specific pattern of neurodegeneration.

Oleuropein aglycone and quercetin-3-*O*-glucuronide significantly reduced the generation of β-amyloid (Aβ) peptides by primary neuron cultures generated from the Tg2576 AD mouse model. Moreover, treatment with myricetin, quercetin, tannic acid, anthocyanin-rich bilberry and black currant extracts, specific grape-derived polyphenolic preparation (comprised of the proanthocyanidin (PAC) catechin, and epicatechin in monomeric (Mo), oligomeric, and polymeric forms), and pomegranate polyphenols showed beneficial effects in amyloid precursor protein/presenilin 1 (APP/PS1) transgenic mice.

Apart from these theories, various theories based on blood–brain barrier dysfunction, immunology, and infectious agents were also proposed. But no or only limited studies were carried out regarding these aspects.

Barriers to Be Crossed

Polyphenols or polyphenol-rich diets provide significant protection against the development and progression of AD. Although several biological effects based on experimental studies can be scientifically explained, the way to bring natural polyphenols into routine clinical application against neurodegeneration seems to be long. This is because

1. Much of the evidence for polyphenol-related disease prevention is derived from in vitro or animal experiments; however, the extrapolation of animal data to humans is controversial. In most of the experimental studies, the neuroprotective effect of polyphenols offered at the doses of 50 mg to 200 mg/kg body weight/day in rodents. If this dose is extrapolated to human beings of 70 kg body weight, then it comes around 3500 mg to 14,000 mg/day. But the average daily intake of polyphenols in the diet is about 1000 mg/day.
2. Most of the natural polyphenols are in the form of esters, glycosides, or polymers which are poorly absorbed via the gastrointestinal tract (GI), highly metabolized, and rapidly eliminated from the body. Although very abundant in our diet, proanthocyanidins are either very poorly absorbed or not absorbed at all, and their action is thus restricted to the intestine. The same appears to be true for anthocyanins, unless some of their metabolites are not yet identified but are well absorbed. Intakes of monomeric flavonols, flavones, and flavanols are relatively low, and their plasma concentrations were very low, because of limited absorption and rapid elimination. Flavanones and isoflavones are the flavonoids with the best bioavailability profiles.
3. Though some important differences may exist between animals and humans in some metabolic processes, especially the conjugation process, more animal studies are needed to investigate intracellular metabolism and the accumulation of polyphenol metabolites in specific organs.
4. As the polyphenols undergo a rapid metabolism and elimination, to maintain a high concentration of these polyphenols in plasma, a repeated ingestion of them is required. Although the maximum concentration in plasma rarely exceeds 1 mM after the consumption of 10–100 mg of a single phenolic compound, the total plasma phenol concentration is probably higher due to the presence of metabolites formed in the body's tissues or by the colonic microbiota. It is generally accepted that the bioavailability of phenolics is rather low and the values of the relative urinary excretion of the intake range from 0.3 % for anthocyanins to 43 % for isoflavones such as daidzin. This bioavailability may be even lower when the food polyphenols have a large molecular weight, as is the case of hydrolyzable and condensed tannins and complex flavonoid conjugates with several sugars and acylated with hydroxycinnamic acids.
5. Metabolic modifications of polyphenols lead to a reduction of their biological properties. For example, glucuronides and O-methylated metabolites of flavonoids have shown a reduced ability to donate hydrogen and less effective free radical scavenging capacity. Moreover, the capacity of polyphenol molecules for metal ion scavenging is limited and usually high concentrations of a polyphenol are needed to produce an effective metal ion scavenging effect in the brain.
6. Another important issue is whether the polyphenols reach the brain in sufficient concentrations, and in biologically active forms. There are two main obstacles for polyphenols to cross the BBB: (1) endothelium of the brain microvessels (BBB) and (2) multidrug resistance-associated proteins (MRPs).

7. There are few reports about adverse effects of polyphenols. Feeding rats with high doses of quercetin (2 or 4 %) caused chronic nephropathy, whereas administration of high-dose green tea polyphenols in the diet (1 %) disrupted kidney function through the reduction of antioxidant enzymes and heat-shock protein expressions in mice (Ebrahimi and Schluesener 2012).

Conclusion

Due to the high diversity in the structure and functions of polyphenols, the notion of bioavailability integrates several variables, such as intestinal absorption, excretion of glucuronides toward the intestinal lumen, metabolism by the microflora, intestinal and hepatic metabolism, plasma kinetics, the nature of circulating metabolites, binding to albumin, cellular uptake, intracellular metabolism, accumulation in tissues, and biliary and urinary excretion. Though the validity of many in vivo and in vitro experiments performed on the neuroprotective effects of polyphenolic compounds is still a matter of debate, a better knowledge of polyphenol bioavailability will facilitate current scientific understanding and offer great hope for the prevention of AD.

Compliance with Ethics Requirements The authors declare that they have no conflicts of interest.

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Cannabinoids: Glutamatergic Transmission and Kynurenines

Ana Laura Colín-González, Gabriela Aguilera, and Abel Santamaría

Abstract The endocannabinoid system (ECS) comprises a complex of receptors, enzymes, and endogenous agonists that are widely distributed in the central nervous system of mammals and participates in a considerable number of neuromodulatory functions, including neurotransmission, immunological control, and cell signaling. In turn, the kynurenine pathway (KP) is the most relevant metabolic route for tryptophan degradation to form the metabolic precursor NAD^+. Recent studies demonstrate that the control exerted by the pharmacological manipulation of the ECS on the glutamatergic system in the brain may offer key information not only on the development of psychiatric disorders like psychosis and schizophrenia-like symptoms, but it also may constitute a solid basis for the development of therapeutic strategies to combat excitotoxic events occurring in neurological disorders like Huntington's disease (HD). Part of the evidence pointing to the last approach is based on experimental protocols demonstrating the efficacy of cannabinoids to prevent the deleterious actions of the endogenous neurotoxin and KP metabolite quinolinic acid (QUIN). These findings intuitively raise the question about what is the precise role of the ECS in tryptophan metabolism through KP and vice versa. In this chapter, we will review basic concepts on the physiology of both the ECS and the KP to finally describe those recent findings combining the components of these two systems and hypothesize the future course that the research in this emerging field will take in the next years.

Keywords Endocannabinoid system • Kynurenine pathway • Neuroactive metabolites • Neuromodulation • Cannabinoid receptors

A.L. Colín-González (✉) • G. Aguilera • A. Santamaría (✉)
Laboratorio de Aminoácidos Excitadores, Instituto Nacional de Neurología y Neurocirugía, Insurgentes Sur 3877, 14269 Mexico, Mexico
e-mail: absada@yahoo.com

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Introduction

The history of humanity has been characterized by the use of natural resources to improve the health and life status. *Cannabis sativus* represented a medicinal alternative for different health problems before the twentieth century, but its use decayed later because of its recreational profile as an illicit drug (Robson 2014). Reconsideration of the use of cannabinoid-based medicine is growing recently due to an integral characterization of the endocannabinoid system (ECS) and its components, as well as the many physiological functions of which it seems to be responsible. It is precisely due to this increased interest for cannabinoid-based medicine that an intense search for cannabinoid drugs has grown up intensely during the last three decades (Sagredo et al. 2012).

Kynurenine pathway is probably the most important metabolic route for tryptophan (Tryp) degradation and synthesis of nicotinamide dinucleotide (NAD^+), a well-known metabolic precursor. This pathway consumes more than 90 % of the free Tryp and has been implicated in different physiological functions as well as in neurological and psychiatric disorders.

Herein, we offer an overview of both themes in order to offer new enlightening information about the present and the future of an emerging line of research combining these fields. We initiate with a general description of the ECS, to further follow with basic concepts of KP, and finally a description of those studies dealing with their interaction.

Endocannabinoid System (ECS)

The ECS is formed by cannabinoid receptors, their ligands, and the enzymes involved in the endocannabinoid metabolism. The ECS regulates a wide range of functions in the CNS such as memory, blood pressure, cognition, movement, immunity, drug addiction, reproduction, sleep, and pain perception (Stella et al. 1997; Martin et al. 1999). Endocannabinoids release is triggered by calcium influx during depolarization or by activation of metabotropic glutamate receptors (mGluR1) and muscarinic acetylcholine receptors (mAChR) (El Manira et al. 2008).

Cannabinoid Receptors

There are two well-characterized CB1 and CB2 receptors associated with GI/G_o-protein and other receptors less studied: G protein-coupled receptor 35 (GPR35), GPR55, peroxisome proliferator-activated receptors (PPARS), and the vanilloid transient receptor potential V1 (TRPV1) (Onaivi et al. 2012). CB1 is found mainly in the CNS while CB2 is located in peripheral tissues, especially in

the immune system (Galiegue et al. 1995; Onaivi 2009). CB1 is mostly located on GABAergic, glutamatergic, dopaminergic, noradrenergic, and serotonergic terminals (Morena et al. 2015).

CB1 Receptor

CB1 is a G protein-coupled receptor whose gene is located on chromosome 6. Two types of NH_2 terminal splice variants have been reported. Several polymorphisms in the central cannabinoid receptor-1 (CNR1) gene have been described and their correlation with various neuropsychiatric features has been examined; some examples are mentioned in Table 1.

CB1 may exist as either a homodimer or heterodimer. The interaction of CB1 and D2 receptors was confirmed by co-immunoprecipitation and in vitro binding experiments. The complex can be formed by a direct protein-protein interaction mediated by the carboxyl terminus of CB1 and the third intracellular loop of the D2 receptor (Khan and Lee 2014). Orexin receptor 1 belongs to the superfamily of G protein-coupled receptors that overlap its distribution with CB1 in hippocampus and hypothalamus, exhibiting "crosstalk" interaction to modulate pain and feeding (Perrey et al. 2014; Ho et al. 2011; Crespo et al. 2008). CB1 also forms heterodimers with the μ-, κ-, δ-opioid receptors, the A_{2A} adenosine receptors, and the β_2-adrenergic receptor (Hudson et al. 2010). The heterodimers formation of CB1 has been widely reported. However, their functional significance has yet to be fully understood.

Table 1 Polymorphisms of the central cannabinoid receptor-1 (CNR1) gene and its correlation with various neuropsychiatric features

Polymorphism	Effect	References
rs2023239	Substance dependence	Hirvonen et al. (2013)
rs806378	Tardive dyskinesia	Tiwari et al. (2012)
rs1535255, rs2023239, and rs1049353	Impulsive behaviors	Ehlers et al. (2007)
CNR1	Psychosis-related disorders Metabolic disorders (obesity, hypercholesterolemia, and insulin resistance)	Eggan et al. (2008, 2012), Brown et al. (2014), Benzinou et al. (2008), Feng et al. (2010)
rs1049353	Predisposition to the hebephrenic schizophrenia subtype	Ujike et al. (2002)
rs2501431 and rs1049353	Depression	Monteleone et al. (2010), Mitjans et al. (2013)
rs12199654 and rs2023239	Reduction in white matter volume	Onwuameze et al. (2013), Schacht et al. (2012)
rs1049353	Reduction in caudate volume	Suárez-Pinilla et al. (2015)
rs2023239	Reduction in thalamic volume Schizophrenia	Suárez-Pinilla et al. (2015)

CB1 is widely and richly expressed in the CNS, showing its major levels in the striatum, thalamus, hypothalamus, cerebellum, and lower brainstem. CB1 is mainly found in presynaptic elements of projecting neurons (Kano et al. 2009). Moreover, CB1 has also been located in the vesicular glutamate transporter (VGLUT)-1 protein, and in serotonin transporter in the mouse and rat frontal cortex (Ferreira et al. 2012), hence supporting an active role in neurotransmission.

CB2 Receptor

Human CB2 is spliced to yield two isoforms CB2A and -B. CB2A is highly expressed in testis and shows some expression in the brain while CB2B is expressed in immune cells and tissues (Miller and Devi 2011). CB2 is expressed in microglial cells and is related to their activation state, as observed in macrophages. However, CB2 levels are higher in microglia than in macrophages. M2-type microglia (induced by IL-4 or IL-13) increase the synthesis of endocannabinoids and enhance the production of CB2, indicating that the signaling pathways associated with CB2 could be critical for the acquisition of an alternative phenotype in microglia (Mecha et al. 2015).

TRPV1 Receptor

The transient receptor potential (TRP) family is a group of nonselective cation channels with a common structure of six transmembrane domains. There are six subfamilies of TRPs: canonical (TRPC1-7), vanilloid (TRPV1-6), melastatin (TRPM1-8), ankyrin (TRPA1), polycystin (TRPP1-3), mucolipin (TRPML1-3), and no-mechano-potential (TRPN). The vanilloid family is one of the best characterized subfamilies of TRP channels (Martins et al. 2014). TRPV1 receptor is a Ca^{2+} channel activated by anandamide (AEA), but not by 2-2-arachidonoyl glycerol (2-AG), two well-known endocannabinoids. TRPV1 has anti-inflammatory and antinociceptive properties, and it has been related to behaviors such as fear, anxiety, stress, thermoregulation, pain, and synaptic plasticity (Edwards 2014).

GPR55 Receptor

G protein-coupled receptor 55 (GPR55) induces intracellular calcium release via G 13/RhoA-mediated pathway. Similarly to GPR35, GPR55 is activated by AEA, virodhamine, cannabidiol, and lysophosphatidylinositols but not by WIN55, 212-2 (WIN), an agonist for CB1 and CB2 (Ryberg et al. 2007). GPR55 mRNA was found in the striatum, hippocampus, forebrain, cortex, and cerebellum (Wu et al. 2013). In the striatum, GPR55 heteromerizes with CB1 receptor and it has been suggested that it plays a role in motor coordination (Wu et al. 2013; Martínez-Pinilla et al. 2014).

Endocannabinoids

Endocannabinoids are released lipids that activate cannabinoid receptors and are inactivated by uptake and hydrolysis. The ECS has two major endogenous ligands: AEA and 2-AG. They are synthesized "on demand" through Ca^{2+}-dependent and -independent mechanisms (Kano et al. 2009). AEA and 2-AG are present in peripheral and central tissues and their administration produces effects similar to those elicited by Δ9-tetrahydrocannabinol (THC) (Martin et al. 1999). Levels of 2-AG in the brain are >100 times greater than AEA (Stella et al. 1997).

In addition to the AEA and 2-AG, there are other active endocannabinoids such as dihomo-γ-linoleoyl ethanolamide, docosatetraenoyl ethanolamide, 2-arachidonyl glycerol ether, *O*-arachidonoylethanolamine, and *N*-arachidonoyldopamine (Kano et al. 2009).

Anandamide (AEA)

Biosynthesis

The synthesis of AEA occurs by several pathways. Liu and coworkers (2008) suggested that there are at least three pathways in which *N*-arachidonoyl phosphatidyl ethanolamine (NAPE) can be converted to AEA: (1) hydrolysis of NAPE by phospholipase D (NAPE-PLD); (2) deacylation of NAPE by α,β-hydrolase 4 (abhd4), a serine hydrolase, which serially removes acyl groups from NAPE to form lyso-NAPE and glycerophospho-arachidonoyl ethanolamide (GP-AEA). GP-AEA is subsequently hydrolyzed by metal-dependent phosphodiesterase (GDE1) and transformed into AEA; and (3) hydrolysis of NAPE by a type-C phospholipase which produces phosphoanandamide (pAEA), then an uncharacterized phosphatase dephosphorylates pAEA to form AEA (Liu et al. 2008; Okamoto et al. 2009). Degradation of AEA is performed by fatty acid amide hydrolase (FAAH), cyclooxygenase, and lipoxygenase (Kano et al. 2009).

Physiological Effects

It was previously believed that the physiological effects of AEA were mediated only by CB1 and CB2 activation. However, several studies have now shown that AEA interacts with discrete binding sites on voltage-sensitive channels, producing wide-ranging effects on channel operation. Also, it has been demonstrated that AEA modulates voltage-gated Ca^{2+} (Oz et al. 2000, 2005; Alptekin et al. 2010), Na^{+} (Nicholson et al. 2003), and K^{+} channels (review by Poling et al. 1996).

AEA directly inhibits the function of voltage-dependent calcium channels and alters the specific binding of calcium channel ligands, inhibiting neuronal Ca^{2+} currents, and showing possible secondary effects on intracellular Ca^{2+} homeostasis (Oz et al. 2000, 2005; Alptekin et al. 2010).

Nicholson et al. (2003) showed that AEA inhibits voltage-gated sodium channels in neuronal preparations. Therefore, AEA could work in the same way as class I antiarrhythmics, anticonvulsants, and anesthetics, which directly inhibit sodium channels (Nicholson et al. 2003; Al kury et al. 2014). AEA also inhibits the function of α subunits in neuronal sodium channels Nav1.2, Nav1.6, Nav1.7, and Nav1.8 (Okura et al. 2014). Thus, AEA may be used as a therapeutic tool in mechanisms related to inflammation and analgesia.

In addition, AEA interacts with voltage-gated K^+ channels in a cannabinoid receptor-independent manner. Moreno-Galindo and coworkers (2010) showed that AEA blocks (IC_{50} = 200 nM) voltage-gated K^+ channels expressed on HEK-293 cells. AEA could interact with Val505 and Ile508 within the S6 domain, residues that form a hydrophobic motif important for the ion conduction pathway (Moreno-Galindo et al. 2010). By contrast, Barana et al. (2010) reported that AEA inhibits voltage-gated K^+ channels when it binds to the external vestibule. However, both reports showed that AEA blocks voltage-gated K^+ channels in a cannabinoid receptor-independent manner, acting as an intracellular messenger capable of modulating channel activity. The fact that AEA can act through cannabinoid receptor-independent manner suggests a direct chemical action of this molecule and a more complex pattern of actions for this cannabinoid, which is not merely related to its actions as an endogenous agonist.

Moreover, it has been demonstrated that AEA modulates the function of several other receptors: serotonin type 3 (Oz et al. 2002; Barann et al. 2002), nicotinic acetylcholine (Oz et al. 2003; Spivak et al. 2007; Butt et al. 2008), and glycine (Lozovaya et al. 2005; Hejazi et al. 2006), once again through a cannabinoid receptor-independent mechanism.

Intravenous administration of AEA increases extracellular dopamine levels in the nucleus accumbens, an effect that can be either cannabinoid receptor-dependent or -independent (Solinas et al. 2006). AEA modulates the activity of the dopamine transporter (DAT) through an unknown mechanism, producing this outcome, in part, by modulating DAT trafficking in a cannabinoid receptor-independent manner.

Age-dependent variations of the AEA have been shown. Under basal conditions, AEA levels, and NAPE-PLD and FAAH activities are higher in the hippocampus of mature (P56-70) compared to young rats (P14). Moreover, cannabinoid receptor binding increases in older rats (Fezza et al. 2014). These age-related brain changes may be related to an altered susceptibility and responsiveness in cerebral disorders.

The age-dependent changes of AEA levels modify the concentration of other endocannabinoids. There is a relationship between AEA and 2-AG concentrations. Higher AEA concentrations control the production of 2-AG by interaction with TRPV1 channels. In turn, the inhibition of AEA degradation reduces the levels, metabolism, and physiological effects of 2-AG (Maccarrone et al. 2008). Therefore, there is a tight correlation between AEA and 2-AG production. In contrast to this observation, a reduction of the AEA was found in the cerebrospinal fluid of patients affected by temporal lobe epilepsy compared with healthy control, whereas 2-AG levels were not affected; however, the role of this dysregulation still remains unclear (Romigi et al. 2010) and deserves detailed investigation.

2-Arachidonoyl Glycerol (2-AG)

Biosynthesis

The 2-AG synthesis began with phosphatidylinositol 4,5-biphosphate (PIP2) metabolism catalyzed by phospholipase C which triggers diacylglycerol (DAG) and inositol triphosphate (IP3). DAG is then converted into 2-AG by diacylglycerol lipase (DAGL). All forms of phospholipase C (σ, β, γ, ε, ζ, and η) require calcium for activation; therefore, the formation of 2-AG is calcium dependent (Stella et al. 1997). 2-AG is rapidly eliminated by intracellular serine hydrolases, principally by monoacylglycerol lipase (MAGL), and to a lesser degree by α, β-hydrolase-6 (ABDH6) and α, β-hydrolase-12 (ABDH12). These three enzymes have different subcellular localization (Navia-Paldanius et al. 2015). Degradation of 2-AG is also catalyzed by cyclooxygenase and lipoxygenase (Kano et al. 2009).

Previous studies have demonstrated a 2-AG levels increase in the adult brain exposed to various insults that triggers astrocyte stimulation and inflammation, which could be a neuroprotective mechanism (Fezza et al. 2014).

Cannabinoids are Retrograde Messengers

Cannabinoids have the ability to mediate retrograde signaling in the brain via CB1 receptors, leading to reduced neuronal excitability. Cannabinoids are released from postsynaptic neuron and act on presynaptic CB receptors to decrease neurotransmitter release. However, cannabinoid signaling can also be CB receptor-independent involving voltage- and ligand-gated ion channels and the Cys-loop receptor superfamily (nicotinic, serotonin, and glycine) (for review see Oz 2006).

The interaction of endocannabinoids with their receptors decreases cyclic AMP levels, inhibits protein kinases A (PKA) activity, activates potassium channels, inhibits voltage-gated calcium channels, and suppresses transmitter release (Howlett et al. 2002). Below there is an explanation on how these molecules modify inhibitory and excitatory transmission using as example glycinergic and glutamatergic transmission.

Modulation of Glycine Receptors

Glycinergic synaptic currents are presynaptically modulated through the retrograde cannabinoid signaling pathway. Cannabinoids exert dual concentration-dependent effects on glycine receptors. This modulation depends on the concentration of glycine; at low doses of glycine ($<EC_{30}$) cannabinoids augment glycine receptors current while at high concentrations ($>EC_{50}$) they suppress it (Lozovaya et al. 2011).

In the locomotor network, activation of postsynaptic mGluR1 induces release of endocannabinoids from both motoneurons and interneurons; in turn, the released endocannabinoids activate CB1 and cause decreased glycinergic transmission, which depress inhibitory synaptic transmission (Kettunen et al. 2005). Lozovaya et al. (2011) found that endocannabinoids can modulate postsynaptic glycinergic synaptic currents in Chinese hamster ovary cells that do not contain endogenous cannabinoid receptors. 2-AG, at physiological concentrations (0.1–1 μM), directly affects the function of recombinant homomeric glycine receptor, inhibiting peak amplitude and enhancing desensitization. These authors suggest that the receptor activity can be downregulated by progressive accumulation of the number of postsynaptic receptors being in a long-lasting desensitization state (Lozovaya et al. 2011). Thus, modulation of glycine receptor by endocannabinoids can be mediated by CB1-dependent and -independent mechanisms.

Cannabinoids and Glutamatergic Synapses

AEA and WIN reversibly induced short-term depression of glutamatergic synapses on motoneurons by a CB1-dependent presynaptic mechanism. These CB receptor agonists reduced the available pool of synaptic vesicles at excitatory terminals. Cannabinoids may modulate glutamatergic transmission, incoming to neurons through the reduction in the probability of quantal release, action potential, and Ca^{2+}-dependent transmitter release (García-Morales et al. 2015).

To emphasize the relevance of CB1 for excitatory transmission, it was demonstrated that mice lacking CB1 in glutamatergic cells showed CA1 pyramidal neurons with increased branching and increased spine density in the apical dendritic region, indicating that the CB1 signaling exerted on excitatory neurons controls not only functional, but structural synaptic plasticity (Monory et al. 2015).

Chiarlone and coworkers (2014) found that only CB1 located in glutamatergic terminals plays an indispensable role in the neuroprotective activity of ECS. They suggested that intense activation of glutamatergic projection triggers the endocannabinoid synthesis on the glutamatergic terminal, inhibiting excess excitatory transmission and decreasing the neurotoxic effects produced by overactivation of NMDA receptors. Glutamatergic-induced excitotoxicity is one of the main mechanisms that mediated degeneration in many neurological diseases. Thus, endocannabinoids may be natural antiexcitotoxic agents with neuroprotective properties.

Neuroprotective Role of Cannabinoids

Increased levels of cannabinoids can be reached by preventing their degradation, stimulating their synthesis, or increasing receptor binding. Pharmacology or genetic blockade of FAAH is the most used strategy in models of neurotoxicity. However, the precise molecular mechanism of neuroprotection is still unknown. For instance,

the administration of R (+) -WIN 55212-2 to animals decreases neuronal loss, infarct volume, and improves functional deficits and survival rates after cerebral ischemia via activation of CB1 (Nagayama et al. 1999; Hayakawa et al. 2009). Like this, several other examples of neuroprotective properties of cannabinoid agents are available in the literature. For practical purposes, we will discuss only those related to excitotoxicity and metabolites of the KP.

The ECS and Huntington's Disease

Huntington's disease (HD) is an inheritable neurodegenerative disease affecting specific populations carrying the mutant huntingtin (mHtt) gene. This autosomal disorder is characterized by a polyQ expansion in mHtt, corticostriatal degeneration, alterations in glutamatergic and GABAergic transmission, excitotoxic events, choreiform movements, and dementia, among other hallmarks (Sagredo et al. 2012). It is the predictive nature of this neurodegenerative disorder that allows the design of therapeutic strategies. Recently, the use of Sativex™, a combination of THC- and cannabidiol-enriched botanical extracts, has recently emerged as a potential alternative for treatment of HD (Sagredo et al. 2012), since this cannabinoid-based design drug exhibits antihyperkinetic, anti-inflammatory, neuroprotective, and neuroregenerative properties at the preclinical level. These authors have addressed the issue of the initiation of clinical trials using this drug in HD patients. Since HD is a typical disorder coursing with excitotoxic events and alterations in the KP (Schwarcz et al. 2012), the use of cannabinoid-based medicine for this disorder prompts the detailed investigation on the mechanisms subordinated to the ECS that may alleviate the disease symptoms. Therefore, the ongoing clinical trials will provide important clues for the use of this therapeutic strategy against neurodegenerative events involving an altered glutamatergic transmission.

Given the relevance of the KP for the physiology and physiopathology in health status and neurodegenerative disorders, next we will briefly describe the most relevant points of this metabolic pathway.

The Kynurenine Pathway

Among several metabolic routes in the brain and other tissues, the KP is the major cascade for the catabolism of tryptophan (Tryp), an essential amino acid (Jones and Brew 2013). The activity of this pathway is responsible for modulating endogenous levels of Tryp, thus influencing serotonin synthesis, a monoamine neurotransmitter which is synthesized from L-Tryp in a two-step mechanism. Overall, and most importantly, KP leads to the production of an essential pyrimidine nucleotide supply in mammals, the nicotinamide adenine dinucleotide (NAD^+), which ultimately plays a key role in cellular metabolism within the mitochondria. In addition, several metabolites with diverse neurological activities are formed throughout this cascade, all of which either participate in inflammation and immunoregulation processes or

exhibit pro/antiexcitotoxic properties. Such compounds include the metabolite L-kynurenine (KYN), the redox modulator 3-hydroxykynurenine (3-HK), the neuroprotectants picolinic acid (PA) and kynurenic acid (KYNA), and the excitotoxin quinolinic acid (QUIN) (Adams et al. 2014).

As depicted in Fig. 1, the catabolism of Tryp is carried out by indoleamine 2,3-dioxygenase 1 (IDO-1), 2,3-dioxygenase 2 (IDO-2), and tryptophan 2,3-dioxygenase (TDO) enzymes—yielding *N*-formylkynurenine (NFK)—all of which entails a rate-limiting step in the KYN synthesis. Independently, these two analogous heme dioxygenases catalyze the same reaction through what is thought to be a very similar mechanism, in order to deplete Tryp and achieve the formation of other KP metabolites. Expression of the monomeric enzyme IDO is constitutive and positively induced by several inflammatory stimuli (such as the release of the proinflammatory cytokine interferon-γ (IFN- γ), tumor necrosis factor (TNF), or lipopolysaccharide (LPS)). In turn, the tetrameric enzyme TDO is known to be expressed in the liver and its activity is upregulated by glucocorticoids and L-Tryp itself. KYN endures three distinct pathways in order to form KYNA, 3-HK, and anthranilic acid (AA). As such, NFK is degraded by a formamidase, yielding KYN, just to suffer irreversible transmission in both astrocytes and neurons by four different subtypes of kynurenine aminotransferases (KAT1 to KAT4), to ultimately form KYNA. Furthermore, kynurenine 3-monooxygenase (KMO) and kynureninase catalyze the degradation of KYN to 3-HK and

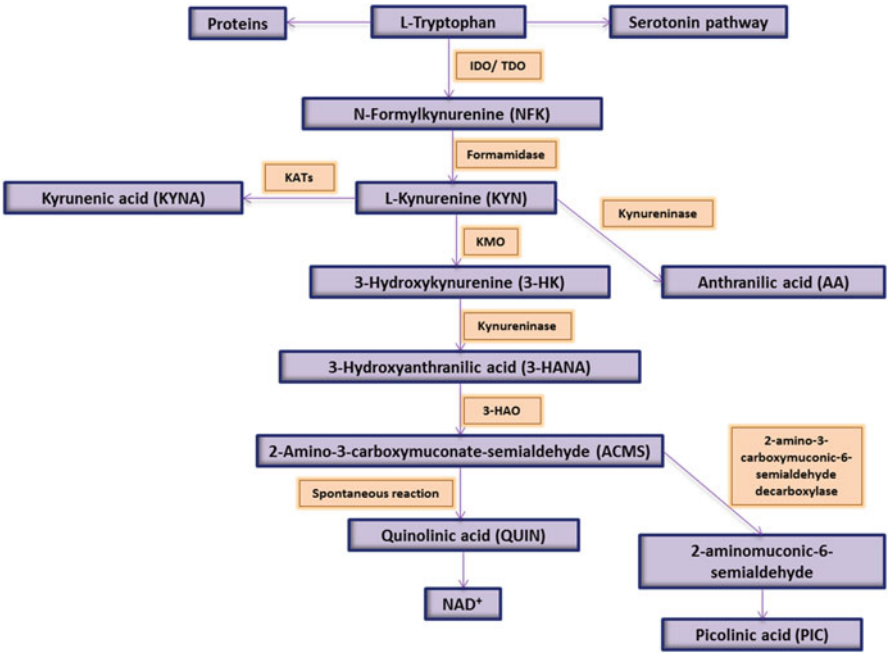

Fig. 1 Complete tryptophan catabolism along the kynurenine pathway (KP)

AA. Then, the 2-amino-3-carboxymuconic-6-semialdehyde spontaneously rearranges to form QA under physiological conditions. In addition, very little amounts of 2-amino-3-carboxymuconic-6-semialdehyde decarboxylase are found in brain tissue, thus diminishing significantly the synthesis flow toward the production of PA. Finally, quinolinate phosphoribosyltransferase activity is remarkably low, and yet carries out the synthesis of NAD^+ (Schwarcz, et al. 2012; Vecsei et al. 2013; Adams et al. 2014; Ball et al. 2014; Yan et al. 2015). While under normal conditions KP runs to form NAD^+, under pathological conditions, such as inflammation, toxic metabolites accumulate in the brain.

Quinolinic Acid (QUIN) Synthesis

To date, the QUIN-induced toxicity has been reviewed extensively, given that it is by far the most prominent neurotoxic metabolite in the KP. In accordance to its narrative profile, QUIN is an *N*-methyl-D-aspartate receptor (NMDAr) agonist often implicated in the pathogenesis of a number of human neurological diseases.

As a by-product of the oxidative metabolism of Tryp, QUIN is an important intermediate metabolite of the KP toward the production of NAD^+. However, taken as a whole, QUIN is vastly involved in several pathophysiological processes acting not only as a neurotoxin but also as a key molecule for the immune response. QUIN is found in nanomolar concentrations in the brain and cerebrospinal fluid, and examples of some of its most relevant targets include presynaptic receptors, oxidative stress, energetic dysfunction, and eventually cell death (Lugo-Huitrón et al. 2013). Consequently, this molecule, as a target, offers a number of potential therapeutic applications for modulators of both its synthesis and degradation processes.

Once kynurenine 3-hydroxylase is attained, 3-HK is produced from L-KYN; these steps constitute the most important subdivision in the whole QUIN synthesis, emphasized in Fig. 1. Under physiological conditions, KYN has been reported to mostly follow metabolism through hydroxylation (rather than the corresponding cleavage) due to a higher affinity, and therefore it metabolizes most of the KYN (Bender and McCreanor 1982). In addition, KYN is cleaved to produce 3-hydroxyanthranilic acid (3-HAA) by kynureninase, followed by catalysis of 3-HA to the aminocarboxymuconic semialdehyde, which rearranges to form QUIN by a nonenzymatic cyclization. Noteworthy, this intermediate can also produce PA, which exhibits protective properties against QUIN-induced toxicity to some extent (Bender and McCreanor 1982; Foster and Schwarcz 1986; Braidy et al. 2009; Costantino 2014).

Toxic Mechanisms of QUIN

The mechanisms by which QUIN induces neurotoxicity have been extensively studied over the past decade. In agreement with what has been previously discussed, QUIN is an endogenous molecule that emerges as part of the Tryp catabolism, and

constitutes a glutamatergic agonist that acts on NMDAr. Under physiological conditions, QUIN is usually present in nanomolar concentrations in brain tissue and micromolar concentrations in cerebrospinal fluid (Pérez De La Cruz et al. 2012; Lugo-Huitrón et al. 2013), and is involved in the degradation of Tryp into NAD^+. However, pathological conditions are associated with its excitotoxic and pro-oxidant properties, which lead to increased concentrations of this metabolite accompanied by inflammatory stimuli (mainly evoked by cytokines); therefore, increased levels of QUIN are often linked with inflammatory responses in several disorders of the central nervous system (Chiarugi et al. 2000).

QUIN exerts its toxic pattern mainly through the overactivation of the NMDAr-ion channel complexes and endogenous glutamate, which in turn trigger strong deleterious events such as mitochondrial dysfunction induced by lysis-enzymes activation (and a consequent decrease in ATP levels), cytochrome C release (with the corresponding apoptosis set off), as well as oxidative stress. In addition, elevated levels of QUIN prompt the formation of reactive oxygen and nitrogen species (ROS and RNS, respectively), further yielding cells to oxidative damage. Overall, such processes finally lead to cell death by apoptotic or necrotic means. In agreement with these events, exposure to exogenous QUIN in experimental in vivo models, in particular in the striatum and cortex, is reported to cause neuronal cell death through excitotoxic mechanisms; interestingly, the toxic mechanisms induced by QUIN are not limited to neurons, but also affect glial cells, which raises attention toward the importance of determining neurological disorders that develop these circumstances (Chiarugi et al. 2000; Pérez-De La Cruz et al. 2012; Lugo-Huitrón et al. 2013). All of the above contribute to the general understanding of the excitotoxic mechanisms exerted by QUIN, as well as to appreciate the relevance of this by-product as a toxin associated with brain damage and the pathophysiology of diseases in the CNS.

Physiological Importance of KP

The fact that Tryp constitutes the major source of the human body stores of NAD^+—a molecule involved in nearly all the biosynthetic metabolism—reveals the overall importance of KP and its link with several physiological events. In addition, aged metabolism itself alters the Tryp catabolism, all of which leads to a decreased nicotinamide biosynthesis over time (Reyes-Ocampo et al. 2014).

Among its numerous intermediates and by-products, the KP of Tryp catabolism involves two molecules exhibiting key neurobiological activities. On one hand, KYNA acts as an endogenous antagonist of glutamate and α-7-nicotinic acetylcholine receptors. On the other hand, QUIN has a well-defined NMDAr agonist activity (Cherian et al. 2014). Given this scenario, further studies and characterization of the elements downstream this cascade were, and remain, crucial when considering that excessive activation of the KP constitutes a consequence of inflammatory processes in a number of neurological diseases such as meningitis (Coutinho et al. 2014). Additional information supports this view, especially when taking into consideration that KYNA is strongly

related to embryonic brain development, in which alterations fall into severe consequences in neuronal morphology, structure, and activity up to maturity (Khalil et al. 2014). On the other hand, imbalances in the KP that enhance the production of KYNA instead of leading to the activation of the long arm of the pathway which is responsible for NAD^+ synthesis are proposed as part of the physiopathology of complex disorders such as schizophrenia (Kegel et al. 2014). Additionally, other elements of the KP have importance from a physiological point of view. For instance, the enzymes responsible for the Tryp degradation in the first steps of the cascade, IDO1, IDO2, and TDO, are accounted to restore antitumor immunity (van-Baren 2015). Also, as much as enzymes IDO1 and IDO2 are linked to Tryp catabolism and to several aspects of immune modulation, few information has been collected in regard to its specific role in physiological conditions, as well as its alterations in disease (Prendergast et al. 2014).

Role of KP in Neurodegenerative Diseases

Regarding the aforementioned metabolites of the KP, whilst KYN and KYNA possess neuroprotective properties, 3-HAA and QUIN are, in general terms, considered as neurotoxic. Accordingly, considerable evidence supports the fact that KP has a role in normal physiology in the brain and is closely connected to the pathology of neurodegenerative diseases, such as Parkinson's disease (PD), Huntington's disease (HD), and others. In some reports, the genetic background was evaluated along the biochemical alterations of the KP; however, genetic elements such as single nucleotide polymorphisms (SNPs) have so far been ruled out of the hypothesis, reflecting that this might not influence the activity of some KP's intermediates (Torok et al. 2015). Up to date, it is known that during the development of inflammatory processes, KP catabolizes Tryp through several steps, in a mechanism that per se contributes to excitotoxic events through the release of QUIN and 3-HAA. Consequently, it is not surprising that such intermediates and products have been strongly related to the onset and development of neurological diseases that encompass degenerative factors. As examples, it is known that KP participates in the regulation of neuroinflammatory events in disorders such as Alzheimer's disease (AD) (Jones and Brew 2013). Moreover, the brain tissue of patients suffering neurological disorders such as schizophrenia often exhibits increased levels of KYNA, which is thought to contribute somewhat to the characteristic cognitive symptoms of such disorders (Cherian et al. 2014). Numerous approaches to the comprehension of QUIN toxicity also lead to important clues and insights on how an altered KP can be harmful. Oligodendrocyte cell lines have a limited threshold to QUIN catabolism in pathological concentrations and from an endogenous source, all of which emerges as an important hypothesis for encephalomyelitis and QA-induced gliotoxicity (Sundaram et al. 2014). Upon these circumstances, burgeoning research about the KP is currently providing new goals for the development of therapeutic approaches to explain and resolve neurodegenerative diseases with increasing incidence, including dementia, as well as disorders with severe impairment of motor and cognitive skills such as multiple sclerosis (MS). Unfortunately, despite the thorough

research that is conducted toward finding a neuroprotective strategy for this matter, the therapeutic approaches are still limited.

The KP and the Endocannabinoid System: Demonstrated and Possible Interactions

Unexplainably, the role of kynurenines on the ECS and vice versa has been poorly explored this far. This is particularly intriguing since, as above mentioned: (1) KP produces at least two neuroactive metabolites with antagonistic profile one over the other (QUIN and KYNA), both acting at glutamate receptors and one of them also exerting modulation of cholinergic receptors; and (2) the ECS exerts intense modulation of neurotransmitter systems, especially on glutamatergic and GABAergic systems. Few reports have explored these interactions in a preliminary manner, constituting the basis for the upcoming studies. In this regard, probably one on the first reports providing key information on these two systems came from the study of Jenny and coworkers (2009). These authors presented evidence suggesting that the suppression of Tryp degradation by the cannabinoids Δ9-tetrahydrocannabinol or cannabidiol (at micromolar concentrations) via indoleamine-2,3-dioxygenase (IDO)—a mechanism that is independent of cannabinoid receptor activation—increased the availability of tryptophan for serotonin biosynthesis, thus contributing to enhance the ability of cannabinoids to improve mood disturbances. This effect is particularly relevant as it contrasts with the effects of the same cannabinoids at nanomolar concentrations, which, in a cannabinoid receptor-mediated mechanism, enhanced Tryp degradation in human blood mononuclear cells. Thus, there seem to be at least two mechanisms for cannabinoid action that might contribute to Tryp metabolism modulation, one related to a direct chemical action of these agents at micromolar ranges with no participation of cannabinoid receptors, and the other regulated by receptors, taking place at nanomolar ranges. From a physiological point of view, these findings are of major relevance as they open contrasting scenarios for the ECS and KP modulation.

In this section, we compile and update the advance on this field through the mention of those studies describing interactions between these two systems at the level of the two neuroactive KP metabolites: QUIN and KYNA.

Kynurenic Acid (KYNA) and Cannabinoids

Unfortunately, most of the studies relating KYNA and the ECS have used the first more as a tool to evidence the role of glutamatergic transmission in different experimental protocols than a real target of the ECS. One of the few studies providing a physiological weight to KYNA after ECS manipulation established that silencing proinflammatory mediators will account for depletion of KYNA synthesis and further preservation of

learning and memory. In 2008, Andrade and coworkers explored the role of glutamatergic and lipid signaling in electroconvulsive therapy-induced retrograde amnesia in rats. Emphasis was paid on the involvement of cyclooxygenase (COX) mechanisms in amnesia, including the NMDAr and ECS systems. After testing different experimental conditions in animals receiving electroconvulsive shocks, the authors demonstrated that the electroconvulsive shock impairs cognition, upregulates the glutamatergic signaling, and generates excitotoxic conditions and hippocampal LTP, all through COX-2-mediated mechanisms involving the depletion of endogenous cannabinoids. The use of the COX-2 inhibitors indomethacin and celecoxib prevented these alterations presumably toward a mechanism partially involving the stimulation of the ECS and the consequent modulation of the glutamatergic system. In addition, while the COX-2-induced increase in the hippocampal levels of KYNA was assumed to compromise the glutamate-dependent NMDAr-mediated learning and memory processes, celecoxib inhibited KYNA synthesis, thus accounting for protection. Although this work did not establish specific interactions between KYNA and the ECS, it suggests for the first time that the modulation of the glutamatergic system and KP by the ECS could reduce the immunological reaction responsible for KYNA formation and further compromise the glutamatergic system. This evidence clearly points to a reduction of KP metabolism mediated by the ECS activation, which represents a topic deserving detailed investigation of further studies, as recently addressed by Hermann and Schneider (2012).

Further evidence on the involvement of CB1 in the modulation of neuronal activity in Substantia nigra pars compacta in rats through the modulation of receptors for excitatory amino acids was collected by Morera-Herreras and coworkers (2008). These authors employed KYNA to specifically test the involvement of NMDAr. Similar experiments conducted by the same authors demonstrated that CB1 activation modulates STN neuronal activity by mechanisms involving glutamatergic and GABAergic neurotransmission (Morera-Herreras et al. 2010). Simultaneously, Farkas and coworkers (2010) demonstrated that retrograde endocannabinoid signaling is capable of reducing GABAergic synaptic transmission to gonadotropin-releasing hormone neurons, emphasizing the suggestion that retrograde ECS signaling is crucial for the regulation of excitatory GABAergic inputs in hypothalamic neurons. Once again, KYNA was merely used as a tool to evidence the involvement of NMDAr-mediated excitatory events.

Interestingly, Zhao and Abood (2013) emphasized the fact that the G protein-coupled receptor GPR35, a protein involved in different physiological responses, including control of pain and inflammation, can act as a receptor for both KYNA and cannabinoids. In principle, this evidence suggests that the ECS and KYNA might, under certain circumstances, share physiological functions oriented to the modulation of the CNS. However, antagonistic actions of KYNA on the cannabinoid responses can be exerted upon other circumstances: recently, upon the concept that α-7-nicotinic acetylcholine receptors (a7nAChRs) modulate the effects of cannabinoids like THC, Justinova and coworkers (2013), carried out a series of experiments testing the effects of the 3-monooxygenase (KMO) inhibitor Ro 61-8048 on the brains levels of KYNA and the extracellular levels of dopamine induced by THC in a self-administration reward-related protocol. Ro 61-8048 augmented the KYNA

levels and reduced the extracellular content of dopamine, hence reducing the cannabinoid-dependent addictive behavior also in squirrel monkeys through a7nAChRs regulation. This important research opens interesting hypothesis about the relationship between KP and the cannabinoid axis, which requires detailed investigation upon both normal and pathological conditions.

Quinolinic Acid (QUIN) and Cannabinoids

QUIN has been used as a tool to produce neurotoxic paradigms where the use of cannabinoids has been explored as potential therapeutic agents. In 2006, Pintor and coworkers used the toxic paradigm produced by QUIN as a model of HD in rats. The synthetic cannabinoid receptor agonist WIN 55,212-2 was tested as a protective tool to investigate the role of the ECS in this paradigm. WIN 55,212-2 dose dependently prevented the QUIN-induced glutamate release and reduced electrophysiological activity induced by QUIN in corticostriatal slices through a CB1-mediated mechanism. Under in vivo conditions, WIN 55,212-2 also prevented the striatal damage induced by QUIN. It was assumed that the stimulation of CB1 was responsible for the inhibition of glutamate release and the preventive actions observed in this study, which in turn could have therapeutic value as an approach to design strategies for HD and other neurodegenerative disorders.

Two years later, De March and coworkers (2008) addressed the issue of an involvement of CB1 in the upregulation of the gene transcription for the protective neurotrophin brain-derived neurotrophic factor (BDNF) in HD. They carried out experiments in rats lesioned with QUIN as the HD model to elucidate the relationship between cannabinoid receptors and BDNF upregulation. These authors found that after 2 weeks of progression of the striatal lesion induced by QUIN, cortical neurons projecting to the striatum contained more BDNF, which in turn coincided with an enhanced expression of CB1. These results were interpreted as a compensatory attempt of CB1 and the ECS to rescue striatal neurons in risk during excitotoxic events, emphasizing the relevance of the ECS as a first line of defense during degenerative events in the CNS. The faith of this early attempt certainly deserves more detailed investigation.

The studies described above established the protective role that CB1 stimulation can exert in the excitotoxic paradigm produced by QUIN; however, the role of CB2 located in glia remained unclear. Palazuelos and coworkers (2009) demonstrated that CB2 can also be neuroprotective against QUIN toxicity. These authors carried out experiments in which they found that CB2 expression was increased in microglia in HD patients and transgenic mouse models. While the genetic ablation of CB2 in the R6/2 transgenic mice stimulated the microglial activation leading to an aggravated HD symptomatology, and the striatal lesion with QUIN to CB2-deficient mice enhanced the nerve tissue damage and neuronal degeneration, microglial activation and inflammatory response, the induction of excitotoxic events with QUIN to wild-type animals administered with selective CB2 agonists prevented all toxic

endpoints. Interestingly, when astrocyte proliferation was selectively prevented in transgenic mice, it resulted clear that the observed CB2 actions were excluded from this population, hence leaving microglia as the cell type responsible for the protective actions of CB2. Therefore, CB2 can also account for an integral protective and a modulator profile of the ECS in neurodegenerative events.

Shortly thereafter, Casteels and coworkers (2010) monitored by PET the brain alterations in CB1 binding in the HD model produced by QUIN in rats in relation to glucose metabolism and D2 dopamine receptor upon the rationale that changes readily occur in the brains of HD patients. All these markers were decreased in the lesioned caudate-putamen. Changes in other brain regions were also detected. Based on their findings, the authors concluded that the changes in the ECS produced by QUIN comprised the caudate-putamen and other distant regions. Since both D2 and CB1 neurotransmission were found enhanced in the contralateral side, functional plasticity was proposed as a compensatory response. This work describes the changes occurring in the ECS during the progression of excitotoxic and neurodegenerative events in the brain and emphasizes the relevance of the ECS for the adequate physiological functioning of the CNS.

More recently, Sánchez-Blazquez and coworkers (2014) formally described in a review what it constitutes a key mechanism of action involving CB1 in the NMDAr-related schizophrenia, but that is also useful to explain neuroprotection mediated by CB1 under excitotoxic episodes. These authors established that, since the ECS controls Ca^{2+} dynamics at the nerve terminal, there must be a physiological role exerted by the cannabinoid receptors to modulate the NMDAr activity, thus decreasing the response of the latter to excitatory stimuli. However, the use of cannabinoids to reduce the function of the glutamatergic system in neurological disorders remains under debate, given the diverse pharmacological properties that these agents exhibit, especially as psychostimulants. Indeed, frequent cannabis consumers have shown a high incidence of psychotic episodes, prompting symptoms of schizophrenia probably through the same mechanism inherent to neuromodulation and neuroprotection: reduction of NMDAr activity. In this regard, cannabinoids are supposed to exert these effects by two main mechanisms: (1) reduction of presynaptic glutamate release through presynaptic cannabinoid receptors, and/or (2) prevention of postsynaptic NMDAr-regulated signaling cascades induced by glutamate. While under normal conditions this modulation contributes to the preservation of homeostasis, under excitotoxic conditions like those prevailing in neurodegenerative disorders, this mechanism accounts for resistance to neurodegeneration; however, upon conditions of enhanced cannabinoid receptor stimulation, this modulation exerts a noxious number hypofunction. Thus, the association between cannabinoid receptors and NMDAr would be at the same time beneficial to prevent excitotoxicity and detrimental to induce schizophrenia-like psychosis. But how this interaction is supposed to act? The precise interaction between CB1 and NMDAr involves the NR1 subunit of the glutamatergic receptor: Once an endocannabinoid or a cannabinoid receptor agonist binds to its receptor, both postsynaptic CB1 C terminus and NR1 C1 segments located at the membrane surface interact through an arm of the histidine triad nucleotide-binding protein 1 (HINT1) homodimeric protein, as previously demonstrated by Vicente-Sánchez et al. (2013) and Sánchez-Blázquez et al. (2013). The complex formed by this interaction

(CB1-HINT1-NR1) is then internalized to be separated in the cytosolic space. While NR1 is submitted to proteasomal degradation, the re-sensitized CB1 returns to the membrane surface to reinitiate the cycle, finding another NR1 subunit to sequester it. This novel mechanism, presented in a summarized manner, represents an elegant explanation on how cannabinoid receptors—particularly CB1—reduce the bioavailability of NMDAr, hence decreasing the glutamatergic transmission with the subsequent positive or negative implications.

Another key contribution in this field has been released recently by Chiarlone and coworkers (2014). These authors addressed a major issue in regard to CB1, the most important G protein-coupled receptor in the mammalian brain. Since CB1 is expressed in both GABAergic and glutamatergic synapses, it is assumed that CB1 activation would be responsible for both excitatory and inhibitory responses. In order to establish the precise contribution of these receptors in neuroprotection, the authors explored their role in toxic models. First, QUIN was used as a tool to induce excitotoxic damage in the brain of mutant mice lacking CB1 in both GABAergic and glutamatergic neurons. In a second experimental protocol, the authors elegantly manipulated corticostriatal glutamatergic projections through a designer drug pharmacogenetic tool to evaluate the alterations in the R6/2 mouse model of HD that were either fully knocked out for CB1 or with a selective deletion of CB1 in corticostriatal glutamatergic or striatal GABAergic neurons. Their findings demonstrated that a restricted population of CB1 located in glutamatergic terminals contacting striatal neurons was in charge of the protective activity of the ECS, hence establishing them as potential therapeutic targets for neuroprotective paradigms. Through this approach, an important step has been given to characterize the neuroprotective profile that the ECS exerts in events involving excitotoxic damage, strongly linking the ECS with the glutamatergic system at specific levels. Immediately after this evidence appeared, our group made a new approach regarding the role of different cannabinoid agonists on the early pattern of toxicity elicited by QUIN in rat brain synaptosomes and striatal cultured cells (Rangel-López et al. 2015). Two synthetic (WIN 55,212-2 and CP 55,940) and one endogenous cannabinoid (anandamide or AEA) were tested as pretreatments in brain synaptic terminals and cultured striatal cells exposed to QUIN for a short time in order to provide key information on the timing and nature of toxic events occurring in the excitotoxic model and the role of the ECS in these early processes. While QUIN induced early loss of cell viability, mitochondrial dysfunction, and oxidative stress in these preparations, the three cannabinoid agents tested (WIN 55,212-2, CP 55-940, and AEA) prevented these effects, with WIN 55,212-2 being the most effective of all. Interestingly, the simultaneous incubation of cannabinoids with QUIN had no positive effects on toxic endpoints, suggesting that these agents shall exert their actions prior to the initiation of the excitotoxic event. Moreover, since WIN 55,212-2 prevented oxidative damage and mitochondrial dysfunction, it cannot be discarded at all that cannabinoid receptor agonists might induce preventive actions through mechanisms that are dependent or independent of cannabinoid receptors, an issue that deserves further detailed investigation.

However, at this point, a question is raised: Is there a real correlation between the ECS and the KP? So far, QUIN has been used merely as a tool to produce a neurodegenerative model with excitotoxic features, and so, the relationship should be considered unilateral, meaning that the ECS would be responsible for prevention mostly at the glutamatergic levels. On the other hand, the evidence showing that the ECS is affected in disorders like HD comprising changes in the KP cannot be ignored at all. Hence, it is true that this far this relationship has not been explored, or at least, it has not appeared reported elsewhere, but in regard to this topic, there is a clue that might help to hypothesize part of this interaction to enlighten the tendency of this relationship, independently of the trend that research will take on this topic: as previously mentioned, Jenny and coworkers (2009) showed that Tryp degradation is blocked by cannabinoids at micromolar concentrations through inhibition of IDO in a cannabinoid receptor-independent mechanism. If this effect inhibits KP, then it can be hypothesized that all metabolites would be decreased at these concentration ranges, whereas at nanomolar concentrations, cannabinoids increased Tryp degradation in human blood mononuclear cells in a cannabinoid receptor-dependent mechanism. Per se, these dual actions are highly suggestive of a possible adaptive modulatory action of the ECS on the KP metabolism; however, in order to validate this mechanism as an event with considerable relevance for the CNS, experimental evidence shall be collected reproducing these effects in brain cells and testing endogenous cannabinoid receptor agonists like AEA at different concentrations. This evidence is highly desirable to explain the many physiological and pathophysiological events that are attributable to the ECS in the human brain. Moreover, how cannabinoids reduce Tryp degradation in a receptor-independent manner? The first explanation for this effect would be linked to the action of other agents, some of which possess anti-inflammatory profiles, reducing the levels of cytokines that regulate IDO's activity. Examples of these agents are Norharmane (Chiarugi et al. 2000), alpha-methyltryptophan (Hou et al. 2007), rosmarinic acid (Lee et al. 2007), and some COX-2 inhibitors (Cesario and Rutella 2011). Detailed investigation is needed on the possible similarities, at the functional and chemical levels, of cannabinoids with all these agents. In addition, it cannot be discarded at all a direct action of cannabinoid agents at IDO or TDO, another branch for future studies. Finally, probably one of the most promising lines for future research is related to other direct actions of cannabinoids, as targets like oxidative stress and mitochondrial function would be revealing issues for the action of these agents. If besides all the mechanisms mentioned above are complemented with direct actions on these events, then the scope of action of these agents will grow up enough to consider new avenues of research with therapeutic perspectives.

Concluding Remarks

The field of research devoted to the actions of cannabinoids at the central level, and more specifically at the neurotransmission level, is gaining attention every day as the ECS constitutes not only a widely distributed modulatory system but also an endogenous system in charge of neurotransmission and regulatory actions in

pathological disorders. The evidence presented in this chapter is intended to provide a wide scope of actions for the intervention and design of pharmacological therapies oriented to modulate the ECS at different levels. Indeed, we have learned from the collected and described evidence that cannabinoid agents are capable of exerting different actions, comprising mechanisms that are either dependent or independent of cannabinoid receptors. The fact that cannabinoid agonist can act through mechanisms not involving their receptors open new and exciting perspectives of research as their properties as protective, immunomodulatory, anti-inflammatory, and antioxidant, among several other properties, raise expectations on their selective use of experimental and clinical protocols. Furthermore, their selectiveness on specific neurotransmission systems, reducing glutamatergic transmission and regulating GABAergic and dopaminergic systems, contributes to their consideration for psychiatric and mood disorders. Most importantly is the possibility of using specific approaches based on the modification of the ECS to attend pathologies linked to alterations in the KP. In this regard, the immediate concept of employing these agents for the treatment of HD and other neurodegenerative disorders has found echo in reviews that offer recent evidence of selective cannabinoid drugs for this purpose. We have also learned recently from the depressive actions seen in Cannabis consumers, reaching the concept that a compromised glutamatergic transmission is also detrimental to humans, leading to schizophrenia-like symptoms. In summary, we are still quite far from understanding the complexity of this fascinating system and the many targets it comprises, but in the next year it is expected that we will collect key information on this topic.

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Carotenoids and Neurobiological Health

J. Guest and R. Grant

Abstract The consumption of carotenoid phytonutrients, largely as part of plant tissue, has been associated with a number of health benefits. Epidemiological and other studies support a link between higher dietary intake and tissue concentrations of carotenoids and lower risk of chronic diseases such as heart disease, diabetes, and some cancers. Evidence also suggests that increased levels of carotenoids can help maintain healthy cognitive function, especially into older age. Carotenoids mediate their beneficial effects via several mechanisms including cell growth regulation and modulation of gene expression and immune activity. However their primary protective mechanism is thought to be due to their potent antioxidant properties that effectively scavenge free radicals and reduce the risk of oxidative damage. This chapter discusses the impact of carotenoids on neurological health by first reviewing their chemical characteristics, dietary sources, and general mechanisms of action before examining in some detail the available evidence for a protective role for various carotenoids in neurodegenerative disease.

Introduction

There is strong scientific evidence in support of the association between diet and chronic diseases such as cardiovascular disease, diabetes, some types of cancer, and neurodegenerative dementias such as Alzheimer's disease. Based on this evidence, dietary guidelines for the prevention of chronic disease promote increased consumption of plant-based foods as a good source of obtaining biologically active phytonutrients. One important class of phytochemicals is the carotenoids made up of over 700 different molecules of which about 6 seem to be important to human health.

J. Guest
School of Medical Sciences, Australasian Research Institute, University of NSW, 185 Fox Valley Rd, 2076 Sydney, NSW, Australia

R. Grant (✉)
School of Medical Sciences, Australasian Research Institute, University of NSW, 185 Fox Valley Rd, 2076 Sydney, NSW, Australia

Sydney Medical School, Australasian Research Institute, University of Sydney, 185 Fox Valley Rd, 2076 Sydney, NSW, Australia
e-mail: rossg@sah.org.au

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While the role of some of the carotenoids in human disease has been known for many decades, in particular β-carotene and its pro-vitamin A activity (Moore 1937), the benefits of other members of the carotenoid class have emerged much later. Mathews-Roth suggested that the photoprotective carotenoids may be a treatment for any human disease in 1984 (Mathews-Roth 1984), while in 1993 Jimenez-Jimenez et al. were the first to report on the relationship between lycopene levels and a neurodegenerative disease (Jiménez-Jiménez et al. 1993). Subsequent epidemiological and other studies support a link between higher dietary intake and tissue concentrations of carotenoids and lower risk of chronic diseases such as heart disease, diabetes, and some cancers (recently reviewed by (Kaulmann and Bohn 2014)). As discussed in this chapter, evidence also suggests that increased levels of carotenoids can help maintain healthy cognitive function, especially into older age.

Carotenoids mediate their beneficial effects via several mechanisms, including cell growth regulation and modulation of gene expression and immune response. However, their primary protective mechanism is thought to be due to their potent antioxidant properties that effectively scavenge free radicals and reduce the risk of oxidative stress.

This chapter discusses the impact of carotenoids on neurological health by first reviewing their chemical characteristics, dietary sources, and general mechanisms of action before examining in some detail the available evidence for a protective role of various carotenoids in neurodegenerative disease.

Chemical Structure and Nomenclature

More than 600 carotenoids have been identified in nature and essentially all possess a polyisoprenoid structure. This is synthesized by the tail to tail linkage of two C_{20} diphosphate molecules. Joined by a long polyene chain containing 3–15 conjugated double bonds, the π electrons are delocalized over the complete length of the system. The resulting $C_{40}H_{56}$ skeleton is near symmetrical and can be considered the backbone of the molecule. Alteration by cyclization at one or both ends, addition of oxygenized functional groups, changes in the level of hydrogenation, or any combination of these processes results in the individual carotenoid types (Britton 1995). Rearrangements or degradations of the carbon skeleton may also occur, so long as the two central methyl groups, joined in a 1,6-positional relationship, remain. As such retinoids are not incorporated within the carotenoid class.

Based on their structure carotenoids are broadly classified into two groups, the parent hydrocarbons, also termed carotenes, and xanthophylls, their oxygenated derivatives. Using this system, well-described carotenoids such as α-carotene and β-carotene are classified as hydrocarbons, while lutein, lycopene, zeaxanthin, and astaxanthin are described as xanthophylls (see Fig. 1) (Namitha and Negi 2010).

Rules for the nomenclature of carotenoids have been published by the International Union of Pure and Applied Chemistry (IUPAC) and the IUPAC-International Union of Biochemistry Commissions on Biochemical

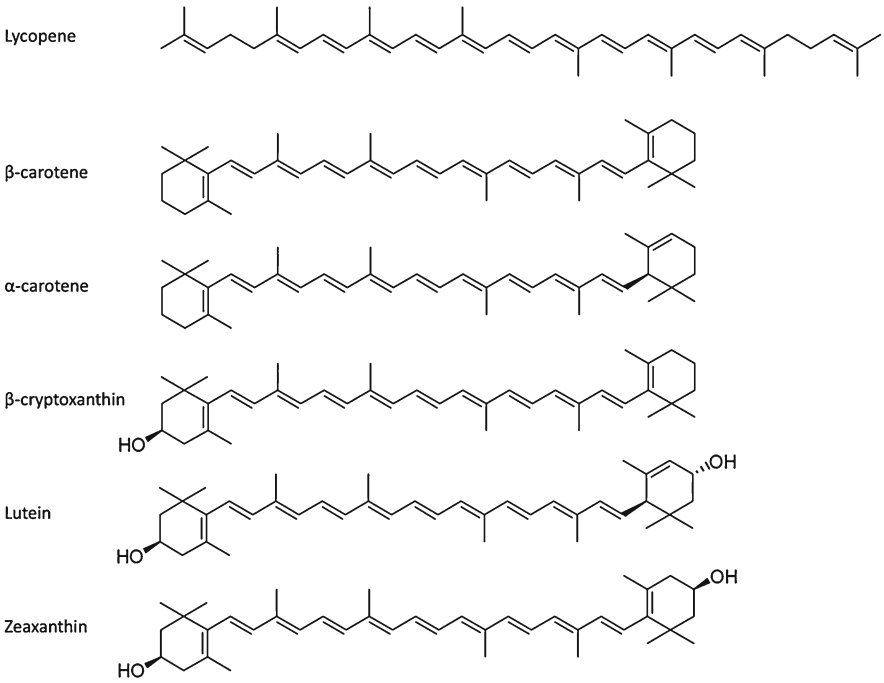

Fig. 1 The chemical structure of some common plasma carotenoids

Nomenclature (1975). Although nonsystematic names are often used for common carotenoids, all semi-systematic names are derived from the stem "carotene" structure and numbered according to Fig. 2. Two Greek letters are used to describe the end groups of the structure while prefixes and suffixes indicate the position of hydrogenation and group substitution (IUPAC Commission on the Nomenclature of Organic Chemistry and IUPAC-IUB commission on Biochemical Nomenclature 1975).

According to the number of double bonds, several *cis*/*trans* configurations are possible for a given molecule. Carotenoids tend to isomerize and form a mixture of mono- and poly-*cis*-isomers in addition to the all-trans form. In general, the majority of the carotenoids occurs as *trans* isomers in nature. However, this may be influenced by a number of factors, including heat and light.

Dietary Sources

As carotenoids cannot be synthesized by humans, or any Animalia, they must be obtained from the diet. The typical human diet incorporates about 40 carotenoids most of which are derived from natural food sources, particularly fruit and vegetables

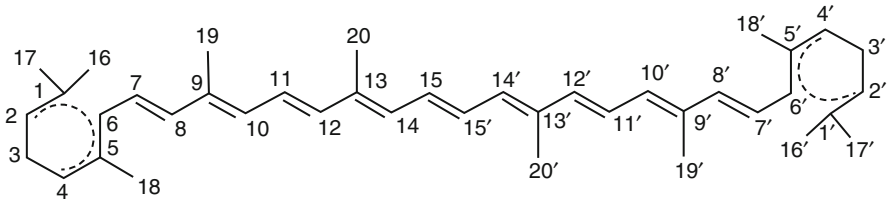

Fig. 2 Carotenoid stem "carotene" structure. All carotenoid semi-systematic names are based on the stem name "carotene," which corresponds to the structure above, where the *broken lines* at the two terminations represent two "double-bond equivalents"

(Nagao 2011), as *trans* isomers. Some animal products, such as eggs and red/pink fish and seafood, may also contain significant carotenoid concentrations; however, this is usually dependant on the quantity of plant matter consumed and their overall contribution to dietary intake is comparatively small. Carotenoids may also be ingested as colorants in manufacturing food products or taken as supplements.

Over the years a number of papers have reported the precise concentration of carotenoids, usually obtained by HPLC, found in particular food products produced under varying conditions. However the results are varied and somewhat confusing. In an effort to overcome this variability, the following provides a broad overview of the carotenoid content in fruit and vegetables according to color.

Green Fruits and Vegetables

All green plant tissue is so colored because of the presence of chlorophyll within chloroplasts. Within chloroplasts carotenoids serve as photosynthetic accessory pigments, absorbing light in the blue spectrum (400–600 mm) and transferring the energy to chlorophylls (Koyama 1991; Thornber et al. 1987). In addition to their photosynthetic function, chlorophyll-related carotenoids also play a vital role in photoprotection, mitigating oxidative damage from excessive light energy by quenching superoxide anion radicals, triplet chlorophylls, and singlet oxygen (Davidson and Cogdell 1981; Krinsky 1979; Niyogi 1999).

The carotenoid composition of chloroplasts is generally consistent and comprised of violaxanthin and neoxanthin (~15 %), β-carotene (25–30 %), and lutein (40–50 %), while small amounts of other carotenoids, such as zeaxanthin, antheraxanthin, and α-carotene, may also be found (Khachik et al. 1986; Žnidarčič et al. 2011). In contrast the quantity of carotenoids in different green fruit and vegetables varies widely; though in general, a darker green color is indicative of high chloroplast numbers and therefore a high concentration. Accordingly broccoli, kale, spinach, and coriander are known to possess high carotenoid concentrations while lighter green fruits and vegetables, such as celery or lettuce, contain comparatively low quantities (see Table 1) (Khoo et al. 2011).

Table 1 Carotenoid content of common fruits and vegetables[a]

	β-carotene (μg/100 g)	α-carotene (μg/100 g)	Cryptoxanthin-β (μg/100 g)	Lycopene (μg/100 g)	Lutein + Zeaxanthin (μg/100 g)
Cauliflower (white)	0	0	0	0	1
Asparagus	449	9	0	0	710
Peas	449	21	0	0	2477
Spinach	5626	0	0	0	12,198
Kale	5927	54	81	0	8198
Broccoli	361	25	1	0	1403
Beans, snap	379	69	0	0	640
Capsicum (green)	208	21	7	0	341
Brussels sprouts	450	6	0	0	1590
Squash	90	0	0	0	290
Corn (yellow)	47	16	115	0	664
Lemon (without peel)	3	1	20	0	11
Banana	26	25	0	0	22
Cantaloupe (orange)	2020	16	1	0	26
Apricot	1094	19	104	0	89
Nectarine	150	0	98	0	130
Orange	87	7	116	0	129
Carrot (orange)	8285	3477	0	1	256
Pumpkin	3100	4016	0	0	1500
Plum	190	0	35	0	73
Watermelon	303	0	78	4532	8
Tomato (red)	449	101	0	2573	123
Capsicum (red)	1624	20	490	0	51
Apple (red)	11	0	11	0	11
Raspberry	12	16	0	0	136
Strawberry	7	0	0	0	26
Cherry	38	0	0	0	85
Grapefruit	686	3	6	1419	5
Beetroot	20	0	0	0	0
Blueberry	32	0	0	0	80
Cabbage (red)	670	0	0	20	329

All concentrations based on raw food variants

[a]Derived from the USDA National Nutrient Database for Standard Reference available at http://ndb.nal.usda.gov/

Red, Orange, and Yellow Fruits and Vegetables

As with those that are dark green in color, many of the red, orange, and yellow fruits and vegetables are a rich source of carotenoids. It is important to note however that these vibrant colors are not always due to the presence of carotenoids, and instead may be attributed to the existence of other phytonutrients such as flavonoids (including anthocyanidins, flavanols, and flavanones).

Within red, orange, and yellow fruits and vegetables, carotenoids mainly exist within chromoplasts (chromo = color). Usually derived from chloroplasts, chromoplasts develop during fruit maturation, resulting in the loss of most photosynthetic machinery and the accumulation of carotenoids in novel cellular structures (Li 2013; Li and Yuan 2013). Due to the absence of chlorophyll, the light-gathering function of chromoplast carotenoids is no longer required. Rather, their key role is to act as attractants for seed dispersal and pollination, as precursors to a range of scents as well as photoprotective compounds (Merzlyak and Solovchenko 2002).

Unlike chloroplasts the carotenoid composition of chromoplasts varies widely between different plant species. For example the major carotenoid present in tomato is lycopene, while the mango fruit chiefly accrues β-carotene. Natural variation in the type and quantity of carotenoid accumulation also occurs between individuals of the same species and is largely influenced by different cultivation practices and location (Ben-Amotz and Fishler 1998; Pott et al. 2003). However despite these variations some distinctive patterns have been recognized. Depending on color, the predominant carotenoid/s found in different fruit/vegetables is generally as follows: (a) red, lycopene; (b) orange, β-carotene and/or its hydroxyl derivatives β-Cryptoxanthin and zeaxanthin; (c) orange to yellow, α-carotene and/or its hydroxyl derivatives, especially lutein; (d) yellow, carotenoid epoxides (see Table 1) (Brown et al. 2008; Wall et al. 2001). Some carotenoids are also characteristic or unique to a particular species, for example capsorubin and capsanthin in red peppers (Shim et al. 2013). In general intense/bright color is also indicative of high chromoplast numbers and therefore a high carotenoid concentration (Khoo et al. 2011; Britton et al. 2009).

A number of useful databases are available that list the food content of common carotenoids. The USDA National Nutrient Database for Standard Reference is particularly useful and available at http://ndb.nal.usda.gov/.

Absorption and Bioavailability

The absorption and bioavailability of carotenoids has recently been well reviewed by others (Nagao 2011; Fernández-García et al. 2012; Nagao 2014) and, as not the focus of this chapter will only be briefly discussed.

Of the more than 700 carotenoids that have been observed in nature, about 40 are present in the human diet and only 20 have been characterized in human blood and tissue. Of those quantifiable in serum, β-Cryptoxanthin, lutein, lycopene, zeaxanthin, α-carotene, and β-carotene are the most abundant (Nagao 2011; Nierenberg and Nann 1992; Parker 1989; Khachik et al. 1991).

Table 2 Concentration and half-life of human plasma carotenoids

Carotenoid	Conc. human plasma (Guest et al. 2014; Olmedilla-Alonso et al. 2005; Talwar et al. 1998; Yeum et al. 1996)	~$t_{1/2}$ (days) (Burri et al. 2001)
α-Carotene	0.03–0.22	45
β-Carotene	0.14–0.69	37
Lycopene	0.41–0.66	26
Lutein	0.22–0.43	76
Zeaxanthin	0.03–0.12	38
β-Cryptoxanthin	0.11–0.37	39

Due to their lipophilicity the absorption of carotenoids follows a similar pathway to that of dietary fats. This is initiated by the release of carotenoids from the food matrix, via chewing and the action of digestive enzymes. The released carotenoids are subsequently incorporated into lipid droplets within gastric emulsions where thereafter, by the action of lipolytic enzymes in pancreatic juice, they become solubilized in mixed micelles comprised of dietary lipids, bile salts, and biliary phospholipids. A portion of solubilized carotenoids is then absorbed by intestinal epithelia, packaged into chylomicrons and secreted into the lymphatic system for transport to various tissues (Nagao 2014).

The bioavailability of carotenoids is highly variable and affected by a variety of dietary and physiological factors. As outlined by in 1998 by Castenmiller and West (Castenmiller and West 1998), these factors include species of carotenoids, molecular linkage, amount of carotenoids consumed in a meal, matrix in which the carotenoid is incorporated, effectors of absorption and bioconversion, nutrient status of the host, and genetic or other host-related factors.

Once in the lymphatic system, carotenoids within chylomicrons are delivered to the peripheral circulation via the thoracic duct. Following ingestion the peak blood concentration usually occurs between 24 and 48 h; however for some carotenoids, such as canthaxanthin, peak blood concentrations may occur as early as 6 and 11 h post-dosing (see Table 2) (Gustin et al. 2004; Kostic et al. 1995; White et al. 1994). This reflects the time required for transport of carotenoid containing chylomicrons to the liver and then the re-excretion of carotin within very low-density lipoproteins into the circulation.

Entry and Distribution of Carotenoids in the Central Nervous System

Transport Systems

Our understanding of the mechanisms involved in the transport of carotenoids into the brain is surprisingly limited. While previously assumed to occur almost exclusively via passive diffusion (Hollander and Ruble 1978), evidence now suggests that by interacting with mixed micelles a number of transporter proteins may facilitate

the uptake of molecules solubilized within them, thereby assisting the passage of carotenoids across the blood brain barrier (BBB). In particular at least four transporter proteins, described below, are thought to function in this manner.

Firstly scavenger receptor class B type 1 (SR-BI; also known as CLA1 for CD36 and LIMPII Analogous-1 in humans) is a transmembrane multiligand lipoprotein receptor known to selectively mediate the uptake of lipids into cells. Expressed in brain capillary endothelial (Goti et al. 2001), its possible role in the transport of carotenoids was first indicated by Reboul et al. in 2005. Using Caco-2 TC-7 monolayers as a model for human intestinal epithelium, Reboul et al. identified that SR-BI was involved in the uptake of lutein (Reboul et al. 2005). Since then, SR-BI has been implicated in the transport of other carotenoids including zeaxanthin, β-carotene, and lycopene (During et al. 2008; Moussa et al. 2011; Van Bennekum et al. 2005). While not directly demonstrated to facilitate the transport of carotenoids into the brain, SR-BI has been found to contribute to the selective uptake of HDL-associated vitamin E in porcine brain capillary endothelial cells and thus may assist in the transport of other lipophilic molecules, such as carotenoids, across the BBB (Goti et al. 2001).

Cluster determinant 36 (CD36; also known as Fatty Acid Translocase), a scavenger receptor akin to SR-BI, is likewise thought to assist the entry of carotenoids into the brain. Expressed on brain microvascular epithelium (Ueno 2011), CD36 has also been located in the membrane of several other cell types including microglia (Coraci et al. 2002). While traditionally considered in the context of immunity CD36, like SR-BI, can interact with a broad variety of ligands, including VLDL, LDL, and HDL, and is involved in lipoprotein ligation/endocytosis (Coburn et al. 2001; Endemann et al. 1993). With regard to carotenoid transport, CD36 has been shown using transfected COS-7 kidney cells, adipocytes, and adipose tissue cultures to be involved in the uptake of β-carotene as well as lycopene and lutein respectively (Moussa et al. 2011; Van Bennekum et al. 2005). Though, like SR-BI, not directly demonstrated to facilitate the transport of carotenoids into the brain, its known interaction with carotenoids and presence in brain capillary endothelia indicates a potential role for this glycoprotein in carotenoid BBB transport.

In addition to the transporter proteins discussed thus far the passage of carotenoids, in particular lutein and zeaxanthin, into the central nervous system (CNS) is also likely assisted by Glutathione S-Transferase Pi 1 (GSTP1) and Human Retinal Lutein-Binding Protein (HR-LBP). Identified within the choroid plexus, vascular endothelium and ventricular lining cells (Carder 1990, MacDonald 1990) of the human brain (Carder et al. 1990; Macdonald et al. 1990), these two lutein-binding proteins are known to be responsible for the selective accumulation of lutein and zeaxanthin in the retina (Bhosale et al. 2004, 2009; Bone et al. 1988; Snodderly et al. 1991). Combined with the fact that xanthophylls account for less than 40 % of carotenoids in plasma and most tissues (Nierenberg and Nann 1992; Yeum et al. 1996; Kaplan et al. 1990), but ~65 % of total brain concentrations (Craft et al. 2004), it can be suggested that the transport of lutein and zeaxanthin across the BBB occurs, at least partially, by active mechanisms.

The known uptake of carotenoids by these transporter proteins, present within cells of the CNS, suggests that the passage of carotenoids across the BBB is likely

Table 3 Carotenoid distribution in various brain regions[a]

	Frontal		Occipital	
Carotenoid	Gray	White	Gray	White
α-Carotene	3.6 ± 1.2	6.3 ± 2.8	3.3 ± 1.2	2.0 ± 0.4
β-Carotene	7.6 ± 3.2	15.2 ± 7.0	7.8 ± 2.3	9.8 ± 3.2
Lycopene	7.9 ± 3.8	11.1 ± 9.0	6.0 ± 1.8	3.4 ± 1.8
Lutein	11.8 ± 2.6	8.7 ± 3.2	8.3 ± 2.3	2.8 ± 1.2
Zeaxanthin	9.2 ± 2.3	7.8 ± 2.8	6.7 ± 2.4	1.8 ± 0.6
β-Cryptoxanthin	17.5 ± 3.2	23.0 ± 8.5	14.6 ± 3.3	7.8 ± 0.9
Total carotenoids	84.2 ± 12.7	121.2 ± 48.5	65.0 ± 9.6	44.0 ± 10.7

[a]Adapted from Craft et al. (2004)

to be assisted. It is theorized that as with other fat soluble vitamins, such as vitamin E, passive diffusion probably occurs at high, pharmacological concentrations, while protein-mediated transport likely predominates at dietary doses (Reboul and Borel 2011; Spector and Johanson 2007). However dedicated experiments are clearly required to confirm this hypothesis and questions regarding the mechanisms of interaction between the transporter and its ligands still remain.

Brain Distribution

While further investigation is required to understand the passage of carotenoids into the CNS, within the brain at least 16 carotenoids have been identified. The major carotenoids include lutein, zeaxanthin, anhydrolutein, α-cryptoxanthin, ß-cryptoxanthin, α-carotene, *cis*- and *trans*-β carotene, and *cis* and *trans* lycopene (Craft et al. 2004). The combined total carotenoid brain concentration ranges from 14.3 to 303 pmol/g. Xanthophylls account for approximately 65 % and significantly exceed that of carotenes in both gray and white matter (see Table 3). Interestingly, age-associated reductions in frontal but not occipital lobe concentrations in total carotenoids have been observed in humans, and, as discussed in section 1.6, may be linked to adverse outcomes (Craft et al. 2004; Mathews Roth et al. 1976).

Mechanisms of Action

Significant evidence indicates that an increased intake of dietary carotenoids reduces the rate of age-related cognitive decline and risk of several neurodegenerative disorders (Johnson et al. 2013; Renzi et al. 2014; Suganuma et al. 2002). Research suggests that these beneficial effects are mediated by a variety of mechanisms activity a includes to their pro-vitamin A activities include antioxidant, anti-inflammatory, and hypocholesterolemic effects.

Antioxidant Activity

In 1956 Harman proposed the oxidative stress theory of aging which suggests that the accumulation of unrepaired oxidative damage results in the typical aging phenotype (Harman 1956). The term "oxidative stress" describes a significant imbalance between antioxidant defences and the bodies' formation of reactive oxygen and/or nitrogen species (RONS). While there are several sources of ROS within the body, the primary source is generally agreed to be the leakage of electrons to ground state oxygen from early components of the mitochondrial electron transport chain, resulting in the production of the superoxide radical (O_2^-) (Kowaltowski et al. 2009; Short et al. 2005). Importantly, at modest concentrations, ROS are used in a variety of normal physiological functions. Although there is the potential for damage, this is kept in check by an intricately connected antioxidant defense and repair system (Pamplona and Costantini 2011). However, under conditions of reduced antioxidant capacity or excess production, ROS can cause indiscriminate damage to cellular constituents (DNA, proteins, and lipids) that, if unrepaired, may lead to cell death and tissue dysfunction.

The brain is particularly vulnerable to oxidative damage as a consequence of its high oxygen demand, high level of both polyunsaturated fatty acids and transition metals, and poor antioxidant defences (Halliwell 2006; Sastry 1985; Schenck and Zimmerman 2004). As we age, the vulnerability of the brain to oxidative damage increases due to reduced integrity of the blood brain barrier and amplified mitochondrial dysfunction (Farrall and Wardlaw 2009; Mecocci et al. 1993). Indeed animal and tissue studies have shown the aging brain to be accompanied by an accumulation of markers of lipid, protein, and DNA oxidative damage (Çakatay et al. 2001; Sattarova et al. 2013). Failure to repair this damage has been demonstrated to cause genomic instability and neuronal apoptosis and is associated with the development of neuropathologies such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Annunziato et al. 2003; Cutler et al. 2004; Dexter et al. 1989; Nunomura et al. 2001; Rothstein 2009).

Singlet Oxygen Quenching

Within the brain, carotenoids are thought to exert a variety of protective effects, including inhibition of oxidative damage through antioxidant mechanisms. The antioxidant properties of carotenoids are largely associated with their exceptional singlet oxygen quenching ($^1O_2$) ability (Ouchi et al. 2010), a process, for the most part, that depends largely on the physical transfer of energy between singlet oxygen and the carotenoid molecule (physical quenching). The resultant triplet state carotenoid then dissipates this energy into the surrounding solvent and subsequently returns intact back to ground state for reuse in preceding quenching cycles (Stahl and Sies 2003).

The effectiveness of a carotenoid to quench singlet oxygen is primarily related to its structure, chiefly the number of conjugated double bonds (which determines its lowest triplet energy level) as well as the presence/absence and structure of a β-ionone ring. Thus, in relatively simple matrices such as soybean oil the total singlet oxygen quenching rate constants for lutein, zeaxanthin, lycopene, and astaxanthin, containing 10, 11, 11, and 13 conjugated double bonds have been reported as ~5.7×10^9, 6.7×10^9, 6.9×10^9, and 9.8×10^9 M^{-1} s^{-1}, respectively. Interestingly in more complex matrices such as plasma, lycopene has been found to be the most efficient singlet oxygen quencher, followed by astaxanthin, α-carotene, β-carotene, zeaxanthin, and lutein (Di Mascio et al. 1989). While the reason for this inconsistency is largely unknown in vivo metabolic compartmentalization and binding will influence carotenoid availability and need to be considered (Nunomura et al. 2001; Rothstein 2009).

Free Radical Scavenging

In addition to their capacity to quench singlet oxygen carotenoids are known to scavenge a number of free radical species including peroxyl, hydroxyl, and superoxide radicals (Takashima et al. 2012; Trevithick-Sutton et al. 2006). As previously discussed by Rice-Evans et al. (1997), this is initiated in vitro by at least three types of reactions, specifically allylic hydrogen abstraction, electron transfer, or radical addition (Burton and Ingold 1984). Kin to the efficacy of carotenoids to quench singlet oxygen, the rate at which these reactions occur depends primarily upon the carotenoid structure. In general the rate of reaction has been found to increase for an increasing number of conjugated double bonds and to decrease in the presence of hydroxy and especially keto groups (Mortensen and Skibsted 1997).

Through these mechanisms carotenoids, at least at dietary derived concentrations, are thought to constrain oxidative processes within the CNS. Indeed all common dietary derived carotenoid species have been found, using various in vitro and in vivo techniques, to inhibit oxidative damage to multiple cellular constituents (Porrini and Riso 2000; Santocono et al. 2006; Sarkar et al. 1995; Wu et al. 2014). As an example, astaxanthin has been observed to alleviate brain aging in rats by attenuating oxidative damage to lipids, protein, and DNA and by restoring the activities of antioxidant enzymes including glutathione peroxidase and superoxide dismutase (Wu et al. 2014). Though limited work has been conducted in humans, work from our own research team has observed that increased plasma levels of α-carotene and β-carotene have also been associated with increased cerebrospinal fluid (CSF) total antioxidant capacity (Guest et al. 2014).

Mitochondrial Localization

As discussed above, the primary source of ROS is generally agreed to be the leakage of electrons from early components of the mitochondrial electron transport chain. Interestingly, studies in cats and dogs have found that dietary β-carotene and

lutein are significantly taken up into the nuclei, microsomes, and mitochondria of circulating lymphocytes, with a maximum accumulation occurring in the mitochondria (Chew et al. 1998, 2000). Thus the close proximity of at least some carotenoids to the mitochondria may serve to augment their ROS quenching/scavenging effects.

Modulation of Enzymes with Pro/Antioxidant Function

In addition to directly interacting with free radicals a number of carotenoids, such as lycopene, have been shown to inhibit oxidative damage by modulating free radical generating enzymes (such as nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), inducible nitric oxide synthase (iNOS), cytochrome P450 enzymes (CYP450), and cyclooxygenase-2 (COX-2)) and by activating protective/antioxidant Phase II enzymes (including NAD(P)H dehydrogenase quinone 1 (NQO1), heme oxygenase-1 (HO-1), and glutathione S-transferase (GSTs)) (Edderkaoui et al. 2010; Lin et al. 2014; Rafi et al. 2007; Wang and Leung 2010). While the mechanisms surrounding these phenomena are not well described, particularly within the context of the CNS, a number of carotenoid species have been found to influence the redox sensitive Keap1-Nrf2 pathway (Ben-Dor et al. 2005).

The Keap1-Nrf2 pathway functions to protect cells against damage caused by oxidative processes. Under homeostatic conditions, Nrf2 is bound to the inhibitory protein Keap1 within the cytosol. Alteration to the conformation of Keap1, however, by various oxidants and electrophiles leads to the liberation of Nrf2 and its translocation to the nucleus where it binds to the antioxidant response element (ARE). This subsequently results in an increased expression of cytoprotective and antioxidant enzymes, including HO-1, NQO1, and GSTs mentioned above (Kansanen et al. 2013; Kim et al. 2010a).

Importantly a number of carotenoids, including lycopene, β-carotene, and astaxanthin, have been demonstrated to stimulate the ARE transcription system. However, as carotenoids lack an electrophilic group, it is theorized these effects are most likely modulated by their oxidized metabolites, which covalently modify Keap1 resulting in activation of Nrf2 and elevated expression of ARE genes.

Potential Pro-oxidant Effects

It is important to note however that at high concentrations, or under conditions of high oxygen partial pressure, carotenoids can exhibit pro-oxidant behavior. While primarily investigated in relation to β-carotene after supplementation was found to increase the incidence of lung cancer in smokers by 18 % (Blumberg and Block 1994), other carotenoids species have been shown to exhibit similar effects. For example at relatively low concentrations (1–3 μM), within the range normally acquired through food consumption (Porrini and Riso 2005), lycopene has been shown to afford protection against DNA damage whereas at high concentrations

(>4 μM) the ability of lycopene to inhibit DNA damage is lost and even increased (Lowe et al. 1999; Guest et al. 2015). Thus while the consumption of carotenoids has demonstrated health benefits and may inhibit oxidative processes within the brain, evidence suggests that under certain circumstances carotenoids may potentiate toxicity and suggest a need for caution when supplementing, particularly among individuals with enhanced oxidative potential.

Anti-inflammatory Effects

(RONS interact with inflammatory mediators in a complex bidirectional manner, leading many to consider oxidative and inflammatory processes interdependent. Certainly inflammatory cytokines have been shown to directly induce RONS formation and cause the activation of various oxidant generating enzymes, including COX-2, iNOS, and myeloperoxidase (MPO) (Keshari et al. 2012; Mohaupt et al. 1995; Wassmann et al. 2004; Wu et al. 2012). On the other hand, RONS facilitated damage to cellular constituents can directly ind.e microglial activation resulting in the . Further release of both RONS and cytokines (Wang et al. 2004). During acute inflammation these processes function to protect the host and are generally beneficial. However, chronic inflammation, even at low levels, may result in the continuous and synergistic activation of these processes causing cell death, tissue dysfunction, and disease (Il'Yasova et al. 2008; Rawdin et al. 2013).

Indeed, chronic neuroinflammation is a well-established feature of the pathology associated with most neurodegenerative disorders (Collins et al. 2012; Evans et al. 2013; Furney et al. 2011). Characterized by upregulated microglial and astrocyte activity, altered production of signaling molecules such as cytokines and increased BBB permeability, its chronic induction causes numerous detrimental consequences within the CNS. Evidence suggests that carotenoids may, at least partially, inhibit these processes.

In addition to the antioxidative activities discussed above, these anti-inflammatory mechanisms include modulation of: (a) inflammatory related cells, including microglia; (b) proinflammatory enzyme activity such as cyclooxygenase; (c) proinflammatory molecule (i.e., cytokine) production; and (d) inflammatory gene expression (see Fig. 3).

Microglia are considered to be the most important innate immune cells of the CNS. Constituting about 5–12 % of the total glial population (Ling and Leblond 1973), they use an array of immune receptors to recognize threatening stimuli. Under pathological conditions, the activation of microglia helps to restore CNS homeostasis. However, uncontrolled activation may have deleterious outcomes. The capacity for carotenoids to influence microglial activation has only been recently recognized and as such the precise mechanisms involved are not completely understood. What has been elucidated, using murine models and in vitro techniques, is that in activated microglia at least some carotenoid species can regulate the production of cytokines. At present this is thought to occur via two main mechanisms, namely downregulation of both the MAPK and NF-κB pathways.

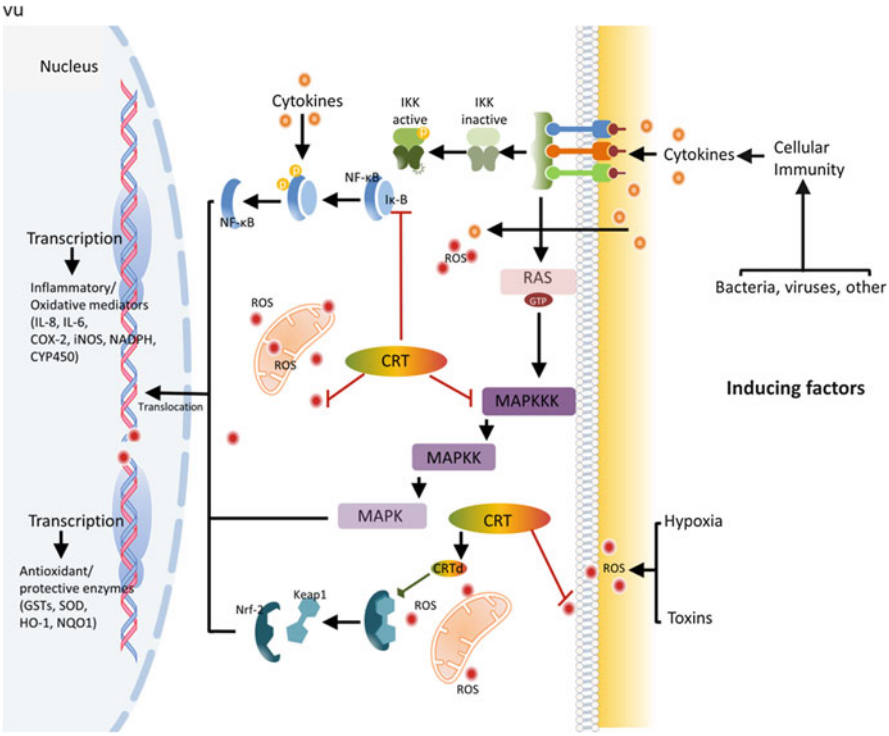

Fig. 3 Inhibition of oxidative and inflammatory processes by carotenoids. In addition to directly quenching/scavenging free radicals, carotenoids inhibit oxidative and inflammatory processes in responses to various inducing factors, by modulating free radical generating/proinflammatory enzymes (such as nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), inducible nitric oxide synthase (iNOS), cytochrome P450 enzymes (CYP450), and cyclooxygenase-2 (COX-2)) and by activating protective/antioxidant Phase II enzymes (including NAD (P) H dehydrogenase quinone 1 (NQO1), heme oxygenase-1 (HO-1), and glutathione S-transferase (GSTs)). The modulating capacity of carotenoids is linked to (a) inhibition of I-kappaB (IκB) kinase, preventing the phosphorylation of IκB inhibitory proteins and thus the translocation of the nuclear factor kappa B (NF-κB) p65 subunit to the nucleus, (b) inhibition of extracellular signal-regulated protein kinases 1/2 and mitogen- and stress-activated protein kinase-1 phosphorylation preventing mitogen-activated protein kinases (MAPK) pathway activation, (c) covalent modification of kelch-like ECH-associated protein 1 (Keap1) resulting in the translocation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) to the nucleus and elevated expression of antioxidant response element genes. Adapted from Kaulmann and Bohn (2014)

Nuclear factor κB plays a key role in the transcription of various genes that modulate inflammatory responses. When cells, such as microglial, are not activated, NF-κB is complexed to an inhibitory protein κB (e.g., IκB-α, IκB -β, IκB-γ, IκB-ε) within the cytosol. Unspecific (RONS) and specific (TNF-α, IL-1β) signals can activate the NF-κB pathway, beginning with the dissociation of the inhibitor from the NF-κB complex. NF-κB then translocates into the nucleus where it binds to DNA promoter sequences and activates the transcription of various immunoregulatory

and inflammatory genes (Kuryłowicz and Nauman 2008; McConnell and Yang 2009). A number of carotenoids have been funded to reduce NF-κB. In particular astaxanthin has been shown in activated BV-2 microglia, to inhibit the activity of IκB kinase, preventing the phosphorylation and degradation of IκB inhibitory proteins, and subsequently the translocation of the NF-κB p65 subunit to the nucleus. This was associated with suppressed IL-6 expression (Kim and Choi 2010; Kim et al. 2010b; Lee et al. 2003; Zhang et al. 2014).

Comparatively few studies have investigated the effect of carotenoid cellular exposure on the targets of the MAPK pathway, fewer still on cells that reside within the CNS. The family of mitogen-activated protein kinases (MAPKs) includes c-Jun NH2-terminal kinase (JNK), p38, and extracellular signal-regulated kinase (ERK). Every MAPK signaling pathway also consists of at least three components, a MAPK 3 kinase (MAPKKK), a MAPK 2 kinase (MAPKK), and a MAPK (Kim and Choi 2010). In response to various stimuli (i.e., Proinflammatory cytokines, including TNF-α and IL-1β and oxidative stress) MAPK pathways are activated, resulting in a variety of cellular consequences. While some of these are beneficial the chronic induction of others, such as microglial and astrocyte activation and the release of proinflammatory mediators, can result in adverse effects (Kim and Choi 2010; Lee et al. 2000; Waetzig et al. 2005). Research suggests that activation of the MAPK pathway may be prevented via several carotenoid species, including astaxanthin, lycopene, and β-carotene (Jang et al. 2009; Kavitha et al. 2013; Kim et al. 2004). While the majority of research has been conducted in systemic cell lineages, astaxanthin has been shown in activated BV-2 microglial to inhibit the phosphorylation of both ERK1/2 and mitogen- and stress-activated protein kinase-1 (MSK1), resulting in reduced expression of the classically proinflammatory cytokine IL-6 (Kim et al. 2010b). Taken together these results suggest that carotenoids, such as astaxanthin, may reduce neuroinflammation by inhibiting the production of proinflammatory cytokines, through a NF-κB and MAPK dependant pathways.

In addition to downregulating both MAPK and NF-κB pathways, carotenoids have also been shown in activated microglia to modulate the activity of COX-2, an inducible enzyme primarily responsible for the production of proinflammatory prostaglandins. Specifically lycopene has been found using primary murine microglia to inhibit the expression of COX-2 by both increasing the phosphorylation of adenosine monophosphate-activated protein kinase (AMPKα) and increasing HO-1 expression (Lin et al. 2014), presumably by stimulation of ARE transcription system as discussed in section 1.5.2 above. Although reports of the modulatory effect of carotenoids on other proinflammatory enzymes within the CNS are scarce, lutein has been shown in macrophage cell lines to competitively inhibit cytosolic phospholipase A2 (Song et al. 2010); while β-carotene has been demonstrated to reduce lipoxygenase activity in rat epidermal cells (Lomnitski et al. 1997). Although further research on the capacity of carotenoids to modulate inflammatory related enzymes is required, particularly within the CNS, there is now strong evidence in support of their anti-inflammatory influence.

Hypocholesterolemic Effects

In addition to their radical scavenging and inflammatory modulating potential as discussed above, carotenoids may aid CNS function through modulation of CNS cholesterol metabolism.

Cholesterol is a pluripotent molecule whose transport and metabolism is tightly regulated between the major brain cells, playing vital roles in axon myelination, lipid raft formation, and neurosteroid synthesis (Hu et al. 1987; Korade and Kenworthy 2008; Saher et al. 2005). However, while essential for brain development and function, if cholesterol levels fall above or below the normal physiological range several metabolic pathways of compensation are activated that, if chronically induced, can result in neurodegeneration. Indeed, altered cholesterol metabolism has been identified in several neurologic disorders (Anchisi et al. 2013). In particular elevated levels of cholesterol have been found in the spinal cord of ALS patients and have been shown to accelerate Alzheimer's amyloid pathology in a transgenic mouse models (Cutler et al. 2002; Puglielli et al. 2001; Refolo et al. 2000).

Recently, in addition to their well-described antioxidant and anti-inflammatory activity, a new mechanism involving a regulation of cholesterol metabolism by carotenoids has been evoked. While several carotenoid species, such as astaxanthin and β-carotene (Fuhrman et al. 1997; Yang et al. 2011), have been found to possess hypocholesterolemic properties, the majority of research conducted to date has focused on the lipid modulatory effect of lycopene. A recent meta-analysis of serum lipids revealed a significant cholesterol-lowering effect of lycopene for total serum cholesterol and low-density lipoprotein (LDL) at daily dosages of ≥25 mg (Ried and Fakler 2011).

The mechanisms through which lycopene exerts its hypocholesterolemic effects are severalfold. In human macrophages lycopene has been shown to decrease cholesterol synthesis through a reduction of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) activity and expression as well as modulation of acyl-coenzyme A: cholesterol acyltransferase (ACAT) and LDL receptor and activity (Fuhrman et al. 1997; Napolitano et al. 2007a, b; Yang et al. 2001). Moreover lycopene has been shown in vitro to enhance civilianrol efflux through an increase in caveolin-1 and ABCA1 (ATP-binding cassette, subfamily A, member 1) expression (During et al. 2005; Fielding and Fielding 2001; Gargalovic and Dory 2003; Oram 2002).

Unfortunately, very few studies have investigated the cholesterol modulating effects of carotenoids within the context of the CNS. Recently, however β-carotene has been found in astrocytes to enhance the expression of genes related to cholesterol regulation, including *Abca1, Abcg1,* and *Hmgcr*. Of note *ApoE* expression was also increased (Yamagata et al. 2013). Within the brain, apolipoprotein E (ApoE) is the main lipid carrier protein mediating the exchange of cholesterol between neuronal and nonneuronal cells. Importantly evidence suggests that ApoE may play a crucial role in lipid clearance and recycling, particularly after an injury, and its impairment has been associated with increased risk of Alzheimer's disease in several studies (Blom et al. 2009; Leoni et al. 2010; Petersen et al. 1995). Thus by both

maintaining efficient cholesterol exchange between cells within the CNS, and possibly through other cholesterol modulatory effects, carotenoids may inhibit neurodegenerative processes.

Carotenoids in Neurodegenerative Disease

Mild Cognitive Impairment

Individuals with mild cognitive impairment (MCI) have difficulties with memory (amnestic MCI) or other essential cognitive function (nonamnestic MCI) that is serious enough to be noticed by themselves and others but are not sufficiently severe to interfere with daily life. While MCI is considered to be the intermediate phase between normal aging and the initial symptoms of Alzheimer disease (AD) not all individuals with MCI progress to dementia and some may even improve (Belleville et al. 2006).

The differential molecular and pathologic manifestations of the aged brain, MCI, and AD are not well established. What is known is that the pathological hallmarks of AD, including deposition of amyloid-β (Aβ) peptides and neurofibrillary tangles (NFT) of hyperphosphorylated tau, are first evident in the temporal lobe before subsequently progressing to other brain regions (Blennow et al. 2006; De Leon et al. 1993). While tentative, a growing body of evidence suggests that these histopathological changes, initiated decades before the onset of clinical symptoms (Braak and Braak 1997; De Leon et al. 2001; Price and Morris 1999), are propagated by increased inflammation and accompanying oxidative damage to proteins, lipids, and DNA (Andersen 2004; Barnham et al. 2004; Marchesi 2011; Sheng et al. 2000). Since exacerbation of these processes appears to be involved during the earliest time points of disease, therapeutic interventions during the prodromal MCI period are more likely to inhibit disease progression than those instigated after signifithe significant neuronal losscurred.

The hypothesis of carotenoids having a preventive role in MCI development and progression of AD is centered on their demonstrated antioxidant and anti-inflammatory properties, discussed previously in sections 1.5.1 and 1.5.4. This is further supported by evidence from several epidemiological studies of an association between increased carotenoid consumption, particularly during midlife, and either improved cognition or reduced risk of cognitive decline (Johnson et al. 2013; Johnson 2012; Kesse-Guyot et al. 2014). Indeed lower plasma, retinal, and brain carotenoid concentrations have been observed in MCI patients compared to controls (Renzi et al. 2014; Johnson 2012). In contrast midlife consumption of a carotenoid-rich diet was found, in a sample of 2983 middle-aged adults participating in the SU.VI.MAX (Supplémentation en Vitamines et Minéraux Antioxydants) study, to be associated with a higher composite cognitive score (Kesse-Guyot et al. 2014). While in centenarians from the Georgia Centenarian Study, serum lutein, zeaxanthin, and β-carotene concentrations were consistently related to enhanced cognition (Johnson et al. 2013).

Although no studies have investigated the effect of carotenoid supplementation, or consumption of carotenoid-rich foods, on MCI progression to AD, a small number have sought to determine the influence of carotenoid ingestion on cognition. In the Physicians Health Study II, 4052 male participants were randomized to consume either 50 mg of β-carotene or a low-dose aspirin placebo every second day for 18 years. From 1997, 1904 additional recruits also consumed either 50 mg of β-carotene or placebo on alternate days for an average of 1 year. After age 65 years, cognitive testing revealed that while short-term β-carotene supplementation did not affect cognitive performance, long-term consumption was associated with enhanced verbal memory and mean global cognitive scores, compared to the placebo (Grodstein et al. 2007). Considered together, evidence from both epidemiological and intervention studies suggests that increased carotenoid consumption may assist in preventing both the development and progression of MCI, most likely by inhibiting oxidative and inflammatory processes.

Alzheimer's Disease

Alzheimer's disease (AD) is the most common form of dementia. In contrast to MCI, where frank disease is not apparent and progressive dysfunction may be inhibited, AD is characterized by progressive deficits in visuospatial and verbal memory, as well as other domains of cognition (e.g., executive function). As previously mentioned the pathological hallmark of disease development includes the extracellular deposition of amyloid-β (Aβ) peptides and intracellular neurofibrillary tangles (NFT) of hyperphosphorylated tau. Upstream drivers of these histopathological changes appear to involve a complex series of partially overlapping events comprising innate immune activation, disturbed cell signaling, excitotoxicity, mitochondrial dysfunction, altered metal ion metabolism, and abnormal glycation, amongst others (for review see Selkoe 2004). Of note all these processes are associated with increased inflammation and oxidative damage (Andersen 2004; Barnham et al. 2004; Marchesi 2011; Sheng et al. 2000).

Although considerable advances have been made in characterizing tissue damage, a clear understanding of the initiating causes of these events remains to be identified. Increased risk of AD in people over 65 has been linked to persons carrying alleles for APOE4, while an increased risk of early onset AD (i.e., < age 65) has been linked to the presence of the PSEN1, PSEN2, TREM2 genotypes or to those carrying an extra copy of chromosome 21 (i.e., Down's syndrome). However, as genetic factors account for only 5–10 % of AD risk, lifestyle factors clearly play the dominant role (Panza et al. 2004).

The consumption of a diet rich in carotenoids has been epidemiologically linked with reduced risk of AD and slower rates of cognitive decline (Kesse-Guyot et al. 2014; Dai et al. 2006). Lower plasma carotenoid concentrati. NS has also been observed in AD patients (Jiménez-Jiménez et al. 1999; Wang et al. 2008; Zaman et al. 1992). Importantly plasma levels of carotenoids have been found to

significantly and inversely correlate with dementia severity. In particular lower plasma levels of lutein and β-carotene have been observed in moderately severe AD patients compared to mild AD patients or controls (Wang et al. 2008). Although this may be indicative of the importance of fruit and vegetable consumption for brain health maintenance, these results do support a protective role of carotenoids the neurodegenerative changes associated with AD.

Unfortunately, no human intervention studies have investigated the sole influence of carotenoid consumption on AD risk or progression. Some studies have, however, explored the potential effect of combination supplementation. As an example, in a 3-year open-label intervention study involving cognitively normal, community-dwelling participants aged 65 years or older, Bun et al. investigated the efficacy of an omega-3, Ginkgo biloba, and lycopene supplement in preventing the development of AD. Bun et al. observed that higher adherence was associated with lower AD incidence in both unadjusted and adjusted models (Bun et al. 2015). In an earlier intervention study, omega-3, Ginkgo biloba, and lycopene supplementation was likewise found to improve cognition. Of interest while cognitive improvements were observed in both non-APOE4 carriers and carriers alike, a comparatively larger effect size was observed in those carrying the APOE4 allele (Yasuno et al. 2012). While the positive effects observed in these studies are not solely attributable to lycopene, these results do suggest that carotenoids, when combined with other beneficial phytonutrients, may prevent AD development, even in those who are genetically susceptible. Further research into this area is undoubtedly required.

Carotenoids may inhibit the development of AD through several mechanisms (as discussed above). In particular by both quenching ROS and upregulating antioxidant enzyme systems carotenoids may inhibit the neurodegenerative changes associated with mitochondrial dysfunction and oxidative stress, both of which are known to be key players in the development and progression of AD (Nunomura et al. 2001; Chen et al. 2006; Moreira et al. 2008). Notably an inverse relationship has been observed between RBC carotenoids, especially lutein, and peroxidized phospholipids concentrations in AD patients (Kiko et al. 2012). In a cell culture model of AD, lycopene has been shown to efficiently attenuate Aβ-induced ROS formation, improve neuron viability, and decrease the rate of apoptosis (Qu et al. 2011). While in a murine model of AD, lycopene administration (2.5 and 5 mg/kg/day) for 21 days has likewise been found to attenuate mitochondrial oxidative damage and improve memory retention (Prakash and Kumar 2014).

Carotenoids have also been shown in murine models to abrogate the neuroinflammatory changes associated with AD. Specifically lycopene (1, 2, and 4 mg/kg/day for 14 days) has been observed in rats to ameliorate Aβ1-42 induced impairments in spatial learning and memory, by inhibiting NF-κβ activity and associated increases in brain proinflammatory cytokines. Similarly, in a separate study chronic lycopene administration (2.5 and 5 mg/kg/day for 21 days) was again observed to improve memory retention, reduce neuroinflammation, and restore brain-derived neurotrophic factor (BDNF) levels in Aβ1-42 treated rats (Prakash and Kumar 2014).

In addition to positively modulating oxidative and inflammatory processes, carotenoids may also prevent AD development and progression through its less known hypocholesterolemic properties. As previously discussed evidence is accumulating for a link between altered brain cholesterol metabolism and AD susceptibility, with cholesterol depletion or loading influencing Aβ deposition both in vivo and in vitro (Howland et al. 1998; Simons et al. 1998). While altered cholesterol metabolism has similarly been shown to promote tau pathology (Glöckner and Ohm 2014). Importantly variation in the ApoE gene, known to modulate lipid metabolism and transport (Leoni et al. 2010; Bu 2009; de Chaves and Narayanaswami 2008), has been identified as a major risk factor for AD (Blom et al. 2009; Petersen et al. 1995). Though very few studies have investigated the cholesterol modulating effects of carotenoids within the context of the CNS, β-carotene, as discussed above, has been found in astrocytes to enhance ApoE expression (Yamagata et al. 2013). Thus preliminary evidence supports the view that carotenoids may prevent cholesterol-mediated AD pathology through ApoE-dependant mechanisms.

Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder behind AD (Lees et al. 2009). Characterized by degeneration of dopaminergic neurons and the formation of Lewy bodies, its pathogenesis has been related to an increase in oxidative and inflammatory processes in numerous studies. Several authors have also reported significant postmortem reductions in antioxidant enzyme activities, you within the substantia nigra (Ambani et al. 1975; Kish et al. 1985; Perry et al. 1982; Riederer et al. 1989), further indicating a possible contribution of redox imbalance in disease development.

Considering no pharmaceutical therapy has been found to cure or slow the progression of disease (Lees et al. 2009), and in light of their potent antioxidant and anti-inflammatory properties, carotenoids thus present a potential therapeutic option. Indeed, while the dopaminergic system is a key target of retinoic acid action, increased intake of both pro-vitamin A (β-carotene, α-carotene, and β-Cryptoxanthin) and non-pro-vitamin A (canthaxanthin, lutein, lycopene, and zeaxanthin) carotenoid species has been associated in a number of epidemiological studies with a reduced risk of PD. However, results are not always significant and a small number of studies have even reported a positive association between carotenoid consumption, in particular lutein, and increased risk of PD. In an attempt to clarify these inconsistencies Takeda et al. recently conducted a meta-analysis to assess the evidence on the association between blood levels or dietary intakes of carotenoids and risk of disease. Based on 13 studies with relatively low potential for selection bias Takeda et al. reported that increased levels of the pro-vitamin A carotenoid species, β-carotene and α-carotene, alone may potentially reduce the risk of development (Takeda et al. 2013).

While epidemiological evidence, therefore, indicates that the chief pathway through which some carotenoids may inhibit PD is centered on their participation in

retinol synthesis and metabolism, a number of in vivo and murine studies suggest other mechanisms may be involved. As an example using a Parkinsonian murine model the ingestion of tomato powder, rich in lycopene, was observed to prevent a decrease in striatal dopamine levels (Suganuma et al. 2002). More recently in a dopaminergic SH-SY5Y cellular model, physiologically relevant concentrations of lycopene were found to suppress methyl-4-phenylpyridinium iodide induced ROS accumulation, lipid peroxidation, and mitochondrial morphological changes as well as reverse reductions in ATP concentration (Yi et al. 2013). Thus although more research is required to determine the efficacy of carotenoid ingestion, particularly those of the non-pro-vitamin A species, for the prevention and inhibition of PD, some evidence does suggest that protection may be afforded through, at least, retinol and antioxidant-dependant mechanisms.

Conclusion

Consistent inverse relationships have been reported over many years between the ingestion of fruits and vegetables and a wide spectrum of diseases. While multiple elements contribute to these health benefits, the carotenoid class of molecules is considered to be key contributors. The most studied carotenoids in humans include α & β-carotene, lycopene, lutein, and zeaxanthin. Importantly the beneficial effects of these carotenoids are thought to be mediated, at least in part, through their role as antioxidants and anti-inflammatory modulators. As oxidative stress and inflammation also play central roles in the pathophysiology of mild cognitive impairment (MCI) and the neurodegenerative disorders, AD and PD, this chapter explored the evidence in support of a beneficial effect of carotenoids in these conditions.

In MCI the accumulated data does endorse the view that increased carotenoid intakes confer a level of reduced risk of cognitive decline. Similarly, in AD, the evidence also supports the view that carotenoids are beneficial, most likely through both antioxidative and anti-inflammatory processes as well as an apparent hypocholesterolemic effect. However, though some evidence does suggest a protective effect of carotenoids for PD patients, more research is required.

In conclusion, it is clear that disorders of the CNS are necessarily complex in relation to both their etiology and pathobiochemistry. Therefore, it is naïve to assume that simple supplementation with one group of molecules, such as the carotenoids, even given their many benefits could, in isolation, resolve these disorders. However, in spite of this, a relatively consistent and growing body of evidence does indicate that increasing carotenoid levels in the CNS are beneficial to neurological health, especially in those disorders characterized by increased oxidative and inflammatory activity.

Compliance with Ethics Requirements The authors declare that they have no conflicts of interest.

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Role of Quercetin Benefits in Neurodegeneration

Preetham Elumalai and Sreeja Lakshmi

Abstract Neurodegenerative disorders are often life threatening and hired as an economic burden to the health-care system. Nutritional interventions principally involving polyphenols were practiced to arrest or reverse the age-related health disorders. Flavonoids, a class of dietary polyphenols, are rising to superstardom in preventing brain disorders with their potent antioxidant defense mechanism. Quercetin is a ubiquitous flavonoid reported to have all-natural myriad of health benefits. Citrus fruits, apple, onion, parsley, berries, green tea, and red wine comprise the major dietary supplements of quercetin apart from some herbal remedies like *Ginkgo biloba*. Appositeness of quercetin in reducing risks of neurodegenerative disorders, cancer, cardiovascular diseases, allergic disorders, thrombosis, atherosclerosis, hypertension, and arrhythmia, to name a few, is attributed to its highly pronounced antioxidant and anti-inflammatory properties. Neurodegeneration, characterized by progressive deterioration of the structure and function of neurons, is crucially accompanied by severe cognitive deficits. Aging is the major risk factor for neurodegenerative disorders in Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) being coequal high hands. Oxidative stress and mitochondrial dysfunction are the key players in triggering neurodegeneration. The upsurge of neurodegenerative disorders is always appalling since there exists a paucity in effective treatment practices. Past few years' studies have underpinned the mechanisms through which quercetin boons the brain health in many aspects including betterment in cognitive output. Undoubtedly, quercetin will be escalating as an arable field, both in scientific research and in pharmacological and clinical applications.

Keywords Polyphenols • Nutraceuticals • Flavonoid • Flavonol • Quercetin • Neuroprotection

P. Elumalai (✉) • S. Lakshmi
Kerala University of Fisheries and Ocean Studies, Panangad, Cochin, Kerala, India
e-mail: epreeth@gmail.com

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Introduction

Polyphenols are highlighted as a broad category of natural products, identified in a vast array of plant products like vegetables, fruits, and cereals (Scalbert and Williamson 2000). Despite protecting plants from UV radiations and aggressive pathogens, they provide plant pigmentation and serve as signaling molecules in growth processes and ripening (Haslam and Cai 1994). A productive tenure of scientific investigation unveiled the potential role of polyphenol-rich diet in preventing diseases like cardiovascular diseases, diabetes, osteoporosis, cancers, and neurodegenerative disorders, which in turn made them an on-top field of clinical research. Polyphenols are also referred as nutraceuticals owing to their antioxidant property, mainly by scavenging the free radicals (Pandey and Rizvi 2009; Kelsey et al. 2010).

Polyphenols comprise four groups—phenolic acids, flavonoids, lignans, and stilbenes—depending on the number of phenol rings they possess and the structural elements that bind the rings together (Scalbert and Williamson 2000; Pandey and Rizvi 2009; Vauzour 2012). The focal point of this chapter being quercetin, a flavonol, the following part will mainly feature flavonoids.

Flavonoids: An Overview

Flavonoids are the most common and highly studied polyphenolic compounds in the human diet. They are ubiquitous secondary plant metabolites with over 8000 structural variants. Flavonoids possess a large extent of structural diversity owing to their acylation, glycosylation, methoxylation, and hydroxylation properties (Scalbert and Williamson 2000). Major dietary sources of flavonoids include vegetables, cereals, fruits, and drinks such as tea, beer, and red wine (Manach et al. 2004).

Flavonoids share a common structural core containing two aromatic rings (rings A and B) that bind together with a linear 3-carbon bridge (C_6–C_3–C_6) forming an oxygenated heterocycle (ring C) (Manach et al. 2004). Flavonoids are classified into six subgroups according to the degree of oxidation of ring C, the hydroxylation pattern of the ring structure, and substitution of the third position. The major dietary groups of flavonoids encompass (1) flavonols, (2) flavones, (3) isoflavones, (4) flavanones, (5) flavanols, and (6) anthocyanins (Manach et al. 2004; Spencer et al. 2009) (Fig. 1).

Flavonols are the prime dietary flavonoids among the whole family, with quercetin and kaempferol as the main representatives (Vauzour 2012; Manach et al. 2004; Spencer et al. 2009). Onions, leeks, and broccoli are the richest sources of flavonols (Table 1). Flavones are found in parsley and celery. Isoflavones are flavonoids with estrogen-like properties and consequently classified as phytoestrogens (Manach et al. 2004). Isoflavones are exclusively present in leguminous plants, apart from soy and soy products being the richest edible sources in the human diet. Flavanones are present in high concentrations in citrus fruits and tomatoes. Flavanols exist in both monomeric and polymeric forms, namely, catechins and proanthocyanidins,

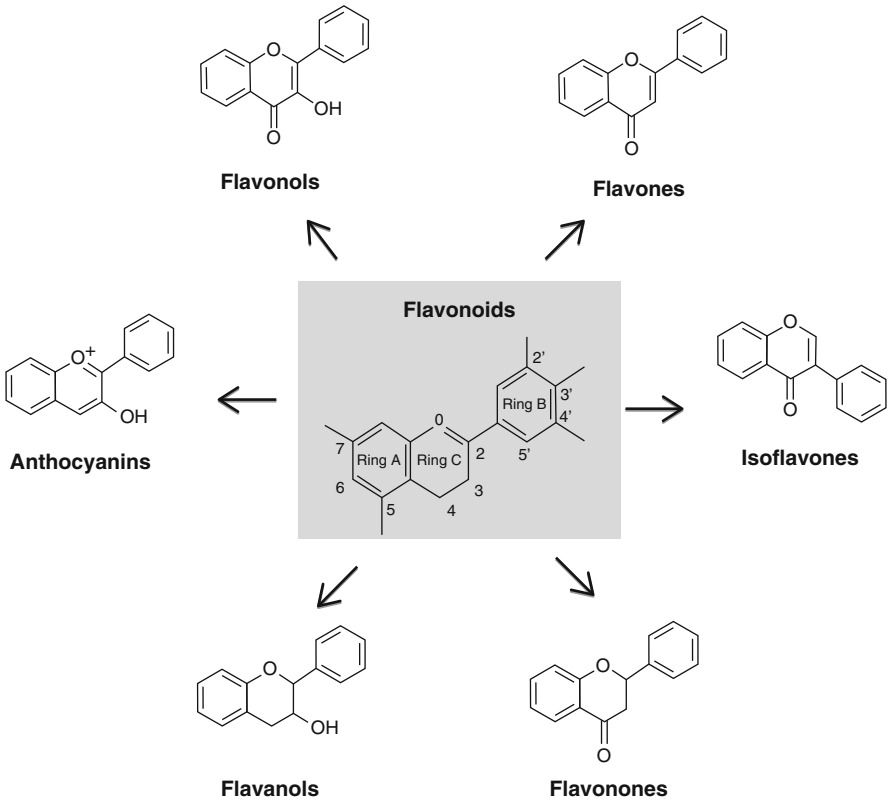

Fig. 1 Structures of major flavonoid classes. The core structure of flavonoid is given in the center. Individual groups differ from each other in a hydroxylation pattern of ring structure, substitution in the third position, and degree of oxidation of ring C

Table 1 Selected dietary sources of flavonoids (selection based on Vauzour 2012, Manach et al. 2004)

Subclasses	Dietary sources	Examples
Flavonols	Onion, leeks, broccoli	Kaempferol, quercetin
Flavones	Parsley, celery	Apigenin, luteolin
Isoflavones	Soy and soy products, legumes	Daidzein, genistein
Flavanones	Citrus fruits, tomatoes	Hesperetin, nanogenin
Flavanols	Green tea, red wine, chocolate	Epigallocatechin gallate (EGCG), catechin
Anthocyanidins	Red wine, berry fruits	Pelargonidin, cyanidin

respectively. Red wine, tea, and chocolate represent good sources of catechins. Proanthocyanidins, either dimers, oligomers, or polymers of catechins, are also referred as condensed tannins. They are responsible for astringency of fruits and beverages and bitterness of chocolates. Anthocyanins are pigments and impart pink, red, blue, or purple colors to the flowers or fruits. Even though fruits are the abundant sources, red wine, certain cereals, and leafy and root vegetables contribute anthocyanins in human diet (Manach et al. 2004; Spencer et al. 2009; D'Archivio et al. 2007; Mazza and Maniati 1994) (Table 1).

Even if the consumption of dietary flavonoids is generally regarded as safe, the advantage and disadvantage of their administration are entirely dose dependent. One should not believe in entire safety in taking flavonoids, since they are natural products. Administration of excess amount may have adverse effects on health, such as negative balance of thyroid function and decrease in the bioavailability of trace elements or vitamins (Egert and Rimbach 2011).

Flavonoids can be found in free or bound forms with sugar moieties, as aglycones and glycosides, respectively (Kozłowska and Szostak-Wegierek 2014). Dietary flavonoids mostly exist as glycosides and to a lesser extent aglycones, and both are extensively conjugated and metabolized during absorption. While traversing the small intestine and in the liver, they form glucuronides, sulfates, and methylated metabolites. Further, in the colon, both flavonoids and their metabolites get metabolized by bacterial enzymes of the gut microflora and then absorbed (Manach et al. 2004; Spencer 2007).

Flavonoids exert multifarious effects in the protection of human body. Being promising candidates for antioxidant therapy, flavonoids have gone through constructive years of research to manifest their influence on the incidence and onset of cardiovascular diseases, cancer, and neurodegenerative disorders. A positive correlation between the consumption of flavonoid-rich diet and reduction of cardiovascular death has been reported (Kozłowska and Szostak-Wegierek 2014). Intake of flavonoid-rich diet is shown to be protective against hypertension and atherosclerosis. They promote the reinforcement of blood vessels and anti-aggregation properties to reduce the risk of clots near the damaged endothelium. Anticarcinogenic potential of flavonoids has been revealed through their role in inhibiting both cancer initiation and progression. They induce apoptosis, inhibit angiogenesis with their antioxidant property, and behave cytotoxic against cancer cells as well (Kozłowska and Szostak-Wegierek 2014).

Relevancy of flavonoids to forestall the neurodegenerative disorders was revealed in recent years, as and when lifestyle factors signed up as saviors to arrest or delay the age-related health disorders. Neurodegeneration is a unified term to define inherited and sporadic conditions characterized by progressive deterioration of neuronal structure and function. Despite a large number of different disorders huddle under the title "neurodegenerative disorders," only a handful of diseases, including PD, AD, HD, and amyotrophic lateral sclerosis (ALS), were always been the peer domineers closely accompanied by loss of cognition and memory (Kelsey et al. 2010). Oxidative stress, mitochondrial dysfunction, protein misfolding or aggregation, neuroinflammation, and genetic mutations underlie the hazardous elements of neurodegenerative disorders (Kelsey et al. 2010) (Fig. 2). Oxidative stress

was always highlighted as a promising performer in the pathogenesis of PD, AD, and HD and has been uniquely figured as an aging-contributing factor. Oxidative stress is mediated by reactive oxygen species (ROS—superoxide anions, hydrogen peroxide, hydroxyl radical, etc.) and reactive nitrogen species (RNS—nitric oxide, peroxynitrite, etc.), belonging to the group of free radicals. Cells maintain a homeostasis of production and antioxidation of ROS/RNS with the aid of effective enzymatic and nonenzymatic antioxidant mechanisms. An imbalance by excess ROS/RNS and decreased cellular antioxidant defense mechanism generates the state of oxidative stress (Kelsey et al. 2010; Oliveira et al. 2014).

Mitochondrial dysfunction is closely accompanied by oxidative stress as mitochondrion is the powerhouse of the cell. ROS formed in the mitochondria discordantly affect different components of ETC, cause ATP depletion, damage mitochondria along with other cellular organelles, and lead to eventual cell death. Apart from being the largest consumer of energy in the body (20 % of O_2 consumption), high vulnerability of the brain to oxidative stress is attributed to its less capacity for cellular regeneration and small amount of antioxidant mechanisms (Oliveira et al. 2014; Dajas et al. 2013). The incidence of neurodegenerative diseases is on the rise, yet paucity of effective therapeutic strategies continues. Nowadays, nutritional therapies including herbal plants are heading as a choice of treatment for neurodegenerative disorders owing to their cut rate and easy access, than conventional pharmacological and hormone therapies, which made the brain disorders an economic burden to the health-care systems.

Flavonoids were mainly known to reduce the risk of cerebral ischemia, PD, and AD. Consumption of dietary flavonoids has positive effects to maintain the cognitive function during senescence, by protecting and enhancing the function and regeneration of neurons through increased cerebrovascular blood flow and initiation of neurogenesis (Letenneur et al. 2007; Youdim and Joseph 2001). Extracts of flavonoids from green tea and *Ginkgo biloba* have been identified to tone the memory and learning processes in the hippocampus. Flavonoids increase the number and quality of synaptic connections

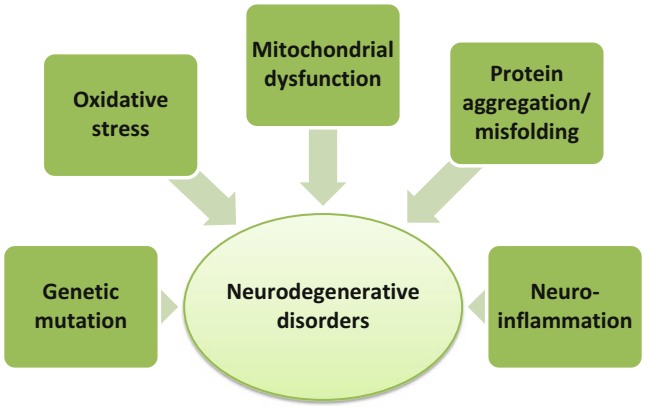

Fig. 2 The risk factors underlying the neurodegenerative disorders (modified from Kelsey et al. 2010)

by interacting with critical signaling pathways mitogen-activated protein (MAP) kinase (MAPK), tyrosine kinase, protein kinase C (PKC), and phosphatidylinositol-3-kinase (PI3K)/Akt and transcription factors like cAMP response element-binding protein (CREB), which in turn increase the synaptic plasticity and long-term potentiation (Spencer et al. 2009; Spencer 2009; Rendeiro et al. 2012). Flavonoids also interact with brain-derived neurotrophic factor (BDNF), an important factor in the protection and survival of neurons (Rendeiro et al. 2012). Studies were done to exhibit the role of flavonoids to attenuate the microglia and astrocyte-mediated neuroinflammation by inhibiting nitric oxide synthase enzyme and subsequent nitric oxide production and ROS (Spencer 2009). Experiments conducted with the brains of animal models of depression have shown the antidepressant-like activity of at least some flavonoids (e.g., quercetin, ellagic acid, apigenin, etc.) by modulating monoaminergic neurotransmission in the suffering brain, highlighting the clinical relevance of flavonoids in the treatment of depression (Pathak et al. 2013).

Quercetin

Quercetin represents the most abundant bioflavonoid whose efficacy in maintaining proper human health has been debated in many aspects over the past years. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a dietary flavonol which exhibits relatively high availability in comparison with other phytochemicals (Russo et al. 2012) (Fig. 3).

Dietary Sources

Quercetin is widely distributed in fruits, vegetables, and nuts. Primary sources of quercetin include citrus fruits, apple, onions, parsley, grapes, blueberries, and blackberries. A notable amount of quercetin is found in green tea and red wine as well

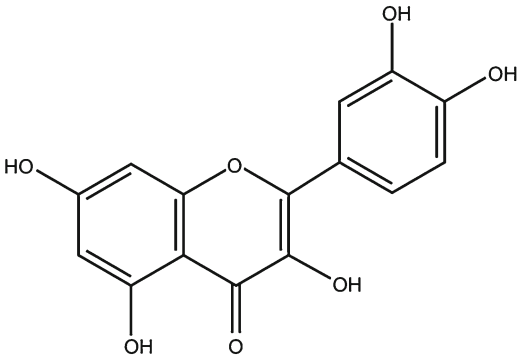

Fig. 3 Structure of quercetin

(Boots et al. 2008; Guo and Bruno 2015). In plants quercetin-3-*O*-glycoside functions as pigment to color fruits, flowers, and vegetables (Miles et al. 2014). Dietary quercetin exists mainly as glycosides (a sugar moiety like glucose, galactose, rutinose, or xylose, attached to one of the hydroxyl groups of the compound) rather than aglycones (without sugar) (Boots et al. 2008; Guo and Bruno 2015). In human diet, level of quercetin can reach as high as 16–25 mg/day (Dajas 2012). Traditional Ayurvedic medicine in India put forward a few selected plants such as *Ginkgo biloba*, kava kava, *Bacopa monnieri*, and *Convolvulus pluricaulis* where quercetin was found to be a component of *Ginkgo biloba*, *Bacopa monnieri*, and *Convolvulus pluricaulis* which are found to enhance cognition and effectively treat brain disorders (Dajas 2012). Standardized leaf extracts of GB are employed in the treatment of AD and PD and in the improvisations of synaptic plasticity and memory (Kelsey et al. 2010).

Metabolism and Bioavailability

Quercetin follows a complex metabolic pathway. Both the glycosidic and aglycone forms of quercetin are absorbed by the small intestine by either passive diffusion or organic anion-transporting polypeptide. The stomach is incapable of absorbing glycosylated quercetin, but a β-glucosidase, lactase phlorizin hydrolase (LPH), at the intestinal brush border, performs deglycosylation and absorbs quercetin in the aglycone form. Following absorption, quercetin gets metabolized in the small intestine, large intestine, liver, and kidney (Russo et al. 2012; Boots et al. 2008). Phase I metabolism mainly involves enzymes of cytochrome p450 metabolism. Phase 2 conjugation comprises the biotransformation and yields glucuronidated, sulfated, and methylated derivatives of quercetin. Phase 3 elimination of the metabolites from the small intestine to the liver is via the portal vein. The uptaken metabolites are further metabolized by the liver, and the products either enter the circulation or directed to biliary excretion. Quercetin is rapidly excreted through urine and feces. Deconjugation of metabolites yields aglycone of quercetin, which undergo bacterial ring fission to form smaller phenolic acids (benzoic acid, hippuric acid, 3-OH phenylacetic acid) and are subsequently eliminated (Russo et al. 2012; Boots et al. 2008; Guo and Bruno 2015).

Bioavailability refers to a fraction of an administered dose of a substance which is absorbed and accessible for exerting physiological function or storage (Guo and Bruno 2015; de Boer and Dihal 2005). Based on pharmacokinetic assessment, bioavailability is divided into absolute and relative, where the former seems to be accurate than the latter (Guo and Bruno 2015). In humans, the level of quercetin proportionally increases from lower to higher level upon a continuous supply of quercetin, and commonly the relative bioavailability is examined (Guo and Bruno 2015). Evaluation of the bioavailability of quercetin can be done by taking the sum of free quercetin and glucuronidated and sulfated

conjugates of the compound. A study by de Boer et al. revealed the target tissues of quercetin, as they observed high accumulation of quercetin and its metabolites in rat lungs and pig liver and kidneys, which in turn helps to understand the biological functions of quercetin in vivo (de Boer and Dihal 2005). Plasma half-life of quercetin and its metabolites ranges from 11 to 28 h (Boots et al. 2008; Miles et al. 2014). In short, metabolism, absorption, and elimination are key factors to determine the bioavailability of a compound (Guo and Bruno 2015).

Quercetin and Neuroprotection

Quercetin is well known for a multitude of health-promoting beneficiaries, comprising reducing risks of neurodegenerative disorders, cancer, cardiovascular diseases, allergic disorders, thrombosis, atherosclerosis, hypertension, arrhythmia, and many more, apart from being well known for its anti-inflammatory and antioxidant effects. Even though quercetin is not that predominant among dietary flavonoids, it is one of the most studied. Quercetin is well accepted for its antioxidant property, which highs up the compound for being one of the main foci in scientific research as well as pharmacological and clinical applications.

A General View on Antioxidant and Anti-inflammatory Properties of Quercetin

The demand of neurodegenerative disorders keeps on escalating both scientifically and economically. Healthy brain activities are potential contributors for the fitness of the body, and, indeed, a healthy brain diet is essential for the protection of brain cells from degeneration. Depletion of endogenous antioxidants and neuroinflammation are the main triggers of age-related neurodegenerative diseases (Boots et al. 2008). A series of factors influence the antioxidant capacity of flavonols like quercetin, apart from direct free radical scavenger-like metal-chelating property, modulation of enzyme systems like nitric oxide synthase, and redox-sensitive transcription factors such as nuclear factor-κB (NF-κB) and NF-E2-related factor-2 (Nrf2), which in turn induces the genes coding for detoxifying and antioxidant proteins (Dajas et al. 2013). Moreover, quercetin interacts and modulates signaling pathways such as PI3K/Akt, tyrosine kinase, PKC, and MAPK, which have been related to cognition, neurogenesis, and neuronal survival (Dajas et al. 2013). On the flip side, structural features also underlie the antioxidant potency, since studies present flavonols with the catechol group as the most effective free radical scavengers. Catechol group in ring B and the third –OH group at A and C rings make the perfect configuration for free radical scavenging of quercetin, as it is the most potent scavenger of ROS and RNS (Boots et al. 2008; Dajas 2012). The state of oxidative stress leads to critical mitochondrial dysfunction as ROS formed inside

the mitochondria adversely affect the components of electron transport chain (ETC) and cause depletion of ATP and subsequent damage of mitochondria and other organelles (Lin and Beal 2006). Undoubtedly, mitochondrial dysfunction contributes to neurodegeneration owing to the high metabolic rate of the brain and its reduced capacity for cellular regeneration. Quercetin manages the redox state of mitochondria by accumulating in it (Fiorania et al. 2010). It enables to inhibit or enhance the mitochondrial permeability transition pore (MPTP) permeability which is critical for cell survival or death. This property of quercetin highlights its pharmacological applicability in treating conditions with mitochondrial dysfunctions (Dajas et al. 2013).

Quercetin exhibits antioxidative and anti-inflammatory effects on an even keel. ROS elicits oxidative stress as well as inflammation when cytokine-like tumor necrosis factor (TNF) α is produced by the activation of transcription factors such as NF-κB and activator protein-1 (AP-1). Hence, prevention of oxidative stress and neuroinflammation occurs as ROS gradually disappears (Boots et al. 2008). Early in vitro studies have shown that quercetin inhibits lipopolysaccharide (LPS)-induced TNFα production in macrophages and IL-8 production in lung cells (Russo et al. 2012). In the central nervous system (CNS), suppression of microglial activation attributes to the protection against inflammation (Spencer et al. 2012). Microglial cells are primary immune cells of CNS functions mainly to promote host defense (Glass et al. 2010). Uncontrolled microglial activation leads to overproduction of ROS and nitric oxide, by increased expression of inducible nitric oxide synthase (iNOS), pro-inflammatory cytokines like TNFα, and all causatives of neuroinflammation-mediated degeneration (Fig. 4). Consequently, mitochondrial ETC in neurons gets disrupted causing depletion of neuronal ATP synthesis and increased ROS production. A study by Kao et al. proposed that quercetin's anti-inflammatory property involves downregulation of signaling pathways (tyrosine kinase, MAPK, PI3K/Akt), suppression of transcription factors (AP-1, STAT-1, NF-κB), and disruption of membrane lipid raft accumulation (Kao et al. 2010). The anti-neuroinflammatory activity of quercetin has also been reported to contribute in improving spatial memory (Lu et al. 2010).

Supplementation of flavonoid-rich diets has showed up with their beneficiaries in preventing or reversing the cognitive deficits (Rendeiro et al. 2012). Mouse models suffering from age-related cognitive impairment were shown a marked improvement in memory and learning on quercetin administration compared with D-galactose (D-Gal)-administered control mice (Lu et al. 2006). The ability of quercetin to activate signaling pathways and induction of vascular effects to promote the hippocampal neurogenesis has been explored (Letenneur et al. 2007; Ossola et al. 2009). Studies in mouse models also revealed the elevation of superoxide dismutase and expression of growth-associated protein 43 mRNA (Lu et al. 2006). Improvement in cognition and alleviation of oxidative stress in the brain, in the presence of quercetin, was observed in rats suffering from colchicine-induced cognitive impairment (Kumar et al. 2008).

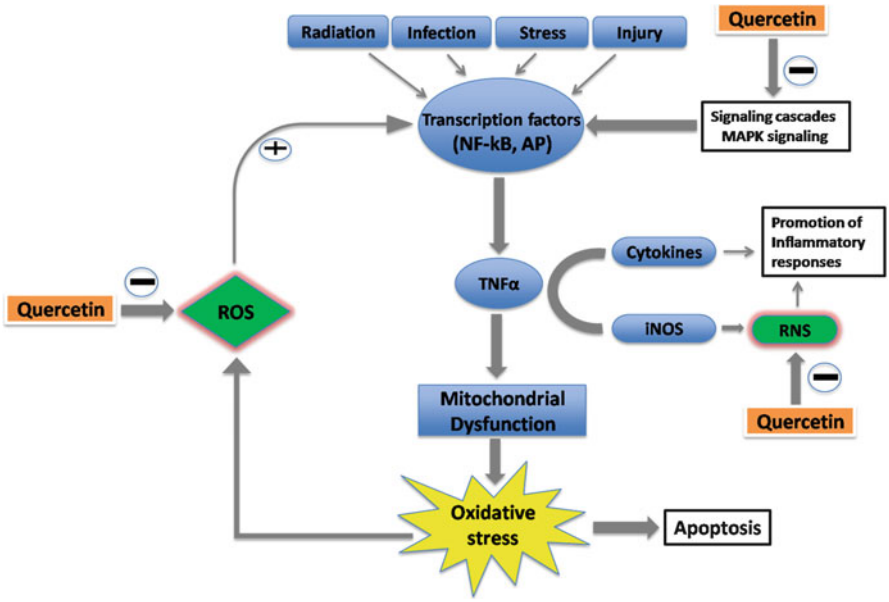

Fig. 4 Antioxidant and anti-inflammatory activities of quercetin. Transcription factors like NF-κB or AP-1 get activated by ROS to induce the production of TNFα, which in turn initiate the production of ROS and promote the inflammatory responses by inducing the production of other cytokines and RNS. Signaling cascades (MAPK signaling) also as well activate NF-κB to initiate the series. Quercetin exhibits antioxidant and anti-inflammatory activities mainly by directly scavenging ROS and RNS and by downregulating signaling cascade. *NF-κB* nuclear factor-κB, *AP-1* activator protein-1, *TNFα* tumor necrosis factor α, *iNOS* inducible nitric oxide synthase, *RNS* reactive nitrogen species, *ROS* reactive oxygen species, *MAPK* mitogen-activated protein kinase (modified from Boots et al. 2008, Spencer et al. 2012, Kao et al. 2010)

Role of Quercetin in Ameliorating Neurodegenerative Disorders

Alzheimer's Disease (AD)

AD is a progressive neurodegenerative disorder and most common form of dementia, where loss of memory and other intellectual abilities keeps on interfering the daily life. A worldwide calculation reported a number of more than 35 million people suffering from AD (Querfurth and LaFerla 2010). AD is characterized by the presence of neuritic plaques and neurofibrillary tangles associated with loss of cortical neurons and synapses (Choi et al. 2014). Uncanny accumulation of β-amyloid (Aβ) peptide, a key component of extracellular neuritic plaques, is one among the major responsible factors for neuronal death in AD (Small and McLean 1999). Oligomeric Aβ (1–42) is considered as highly toxic among the Aβ species, and Aβ (1–42)-induced oxidative stress tightly floors the pathogenesis of AD (Pocernich et al. 2011). Currently, the concept of slowing down the neuronal damage induced by oxidative stress is more

dependent on the portal of brain-accessible antioxidants from dietary sources. Studies in vitro feature quercetin as one of the prominent inhibitors of Aβ (1–42)-mediated fibril formation and oxidative stress (Ansari et al. 2009; Kim et al. 2005). A dose-dependent administration of quercetin displayed its protective effects at lower doses, whereas it goes toxic at high concentration (Ansari et al. 2009). The role of quercetin was also disclosed in maintaining effective membrane integrity and fluidity which can be damaged by lipid peroxidation caused by oxidative stress, since brain membrane lipids are rich in polyunsaturated fatty acids (Prasad et al. 1998). Thiols, mainly glutathione (GSH), a prime nonenzymatic antioxidant defense mechanism in cells, protect neuronal damage induced by Aβ (1–42), peroxynitrite, or hydroxyl radicals (Sen 1998). 4-Hydroxynonenal, an α, β-unsaturated hydroxyalkenal, formed via lipid peroxidation, induces oxidative stress and damages biomembranes and biomolecules in AD brain. They even react with GSH and cause GSH depletion followed by apoptosis (Mark et al. 1997). Quercetin increases intracellular GSH levels in AD brain and reverses the above condition (Kim et al. 2005). The latest study by Sabogal-Guáqueta et al., in an aged triple-transgenic AD model mouse, has explored quercetin in reversing β-amyloidosis, astrogliosis, and microgliosis in the hippocampus and amygdala via reducing Aβ (1–40) and Aβ (1–42) levels. In parallel, an improvement in cognition and emotional function in the mouse model to quercetin intake was also observed (Sabogal-Guáqueta et al. 2015). Reduced number of acetylcholine (ACh) receptors and increased activity of acetylcholinesterase (AChE) cause depletion of ACh in AD. This leads to hyperphosphorylation of Tau protein and results in reduced secretion of soluble amyloid precursor protein (APP) and increased synthesis of Aβ (Islam et al. 2013; Jung and Park 2007). Obviously, one of the best treatment practices to restore the neurotransmission between cholinergic neurons is the use of AChE inhibitors. A great interest in finding AChE inhibitors from herbal medicinal plants was achieved since conventional drugs as AChE inhibitors like tacrine, galantamine, etc. exert side effects like hepatotoxicity (Islam et al. 2013). High binding affinity to AChE and less toxic nature of quercetin extracted from its natural source, *Agrimonia pilosa*, make quercetin superior to the aforementioned drugs in treating AD (Islam et al. 2013; Jung and Park 2007). Quercetin fulfills the natural requirements for being employed as a potential candidate in drug therapy for effective treatment of AD.

Parkinson's Disease (PD)

PD is the second most prevalent neurodegenerative disorder after AD, usually suffered by people over the age of 60 (Agim and Cannon 2015; Di Giovanni et al. 2010). PD is characterized by the loss of dopamine-producing neurons in substantia nigra and the development of Lewy bodies followed by striatal dopamine depletion resulting in postural instability, bradykinesia, rigidity, and tremors (Agim and Cannon 2015; Beitz 2014). An approximation of 10 % of PD onset is known from genetic mutations and the rest 90 % arise from unknown causes. Environmental factors like pesticide exposure too play a crucial role in the progression of PD (Singleton et al. 2013; Cannon and Greenamyre 2011). The transient symptomatic therapeutic strategy employed for

treating PD is dopamine replacement therapy, restoring the loss of dopamine, which is currently accomplished by administration of L-DOPA (Di Giovanni et al. 2010). Flavonoids gather assiduity in offering protection from PD owing to their antioxidant and anti-inflammatory properties. A bunch of experimental evidences support the protective role of quercetin on CNS through a series of processes like reducing lipid peroxidation, preventing GSH depletion, enhancing the activity of superoxide dismutase and catalase, etc. (Sriraksa et al. 2012). It has been shown that a standardized GB extract containing a high amount of quercetin exerts neuroprotective effects against 6-hydroxydopamine (6-OHDA)-induced oxidative damage (Kim et al. 2004). 6-OHDA is a common catecholaminergic neurotoxin used in experiments to model nigral dopaminergic degeneration in vivo (Ossola et al. 2009). 6-OHDA-induced oxidative stress via the production of hydroxyl radicals causes impairment in memory and neuronal death. Quercetin improved the 6-OHDA-induced cognitive impairment (Kim et al. 2004). Like in AD, in PD as well, a high dose of quercetin decreases the activity of AChE to provide enough acetylcholine in the synapses between the cholinergic neurons. Increased rate of lipid peroxidation and decreased amount of free radical scavenging enzymes are always seen in PD. The antioxidant effect of quercetin promotes the survival of neurons in the hippocampus by enhancing the activity of free radical scavenging enzymes, thereby protecting neurons from oxidative damage (Kim et al. 2004).

Huntington's Disease (HD)

HD is a progressive neurodegenerative disorder whose symptoms can begin at any age from infancy to old age (Vonsattel and DiFiglia 1998). The disease is a result of autosomal dominant mutation of a gene called Huntingtin. Expansion of the trinucleotide triplet cytosine-adenine-guanine (CAG) in the Huntington results in corresponding mutant Huntingtin (mHtt) protein, which in turn causes neuronal damage (Vonsattel and DiFiglia 1998; Sandhir and Mehrotra 1832). HD is characterized by chorea, cognitive decline, and behavioral and coordination abnormalities (Vonsattel and DiFiglia 1998). Pathogenesis of HD has a significant backup from mitochondrial dysfunction and ROS generation. Deficits in the activities of succinate dehydrogenase complex (complex II), complex III, and complex IV of ETC and decreased aconitase and pyruvate dehydrogenase activities were observed in HD patients (Jin and Johnson 2010). A recent study has shown the protective effect of quercetin against 3-nitropropionic acid (3-NP), an irreversible inhibitor of succinate dehydrogenase, and induced mitochondrial dysfunction followed by oxidative stress leading to neurological and behavioral deficits (Sandhir and Mehrotra 1832). Quercetin ameliorated the inhibitory effect of 3-NP on ETC, which in turn managed normal function of ETC enzymes, restoration of the ATP level, and prevention of oxidative stress and mitochondrial swelling. Quercetin also improved the functioning of catalase and superoxide dismutase, core components of the enzymatic antioxidant defense system, in 3-NP-treated animals (Jin and Johnson 2010). The condition of reduction in mitochondrial thiols inducing mitochondrial swelling followed by apoptosis seen in HD gets reversed in the presence of quercetin by preventing the

decrease in mitochondrial thiols through neutralizing ROS (Sandhir and Mehrotra 1832; Lin et al. 2002; Marchetti et al. 1997). Improvement of cognition and motor functions in HD cases is also influenced by quercetin. Structure of quercetin with the catechol ring has a significant contribution to the antioxidant property of the compound (section "A General View on Antioxidant and Anti-inflammatory Properties of Quercetin").

Quercetin on Spinal Cord Injury, Brain Trauma, and Cerebral Ischemia

Bioavailability and permeability through BBB are the two ardent hands for an effective concentration of quercetin to reach out neurons and exert protective effects. Liposomes were shown to carry quercetin to impart antioxidant enzyme activity and inhibition of edema (Dajas 2012; Ossola et al. 2009). A study by Schultke et al. in a rat model of acute traumatic spinal cord injury revealed a significant betterment of motor function supported by daily quercetin administration of 25 μmol/kg (Lee et al. 2011). A notable recovery from pathological conditions associated with brain trauma on quercetin intake was also reported following the inhibition of myeloperoxidase activation in rat models (Ossola et al. 2009). The significance of quercetin against cerebral ischemia has also been confirmed by experimental evidences. Breakdown of BBB by activated matrix metalloproteinases (MMPs) is a common event during ischemic damage. Quercetin was found to decrease the overexpression of MMP-9 within 48 h postischemic injury when studied in a focal ischemic rat model induced by photothrombosis, which points to the fact that inhibition of MMPs can be considered as a protective tool against ischemia. Quercetin dihydrate confers protection against cerebral ischemic neuronal damage in MCAO (transient middle cerebral artery occlusion) rats and upregulates antioxidant status (Awad et al. 2002). Quercetin protects against selective hippocampal injury, especially in CA1 area, in global ischemia, by a complete inhibition of the MMP-9 elevation (Ossola et al. 2009).

Miscellaneous: Quercetin is a promising metal chelator, and this property found the base for suppression of lead (Pb)-induced toxicity in the brain by reducing the concentration of Pb in the brain as well as in the blood (Schultke et al. 2003). Pb toxicity exerts adverse effects like the disruption of the BBB, inhibition of LTP, memory and learning impairment, and growth retardation. Experiments in Pb-treated mice suggest that quercetin may protect the mouse brain by increasing NO production and CREB phosphorylation. Quercetin attenuated Pb-induced oxidative stress by inhibiting ROS in mouse brain (Ossola et al. 2009; Schultke et al. 2003).

Diet rich in quercetin and its glycosides are good candidates for antidepressant-like activity. A study with animals subjected to water immersion restraint stress showed an elevated hypothalamic-pituitary-adrenal (HPA) axis which was normalized with quercetin treatment (Pathak et al. 2013). Quercetin treatment reversed the effect of corticotrophin-releasing factor (CRF)-induced anxiety and depression-like stage. The ability to modulate BDNF level is also contributing to the antidepressant-like activity of quercetin and thereby makes it a promising candidate in antidepressant drug therapy (Pathak et al. 2013).

Over and above, incongruities regarding cytotoxicity of quercetin have been reported on the basis of the formation of toxic oxidation products of quercetin, most important being orthoquinone (quercetin-quinone, QQ), during its antioxidant activities. QQ is highly reactive toward thiols and interacts with glutathione (GSH) or sulfhydryl (–SH) groups of enzymes and causes functional impairments (Boots et al. 2005, 2008). GSH-QQ is an unstable compound susceptible to easy dissociation and QQ released interacts with endogenous thiols (Liu et al. 2013). It was suggested that quercetin toxicity can be prevented by administering quercetin together with dithiol which in turn detoxifies the toxic oxidation products (Russo et al. 2012). Therefore, investigations are mandatory, seeking the safety of quercetin supplementation when one should take a high-dose or long-term quercetin intake (Boots et al. 2008; Pocernich et al. 2011).

Conclusion

The emergence of lifestyle-based treatment practices for the most prevailing health disorders like cancer and cardiovascular and neurodegenerative diseases brought polyphenols to light. Flavonoids, a subgroup of polyphenols, have seen many constructive years of research to manifest their influence on reducing the risk of aforesaid diseases. Owing to high-abundance and multifarious health effects, quercetin holds its own space in scientific research as well as therapeutic applications. Studies of quercetin were always vocal about its antioxidative and anti-inflammatory properties, in ascertaining health beneficiaries. Senescence and neurodegenerative disorders are typically accompanied by impairment in cognitive functions, and quercetin exhibited healthful effects in reversing or preventing the same. Observations till date support the development of flavonoid molecules, including quercetin, in launching a new generic drug to offset the neurodegenerative disorders. Yet investigations paving toward the credibility and efficacy regarding high-dose and long-term supplementation of quercetin are highly demanding in future research.

Compliance with Ethics Requirements The authors declare that they have no conflicts of interest.

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Role of Omega-3 PUFAs in Neurobiological Health

R. Grant and J. Guest

Abstract The importance of the essential, dietary-derived, polyunsaturated fatty acids (PUFAs) omega-6 and omega-3 to human health was first reported over 85 years ago. Subsequent research has revealed many beneficial effects of the omega-3 PUFAs in particular. This has been linked to their involvement in multiple biochemical functions, including synthesis of inflammatory mediators, cell membrane fluidity, intracellular signalling and gene expression. Through these pathways, the omega-3 PUFAs help modulate aspects of inflammation and immunity, cell growth and tissue repair. While a detailed understanding of the mechanisms involved in the role of omega-3 PUFAs to health in the central nervous system (CNS) is still to be elucidated, a role for both inflammatory modulation and a direct impact on neuronal membrane fluidity and receptor function is apparent. At least partially through these mechanisms, low omega-3 levels have been associated with CNS-linked disorders such as poor cognition, depression, anxiety disorders, poor anger control, attention deficit hyperactivity disorder (ADHD) and accelerated neurodegeneration in the elderly.

Following a brief introduction to the history and chemistry of the omega-3 family of PUFAs, this chapter will provide an overview of the omega-3 fatty acids and how various members of this PUFA family influence central nervous system function leading towards either health or disease.

Keywords Omega-3 • Inflammation • Neurodegenerative • Depression • Dementia • CNS

R. Grant (✉)
Australasian Research Institute, Sydney, NSW, Australia

School of Medical Sciences, University of NSW, Sydney, NSW, Australia

Sydney Medical School, University of Sydney, Sydney, NSW, Australia
e-mail: rossg@sah.org.au

J. Guest
Australasian Research Institute, Sydney, NSW, Australia

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Introduction

The importance of the essential, dietary-derived, polyunsaturated fatty acids (PUFAs) omega-6 and omega-3 to human health was first reported by Burr and Burr in two landmark publications in 1929 and 1930, over 85 years ago (Burr and Burr 1973, 1981).

Although the potential health benefits of the long-chain omega-3 fatty acids were noted in the 1970s by researchers studying the Greenland Inuits, interest in these essential PUFAs remained relatively low until the mid-1980s when their benefits to diseases like heart disease and insulin sensitivity began to be realised (Lardinois 1987; Popp-Snijders et al. 1987; von Schacky 1987). Omega-3 research saw a modest increase in research interest rising from around 5 to 10 published articles per year in the mid-1980s to around 50 per year in the late 1990s. However, from the early 2000s, a rapid rise in the number of studies investigating the role of omega-3 in human disease has occurred increasing from just 50 in the year 2000 to over 400 published papers in 2014.

Not coincidentally, the role of oxidative stress in disease and its relationship to increased inflammatory activity also began to increase dramatically around this time. With the inflammatory modulating potential of the longer chain omega-3 molecules being reported in the late 1990s (Mori et al. 1999), interest in the role of omega-3 status to inflammatory-related disorders accelerated.

The many beneficial effects attributed to the omega-3 PUFAs are arguably linked to their involvement in the synthesis of eicosanoid inflammatory mediators. However, their influence on other biochemical functions such as cell membrane fluidity, intracellular signalling and gene expression, is also relevant. Through these pathways, omega-3s help modulate aspects of inflammation and immunity, cell growth and tissue repair. As omega-3 metabolites affect inflammatory activity, they have been shown to improve the pathology associated with inflammatory conditions such as rheumatoid arthritis, psoriasis and cardiovascular disease. As omega-3 intake has been linked mechanistically with vascular health, low levels have also been included as a risk factor on both cardio- and cerebrovascular diseases (Siegel and Ermilov 2012).

In addition to its important effects on systemic organs like the heart and peripheral vasculature, omega-3 PUFAs have also been shown to play an important role in maintaining health within the central nervous system (CNS). While a detailed understanding of the mechanisms involved is still to be elucidated, evidence supports a role for both inflammatory modulation and a direct impact on neuronal membrane fluidity and receptor function. At least partially through these mechanisms, low omega-3 levels have been associated with CNS-linked disorders such as poor cognition, depression, anxiety disorders, poor anger control, ADHD and accelerated neurodegeneration in the elderly.

This chapter will provide an overview of the omega-3 fatty acids and how various members of this PUFA family influence central nervous system function leading towards either health or disease.

The Chemistry of Omega-3 PUFAs

The omega-3 fatty acids are a family of polyunsaturated fatty acids with either 3, 5 or 6 double bonds in a carbon chain of 18, 20 or 22 carbon atoms. The major omega-3 PUFAs include alpha-linolenic acid (ALA; 18:3, $n-3$), eicosapentaenoic acid (EPA; 20:5, $n-3$), docosahexaenoic acid (DHA; 22:6, $n-3$) and docosapentaenoic acid (DPA; 22:5, $n-3$), where ALA is the only omega-3 PUFA found in plants.

Oxidisability of Omega-3 PUFAs

The omega-3 and omega-6 PUFAs in cellular membranes along with low-density lipoprotein (LDL) cholesterol are the major sites of oxidative attack in vivo, causing widespread cellular and tissue damage (Shahidi and Zhong 2010).

Omega-3 PUFAs are chemically susceptible to oxidation due to their relatively large number of double bonds and the position of those double bonds within the fatty acid chain (Fig. 1). The longer chain omega-3 PUFAs such as EPA and DHA have 4 and 5 bis-allylic carbon atoms, respectively (i.e. those between two double-bonded carbon atoms). This makes them more susceptible to oxidation compared to the shorter chain omega-3 PUFA, alpha-linolenic acid which only has 2 or the omega-6 PUFAs like arachidonic acid which has 3 bis-allylic carbon atoms (Albert

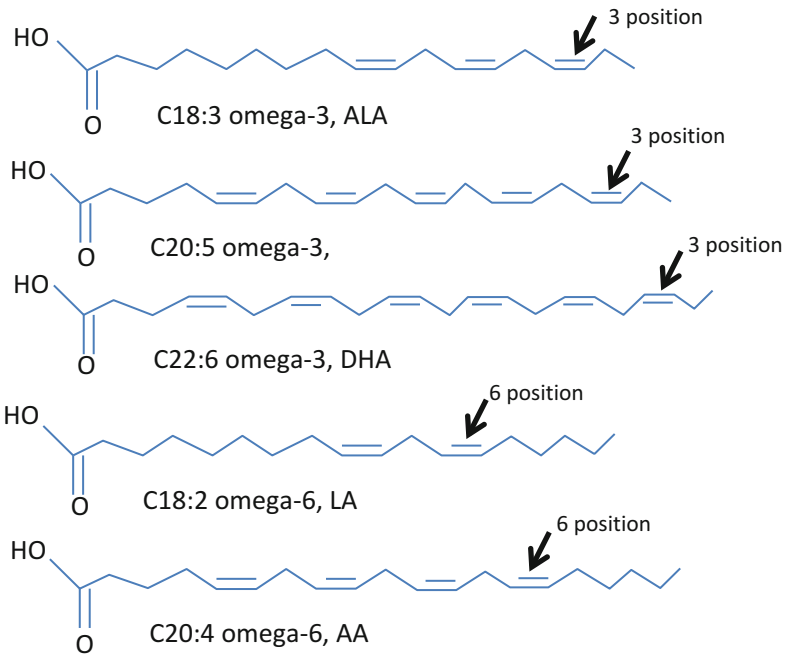

Fig. 1 Chemical structures of the major omega-3 and omega-6 PUFAs

et al. 2013). Oxidation of omega-3 PUFAs produces a number of different peroxide compounds depending on the position of the oxidised carbon. For example, F3 isoprostanes are formed from the oxidation of EPA and F4 isoprostanes from DHA. These may be physiologically relevant as both possess some prostaglandin-like activity. These and other lipid peroxides are themselves unstable and are likely to undergo secondary oxidation to products such as 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) both secondary oxidation products of arachidonic acid (Esterbauer et al. 1993). Quantitation of the isoprostanes and/or MDA by various methods is used widely as an index of 'oxidative' activity within cellular and whole-body systems (Ayala et al. 2014; Janicka et al. 2010).

Sources of Omega-3 PUFAs

As previously mentioned, the short-chain omega-3, alpha-linolenic acid (ALA; 18:3, $n-3$), is the only omega-3 available from plant sources. Plant foods rich in omega-3 relative to omega-6 include green, leafy vegetables, flaxseeds, chia seeds and cauliflower (Table 1.)

Green, leafy vegetables, though not high in oil content, have a favourable omega-3:omega-6 ratios. These include spinach, lettuce (romaine or cos), purslane and basil.

It is well understood that plant-derived ALA must be converted to the longer chain EPA and DHA for many of the health benefits of the omega-3s to be realised. However, EPA and DHA are synthesised de novo in a variety of marine microalgae such as *S. mangrovei*, *Y. lipolytica* or *N. oculata* and the marine crustacean krill (Euphausiacea) (Jiang et al. 2004; Kagan et al. 2013; Xie et al. 2015). Each of these has been explored for their commercial benefit in meeting the growing consumer demand for omega-3 PUFAs. Selected varieties of microalgae are now being developed into bio-factories for production of commercial quantities of the omega-3 fatty acids EPA and DHA (Table 2).

Though some omega-3 PUFAs can be obtained from eating grass-fed beef and fowl, there is generally little in grain or feedlot-fed animals. It is well recognised that the best animal sources of the longer chain omega-3 PUFAs are obtained from eating oily fish such as tuna, salmon or trout (Table 3). However, with a growing global population fuelling increased demand for table fish, the commercial incentive for aquaculture is significant. Aquaculture now contributes about the same as wild-catch harvests (Nichols et al. 2014).

Importantly, evidence is now also emerging that the percentage of omega-3 in these farmed animals is decreasing due to the limited availability of essential oils in their food supply (Nichols et al. 2014). So while the oily fish is still an excellent source of EPA and DHA, if the omega-3 PUFA content is diminished, adequate in vivo levels may not be achieved, and therefore, health benefits may not be realised without additional supplementation.

Table 1 Plant sources rich in omega-3 (relative to omega-6)

Plant food[a]	*n*−6:*n*−3 ratio	ALA (mg/100 g)	EPA (mg/100 g)	DHA (mg/100 g)
Seeds				
Flaxseed	1:4	22,813	–	–
Chia seed	1:3	17,553	–	–
Sesame seed	***56:1***	541	–	–
Nuts				
Walnuts	***4:1***	9080	–	–
Pecans	***21:1***	986	–	–
Hickory nuts	***20:1***	1047	–	–
Pine nuts	***32:1***	787	–	–
Oils				
Flaxseed oil	1:4	53,304	–	–
Chia seed oil	1:3	17,552	–	–
Soya bean oil	***7.4:1***	6789	–	–
Canola oil	***4:1***	5798	–	–
Sunflower oil (high oleic)	***18:1***	192	–	–
Vegetables				
Spirulina (raw, dried)	***5.2:1***	922	–	–
Leeks (bulb, lower leaf)	1:4.8	88		
Cauliflower	1:3.4	37	–	–
Broccoli	1:1.2	5.9	–	–
Spinach	1:5.3	138	–	–
Brussels sprouts	1:2.2	99	–	–
Kale	1:1.3	121	–	–
Purslane[b]	1:100	400	1	–
Basil	1:4.4	17	–	–
Chinese cabbage	1:1.3	55	–	–
Cabbage (red)	1:1.3	45	–	–
Lettuce (cos, romaine)	1:2.4	7	–	–

[a]Nutrition data: http://nutritiondata.self.com/facts/nut-and-seed-products/3061/2

[b]Uddin et al. (2014)

Table 2 Algal sources rich in omega-3 (relative to omega-6)

Algae source[a]	*n*−6:*n*−3 ratio	ALA (mg/100 g)	EPA (mg/100 g)	DHA (mg/100 g)
Schizochytrium mangrovei (algal oil)	No omega-6	–	14,000	28,000

[a]Compositional guideline for DHA/EPA rich *Schizochytrium algal oil*. Australian Govt

Table 3 Animal sources rich in omega-3 (relative to omega-6)

Fish[a]	*n*−6:*n*−3 ratio	ALA (mg/100 g)	EPA + DHA/100 g
Salmon	1:12	–	2018
Tuna	1:24	–	1298
Trout	1:3	–	812

[a]Nutrition data: http://nutritiondata.self.com/facts/nut-and-seed-products/3061/2

Broad Role of Omega-3 PUFAs in Systemic Inflammatory Diseases

Outside of their contribution to health within the CNS which is the later focus of this chapter, it is worth noting the positive benefits of maintaining adequate omega-3 levels in a variety of peripheral diseases.

Heart disease: Omega-3 PUFAs have long been thought to positively alter the course of coronary artery disease. Supplementation studies with EPA and DHA have shown positive effects in key biochemical and physiological parameters including decreased platelet aggregation (Iwase et al. 2014; Mehta et al. 1988), reduced neutrophil chemotactic function (Mehta et al. 1988), reduced arachidonic acid and thromboxane production and reduced serum homocysteine and triglyceride and VLDL concentrations (Mehmetoglu et al. 2012; Zhang et al. 1997). ALA supplementation has also been shown to significantly reduce both systolic and diastolic blood pressure (Caligiuri et al. 2014).

Rheumatoid arthritis: Rheumatoid arthritis is a common autoimmune disease characterised by chronic inflammation in susceptible joints through increased synthesis of inflammatory eicosanoids by the cyclooxygenase 1 (COX-1) and 2 (COX-2) enzymes. This leads to synthesis of the pro-inflammatory cytokines IL-1 and TNF-α and increased aggrecanase activity resulting in loss of cartilage proteoglycan and degeneration of the joint (Curtis et al. 2000). While the omega-6 PUFA arachidonic acid stimulates inflammatory activity in this condition, the omega-3 PUFAs, EPA and DHA, promote inflammatory resolution. EPA in particular gives rise to 3-series prostaglandins and 5-series leukotrienes that downregulate inflammatory activity, and both EPA and DHA produce anti-inflammatory resolvins (Miles and Calder 2012).

A recent meta-analysis concludes that use of omega-3 PUFAs at dosages >2.7 g/day for >3 months can significantly improve symptoms and reduce the need for consumption of the non-steroidal anti-inflammatory drugs (NSAID) by patients (Lee et al. 2012).

Psoriasis: Psoriasis is another autoimmune disorder where unwanted activation of T-cells produces large amounts of the omega-6 PUFA arachidonic acid leading to the generation of pro-inflammatory mediators such as 2-series prostaglandins and 4-series leukotrienes in the skin. These result in dilation of blood vessels and an ongoing cycle in which new skin cells mature and move to the outermost layer of the skin within days rather than the usual weeks. As a result, dead skin cells and white blood cells

accumulate at the skin's surface resulting in a build-up of thick, scaly patches causing significant discomfort. Again, supplementation with the omega-3 fatty acids EPA and DHA has been shown to inhibit synthesis of the omega-6-derived pro-inflammatory mediators in a dose-dependent manner, dampening inflammation and significantly improving resolution of the skin abnormalities (Rahman et al. 2013).

General Mechanisms of Action

As briefly discussed above, the omega-3 family of lipids are positively associated with improved clinical outcomes in conditions associated with increased inflammatory activity. At least partial replacement of omega-6 PUFA with omega-3 PUFA in cell membranes results in a decreased cellular response to mitogenic and inflammatory stimuli (Bagga et al. 2003). However, the mechanisms behind omega-3's inflammation-resolving abilities are complex. Though a comprehensive review of the mechanisms is beyond the scope of this chapter, a brief summary of the multiple effects is given.

The anti-inflammatory action of omega-3 PUFAs may be attributed to a variety of mechanisms including:

1. The suppressive effect of higher levels of omega-3 PUFAs on the formation of the omega-6 PUFA arachidonic acid-derived proinflammatory prostaglandins and leukotrienes.
2. Providing alternative substrate for the 5-lipoxygenase enzymes increasing the production of the less potent 5-series leukotrienes
3. Providing alternative substrate for COX enzymes for conversion to the antithrombotic 3-series prostanoids rather than the prothrombotic 2-series prostaglandins (Fig. 2).
4. Down regulation of COX-1 activity by DHA (Martinez-Micaelo et al. 2012).
5. Activation of the G-protein receptor, GPR120, by EPA and DHA, reducing NF-κB activation through EPA and DHA competing with LPS and saturated fats for binding to toll-like receptor TLR-4 and further reduction of NF-κB activation through the binding of ALA, EPA and DHA to the nuclear fatty acid biosensor PPARγ (Fig. 3) (Im 2012).
6. Additional inflammation-suppressing and resolving actions occur through omega-3-derived resolvins and protectins. These hydroxylated derivatives of EPA (E-resolvins) and DHA (D-resolvins) make a significant contribution to resolution of the inflammatory process (Weylandt et al. 2012).

The weight of evidence from a range of inflammatory conditions strongly supports the view that either reduction or dysregulation of these omega-3-derived pro-resolving molecules promotes inflammatory disease (Kohli and Levy 2009; Serhan et al. 2008).

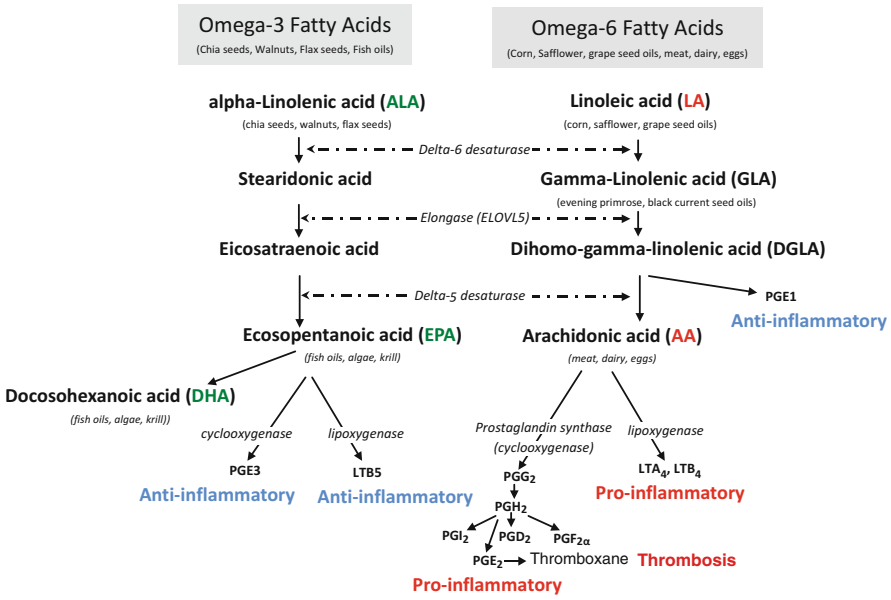

Fig. 2 Summary of the relationship between the omega-3 PUFA (anti-inflammatory) and omega-6 PUFA (proinflammatory) synthetic pathways. *PGE* prostaglandin E3, *LTB5* leukotriene B5, *PGG2* prostaglandin G2, *PGH2* prostaglandin H2, *PGI1* prostaglandin I2, *PGD2* prostaglandin D2, *PGF2α* prostaglandin F2alpha, *PGE2* prostaglandin E2, *LTA4* leukotriene A4, *LTB4* leukotriene B4

Omega-3 PUFAs and the Central Nervous System (CNS)

The human brain and CNS are rich in lipid content. Up to 60 % of the brain by weight is made up of fats which play crucial roles in the organs' structural characteristics and functional integrity. One of the most abundant fats in the brain is the essential omega-3 PUFA, DHA, which makes up around 30 % of the lipid fraction of adult cerebral grey matter (Svennerholm 1968). For contrast, EPA makes up just 1 % and ALA only 0.3 % of the fatty acid content of the brain. The prevalence of DHA in the brain suggests that maintaining adequate levels of this PUFA is essential for optimum brain function and development.

Omega-3 and Brain Development

Development of the human brain is a prolonged and intricate process that begins in the third week of gestation and extends to at least late adolescence and possibly into the early 20s. The processes contributing to brain development include genetic

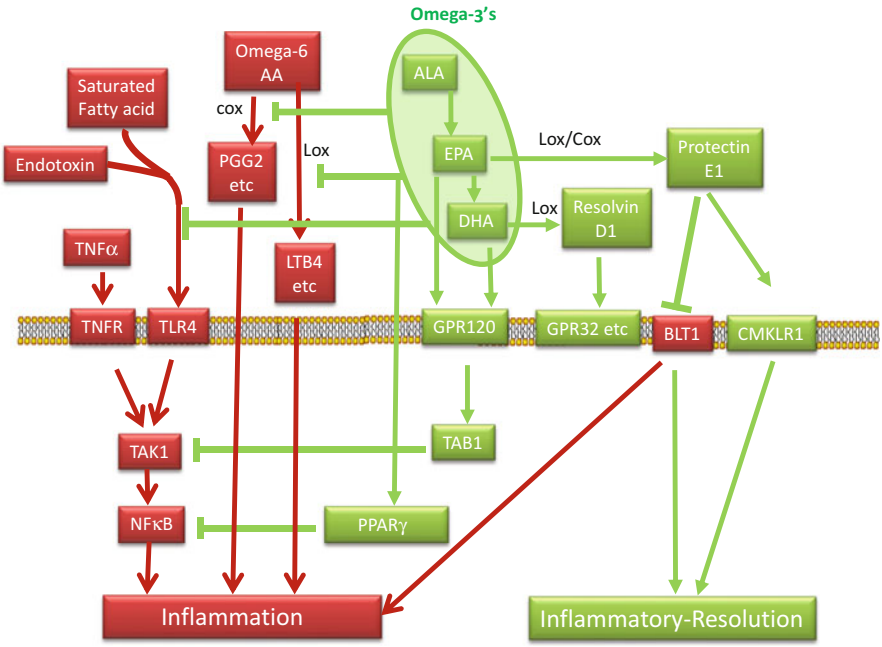

Fig. 3 Impact of omega-3 PUFAs on inflammation—multiple mechanisms. Adapted from Im (2012) (Bagga et al. 2003). *TNF-α* tumour necrosis factor alpha, *TNFR* tumour necrosis factor receptor, *TLR4* toll-like receptor-4, *TAK1* transforming growth factor activated kinase-1, *NF-κB* nuclear factor-kappa B, *AA* arachidonic acid, *PGG2* prostaglandin G2, *LTB-4* leukotriene B4, *ALA* alpha-linolenic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *GPR120* G-protein-coupled receptor 120, *TAB1* TGF-beta-activated kinase 1 and MAP3K7-binding protein 1, *PPARγ* peroxisome proliferator-activated receptor gamma, *GPR32* G-protein-coupled receptor 32, *BLT1* leukotriene B4 receptor, *CMKLR1* chemokine-like receptor 1. Enzymes: *LOX* lipoxygenase, *COX* cyclooxygenase

drivers of molecular activity, environmental stimuli and nutrient (i.e. raw biological material) availability.

These various elements interact to promote the series of highly coordinated events that result in development of the neural structures and functions of the mature brain.

In the early stages of development, the basic structures of the brain and central nervous system are complete by the end of the embryonic period around day 56 post conception (pc). Neurons begin to form by day 42 pc and establish connections with other neurons forming basic neural networks with major communication pathways (e.g. thalamocortical) which are complete by the end of the prenatal period.

Importantly, brain development continues after birth increasing in size by four-fold by age 6, by which time it is around 90 % of the adult brain volume.

While the rate of synaptic connectivity in early childhood (0–6 years) far exceeds that of adults, structural changes continue to occur throughout childhood and adolescence paralleling the brain's functional development which is reflected in maturing behaviour (for a review, see Stiles and Jernigan 2010).

During its growth, the brain goes through critical stages of neural development where selective synaptic elimination and neural network remodelling occur. Two critical periods for this process are from the immediate postnatal period up to age 6 and then through adolescence.

The success of this complex process of neurogenesis and synaptic connection and pruning can be influenced by both environmental and neurobiological factors which include omega-3 PUFA availability. As already mentioned, DHA is present in one of the highest concentrations of any lipid in the human brain. During development, DHA progressively increases in concentration in brain cell membranes from just 11 % in a 12-week-old foetus to 22 % at 4 years before reaching near adult levels in adolescents (16 years). For comparison, the levels of both ALA and EPA remain unchanged throughout development at ~0.3 % and 0.5 % of membrane fatty acid content, respectively (Svennerholm 1968). These observations correlate well with later findings that the foetal brain selectively accumulates DHA in the third trimester paralleling the increase in maternal–foetal transfer of DHA across the placenta (Clandinin et al. 1980; Larque et al. 2003).

While the prenatal period serves to establish the core compartments of the developing nervous system, environmental stimuli supported by optimal nutrition help determine the growth potential of the brain in the early postnatal years (up to age 6).

In spite of an incomplete understanding of how the observed changes in DHA levels affect neuronal function, insufficient dietary intakes of DHA during the early postnatal period have been associated with a variety of neurological deficiencies including reduced motor function, visual acuity, mental processing and IQ at age 4 and 7 years (Clandinin et al. 1980; Birch et al. 2010; Colombo et al. 2004; Helland et al. 2003, 2008; Luxwolda et al. 2014).

Importantly, during adolescence, the brain again goes through a critical period of neural development (Giedd et al. 1999), where a dynamic remodelling of the neural network occurs in parallel with the hormone-driven sexual development. The success of this 'brain rewiring' is also potentially susceptible to influences from neurobiological factors such as omega-3 PUFA availability. In support of this, recent studies have observed altered cortical attention networks associated with lower DHA levels in healthy boys (McNamara et al. 2013). The normal brain requires development of a coordinated network linking all of the major neurological systems. When specific elements of these inputs are lacking, alternative patterns of brain organisation can emerge. Low omega-3 PUFA availability during adolescence may therefore predispose susceptible children to a more disordered network in key brain regions (Fu et al. 2013), reducing their intellectual potential and increasing their risk of neurological disease. Consistent with this is the observation that fish consumption (a good source of DHA and EPA) in adolescence has been positively correlated with advanced vocabulary, higher grades and intelligence at early adulthood (Åberg et al. 2009; De Groot et al. 2012; Kim et al. 2010).

Finally, omega-3 PUFA intake is also important in the maintenance of optimum function of the developed adult brain. As previously mentioned, DHA makes up around 30 % of the lipid fraction of adult cerebral grey matter (Svennerholm 1968).

There is now considerable evidence supporting the view that increased intakes of omega-3 PUFAs are beneficial for a variety of neurological and neurodegenerative conditions. The following section provides a brief overview of the links and benefits between the main omega-3 PUFAs and neurological health.

Neurological Disorders Associated with Altered Omega-3 Levels

Clinical observation studies have related imbalanced dietary intake of fatty acids to impaired brain performance for a variety of diseases.

Depression

Depression is a universal disorder affecting around 350 million people worldwide (WHO Fact Sheet 2012). In a recent survey of chronic conditions managed by doctors in general practice (GP) in Australia, depression rated fourth after hypertension, hyperlipidaemia and osteoarthritis (Harrison et al. 2013). While the clinical manifestation of depression is well defined, its pathophysiology is not clearly understood. Beginning with early epidemiological reports that lower consumption of fish was associated with an increased prevalence of depression (Hibbeln 1998), a considerable body of data is now available linking depression with omega-3 status of both adults and now also adolescent children (Deacon et al. 2015; Grant et al. 2013; Grosso et al. 2014; Mello et al. 2014; Nemets et al. 2006).

As half of all lifetime mental disorders such as depression are initiated by mid-teens and three quarters by the early 20s, timely effective intervention is critical. In light of this and the growing recognition of its benefits, correction of omega-3 deficits as a way of preventing or minimising development of the depressive phenotype in this susceptible age group is being seriously investigated (Rice et al. 2014).

Early reports noted a negative association between fish consumption and the incidence of depression (Hibbeln 1998). These broad epidemiological observations have been corroborated in a number of cross-sectional studies in which higher fish intake has also been associated with reduced risk of depression (Barberger-Gateau et al. 2005; Bountziouka et al. 2009; Silvers and Scott 2002; Smith et al. 2014; Tanskanen et al. 2001). As omega-3 fatty acids are the elements in fish that are thought to provide the beneficial effects on mental health, in particular EPA and DHA, additional omega-3 intervention trials have been conducted (Su 2009).

While some studies have produced inconclusive results (Jacka et al. 2004; Kyrozis et al. 2009), a general consensus of the many studies now available provides significant support for a role of the omega-3 PUFAs, EPA and DHA, in positively modulating neuronal function in a fashion that reduces the risk or/and experience of depression (Deacon et al. 2015; Grosso et al. 2014; Mello et al. 2014; Martins 2009). Consistent with this are at least three meta-analyses that conclude that clinical trials of the use of omega-3 PUFAs in the treatment of

depression produce outcomes comparable to that of conventional antidepressants (Freeman et al. 2006; Ross et al. 2007; Lin and Su 2007).

But how are these neurological benefits obtained by increasing available omega-3 PUFAs? Although the evidence for a positive benefit of omega-3 in the prevention and resolution of depression in both adulthood and adolescence is compelling, the underlying mechanisms are not clearly defined and likely involve multiple pathways.

As both EPA and DHA are highly unsaturated, increased concentrations of these PUFAs have the effect of increasing membrane fluidity potentially increasing the efficiency of receptor-mediated neural transmission (Calder et al. 1994). Low membrane omega-3 levels have also been linked to increased serotonin 2 (5-HT 2) and decreased dopamine 2 receptor density in the frontal cortex which has been linked to depression (Delion et al. 1996).

Hibbeln et al. (1998) also reported that higher DHA concentrations are predictive of increased serotonergic neurotransmission as evidenced by higher cerebrospinal fluid (CSF) 5-HIAA levels in healthy adults.

More recent studies in animals have shown that a deficiency in omega-3 PUFAs also induces a hypothalamic–pituitary–adrenal (HPA) axis hyperactivity resulting in atrophy of selected pathways in the prefrontal cortex consistent with a developing depressive phenotype, leading to depressive behaviour (Larrieu et al. 2014).

In addition to the direct effects on membrane function and neurotransmission, increased levels of omega-3 PUFAs may indirectly foster healthier cell communication by helping resolve chronic central inflammatory activity and accompanying oxidative damage. As discussed above, the omega-3 PUFAs help downregulate expression and activity of pro-inflammatory cytokines such as IL-6, IFN-γ and the synthetic enzymes cyclooxygenase and lipoxygenase (Fig. 2).

Taken together, there appears to be a robust body of evidence in support of the view that ensuring and maintaining adequate omega-3 PUFA levels positively impact both the risk of developing depression and its resolution. In addition to its documented benefits in depression, a number of other neurological disorders also appear to be positively influenced by increased omega-3 PUFA levels.

Anxiety

Anxiety is common in modern society. As a group of psychiatric disorders, they are generally characterised by exaggerated feelings of worry, nervousness or unease. While chronic anxiety impacts negatively on a person's wellbeing and capacity to function within the community, it also increases the risk of mood disorders such as depression (Kessler et al. 2005). The most common anxiety disorders include phobias, post-traumatic stress disorder, generalised anxiety disorder, panic disorder and obsessive–compulsive disorder (Kessler et al. 2005). As there are clear associations between anxiety and mood disorders, it is reasonable to predict that correcting omega-3 PUFA deficiencies may also provide clinical benefit in this condition.

Though the number of studies in this area is limited, there are data that support this hypothesis. In a preclinical study, Song et al. (2003) observed that increasing EPA in the diet could reduce anxiety-like behaviours of rats. Consistent with this finding was

a cross-sectional study of 27 untreated persons with social anxiety disorder (SAD). A significant inverse correlation was observed between their individual levels of omega-3 PUFA and their anxiety score (Green et al. 2006). Interventional studies also provide support. A recent 12-week randomised controlled trial (RCT) reported that anxiety levels decreased in 68 students who were supplemented with omega-3 PUFAs (2.5 g/day, EPA and DHA) for 12 weeks (Kiecolt-Glaser et al. 2011).

While further studies are needed to confirm these benefits in anxiety disorders, the accumulating evidence linking omega-3 PUFA levels to neurological conditions associated with disordered thinking suggests that correcting deficits of these essential fatty acids may have benefit in other conditions also.

ADHD

ADHD is a condition in which the sufferer finds it difficult to control certain behaviours due to poor processing of selected neural inputs. This manifests as an inability to focus attention on a subject or task and overactive behaviour, outside that expected for a person's age and development. The condition is more prevalent in boys than girls.

Current pharmacological treatments for ADHD involve the use of stimulants such as methylphenidate and dexamphetamine. Other treatments include behaviour modification and individual or family counselling. While ADHD has heritable links, its development is likely to be consequence of environmental factors (including nutrition) acting on a susceptible genetic background (Nigg et al. 2010). Though stimulant pharmacotherapies are effective, there is concern over the use of these medications in younger patients in particular. The long-term impact of ADHD medication on the developing brain is not yet understood. As the brain continues to develop into at least late teens, the potential exists that chronic use of psychoactive medications in children and teens could interfere with normal brain development.

As discussed previously, omega-3 PUFAs play a significant role in neural development and synaptic networking. Beginning in the late 1980s, cross-sectional studies have reported reduced levels of omega-3 PUFAs in children with ADHD (Burgess et al. 2000; Hawkey and Nigg 2014; Mitchell et al. 1987). This may, at least in part, be due to higher rates of oxidative turnover of omega-3 PUFAs compared to the general population (Ross et al. 2003). Whatever the reason for the reduced levels, an association between low omega-3 PUFAs and abnormal emotion processing and callous and unemotional traits in adolescent boys and aggression in adults with ADHD has been reported (Gow et al. 2013; Meyer et al. 2015). Interventional studies provide support for a positive role for omega-3 PUFAs in ADHD. Vaisman et al. (2008) recently observed that supplementation with EPA and DHA was able to increase visual sustained attention in children with attention deficits. A recent 16-week RCT found that EPA and DHA supplementation improved working memory in children with ADHD, while Dashti et al. (2014) reported significant improvement in both hyperactivity–impulsivity and combined type (Dashti et al. 2014; Widenhorn-Muller et al. 2014).

The consensus at this stage from three recent meta-analyses concludes that while modest compared to available pharmacotherapies, omega-3 PUFA supplementation, particularly with EPA, is effective in moderating symptoms in ADHD and may be a useful adjunct treatment (Hawkey and Nigg 2014; Bloch and Qawasmi 2011; Puri and Martins 2014).

Mild Cognitive Impairment (MCI)

The normal ageing process results in a progressive decline in cellular and tissue function involving all the organs of the body. While the rate of ageing differs between individuals, there will always be a degree of decline in cellular integrity and utility. As a result, the individual no longer enjoys the same level of resilience to various challengers and stressors it once did. This is true of the brain as much as any of the other organs.

In the brain, advancing age is reflected in reduced volume, neuronal density and neurotransmitter concentrations resulting in impaired synaptic activity and reduced signal efficiency. These degenerative changes occur, at least in part, due to reduced membrane fluidity resulting from high levels of cholesterol and reduced desaturase activity and the generally widespread increase in inflammatory and oxidative activity (Guest et al. 2014).

While the factors leading to these changes are many, what is clear is that any therapy in which membrane fluidity is increased and inflammation is reduced is likely to translate into healthier brain function. Importantly, elevated omega-3 PUFA levels have been shown to improve membrane fluidity and improve inflammation generally and also reduce oxidative damage in the brain of those with MCI (Mori et al. 1999; Calder et al. 1994; Lee et al. 2013).

MCI is characterised by reduced mental processing significant enough to be noticed (by the individual or others), but not severe enough to interfere with independent living. Importantly, those with MCI have an increased risk of progression to frank dementia. A recent review concluded that there was no consistent evidence that any of the current pharmacological interventions including cholinesterase inhibitors and non-steroidal anti-inflammatory drugs were ineffective in reducing MCI and progression to dementia (Cooper et al. 2013). Though still few in number, interventional trials using omega-3 PUFAs seem to provide more positive effects. Chiu et al. (2008) reported that supplementation with 1.8 g/day resulted in improved cognition scores after 24 weeks. However, these benefits were not observed in patients already diagnosed with Alzheimer's dementia. Sinn et al. (2012) found that while supplementing with either 1.7 g EPA or 1.6 g DHA for 6 months improved depressive symptoms, improvements in verbal fluency scores were only observed in those in the DHA group.

As with each of the conditions discussed so far, the mechanisms behind these reported benefits are not completely understood. As omega-3 PUFA levels are linked to improved membrane fluidity and resolution of inflammatory activity, these almost certainly play a role in any clinical benefits. Consistent with this is a recent report by Fiala et al. (2015) who found that omega-3 supplementation was

able to increase β-amyloid (Aβ) phagocytosis while concomitantly increasing resolving D1 by macrophages in a cohort of MCI patients.

Though further studies are required to more clearly describe the benefits of correcting omega-3 levels in MCI, the situation is less clear for those patients who have progressed to frank dementia, in particular Alzheimer's dementia.

Neurodegenerative (Alzheimer's Disease)

AD is the most prevalent form of dementia accounting for 60–80 % of all dementia cases worldwide. A provisional diagnosis of AD is made through a set of clearly defined clinical and behavioural criteria (McKhann et al. 1984). However, a definitive diagnosis of AD can only be made at autopsy where the histological hallmarks of AD including increased deposition of β-amyloid plaques and neurofibrillary tangles, associated with increased inflammation and oxidative stress, are identified. The histological features of AD reflect the complex pathophysiology of the disease that results in marked brain atrophy, with significant neuronal loss.

Although plaques and tangles are seen in other neurodegenerative conditions and even normal individuals, when present with significant inflammation, Alzheimer's dementia is the clinical result (Lue et al. 1996). This observation underpins the central role played by inflammatory processes in the development of AD. With this in mind and with an appreciation of how increased levels of omega-3 PUFAs can significantly modulate elements of the inflammatory process, it is reasonable to ask the question: Can elevation of any one or all of the omega-3 PUFAs help prevent, reduce or reverse the incidence of AD? A complete review of the literature in this section is beyond the scope of this chapter; however, an overview of key findings is provided.

Reviewing preclinical studies, Hooijmans et al. (2012) concluded that long-term omega-3 PUFA supplementation (for >10 % of the lifespan) could reduce β-amyloid in animal models of AD and improve cognitive function. However, this effect was greater in rats than mice and in males compared to females.

The reported clinical benefit in humans is less clear. This is not surprising given the number of altered variables between each study such as stage of the disease, environmental background, genetics of the selected cohort, omega-3 dosage level, length of intervention and selection of different outcome measures. However, in spite of this, there are findings that provide general support to altered lipid metabolism in AD. Epidemiological and cross-sectional studies generally agree that omega-3 (in particular DHA) levels are lower in the brain of AD patients compared to non-demented controls (Grimm et al. 2013; Lin et al. 2012; Mohajeri et al. 2015). In their meta-analysis of the relationship between omega-3 PUFAs and fish intake with risk of developing AD, Wu et al. (2014) concluded that intake of long-chain omega-3 PUFAs did not significantly reduce risk (RR = 0.89, CI 0.74–1.08) of developing AD (Wu et al. 2015). However, higher intakes of fish were associated with a 36 % lower risk (Wu et al. 2015). Why a clear protective benefit was seen for fish but not for omega-3

intake is unclear but may be linked to a potential difference in omega-3 oxidation levels between supplements and fresh fish as discussed later in this chapter.

Unlike the modest but significant benefits reported for patients with MCI, the benefits of correcting omega-3 PUFA levels in those with AD through supplementation are unclear. A recent intervention study in which 40 AD patients were given 2.3 g of EPA and DHA/day for 6 months showed no clear reduction in oxidative stress in these patients (Freund-Levi et al. 2014). This is consistent with a more recent 12-month RCT where no significant difference in oxidative stress markers was observed between the placebo and omega-3 PUFA supplementation groups (Shinto et al. 2014). In spite of this, however, this same study observed a significant reduction in the rate of decline in the mini-mental state examination (MMSE) and activities of daily living test (Shinto et al. 2014). This variation in outcomes is reflected in a recent review which concluded that the available evidence does not provide strong evidence that omega-3 PUFA supplements protect against cognitive decline or dementia and that RCTs generally report no benefit.

However, the bulk of the epidemiologic literature and some RCTs support the view that raising omega-3 PUFA levels can provide cognitive benefit and potentially slow decline (Dacks et al. 2013).

The discrepancy between preclinical and epidemiological evidence on the one hand and RCTs on the other is a conundrum requiring future studies to unravel. However, some observations that may help identify the source of the controversy include:

1. RCTs have not targeted people in the lowest quartile of omega-3 PUFA status and thus, based on epidemiology, at increased risk of dementia or cognitive decline (Dacks et al. 2013).
2. Cognitive protection by omega-3 PUFA may be limited to the APOE-4 (high-risk) genotype (Dacks et al. 2013).
3. As omega-3 PUFAs are highly oxidisable and as the biological activity of oxidised omega-3 PUFAs will be altered and can have harmful effects, oxidation of supplemental oils used in the various trials may be responsible for the discrepant results not only between fish consumption and omega-3 supplements (see discussion above) but also between the various trials.

In respect of point (3) above, it is noteworthy that oxidised oils do not taste pleasant (i.e. they are rancid); therefore, for those eating fish, only fresh fish, containing negligible oxidised oils, should be eaten. However, oxidised oils may readily be consumed as a capsule supplement as the oil will not contact taste or olfactory receptors.

Unfortunately, there is good evidence of a high level of oxidation in omega-3 supplements with a recent study finding that 83 % of the omega-3 supplements tested exceeded the recommended levels of oxidation markers (Albert et al. 2015).

Thus, the benefits for omega-3 PUFA supplementation in AD are mixed, possibly due to high oxidation of the supplement, either pre-administration (i.e. oxide supplement) or in vivo due to a high systemic or central oxidative environment in the patient. Taking at least these three considerations into account may go some way to explaining why omega-3 trials in MCI patients are generally more positive than those reported for AD, a condition characterised by more advanced inflammatory and oxidative activity.

Traumatic Brain Injury (TBI)

TBI is a frequent cause of death and disability worldwide resulting from falls, motor vehicle accidents, sporting injuries or social or domestic violence. Damage to the brain occurs as it rapidly decelerates against the skull or is crushed by an external force. Under these circumstances, the brain may be compressed, contorted, pierced or ripped. This causes a disruption to cerebral blood flow and at a cellular level, damage to the brain's neural pathways, disrupting both axonal and dendritic connections and glial and vascular support networks.

The stretching and disruption of the axons result in loss of ionic regulation (Na^+/K^+/Ca^{2+}) resulting in a massive influx of Ca^{2+} and an efflux of K^+ in and from the cell bodies. The subsequent membrane depolarization results in uncontrolled release of excitatory neurotransmitters, such as glutamate, causing over activation of *N*-methyl-D-aspartate (NMDA) receptors and altered neuronal firing affecting processes such as learning and memory (Barkhoudarian et al. 2011).

The molecular mechanisms involved in the repair of a TBI are extremely complex but, if it is to be successful, involve at least the coordinated process of immune/inflammatory activation and resolution coupled with synaptogenesis and angiogenesis.

As a result of the ionic dysregulation, ATP-dependent membrane ionic pumps are activated to restore ionic homeostasis and reduce the excitotoxicity. As a result of accompanying mitochondrial dysfunction, the increased demand for ATP is met through glycolysis resulting in excessive lactic acid production and oxygen free radical production. This leads to oxidative stress and accelerated damage to DNA, proteins and phospholipids (Barkhoudarian et al. 2011). As glucose stores become diminished, a hypometabolic state can develop which may last for several days or even months in severe cases (Barkhoudarian et al. 2011).

In an effort to clear the debris from the structural damage to the axons and cell bodies etc., the brain's resident macrophages (i.e. microglial) are activated playing an integral part in the inflammatory response.

This inflammatory component can begin as early as 1 h after injury and can last up to 1 month (Blaylock and Maroon 2011). When activated, these microglia release chemical signals (e.g. cytokines) that initiate a cascade of events that, when working well, assist in the clearance and repair of the injury. However, if this inflammatory element fails to 'switch off', additional collateral damage to surrounding neurons and other cells occurs.

Overall, a combination of the initial trauma, resultant metabolic and mitochondrial dysfunction and resulting oxidative stress and inflammation, if not resolved appropriately, can impair neuronal repair and exacerbate the cognitive dysfunction and final neurological deficits.

In preclinical studies of mild traumatic brain injury (mTBI), supplementation with DHA either before or after injury has been shown to consistently improve functional outcomes in animal models. This included improved memory, cognitive function, spatial learning and body weight recovery after injury (Barrett et al. 2014). The multiple mechanisms through which these were achieved included increased resolution of inflammatory signalling, induction of antioxidant enzymes (Mn-SOD) and reduced oxidative stress, normalisation of mitochondrial activity and energy

metabolism, restoration of neurotransmitter (e.g. dopamine) release and maintenance of brain-derived neurotrophic factor (BDNF) concentrations (Barrett et al. 2014; Hasadsri et al. 2013; Kumar et al. 2014).

While the preclinical evidence from animal models of TBI is compelling, little data from humans is yet available. Lewis et al. (2013) report that from their experience in emergency medicine, aggressively adding substantial amounts of omega-3 PUFAs to optimise the nutritional foundation of severe TBI patient is beneficial for recovery. An earlier report by Roberts et al. (2008) in reference to the recovery of a mining accident TBI victim also concluded that the relatively good outcomes in the patient could be attributed to aggressive nutritional repletion and supplementation with omega-3 fatty acids, in addition to good clinical care.

However, though preclinical animal models provide good data in support of the positive benefit of including omega-3 supplementation in the management of mTBI, further research using human trials is required to demonstrate the efficacy and clinical utility in the human TBI context.

Maintenance of Omega-3 Levels in the CNS

In the body as a whole, it is widely accepted that the liver is the primary site in which ALA is converted to DHA, from where it is packaged into triacylglycerols for transport to various systemic tissues. Therefore, while the brain is unlikely to be the major user of omega-3 PUFAs, as discussed above, it has a functional requirement for DHA in particular throughout the lifespan. From embryo to older age, the adult human brain is estimated to turn over around 5 mg DHA per day, while the breast-fed infant is about twice this amount (i.e. 10 mg/day) (Cunnane et al. 2000; Rapoport et al. 2007). A relevant question to be answered is: Does the daily supply of DHA synthesised within the CNS form ALA and/or EPA or is it predominantly taken up from the systemic circulation?

ALA to DHA Synthesis Within the CNS

As outlined schematically in Fig. 2, beginning with the plant-derived shorter chain-length alpha-linolenic acid (ALA, C18:3), the carbon chain can be progressively elongated using elongase and the delta-5 and delta-6 desaturase enzymes to the more bioactive eicosapentaenoic acid (EPA, C20:3) and docosahexaenoic acid (DHA, C22:6) omega-3 PUFAs. Though ALA does have some biological function (see Fig. 3), in the central nervous system (CNS) the focus of attention is on EPA and DHA. There has been much speculation on whether a diet supplying largely ALA is sufficient to supply the brain with enough DHA for optimal health, in particular due to questions over the efficiency of this conversion.

A recent preclinical study investigated whether rats fed with only ALA could maintain adequate DHA levels in the brain (over 15 weeks) compared to animals

fed with DHA (Domenichiello et al. 2014). This study concluded that there was no difference in brain DHA levels irrespective of diet. DHA synthesis from ALA is therefore likely sufficient to maintain brain levels at least in the rat.

Whether synthesis of longer chain omega-3 PUFAs from ALA is comparable in the human is still open to challenge (Domenichiello et al. 2014). Brenna et al. (2009) concluded that ALA supplementation in humans, while still able to increase DHA levels, was not sufficient to produce an increase in DHA levels in the blood equivalent to that of direct DHA supplementation.

Others agree that dietary ALA provides a robust dose–response relationship with increased brain DHA levels (but not the heart and liver) suggesting direct CNS conversion of ALA to DHA occurs in the brain (Barceló-Coblijn and Murphy 2009). Thus, while the brain is not the major source of DHA for the body in general, it likely has the synthetic capacity to meet its daily needs for DHA synthesis through the conversion of plasma-derived ALA.

BBB Transport from Systemic Circulation

There is currently no complete agreement on the mechanisms through which omega-3 PUFAs and DHA in particular are trafficked into the CNS although, as discussed above, synthesis from centrally available ALA is operational.

Preclinical studies have demonstrated that unesterified ALA and DHA can cross the blood–brain barrier (BBB) from the plasma lipid pool. However, it is still not known whether this occurs via an active protein-mediated transport process or predominantly via passive diffusion. Pelerin et al. (2014) favour protein-mediated transport, recently reporting that several fatty acid transporters are expressed in the BBB where their expression levels were high during the period of active omega-3 PUFA accretion into the brain of rat pups. However, they also noted that no significant changes in expression occurred for these transporters if rats were fed with diets either deficient in DHA or provided in excess. This would suggest that these transporters may be used for other purposes and that DHA (and other omega-3 PUFAs) may still access the brain via a passive process (Pelerin et al. 2014).

A recent study from our group in humans observed a positive correlation between systemic (blood) and central (CSF) omega-3 PUFA concentrations and docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) in particular (Guest et al. 2013), suggesting passive BBB transport of at least these PUFAs.

However, substantial work is still needed to clearly determine whether DHA and other PUFAs require facilitated transport into the CNS in humans or if in fact this is a predominantly non-saturable, passive process. The importance of this may be appreciated when it is recognised that a predominantly passive transport system at least provides the possibility that excessive supplementation may result in toxic CNS levels of DHA.

Ideal Omega-3 Levels in the Peripheral Body and CNS

Given the overall finding that omega-3 PUFAs in general and DHA in particular are beneficial for CNS function and a range of neurological conditions, it is relevant to ask the question, how much omega-3 is needed to realise these health benefits? Though DHA turnover in the normal adult brain is only around 5 mg/day for an adult (Rapoport et al. 2007), how much supplemental DHA (or ALA/EPA) is needed to supply this amount as the final amount supplied is dependent on (a) the oral bioavailability of DHA (or precursor PUFAs) and (b) the degree to which the respective PUFAs are used for replacement of systemic omega-3 PUFAs and energy metabolism.

Unfortunately, the bioavailability of the omega-3 PUFAs is complex and has not been clearly characterised. Their absorption is variable depending on their chemical structure (i.e. ALA, EPA or DHA), form (i.e. free fatty acid, triglycerides, phospholipids, esterified, etc.) and delivery associations (i.e. is it a supplement, taken with food, with other oils, etc.). Though no 'true' bioavailability (i.e. AUC oral/AUC1.v.) data is currently available for any of the omega-3 PUFAs, there is considerable data on relative comparisons of various formulations and the effect of different doses on clinical outcomes. Therefore, the final doses selected for any neurological condition are arrived at empirically.

As discussed above, how omega-3 PUFAs are trafficked across the BBB is not definitively understood. However, some studies have observed that central (e.g. CSF) and peripheral (i.e. blood) omega-3 PUFAs do correlate inferring passive transport (Guest et al. 2013). Therefore, while it is not yet known what levels of supplementation are beneficial for the CNS, it may be presumed that achieving at least those levels in the blood that are beneficial for cardiovascular disease would serve as a minimum target.

Block et al. (2008) showed a significant association between acute coronary disease and red blood cell EPA and DHA levels, expressed as a percentage of total membrane fatty acids, known as the omega-3 Index. Cut points for cardiovascular risk were therefore determined on the basis of these and other observations to be <4 = high risk, 4–8 = intermediate risk and >8 = low risk.

The value of these omega-3 Index cut points has since been argued convincingly as both a risk marker and risk factor for cardiovascular disease (Harris 2009). Subsequent work by Baghai et al. (2011) provides additional support for an association between low omega-3 index (<4) and risk for both cardiovascular and major depressive disorder (MDD) in adults. However, comparable, disease–risk cut points based on omega-3 red cell status for neurological or neurodegenerative diseases in adults, children or neonates are still to be developed.

Importantly, with a growing knowledge of the benefits of omega-3 PUFAs to both central and systemic health has also come to the realisation that a typical Western diet appears generally inadequate to supply the need. While Western dietary patterns generally supply substantial amounts of omega-6 through ingestion of manufactured foods containing oils such as corn, safflower, grapeseed, sunflower, peanut and soya bean, this same diet has limited capacity to provide

omega-3 resulting in a majority of the population being omega-3 PUFA deficient (Grant et al. 2013; Blasbalg et al. 2011). Given the widespread benefits attributable to adequate omega-3 PUFA status and both the ease with which its tissue status can be tested and deficiencies corrected (i.e. through either supplementation or dietary adjustment), it is surprising omega-3 status assessments are not routine.

Cautions Regarding Omega-3 Supplementation

It is now widely appreciated by the research, clinical and general communities that maintaining optimal omega-3 PUFA levels in the body is beneficial to health. However, as with most biomolecules, certain cautions should be heeded to ensure maximum benefit.

1. *Potential toxicity*: While no studies have yet identified any consistent level of toxicity for supplementation with DHA (up to 7.5 g/day) (Lien E, 2009) 2009), or EPA (600 mg/kg rat, equivalent to 42 g/day, 70 kg man), common sense would suggest that megadoses of these essential fatty acids could still produce undesirable effects in the longer term (Collins et al. 2014).
2. *Oxidised omega-3 PUFAs*: All omega-3 PUFAs have highly oxidisable structures as discussed above. As such, any supplement or food source containing significant quantities of these fatty acids must be stored in a manner to prevent their oxidation. Increased oxidation of omega-3 PUFAs can occur in the presence of microbial action, UV light, heat, metals and metalloproteins leading to generation of free radicals and resulting damage to cellular structures and metabolic components (Shahidi and Zhong 2010; Albert et al. 2013). A recent study by Albert et al. (2015) found that 83 % of supplements tested exceeded the recommended level of oxidation products for at least one marker. The direct health implication for potential widespread supplementation with 'already'-oxidised omega-3 PUFAs is unknown, requiring investigation. However, it is reasonable to assume, based on the available chemistry, that the outcome is likely to favour the exacerbation of oxidative imbalance and, depending on the degree of product oxidation, exacerbation of oxidative (and secondary inflammatory) processes in those already suffering from disease. This may be one reason why, in conditions where the oxidative inflammatory background is already high (e.g. Alzheimer's disease), omega-3 PUFA supplementation has mixed benefits.

Conclusion

A convincing body of evidence now supports the view that promotion of higher levels of omega-3 PUFAs is required to obtain and maintain optimum systemic and neurobiological growth and health throughout life (Bhatia et al. 2011; Larque et al. 2012; Swanson et al. 2012).

Maintaining adequate omega-3 PUFA levels has been shown to be beneficial in reducing the incidence of a range of chronic systemic, inflammatory-associated conditions including cardiovascular disease (Harris 2009), diabetes (Popp-Snijders et al. 1987), psoriasis (Rahman et al. 2013) and rheumatoid arthritis (Curtis et al. 2000). Evidence is now growing that in the brain, omega-3 PUFAs are also beneficial in preventing or alleviating the symptoms of a range of neurological conditions including depression (Deacon et al. 2015; Grosso et al. 2014; Mello et al. 2014), anxiety (Kiecolt-Glaser et al. 2011), ADHD (Hawkey and Nigg 2014), MCI and TBI (Chiu et al. 2008; Barrett et al. 2014). Benefits for omega-3 PUFA supplementation in AD are mixed, possibly due to the lack of attention given to the generally high in vivo oxidative potential of the trial population.

While the mechanisms underlying these benefits are not completely clear for any of these conditions, the roles of DHA in influencing membrane dynamics and the omega-3 PUFAs as a group in modulating inflammatory activity at a number of levels (Figs. 2 and 3) are likely central elements.

Compliance with Ethics Requirements The authors declare that they have no conflicts of interest.

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Neuroprotective Activities of Saffron and Crocin

Shinji Soeda, Kosuke Aritake, Yoshihiro Urade, Hiroshi Sato, and Yukihiro Shoyama

Abstract We first considered that saffron is really safety food because it has a long-use history. The neuroprotective activities of saffron and its major constituent, crocin, are separately discussed in vitro and in vivo. We reviewed the inhibitory activities of crocin against PC-12 cell apoptosis. The oxidative stress decreased the cellular levels of glutathione (GSH) which is an inhibitor of neutral sphingomyelinase (N-SMase). Therefore, the level of GSH was assayed by the addition of crocin resulted in the activation of glutathione reductase (GR). It became evident that crocin treatment prevents the N-SMase activation resulting in the decrease of ceramide release. From these evidences we summarized the role of crocin for neuronal cell death. We used the ethanol-blocking assay system for learning and memory activities. The effect of saffron and crocin on improving ethanol-induced impairment of learning behaviors of mice in passive avoidance tasks has been clear. Further, we did make clear that saffron and crocin prevent the inhibitory effect of ethanol on long-term potentiation (LTP) in the dentate gyrus. Finally we found that 100 mg/kg of crocin gave non-rapid eye movement sleep (non-REM sleep) although mice were started to be active during night time.

Keywords Saffron • *Crocus sativus* • Crocin • Neuroprotective activity • Learning and memory • Non-REM sleep

S. Soeda
Faculty of Pharmaceutical Science, Fukuoka University, Fukuoka, Japan
e-mail: ssoeda@fukuoka-u.ac.jp

K. Aritake • Y. Urade
International Institute for Integrative Sleep Medicine, Tsukuba University, Tsukuba, Japan
e-mail: aritake.kosuke.gt@u.tsukuba.ac.jp; urade.yoshihiro.ft@u.tsukuba.ac.jp

H. Sato • Y. Shoyama (✉)
Faculty of Pharmaceutical Science, Nagasaki International University,
Sasebo, Nagasaki, Japan
e-mail: satoh@niu.ac.jp; shoyama@niu.ac.jp

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Abbreviation

DMEM	Dulbecco's modified Eagle's medium
ELISA	Enzyme-linked immunosorbent assay
FB1	Fumonisin B1
c-GCS	c-Glutamylcysteinyl synthase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
IL-6	Interleukin-6
JNK	c-Jun kinase
LTP	Long-term potentiation
MAb	Monoclonal antibody
NGF	Nerve growth factor
NMDA	*N*-Methyl-D-aspartate
Non-REM sleeping	Non-rapid eye movement sleep
N-Smase	Neutral sphingomyelinase
PS	Phosphatidylserine
SAPK	Stress-activated protein kinase
SD	Step-down
SM	Sphingomyelin
SOD	Superoxide dismutase
ST	Step through
TNF-a	Tumor necrosis factor
TCM	Traditional Chinese medicine

Introduction

Japan has been running into super aging society resulting in rapidly increasing lifestyle disease including dementia which is the most serious problem in recent Japan. Japanese patient survey by Ministry of Health, Labour, and Welfare reported 462,000 of dementia patients in 2012 and quickly increasing to 700,000 in 2025 in Japan meaning that the estimate of national medical care expenditure swells up, and therefore the Japanese health insurance system is considered to be confronted with the brink of collapse. This is the reason why the natural products having preventive activities for brain disease are particularly desirable in Japan. Considering such recent healthy circumstances in Japan, we select saffron as a neuroprotective natural product, and its function will be reviewed in this chapter.

Crocus sativus L. (Iridaceae) is a perennial herb that is widely cultivated mainly in Iran, where 90 % of saffron has been produced, and the other countries like Greece, Spain, and Morocco for its red stigmatic lobes that constitute saffron from 3500 years ago. This plant blooms only once a year, and the man-

ual harvest of stigmas should be performed within a very short time (Trease and Evans 2002).

The manual cultivation methods practiced with saffron crocus contribute greatly to its high price. About 100,000 flowers give about 1000 g of the dried saffron. Since weather conditions affect the quality of saffron, an indoor cultivation system was established in Japan from 100 or more years ago in Oita Prefecture in Japan (Fig. 1, upper). The stigmas can be collected from full blooming *C. sativus* in the room. This is the reason why the indoor cultivation method is advantageous for the achievement of a homogenous quality of saffron and for saving time (Morimoto et al. 1994). We confirmed that the concentration of crocin is increasing until full blooming and then decreased. Therefore, stig-

Fig. 1 Cultivation of *Crocus sativus* indoor and saffron corrected

mas can be collected in full blooming season in general in order to keep the higher concentration of crocin (Morimoto et al. 1994).

Saffron finds its use as folk medicines and traditional Chinese medicine (TCM) as well as a flavoring and a coloring agent. Saffron has three main chemical components, the bright yellow coloring carotenoids, a bitter taste, picrocrocin, and a spicy aroma, safranal. The carotenoid pigments consist of crocetin-diglucoside, crocin-2, crocin-3, crocin-4, and crocetin di-(β-D-digentiobiosyl)-ester (crocin) (Fig. 2). More recently we succeeded to isolate a novel crocetin glycoside, *trans*-crocetin-1-al 1-*O*-β-gentiobiosyl ester (Fig. 2) (Tung and Shoyama 2013). We confirmed that drying is important because an endogenous β-glucosidase is still active when moisture remains (Morimoto et al. 1994). Therefore, drying is completed in about 30–45 min, after which the drug is cooled and stored under dry condition (Morimoto et al. 1994).

It is well known that saffron has anticancer activities against several cancer cell lines and in vivo investigations. We also investigated the anticancer activity of saffron and its constituent, crocin. In the first investigation, we tested the

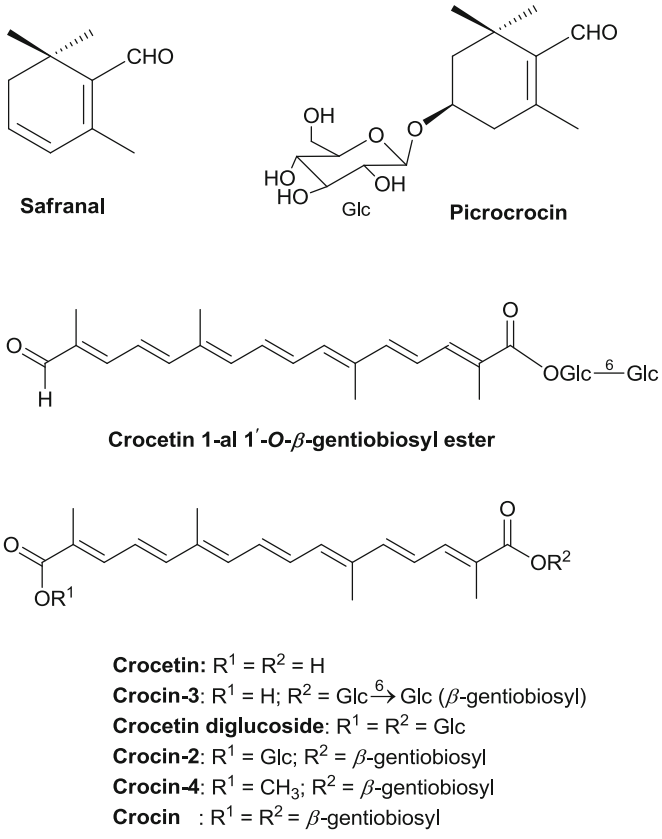

Fig. 2 Major constituent in saffron

inhibitory activity for skin tumor promoted by chemicals by using saffron and crocetin glucosides. When crocin was applied before each 12-*O*-tetradecanoylphorbol-13-acetate treatment, it delayed the formation of papillomas; only 10 % of mice bore papillomas at 9 weeks of promotion. The effect of crocin was not mimicked by gentiobiose or glucose alone (Konoshima et al. 1998).

Furthermore, we investigated in vitro anticancer-active evidences of saffron extract and its constituent, crocin, using several cancer cell lines like HTC-116, SW-480, and HT-29. Saffron extract and crocin significantly inhibited the growth of colorectal cancer cells while not affecting normal cell (Aung et al. 2007). From these data we started in vitro experiments using mice. The development of colonic adenocarcinomas in mice was induced by azoxymethane and dextran sodium sulfate. Crocin significantly inhibited the colonic adenocarcinomas depending on the inhibition of inflammation phenomenon resulting in the prevention of colitis and inflammation-associated colon carcinogenesis (Kawabata et al. 2012).

Saffron can be used as an antispasmodic, anticatarrhal, and nerve sedative ingredient and is reported to be useful in treating various human disorders such as heart and blood disorders (Konoshima et al. 1998; Aung et al. 2007; Kawabata et al. 2012; Lee et al. 2005). Crocin has a wide range of activities including antioxidant (Aung et al. 2007; Kawabata et al. 2012; Lee et al. 2005; Ochiai et al. 2004; Rigobello et al. 2002), anticancer (Konoshima et al. 1998; Ochiai et al. 2004; Chryssanthi et al. 2007; Abdullaev 2002), hypolipidemic (Aung et al. 2007; Rigobello et al. 2002; Sheng et al. 2006), anti-atherosclerotic (Chryssanthi et al. 2007; Abdullaev 2002; Sheng et al. 2006; Xu et al. 2005, 2006), and anti-inflammatory effects (Xu et al. 2005, 2006, 2009). The neuroprotective activities of crocin have also been demonstrated in various experimental animal models of brain disorders, such as cerebral ischemia (Xu et al. 2005; Ochiai et al. 2007), Alzheimer's disease (Xu et al. 2009; Papandreou et al. 2006), depression (Ochiai et al. 2007; Lechtenberg et al. 2008), and memory impairment (Papandreou et al. 2006; Lechtenberg et al. 2008; Abe et al. 1998; Sugiura et al. 1995a, b).

Neuronal cell death is required for the development of the nervous system. However, recent studies suggest that neurons die from programmed cell death (apoptosis) in the brains deprived of oxygen by stroke (Crowe et al. 1997; Zhang et al. 1994a) and trauma (Hill et al. 1995; Sugiura et al. 1995c) and in the brains of Alzheimer's patients (Pettmann and Henderson 1998; Xuan et al. 1999). Therefore, prevention of neuronal apoptosis has been considered to be a desirable therapeutic strategy for treating such neurodegenerative diseases, although the value of this approach is not yet evident. This review discusses the value of folk medicines in terms of learning and memory and also in modulating apoptotic cell death, together with our recent data of crocin's effect on neuronal cell death.

The development of natural products with properties for alleviating the symptoms of learning and memory impairments has been expected by clinicians and researchers in the field. In the brain, the hippocampus is a very important

region in the learning and memory processes, and the LTP induced from the brain tissue is closely related to learning and memory (Hill et al. 1995; Ishiyama et al. 1991). In earlier publications, we reported the effects of an ethanol extract of *C. sativus* and its purified components on the central nervous system in terms of learning behaviors in mice and LTP in the dentate gyrus of hippocampus in anesthetized rats and in the CA1 region of rat hippocampus slices (Pettmann and Henderson 1998; Ishiyama et al. 1991; Abe et al. 1991; Zhang et al. 1994a; Sugiura et al. 1995c).

Preparation of Monoclonal Antibody (MAb) Against Crocin and Confirmation for Incorporation of Crocin into PC-12 Cells by Immunostaining

In the first stage of neuronal investigations, we prepared monoclonal antibody (MAb) against crocin (Xuan et al. 1999). In the first step for preparation of MAb against crocin, the conjugate of crocin with carrier protein for immunization is necessary. Therefore, crocin was treated with $NaIO_4$ to cut sugar moiety releasing aldehyde in a molecule following addition of carrier protein. As the other way, the crocin hemisuccinate was prepared first and then conjugated with BSA to give crocin hemisuccinate-BSA conjugate as indicated in Fig. 3. The molecular weight of prepared schiff base was analyzed by MALDI-TOF mass spectrometry to determine the hapten number in the conjugate for suitability of immunization. Since the hapten number in crocin hemisuccinate-BSA conjugate was determined to be 8.6 which was suitably enough for immunization rather than that of crocin-BSA conjugate prepared by $NaIO_4$ treatment, the former was used as an antigen. Hybridoma-producing MAb reactive to crocin was obtained by general procedure and classified into IgG2a which had λ light chains. The reactivity of IgG-type MAb 12a was tested by varying antibody concentration and by performing a dilution curve, and then the antibody concentration was selected for competitive ELISA. The measuring range of this ELISA system extends from 10 to 200 ng/ml of crocin (Xuan et al. 1999).

In order to confirm the incorporation of crocin and the localization of crocin into PC-12 cells, we immunostained cells using the anti-crocin MAb prepared. Clear incorporation of crocin into PC-12 cells was confirmed after 30 min comparing with the control cells as indicated in Fig. 4 (Ochiai et al. 2004). The incorporation after addition of crocin in the medium was not enough after 15 min (Fig. 4b). From 30 min later, the clear staining occurred (Fig. 4c, d). From this evidence we confirmed that crocin can be incorporated into the cell and be functioned.

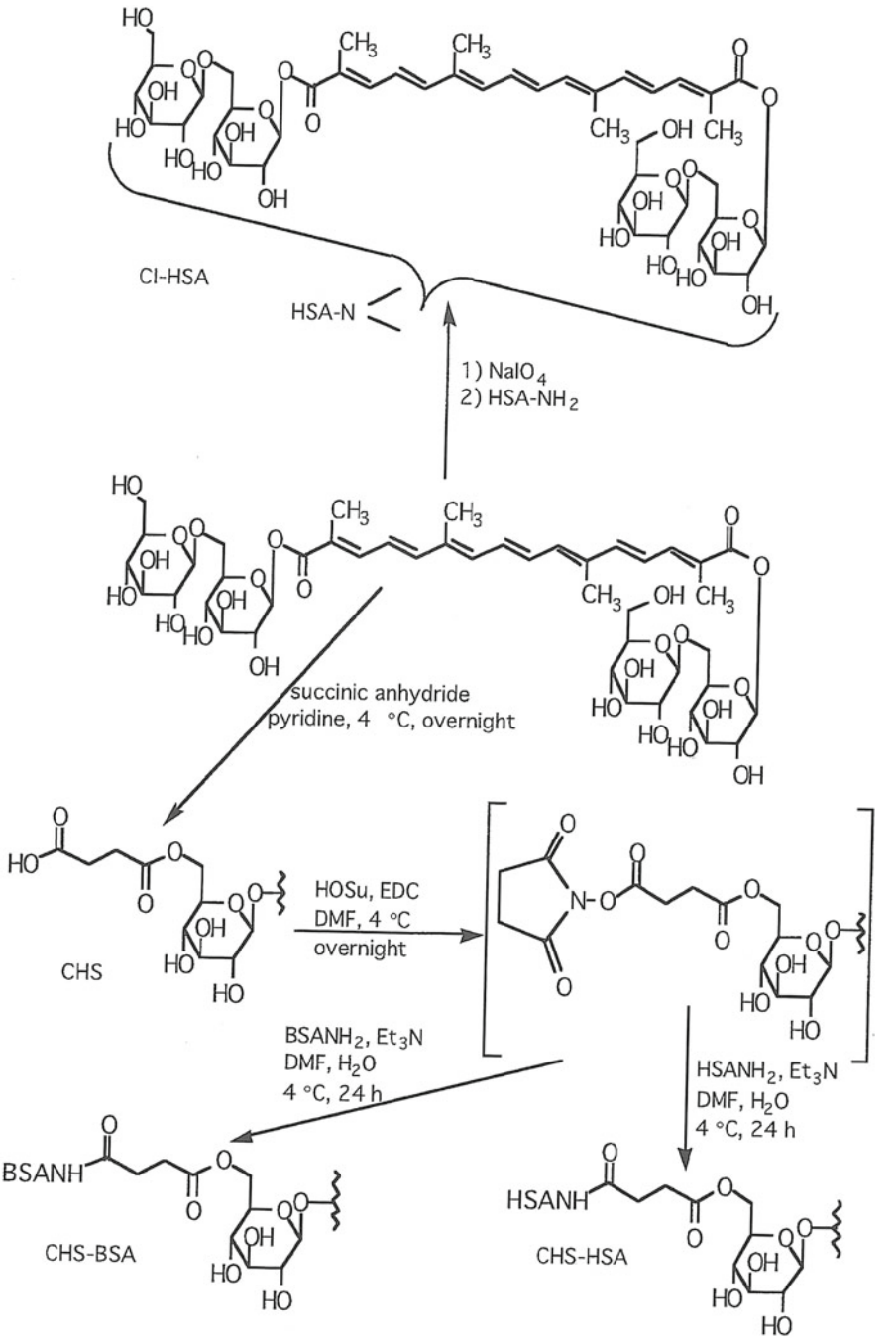

Fig. 3 Synthetic pathway for hapten-carrier protein conjugate of crocin

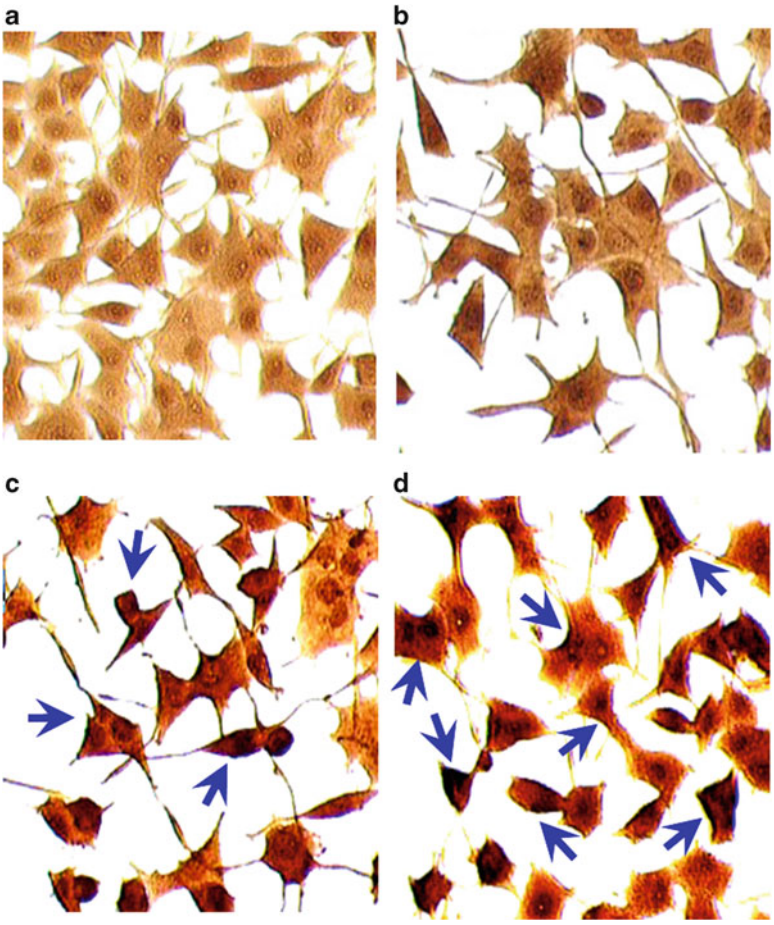

Fig. 4 Immunostaining of crocin using anti-crocin monoclonal antibody in PC-12 cells. (**a**) Stained after 0 min; (**b**) after 15 min; (**c**) after 30 min; (**d**) after 1 h. *Arrows* indicated stained cells by immunostaining using anti-crocin MAb

Neuroprotective Activity of Crocin In Vitro

Antioxidant Activity of Crocin in Preventing Neuronal Cell Death

First of all we examined the effects of crocin on PC-12 cells deprived of serum/glucose in comparison with those of α-tocopherol (Soeda et al. 2001). Depriving the PC-12 cells of serum/glucose caused changes in the morphology and peroxidation of their membrane lipids and decreased intracellular superoxide dismutase (SOD) activity. The oxidative stress transferred the phosphatidylserine (PS) residues into

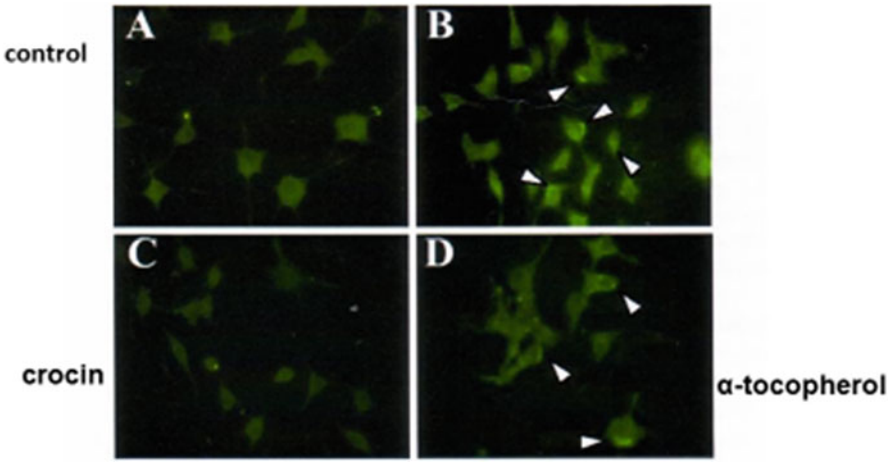

Fig. 5 Annexin V staining of PC-12 cells exposed for 3 h in serum/glucose-deprived medium. (**a**) Control cells in serum/glucose (+) DMEM; (**b**) cells in (−) DMEM alone; (**c**) cells in (−) DMEM plus 10 μM crocin; (**d**) cells in (−) DMEM plus 10 μM α-tocopherol. *Arrows* indicate ring-like stains

the outer membrane, although they are usually consisted in the inner membrane, and resulted that PS externalization can be used as an early marker of apoptotic induction. Annexin binds to the negatively charged PS, and the conjugated FITC shows a ring-like stain along the cellular boundary (Mukhopadhyay et al. 2007). The cells deprived of serum/glucose show strong ring-like stains compared to the control cells. Crocin kept the cell's morphology more intact than α-tocopherol. In PC-12 cells deprived of serum/glucose for 6 h, the level of peroxidized lipid membrane increased 1.8-fold in comparison to the control cells, and SOD activity decreased to 14 % of that in the control cells. However, crocin significantly decreased the formation of peroxidized membrane lipids and restored SOD activity compared to α-tocopherol activity. The restoration of SOD activity suggests that crocin has an important role in modulating antioxidative effects. Crocin also suppressed the activation of caspase-8 caused by serum/glucose deprivation; this activation was suppressed in a concentration-dependent manner (0.1–10 μM). Crocin did not inhibit caspase-8 activity in the cell lysates, and its inhibitory effect may be caused indirectly by the antioxidant activity (Fig. 5).

Inhibitory Activity of Crocin for PC-12 Cell Death Induced by Serum/Glucose Deprivation

Cells cultured in serum/glucose-containing Dulbecco's modified Eagle's medium [DMEM (+)] had a normal morphology at 24 h, while those cultured in the serum- and glucose-free medium [DMEM (−)] for 24 h were round in shape and showed

the characteristic properties of necrotic and/or apoptotic cells. We confirmed that approximately 60 % cell death had occurred in the latter culture c exclusion method. The addition of crocin (10 μM) significantly suppressed both the morphological changes and the PC-12 cell death induced by the DMEM (−) conditions as crocin inhibited TNF-a-induced PC-12 cell death (Oppenheim 1991), resulting in 85 % survival. It is well known that serum or nerve growth factor (NGF) (Batistatou and Green 1991; Rukenstein et al. 1991; Mesner et al. 1992; Pittman et al. 1993) deprivation induces apoptosis in PC-12 cells. Colombaioni et al. (2002) demonstrated that serum deprivation increased the intracellular ceramide levels in undifferentiated HN9.10e cells, resulting in apoptosis. These findings easily suggest a possibility that ceramide levels increase in PC-12 cells under DMEM (−) conditions. PC-12 cells cultured for 3 h in DMEM (−) showed a significant increase (3.5-fold increase) in the level of ceramide compared to the basal level in cells cultured in DMEM (+) conditions. The suppressive effect of crocin was dose dependent. We also tested the effect of fumonisin B1 (FB1), which inhibits de novo ceramide synthesis in cells at a concentration of 10–30 μM (Wang et al. 1991; Merrill et al. 1993). However, FB1 had no significant effect on ceramide levels, suggesting that the accumulation of ceramide through an enhancement of de novo synthesis following a 3-h culture in DMEM (−) was in itself not sufficient to explain the increase. It has been suggested that the sphingomyelin (SM) pathway and SAPK/JNK signaling systems may function together (Verheij et al. 1996) in stress-induced apoptosis of U937 cells and BAE cells. Since the environmental stress under DMEM (−) conditions may activate the stress-activated protein kinase (SAPK)/JNK cascade in PC-12 cells, we compared the amounts of phosphorylated JNK in the cells cultured in DMEM (+) and DMEM (−) for 6 h. The DMEM (−) conditions stimulated the phosphorylation of JNK in the cells by approximately 3.7-fold relative to the control cells.

Inhibitory Effect of Crocin on the Activation of N-SMase Induced in Serum/Glucose-Deprived PC-12 Cells

In order to confirm the resource of the accumulated ceramide, we measured the activity of magnesium-dependent N-SMase in the PC-12 cell homogenate. N-SMase activity in cells cultured in DMEM (−) reached a maximum at 1 h and decreased to around the level of the control cells at 3 h. However, there was no time-dependent change in the N-SMase activity during a 3-h culture in DMEM (−). This assay method can detect N-SMase activity in these supernatants by substitution of the reaction medium for 50 mM sodium acetate buffer (pH 5.6). The results demonstrated that the activity of N-SMase in PC-12 cells was unaffected by serum/glucose deprivation for at least for 3 h. The addition of crocin in the culture medium suppressed the enzyme activities at 1 and 2 h in a dose-dependent manner. To determine

whether or not the inhibition of N-SMase is a direct action of crocin on the enzyme, we added crocin to the reaction medium, whose cells had been cultured in DMEM (−) for 2 h. The addition of 1 or 10 μM crocin had no inhibitory effect on N-SMase activity in the reaction medium. However, the addition of GSH at concentrations of 1 and 10 mM inhibited the enzyme activity dose dependently. Earlier reports indicate that GSH is a physiological inhibitor of magnesium-dependent N-SMase in plasma membranes (Yoshimura et al. 1998, 1999; Liu and Hannun 1997). N-SMase is inactive in the presence of physiological concentrations (1–20 mM) of GSH. Therefore, these results suggest that the N-SMase activity in the reaction medium is derived from magnesium-dependent N-SMase contained in plasma membranes and that the observed N-SMase inhibition by crocin does not occur through its direct action on the enzyme.

Increase of Intracellular GSH Levels in Serum/Glucose-Deprived PC-12 Cells Through Activation of GR and c-GCS by Crocin

In an investigation aimed at testing the above-mentioned hypothesis, we examined the effect of crocin on intracellular GSH levels in serum/glucose-deprived PC-12 cells. The GSH levels in PC-12 cells exposed for 3 h to serum/glucose-free DMEM decreased to half than that found in the control cells and thereafter remained constant. However, the addition of crocin to the medium increased the intracellular GSH level dose dependently, maintaining it at the 3-h time point at a higher level. The most significant effect of crocin occurred at a concentration of 10 μM. The concentration of GSH was high enough to inactivate N-SMase. We then investigated the mechanism by which crocin increased the GSH levels. The GR activities in serum/glucose-deprived PC-12 cells decreased in a time-dependent fashion, whereas the copresence of 10 μM crocin enhanced GR activity each hour (approximately fourfold elevation at 6 h). This result indicates that crocin has no significant effect on the GPx activity in the cells. GSH synthesis is regulated by the rate-limiting enzyme *c*-GCS. This enzyme is thought to be regulated by several mechanisms. In mouse endothelial cells, the TNF-a- or IL-1b-induced increase in *c*-GCS activity is associated with an increase in mRNA expression (Urata et al. 1996). IL-6 also stimulates the expression of *c*-GCS mRNA and increases the activity of this enzyme, which leads to increased GSH levels in PC-12 cells. In contrast, Nakajima et al. (2002) reported that NGF had an ability to increase the activity of *c*-GCS at the transcription level by extending the half-life of *c*-GCS mRNA. The addition of crocin (10 μM) doubled *c*-GCS mRNA expression in PC-12 cells in serum/glucose-free DMEM, while it had no effect on the mRNA levels of the control PC-12 cells. The crocin-induced increase in *c*-GCS mRNA expression is reflected in an increase in the activity of this enzyme in the cells. These results suggest that crocin can increase GSH levels by increasing the 1 h DMEM.

Neuroprotective Activity of Crocin In Vivo

Inhibitory Activity of Crocin for Infarcted Areas Caused by Occlusion of the Middle Cerebral Artery in Mice Brain

We investigated the effect of crocin on an infarcted area caused by occlusion of the middle cerebral artery (MCA) in mice. Crocin was administrated immediately before and 3 h after the MCA occlusion. The administration of crocin (10 mg/kg) reduced the infarct volume significantly resulting in half compared to vehicle as indicated in Fig. 3. Recently Vakili et al. reported the protective effect of crocin against cerebral ischemia in a dose-dependent manner using a rat model (Vakili et al. 2014). These data had a good correlation with our data although the model animals were different. Furthermore, it has been reported that a ginseng saponin, ginsenoside Rb1, also has the protective effects against ischemic hippocampal neurons (Lim et al. 1997) although these structures are completely different from each other having strong antioxidant activities. These evidences suggest that it may be possible to search and find out new active compounds against infarct induced by occlusion of the MCA from natural products reaching to preventive medicine because the infarct patients are 260,000 in Japan and quickly increasing (Fig. 6).

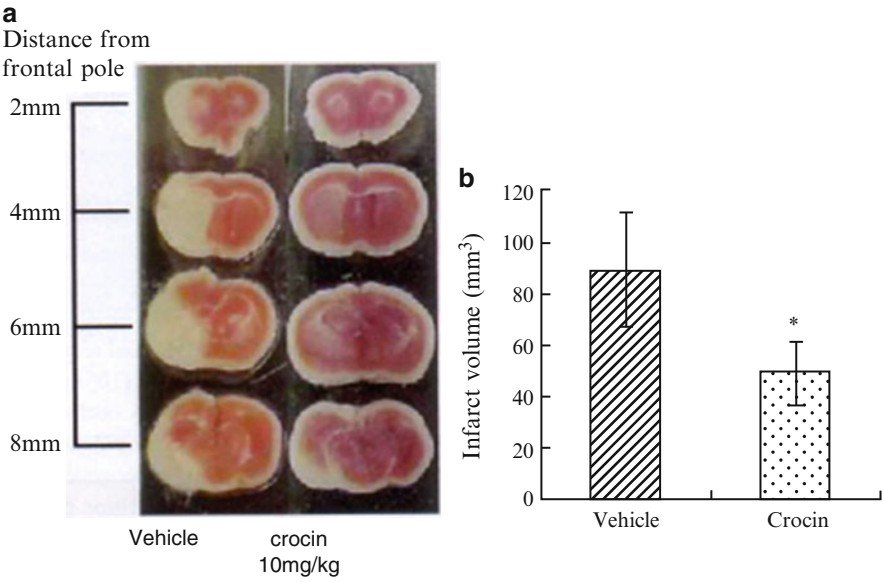

Fig. 6 The effects of crocin on the infarct volume induced by the middle cerebral artery occlusion in mice. Crocin was injected i.v. immediately before and 3 h after the middle cerebral artery occlusion. Slices were immediately stained with 2 % 2,3,5-triphenyltetrazolium chloride. (**a**) Representative infarcted area caused in each group of mice. (**b**) Each bar represents the mean ± S.E. of five to seven mouse brains

Learning and Memory by Saffron Extract and Crocin

Although the saffron extract had no effect on memory registration in normal mice, the crude extract of saffron prevents the ethanol-induced impairment of memory acquisition in step-through (ST) and step-down (SD) tests (Sugiura et al. 1995d). On the basis of these results, it is suggested that some components of saffron are capable of antagonizing the blocking effect of ethanol for memory acquisition. Activity-guided fractionation of the crude extract revealed that crocin is actual active component in saffron.

Single oral administration of crocin had no effect on mice in passive avoidance tasks. Oral administration of 30 % ethanol induced an impairment in memory acquisition in ST and SD tests. However, the subsequent oral administration of crocin (50 mg/kg) improved the impairment of memory acquisition in both tests in a dose-dependent manner (Sugiura et al. 1996; Zhang et al. 1994b).

Effect of Saffron and Crocin on LTP

We already discussed that the saffron crude extract and crocin can improve the blocking effect of ethanol on learning and memory dose dependently. In order to continue the story, the saffron extract was injected intracerebroventricularly, and the blocking effect of ethanol on the LTP decreased dose dependently. These results led us to hypothesize that crocin might antagonize the blocking effect of ethanol on the induction of LTP. Following the activity-guided separation from the crude extract, we confirmed that crocin is the actual active component in saffron. When a 50 mg/kg dose of crocin was injected 5 min before the administration of ethanol, LTP was induced at 84 % than that of control, suggesting that the LTP-blocking effect of ethanol was improved dose dependently with the administration of crocin (Sugiura et al. 1995a, b, c). The activities of the crocetin gentiobiose glucose ester and crocetin di-glucose ester, which are analogs of crocin on the LTP-blocking effect of ethanol, were investigated at the same dose scale. These activities were found to be distinctly lower than that of crocin. The active improvement effect against blocking was clearly proportional to the number of glucoses because crocin, which possesses four glucoses in a molecule, showed the highest improvement effect, while the activity of crocetin di-glucose ester was almost the same as the control (Sugiura et al. 1995b).

Sleep-Promoting Effect of Crocin

It is known that saffron with traditional Chinese medicines (TCM) and/or Japanese Kampo medicine promotes the sleep activity in the field of therapy of mental disorders and is used for sleep promotion as a folk medicine in Japan. From these evidences we

started to search the activity of saffron and its component, crocin. We examined the sleep-promoting effect of crocin on mice after an intraperitoneal administration at 20:00 during the wake period. Figure 7a shows time courses of the hourly amounts of non-REM sleep after the administration of vehicle or crocin (100 mg/kg). During the period of 20:00 to 01:00, mice with vehicle treatment spent more time in wake than in sleep. When 100 mg/kg of crocin was injected on the experimental day, the amount of non-REM sleep was increased immediately after the injection, and the effect was statistically significant from 2 to 4 h after the administration. Crocin did not change the REM sleep after the administration (Masaki et al. 2012). This augmentation effect on non-REM sleep time was accompanied by reduction in wakefulness. The increase in non-REM sleep and decrease in wake lasted more than 4 h after the injection. There was no further disruption of the sleep architecture during the subsequent period (8:00 to 20:00). These data indicated that crocin induces non-REM sleep without occurrence of adverse effects, such as rebound insomnia after the sleep induction. Similar time-course profiles were observed with a low dose of 10 mg/kg, but the effect on sleep was small and lasted only about 1–2 h after the injection.

We calculated the total time spent in non-REM and REM sleep and wakefulness for 4 h after the crocin injection (Fig. 7b). Crocin at 10 mg/kg did not affect the cumulative amounts of non-REM and REM sleep and wakefulness for 4 h after injection. Crocin given at 30 and 100 mg/kg statistically significantly increased the total amount of non-REM sleep by 160 % and 270 %, respectively, and

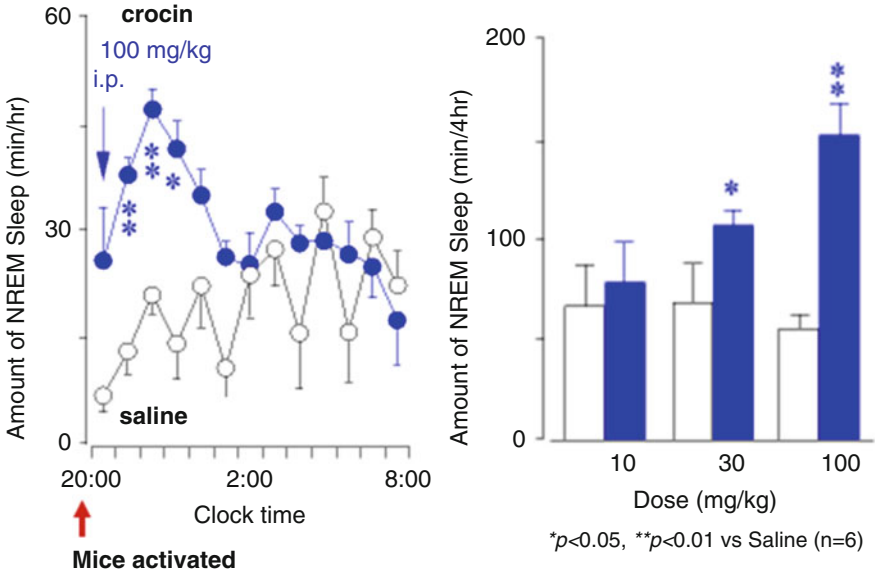

Fig. 7 Increase in non-REM sleep by crocin. (**a**) Time courses of non-REM sleep after an intraperitoneal administration of vehicle (*open circle*) or crocin (*filled circle*) at a dose of 100 mg/kg. (**b**) Total time spent in non-REM sleep for 4 h after the administration of vehicle (*open column*) or crocin (*closed column*) at a dose of 10, 30, or 100 mg/kg, respectively

decreased the total amount of wakefulness by 20 % and 50 %, respectively, without changing the amount of REM sleep during a 4-h period as compared with the vehicle control.

Conclusion

Since we do not have preventive medicine in Japan, some preventive natural products are strongly required instead of medicines. There are thousands of health food in Japan; however safeties against many of them are not clear enough. From these circumstances Consumer Affair Agency, Government of Japan, started a new system, Functional Labeling System on health foods, from April 2015. This system is basically focusing to confirm the safety and to make sure the real function on health food. Regarding safety of functional food, the most important point is how much longer the eating experiences and nothing of toxicity of major constituents contained in the functional food. From these viewpoints we selected saffron because saffron has been used from approximately 3000 years ago as a spice and coloring and a medicine as listed in materia medica 2000 years ago. In fact saffron is known well as safety food because oral administration of saffron extract at concentrations of up to 5 g/kg is still nontoxic in mice (Abdullaev 2002). Therefore, we have been searching the neuroprotective activities in saffron in this chapter.

The main constituent in saffron, crocin, increases the intracellular glutathione level and prevents PC-12 cell death in serum deprived by its antioxidant property. The antioxidant capacities of crocin have been reported in association with a variety of neuroprotective potentials. In PC-12 cells, a cell culture model for brain ischemia, the generation of ROS activates neutral SMase to generate ceramide, which induces cell death. Glutathione directly inhibits the activation of the SMase. Therefore, we hypothesized that crocin might prevent the activation of N-SMase in serum/glucose-deprived PC-12 cells by a GSH-dependent inhibition mechanism. Ceramide releases and activates the caspase family as discussed already. More recently we reported the inhibitory effects of crocin on Bcl-2, Bax, and caspase-3 expression of PC-12 cells injured by H_2O_2 (Cui et al. 2015). Moreover, we found the protective effect of crocin on an infarcted area induced by MCA occlusion of the middle cerebral artery in mice. This evidence also helps the brain health care by saffron and/or crocin.

We reported that crocin is the actual active component involved both in the improvement of learning and memory and with the preventive effect of LTP blocked by ethanol in vivo although oral administrations of saffron and/or crocin had no effect on memory acquisition in normal mice. Recently Naghibi et al. investigated the effect of saffron extract on morphine-induced memory impairment and concluded that the saffron extract attenuated morphine-induced memory impairment (Naghibi et al. 2012). We further demonstrated for the first time that crocin selectively antagonizes the inhibitory effect of ethanol on *N*-methyl-D-aspartate (NMDA)-receptor-mediated responses in hippocampal neurons (Abe et al. 1998). The sugar numbers in crocetin glycosides reflected the LTP-blocking activity by ethanol like that of crocin having four glucoses in a molecule attenuated

strongest compared to the other smaller molecules. This tendency is a good agreement with the previous reports that the sugar residues are important for the activities of some drugs like cardiac steroids (Shimada et al. 1986), streptozotocin (Gunnarsson et al. 1974), ginsenosides (Takemoto et al. 1984), and so on.

Twenty-five percent of population have some sleeping problems in Japan resulting in brain diseases. Saffron has been widely prescribed with Japanese Kampo medicine and/or TCM for mental disorder patient having sleeping problem in Japan. Therefore, crocin was tested for sleep promotion, and we found crocin increased the total time of non-REM sleeping. This finding promotes the clinical use of saffron since saffron has been used as safety food from thousands ago. Moreover, crocin was approved by the State Food and Drug Administration for the clinical trial in 2006 and now a drug for angina in China. From various kinds of phenomena described in this review, saffron and crocin have a multifunctional natural product in the brain.

Compliance with Ethics Requirements The authors declare that they have no conflicts of interest.

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The Advances in Neurobiology

M. Ishwarya and R.T. Narendhirakannan

Abstract Neurological disorders are diseases of the brain, the spine, and the nerves that connect them. There are more than 600 diseases of the nervous system, such as brain tumors, epilepsy, Parkinson's disease, and stroke as well as less familiar ones such as frontotemporal dementia, Alzheimer's disease, and other dementias; cerebrovascular diseases including stroke, migraine, and other headache disorders; multiple sclerosis; neuroinfections; brain tumors; traumatic disorders of the nervous system such as brain trauma; and neurological disorders as a result of malnutrition. More than a decade of research worldwide has shown that berries support cognitive health by protecting nerves and help brain cells communicate with each other and improve the flexibility of nerve structures. Berries help nerves tolerate stress, including the stress of toxic exposure. They also support the healthy function of glial cells in the brain, essential for optimum brain function. Polyphenols, namely, anthocyanins, found in berries may slow cognitive decline through antioxidant and anti-inflammatory properties in experimental animals. Based on the previous reports, this review explains the beneficial effects of the phytochemicals present in nine varieties of berries on neurodegenerative diseases.

Keywords Berries • Neurodegenerative diseases • Neuroprotective • Natural products

Introduction

Neurological disorders are diseases of the central and peripheral nervous system (the brain, spine, and nerves that connect them). There are more than 600 diseases of the nervous system. These disorders include epilepsy; Alzheimer's disease and other dementias; cerebrovascular diseases including stroke, migraine, and other

M. Ishwarya • R.T. Narendhirakannan, Ph.D., F.I.S.C.A., D.C.S. (✉)
Department of Biotechnology, School of Biotechnology and Health Sciences,
(Karunya Institute of Technology and Sciences) Karunya University,
Karunya Nagar, Coimbatore 641 114, Tamil Nadu, India
e-mail: bionaren_phd@yahoo.co.in

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headache disorders; multiple sclerosis; Parkinson's disease; neuroinfections; brain tumors; traumatic disorders of the nervous system such as brain trauma; and many other neurological disorders.

Hundreds million people worldwide are affected by neurological disorders. Approximately 6.2 million people die because of stroke each year; over 80 % of deaths take place in low- and middle-income countries. More than 50 million people have epilepsy worldwide. It is estimated that there are globally 35.6 million people with dementia with 7.7 million new cases every year—Alzheimer's disease is the most common cause of dementia and may contribute to 60–70 % of cases. The prevalence of migraine is more than 10 % worldwide (WHO 2014).

Physical symptoms of neurological disorders that may sustain for a short-term or long-term duration include partial or complete paralysis, muscle weakness, loss of sensation, seizures, difficulty in vocabulary, poor cognitive abilities, and lack of alertness (Psychguides.com).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) play an important role in neurodegenerative disorders by oxidizing the macromolecules like proteins, DNA, and lipids leading to the common final pathway for cell death (Sohal et al. 1995). According to the study conducted by Andersen (2004) and Ramassamy (2006), it is evident that there was an increased level of ROS markers observed in tissues from patients with neurodegenerative disorders.

The excessive production of reactive oxygen species, nitric oxide, and tumor necrosis factors may initiate neuronal apoptosis (Iadecola et al. 1996; Chan 2001) after cerebral ischemia-reperfusion injury (White et al. 2000; Sugawara et al. 2014; Abas et al. 2010). Oxidative damage has been found in all classes of organic molecules that are critical for maintaining neuronal structure and function. Excessive lipid, protein, and DNA peroxidation have all been studied in neurodegenerative disorders (Smith et al. 2000).

There are more than 600 neurological disorders that strike millions of people each year. These diseases and disorders inflict great pain and suffering for millions of patients and their families.

Key Treatments Available for Neurodegenerative Diseases

Hybrid PET/MRI testing Brain mapping Cyber Knife Stem cell therapy (Lindvall and Kokaia 2006) Deep brain stimulationGamma Knife Parkinson's disease or epilepsy has shown a considerable effect in the treatment of attention deficit hyperactivity disorder (ADHD), anxiety and other mood disorders, and a range of primary psychogenic impairments (Tarazi et al. 2014; Fasano and Lozano 2015).

Epigenetics and Neurodisorders

The epigenetic changes in various neurological diseases, the first issue to consider is whether the normal course of maturation and aging is associated with changes in the brain's epigenome.

Age-related epigenetic drifts could impact vulnerability to neurodegenerative disease. For example, in the mouse cerebellum, the levels of the mC5 derivative, 5-hydroxymethyl-cytosine (5hmC), are subject to a tenfold increase from postnatal week 1 to adulthood. Notably, among the genes that are affected by increasing 5hmC amounts at their promoters during cerebellar maturation, pathways for age-related neurodegenerative diseases and angiogenesis were overrepresented and included at least 15 genes linked to hereditary forms of spinocerebellar ataxia, a neurological syndrome defined by severe motor dysfunction with the degeneration of cerebellar Purkinje neurons and other systems (Szulwach et al. 2011).

Drawbacks of Neurological Drugs

Many drugs can cause confusional states, including amphetamines, anticonvulsants, antidepressants, antituberculous drugs, antimalarials, anti-inflammatories, cardiac glycosides, diuretics, hypotensive agents, H2 antagonists, neuroleptics, opiates, sympathomimetics, and sedatives. Agitation and confusion may be part of a withdrawal syndrome from addiction of drugs or alcohol. Central neurotoxicity can result from chemotherapy (particularly methotrexate, cytarabine, and ifosfamide used in the treatment of acute leukemias) ranging from minor cognitive impairment to encephalopathy (Verstappen et al. 2003). Confusion, cognitive impairment, and hallucinations are manifestations of relatively reduced cholinergic activity. Antiparkinsonian medications, particularly anticholinergics and dopamine agonists, may induce such adverse effects that necessitate dose reduction although discontinuation is often required. Psychosis occurs more rarely. Ataxia, dysarthria, and nystagmus can be a consequence of phenytoin toxicity. Akathisia (restlessness) may be induced by antidepressants, antipsychotics, antihistamines, calcium channel blockers, carbamazepine, or metoclopramide (Grosset and Grosset 2004).

Neuroprotective Medicine from Natural Source

It is widely accepted that a healthy diet is an important factor in reducing the risk of several diseases including cardiovascular diseases (Willcox et al. 2003) and certain cancers (Giovannucci 1999) and development of age-related neurodegenerative diseases (Lau et al. 2005), as has been demonstrated in a large number of epidemiological studies. Vegetables and fruits are of great importance in the human diet as they provide a major source of bioactive substances. Polyphenols, ubiquitously present in them, have been considered as the major responsible elements for the beneficial effect observed (Arts and Hollman 2005). Higher consumption of components/nutrients with antioxidant capabilities has been associated with lower frequency of numerous human morbidities or mortalities as per many epidemiological studies (Bhandari and Kamdod 2012).

Role of Berries

Considering the limits of existing prevention methods, intervention strategies using antioxidant and flavonoid-rich natural products such as fruits, vegetables, and nuts are of paramount importance (Essa et al. 2012). Considerable research has been directed at the potential health benefits of eating berries. As well as being a good source of vitamin C, dietary fiber, and minerals, berries contain high levels of natural polyphenol components that act as potent antioxidants. Berry extracts, rich in polyphenols, have a range of biological effects that can have beneficial outcomes on human health (Battino et al. 2009).

Active Compounds in Berries

1	Tomato (*Solanum lycopersicum*)	Carotenes, phenolic compounds	Hertog et al. (1992), Clifford (1999), Khachik et al. (1995), Clinton (1998), Nguyen and Schwartz (1999)
2	Gooseberry (*Phyllanthus emblica*)	Ascorbic acid, tannoids (emblicanin A and B, punigluconin)	Vasudevan and Parle (2007)
3	Grape (*Vitis vinifera*)	Resveratrol, anthocyanins, and other phenolics, Flava-3-nols (i.e., catechins), ellagic acid, myricetin, quercetin, kaempferol, and trans-resveratrol	Cantos et al. (2002), Pastrana-Bonilla et al. (2003)
4	Berberry (*Berberis vulgaris*)	Isoquinoline alkaloid	Host'álková et al. (2013), Singh et al. (2015)
5	Strawberry (*Fragaria ananassa*)	Flavanoids like anthocyanins, flavanols, flavonols, and phenolic acids, such as hydroxybenzoic acid and hydroxycinnamic acid, ellagic acid, ellagic acid glycosides, and ellagitannins	Giampieri et al. (2012), Aaby et al. (2005)
6	Blackberry (*Rubus laciniatus*)	Vitamin C and vitamin K, polyphenols, flavonoids, anthocyanins, salicylic acid, ellagic acid, and fiber	Zia-Ul-Haq et al. (2014), Sellappan et al. (2002)
7	Raspberry (*Rubus idaeus*)	Anthocyanin pigments, ellagic acid (from ellagitannins, see, for instance, the polyphenol ellagitannin), quercetin, gallic acid, cyanidins, pelargonidins, catechins, kaempferol, and salicylic acid	International Berry Health Benefits Symposium

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8	Apple (*Malus domestica*)	Flava-3-nols (e.g., catechin, procyanidins), hydroxycinnamates (e.g., chlorogenic acid, caffeic acid, coumaroylquinic acid), flavonols (e.g., quercetin conjugates, dihydrochalcones, anthocyanins)	Kahle et al. (2005), Gerhauser (2008), Vrhovsek et al. (2004)
9	Goji berry (*Lycium barbarum*)	Ascorbic acid, beta-carotene, polysaccharides, lutein, zeaxanthin, B and E vitamins, minerals	Teng et al. (2013)

Tomato

The oral supplements of tomato seed aqueous extract significantly reduce rotenone-induced oxidative impairments and damage to the dopamine system. It is likely that acetylcholinesterase inhibition by seed extract may play a significant protective role in inhibiting rotenone-mediated apoptosis resulting in protection of dopaminergic neurons from demise in this model (Gokul and Muralidhara 2014).

Tomatine, a known steroidal glycoalkaloid, was extracted from the Chinese tomato *Solanum cathayanum*. The pretreatment with tomatine inhibits the release of cellular lactate dehydrogenase and increases SOD, CAT, and GPx activity and GSH content; it also reverses the downregulated protein expression of the brain derived neurotrophic factor (BDNF) and inhibits expression of Bax and activations of caspase-3 and caspase-9 in hydrogen peroxide-induced SH-SY5Y (human neuroblastoma) cells (Huang et al 2014).

Lycopersicon esculentum Mill. (tomatine and tomatidine) and the extracts from tomato leaves showed AChE and BChE inhibition capacity. Additionally, compounds/extracts revealed neuroprotective effects on glutamate-induced toxicity in SH-SY5Y neuroblastoma cells, without gastrointestinal toxicity, by preserving the mitochondrial membrane potential and reducing oxidative species (Taveira et al. 2014).

Di Matteo et al. (2009) proved that that repeated intake of a transgenic tomato fruit rich in beta-carotene will increase striatal dopamine and 3,4-dihydroxyphenylacetic acid levels and that the tomato-enriched diet exerted a positive effect against 6-hydroxydopamine-induced nigrostriatal lesions (Parkinson's disease) in rats. The beneficial effect of tomato is most likely due to the great lycopene content of the RS-enriched diet.

Tomato contains various agents that attenuate the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurodegenerative disease in mice (Suganuma et al. 2002).

Gooseberry

The anwala (amla) churna proved to reduce the brain cholinesterase activity and total cholesterol levels in a study conducted by Vasudevan and Parle (2007). Anwala churna may be confirmed as a useful remedy for the management of Alzheimer's

disease on account of its multifarious beneficial effects such as memory-improving property, cholesterol-lowering property, and anticholinesterase activity (Dasaroju and Gottumukkala 2014).

The tannoids of *Emblica officinalis* exert a protective effect against neuroleptic-induced tardive dyskinesia that is likely to be due to its earlier reported antioxidant effects in rat brain areas, together with the striatum (Bhattachary et al. 2000). The aqueous extract of *E. officinalis* showed antidepressant-like activity probably by inhibiting monoamine oxidase A and gamma-aminobutyric acid, along with its antioxidant property (Dhingra et al. 2012).

The solvent extracts of *E. officinalis* completely eliminated the generalized tonic seizures and also improved the retention latency in the passive avoidance task. In a dose-dependent manner, it also ameliorated the oxidative stress induced by pentylenetetrazol. These findings suggest the potential of hydroalcoholic extract of amla to be used as an adjuvant for treatment with antiepileptic drugs (Golechha et al. 2010).

Amla churna produced a dose-dependent improvement in memory of young and aged rats. It reversed the amnesia induced by scopolamine and diazepam. It may prove to be a useful remedy for the management of Alzheimer's disease due to its multifarious beneficial effects such as memory improvement and reversal of memory deficits (Vasudevan and Parle 2007).

Grapes

Grapes are one of the oldest cultivated plants. They are classified as true berries because the fruit wall or pericarp is fleshy all the way through. As results obtained from the study conducted by Balu et al. (2006), administration of grape seed extract for 30 days significantly reduced the level of DNA-protein cross-links in aged rats when compared to aged control rats. They revealed that grape seed extract has an inhibiting effect on the accumulation of age-related oxidative DNA damages in the spinal cord and in various brain regions such as the cerebral cortex, striatum, and hippocampus.

Grape seed extract attenuated the formation of oxygen free radicals, as measured by 8-isoprostaglandin F2 alpha and thiobarbituric acid-reacting substances in the hypoxic ischemic rat pup model. Grape seed extract improved the histopathologic brain score in the cortex, hippocampus, and thalamus (Feng et al. 2005).

Grape, which is easily available as juice form in the market, contains many flavonoids, polyphenols, and proanthocyanidins with antioxidant properties (Bagchi et al. 2000). Moreover, it has been reported that resveratrol, a polyphenolic antioxidant, is present in red wine and an active component in terms of the neuroprotective effect during cerebral ischemic injury (Huang et al. 2001; Wang et al. 2002).

GSE protected the delayed neuronal death of CA1 pyramidal cells by inhibiting oxidative DNA damage following transient forebrain ischemia (Hwang et al. 2004).

Rodrigues et al. (2012) demonstrated that both organic and conventional grape juices show important neuroprotective effects against pentylenetetrazol-induced oxidative damage in rats. This effect could be important in reducing neuronal

damage and, therefore, allow for a better quality of life for epileptic patients. Both juice types were able to protect from lipid and protein oxidative damage, decrease nitric oxide content, and increase enzymatic (superoxide dismutase and catalase) and nonenzymatic (sulfhydryl protein) antioxidant defenses in brain tissues.

Berberry

Berberine is an isoquinoline alkaloid that is found in some plants principally *Berberis*. It has some beneficial effect on anxiety, nociception, inflammation, psychosis, depression, and amnesia (Imanshahidi and Hosseinzadeh 2008; Kulkarni and Dhir 2008, 2010).

Mojarad and Roghani (2014) reported that berberine reduces NMDA receptor binding and inhibits NMDA receptor channel current in the brain. In addition, berberine protects neuronal cells from brain ischemia such as NMDA receptor antagonists (Cui et al. 2009).

Yoo et al. (2006) observed that the extract of berberine has protective effects against ischemic damage after ischemia/reperfusion. They investigated the chronological changes of NR1 and NR2A/B immunoreactivity in the hippocampal CA1 region early time after ischemia/reperfusion. Berberine pretreatment could attenuate spontaneous recurrent seizures. Since administration of berberine has decreased lipid peroxidation in kainite rats, it seems that the berberine favorable effect is due to its effectiveness in lessening of oxidative stress in rat. Their study indicates that berberine extract confers neuroprotection against transient ischemic brain injury through a mechanism that involves the reduction of NR1 expression.

Berberine has been shown to protect against ischemic brain injury by decreasing intracellular reactive oxygen species levels and subsequently inhibiting the mitochondrial apoptotic pathway (Zhou et al. 2008). Berberine also prevents changes in oxidative stress and choline esterase activity and consequently can improve the memory impairment seen in streptozotocin-induced diabetic rats (Bhutada et al. 2011).

In a study by Kim et al. (2014), ischemia showed increased glial fibrillary acidic protein (GFAP) and CD11b expression in the hippocampal CA1 region. On oral administration of berberine extracts, they observed suppression in ischemia-induced increments of GFAP and CD11b expression, showing that berberine can attenuate ischemic injury by inhibiting reactive astrogliosis and microglia activation.

Strawberry

A neurodegeneration cell model was used to evaluate the neuroprotective effect of the strawberry tree phenolics. The total phenolic content was estimated to be at 16.46 ± 3.66 (mg GAE g^{-1} dew). The neuroblastoma cell line SK-N-MC was subjected to an oxidative stress after preincubation with the fruit extracts (Fortalezas et al. 2010).

Heo and Lee (2005) performed the cell viability test using the 3-(4,5-dimethylth iazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay which showed that strawberry phenolics significantly reduced oxidative stress-induced neurotoxicity, because oxidative stress is also known to increase neuronal cell membrane breakdown. The protective effects appeared to be due to the higher phenolic contents, including anthocyanins as a major part.

Blackberry

Tavares et al. (2012) assessed the efficacy of blackberry polyphenolics and metabolites in a neurodegeneration cell model before and after simulated gastrointestinal digestion. It enhanced GSH levels and reduced ROS production. The digested polyphenol metabolites were able to maintain cell membrane integrity, protecting neurons from death.

The wild blackberries, *Rubus brigantinus* and *Rubus vagabundus*, presented neuroprotective effects in a study conducted by Tavares et al. (2013). Digested metabolites from these blackberries, at levels that could be found in human plasma, activated adaptive cellular stress response pathways such as caspase activation, GSH modulation, and also ROS diminishment. These effects protected neuronal cells against oxidative injury, one of the most important features of neurodegeneration.

Raspberry

Rubus coreanus, called as Korean black raspberry, belongs to the species of raspberry. The anthocyanins alleviated intracellular oxidative stress, as assayed by in vitro fluorescent measurements. The anthocyanins of *Rubus coreanus* showed neuroprotective effects on PC-12 cells in vitro against oxidative stress in a dose-dependent manner. Triple quadrupole LC/MS and Q-TOF LC/MS analyses revealed four major anthocyanins: cyanidin-3-*O*-sambubioside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-xylosylrutinoside, and cyanidin-3-*O*-rutinoside. The protective effect on neuronal cells in vitro may be associated with their considerable antioxidant capacity.

Wang et al. (2012) studied the neuroprotective effects and mechanisms of action of total saponins from *Rubus parvifolius* L. (TSRP) on focal cerebral ischemia and reperfusion injury in rats. The ratio of Bax to Bcl-2 increased during hypoxia-induced neuronal death, which supports the hypothesis that the balance in protein expression of these two crucial factors determines cell survival or death following an apoptotic stimulus. Total saponin extract of *Rubus parvifolius* L. protects against ischemia/reperfusion injury. It reduces the cerebral infarct volume; and it may do so by increasing Bcl-2 expression and decreasing Bax expression, consequently inhibiting apoptosis.

Apples

Apple skin contains approximately 46 % of the total phenolics (McGhie et al. 2005), and specific flavonoids such as quercetin glycosides and cyanidin-3-*O*-galactoside are not found in the flesh of apples. From the study carried out by Keddy et al. (2012), it was proved that a flavonoid-rich fraction A4F isolated from Spy apples tends to posses maximum neuroprotective effect. Oral dosing of minimum 25 mg/kg, once daily for 3 days, reduced neuronal cell loss in the dorsal hippocampus and striatum of mice subjected to a model of HI-induced brain damage. It even shows synergistic actions between different phenolic compounds in this fraction that interact with functionally distinct targets.

One of the standardized model of neurodegeneration in which aged mice exhibit impaired cognitive performance and increased oxidative parameters in the brain tissue when subjected to a prooxidant diet (deficient in vitamin E and folate). However, when these mice received apple juice concentrate diluted in drinking water (0.5 %) for 1 month, there was a significant improvement in cognitive-related performance and reduced prooxidative status compared to controls (Tchantchou et al. 2005).

Apple juice concentrate prevents the characteristic decline in acetylcholine associated with aging and oxidative stress (Chan et al. 2006). Because cholinergic depletion is associated with impaired memory and reduced cognitive performance and acetylcholine reduction, in particular, is associated with Alzheimer's disease, there is potential importance in the ability of apple juice to maintain levels of this neurotransmitter.

Apple juice concentrate may work by other mechanisms, including the ability to suppress overexpression of presenilin-1, which is linked to the production of amyloid b peptide, a hallmark of Alzheimer's disease (Chan and Shea 2006, 2009). Apple juice also attenuated the neurotoxicity of amyloid beta peptide in vitro (Chan and Shea 2007). They propose that the content of *S*-adenosylmethionine in apple juice concentrate might account in part for these effects, because comparable effects were observed with *S*-adenosylmethionine alone.

Goji Berry

Promising research on the goji berry (*Lycium barbarum*) has shown a positive correlation between consumption of the fruit and neuroprotective benefits that minimize the ravaging effects of Alzheimer's (Teng et al. 2013). A staple in traditional Chinese medicine (TCM), goji, is an exceptional superfood that safeguards the health of both body and mind.

Goji berry is packed with nutrients like ascorbic acid, beta-carotene, polysaccharides, lutein, zeaxanthin, and B and E vitamins along with trace minerals such as zinc, copper, calcium, and selenium.

A study at the University of Hong Kong found aqueous extracts of goji disrupt the neurotoxic qualities of proteins within the brain which are associated with

Alzheimer's disease. Preliminary research has discovered that goji berry guards against the formation of specific compounds typically found in the brains of Alzheimer's patients. Using a laboratory model of Alzheimer's, scientists found that goji protected brain cells from the harmful effects of amyloid beta peptides, damaging agents that are linked to the pathological changes seen in the brains of Alzheimer's patients. These findings suggest that goji just might help prevent this memory-robbing disease. They showed treatment with 600 μg/ml goji berry effectively suppressed the activation of microglia. Microglia defend the brain by destroying invading pathogens in the innate immune response of the CNS (Kreutzberg 1996).

The researchers believe goji berry extract may play a pivotal role in creating future treatments for Alzheimer's.

Conclusion

The neuroprotective actions of dietary foods involve a number of effects within the brain, including a potential to protect neurons against injury induced by neurotoxins, an ability to suppress neuroinflammation, and the potential to promote memory, learning, and cognitive function. This multiplicity of effects appears to be underpinned by two common processes. Firstly, they interact with important neuronal signaling cascades in the brain, leading to an inhibition of apoptosis triggered by neurotoxic species and to a promotion of neuronal survival and differentiation. These include selective actions on a number of protein kinase and lipid kinase signaling cascades, most notably the PI3 K/Akt and MAP kinase pathways which regulate pro-survival transcription factors and gene expression. It appears that the concentrations of flavonoids and phenolics encountered in the brain may be sufficiently high to exert such pharmacological activity of receptors, kinases, and transcription factors. Secondly, they are known to induce beneficial effects on the peripheral and cerebral vascular system, which lead to changes in cerebrovascular blood flow. These changes are likely to induce angiogenesis, new nerve cell growth in the hippocampus, and changes in neuronal morphology, all processes known to be important in maintaining optimal neuronal function and neurocognitive performance. The consumption of flavonoid-rich foods, such as berries and cocoa, throughout life holds a potential to limit neurodegeneration and prevent or reverse age-dependent deteriorations cognitive performances.

Berries are a natural source of food that helps to treat various diseases. On regular berry diet, many studies have been proven to show rich antioxidant property and improve the body regulation. A diet fortified with either blueberry or strawberry extract prevented the radiation-induced damage to the brain. Interestingly, strawberries had the most impact on spatial placement, whereas blueberries had the most impact on learning—indicating that various different berries may have different benefits to various brain regions in terms of protection. Numerous natural antioxidant/anti-inflammatory compounds found in plant food matrices, like fruits, especially berries (such as strawberry, blueberry, black currant, blackberry, blueberry, and mulberry), can offer neuroprotective effects (Essa et al. 2012).

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Brain Food at High Altitude

Vishal Jain

Abstract Scenic view at high altitude is a pleasure to the eyes, but it has some shortcoming effects as well. High altitude can be divided into different categories, i.e., high altitude (3000–5000 ft), very high altitude (5000–8000 ft), and extreme altitude (above 8000 ft). Much of the population resides at high altitude, and others go there for tourism. Military personnel are also posted there to defend boundaries. As we ascent to high altitude, partial pressure of oxygen reduces, whereas concentration remains the same; this reduces the availability of oxygen to different body parts. This pathophysiological condition is known as hypobaric hypoxia (HH) which leads to oxidative stress and further causes cognitive dysfunction in some cases. Hypoxia causes neurodegeneration in different brain regions; however, the hippocampus is found to be more prone in comparison to other brain regions. As the hippocampus is affected most, therefore, spatial memory is impaired most during such condition. This chapter will give a brief review of the damaging effect of high altitude on cognition and also throw light on possible herbal interventions at high altitude, which can improve cognitive performance as well as provide protection against the deteriorating effect of hypobaric hypoxia at high altitude.

Keywords High altitude • Hypobaric hypoxia • Cognition • Oxidative stress • Memory impairment • Brain food • Nutraceuticals

High Altitude

A significant portion of the world's geography lies above 10,000 ft elevation. Some of this high-altitude land is populated by native people who have adapted to this atmosphere. These native people have evolved mechanisms to manage with available atmospheric condition with their physiology and metabolism (Hoppeler and Vogt 2001). Large numbers of people travel to high altitudes in search of recreational activities, i.e., mountaineering, trekking, skiing, etc. Not only the deployment of military personnel to high-altitude areas, particularly in Asia as a part of security concern and war situation, but also regional conflicts in Kashmir and Afghanistan have become a focus of attention. Those who travel acutely to altitude

V. Jain (✉)
Vallabhbhai Patel Chest Institute, Delhi University, Delhi 110007, India
e-mail: vishal6784@gmail.com

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for short to moderate periods of time (e.g., skiers, trekkers, mountain climbers) or those who travel intermittently to altitude (e.g., highway construction workers, observatory workers, soldiers) typically experience the reduced performance level associated with hypoxia (Brooks et al. 1999; Hoppeler and Vogt 2001; Schoene 2001). Ascent to high altitude results in acute and chronic physiological changes that may also affect the nutritional requirements of individuals. Some, who travel too high too fast, may experience acute mountain sickness (AMS) (Roach and Hackett 2001). Physical training at altitude has been shown to eventually improve performance at altitude, but may not be an effective means of enhancing performance upon return to sea level (Bailey and Davies 1997). Additionally, exercise at altitude may exacerbate AMS (Roach et al. 2000). The added stress of exercise and hypobaric hypoxia may potentiate free radical-mediated oxidative tissue injury, which could exert both short- and long-term consequences for health and performance (Bailey and Davies 1997; Bailey et al. 2000a). Indeed, some investigators have suggested that oxidative stress and some of the cumulative effects of hypoxic exposure may persist for some time after return to sea level (Hornbein 2001; Joanny et al. 2001; Neubauer 2001).

Several environmental challenges are present at high altitude. Physiological stresses from hypoxia, cold, wind, UV rays from the sun, dehydration, and a lack of antioxidant nutrients in the diet lead to decreased physical and mental performance at high altitude (Huey and Eguskitza 2001). Significant physical exertion usually occurs at high altitude due to the exhausting activities needed at such extreme conditions. The reduced pressure of atmospheric oxygen limits energy generation during crucial requirements. The reduced partial pressure of oxygen at altitude has several consequences for the oxygen economy of the body. Much greater volume of air must be moved from the lungs to compensate for the effect reduced oxygen availability of the inspired air. The reduced barometric pressure at altitude reduces the ability of the oxygen to diffuse from the air to the blood across the alveolar–capillary membrane, thus accentuating the resultant hypoxia (Schoene 2001). Some consequences of high-altitude exposure are potentially more serious than a general impairment of physical performance capacity.

High-altitude physiology may be divided into the study of short-term changes that occur with exposure to high altitude and studies of longer-term acclimatization and adaptation. Acute exposure to the atmosphere at extreme altitude is rapidly fatal and causes many physiological damages (Ward 2003). These changes tend to reduce the gradient of oxygen partial pressure of ambient air to the tissues (classical oxygen cascade) (Fig. 1) and are distinct from the pathological changes that lead to altitude illness. On the other hand, during acclimatization lowland humans respond to a reduced-inspired partial pressure of oxygen as an adaptive mechanism. Adaptation to high altitude describes changes that have occurred over a number of generations as a result of natural selection in a hypoxic environment, and this can be observed in some groups of high-altitude residents. The physiological response to acute hypoxia environment at high altitude such as an increase in ventilation, cardiac output, and hemoglobin concentrations (hemoglobin concentration increases initially by the hemoconcentration and later as a result of increased erythropoiesis) helps to increase oxygen delivery to the tissues.

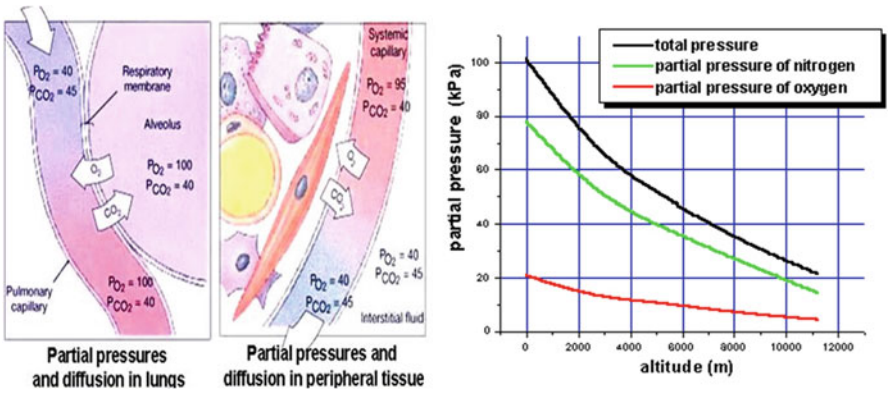

Fig. 1 Oxygen cascade at different altitudes (Courtesy: Heath and Williams 1977)

Hypobaric Hypoxia: Condition Explicit to High Altitude

The number of people traveling to the high-altitude regions, especially South America, Nepal, and India, has risen enormously in the past 10 years. These sojourners (trekkers, tourists, and especially troops) as well as people staying at high altitude (HA) such as local residents are exposed to hypobaric hypoxia. Ascent to high altitude leads to decreased partial pressure of oxygen in the atmosphere and resultant decrease in alveolar partial pressure of oxygen. This reduces oxygen diffusion to the pulmonary artery and causes a subsequent reduction in oxygen saturation in the capillaries. Hence, the oxygen delivery to peripheral tissues is decreased, leading to hypobaric hypoxia (Heath and Williams 1977). In brief it can be quoted that as human beings ascend to altitudes above sea level, changes in atmospheric pressure lead to decreased oxygen tension of inspired air, such that at 4500 m arterial oxygen tension is less than half (44 mmHg) what it is at sea level (94 mmHg). The concentration of oxygen in air remains constant, but, as the barometric pressure decreases, the partial pressure of oxygen decreases proportionately. This condition is referred to as hypobaric hypoxia.

The people who are exposed to hypobaric hypoxia are commonly confronted with mild problems such as acute mountain sickness (AMS), dizziness, nausea (Bahrke and Shukitt-Hale 1993), hypophagia (Singh and Selvamurthy 1993), and motor impairment (Hamilton et al. 1991) or severe problems such as HA pulmonary edema (HAPE) and HA cerebral edema (HACE) (Bailey and Davies 2001; Baumgartner et al. 2002; Chao et al. 1999). Further, many of them experience mental dysfunction and memory deficit. These problems become more severe due to lack of medical care at high altitude.

Hypobaric hypoxia (HH) is an adverse environmental condition that appears to influence several physiological parameters leading to a wide range of mild physiological disorders. It is one of the major problems encountered by people staying at

high altitude (HA). HA exposure is considered as an extreme physiological stress inducing wide range of deleterious effects at the cellular level. Previous findings pointed out that exposure to severe hypoxia could cause increased cellular oxidative stress with consequent damage to lipids, proteins, and DNA and lead to neurodegeneration (Dosek et al. 2007). Previously it was shown that the occurrence of oxidative stress in hypobaric hypoxia (Adcock et al. 2002) might play a key role in memory impairment (Maiti et al. 2006). It has a deleterious effect on higher brain functions such as memory and cognition (Kramer et al. 1993; Shukitt et al. 1998). Altered neurotransmitter synthesis, elevated level of corticosterone, glutamate excitotoxicity, altered calcium homeostasis, and reduced cholinergic transmission have also been implicated in hypoxia-induced memory loss (Hota et al. 2008; Barhwal et al. 2009; Muthuraju et al. 2010; Baitharu et al. 2012).

Hypobaric hypoxia is known to cause cognitive dysfunctions owing to the high oxygen dependency of the brain (Shukitt-Hale et al 1994). Cognitive and motor deficits have been reported to occur on chronic exposure to hypobaric hypoxia (Shukitt-Hale et al 1998). Both acute and chronic exposure to hypobaric hypoxia result in reduced psychomotor performance, learning abilities, mood disorders, and memory impairment (Hornbein et al. 1989; Nicolas et al. 1999; Bolmont et al. 2000; Li et al. 2000). A decline in visual and verbal long-term memory was also observed in mountaineers exposed to altitudes ranging from 5488 to 848 m for 1–30 days and in volunteers exposed to simulated altitude conditions (Hornbein et al. 1989). Impairment in learning abilities and spatial memory on exposure to altitudes above 5000 m has also been reported by several workers (Nelson and Gutmann 1982; Nelson et al. 1990; Cavaletti et al. 1990; Nicolas et al. 1999; Li et al. 2000). The physiological manifestations of hypobaric hypoxia are, however, altitude dependent and more pronounced on exposure to very high altitude, i.e., 12,000–18,000 ft (3658–5487 m), or extremely high altitude, i.e., above 18,000 ft (above 5487 m) (Shukitt-Hale et al. 1996). MRI studies of high-altitude sojourners showed the occurrence of loss of gray matter and atrophy in several brain regions along with shrinkage in the hippocampus indicating neuronal damage on exposure to high altitude (Shukitt-Hale et al. 1996).

Consequences of Hypobaric Hypoxia Exposure

A considerable body of literature exists documenting an increased production of indicators of oxidative stress in breath, blood, urine, and tissue of laboratory rats in response to hypoxia (Yoshikawa et al. 1982; Chang et al. 1989; Radák et al. 1994; Nakanishi et al. 1995; Mohanraj et al. 1998; Arteel et al. 1999; Risby et al. 1999; Hoshikawa et al. 2001; Ilavazhagan et al. 2001). Similar results have been found with humans exposed to hypoxia (Simon-Schnass and Pabst 1988; Rokitzki et al. 1994; Vasankari et al. 1997; Chao et al. 1999; Pfeiffer et al. 1999; Bailey et al. 2000b, 2001a, b; Joanny et al. 2001; Møller et al. 2001; Peltonen et al. 2001; Schmidt et al. 2002; Wing et al. 2002). A limited number of human hypobaric

hypoxia studies have been conducted investigating hypoxia and oxidative stress. These studies indicate that oxidative stress is increased under both hypobaric hypoxia (field) conditions (Simon-Schnass and Pabst 1988; Chao et al. 1999; Pfeiffer et al. 1999; Bailey et al. 2001a; Schmidt et al. 2002), as well as in a laboratory situated at altitude (Møller et al. 2001). Simulated altitude in a hypobaric chamber (Joanny et al. 2001) or normobaric-simulated altitude breathing a gas mixture containing reduced oxygen content (Wing et al. 2002) also points to increased oxidative stress as a function of hypoxic exposure separate from exercise. Møller et al. (2001) have posed the question: does exercise under hypoxic conditions exacerbate oxidative stress compared to either exercise or hypoxia exposure alone? Using a sensitive comet assay to detect DNA strand breaks, Møller et al. (2001) found that hypoxic exercise produces more DNA strand breaks than normoxic exercise. They concluded that hypoxia, in some manner, depletes the body's antioxidant capacity to withstand oxidative stress. Wing et al. (2002) observed a significant increase in serum lipid peroxides (LPOs) with only 60 min of simulated altitude hypoxic exposure at rest, an observation not well explained by an "acute depletion of antioxidants," but perhaps more indicative of acutely increased peroxidation of red blood cell lipids secondary to hypoxic exposure. Simon-Schnass and Pabst (1988) have shown that red blood cells become peroxidized at altitude, reducing their flexibility.

Memory at High Altitude

Hypobaric hypoxia is known to cause cognitive dysfunctions owing to the high oxygen dependency of the brain (Shukitt-Hale et al. 1994). Cognitive and motor deficits have been reported to occur on chronic exposure to hypobaric hypoxia (Shukitt-Hale et al. 1998). Both acute and chronic exposure to hypobaric hypoxia result in reduced psychomotor performance, learning abilities, mood disorders, and memory impairment (Hornbein et al. 1989; Nicolas et al. 1999; Bolmont et al. 2000; Li et al. 2000). A decline in visual and verbal long-term memory was also observed in mountaineers exposed to altitudes ranging from 5488 to 8848 m for 1–30 days and in volunteers exposed to simulated altitude conditions (Hornbein et al. 1989). Impairment in learning abilities and spatial memory on exposure to altitudes above 5000 m has also been reported by several workers (Nelson and Gutmann 1982; Nelson et al. 1990; Cavaletti et al. 1990; Nicolas et al. 1999; Li et al. 2000). The physiological manifestations of hypobaric hypoxia are, however, altitude dependent and more pronounced on exposure to very high altitude, i.e., 12,000–18,000 ft (3658–5487 m), or extremely high altitude, i.e., above 18,000 ft (above 5487 m) (Shukitt-Hale et al. 1996).

Exposure to simulated HA affects the CNS and results in several pathological changes including cerebral palsy, mental retardation, learning disability and memory impairment, epilepsy, and other neurophysiological disturbances such as insomnia, dizziness, alteration of mood, and psychomotor performance

(Rodway et al. 2003; Roach and Hackett 2001; Shukitt-Hale et al. 1996, 1991). It is assumed that chronic HA hypoxia (>5000 m) resulted in neuronal damage in the human brain (Shukitt-Hale et al. 1996) which persisted even after returning to sea level up to a year or longer (Cavaletti and Tredici 1992; Cavaletti et al. 1990; Hornbein et al. 1989), and the performance of human short-term memory is also decreased following exposure to acute, mild, and moderate hypoxia for 1 h at 4400 m (Shukitt-Hale et al. 1996). Exposure to HA at 4700 m for 5–7 h adversely affects the mental performances causing acute mountain sickness (Shukitt-Hale et al. 1991). Further a significant impairment of spatial memory in mountaineers has been reported at altitudes greater than 5000 m and persistent learning impairments up to 75 days following an HA ascent to 4000–5000 m reported by Cavaletti et al. (1990). A decline in both visual and verbal long-term memory was observed in mountaineers in exposed to HH for 1–30 days at altitudes ranging from 5488 to 8848 m and in volunteers exposed to a simulated altitude of the same range (Hornbein et al. 1989). Nelson et al. (1990) found learning impairment in intact retrieval function in a group of mountaineers returning to sea level from an altitude of 6000 m. Cognitive functions like learning and memory are adversely affected by exposure to hypobaric hypoxia (Cavaletti and Tredici 1992). In a similar situation, perinatal hypoxic–ischemic shock to the brain subsequently led to mental retardation and deficit in cognitive abilities such as learning and memory in the rats and human beings alike (Askew 2002; Arteni et al. 2003; Kumral et al. 2004). MRI studies of high-altitude sojourners showed the occurrence of loss of gray matter and atrophy in several brain regions along with shrinkage in the hippocampus indicating neuronal damage on exposure to high altitude (Shukitt-Hale et al. 1996). Learning and memory functions are an important attribute of the hippocampus (Cervós-Navarro and Diemer 1991; Pulsinelli 1985).

Though transient hypoxia induces morphological change and permanent neuronal damage in the rat's brain (Kirino 1982), the extent of hypoxic damage was a function of the degree and duration of exposure (Shukitt-Hale et al. 1996). It has been demonstrated that severe and chronic (N 5500 m, for 3–4 days) hypoxia/ischemia caused neuronal death in the deep and peripheral brain structures, like CA3, CA4, and dentate gyrus of the hippocampus, and the thalamus, cerebral cortex, and striatum (Freyaldenhoven et al. 1997; Gibson et al. 1981; Naghdi et al. 2003; Smith et al. 1993). Indeed, the deep brain hippocampal neurons were highly susceptible to hypoxic injury (Beal 1995; Cervós-Navarro and Diemer 1991; Choi 1996; Pulsinelli 1985). Cell death in CA1 subfield of the hippocampus has been associated with memory loss without any neurological or neuropathological presentation (Naghdi et al. 2003; Sinden et al. 1997). Transient forebrain ischemia for only 5 min was enough to cause irreversible damage to CA1 pyramidal neurons in the rat brain. However, damages were visible only after 4 days (Kirino 1982). The CA3 neuronal networks are thought to play crucial role in memory processes and in the generation of synchronous neuronal activities (Hasselmo and Wyble 1997; Lisman 1999; Lorincz and Buzsaki 2000; McNaughton and Morris 1987). It was shown that hypoxic ischemia resulted in apoptotic death of the neurons in the CA3, dentate gyrus, and lateral thalamus of the newborn rats (Nakajima et al. 2000).

From these reports, it is conceivable that degenerative changes in the hippocampus could lead to serious cognitive deficits (Maiti et al. 2006). Remedies against hypobaric hypoxia include several antioxidants, inhibitors/blockers against elevated glutamate excitotoxicity and corticosterone levels, but have several limitations; therefore, there is a need for some intervention with high-efficacy and less side effects.

Nutraceuticals for Cognition at High Altitude

Withania somnifera

Withania somnifera (Linn.) is a member of the plant family Solanaceae and is also known as ashwagandha, Indian ginseng, or winter cherry. It is an important component of the ayurvedic pharmacopoeia of India, where it has been used for hundreds of years (Henderson and Anderson 1966; Ahmad et al. 2005; Glaser 1988). The chemistry of *W. somnifera* has been extensively studied, and over 35 chemical constituents have been identified, extracted, and isolated. The biologically active chemical constituents are alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins), saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 (sitoindoside IX and X). *W. somnifera* is also rich in iron. The two most active withanolide components, withaferin A and withanolide D, are found in methanol extracts (Kuboyama et al. 2002; Ganzera et al. 2003; Choudhary et al. 2004). Also, the main antioxidant activity of *W. somnifera* was found in the methanol fraction (Parihar et al. 2004). Although water extracts are also known to have great medicinal potential, the methanol extracts seem to extract the active components the best. *W. somnifera* is one of the widely used medicines in ayurvedic as a memory enhancer and antistress drug to cure many diseases. *W. somnifera* root extract is also capable of reducing the stress-induced elevation in corticosterone levels. Baitharu et al. showed the efficacy of root extract against hypobaric hypoxia in rat model (Baitharu et al. 2013). Its active component, i.e., withanolide, is found to modulate the glutathione biosynthesis, one of the major antioxidants in metabolism (Baitharu et al. 2014)

Bacopa monnieri

Bacopa monnieri (also known as Brahmi, water hyssop, *B. monniera*, and *Herpestis monniera*) is a creeping perennial with small oblong leaves and purple flowers, found in warm wetlands, and native to Australia and India. Commonly found as a weed in rice fields, BM grows throughout East Asia. The entire plant is used medicinally. BM finds mention in traditional Indian medicine as a cure for mental diseases,

especially its ability to improve mental potentialities like augmentation of memory, sharpening of intellect, and facilitating the acquisition of newer information and promoting learning. Bacopa extract has been previously reported to facilitate cognitive functions as well as augment mental retention capacity (Singh and Dhawan 1997). Several research groups formulate bacoside-standardized BM extract for clinical use, and the herb is widely used in India, the United States, and Australia. The BM has been applied in rodents and cell culture for the following uses:

- Anticonvulsant
- Antioxidant
- Antidepressant
- Analgesic
- Anti-inflammatory
- Antimicrobial
- Anxiolytic
- Adaptogenic
- Antineoplastic
- Hepatoprotective
- Immunostimulatory

Physostigmine (PHY)

PHY (also known as eserine) is a parasympathomimetic, specifically, a reversible AChEI obtained from the Calabar bean. By interfering with the metabolism of ACh, PHY indirectly stimulates both nicotinic (Mozayan et al. 2006) and muscarinic receptors (Barak and Weiner 2007). The half-life of PHY in plasma is approximately 11 min (Somani and Khalique 1987; Somani et al. 1987). AChEI, such as PHY, may have a dual action at the cholinergic synapse by inhibiting the AChE activity and increasing the level of ACh. In animal studies, PHY significantly increased cortical ACh levels. Second, PHY has also been shown to act as agonists at muscarinic receptors and enhance the release of ACh via a muscarinic receptor-dependent mechanism, particularly under conditions of impaired cholinergic function.

In addition, research on the neurochemical deficits that accompany AD has sparked interest in the possible involvement of cholinergic systems in learning and memory. PHY has been reported to improve memory functions in AD patients. Evidence for the role of the central cholinergic system in AD is now overwhelming and has been reviewed by a number of authors (Bartus et al. 1982).

Early studies showed that supplementation of cholinergic agents such as PHY restored spatial working memory significantly by increasing ACh level in the hippocampus and entorhinal cortex (Nilsson et al. 1987). Cuadra et al. (1994) reported that PHY causes marked and long-lasting inhibition of cerebral cholinesterase leading to a prolonged elevation of ACh levels in the rat cerebral cortex.

Galantamine (GAL)

GAL acts as an allosteric-potentiating ligand (APL) at the nicotinic cholinergic receptors (nAChRs) and also displays properties of a weak, reversible acetylcholinesterase (AChE) inhibitor (Thomsen et al. 1991; Santos et al. 2002). It is currently approved for the treatment of Alzheimer's disease, a common dementia disorder strongly associated with reduced expression of nAChRs in the frontal and temporal cortex and hippocampus (Kihara et al. 2004). Galantamine acts by binding at a site on the alpha-subunit of nAChRs, which is conserved across species and is close to the acetylcholine-binding site (Samochocki et al. 2000). Based on conservation of the sequence of the APL-binding region present on known α-subunits, it has been postulated that galantamine may bind to most, if not all, nAChRs (Maelicke and Albuquerque 2000).

The allosteric action of GAL occurs only within a specific concentration range. At lower concentrations (0.02–2.0 μM), GAL has been shown to enhance acetylcholine-driven depolarization of human embryonic kidney-293 cells transfected with human α4β2 nAChRs. In contrast, 10 μM GAL inhibited this response to acetylcholine, apparently by direct blockade of the ion channel (Samochocki et al. 2000).

GAL, a well-characterized nAChR APL (Pereira et al. 1994; Samochocki et al. 2000), is one of several AChEI currently used in the treatment of AD. It induces single-channel activity of nAChRs through a process not blocked by classic competitive antagonists (Pereira et al. 1993, 1994; Storch et al. 1995). Although GAL and other APLs fail to elicit discernible macroscopic currents themselves, they potentiate whole-cell currents evoked by submaximal concentrations of nAChR agonists (Storch et al. 1995; Schrattenholz et al. 1996; Santos et al. 2002). At present, the neurochemical and cellular consequences of this allosteric interaction remain unknown, although they are assumed to be clinically relevant (Woodruff-Pak et al. 2002).

Emblica officinalis *(Amla)*

Amla (*Emblica officinalis*) is a fruited plant that has been recognized for its medicinal value and has been used since ancient times in the Indian traditional system of medicine "Ayurveda" for treating several diseases (Tan et al. 2005; Jose et al. 2001; Ngamkitidechakul et al. 2010). The fruit part of this plant contains 12 well-known medicinally relevant compounds such as gallic acid, ellagic acid, 1-*O*-galloyl-beta-D-glucose, 3,6-di-*O*-galloyl-D-glucose, chebulinic acid, quercetin, chebulagic acid, corilagin, 1,6-di-*O*-galloyl-beta-D-glucose, 3-ethylgallic acid, isostrictinin, and ascorbic acid (Tan et al. 2005). Additionally branches of this plant contain six bioactive compounds—geraniin, phyllanemblinins C and E, prodelphinidin B1, (2)-epigallocatechin 3-*O*-gallate, and (*S*)-eriodictyol 7-[6-*O*-(*E*)-*p*-coumaroyl]-*b*-D-glucoside (Zhang et al. 2003). Out of all these compounds, gallic acid, ellagic acid,

1-*O*-galloyl-beta-D-glucose, chebulinic acid, quercetin, chebulagic acid, corilagin, ascorbic acid, and geraniin have established strong anticarcinogenic properties individually and explain the anticancer properties of the whole Amla extract (AE) (Tan et al. 2005; Chopra 1958). AE has been shown to inhibit proliferation of a variety of cancer cells in vitro and also has demonstrated antiproliferative effects in vivo (Tan et al. 2005; Jose et al. 2001; Ngamkitidechakul et al. 2010). Recently triphala, an herbal preparation containing AE, has also shown significant antiangiogenesis properties (Shivananjappa & Joshi., 2012). AE has demonstrated significant amelioration against hypobaric hypoxia-mediated oxidative damage in animal model (data under review).

Conclusion and Future Prospects

Natural products and natural product-derived compounds have contributed significantly as important molecular probes to elucidate the process of oxidative stress-mediated stress and may ultimately serve as therapeutically useful antioxidants. In the long history of development of herbal medicines, many natural products have been discovered and employed to treat several neurodegenerative diseases. In recent decades, with the advancement of chromatographic and spectroscopic techniques, a number of agents have been isolated from natural products, and their efficacies against different diseases have been tested both in vitro and in vivo conditions. The activities, on one hand, illustrated the principle of evidence-based medicine to clinically use these medicines to treat neurodegenerative disorders and, on the other hand, discovered many monomer compositions as promising drugs or lead compounds for drug design in the treatment of these diseases. The currently used medications for the treatment of neurological disorders are mainly symptom-management drugs. Although they do improve symptoms and play a key role in the treatment of disease at present, these drugs are not capable of reversing the progress of disorders. Hypobaric hypoxia has multifactorial responses which limit the efficacy of different drugs whose mechanism of action is specific target based. Therefore, there is a need for some drugs derived from natural products which have maximum efficacy with less or no side effects. Further research is required to explore the possible natural products and their derivatives to prevent as well as cure the damaging effect of neurodegenerative diseases like hypobaric hypoxia which involves oxidative stress in their pathophysiology.

Compliance with Ethics Requirements The author declares that he/she has no conflicts of interest.

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Indian Herbs for the Treatment of Neurodegenerative Disease

Padmanabhan Mannangatti and Kamalakkannan Narasimha Naidu

Abstract Ayurveda, an ancient system of medicine that is indigenous to India, is believed to be the world's oldest comprehensive health-care system and is now one of the most recognized and widely practiced disciplines of alternative medicine in the world. Medicinal herbs have been in use for treating diseases since ancient times in India. Ayurvedic therapies with medicinal herbs and herbomineral products generally provide relief without much adverse effects even after prolonged administration. Neurodegenerative disorders are a major cause of mortality and disability, and increasing life spans represent one of the key challenges of medical research. Ayurvedic medicine describes most neurodegenerative diseases and has defined a number of plants with therapeutic benefits for the treatment of neurodegenerative diseases having antioxidant activities. In this chapter, the role of four important Ayurvedic medicinal plants, viz., *Withania somnifera* (ashwagandha), *Bacopa monnieri* (brahmi), *Centella asiatica* (gotu kola), and *Mucuna pruriens* (velvet bean), on neurodegenerative diseases are discussed.

Keywords Ayurveda • Neurodegenerative disorders • Withania somnifera • Bacopa monnieri • Centella asiatica • Mucuna pruriens

Ayurveda: The Indian System of Medicine

The practice of herbal medicine dates back to the very earliest periods of known human history. There is evidence of herbs having been used in the treatment of diseases and for revitalizing body systems in almost all ancient civilizations—the Indian, the Egyptian, the Chinese, and even the Greek and Roman civilizations. Plants were the mainstay of medicine and credited with mystical and almost supernatural powers of healing (Bakhru 2001). Ayurveda, "science of life," is believed to be the world's oldest comprehensive health-care system and is indigenous to India. It is now one of the most recognized and widely practiced disciplines of alternative medicine in the world.

In Ayurveda, substances of natural origin, including whole plants or their parts, animal parts, and minerals, are used as medicines, either alone or in combination.

P. Mannangatti (✉) • K.N. Naidu
Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, USA
e-mail: Padmanabhan.Mannangatti@vcuhealth.org

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In addition, various other measures are used in an attempt to maintain health in a healthy person and alleviate disorders of the body and mind. The belief in Ayurvedic medicine is that a disease is the product of an imbalance in the body and mental elements that reduce the body's resistance to diseases. If the imbalance is corrected and the body's defense mechanisms are strengthened by herbal formulas, lifestyle changes, and diet, then the body will resist a disease with a goal of eliminating it (Mishra 2004).

Herbal and herbomineral products regularly used in Ayurveda are believed to strengthen the body's defenses. These substances act on the principles of *samanya* (homologous) and *visesha* (antagonistic) action. Substances possessing homologous properties and actions increase the relevant elemental properties or constituents of the body, while those having antagonistic properties or actions decrease those properties or constituents. In cases of disease or imbalance of *dosha* (humor), *dhatu* (seven body tissues, the lymph, blood, muscle, adipose tissue, bone, bone marrow, semen), and *mala* (feces, urine, and other waste products), the rational use of naturally available substances aims to restore normality (WHO 2010).

Ayurvedic medicine has benefited from the advances in science and technology. These advances facilitated the understanding of diseases, the development of better pharmaceutical products, and the implementation of diagnostic techniques. Ayurvedic therapies generally provide relief without much adverse effect even after prolonged administration. Ayurvedic herbs and formulas often have a wide spectrum of therapeutic activity. The pharmacological activities of herbs may not be confined to one specific chemical constituent. Ayurvedic therapies are known to be relatively economical, and the Ayurvedic formulas are time tested for safety (Mishra 2004).

Medicinal herbs have been in use for treating diseases since ancient times in India. Herbs are used in many different ways. However, the ultimate objective of their use is that they should interact directly with our body chemistry. They may be used in various forms like food, medicine, cosmetics, or fragrance, but in all cases, their active constituents must be absorbed into the body for deriving the required benefits. Once they are absorbed into the bloodstream, they circulate to influence our whole system. The active constituents of the herb can enter the body in several ways. These include consuming the herb orally or application on the skin or even smelling the aroma through the nose (Bakhru 2001). During the past 100 years, several hundred Ayurvedic herbs have been investigated with respect to plant chemistry, active chemical constituents, pharmacological effects, safety, and efficacy. A single herb is rarely administered to a patient in Ayurveda. Herbal formulas are favored because the founders of Ayurveda recognized the possible synergistic and counterbalancing effects of herbs.

Neurodegenerative Disorders

The progressive loss of structure and/or function of neurons, including their death, is referred to as neurodegeneration. This loss can be inherited and determined at birth or soon after birth or can even be due to an injury (Bredesen et al. 2006). The

number of neurodegenerative diseases is estimated to a few hundred, and among these, many appear to overlap with one another clinically and pathologically. Neurodegenerative disorders are a major cause of mortality and disability, and increasing life spans represent one of the key challenges of medical research. The mechanisms of neurodegeneration vary in nature, but in simpler terms, it could be due to (1) excitotoxicity, (2) apoptosis, (3) amyloid cascade, (4) oxidative stress, and (5) sodium nitrite-mediated neurodegeneration (Pandit 2011).

Reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$), hydroxyl radicals ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2) produced endogenously play certain beneficial roles in cellular functions, but they also participate in harmful events such as in neurodegeneration. Much importance has been given to neurodegenerative diseases such as Alzheimer's, Huntington's, and Parkinson's diseases and amyotrophic lateral sclerosis, and these diseases have been shown to be tightly linked to mitochondrial dysfunction affected by ROS (Ahmad 2012). Any therapeutic strategy that prevents neurons from dying either a drug or treatment is termed as neuroprotection. The goal of neuroprotection is to limit neuronal dysfunction after injury and attempt to maintain the possible integrity of cellular interactions in the brain resulting in an undisturbed neural function. Antioxidants neutralize free radicals and are effective in suppressing or preventing these disorders.

The Ayurvedic Way of Treating Neurodegenerative Diseases

Ayurveda is practiced through its eight specialized branches: *Kayachikitsa* (internal medicine), *Shalya* (surgery), *Shalakya* (ophthalmology and ENT), *Kaumarbhritya* (pediatrics), *Aagada* (toxicology), *Bhuta Vidya* (psychology), *Rasayana* (rejuvenation), and *Bajikarana* (or *Vajikarana*, sexology). Among these disciplines, *Rasayana tantra* regards the treatments to improve longevity and memory, to attain youthful appearance, and to maintain cognitive performance and physical strength (Singh et al. 2008).

Ayurvedic medicine describes most neurodegenerative diseases as being due to significant Vata (the humor of space and air) imbalance in the body and mind, particularly in one of the seven tissues of the body known as majja, or marrow, and of the brain. Imbalance can occur in many different ways and to various different extents. How it occurs is what differentiates one neurodegenerative disease from the other and from one individual to another. The imbalance of Vata in the brain means that the brain "tissue" dries up due to lack of nourishment, whether from a physical or emotional perspective or both. Ayurveda has recognized the need for the brain to remain nourished and accomplishes this through preventative and responsive measures.

Ayurveda has defined a number of plants with therapeutic benefits for the treatment of neurodegenerative diseases having antioxidant activities. This chapter gives details about four important Ayurvedic medicinal plants, viz., *Withania somnifera* (ashwagandha), *Bacopa monnieri* (brahmi), *Centella asiatica* (gotu kola), and *Mucuna pruriens* (velvet bean), and their role in neurodegenerative diseases.

Withania somnifera

Withania somnifera Dunal, commonly known as ashwagandha, belongs to the Solanaceae family and is widely distributed in the drier parts of tropical and subtropical zones, ranging from the Canary Islands, the Mediterranean region, and Northern Africa to Southeast Asia (Warrier et al. 1996). It is an important medicinal plant used in Ayurvedic and indigenous medicine for over 3000 years. The whole plant as well as its specific parts such as roots, stems, and leaves and also the plant extract and its constituents have been used in the treatment of various ailments. The main constituents of ashwagandha are alkaloids and steroidal lactones. Withanine, somniferine, somnine, somniferinine, and withananine are some of the chemical constituents present in it (Rani et al. 2012).

Withania is widely claimed to have potent aphrodisiac, sedative, rejuvenating, and life-prolonging properties. It is also used as a general energy-enhancing tonic known as Medhya Rasayana, which means "that which promotes learning and a good memory," and in geriatric problems (Nadkarni 1976). The roots are also used in constipation, senile debility, rheumatism, general debility, nervous exhaustion, loss of memory, loss of muscular energy, and spermatorrhea (Singh and Kumar 1998).

Withanamides are a novel class of compounds isolated from the fruit of *W. somnifera*. Jayaprakasam et al. (2004) have isolated the major withanamides A and C from *W. somnifera* fruit and tested their ability to inhibit lipid peroxidation. The free radical that initiated damage of the brain tissue is one of the major mechanisms considered in the pathology of AD. The withanamides were found to possess antioxidant activity. They also tested the ability of these withanamides in protecting the rat pheochromocytoma (PC-12) cells from β-amyloid peptide (BAP)-induced cytotoxicity as BAP plays a significant role in the development of AD. They found that withanamide A was the most active, and it completely neutralized the toxic effect of BAP on PC-12 cells. Withanamide C was found to protect the PC-12 cells from cell death due to the BAP (Jayaprakasam et al. 2010).

The active constituents of *W. somnifera* such as withanolide A, withanoside IV, and withanoside VI were shown to restore presynapses and postsynapses in addition to both axons and dendrites in cortical neurons after amyloid-β (Aβ)25–35-induced injury. Oral administration of withanolide A, withanoside IV, and withanoside VI (10 μmol/kg/day for 12 days) improved Aβ25–35-induced memory impairment, neurite atrophy, and synaptic loss in the cerebral cortex and hippocampus in mice. These observations by Tohda (2008) suggest the therapeutic potential of these *Withania* constituents in the treatment of neurodegenerative diseases.

The methanol extract of ashwagandha containing the active principles withanolide A, withanoside IV, and withanoside VI showed neurite outgrowth-promoting activity in human neuroblastoma SK-N-SH cells (Tohda et al. 2000). In an in vitro axonal atrophy model, withanolide A, withanoside IV, and withanoside VI were individually treated to the neurons, and each of these three compounds induced axonal growth in the presence of Aβ25–35 (Tohda et al. 2005; Kuboyama et al. 2005; 2006). Moreover, posttreatment with withanolide A, withanoside IV, or withanoside VI increased the synaptic densities in an in vitro synaptic degeneration

model established by the formation of synapses from rat cortical neurons and then treated with Aβ25–35 which resulted in losses of densities of presynapses and postsynapses (Kuboyama et al. 2005).

Scopolamine is a muscarinic receptor antagonist that induces amnesia in rodents (Klinkenberg and Blokland 2010). Scopolamine also influences the expression of genes related to muscarinic receptor signaling pathways, apoptosis, and cell differentiation in the rat brain (Hsieh et al. 2003). Konar et al. (2011) investigated the effects of the alcoholic extract of ashwagandha leaves on scopolamine-induced cell damages. Pretreatment with this alcoholic extract protected scopolamine-induced cell deaths in cultured human neuroblastoma IMR32 cells and cultured rat glioma C6 cells. Scopolamine treatment induced DNA damage and oxidative stress in C6 cells, but treatment with the alcoholic extract prevented the damage. Withaferin A and withanone were identified as the major constituents in this alcoholic extract. Withanone showed more preventive effects than withaferin A.

A study carried out by Sehgal et al. (2012) reports the reversal of Alzheimer's disease (AD) pathology in APP/PS1 mice by a partially purified *Withania somnifera* extract consisting of 75 % withanolides and 20 % withanosides by increasing the clearance of the toxic Aβ peptide from the brain, promoting its sequestration in plasma and ultimately its degradation in the periphery. These beneficial effects are associated with reversal of behavioral deficits and reduced AD pathology in very old AD-Tg mice (22–24 months old). The therapeutic benefits of *W. somnifera* on behavioral deficits and Aβ pathology are also confirmed in APPSwInd J20 mice, another AD mouse model. Oral administration of *W. somnifera* proved highly effective in reversing the behavioral deficits and pathological features in two mouse models of AD.

In a clinical trial involving chronically stressed humans, the effects of *W. somnifera* root and leaf extract were investigated. This extract contained withanolide glycosides, withaferin A, oligosaccharides, alkaloids, and polysaccharides with no scopolamine. It was reported that the consumption of this extract significantly reduced experiential and biochemical indicators of stress without adverse effects. The therapeutic activity of the extract was attributed to its effect on the hypothalamic-pituitary-adrenal axis, which regulates serum cortisol concentration (Auddy et al. 2008).

In an experimentally validated Alzheimer's disease model, where the syndrome was induced by ibotenic acid (IA) lesioning of the nucleus basalis magnocellularis (NBM) in rats, glycowithanolides withaferin A and sitoindosides VII–X that were isolated from the roots of ashwagandha were tested by administering orally with equimolar amounts of sitoindosides VII–X and withaferin A. The authors found a significantly reversing effect of IA-induced cognitive deficits in these rats on treatment with sitoindosides VII–X and withaferin A (Bhattacharya et al. 1995).

Bacopa monnieri

Bacopa monnieri (L.), commonly known as brahmi, is found throughout the plains of India in damp marshy areas and also in Sri Lanka, Pakistan, and Bangladesh (Khare 2007). Brahmi is used in the Indian Ayurvedic system for its memory-enhancing,

anti-inflammatory, analgesic, antipyretic, sedative, and antiepileptic properties (Kirtikar and Basu 1935). Phytochemical studies on brahmi have shown that it contains many active constituents, including alkaloids, brahmine, herpestine, saponins (bacosides A, A3, and B and bacopasaponin A to F), D-mannitol, betulinic acid, β-sitosterol, and stigmasterols (Nathan et al. 2001). But the major constituents are the steroidal saponins, bacosides A and B (Chatterji et al. 1965). Research has been conducted on both animals and human volunteers to study the efficacy of *Bacopa monnieri*, especially on its neuroprotective and memory-enhancing effects.

The neuroprotective effects of brahmi were determined in normal and in β-amyloid protein (25–35) and glutamate-induced neurotoxicity in primary cortical cultured neurons. Brahmi extract was found to protect neurons from β-amyloid-induced cell death, but not in glutamate-induced excitotoxicity, and this neuroprotection was possible due to the extract's ability to suppress cellular acetylcholinesterase activity. This extract also promoted cell survival and exhibited both reducing and lipid peroxidation inhibitory activities (Limpeanchob et al. 2008). Brahmi was evaluated for its antioxidant activity in 3-month-old female Wistar rats for 10 days and found to protect the central and peripheral neuronal systems through its unique effects on the antioxidant enzyme activities and intracellular signaling pathways (Priyanka et al. 2013). A study was carried out to test the ability of brahmi leaf powder on neuronal oxidative stress in prepubertal mice. *Bacopa monnieri* modulated endogenous markers of oxidative stress in brain tissue of these animals (Shinomol and Muralidhara 2011).

Bacosides from the alcoholic extract of brahmi were tested on experimentally induced amnesia by scopolamine, sodium nitrite, and BN52021 in mice. The results showed that bacosides facilitate anterograde memory and attenuate anterograde experimental amnesia in mice (Kishore and Singh 2005). In a study involving human healthy volunteers and SDAT patients of 60–75 years, a poly-herbal formulation containing brahmi was given for a period of 12 months. The authors evaluated the cognitive functions, inflammatory markers, and oxidative stress in these healthy volunteers and SDAT patients and found an improvement in cognitive measures and a reduction in inflammation and oxidative stress levels (Sadhu et al. 2014). A standardized whole plant dry extract of *B. monnieri* was evaluated for its cognitive function and effect and its safety and tolerability in healthy elderly participants of 65 years or older. The results showed that the standardized extract has potential for enhancing cognitive performance in aging (Calabrese et al. 2008).

There is a significant correlation between paraquat exposure and increased risk for Parkinson's disease in humans. A standardized extract of brahmi was tested on acute paraquat-induced oxidative stress in different brain regions of prepubertal mice. In this study, brahmi supplementation restored the activities of cholinergic enzymes and also the striatal dopamine levels of paraquat-treated mice (Hosamani et al. 2014). These studies show that *B. monnieri* protects against oxidative damage, possibly by enhancing the activities of antioxidative enzymes and improving redox status. In an experiment by Jansen et al. (2014), fruit flies (*Drosophila melanogaster*) that served as a model of Parkinson's disease (based on loss of function of phosphatase and tensin-induced putative kinase 1 (PINK1)) were cultured for food

containing brahmi and a combination of herbs that included brahmi. They observed a significant improvement in the climbing ability of PD flies treated with brahmi alone and in combination with other herbs. Jadiya et al. (2011) tested the effects of *B. monnieri* on transgenic and pharmacological *Caenorhabditis elegans* models of Parkinson's disease. Their results showed that *B. monnieri* reduced α-synuclein aggregation, prevented dopaminergic neurodegeneration, and restored the lipid content in *C. elegans* proving *B. monnieri* as a possible antiparkinsonian agent.

In an animal model of Alzheimer's disease induced by ethylcholine aziridinium, the effect of the alcoholic extract of *B. monnieri* was analyzed. This extract mitigated the memory impairment and the degeneration of neurons in the hippocampus in this animal model by improving acetylcholine levels and cerebral blood flow, suggesting the cognitive enhancing and neuroprotection of brahmi in Alzheimer's disease (Uabundit et al. 2010). Another study by Saini et al. (2012) evaluated the neuroprotective potential of *B. monnieri* against cognitive impairment in colchicine-induced dementia in rats as this is an accepted model of sporadic dementia of Alzheimer's disease. *B. monnieri* administration attenuated the oxidative damage by colchicine by decreasing lipid peroxidation and protein carbonyl levels and restoring the antioxidant enzymes along with the membrane-bound enzymes in the brain suggesting the therapeutic potential of *B. monnieri* in the treatment of AD-associated cognitive decline. In a study by Zhang et al. (2013), the role of *B. monnieri* in improving memory performance and protection against AD by increasing expression or activity of Na^+/K^+-ATPase was highlighted. The effect of bacosides, the active saponins of *B. monnieri*, was investigated against age-associated neurodegeneration in aged female Wistar rat brain and was found to act as a potential therapeutic intervention in forestalling the deleterious effects of aging and preventing the age-associated pathologies like senile dementia of Alzheimer's type (SDAT) (Rastogi et al. 2012a). These authors (Rastogi et al. 2012b) further tested backsides against age-related chronic neuroinflammation in the Wistar rat brain for 3 months as neuroinflammation finds importance in age-associated neurodegeneration and SDAT. The results of this study demonstrated a significant attenuation of age-dependent elevation of pro-inflammatory cytokines, iNOS protein expression, and total nitrite and lipofuscin content in middle-aged and aged rat brain cortex on long-term administration of bacosides.

Centella asiatica

Centella asiatica (L.), commonly known as Indian pennywort, belongs to the family Apiaceae. It is a perennial creeper and a valuable medicinal herb. It is distributed throughout the tropical and subtropical regions of India, China, Nepal, Madagascar, Sri Lanka, and Indonesia. It has been used as a medicinal herb for thousands of years in India, China, Sri Lanka, Nepal, and Madagascar. *C. asiatica* is one of the chief herbs used for treating skin problems, to heal wounds, for revitalizing the nerves and brain cells, to increase attention span and concentration,

and to combat aging (Brinkhaus et al. 2000). In India, *C. asiatica* is valued as an ethnomedicine in Ayurveda and Unani, for treating different ailments like asthma, skin disorders, ulcers, and body aches and for improving memory, and also as a nervine tonic (Singh et al. 2010). *C. asiatica* contains a broad spectrum of phytochemicals that include triterpenoids, volatile fatty acids, glycosides, flavonoids, alkaloids, and tannins (Das 2011).

Fresh leaf extracts of *C. asiatica* were found to facilitate the dendritic growth in the hippocampal CA3 neurons. The active components of *C. asiatica*, asiatic acid, asiaticoside, and SM2, exhibited neuroprotective property and inhibited β-amyloid and free radical-induced cell death in B103 cell cultures and hippocampal slices (Rao et al. 2006). *Centella asiatica* were effective in preventing the cognitive deficits, as well as the oxidative stress caused by the intracerebroventricular administration of streptozotocin, indicating that *the C. asiatica* can act as a free radical scavenger (Kumar and Gupta 2003).

Glutamate treatment for rats can lead to excitation and oxidative stress resulting in neurodegeneration (Mates et al. 2002). Glutamate-administered rats were treated with a standardized extract of *C. asiatica* containing asiaticosides and found to attenuate the glutamate-induced excitation and oxidative stress (Ramanathan et al. 2007). Xu et al. (2012) investigated the neuroprotective effect of asiatic acid both in vitro (human neuroblastoma SH-SY5Y cells) and in vivo (neonatal mice) of glutamate toxicity. Pretreatment of the cells with asiatic acid attenuated glutamate toxicity by decreasing apoptotic cell death and reducing reactive oxygen species and stabilizing mitochondrial membrane potential. In mice, asiatic acid administration attenuated cognitive deficits and lipid peroxidation and glutathione in the hippocampus and cortex. It also attenuates neuronal damage of the pyramidal layer in the CA1 and CA3 regions.

Asiaticoside isolated from *C. asiatica* was investigated on memory impairment and inflammatory cytokine expression induced by transient cerebral ischemia and reperfusion in mice. Asiaticoside was found to be neuroprotective in these mice, and this effect could be associated with the anti-inflammatory effect of asiaticoside via inhibiting overactivation of p38 MAPK pathway (Chen et al. 2014). The derivatives of asiaticoside were tested for their potential protective effects against Aβ-induced cell death of B103 cells. Three of such derivatives, asiatic acid, asiaticoside 6, and SM2, showed strong inhibition of Aβ-induced death of B103 cells. Further, these three derivatives reduced H_2O_2-induced cell death and lowered intracellular free radical concentration (Mook-Jung et al. 1999). Lee et al. (2000) attempted to prepare neuroprotective compounds that were more efficacious than asiatic acid by modifying the chemical structure of asiatic acid. In an in vitro study using primary cultures of rat cortical neurons treated with glutamate, three of those asiatic acid derivatives were found to protect neurons from the oxidative damage caused by exposure to excess glutamate.

Tabassum et al. (2013) evaluated the preventive role of the ethanolic extract of *C. asiatica* in middle cerebral artery occlusion (MCAO) in rats. The administration of this extract revealed improved neurobehavioral activity and diminished infarction volume along with restored histological morphology of the brain in these rats.

Further, this extract reduced the levels of free radicals and improved the antioxidant status in MCAO rats. The authors attribute this antioxidant activity of *C. asiatica* extract to the presence of bioactive triterpenes, asiatic acid, asiaticoside, madecassic acid, and madecassoside. A water extract of *C. asiatica* was examined in a murine model of AD with high amyloid burden and found to attenuate the β-amyloid-associated behavioral abnormalities in these mice. In an in vitro study, this extract protected the SH-SY5Y cells and MC65 human neuroblastoma cells from β-amyloid toxicity (Soumyanath et al. 2012).

The prevention of aggregation of α-synuclein is a potential therapeutic intervention for preventing PD. In a study by Ruben et al. (2014), an aqueous leaf extract of *C. asiatica* was tested for amyloid-β (Aβ) levels in the brain and Aβ aggregation. In this in vitro study, *C. asiatica* extract inhibited α-synuclein aggregation and inhibited the formation of oligomer to aggregates indicating the therapeutic potential of *C. asiatica* in PD. In a rat model of the early phase of parkinsonism, madecassoside isolated from *C. asiatica* was tested by Xu et al. (2013). Treatment with madecassoside was found to improve locomotor dysfunction and to protect dopaminergic neuron by antagonizing 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity. Madecassoside was effective in recovering MPTP-induced early signs of parkinsonism via its neuroprotective effects, including reversing the depletion of dopamine, antioxidant activity, increasing ratio of Bcl-2/Bax, and increasing protein expression of brain-derived neurotropic factor (BDNF). In another study, *C. asiatica* extract was administered to MPTP-induced neurotoxicity in aged Sprague-Dawley rats. The authors (Haleagrahara and Ponnusamy 2010) found that this extract significantly increased total antioxidants and antioxidant enzymes in the corpus striatum and hippocampus showing the neuroprotective effect of *C. asiatica*.

Mucuna pruriens

Mucuna pruriens L. (velvet bean), belonging to the family Fabaceae, is a vigorous annual climbing legume originally from eastern India and southern China (Duke 1981). It is widely distributed in tropical and subtropical regions of the world and was once widely cultivated as a green vegetable crop due to its high protein concentration (Pugalenthi et al. 2005). The plant has long been used in Ayurveda as a powerful aphrodisiac (Amin et al. 1996), to treat nervous disorders and arthritis (Jeyaweera 1981), and also for treating parkinsonism (Sathiyanarayanan and Arulmozhi 2007). The seed, root, and stem of *M. pruriens* possess valuable medicinal properties (Suresh et al. 2012).

The therapeutic effects of aqueous extract of *Mucuna pruriens* seed were evaluated in paraquat-exposed parkinsonian mouse model. Treatment of these mice with *M. pruriens* seed extract reduced paraquat-induced neurotoxicity by decreasing oxidative damage, physiological abnormalities, and immunohistochemical changes in this mouse model (Yadav et al. 2013). The neuroprotective effect of an

ethanolic extract of *M. pruriens* seed was evaluated in the MPTP model of PD. The extract recovered the number of tyrosine hydroxylase-positive cells in the substantia nigra and striatum and reduced the expression of iNOS and glial fibrillary acidic protein (GFAP) in the substantia nigra and increased the levels of dopamine. The extract also downregulated nitric oxide production, neuroinflammation, and microglial activation which can be contributed to its neuroprotective activity (Yadav et al. 2014).

Manyam et al. (2004a) evaluated the neurorestorative effect of *Mucuna pruriens* cotyledon powder on the nigrostriatal tract of 6-hydroxydopamine (6-OHDA)-lesioned rats, a model of PD. The cotyledon powder significantly restored the endogenous levodopa, dopamine, norepinephrine, and serotonin content in the substantia nigra. In a series of tests conducted by Kasture et al. (2009) in a subchronic mouse model of MPTP-induced dopamine neuron degeneration, *M. pruriens* seed extract was found to be antiparkinsonian but not neuroprotective. They attributed this effect to the presence of L-DOPA (dihydroxyphenylalanine) in the seed extract.

In a study involving eight Parkinson's disease patients, the seed powder of *Mucuna* and standard L-dopa/carbidopa (LD/CD) were administered. The authors conclude that *Mucuna* seed powder is better on tests performed than the standard LD/CD (Katzenschlager et al. 2004). Nagashayana and coworkers (2000) evaluated the efficacy of Ayurveda treatment (a concoction in cow's milk of powdered *Mucuna pruriens* and *Hyoscyamus reticulatus* seeds and *Withania somnifera* and *Sida cordifolia* roots) in 18 clinically diagnosed parkinsonian patients for a period of 84 days. They underline the importance of cleansing therapy in Ayurveda medication prior to palliative therapy based on their results. *M. pruriens* in the form of a powder (HP-200) was mixed with water and given orally to 60 patients with Parkinson's disease for a period of 12 weeks and found to be an effective treatment for these patients (HP-200 in Parkinson's Disease Study Group 1995). In a long-term study (52 weeks) in rats, HP-200 was administered to elucidate the levels of monoaminergic neurotransmitters and its metabolite in various regions of the brain. Oral administration of HP-200 had a significant effect on dopamine content in the cortex, but no significant effect on the other neurotransmitters and their metabolites in the nigrostriatal tract (Manyam et al. 2004b).

Mucuna pruriens was co-treated with *Withania somnifera* to parkinsonian mice induced by chronic exposure to paraquat. This co-treatment was effective in behavioral and antioxidant recovery, which is an indicator of the neuroprotective effects of both these plants (Prakash et al. 2013). *M. pruriens* is reputed to provide antiparkinsonian benefits without inducing drug-induced dyskinesias. Lieu et al. (2010) studied the effects of *M. pruriens* seed extract alone or in combination with dopa-decarboxylase inhibitors in the hemiparkinsonian rat model of PD. They found that the seed extract administered chronically provided long-term antiparkinsonian benefits without causing drug-induced dyskinesias. These authors (Lieu et al. 2012) further tested the *M. pruriens* endocarp powder in rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) hemiparkinsonian monkeys. They observed that the seed powder significantly ameliorates behavioral deficits in these animals, and this action could be attributed not only to the presence of L-dopa but to several other constituents in this extract.

Other Important Ayurvedic Medicinal Plants on Neuroprotection

The active component of *Curcuma longa*, curcumin, is a polyphenolic compound, and it has been reported to have protective effects in neurodegenerative disease by either reducing inflammation or oxidative damage in AD (Begum et al. 2008). Curcumin provided protection against α-synuclein-induced cytotoxicity in SH-SY5Y neuroblastoma cells by decreasing cytotoxicity, reducing intracellular ROS, inhibiting caspase-3 activation, and ameliorating signs of apoptosis (Wang et al. 2010). Other plants such as *Acorus calamus* (Zanoli et al. 1998) and *Glycyrrhiza glabra* (Dhingra et al. 2004) are also reported to have neuroprotective activity.

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Avocado as a Major Dietary Source of Antioxidants and Its Preventive Role in Neurodegenerative Diseases

Kalandar Ameer

Abstract Avocados have a high content of phytochemicals especially antioxidants with potential neuroprotective effect. Aging is the major risk factor for neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. A large body of evidence indicates that oxidative stress is involved in the pathophysiology of these diseases. Oxidative stress can induce neuronal damages and modulate intracellular signaling, ultimately leading to neuronal death by apoptosis or necrosis. There is evidence for increased oxidative damage to macromolecules in amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, and Alzheimer's disease. Thus, antioxidants have been used for their effectiveness in reducing these deleterious effects and neuronal death in many in vitro and in vivo studies. The critical review results indicate that compounds in avocado are unique antioxidants, preferentially suppressing radical generation, and thus may be promising as effective neuropreventive agents. The diverse array of bioactive nutrients present in avocado plays a pivotal role in the prevention and cure of various neurodegenerative diseases.

Keywords Avocado • Neuron • Alzheimer's disease (AD) • Antioxidants

Introduction

The brain can be described as the most complex structure in the human body. It is made up of neurons and neuroglia, the neurons being responsible for sending and receiving nerve impulses or signals. The microglia and astrocytes are essential for ensuring the proper functioning of neurons. They are quick to intervene when neurons become injured or stressed. As they are sentinels of neuron well-being, pathological impairment of microglia or astrocytes could have devastating consequences for brain function. It is assumed that neuroglial activation is largely

K. Ameer (✉)
Department of Medical Biochemistry, School of Medicine, College of Health and Medical Sciences, Haramaya University, PO Box 91, Dire Dawa, Ethiopia
e-mail: mailkalandar@gmail.com

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determined by neuronal signals (Halliwell 1992). Acute injury causes neurons to generate signals that inform neuroglia about the neuronal status. Depending on how severe a degree of neuronal injury, neuroglia will either nurse the injured neurons into regeneration or kill them if they are not viable. These types of neuroglial responses are considered to represent normal physiological and neuroprotective responses. In contrast, some processes that are chronic in nature persistently activate neuroglia eventually causing a failure in their physiological ability to maintain homeostasis. This could have detrimental consequences and may lead to bystander damage due to neuroglial dysfunction.

Neurodegenerative diseases: Neurodegenerative diseases are a group of conditions characterized by progressive dysfunction, degeneration, and death of specific populations of nerve cells, which are often synaptically interconnected. Neurons are the building blocks of the nervous system which include the brain and spinal cord. Neurons normally do not reproduce or replace themselves, so when they become damaged or die, they cannot be replaced by the body. Examples of neurodegenerative diseases include Alzheimer's disease (AD) and other dementias, Parkinson's disease (PD) and PD-related disorders, motor neuron diseases (MND), Huntington's disease (HD), spinocerebellar ataxia (SCA), spinal muscular atrophy (SMA), and several other cerebral abnormalities.

According to a consensus that was developed using the Delphi method, the prevalence of AD is on the rise, and an estimated 26.6 million patients with AD are reported worldwide. Besides, this number is estimated to increase to 106.2 million by 2050 (Brookmeyer et al. 2007). The global prevalence of Parkinson's disease (PD) is estimated to be 6.3 million patients, with 1.2 million patients in Europe (Rajput 1992). The frequency of HD was found to be 4–8 in 100,000 people in Europe (Harper 1992), and the prevalence rate of amyotrophic lateral sclerosis (ALS) was determined to be around 2–7 in 100,000 people in the USA (Kurtzke 1982). These neurodegenerative diseases share common symptomatological features at different stages of disease progression. The main physiological symptoms of degenerative diseases include elevated oxidative/nitrosative stress, mitochondrial dysfunction, protein misfolding/aggregation, synapse loss, and decreased neuronal survival (Winner et al. 2011; Finkel 2011). When neurons and immune cells are exposed to toxic proteins, a large amount of energy is needed to defend against the accumulated oxygen and nitrogen species that induce stress in the surrounding environment. This results in mitochondrial malfunction with the release of cytochrome C and other mitochondrial proteins, which pave the way toward apoptosis (Finkel 2011). This overabundance of protein aggregation affects cellular signaling and neuronal function and is a key cause of neuronal loss (Nakamura and Lipton 2007).

AD is perhaps the typical degenerative disease affecting the central nervous system. It is a chronic progressive disease characterized by memory loss and deficits in one or more of the following cognitive domains: aphasia (language disturbance), agnosia (failure to recognize people or objects in the presence of intact sensory function),

apraxia (inability to perform motor acts in the presence of intact motor system), or executive function (plan, organize, and sequence actions or form abstractions).

AD is also associated with elevated rates of oxidative stress that contribute to increased rates of genomic instability events such as an increased frequency in micronucleus (MN) formation, chromosomal aberrations, and alterations in telomere length and levels of apoptosis (Mecocci et al. 1998; Bresgen et al. 2003; Petrozzi et al. 2002). The characteristic hallmarks of AD involve the abnormal deposition of proteins leading to amyloid plaques and neurofibrillary tangles in the brain and a progressive loss of cognitive function (Gotz et al. 2001; St. George-Hyslop 2000). These pathological features have also been linked with oxidative stress (Moreira et al. 2005; Christen 2000). It has been suggested that both plaques and tangles are produced in order to protect sensitive areas of the brain from the effects of oxidative stress-related injury (Moreira et al. 2005).

Oxidative stress (OS) is thought to play a key role in the early stages of AD pathology (Migliore et al. 2005; Nunomura et al. 2001). OS results from an imbalance between free radical formation and the counteractive endogenous antioxidant defense systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Reactive oxygen species (ROS) are damaging to cells, leading to the oxidation of essential cellular components such as proteins and lipids, leading to eventual genomic instability events such as MN formation and telomere shortening (Bresgen et al. 2003; Petrozzi et al. 2002).

Parkinson's disease is the second most common neurodegenerative disorder and is characterized by abnormalities of motor control, as opposed to intellectual and personality changes. PD is characterized by resting tremors, bradykinesia (slowness of voluntary movement), rigidity, and a loss of postural reflexes. Patients with PD typically have a flat, expressionless face and walk with a stooped gait characterized by small steps. Many patients also experience severe depression.

HD is a neurodegenerative genetic disorder that affects muscle coordination and leads to mental decline and behavioral symptoms.

ALS, also known as motor neuron disease or Lou Gehrig's disease, is a rare neuromuscular disease with an incidence rate of about 1 in 100,000. It is characterized by muscular weakness from the degeneration of motor neurons, and like PD, intellect and personality are often unaffected.

Process of Neurodegeneration

The process of neurodegeneration is very complex and has multiple causes. Many evidences suggest that mitochondria have a central role in aging-related neurodegenerative diseases. Mitochondria are critical regulators of cell death, a key feature of neurodegeneration. Mutations in mitochondrial DNA and oxidative stress both contribute to aging, which is the greatest risk factor for neurodegenerative diseases.

In all major examples of these diseases, there is strong evidence that mitochondrial dysfunction occurs early and acts causally in disease pathogenesis. Neurodegeneration is a process involved in both neuropathological conditions and brain aging. It is known that brain pathology in the form of cerebrovascular and neurodegenerative disease is a leading cause of death all over the world, with an incidence of about 2/1000 and an 8 % total death rate (KolominskyRabas et al. 1998).

Oxidative Stress and Neurodegenerative Diseases

Free radicals are molecules with an unpaired electron in their outer orbit. Oxygen radicals are involved in many biochemical activities of cells, such as signal transduction, gene transcription, and regulation of soluble guanylate cyclase activity. Nitric oxide (NO) is an important signaling molecule that essentially regulates the relaxation and proliferation of vascular smooth muscle cells, leukocyte adhesion, platelet aggregation, angiogenesis, thrombosis, vascular tone, and hemodynamics (Zheng and Storz 2000). The brain is constantly exposed to free radicals through a variety of mechanisms. The most common reported cellular free radicals are hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{-\bullet}$), and nitric monoxide ($NO^{\bullet}$). Even some other molecules like hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^{-}$) are not free radicals; they are reported to generate free radicals through various chemical reactions in many cases (Gilgun-Sherki et al. 2001). Cells exposed to an environment fortified with oxygen continuously generate oxygen free radicals (OFR). The human body produces oxygen free radicals and other reactive oxygen species as by-products through numerous physiological and biochemical processes. Oxygen-related free radicals (superoxide and hydroxyl radicals) and reactive species (hydrogen peroxide, nitric oxide, peroxynitrite, and hypochlorous acid) are produced in the body, primarily as a result of aerobic metabolism.

Overproduction of free radicals can cause oxidative damage to biomolecules (lipids, proteins, DNA), eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, postischemic perfusion injury, myocardial infarction, cardiovascular diseases, chronic inflammation, stroke, and septic shock, aging, and other degenerative diseases in humans (Freidovich 1999; Yun-Zhong et al. 2002). ROS are particularly active in the brain and neuronal tissue as the excitatory amino acids and neurotransmitters, whose metabolism is a factory of ROS, which are unique to the brain and serve as sources of oxidative stress. ROS attack glial cells and neurons, which are postmitotic cells, and therefore, they are particularly sensitive to free radicals, leading to neuronal damage (Gilgun-Sherki et al. 2001). It has been reported that the deleterious effects of ROS on human cells may end in oxidative injury leading to programmed cell death (apoptosis) (Salganik 2001).

Oxidative stress takes part in the generation of ROS and free radicals; those are potentially toxic to neuronal cells. Free radicals have been reported for their great contribution to neuronal loss in cerebral ischemia, seizure disorders, schizophrenia,

PD, and AD (Demopoulos et al. 1980; Youdim and Lavie 1994). ROS are extremely reactive to different fundamental molecules in cellular pool and initiate a cascade of reactions at the same time that leads to neuronal cell death. Oxidative overload in neuronal micro-environment causes oxidation of lipids, proteins, and DNA and generates many by-products such as peroxides, alcohols, aldehydes, ketones, and cholesterol oxide.

Oxidative stress-related neurodegeneration is not only caused by disturbed metal metabolism, but genetic evidences suggest that persons associated with certain types of genetic mutations are more susceptible to gain neurological pathologies compared to those with normal genetic profiles. Metal metabolism is the combined interplay between genes related to the synthesis of metalloenzymes and dietary metal supplement. Any imbalance in this interaction favors dysregulated cellular metallobiology that subsequently leads to neurodegeneration.

However, when levels of free radicals exceed, the normal clearing capacity of the cellular oxidative stress follows resulting in potential cellular and genome damage (van Rensburg et al. 2006). Damage to the genome could lead to altered gene dosage and gene expression as well as contribute to the risk of accelerated cell death in neuronal tissues. Oxidative stress has also been shown to be related to telomere shortening, which has also been identified in both lymphocytes and buccal cells of patients clinically diagnosed with AD (Thomas et al. 2008; Von 2009). Recent studies have suggested the positive effects of dietary antioxidants as an aid in potentially reducing somatic cell and neuronal damage by free radicals (Lim et al. 2001; Joseph et al. 1999).

It is clear from current neurobiology research that oxidative stress is one of the major causes of neuronal cell death. Any mutations in mtDNA or metal overload in the aged brain lead to oxidative stress and free radical-mediated pathological changes in neurons. Neuronal proteins and structural components get modified due to the OS in different neurological disorders leading to neuroinflammation and loss of cognitive function in AD, PD, MS, and ALS. The OS has been defined as a principal pathological cause of neurodegeneration; antioxidants are proposed as therapeutic options to combat the free radical generation and maintenance.

Avocado as a Potential Source of Antioxidants

Avocados: The avocado (*Persea americana*) originated in Mexico, Central or South America, and was first cultivated in Mexico as early as 500 BC (Duster 2000; Rainey et al. 1994). The first English language mention of avocado was in 1696. Its fruit is highly consumed (1–2 per day) in various parts of Latin America without reports of any associated toxicity (Growers 2012). In fact, avocados are used in traditional herbal medicine for the treatment of various illnesses including hypertension, stomachache, diarrhea, and diabetes (Yasir et al. 2010).

The avocado is also known as an alligator pear or bitter fruit; the versatile avocado is the only fruit that provides a substantial amount of monounsaturated fat (the healthy kind). Fat is essential for every single cell in your body. In fact, over

60 % of your brain is made of fat. Avocados are a naturally nutrient-dense food and contain nearly 20 vitamins and minerals. Avocados are a great source of vitamins C, E, K, and B6, as well as riboflavin, niacin, folate, pantothenic acid, magnesium, and potassium. They also provide lutein, beta-carotene, and omega-3 and omega-6 fatty acids.

The nutrition and phytochemical composition of Hass avocados is summarized in Tables 1, 2, 3, and 4. One-half an avocado is a nutrient- and phytochemical-dense food consisting of the following: dietary fiber (4.6 g), total sugar (0.2 g), potassium (345 mg), sodium (5.5 mg), and magnesium (19.5 mg). It also contains vitamin A (5.0 μg RAE), vitamin C (6.0 mg), vitamin E (1.3 mg), vitamin K1 (14 μg), folate (60 mg), vitamin B6 (0.2 mg), niacin (1.3 mg), pantothenic acid (1.0 mg), and riboflavin (0.1 mg). In addition, it is rich in choline (10 mg), lutein/zeaxanthin (185 μg), cryptoxanthin (18.5 μg), and phytosterols (57 mg), is high in monounsaturated fatty acids (6.7 g), and contains 114 kcal or 1.7 kcal/g (after adjusting for insoluble dietary fiber), which may support a wide range of potential health effects (ADA 2009; USDA 2011).

Table 1 Hass avocados (Persea americana) composition of edible portion (USDA, 2011)

Nutrient/phytochemical Proximates	Value per 100 g
Water (g)	72.3
Energy (kcal)	167
Energy (kcal)(insoluble fibre adjusted)	148
Protein (g)	1.96
Total fat (g)	15.4
Ash (g)	1.66
Carbohydrate, by deference (g)	8.64
Fibre, total dietare (g)	6.80
Sugars total(g)	0.30
Starch (g)	0.11

Table 2 Hass avocados (Persea americana) minerals composition of edible portion (USDA, 2011)

Minerals	Value per 100 g
Calcium (mg)	13.0
Iron (mg)	0.61
magnesium(mg)	29.0
phosphorus (mg)	54.0
Potassium (mg)	507.0
Sodium (mg)	8.0
Zinc (mg)	0.68
Copper (mg)	0.17
Manganese (mg)	0.15
Selenium (μg)	0.40

Table 3 Hass avocados (Persea americana) Vitamins and Phytochemicals composition of edible portion (USDA, 2011)

Vitamins and Phytochemicals	Value per 100 g
Vitamin C (mg)	8.80
Thiamin (mg)	0.08
Riboflavin (mg)	0.14
Niacin (mg)	1.91
Pantothenic acid(mg)	1.46
Vitamin B-6(mg)	0.29
Folate food(μg)	89.0
Vitamin A (μg RAE)	7.0
Carotene, beta (μg)	63.0
Carotene, alpha (μg)	24.0
Cryptoxanthin, beta (μg)	27.0
Lutein + zeaxanthin (μg)	271.0
Vitamin E (alpha-tocopherol), mg	1.97
Tocopherol, beta (mg)	0.04
Tocopherol, gamma (mg)	0.32
Tocopherol, delta (mg)	0.02
Vitamin K1 (phylloquinone) (μg)	21.0
Choline total (mg)	14.2
Betaine (mg)	0.7

Table 4 Hass avocados (Persea americana) Lipids composition of edible portion (USDA, 2011)

Lipids	Value per 100 g
Fatty acids, total saturated (g)	2.13
16:0 (g)	2.08
Fatty acids, total monounsaturated (g)	9.80
18:1 (g)	9.07
Fatty acids, total polyunsaturated (g)	1.82
18:2 (g)	1.67
18:3 (g)	0.13
Stigmasterol (mg)	2.0
Campesterol (mg)	5.0
Beta-sitosterol (mg)	76.0
Cholesterol (mg)	0

Further, these compound classes may be divided into alkanols (also sometimes termed “aliphatic acetogenins”), terpenoid glycosides, various furan ring-containing derivatives, flavonoids, and coumarin. 1,2,4-trihydroxyheptadec-16-ene, 1,2,4-trihy droxyheptadec-16-yne, and 1,2,4-trihydroxynonadecane, 1,2,4-trihydroxyheptadec -16-ene, persin, persenones A and B, The glycosylated abscisic acid derivatives, furanoid constituents . avocadofurans. Several flavonoids such as quercetin, afzelin and quercetin 3-*O*-D-arabinopyranoside, 2,2′-azobis(2,4-dimethylvaleronitrile), catechin and epicatechin, coumarin, scopoletin.

Avocados contain an amazing array of phytonutrients. Included are phytosterols (especially beta-sitosterol, stigmasterol, and campesterol), carotenoids (beta-carotene, alpha-carotene, lutein, neochrome, neoxanthin, chrysanthemaxanthin, beta-cryptoxanthin, zeaxanthin, and violaxanthin), flavonoids (epicatechin and epigallocatechin 3-*O*-gallate), and polyhydroxylated fatty alcohols. Alpha-linolenic acid (an omega-3 fatty acid) and oleic acid are key fats provided by avocado.

Consuming fruits and vegetables of all kinds has long been associated with a reduced risk of many lifestyle-related health conditions. Many studies have suggested that increasing the consumption of plant foods like avocados decreases the risk of obesity, diabetes, heart disease, neurodegenerative diseases, and overall mortality while promoting a healthy complexion and hair, increased energy, and overall lower weight. During the past decade, the traditional systems have gained importance in the field of medicine. The World Health Organization estimates that four billion people, 80 % of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous people's traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional, oriental, and Native American and Indian medicine.

The American Academy of Pediatrics recommends that solid foods be introduced to infants between 4 and 6 months of age and that for the first year foods be mashed or puréed. The creamy consistency and mild taste of avocados make it one of the best first fresh fruits a baby can enjoy. One-fifth of a medium avocado, or about 1 ounce, has 50 cal, along with 3.5 g of unsaturated fats, which are known to be important for normal growth and development of the central nervous system and brain.

Diabetes-Related Neurodegeneration and Avocado

Diabetes is characterized by a constant state of hyperglycemia leading in the long term to severe damage to several systems (Alberti and Zimmer 1998), including the central nervous system (CNS). Diabetes has been involved in several brain conditions such as cerebral ischemia, macrovascular disease, microangiopathy, cognitive decline, and brain atrophy (Moreira et al. 2004). However, the mechanisms underlying neuronal damage in the CNS, known as diabetic encephalopathy, are still unclear (Ola et al. 2014). Mitochondrial dysfunction has been hypothesized to be a key factor in the progression of hyperglycemia-mediated neuronal damage (Vincent et al. 2005; Ceretta et al. 2010). This is related to the large demand of the cells from the CNS for ATP to allow neurotransmission. For this reason, the maintenance of oxidative phosphorylation capacity is extremely important in the CNS since about 90 % of the ATP required for the normal function of neurons is provided by mitochondria (Moreira and Oliveira 2011). Therefore, mitochondrial dysfunction may contribute to the loss of neuronal metabolic control and, consequently, to neurodegeneration (Toth 2014).

Mitochondrial alterations related to diabetic encephalopathy include increased mitochondrial fission, excessive ROS levels (Edwards et al. 2010), augmented levels of both lipid peroxidation and nitrite, and decreased levels of total antioxidant (Acar

et al. 2012). In addition, it has been suggested that diabetes-induced oxidative stress increases the levels of pro-inflammatory cytokines, which enhances neuronal degeneration (Ola et al. 2014). Therefore, mitochondrial oxidative damage contributes, at least in part, to the development of diabetic encephalopathy (Ceretta et al. 2012).

Avocado oil prevented renal mitochondrial dysfunction in streptozotocin-induced type I diabetic rats by preserving the activity of the complex III of the electron transport chain (ETC) and attenuating ROS levels due to the protection of the integrity of cytochromes $c + c1$ (Ortiz-Avila et al. 2013). Improvement in glycemic control, plasma lipid profile, and atherogenic index has been observed in diabetic patients consuming avocado in their diets (Carranza-Madrigal et al. 2008). A candidate belonging to this group of nutraceuticals is avocado, as this fruit contains a wide variety of antioxidants, including carotenoids, tocopherols, chlorophylls, vitamins, and oleic acid (C18:1) as the main fatty acid (Dreher and Davenport 2013).

Avocado oil improves brain mitochondrial function in diabetic rats by preventing the impairment in mitochondrial respiration and $\Delta\Psi m$ induced by diabetes, besides increasing complex III activity. This may be related to decreased ROS levels and improved redox status in diabetic rats as reflected by a higher GSH/GSSG ratio (Ortiz-Avila et al. 2015).

Neuroprotection

Neuroprotection refers to the strategies and relative mechanisms able to defend the central nervous system (CNS) against neuronal injury due to both acute (e.g., stroke or trauma) and chronic neurodegenerative disorders (e.g., Alzheimer's disease and Parkinson's disease) (Harper 1992; Kumar 2006). Phytochemicals present in vegetables and fruits are believed to reduce the risk of several major diseases including cardiovascular diseases, cancers, as well as neurodegenerative disorders. Therefore, people who consume higher amounts of vegetables and fruits may be at reduced risk for some of diseases caused by neuronal dysfunction (Selvam 2008; Lobo et al. 2010).

Herbal medicine has long been used to treat neural symptoms. Although the precise mechanisms of action of herbal drugs have yet to be determined, some of them have been shown to exert anti-inflammatory and/or antioxidant effects in a variety of peripheral systems. Now, as increasing evidence indicates that neuroglia-derived chronic inflammatory responses play a pathological role in the central nervous system, anti-inflammatory herbal medicine and its constituents are being proved to be a potent neuroprotector against various brain pathologies. Structural diversity of medicinal herbs makes them a valuable source of novel lead compounds against therapeutic targets that are newly discovered by genomics, proteomics, and high-throughput screening.

Nootropics is a term used by proponents of smart drugs to describe medical drugs and nutritional supplements that have a positive effect on brain function; "nootropic" is derived from Greek and means acting on the mind. They act by selective enhancement of cerebral blood flow, cerebral oxygen usage metabolic rate, and cerebral glucose metabolic rate in chronic impaired human brain function, i.e.,

multi-infarct (stroke) dementia, senile dementia of the Alzheimer's type and pseudodementia, and ischemic cerebral (poor brain blood flow) infarcts.

In traditional practices of medicine, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases such as AD and other memory-related disorders. Herbal products contain complicated mixtures of organic chemicals, which may include fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, tannins, and terpenes, and most of them are present in avocados. Identification and characterization of new medicinal plants to cure neurodegenerative diseases and brain injuries resulting from stroke is the major and increasing scientific interest in recent years. There are more than 120 traditional medicines that are being used for the therapy of CNS disorders in Asian countries (Kumar 2006).

Antioxidants are classified as exogenous (natural or synthetic) or endogenous compounds, both responsible for removal of free radicals, scavenging ROS or their precursors, inhibiting formation of ROS, and binding metal ions needed for catalysis of ROS generation (Joseph et al. 1999).

Important Phytochemicals of Avocado and the CNS

Dr. Daniel G. Amen, author of *Change Your Brain, Change Your Life*, considers avocados as one of the top brain-healthy foods that can help prevent AD. The folate in avocado contributes to preventing the formation of brain tangles that are considered a factor for Alzheimer's. Avocados combine brain-healthy omega-3 fatty acids with natural vitamin E, which has been clinically proven to prevent AD from progressing and even reversing it in its earliest stages.

Role of Fatty Acids in Brain Function

Avocados are very rich in monounsaturated fatty acids and other polyunsaturated fatty acids (PUFAs). Consuming monounsaturated fatty acids and PUFAs was shown to slow cognitive decline in humans. The majority of studies have been focused on the omega-3 and omega-6 PUFAs found in avocado and nuts, such as walnuts which contain the monounsaturated fatty acid oleic acid (8:1) and the polyunsaturated fatty acids linoleic acid (LA) and α-linolenic acid (ALA) (Ceretta et al. 2010; Crews et al. 2005). Omega-3 fatty acids are essential for brain health and are thought to play an important role in cognitive (memory and performance) and behavioral functions.

Numerous studies have shown that consuming diets deficient in α-linolenic acid will impair cognitive functioning (McCann and Ames 2005). The structure of neurons is critical to their function as the cells must maintain appropriate electrical gradients across the membrane, with normal anchor receptors and the ion channels in proper position to communicate with other cells, and be able to release and

reabsorb unmetabolized neurotransmitters. These properties depend on the fatty acid composition of the neuronal membrane (Yehuda et al. 2002). The fatty acid composition of neuronal membranes declines during aging, but dietary supplementation with essential fatty acids was shown to improve membrane fluidity and PUFA content. In addition to affecting membrane biophysical properties, PUFAs in the form of phospholipids in neuronal membranes can also directly participate in signaling cascades to promote neuronal function, synaptic plasticity, and neuroprotection (Yehuda et al. 2002).

DNA Damage Protection

Several clinical studies suggest that xanthophylls, similar to those found in avocados, may have antioxidant and DNA-protective effects with possible healthy aging protective effects. One study was conducted involving 82 male airline pilots and frequent air travelers who are exposed to high levels of cosmic ionizing radiation known to damage DNA, potentially accelerating the aging process (Young et al. 2009). There was a significant and an inverse association between intake of vitamin C, beta-carotene, β-cryptoxanthin, and lutein-zeaxanthin from fruits and vegetables and the frequency of chromosome translocation, a biomarker of cumulative DNA damage ($p < 0.05$). In another trial, lipid peroxidation (8-epiprostaglandin F2a) was correlated inversely with plasma xanthophyll levels (Haegele et al. 2000). In other studies, inverse correlations were found between lutein and oxidative DNA damage as measured by the comet assay and in contrast to beta-carotene (Hughes et al. 2009).

Other Phytochemicals of Avocados and Their Role

The master antioxidant glutathione supports the liver and the nervous system. It is responsible for replenishing and recycling other antioxidants in the body. It is vital for a strong immune system, and avocado is one of the few foods that contain a considerable amount of glutathione (http www naturalnews com 040067 avocado cancer prevention superfood html ixzz2Rb7Hjbxs).

Aliphatic acetogenins, also known as "alkanols," are a class of compounds almost exclusively isolated from avocado. Among these compounds, persenone A was found to inhibit nitric oxide synthase and cyclooxygenase in a mouse macrophage cell line (Kim et al. 2000a) and reduced nitric oxide and superoxide generation in inflammatory leukocytes (Kim et al. 2000b). Compound [(2*S*,4*S*)-2,4-dihydroxyheptadec-16-enyl acetate] and [(2*S*,4*S*)-2,4-dihydroxyheptadec-16-ynyl acetate] showed inhibition of acetyl-CoA carboxylase (Hashimura et al. 2001).

A methanol extract of avocado fruits showed potent inhibitory activity against acetyl-CoA carboxylase, a key enzyme in fatty acid biosynthesis (acetyl-CoA carboxylase inhibitors are isolated from avocado fruit, and it is important in decreasing fatty acid synthesis, which is generally helpful in atherosclerosis). Recent studies

have suggested the positive effects of dietary antioxidants as an aid in potentially reducing somatic cell and neuronal damage by free radicals (Lim et al. 2001; Joseph et al. 1999).

Folate and Neural Tube Defects (NTDs)

Folate is extremely important for pregnant women. Adequate intake protects against miscarriage and neural tube defects. Recent research has also shown that a father's folate status before conception may be just as important. In a study from McGill University, paternal folate deficiency in mice was associated with a 30 % higher number of various birth defects than in offspring with no paternal folate deficiencies.

NTDs result in malformations of the spine (spina bifida), skull, and brain (anencephaly). They are the most common major congenital malformations of the central nervous system and result from a failure of the neural tube to close at either the upper or lower end during days 21–28 after conception (Wilson et al. 2007). The incidence of NTDs varies from 0.5 to 4.0 per 1000 births in North America (Wilson et al. 2007). Rates of spina bifida and anencephaly (the two most common types of NTDs) are highest among Hispanic women and lowest among African-American and Asian women (Raider and Schneeman 2006).

Due to its role in the synthesis of DNA and other critical cell components, folate is especially important during phases of rapid cell growth (Lamers 2011). Clear clinical trial evidence shows that when women take folic acid periconceptionally, a substantial proportion of NTDs is prevented (Wilson et al. 2007; Scott 2001). Scientists estimate that periconceptional folic acid use could reduce NTDs by 50–60 %.

Folate and Lowered Risk of Depression

Another benefit of foods with high levels of folate is a lowered risk of depression. Folate helps to prevent an excess of homocysteine forming in the body, which can block blood and other nutrients from reaching the brain. Excess homocysteine can interfere with the production of the feel-good hormones serotonin, dopamine, and norepinephrine, which regulate not only mood but sleep and appetite as well.

Folate in Dementia, Cognitive Function, and Alzheimer's Disease

Most observational studies show positive associations between elevated homocysteine levels and the incidence of both AD and dementia (Seshadri et al. 2002). Some, but not all, observational studies have also found correlations between low serum

folate concentrations and both poor cognitive function and higher risk of dementia and AD (Seshadri et al. 2002). A secondary analysis of a study conducted in Australia (which did not have mandatory folic acid fortification at that time) found that daily supplementation with 400 mcg folic acid plus 100 mcg vitamin B12 for 2 years improved some measures of cognitive function, particularly memory, in 900 adults aged 60–74 years who had depressive symptoms (Walker et al. 2012).

Avocado and Brain Function

Blood supply: Avocados improve blood supply and oxygenation to the brain. A combination of avocado and soybean fats prevented oxidation and protected against nerve damage after the animals were exposed to low-oxygen conditions. Benefits were noted in the prefrontal cortex, the area of the brain responsible for such functions as planning and critical thinking.

Brain signaling: Healthy unsaturated fats in avocados help keep your brain cell membranes flexible. The Federation of American Societies for Experimental Biology found that monounsaturated fatty acids helped protect nerve cells in the brain known as astrocytes, which provide support to information-carrying nerves. In the laboratory animal study, monounsaturated fats improved the brain's ability to control muscles in animals with impaired astrocyte function. Fish oil, also tested in this study, did not provide the same benefits. Researchers concluded that monounsaturated fats may be helpful in the treatment of certain brain disorders that involve problems with the ability of astrocytes to properly utilize lipids.

Stroke: Avocado leaf extract may offer potential benefits for reducing seizures. In the laboratory animal study, avocado leaf extract in doses ranging from 100 to 800 mg per kg of body weight helped prevent seizures in response to several substances known to induce seizures. Researchers noted that the avocado leaf extract worked by improving the transmission of the calming neurotransmitter GABA and may be useful for management of childhood epilepsy.

Vitamin K and Stroke

Coagulation is the process of blood clot formation, and vitamin K is essential for proper blood clotting. But it is also involved in the hardening of blood vessels. Insufficient dietary vitamin K may increase the risk of hardening, thereby increasing the risk of stroke. Half of an avocado contains 14 μg of vitamin K.

Avocado has clearly been shown to provide a wide variety of antioxidant and anti-inflammatory nutrients. Included here are both conventional nutrients like manganese, vitamin C, and vitamin E, as well as phytonutrients like unique carotenoids, flavonoids, and phytosterols. Most of the larger-scale, human research studies that we have seen focus on the cardiovascular system and risk of oxidative stress and

inflammation in this system. In terms of the whole body, however, and its many key physiological systems, the antioxidant and anti-inflammatory benefits of avocado have been tested primarily in the lab or in animal studies. For example, numerous animal studies have looked at the impact of avocado intake on risk of inflammation in connective tissue and have speculated about the potential benefits of avocado for reducing human arthritis risk. Because of the promising nature of these preliminary studies, we look forward to new research involving large numbers of human participants and intake of avocado in a weekly meal plan.

In today's marketplace, the largest producers of avocados are Mexico, Chile, the USA, Indonesia, the Dominican Republic, Columbia, Peru, Brazil, China, and Guatemala. Mexico is an especially large exporter of avocado into the USA, with about 500,000 metric tons of avocado being sent from Mexico to the USA each year. About 200,000 tons of avocado are produced in the state of California each year and another 35,000 tons in the state of Florida. As a result of the above global production, you are most likely to find avocados in the supermarket that were grown either in Mexico, California, Florida, or a Central American or South American country. Because of the greater total volume and slightly longer marketing season, you are also more likely to find California versus Florida avocados in the supermarket among domestic varieties.

Avocado and Absorption of Brain Health Nutrients

Fat-soluble vitamins and carotenoids, orange-yellow pigments, offer you outstanding health benefits—but only if they are absorbed up into your cells. Intake of fat along with carotenoids greatly helps to improve their absorption. However, many of our best foods for obtaining carotenoids—for example, sweet potatoes, carrots, and leafy greens—contain very little fat (less than 1 g per serving). As a special step in improving carotenoid absorption from carotenoid-rich foods, researchers have experimented with the addition of avocado to meal choices including salads, side servings of leafy greens, side servings of carrots, or tomato sauce. The amount of avocado added has varied from study to study, but averages approximately 1 cup or 1 small/medium avocado providing 20–25 g of total fat. As expected, this added avocado has been shown to increase carotenoid absorption from all of the foods listed above. Anywhere from two to six times as much absorption was found to occur with the added avocado! But in addition to this increased absorption was a much less anticipated result of a recent study: not only did avocado improve carotenoid absorption, but it also improved conversion of specific carotenoids (most importantly, beta-carotene) into active vitamin A (this unexpected health benefit of increased conversion was determined by the measurement of retinyl esters in the bloodstream of participants, which were found to increase after consumption of carrots or tomato sauce in combination with avocado). Avocados do contain carotenoids, in and of themselves.

Conclusion

A number of studies have shown that dietary intake of antioxidants from fruits and vegetables significantly reduces the risk of developing cognitive impairment. Vitamin E and vitamin C and beta-carotene inhibit the production of free radicals. The best antioxidant fruits and vegetables (from the US Department of Agriculture) are blueberries, blackberries, cranberries, strawberries, spinach, raspberries, Brussels sprouts, plums, broccoli, beets, avocados, oranges, red grapes, red bell peppers, cherries, and kiwis.

The phytochemicals present in avocado particularly antioxidants offer a safe approach to protect against the neuronal damage caused by several factors, particularly oxidative stress, and can prevent or slow down the onset of degenerative diseases. Furthermore, they may protect against neuronal loss in patients with neurodegenerative disease. Phytochemicals may be an alternative to other conventional treatment methods. Phytochemicals may control several pathological pathways. Because they are largely innocuous, neuroprotective phytochemicals derived from fruits and vegetables are attractive alternatives to pharmaceuticals such as NSAIDs and anti-degenerative molecules, which lack conclusively demonstrated clinical efficacy and are associated with significant safety concerns. Although there are clear limits for their immediate widespread use, dietary polyphenolic phytochemicals hold great promise as safe, inexpensive, and readily available prophylactic agents for AD and other neurodegenerative diseases. The continued effort to extrapolate in vitro and in vivo results in the human situation through properly designed clinical trials should help to realize the potential of this class of compounds. The diverse array of bioactive nutrients present in avocado may play a pivotal role in the prevention and cure of various neurodegenerative diseases, such as AD, PD, and other neuronal dysfunctions.

Compliance with Ethics Requirements The author declares that he/she has no conflicts of interest.

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Detoxification of Carbonyl Compounds by Carbonyl Reductase in Neurodegeneration

Mohammad Abdur Rashid, Mahmuda Haque, and Mohammed Akbar

Abstract Oxidative stress in the brain is the major cause of neurodegenerative disorders, including Alzheimer's, Parkinson's, Huntington's, and Creutzfeldt–Jakob diseases or amyotrophic lateral sclerosis. Under conditions of oxidative stress, the production of highly reactive oxygen species (ROS) overwhelms antioxidant defenses, resulting in the modification of macromolecules and their deposition in neuronal cell tissues. ROS plays an important role in neuronal cell death that they generate reactive aldehydes from membrane lipid peroxidation. Several neuronal diseases are associated with increased accumulation of abnormal protein adducts of reactive aldehydes, which mediate oxidative stress-linked pathological events, including cell growth inhibition and apoptosis induction. Combining findings on neurodegeneration and oxidative stress in *Drosophila* with studies on the metabolic characteristics of the human enzyme CBR1, it is clear now that CBR1 has a potential physiological role of neuroprotection in humans. Several studies suggest that CBR1 represents a significant pathway for the detoxification of reactive aldehydes derived from lipid peroxidation and that CBR1 in humans is essential for neuronal cell survival and to confer protection against oxidative stress-induced brain degeneration. Recently, it was discovered that HIF1alpha, AP-1, and Nrf2 could all regulate CBR1 at the transcriptional level. Nrf2 is known to regulate the transcription of antioxidant enzymes, and CBR1 functions as an antioxidant enzyme, suggesting that transcriptional regulation of CBR1 is a major contributor to the control of oxidative stress in neurodegeneration.

Keywords Carbonyl reductase in neurodegeneration • Oxidative stress • Reactive lipid aldehyde

M.A. Rashid, Ph.D. (✉)
Laboratory of Molecular Signaling, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Rockville, MD 20852, USA
e-mail: rashid_biochem@yahoo.com

M. Haque
Department of Pharmacy, Southeast University, Dhaka 1213, Bangladesh

M. Akbar
Laboratory of Molecular Pharmacology and Toxicology, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Rockville, MD 20852, USA

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Introduction

Carbonyl reductase (CBR1, CBR2, and CBR3) is an NADPH-dependent, monomeric, and cytosolic enzyme belonging to a family of short-chain dehydrogenases/reductases. The human enzyme consists of 277 amino acid residues and was named carbonyl reductase 1 (CBR1) owing to its properties to reduce efficiently various endogenous and xenobiotic carbonyl compounds. CBR1 is widely distributed in human tissues such as liver, epidermis, stomach, small intestine, kidney, neuronal cells, and smooth muscle fibers (Forrest and Gonzalez 2000). The best substrates of CBR1 are quinones, including ubiquinone-1 and tocopherol quinone (vitamin E). Ubiquinones (coenzyme Q) are constitutive parts of the respiratory chain, and tocopherol quinone protects lipids of biological membranes against lipid peroxidation, indicating that CBR1 may play an important role as an oxidation–reduction catalyst in biological processes (Wermuth 1981). Furthermore, CBR1 inactivates highly reactive lipid aldehydes, such as 4-oxonon-2-enal (ONE), 4-hydroxynon-2-enal (HNE), and acrolein, which are able to modify protein and DNA damage within cells (Oppermann 2007). A mutation in the gene encoding a homolog of CBR1 causes oxidative stress-induced neurodegeneration in *Drosophila melanogaster* (Botella et al. 2004), and overexpression of the human CBR1 in NIH3T3 cells protects from ROS-induced cellular damage (Kelner et al. 1997), and CBR1 was also shown to regulate apoptosis and cell survival in insulin-secreting cells by reducing oxidative stress (Rashid et al. 2010). Recently, it was discovered that HIF1alpha, AP-1, and Nrf2 could all regulate CBR1 at the transcriptional level (Jang et al. 2012; Tak et al. 2011). Collectively, these findings indicate that transcriptionally regulated CBR1 is a major contributor to the control of oxidative stress.

CBR1 Metabolism of Xenobiotic Carbonyls and Quinones

CBR1 metabolizes a broad spectrum of substrates, which generally results in detoxification or inactivation of the more chemically reactive carbonyl groups. The ability to metabolize aromatic ketones and quinones is a characteristic of CBR1. CBR1 is involved in prostaglandin and steroid metabolism (Wermuth 1982; Lee and Levine 1974). Wermuth showed that quinones derived from polycyclic aromatic hydrocarbons (PAH) were much better substrates than prostaglandins or steroids. The xenobiotic substrates shown to be metabolized by CBR1 include o-quinones derived from PAH or *p*-quinones, such as menadione (Wermuth et al. 1986), as well as an extraordinarily wide spectrum of xenobiotic carbonyls, such as anthracyclines, metyrapone, or the carcinogen 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (Atalla et al. 2000; Wermuth 1981). Accordingly, the enzyme fulfills an important role in the phase I metabolism of xenobiotics. Human CBR1A is the major hepatic reductase of PAH-derived quinones (Wermuth et al. 1986), suggesting a major role in detoxification of these compounds. This is in contrast to the metabolic preference in rat liver, where NADPH-dependent quinone reductase (NQO1) is the major

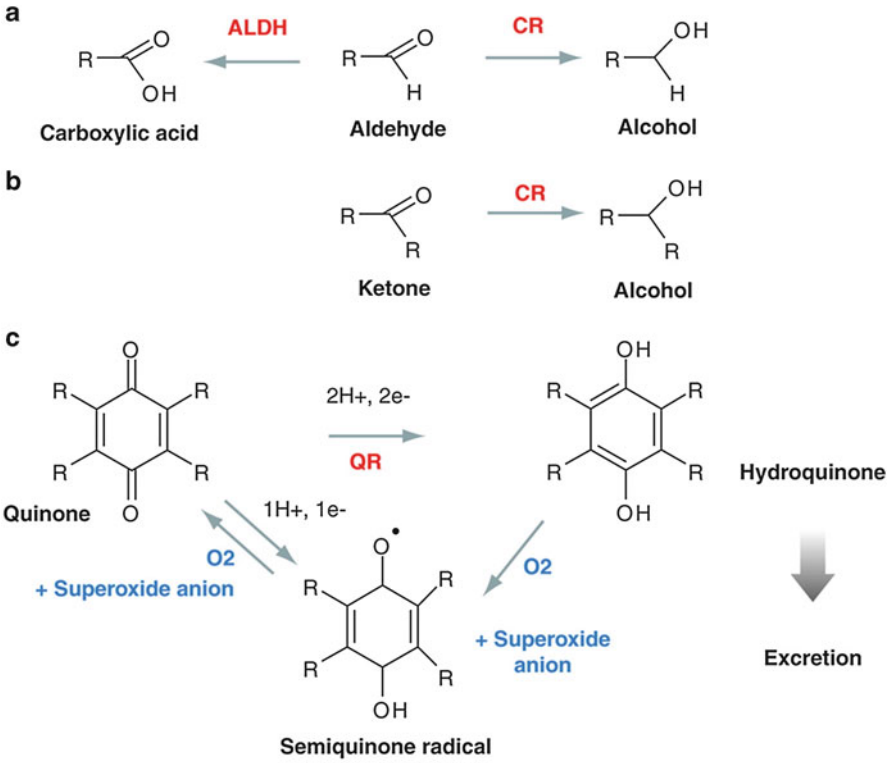

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Fig. 1 Metabolic conversion of carbonyl compounds. (**a**) Aldehydes can be converted by aldehyde dehydrogenases (ALDHs) to carboxylic acids or by carbonyl-reducing (CR) enzymes to the primary alcohol. (**b**) Ketones are reduced to the corresponding alcohol by CR. (**c**) Quinone reduction and redox cycling. Quinone reduction by one-electron transfer yields the semiquinone radical. Two-electron reduction of quinones by quinone-reducing (QR) enzymes yields the hydroquinone, which can be excreted or be oxidized to the semiquinone radical. Both the hydroquinone and semiquinone states can be oxidized in the presence of molecular oxygen (O_2), yielding superoxide anion, thereby sustaining oxidative stress

quinone reductase. However, in the absence of superoxide dismutase (SOD), quinone reduction by CBR1 leads to redox cycling, with generation of superoxide anion and semiquinone radicals mediated through one-electron transfer from the reduced hydroquinones to molecular oxygen (Fig. 1) (Jarabak and Harvey 1993). Thus, CBR1 is an important determinant in the metabolism of PAH quinones; however, a possible protective role against quinone toxicity exerted by CBR1 depends on expression and activity of SOD and further metabolism, reactivity, and excretion of the hydroquinone formed. The CBR1-mediated metabolism of the anthracycline cytostatic agent, doxorubicin, to its alcohol metabolite constitutes an important

determinant in the observed cardiotoxicity because mice heterozygous for a CBR1-null allele show decreased sensitivity to the cardiotoxic effects of the anthracycline alcohol metabolite (Olson et al. 2003). A recent study indicates that CBR1 is involved in tumor metastasis, and mouse lung adenocarcinoma showed distinct metastatic properties that were correlated with CBR1 expression levels. These effects were attributed to the prostaglandin-modulating activities of CBR1; however, further experimental proof is needed. Endogenous substrates for CBR1 comprise 5α-reduced steroids and prostaglandins (Wermuth 1981), but the observed relatively low kinetic constants obtained in vitro for steroid and eicosanoid conversion argue against a physiological role in the metabolism of these lipid hormones and mediators in humans, although this issue remains controversial (Kelner et al. 1997).

Role of CBR1 in Oxidative Stress and Apoptosis

Many recent studies could provide essential clues as to the physiological role of CBR1. First, a screen of viable P-element insertions in the fruit fly *Drosophila melanogaster* resulted in the discovery of a novel hypomorphic mutant, *Sniffer*, which shows age-related severe neurological damages and increased apoptosis in the CNS (Botella et al. 2004). The *Sniffer* gene represents the *Drosophila* ortholog of CBR1, and subsequent structure determination provided a model consistent with results on the mammalian structures (Tanaka et al. 2005; Sgraja et al. 2004). Notably, the *Sniffer* is essential to the prevention of oxidative stress-induced neurodegeneration and cell death, and overexpression of the *Sniffer* gene results in neuronal protection against oxygen-induced apoptosis (Botella et al. 2004). These data are consistent with a role of *Sniffer*/CBR1 in the detoxification of a metabolite produced under conditions of oxidative stress. A possible experimental proof for this concept was provided by a study demonstrating in vitro that recombinant human CBR1 metabolizes and inactivates lipid aldehydes (Doorn et al. 2004), such as the highly reactive and genotoxic 4-oxonon-2-enal product, formed during oxidative stress, to less reactive metabolites by reducing the carbonyl groups and the double bond, yielding 4-hydroxynon-2-enal, 1-hydroxy-2-en-4-one, and 4-oxononanal (Doorn et al. 2004). These metabolites are thought to be less reactive than the parent compound (Doorn et al. 2004; Doorn and Petersen 2003), and 4-keto reductase activity was also observed with the glutathione adduct. Additionally, results with murine NIH3T3 cells transfected with human CBR1 showed increased resistance against paraquat, a toxic herbicide known to generate ROS (Kelner et al. 1997). These results, as well as other protective effects reported for CBR1 toward quinones, could be due to inactivation of reactive lipid aldehydes formed through oxidative stress. Combined, these data suggest the neuroprotective role of CBR1 against aging and neurodegeneration, such as Alzheimer's or Parkinson's disease (Fig. 2). In another study on selective CBR1 inhibitors or CBR1-directed RNA revealed that CBR1 plays an essential role in serum withdrawal-induced apoptosis in A549 cells (Tanaka et al.

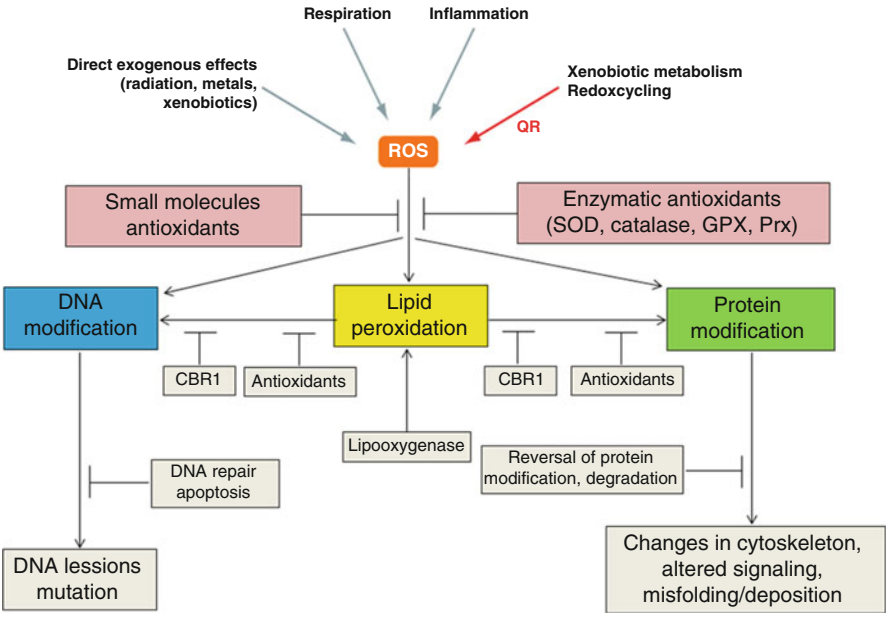

Fig. 2 Schematic overview on the involvement of quinone (QR) and carbonyl reductases (CBR1) in oxidative stress. ROS are produced through different initiating events, such as direct exogenous effects, aerobic metabolism, inflammation, and redox cycling of quinones. The ROS generated cause DNA lesions and mutations (eventually leading to neoplastic transformation) or protein modifications with altered cytoskeleton, protein degradation, misfolding and deposition, or signaling (eventually leading to degenerative diseases). ROS can also initiate lipid peroxidation if not inactivated by primary antioxidants, such as α-tocopherol, glutathione, or ascorbic acid. Inactivation of ROS is also achieved through enzymatic inactivation by superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), or peroxiredoxins (Prx). Lipid peroxidation products comprise highly reactive lipid aldehydes, such as 4-oxonon-2-enal, 4-hydroxynon-2-enal, and acrolein, which are able to modify proteins and DNA. The reactive lipid metabolites can be inactivated by a variety of CBR1, as discussed in the text

2005). Treatment with the inhibitor 3-hydroxy-PP-Me resulted in a dose-dependent, nearly complete reversal of apoptosis induced by serum starvation, indicating a pro-apoptotic function of CBR1, which may be metabolizing a yet unidentified physiological substrate that is formed upon serum withdrawal. This pathway is apparently not directly linked to p53 function or interaction because no p53 increase or ubiquitination was observed (Tanaka et al. 2005). These results could further indicate that the observed nonviability of the CBR1-null mouse is due to developmental deregulation related to lack of elimination of progenitor cells due to a missing proapoptotic function of CBR1 (Olson et al. 2003). Obviously, despite the clear indications for a neuroprotective role of CBR1 of the *Sniffer* model, further studies are necessary to clarify and correlate the roles of CBR1 in neurodegeneration and development with the observed proapoptotic function.

Reactive Aldehydes Derived from Lipid Peroxidation Are Downstream Effectors of Oxidative Stress

Lipid peroxidation by ROS plays a central role in the pathogenesis of oxidative stress (Circu and Aw 2010). Lipid peroxidation products comprise highly reactive lipid aldehydes, such as ONE, HNE, MDA, and acrolein, which are able to cause protein and DNA damage within cells. Furthermore, lipid peroxidation contains an α, β-unsaturated aldehyde capable of reacting with cysteine, histidine, and lysine in proteins via Michael addition to the C=C bond. Primary amines may alternatively react with the carbonyl group to form Schiff bases (Nadkarni and Sayre 1995). It has been shown that under pathological conditions, these lipid-derived reactive aldehydes are downstream effectors of free radicals. Importantly, a large number of disease states, including Parkinson's and Alzheimer's disease, CNS trauma, diabetes, atherosclerosis, hypertension, ethanol toxicity, and HIV dementia, are associated with increased accumulation of protein adducts derived of reactive aldehydes produced from lipid peroxidation (Shapiro 1998).

Lipid-derived reactive aldehydes react with multiple cell surface and intracellular sites for triggering a network of signal transduction that ultimately focuses on the suppression of cellular functions (Lin et al. 2005). Apparently, these protein adducts mimic ligand cell surface receptor binding, thereby activating tyrosine kinases such as epithelial growth factor receptor (EGFR). Intracellular, reactive aldehyde-mediated scavenging of glutathione leads to the activation of caspases and to DNA fragmentation through a FAS-independent and mitochondria-linked proapoptotic signal pathway. By direct covalent modification and inactivation of IκB, reactive aldehydes are suggested to downregulate the NF-κB-mediated cellular response. In various cell lines, reactive aldehyde-induced apoptosis was accompanied by *c-Jun* N-terminal kinase and caspase-3 activation. In NT2 neurons, lipid aldehyde-mediated βPKC activation induced an increase in intracellular amyloid production. A formation of 4-hydroxyalkenals due to lipoprotein oxidation is also a central feature of atherosclerosis and has been shown to drive macrophage uptake and subintimal accumulation of oxidized lipids and lesion progression (Lusis 2000). Therefore, understanding the toxicity and metabolism of reactive aldehydes is of high clinical interest and may help in identifying genetic elements that confer protection against oxidative stress-induced neurodegeneration.

Detoxification of Reactive Aldehydes Occurs Through Carbonyl Reduction

Importantly, the different response with respect to the endpoints of toxicity most probably depends on the different metabolizing capacities and thus the action of different metabolites of reactive lipid aldehydes. It is clear that degradation pathways for these compounds are an important part of the secondary antioxidative defense system in order to protect mammalian cells against aldehydic lipid peroxidation products (Siems and Grune 2003). In general, routes of metabolism for these

carbonylic lipids include spontaneous and enzyme-catalyzed glutathione (GSH) conjugation and either oxidation by aldehyde dehydrogenase or carbonyl reduction catalyzed by enzymes from two different protein superfamilies, the aldo-keto reductases (AKR) (Jez et al. 1997) and the short-chain dehydrogenases (SDR) (Jornvall et al. 1995). CBR1 is a member of the superfamily of SDR, and it plays an important role in the detoxification of reactive lipid aldehydes (Rashid et al. 2010).

CBR1 has been first isolated and cloned from the human brain, suggesting that the brain may be an important site of CBR1 distribution. The enzyme was found to have acted toward physiologic compounds such as steroids and prostaglandins, but the low catalytic efficiency reported raised skepticism about these ketone-containing molecules as being endogenous substrates (Wermuth 1981). Since CBR1 catalyzes the NADPH-dependent reduction of a wide variety of xenobiotic carbonyl compounds, it has been attributed a significant role in the protection against deleterious aldehydes and ketones (Oppermann and Maser 2000). Interestingly, the *Sniffer* gene is expressed within the entire cortex of the fly brain, where all neuronal cell bodies are localized. The *Sniffer* is, so far, the only known functional carbonyl reductase in *Drosophila melanogaster* which emphasizes the importance of this enzyme as a neuroprotective agent (Botella et al. 2004).

Carbonyl Reductase Plays a Key Role in the Detoxification of Reactive Aldehydes Derived from Lipid Peroxidation

CBR1 was confirmed for the first time to have activity toward a product of lipid peroxidation in that it catalyzed the reduction of 4-oxonon-2-enal (ONE) (Doorn et al. 2004). ONE was found to be a major product of lipid peroxidation and is a mainly potent genotoxin, which reacts with DNA bases to form substituted etheno-adducts. Failure to repair these DNA lesions can lead to mutations or apoptosis (Dawson and Dawson 2003). Furthermore, ONE is highly reactive toward the protein nucleophiles cysteine, histidine, lysine, and arginine and also toward thiols, suggesting that it may also play a role in lipid peroxidation-mediated cross-linking of proteins. Importantly, ONE has been found to be both more neurotoxic and more protein reactive than HNE (Lin et al. 2005). NADPH-dependent ONE ketone reduction resulted in the production of HNE, and ONE aldehyde reduction yielded 1-hydroxynon-2-en-4-one (HNO) (Doorn et al. 2004). In addition, CBR1 was demonstrated for the first time to catalyze the hydride transfer to the C=C double bond of ONE, resulting in 4-oxononanal (ONA). Interestingly, the glutathione adduct of ONE (GS-ONE) was also found to be a substrate for CBR1, resulting in the production of GS-HNE. At a first glance, compared to CBR1, the enzyme AKR1B1 (aldose reductase) from the AKR superfamily exhibits a higher k_{cat}/K_m (due to a lower K_m) for ONE and GS-ONE reduction (Doorn et al. 2003). In addition, the fact that CBR1 produces another reactive lipid aldehyde, HNE, from ONE may indicate that AKR1B1 is the most important catalyst for ONE and GS-ONE reduction in several tissues. However, several studies suggest that CBR1 may represent the superior enzyme for the metabolism of lipid aldehydes.

First, ONE, but not HNE, is the major product of the breakdown of lipid hydroperoxides, and covalent modifications of DNA bases were identified as a result from ONE rather than from HNE (Lee et al. 2001). In addition, ONE is both more neurotoxic and more protein reactive than HNE (Lin et al. 2005).

Second, ONA lacks an α, β-unsaturated carbonyl, is therefore not a Michael acceptor, and may represent a final detoxification product of ONE. There is also strong evidence that ONA undergoes subsequent reduction catalyzed by CBR1 to form 4-hydroxynonanal or 1-hydroxynonan-4-one which most probably exists primarily as a lactol and, therefore, may not have a free aldehyde to react with amines.

Third, the characteristic of CBR1 to enzymatically generate products that are potential substrates for further reduction indicates the existence of a pathway in which products of lipid peroxidation are sequentially detoxified. HNO and HNE are much less reactive toward thiols than ONE (i.e., 55-fold for HNO and 110-fold for HNE) but are still electrophilic Michael acceptors (Doorn and Petersen 2002). HNE in turn is a substrate for AKR1B1 (carbonyl reduction) and/or ALDH (aldehyde dehydrogenation). Both reactions yield inactive products, namely, the diol (DHN) and/or acid (HNA). By catalyzing ONE carbonyl reduction, CBR1 initiates the detoxification of reactive aldehydes and therefore seems to play a unique role in this pathway (Fig. 3).

Fourth, CBR1 expression has been found to be upregulated in brains suffering from Alzheimer's disease, suggesting a role in the oxidative stress-induced detoxification of highly reactive carbonyl groups (Balcz et al. 2001). Finally, the finding

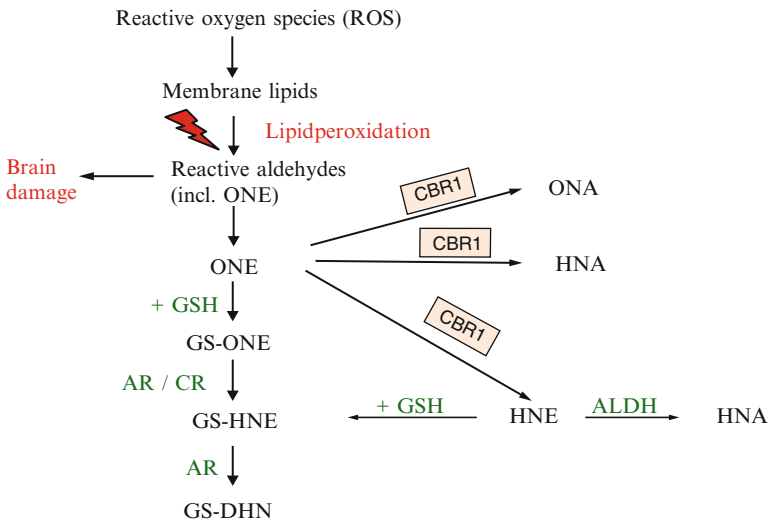

Fig. 3 CBR1 plays an important role in detoxification of reactive aldehydes. Scheme on the metabolic inactivation of the lipid peroxidation product 4-oxonon-2-enal. Abbreviations: *DHN* 4-dihydroxynonene, *HNA* 4-hydroxynonan-acid, *HNE* 4-hydroxynon-2-enal, *HNO* 1-hydroxynon-2-en-4-one, *ONA* 4-oxononanal, *ONE* 4-oxonon-2-enal, *GSH* reduced glutathione, *AR* aldose reductase (AKR1B1), *ALDH* aldehyde dehydrogenase, *CBR1* carbonyl reductase

that the CBR1 homolog in *Drosophila*, Sniffer, protects against brain degeneration induced by oxygen underlines the importance of this enzyme against oxidative stress in neurons.

Transcriptional Regulation of CBR1 Is a Major Contributor to the Control of Oxidative Stress

The gene expression of *CBR1* is known to be regulated by the aryl hydrocarbon receptor, HIF-1α, and AP-1 (Lakhman et al. 2007; Kalabus et al. 2012; Tak et al. 2011; Jang et al. 2012). Furthermore, because the expression of CBR1 protein increased on treatment with clofibric acid, which is an agonist of peroxisome proliferator-activated receptors (PPARs) α, the CBR1 activity may also be related to PPAR activity. In addition, luciferase gene reporter's observations revealed that clofibric acid induced the promoter activity of human CBR1 in HepG2 cells. It has been found that Nrf2 is a novel transcriptional regulator of CBR1 genes in humans and the Chinese hamster. Nrf2 is known to regulate the transcription of antioxidant enzymes, and CBR1 functions as an antioxidant enzyme, which suggests the existence of a link between the physiological function and transcriptional regulation of CBR1 (Giudice et al. 2010; Martin et al. 2011; Rashid et al. 2010).

Conclusion

Carbonyl groups (ketones, aldehydes) are formed during endogenous metabolism or are found in xenobiotics. Reduction of xenobiotic carbonyls is a significant metabolic route to produce more soluble and often less toxic compounds, which can be conjugated and excreted. The major xenobiotic metabolizing enzymes of CBR1 participate in the detoxification of reactive lipid aldehydes, formed during oxidative stress and lipid peroxidation, and play a protective role in neurodegeneration. Until recently, a causal relationship between the generation of high concentrations of reactive lipid aldehydes in neuronal tissues during oxidative stress and tissue injury has been difficult to establish. The detection of the *Sniffer* gene in *Drosophila* was the first direct link between oxidative stress and neurodegeneration at the molecular level. This finding was extended by results showing that the human homolog, CBR1, catalyzes the detoxification of lipid peroxidation products, thereby unraveling the neuroprotective mechanism of this enzyme. These findings open up new avenues for further understanding of the pathology of oxidative stress-induced brain dysfunction as well as providing the base for the identification of putative neuroprotective agents. Studies with CBR1 knockout systems in vivo and in vitro may further underline the importance of this enzyme. In addition, interindividual differences (genetic polymorphisms) in the expression of CBR1 could help to explain the susceptibility of people suffering from neurodegenerative

diseases. Importantly, Nrf2 is known to regulate the transcription of antioxidant enzymes, and CBR1 functions as an antioxidant enzyme, suggesting that transcriptional regulation of CBR1 is a major contributor to the control of oxidative stress in neurodegeneration. As a long-term goal, carbonyl-trapping agents and/or CBR1 enzyme inducers may offer new opportunities for improved clinical management of neurodegenerative disorders.

Compliance with Ethics Requirements The authors declare that they have no conflicts of interest.

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Role of Polyunsaturated Fatty Acids and Their Metabolites on Stem Cell Proliferation and Differentiation

Mohammad Abdur Rashid, Mahmuda Haque, and Mohammed Akbar

Abstract The nervous system is highly enriched with long-chain polyunsaturated fatty acids (PUFAs). Essential fatty acids, namely, ω-6 ($n-6$) and ω-3 ($n-3$) PUFA, and their metabolites are critical components of cell structure and function and could therefore influence stem cell fate. The available supporting experimental data reveal that $n-6$ and $n-3$ PUFA and their metabolites can act through multiple mechanisms to promote the proliferation and differentiation of various stem cell types. PUFAs and their mediators regulate several processes within the brain, such as neurotransmission, cell survival and neuroinflammation, and thereby mood and cognition. PUFA levels and the signaling pathways that they regulate are altered in various neurological disorders, including Alzheimer's disease and major depression. Therefore, elucidating the role of PUFAs and their metabolites in stem cell fate regulation is important for stem cell biology as well as stem cell therapy. PUFA-based interventions to generate a positive environment for stem cell proliferation or differentiation might be a promising and practical approach to controlling stem cell fate for clinical applications.

Keywords Polyunsaturated fatty acids • Stem cell proliferation and differentiation

Introduction

Polyunsaturated fatty acids (PUFAs) are essential dietary nutrients because they constitute part of the cell membrane, are a source of energy, and function as signaling molecules. Metabolites of PUFAs are strong lipid mediators and play a role in

M.A. Rashid, Ph.D. (✉)
Laboratory of Molecular Signaling, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Rockville, MD 20852, USA
e-mail: rashid_biochem@yahoo.com

M. Haque
Department of Pharmacy, Southeast University, Dhaka 1213, Bangladesh

M. Akbar
Laboratory of Molecular Pharmacology and Toxicology, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Rockville, MD 20852, USA

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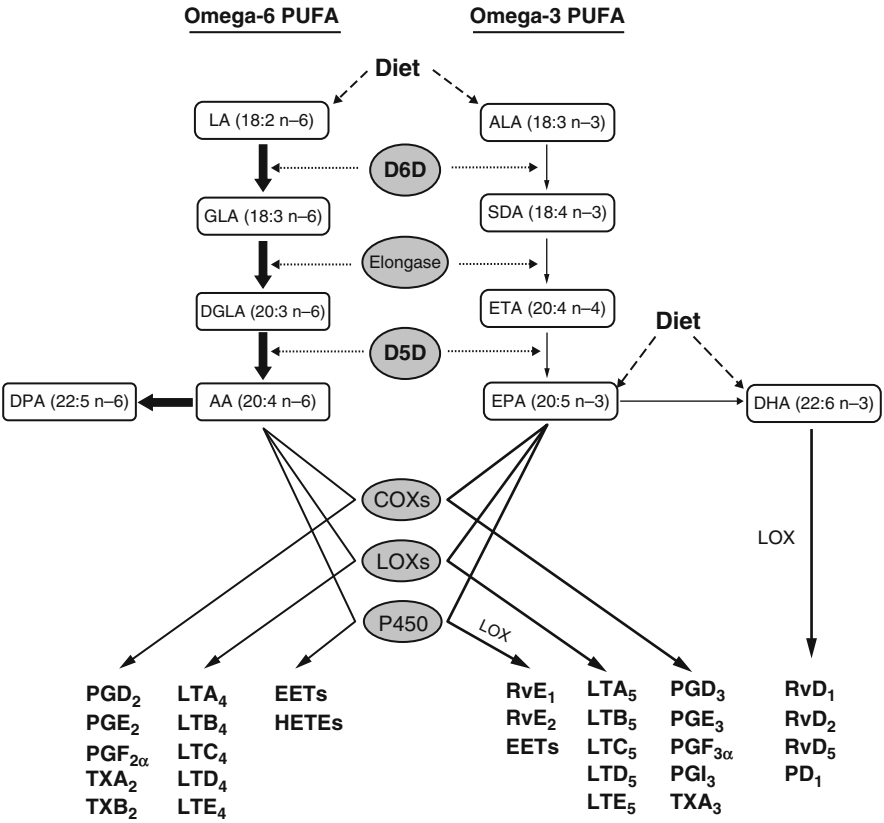

Fig. 1 The metabolic pathways of PUFA. The metabolic conversion for $n-6$ PUFA and $n-3$ PUFA. COX, LOX, and cytochrome P450 convert $n-6$ arachidonic acid and $n-3$ EPA into eicosanoids, including PG, LT, TX, and HETE. The $n-3$ DHA can be converted into Rv and PD. Abbreviations: *ALA* a-linolenic acid, *COX* cyclooxygenases, *DHA* docosahexaenoic acid, *DGLA* dihomo-gamma-linolenic acid, *EPA* eicosapentaenoic acid, *ETA* eicosatetraenoic acid, *GLA* gamma-linolenic acid, *HETE* hydroxyeicosatetraenoic acid, *LA* linoleic acid, *LOX* lipoxygenases, *LT* leukotrienes, *PD* protectins, *PG* prostaglandins, *PUFA* polyunsaturated fatty acid, *Rv* resolvins, *SDA* stearidonic acid, *TX* thromboxanes

regulating inflammation. However, increasing evidence indicates that lipids, particularly the ω-6 ($n-6$) and ω-3 ($n-3$) PUFA, play significant roles in cell signaling and gene expression and are thereby linked to many physiological and pathological processes. The $n-6$ linoleic acid (LA, 18:2$n-6$) and $n-3$ α-linolenic acid (ALA, 18:3$n-3$) are essential fatty acids, as they cannot be synthesized de novo in mammals and must therefore be obtained from the diet (Kang 2011). Omega-6 and $n-3$ PUFA are not interconvertible (Kang 2011). LA and ALA share the same metabolic enzymes and are converted through a series of desaturation and elongation reactions into their respective 20-carbon products: arachidonic acid (AA; 20:4$n-6$) and eicosapentaenoic acid (EPA; 20:5$n-3$) (Fig. 1). AA and EPA serve as the primary precursors for the synthesis of lipid mediators, including prostaglandins (PG), leukotrienes

(LT), thromboxanes (TX), resolvins (Rv), protectins, and epoxyeicosatrienoic acids, through three major pathways: cyclooxygenase (COX), lipoxygenase, and cytochrome P450 (Fig. 1). Another important $n-3$ PUFA, docosahexaenoic acid (DHA, 22:6$n-3$), can be derived from EPA or from the diet and is a main constituent of the brain and nervous system that can be metabolized to form key lipid mediators, such as Rv and protectins (Ariel and Serhan 2007). Lipid mediators derived from $n-6$ and $n-3$ PUFA are metabolically distinct and often have opposing physiological and pathological functions; for example, $n-6$ PUFA-derived eicosanoids tend to promote inflammation, while $n-3$ PUFA-derived lipid mediators largely inhibit inflammation (James et al. 2000).

Essential fatty acids and their metabolites can exert their biological effects via multiple mechanisms. PUFAs can be readily incorporated into membrane phospholipids, altering the chemical and physical properties of cell membranes and lipid rafts and thereby modulating the activity of membrane-associated functional proteins, such as ion channels and receptors (Turk and Chapkin 2013; Quest et al. 2004). The eicosanoids and lipid mediators derived from PUFAs can act as cell signaling messengers by binding to corresponding receptors and initiating signal transduction and gene expression; for example, prostaglandin E2 (PGE2), derived from $n-6$ AA, can bind to the EP2 receptor to activate pathways related to cell growth and proliferation (Yun et al. 2011). More importantly, PUFA-derived eicosanoids and lipid mediators can serve as ligands or coactivators for a number of key transcriptional factors, such as peroxisome proliferator-activated receptors (PPARs), sterol regulatory element-binding proteins, nuclear factor kappa B, and activator protein-1 (Rajasingh and Bright 2006; Liu et al. 2007; Chapkin et al. 2009; Iwahashi et al. 2000). Activation of these transcriptional factors has profound effects on cell proliferation and differentiation. PUFAs can also influence the structure of lipid rafts in the cell membrane and subsequently modify cellular processes such as receptor-mediated signal transduction (Grimm et al. 2011; Langelier et al. 2010; Shaikh 2012). Lipid rafts have been reported to play an important role in regulating stem cell self-renewal, cell cycle, survival, and induction of apoptosis (Lee et al. 2010; Yamazaki et al. 2006). In addition, essential fatty acids and their metabolites are involved in energy metabolism and interact with other functional proteins or genes to affect cellular processes (Lands 2012). Overall, it is highly conceivable that PUFAs and their metabolites may play a significant role in stem cell proliferation and differentiation.

Effect of PUFAs on Stem Cell Proliferation

The effect of PUFAs on stem cell proliferation has mainly focused on eicosanoids. Studies on the role of PGs, the major PUFA metabolites derived through the COX-2 pathway, in stem cell proliferation date back to the 1970s, when E-type PGs were shown to stimulate proliferation of hematopoietic stem cells (HSCs) (Feher and Gidali 1974; Gidali and Feher 1977). Since then, numerous studies have supported regulatory roles for PGs and other eicosanoids in various types of stem cells,

including hematopoietic, embryonic, mesenchymal, and NSCs. PGE2, the most abundant eicosanoid derived from $n-6$ AA, has been shown to have profound effects on stem cell proliferation. Indeed, treatment with a long-acting PGE2 analog after irradiation resulted in enhanced recovery of hematopoiesis and increased survival of severely irradiated mice (Hoggatt et al. 2013; Porter et al. 2013). PGE2 has also been shown to directly regulate Wnt activity through cAMP/PKA-mediated regulation of β-catenin protein stability in vivo in HSCs and the hematopoietic niche during vertebrate development and organ regeneration (Goessling et al. 2009). Overall, PGE2 seems to have a proliferative effect on HSCs. PGE2 has also been shown to affect other types of stem cells, such as ESCs and mesenchymal stem cells (MSCs). PGE2 may increase the proliferation of ESCs through upregulating the expression of cell cycle regulatory proteins and the percentage of cells in the S phase, mediated by EP1 receptor-dependent protein kinase C (PKC) and epidermal growth factor receptor-dependent phosphoinositide 3-kinase (PI3K)/Akt signaling pathways (Yun et al. 2009). Furthermore, PGE2 may stimulate human umbilical cord blood-derived MSC proliferation through β-catenin-mediated c-Myc and vascular endothelial growth factor expression via exchange protein directly activated by cAMP (Epac1)/Ras-related protein 1 (Rap1)/Akt and PKA cooperation (Jang et al. 2012) and through interaction of profilin-1 (Pfn-1) and filamentous actin (F-actin) via EP2 receptor-dependent β-arrestin-1/JNK signaling pathways. In addition, numerous studies have shown that inhibitors of COX-2, an inducible enzyme for PG and TX synthesis, suppressed neurogenesis in the injured brain (Jung et al. 2006; Goncalves et al. 2010; Sasaki et al. 2003), suggesting that PGE2 also enhances the proliferation of NSCs. Other classes of eicosanoids, including LT and TX, have also been investigated for their effects on stem cell proliferation. Physiological levels of LTB4 were shown to promote NSC proliferation, while excessive LTB4 levels inhibited NSC growth (Wada et al. 2006), suggesting a critical concentration range of LTB4 for NSC survival and proliferation. LTB4 was also shown to induce proliferation of HSC through interacting with BLT2 (the low-affinity LTB4 receptor) and exerting anti-apoptotic effects on the stem cells (Chung et al. 2005). LTD4, which plays a key role in paracrine or autocrine regulations of embryonic and fetal functions (Sato et al. 2008), has been shown to stimulate mouse ESC proliferation and migration through the signal transducer and activator of transcription-3, PI3K/Akt, Ca^{2+}–calcineurin, and glycogen synthase kinase 3β/β-catenin pathway (Kim et al. 2010). Omega-6 LA was reported to enhance mouse ESC proliferation via Ca^{2+}/PKC, PI3K/Akt, and mitogen-activated protein kinase (MAPK) signaling pathways (Kim et al. 2009a, b). The long-chain PUFA $n-6$ AA and $n-3$ DHA have been more thoroughly investigated for their role in NSC regulation, as they are critical for neural regeneration. Many studies have been reported that $n-6$ AA and $n-3$ DHA can increase the proliferation of NSCs (He et al. 2009; Sakayori et al. 2011; Kawakita et al. 2006; Sakamoto et al. 2007). In particular, DHA has been reported to promote neurogenesis in vitro and in vivo (Beltz et al. 2007). Many studies suggest that essential fatty acids and their metabolites, especially $n-6$ PUFA-derived mediators, have profound effects on the proliferation of different stem cell types.

Effect of PUFAs on Stem Cell Differentiation

The major effect of $n-3$ PUFA, especially DHA, promotes NSC differentiation. DHA has also been shown to enhance the differentiation of ESCs into neurons and promote neuritogenesis in vitro and in vivo, as shown by increased neurite length, neurite number, and number of neurite branches, which are hallmarks of neuronal differentiation (Vaca et al. 2008). DHA and EPA have also been reported to induce neuronal differentiation through Hes1 and/or Hes6 pathways, which is critical for regulating NSC differentiation (Katakura et al. 2009). While $n-6$ AA alone was found to have no effect on NSC differentiation, combined supplementation of DHA and AA appeared to enhance neuronal differentiation of bone marrow-derived MSCs, suggesting that DHA and AA may have a synergistic effect on NSC differentiation (Kan et al. 2007). In addition, on the role of essential fatty acid-derived eicosanoids in stem cell differentiation, studies have mainly focused on the effects of $n-6$ PUFA-derived metabolites, including PGE2, LTB4, and TXA2. PGE2 has been shown to promote endothelial differentiation from bone marrow-derived cells through AMP-activated protein kinase (AMPK) activation (Zhu et al. 2011), osteogenic differentiation of rat tendon stem cells via PI3K/Akt signaling (Liu et al. 2012), and differentiation of dendritic cells from CD34+ HSCs. LTB4 has been shown to promote differentiation of NSCs into neurons, as indicated by enhanced neurite outgrowth upon exposure to LTB4 (Wada et al. 2006), while LT synthesis is reported to be critical for the hedgehog-dependent neural differentiation of embryoid bodies (Bijlsma et al. 2008). TXA2 appears to be capable of inducing differentiation of human adipose tissue-derived MSCs into smooth-muscle-like cells (Kim et al. 2009a, b). These findings show that the $n-6$ PUFA-derived eicosanoids PGE2, LTB4, and TXA2 have promoting effects on both the proliferation and differentiation of stem cells. It has been shown that inhibition of the eicosanoid synthesis pathway promotes the pluripotent state of ESCs and that supplementation with the DHA-derived neuroprotectin D1 (NPD1), but not LTB4 or LTC4, promotes neuronal differentiation (Yanes et al. 2010).

Effect of Synaptic Function of PUFAs

PUFAs and their metabolites act in the brain through several potential mechanisms. One of the mechanisms involves the activation of receptors and consequent activation of cell signaling pathways. For example, unesterified PUFAs and their mediators are agonists for the oxysterol receptor LXR, peroxisome proliferator-activated receptor (PPAR), hepatic nuclear factor 4A (HNF4A, also known as NR2A1), chemokine-like receptor 1 (also known as CHEMR23), G protein-coupled receptor 32 (GPR32), and lipoxin receptor ALX/FPR2, and they can activate protein kinase C (PKC) and inhibit nuclear factor-κB (NF-κB) (Green et al. 2008; Rapoport 2014; Serhan 2014; Rao et al. 2008).

PUFAs can also influence brain function through modulation of the endocannabinoid system. The endocannabinoids include the fatty acid ethanolamide anandamide (AEA), synaptamide (also known as docosahexaenoyl ethanolamide), oleylethanolamide, and palmitoylethanolamide, as well as 2-arachidonoylglycerol (2-AG) (Piomelli and Sasso 2014). The most abundant endocannabinoids in the brain are the ARA metabolites AEA and 2-AG, which bind to cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2). Neurons and glial cells, including astrocytes and microglia, produce endocannabinoids and express cannabinoid receptors (Stella 2009). Endocannabinoids are important regulators of synaptic function; they suppress neurotransmitter release (including the release of glutamate, GABA, monoamine neurotransmitters, opioids, and acetylcholine) by acting as retrograde messengers at presynaptic CB1s (Castillo et al. 2012). Recent studies have shown that endocannabinoids can also modulate synaptic transmission through TRPV1 (transient receptor potential cation channel subfamily V member 1), which is located postsynaptically, and through CBs expressed in astrocytes (Grueter et al. 2010). In addition to regulating endocannabinoid levels and metabolism, in mice, $n-3$ PUFAs also regulate CB1 activity and CB1-associated signaling pathways in the prefrontal cortex and nucleus accumbens. For example, as a consequence of chronic exposure to low levels of dietary $n-3$ PUFAs, which leads to low DHA levels in the brain, endocannabinoid-dependent LTD is impaired in these brain regions, and mice develop depression-like and anxiety-like behavior (Larrieu et al. 2012; Lafourcade et al. 2011).

DHA, which is particularly enriched in synapses, also has a role in synaptic integrity and the assembly of the SNARE complex. DHA has been shown to attenuate the altered expression of postsynaptic dendritic proteins (including drebrin, postsynaptic density 95 (PSD95), NMDA receptors, and Ca^{2+}–calmodulin-dependent protein kinase 2 (CAMK2)) in a mouse model of Alzheimer's disease (Calon et al. 2004; Calon et al. 2005). DHA also regulates the SNARE fusion machinery involved in presynaptic exocytosis and endocytosis (Davletov et al. 2007). Syntaxin 3 is activated by both ARA and DHA in vitro, and DHA promotes syntaxin 3 incorporation into SNARE complexes and, thereby, regulates rod photoreceptor biogenesis in the retina. DHA also impairs syntaxin binding of the SNARE complex in PC12 cells, but has no effect on synaptobrevin mRNA and protein expression in an SH-SY5Y neuroblastoma cell line (Mathieu et al. 2010).

Synaptamide, an endogenous metabolite of DHA, is a member of the *N*-acylated amino acid or neurotransmitter class of lipid signaling molecules with endocannabinoid-like structure (Connor et al. 2010). A recent study showed that synaptamide is a potent mediator of neurite growth, synaptogenesis, and neurogenic differentiation of neural stem cells (NSCs) (Kim et al. 2011a, b; Rashid et al. 2013). The endogenous receptor mediating synaptamide effects has not been studied well. There are fewer receptors for synaptamide-induced differentiation that might be considered such as G protein-coupled receptor (GPR)40, GPR41, GPR43, GPR84, GPR110, GPR120, cannabinoid receptor type 1 (CB1), retinoid X receptor (RXR), and peroxisome proliferator-activated receptors

(PPARs). It has been shown that GPR40 is expressed in the brain and pancreas in the human and monkey, and its ligands are medium- and long-chain fatty acids including DHA, EPA, and AA. GPR40 is also expressed in the subgranular zone of the adult monkey hippocampus, where neurogenesis is stimulated by GPR40–CREB signaling (Ma et al. 2008). Although DHA has been demonstrated to promote neuronal differentiation in rat NSCs transfected with GPR40 (Ma et al. 2010), GPR40 expression has not been known in rodents thus far. GPR120 is a functional omega-3 fatty acid receptor/sensor and facilitates potent insulin-sensitizing and antidiabetic effects in vivo by suppressing macrophage-induced tissue inflammation (Oh et al. 2010).

Role of PUFAs in Neurogenesis and Neuroprotection

DHA is an omega-3 fatty acid highly enriched in the brain and is essential to maintain proper brain function (Salem et al. 2011; McNamara and Carlson 2006). Both human and animal studies have indicated that accretion of DHA at an early stage is particularly critical for optimal neurodevelopment. Several studies have demonstrated that dietary administration of DHA improves spatial learning ability in young and aged rats. DHA promotes neurogenesis both in vivo and in vitro (Kawakita et al. 2006). DHA, the main PUFA in phosphatidylserine, enhances phosphatidylserine synthesis in vitro (Garcia et al. 1998), and depletion of DHA from the membrane impairs phosphatidylserine-mediated AKT and RAF1 translocation and activation, which are important for promoting neurogenesis. The DHA mediator synaptamide (Kim and Spector 2013) is sufficient to promote neuronal differentiation and is a much more potent promoter of neurite growth, synaptogenesis, and synaptic function than DHA itself (Kim and Spector 2013; Kim et al. 2011a, b; Rashid et al. 2013). The brains of mice that are fed high levels of DHA have increased synaptamide levels and show increased neuronal differentiation of neural stem cells (Rashid et al. 2013). The LOX-synthesized DHA mediator NPD1 may promote neuronal survival by upregulating genes encoding the anti-apoptotic proteins B-cell lymphoma 2 (BCL2), BCLXL, and BCL2-related protein A1 (BCL2A1, also known and BFL1) and downregulating genes encoding the pro-apoptotic proteins BCL2-associated agonist of cell death (BAD), BAX, BH3-interacting domain death agonist (BID), and BCL2-interacting killer (BIK) in vitro and in the brain in vivo (Lukiw and Bazan 2008; Lukiw et al. 2005). Furthermore, increased brain DHA levels have been shown to normalize brain-derived neurotrophic factor (BDNF) levels in rats exposed to traumatic brain injury (Wu et al. 2004); consistent with this, the addition of DHA increased BDNF levels in astrocytes in vitro, and DHA deprivation decreased BDNF levels in the rodent brain (Rao et al. 2007). In addition, during ischemia, unesterified DHA is released from the phospholipid membrane and is converted to NPD1, which may promote anti-apoptotic signaling. Increasing brain DHA levels through dietary supplementation, intravenous infusion, or intracerebroventricular injection augments anti-apoptotic and anti-inflammatory signaling in rodent

brains (Orr et al. 2013). Interestingly, an aspirin-induced stereoisomer of NPD1 is produced in the brain upon ischemia followed by reperfusion, and co-administration of both DHA and aspirin decreases lesion volume and improves functional outcomes after experimental stroke in rats (Bazan et al. 2012). Intracerebroventricular administration of DHA or NPD1 also decreases stroke volume and attenuates the induction of the pro-inflammatory signaling proteins NF-κB and COX2. Thus, targeting the brain with DHA and its mediators is not only therapeutic when done before ischemia but can also have beneficial effects after ischemic injury.

Role of PUFAs in Inflammation in the Nervous System

DHA and its mediators have potent anti-inflammatory and pro-resolving properties in peripheral tissues. In humans, higher dietary intakes of DHA (from consuming fish) are associated with a lower risk of neurological disorders that have an inflammatory component, including Alzheimer's disease, Parkinson's disease, and major depression (Laye 2010).

Indeed, the expression of pro-inflammatory cytokines in the brain following systemic lipopolysaccharide (LPS) administration, brain ischemia–reperfusion, spinal cord injury, and aging is reduced in rodents with high levels of brain DHA (Mingam et al. 2008; Lalancette-Hébert et al. 2011; Huang et al. 2007; Minogue et al. 2007; Orr et al. 2013). Furthermore, $n-3$ PUFAs can improve behavior and neurophysiological systems affected by neuroinflammation. For example, short-term exposure to EPA through the diet reduced spatial memory deficits and anxiolytic behavior induced by central administration of interleukin-1β (IL-1β) (Song et al. 2004) and improved inhibition of long-term potentiation (LTP) by LPS and amyloid-β in rats (Minogue et al. 2007). In addition, aging-associated microglia activation, and the associated production of IL-1β and alterations in hippocampal LTP, could be attenuated by dietary EPA supplementation in rats (Lynch et al. 2007; Martin et al. 2002). Moreover, diet supplementation with EPA and DHA increased brain DHA levels, attenuated pro-inflammatory cytokine expression and astrogliosis, and restored spatial memory deficits and FOS expression upon memory test exposure in the hippocampus of aged mice. The anti-inflammatory effects of DHA could be due to a direct effect of DHA on invading macrophages or microglia. Indeed, in vitro and in vivo data have shown that DHA blocks macrophage- and microglia-induced activation of NF-κB in the CNS of rodents with neuroinflammation (De Smedt-Peyrusse et al. 2008). Moreover, DHA promotes the switching of microglia to an anti-inflammatory M2 phenotype that shows increased phagocytosis of amyloid-β isoform 42 (Aβ42) in vitro (Hjorth et al. 2013). DHA mediators are also crucial components of inflammation resolution, and DHA downregulates the expression of several enzymes of the ARA cascade (including COX2) in the brain, both under basal conditions and in response to neuroinflammation. DHA has been shown to decrease infarct volume; it is difficult to separate the neuroprotective effects of DHA from the anti-inflammatory effects in animal models of disease (Orr et al. 2013). Cell culture studies show that

acute administration of DHA or its mediators attenuates markers of neuroinflammation (Orr et al. 2013; Hjorth and Freund-Levi 2012) and pro-inflammatory signaling. Furthermore, unesterified DHA infused into the ventricle is protected in an ischemia–reperfusion model of stroke and in a mouse model of LPS-induced neuroinflammation, and both DHA and its mediator NPD1 downregulate pro-inflammatory cytokine signaling and decrease the activation of microglia. These data suggest that unesterified DHA and/or its mediators are responsible for at least some of the anti-inflammatory effects that have been attributed to DHA.

Role of PUFAs in the Regulation of Brain Glucose Uptake

DHA may have a role in the regulation of brain glucose uptake. In rodents, low brain levels of DHA are associated with decreased cytochrome oxidase activity and decreased endothelial glucose transporter 1 (GLUT1)-mediated glucose uptake (Pifferi et al. 2005). In addition, DHA supplementation can rescue decreased GLUT1 levels induced by low DHA and increase GLUT1 density in rat brain endothelial cells (Pifferi et al. 2007). These results suggest that DHA may have a direct effect on brain glucose uptake.

Conclusions and Future Directions

Understanding the role of PUFAs and their metabolites in stem cell fate regulation is important for stem cell biology as well as stem cell therapy. Overall, $n-6$ and $n-3$ PUFA and their metabolites emerge to reveal promoting effects on the proliferation and differentiation of various stem cells. The available evidence suggests that the role of $n-6$ PUFA-derived metabolites in stem cell fate is mainly to develop cell proliferation, while $n-3$ DHA is a promoting factor of NSC differentiation. The DHA mediator synaptamide is sufficient to promote neuronal differentiation and is a much more potent promoter of neurite growth, synaptogenesis, and synaptic function than DHA itself. These two classes of PUFA can produce a variety of metabolites with distinct functions, and they act through multiple mechanisms; their overall impact is dependent on the fatty acid composition (such as the $n-6/n-3$ PUFA ratio) and metabolite interaction. Various molecular and cellular signaling pathways that are regulated by PUFAs have been identified. However, major questions and areas of contention remain regarding how to target brain PUFA metabolism, including the delivery of PUFAs to the brain. Future studies aimed at identifying how PUFA signaling changes in brain disorders and in developing methods to return PUFA metabolism to homeostasis may offer novel therapeutic approaches for diseases of the brain.

Compliance with Ethics Requirements The authors declare that they have no conflicts of interest.

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Choline and the Brain: An Epigenetic Perspective

Rola Aldana Bekdash

Abstract Choline is an essential nutrient that is required for normal development of the brain. Via its metabolite betaine, it participates in the synthesis of *S*-adenosylmethionine, a major methyl donor for histone and DNA methylation, two epigenetic mechanisms that regulate gene expression and may alter brain function. Besides its role in methyl group metabolism, choline also has pivotal functions, including the maintenance of structural integrity of membranes and modulation of cholinergic neurotransmission, functions that are often dysregulated in some neurodegenerative disorders. Emerging evidence suggests that environmental factors, including lifestyle or diet, sometimes cause epigenetic changes in the expression of neuronal genes resulting in long-term changes in brain function. Recently, choline has been implicated as an epigenetic modifier of the genome that may alter gene methylation, expression, and cellular function. Abnormal level of choline during fetal or early postnatal life has been shown to alter memory functions during adulthood. It may also contribute to the etiology of stress-related disorders and age-related decline in memory later in life. Conversely, rodent studies suggested that perinatal choline supplementation enhances performance in memory-related tasks during adulthood. In this chapter, we will focus on the impact of choline-gene interaction on brain function in early life and during adulthood. In particular, we will emphasize the potential role of choline as a neuroprotectant that may mitigate some of the adverse effects of neurodegenerative disorders and protect mental health across the lifespan.

Keywords Brain • Choline • Environment • Epigenetics • Gene • Memory • Neurodegenerative diseases

Abbreviations

Ach	Acetylcholine
AchE	Acetylcholine esterase
APP	Amyloid precursor protein

R.A. Bekdash (✉)
Department of Anesthesiology, Columbia University Medical Center, New York, NY, USA
e-mail: rola.bekdash@gmail.com

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ChAT	Choline acetyltransferase
Dnmts	DNA methyltransferases
E	Embryonic day
HDACs	Histone deacetylases
HMTs	Histone methyltransferases
HPA	Hypothalamic-pituitary-adrenal
IGF	Insulin-like growth factor
5MTHF	5-Methyltetrahydrofolate
MTHFR	Methyltetrahydrofolate reductase
NPC	Neural progenitor cell
PC	Phosphatidylcholine
PEM	Phosphatidylethanolamine
POMC	Proopiomelanocortin
RE1	Repressor element-1
SAH	*S*-Adenosylhomocysteine
SAM	*S*-Adenosylmethionine
THF	Tetrahydrofolate

Introduction

Choline is an essential nutrient that is required for normal functioning of major organ systems in the body including the brain. It acts as a precursor for major components of cellular membrane phospholipids such as phosphatidylcholine (PC) and sphingomyelin, and it affects cholinergic neurotransmission via the synthesis of the neurotransmitter acetylcholine (Ach). Besides these essential physiological functions, choline has been shown lately to play a role in histone protein and DNA methylation, two epigenetic processes that regulate gene expression and may alter brain function (Zeisel 1997, 2004; Zeisel et al. 1991). Via its metabolite betaine, choline contributes to the synthesis of *S*-adenosylmethionine (SAM), a major methyl donor for methylation pathways including DNA methylation (Niculescu and Zeisel 2002). DNA methylation is an epigenetic modification that has been shown to be indispensable to many physiological processes including normal brain development (Robertson and Wolffe 2000; Ordway and Curran 2002; Hermann et al. 2004). Epigenetics is defined as a process that leads to changes in gene expression without changes in DNA sequence and that these changes are induced by environmental factors. This process is often mediated by alterations in DNA methylation and/or histone modifications that may result in long-term changes in cellular functions (Jaenisch and Bird 2003). For example, modulation in global or gene-specific DNA methylation has been lately linked to many neurological diseases or disorders including stress, drug addiction, neuropsychiatric disorders, and neurodegenerative disorders, including impairments in memory functions (Klengel et al. 2014; Connor and Akbarian 2008; Mill et al. 2008; Sillivan et al. 2015; Jirtle and Skinner 2007; Liu et al. 2009).

Emerging evidence suggests that environmental factors including repeated exposure to substances of abuse, stress, lifestyle, nutrition, or maternal diet could sometimes cause epigenetic dysregulation in the expression of genes resulting in many diseases or disorders (Jirtle and Skinner 2007; Nestler 2014; Choi and Friso 2010) including neurological disorders (Van den Veyver 2002). In the context of central nervous system function, the availability of choline during fetal life is pivotal to brain growth, structure, and function (Zeisel and Niculescu 2006). It has been suggested that prenatal choline supplementation could alter fetal epigenome and cause developmental programming of specific phenotypes during adulthood and may impact the fetus long-term health and vulnerability to a wide range of diseases later in life. For example, rodent studies showed that prenatal choline supplementation exerts beneficial effects on memory functions and behavior during adulthood (Zeisel 2000; Schulz et al. 2014; Meck et al. 2008; Meck and Williams 1997a) and may attenuate some of the adverse effects of prenatal stress and exposure to drugs of abuse such as prenatal alcohol in adult offspring (Ryan et al. 2008; Thomas et al. 2000, 2007, 2009, 2010; Bekdash et al. 2013).

Besides the effects of prenatal choline supplementation on fetal programming and health, choline has been suggested to have a neuroprotective role against age-related memory decline and brain insults including seizures (Yang et al. 2000; Holmes et al. 2002; Wong-Goodrich et al. 2011). It could also mitigate some of the symptoms of genetically related neurodegenerative disorders such as Down's syndrome (Moon et al. 2010), Rett syndrome (Nag et al. 2008; Ward et al. 2009; Nag and Berger-Sweeney 2007) or Alzheimer's disease (Sezgin and Dincer 2014; Wurtman 1992). Considering the widespread physiological functions of choline in health and disease of the brain, we will summarize in this chapter recent animal and select human studies that showed the potential impact of gene-choline interaction during early life on brain function during adulthood. In particular, we will focus on the effects of prenatal choline supplementation on fetal brain and the potential significance of this neuroprotective nutrient in attenuating some of the symptoms that are often associated with neurodegenerative disorders during adulthood such as decline in memory and cognitive functions.

Physiological Functions of Choline: General Overview

Choline is a quaternary ammonium compound that has three methyl groups. It was first isolated in 1862 from the bile of the pig by Adolph Strecker then chemically and structurally characterized in 1866. It was not until 1868 that Strecker found that choline is a precursor of PC and sphingomyelin, two main structural components of cellular membrane phospholipids (Zeisel 2012). These choline-derived phospholipids are needed for rapid cell division and nerve cell myelination, two processes that are required for proper development and functioning of the brain. Choline is also a precursor for the neurotransmitter Ach that plays a critical role in cholinergic signaling (Blusztajn and Wurtman 1983), a function that is often dysregulated in some

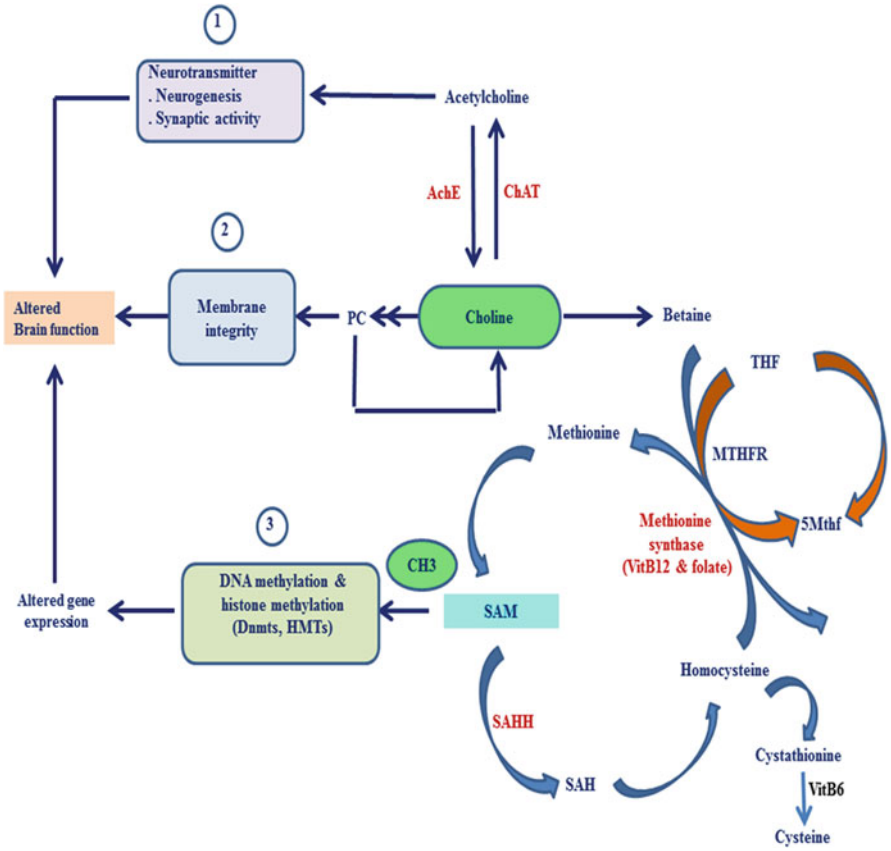

Fig. 1 Physiological functions of choline. Choline is a precursor for the formation of acetylcholine, a major neurotransmitter in the brain. Via its metabolite betaine, it participates in the formation of SAM, a major methyl donor for DNA methylation that is catalyzed by Dnmts and histone methylation that is catalyzed by HMTs, two epigenetic processes that regulate gene expression. These processes in turn alter brain structure and function. Choline, betaine, and folate participate in one-carbon metabolism. Choline is acetylated to acetylcholine by ChAT. Acetylcholine is degraded by AchE

neurodegenerative diseases such as Alzheimer's disease (Geula et al. 2008; Wurtman 1992). It also plays roles in synapse formation and neurogenesis (Fig. 1). In 1998, the Food and Nutrition Board of the Institute of the National Academy of Sciences of the United States issued a report with specific guidelines for choline daily intake in humans suggesting an adequate intake (AI) value of 7.5 mg of choline daily per kg of body weight (National Academies Press 1998). These requirements for choline intake are not uniform among individuals but are mainly affected by several factors including sex, age, genetic factors, and environmental factors (Da Costa

et al. 2006; Fischer et al. 2007). For example, the adequate intake of choline is often elevated in pregnant women to support fetal growth and development. Our sources of choline are often derived from a wide variety of foods including eggs, peanuts, liver, wheat germ, beans, fish, some meats, and vegetables.

Choline is not only derived from food but also can be de novo synthesized from PC in the liver and in other tissues as well including the brain. In mammals, the de novo synthesis of choline is catalyzed by the activity of phosphatidylethanolamine-*N*-methyltransferase (PEMT) via the sequential methylation of phosphatidylethanolamine (PEM) using SAM as a methyl donor (Vance and Ridgway 1988). A significant portion of choline can be oxidized into betaine to participate in the formation of SAM, a major methyl donor for methylation pathways (Fig. 1). A small portion of dietary choline is usually acetylated to Ach by choline acetyltransferase (ChAT). It has been demonstrated that choline reaches the brain from the systemic circulation via carriers or transporters that are located at the blood-brain barrier where it is usually stored as membrane PC for Ach biosynthesis and for support of cholinergic functions. Ach can in turn be degraded into choline and acetic acid by acetylcholinesterase (AchE) or recycled back for reuse by presynaptic neurons (Wurtman 1992).

Animal and human studies showed that depletion of choline from the diet during adulthood may cause organ dysfunctions such as fatty liver, liver damage, or abnormalities in muscle function (da Costa et al. 2005; Da Costa et al. 2004). Other supporting studies also revealed that deficiency in choline levels has been linked to many diseases including liver dysfunction, cardiovascular disease, cancer (Xu et al. 2008; Cho et al. 2010; Ciappio et al. 2011), and neurological disorders (Moon et al. 2010; Ward et al. 2009; Nag and Berger-Sweeney 2007; Geula et al. 2008; Ross et al. 2013). Choline's most pivotal role is evident in its activity in the central nervous system where it subserves vital physiological functions for proper functioning of the brain. For example, via its oxidation to PC, choline participates in maintaining the integrity of cellular membranes. It also affects cholinergic neuron signaling via modulation in Ach biosynthesis (Montoya et al. 2000; Cermak et al. 1999). One of the hallmarks of Alzheimer's disease, a progressive debilitating neurodegenerative disorder, is the gradual degeneration of cholinergic neurons and a resulting deficit in cholinergic neurotransmission in the aging brain. This neurodegeneration may be partly induced by a decrease in membrane PC and PEM levels (Nitsch et al. 1992), suggesting the potential role of choline in boosting brain activity and probably delaying cognitive and memory functions that are often associated with aging.

Choline is not only a precursor of PC but also of sphingomyelin, another structural component of membranes. Sphingomyelin supports proper myelination of nerve cells, a process that is often accelerated during fetal life and is essential for proper neuronal signaling in the brain (Oshida et al. 2003). It is then not surprising that abnormal levels of choline in the brain would affect the availability of this structural component and potentially could have adverse effects on brain circuitry and alter brain function across the lifespan.

Epigenetic Mechanisms of Choline Effects on the Brain

The normal development of the central nervous system is partly affected by the availability of choline during fetal life (Zeisel 2006). Choline may also attenuate age-related memory decline or memory impairments that may be induced during adulthood (Klein 2000; Borges et al. 2014; Park et al. 2012). Animal and human studies have implicated choline deficiency with the etiology of neural tube defects (Shaw et al. 2004; Fisher et al. 2002) and possible decline of mental capabilities with age (Meck and Williams 1997b), suggesting that this fundamental nutrient is critical for proper functioning of the developing and the aging brain. Several studies also suggested that choline intake during critical period of brain development may accentuate brain function during adulthood and attenuate age-related decline of memory (Meck et al. 2008; Blusztajn 1998; McCann et al. 2006). So, understanding the molecular mechanisms of choline at different stages of brain development could be key to our understanding of the physiological, structural, and behavioral changes that have been associated with changes in its levels, thereby providing a potential new avenue for the safe use of this nutrient to improve or protect mental capabilities throughout life.

The role of choline in promoting health in the adult stage has been documented (Zeisel et al. 1991; Zeisel and da Costa 2009), and accumulating evidence points to the vital role of early life nutritional environment on the fetal epigenome and in the induction of long-term cellular and/or behavioral changes. In this section, we will discuss the effects of choline on both the fetal brain and the aging brain and summarize current knowledge of the potential molecular mechanisms that could be involved.

Choline and the Fetal Brain

Fetal life is a critical developmental period that is characterized by a remarkable plasticity and the ability to respond to various environmental factors including maternal diet. These factors could elicit favorable or unfavorable lifelong changes on health during adulthood. This developmental period necessitates the availability of essential nutrients including methyl group donors such as choline to support multiple brain processes such as neurogenesis, neural connectivity, and neuroplasticity that are related to learning, cognitive, and memory functions across the lifespan. Rodent studies showed that choline supplementation during early life attenuated the severity of memory decline in adult animals (Meck and Williams 1997a, c, 1999; Jones et al. 1999; Meck et al. 1988, 2007). Human studies also showed that choline supplementation may be useful in attenuating symptoms of dementia (Meck et al. 2007). It has been proposed that the effects of choline on fetal brain are epigenetically mediated and may be induced by the exposure of fetus microenvironment to many factors. Betaine, a choline derivative, plays a role in the remethylation of homocysteine to methionine then SAM. SAM acts as a major methyl donor for the components of the epigenetic machinery such DNA methyltransferases (Dnmts) or histone methyltransferases (HMTs) that catalyze the methylation of DNA or

histones (Davison et al. 2009) to modulate gene expression (Fig. 1). So, changes in the level of choline and its derivatives during fetal life may modulate the activity of these enzymes and alter fetal gene expression that may lead to long-term changes in cellular function and brain function.

Several in vitro and in vivo studies suggested that abnormal changes in prenatal choline levels may cause molecular, structural, functional, or behavioral changes at the organismal level. A low maternal intake of choline has been linked to the development of neural tube defect in humans and in rodents (Shaw et al. 2004; Fisher et al. 2002) and has been shown to negatively impact neuronal migration, survival, and differentiation (Zeisel 2011; Craciunescu et al. 2003). For example, choline deficiency reduced neural progenitor cell (NPC) proliferation and migration in fetal hippocampus (Holmes-McNary et al. 1997). At the behavioral level, choline deficiency during prenatal or early postnatal life has been shown to cause impairments in certain aspects of memory formation in specific memory-related tasks, whereas its supplementation from embryonic days E11–E17 induced cell proliferation in fetal brain and enhanced memory or cognitive functions with age (Meck and Williams 1997a, c; Meck et al. 1988, 2007).

Alteration in choline levels in the brain has been shown to cause epigenetic changes such as changes in global or gene-specific histone methylation (Davison et al. 2009; Mehedint et al. 2010a) and/or DNA methylation (Niculescu et al. 2006; Kovacheva et al. 2007) in a tissue-specific manner. For example, choline deficiency altered hippocampal development in fetal brain and in cultured NPC by causing gene-specific DNA methylation and an alteration in chromatin landscape. In vivo studies also showed that choline deficiency at E17 resulted in a decrease in the expression of G9a histone methyltransferase and its associated marks H3K9me1 and H3K9me2 in the subventricular zone and the ventricular zone of the hippocampus with no changes in global levels of these histone marks in the whole mouse fetal brain. These histone modifications correlated with a decrease in the binding of the repressor element 1-silencing transcription factor (REST) on the repressor element-1 (RE1) site of the calbindin gene (Calb1) promoter and an increase in *Calb1* expression in NPC, both in vivo and in vitro. Choline-induced changes in histone marks created an environment that is conducive for transcription upstream of the RE1 site of this gene. It has been suggested that there is a correlative relationship between DNA methylation and the predominance of the methylated repressive mark H3K9 in causing gene repression (Zhang and Reinberg 2001). Additionally, choline deficiency increased the methylation of one CpG site along the Calb1 gene promoter in cultured NPC with no changes in total methylation of the CpG island of this gene (Mehedint et al. 2010b). Collectively, these data suggest that an alteration in choline levels during fetal life may have altered hippocampal neurogenesis by causing epigenetic changes in fetal brain. These changes could alter memory functions later in life and accelerate the decline of memory functions with aging.

Another study assessed the effects of gestational choline on changes in the expression of histone-methylating enzymes and on the methylation status of their respective histone marks in the cortex and the liver of E17 fetuses of choline-deficient rats. Choline deficiency increased the methylation of the promoter of

histone methyltransferases G9a and Suv39h1 genes. This hypermethylation correlated with a downregulation in the expression of G9a and a reduction in the levels of the repressive marks H3K9me2 and H3K27me3, histone marks that are catalyzed by G9a and are often associated with transcriptional repression. Additionally, choline deficiency downregulated Suv39h1 expression resulting in a decrease in the levels of the repressive mark H3K9me3 (Davison et al. 2009). It has been suggested that choline-induced epigenetic changes may modulate the expression of key genes that subserve vital functions in these tissues.

Prenatal choline supplementation has been shown to alter brain plasticity that is related to memory and cognitive functions throughout life (Glenn et al. 2007). Memory functions most often involve a series of complex molecular changes that modulate gene expression in the brain. While these molecular changes could be induced by specific transcription factors or intracellular signaling factors, considerable evidence suggests that choline, as an epigenetic modifier, could also modulate gene expression and brain function in response to neuronal activity and environmental factors (Bekdash et al. 2013; Niculescu et al. 2006; Mehedint et al. 2010b). For example, on a gene global level, microarray experiments demonstrated that prenatal choline supplementation caused remarkable changes in the expression of memory-related genes in both the hippocampus and the cerebral cortex (Mellott et al. 2007), suggesting a correlative relationship between the availability of choline during early life and these induced molecular changes. It also enhanced neurogenesis in the dentate gyrus of rat hippocampus and elevated the levels of the brain-derived neurotrophic factor (BDNF). Other studies showed that prenatal choline supplementation elevated the levels of the insulin-like growth factor (IGF-1) in the rat brain and differentially altered the performance of these rats in specific memory-related tasks (Wong-Goodrich et al. 2008a). It also elevated IGFII and its receptor (IGFII-R) in the rat hippocampus and the frontal cortex, suggesting a vital role of choline in regulating cholinergic functions (Napoli et al. 2008). Prenatal choline supplementation also increased the size of cell bodies of basal forebrain neurons (Williams et al. 1998), induced the release of Ach by cholinergic neurons (Cermak et al. 1998), and reduced the activity of AchE in the hippocampus (Cermak et al. 1999). Recent study conducted in the hippocampus of adolescent mice showed that methyl donor deficiency including folate, methionine, and choline caused molecular and behavioral changes that are linked to learning and memory (Tomizawa et al. 2015). Collectively, these studies suggest that choline supplementation during early development may have long-term beneficial effects on cognition and memory processes in the brain.

Choline has been recently implicated in the effects of substance abuse such as alcohol abuse on the brain and in the programming of the hypothalamic-pituitary-adrenal (HPA) stress axis function by epigenetic mechanisms. For example, prenatal choline supplementation during alcohol exposure in pregnant rats altered the methylation of the histone activation mark H3K4me3 and the histone repressive mark H3K9me2 in β-endorphin-producing proopiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus of exposed offspring. Prenatal choline supplementation also decreased the methylation pattern along Pomc gene, elevated

its mRNA expression in the hypothalamus, and decreased the levels of the stress hormones adrenocorticotropic hormone (ACTH) and corticosterone in adult offspring. These data suggest a potential role of choline in attenuating some of the adverse effects of prenatal alcohol exposure such as an increased stress reactivity in the adult stage (Bekdash et al. 2013). In the context of stress axis regulation, a human study also showed that maternal choline supplementation attenuated the HPA stress axis reactivity in offspring. In particular, it altered the methylation status along the promoter of some cortisol-regulating genes including the corticotropin-releasing hormone (*Crh*) gene and glucocorticoid gene *Nr3c1* in the placenta and in the cord blood resulting in a lower circulating levels of the stress hormone cortisol in cord plasma (Jiang et al. 2012). A more recent study pointed out the long-term beneficial effects of prenatal choline supplementation in attenuating autism-related symptoms including deficit in social interaction and anxiety in an autistic mouse model (Langley et al. 2015). Collectively, these findings suggest a potential role of choline in altering the fetus epigenome and in attenuating the adverse effects of prenatal alcohol exposure or prenatal stress on the behavioral and neuroendocrine development of adult offspring, potentially protecting their mental health throughout life.

Choline and the Aging Brain

With the aging of the population, the prevalence of neurodegenerative disorders is on the rise worldwide which imposes a huge burden on society and individuals. Alzheimer's disease is an example of a neurodegenerative disease that is multifactorial and polygenic. The causes of this mental illness are not very well understood, but emerging evidence suggests that impairments in brain function are probably induced by genetic and epigenetic factors (Park et al. 2012; Gordon 1997; Dauncey 2012). These factors may have orchestrated changes in the expression of many genes in the brain later in life, altered specific brain circuitry, and resulted in multifaceted behavioral changes including decline in cognitive and memory functions.

An increasing attention has been lately devoted into understanding the underlying mechanisms of cognitive and memory dysfunctions with aging in the goal of identifying effective treatments to promote brain longevity and increase individual's productivity. Identifying these mechanisms has been proven to be quite challenging and would require to redirect our effort toward spotting changes in the expression of a network of genes in the brain or changes in their epigenetic landscape, events that often regulate gene activities and may affect brain circuitry and function. Although some genes were identified as potential causative agents of Alzheimer's disease, a cure for this disorder is still far from our reach suggesting that the onset and/or progression of this disorder are heterogeneous and may be induced not only by the individual's genetic makeup but also exposure to environmental factors including nutrition and lifestyle (Sezgin and Dincer 2014).

Earlier studies showed that mutations in the presenilin gene and the amyloid precursor transmembrane protein (APP) gene (Lippa 1999) are so far consistent molecular changes that have been identified in patients with Alzheimer's disease. Recently, it has been proposed that alteration in memory formation may be linked to epigenetic mechanisms such as changes in DNA methylation (Holliday 1999). For example, a mutation in the methyl-CpG-binding protein (MeCP2) has been identified as a causative agent in the etiology of Rett syndrome (Van den Veyver and Zoghbi 2001; Amir et al. 1999). Changes in histone marks in the astrocytes of Alzheimer's disease patients were evident in the cerebral cortex and the hippocampus (Myung et al. 2008). Hypomethylation of the APP gene promoter (West et al. 1995) has been identified in many Alzheimer's disease patients. This may suggest that this neurodegenerative disorder could also be an epigenetic disorder.

The link between nutrition and changes in cognitive functions has been recognized (Gordon 1997; Lieberman 2003; Kretchmer et al. 1996) suggesting that the quality of an individual's diet and lifestyle may play a role in shaping mental capabilities in beneficial or detrimental ways throughout an individual's life. Several dietary components or natural neuroprotective supplements including choline have been identified to affect brain development and function (Niculescu et al. 2006; Teather and Wurtman 2005), and a decrease in choline levels has been reported with aging (Cohen et al. 1995), suggesting choline's role in health and in prevention of some neurodegenerative disorders to enhance cognition, memory, and other impaired functions that are associated with aging. How changes in choline levels may contribute to the etiology of some neurodegenerative disorders? The effects of nutritional factors on the brain have been manifested as transient or long-term changes in gene expression that could be partly induced by changes in DNA methylation, histone modifications, or changes in the expression of noncoding RNAs such as microRNAs (Dauncey 2012). These epigenetic mechanisms have been suggested to play a role in the plasticity of the brain and in many neurological disorders (Hunter 2012; Jiang et al. 2008; Sun et al. 2013; Mendell and Olson 2012) and may play a role in the etiology of neurodegenerative disorders. For example, several nutrients or dietary methyl groups including choline, betaine, folate, VitB12, and methionine have been shown in several studies to enhance memory and cognitive functions later in life especially if supplemented during critical period of brain development (Zeisel 2013; Niculescu and Zeisel 2002; Ueland 2011; Kalhan and Marczewski 2012). Additionally, the levels of the methyl donor SAM and the expression of many Alzheimer-related genes have been shown to be altered in postmortem Alzheimer's disease patients (Scarpa et al. 2003; Fuso et al. 2005, 2012; Morrison et al. 1996). Few human studies also showed that higher choline intake positively correlated with better performance in memory tests (Poly et al. 2011).

Other underlying mechanisms indicate that alteration in choline levels and/or its metabolites in the brain may be linked to the etiology of Alzheimer's disease. A hypofunction of cholinergic neurons in the cerebral cortex and in other brain areas has been also identified (Lüth et al. 2003). A decrease in Ach synthesis has been shown to correlate with significant cognitive impairments in Alzheimer's disease (Wilcock et al. 1982; Sims et al. 1983). A dysfunction of glutamatergic

signaling that is mediated by NMDA receptors was identified as another plausible mechanism that participates in the etiology of Alzheimer's disease (Lin et al. 2014; Fayed et al. 2011). Collectively, these findings suggest that normal level of choline in the diet and in the brain may attenuate mental decline with age.

Membrane remodeling has been also acknowledged as a potential mechanism in the etiology of Alzheimer's disease. Abnormal change in membrane structure and function, a hallmark of many neurodegenerative disorders (Svennerholm and Gottfries 1994), is now accepted as a physiological process that could negatively impact cellular viability and cause defect in neurotransmission in different areas in the brain. Thus, inhibition or attenuation of this process of membrane breakdown may be beneficial for the treatment of some neurodegenerative disorders. In particular, changes of choline-containing phospholipids have been suggested to play a role in membrane breakdown and affect brain function. For example, postmortem studies demonstrated a reduction in the levels of membrane phospholipids in aging and demented human brains (Söderberg et al. 1991, 1992). Earlier studies suggested that PC breakdown or a decrease in the levels of choline metabolites may contribute to the degeneration of cholinergic neurons in Alzheimer's disease (Wurtman 1992; Wurtman et al. 1985). A reduction in the activity of ChAT in cholinergic neurons was also detected in several brain regions of Alzheimer's disease patients (Geula et al. 2008; Mufson et al. 2003; Ikonomovic et al. 2011), whereas inhibitors of AchE have been proven to have beneficial effects (Harold et al. 2006). At the molecular level, other studies showed a correlative relationship between the reduction in the activity of ChAT and the elevation in the expression of the neuronal restrictive silencing factor (NRSF) (González-Castañeda et al. 2013). A conserved neuronal restrictive silencing element (NRSE) site has been identified in human and rat cholinergic gene loci suggesting that this site is required for the regulation of ChAT gene expression (Hahm et al. 1997). Additionally, three cortical brain regions of postmortem brain of Alzheimer patients showed a significant depletion in the levels of two main components of the phospholipid bilayer such as PC and PEM and a correlative decrease in the levels of their precursors such as choline and ethanolamine (Nitsch et al. 1992). Taking into account these findings, choline could be a nutritional therapeutic approach that could mitigate the severity of Alzheimer's disease symptoms or possibly the symptoms that are associated with other neurodegenerative disorders.

Additional studies supported the idea that the etiology of some neurodegenerative diseases may be induced by epigenetic changes in different areas of the brain, thus providing an avenue for spotting novel molecular targets for the treatment of this brain disease. Reduction in global DNA methylation in the brain has been correlated with a decline in memory and learning functions (Liu et al. 2011). For example, several studies showed that Alzheimer-related genes displayed epigenetic changes by DNA methylation, changes in histone modifications or changes in the expression of specific microRNAs (Zawia et al. 2009; Fuso et al. 2008; West et al. 1995; Tohgi et al. 1999; Wong et al. 2013). In particular, global reduction in DNA or RNA methylation has been reported in the entorhinal cortex layer II in Alzheimer's disease patients (Mastroeni et al. 2010). Changes in histone marks (Ogawa et al.

2003) or changes in the levels of specific types of histone deacetylases (HDACs) including HDAC2 and Sirtuin1 (Gräff et al. 2012; Julien et al. 2009) were also detected in the hippocampus of postmortem Alzheimer patients. This necessitates perhaps the need to time these epigenetic changes during the progression of this debilitating disease in the goal of identifying therapeutic intervention that temporally targets these epigenetic changes to delay or attenuate the decline in brain cognitive and memory functions.

Changes in choline levels have been also linked to other brain insults that may accelerate the development and/or progression of some neurodegenerative disorders. For example, brain choline levels were altered under different pathological conditions including hypoxia (Klein et al. 1993), ischemia (Kozuka 1995; Scremin and Jenden 1989), and seizures (Jope and Gu 1991). It has been shown in several studies that the development of seizure and its severity could have long-term adverse effects on brain function including changes in behavior, learning, memory, and cognitive functions (Holmes 1997; Liu et al. 1994). Recent study demonstrated that oral administration of choline to ischemic rats increased CA1 hippocampal neuron survival (Borges et al. 2015). Prenatal choline supplementation attenuated status epilepticus-induced memory impairment in adult rodents (Yang et al. 2000; Wong-Goodrich et al. 2008b, 2011). Whether these findings in animal models could be extrapolated to human conditions remain to be determined. Collectively, these studies suggest that nutritional intervention such as choline supplementation may attenuate the vulnerability of cognitive and memory functions to various brain insults later in life.

Conclusion

Despite a lack of evidence on a causal relationship between choline dietary intake and boosting brain function during adulthood, substantial evidence derived from human and animal studies indicates that choline supplementation during fetal and early postnatal life could have beneficial long-term effects on brain function including memory and cognitive functions. Choline has emerged recently as an epigenetic modulator of the genome that may alter neuronal gene expression by modulating global or gene-specific epigenetic changes resulting in long-term phenotypic changes. These epigenetic mechanisms have been suggested to play a role in the etiology of many diseases such as drug addiction, cancer, metabolic disorders, and neurological disorders. The role of these mechanisms in the etiology of neurodegenerative diseases or disorders is still quite not very well elucidated but emerging. Whether nutritional intervention such as choline supplementation either alone or in combination with other interventions could promote the longevity of the brain or attenuate symptoms of some neurodegenerative disorders is a novel potential avenue that remains to be explored.

Compliance with Ethics Requirements The author declares that he/she has no conflicts of interest.

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Modulatory Effects of Dietary Amino Acids on Neurodegenerative Diseases

Senthilkumar Rajagopal, Supraj Raja Sangam, Shubham Singh, and Venkateswara Rao Joginapally

Abstract Proteins are playing a vital role in maintaining the cellular integrity and function, as well as for brain cells. Protein intake and supplementation of individual amino acids can affect the brain functioning and mental health, and many of the neurotransmitters in the brain are made from amino acids. The amino acid supplementation has been found to reduce symptoms, as they are converted into neurotransmitters which in turn extenuate the mental disorders. The biosynthesis of amino acids in the brain is regulated by the concentration of amino acids in plasma. The brain diseases such as depression, bipolar disorder, schizophrenia, obsessive-compulsive disorder (OCD), and Alzheimer's (AD), Parkinson's (PD), and Huntington's diseases (HD) are the most common mental disorders that are currently widespread in numerous countries. The intricate biochemical and molecular machinery contributing to the neurological disorders is still unknown, and in this chapter, we revealed the involvement of dietary amino acids on neurological diseases.

Keywords Alzheimer's disease • Amino acids • Calcium • Huntington's diseases • Ketogenic diet • Parkinson's disease

Introduction

Amino acids can serve as the most powerful compounds in the treatment of neurological diseases in the upcoming years. Amino acids ensure regeneration and reorganization processes of neurons (Morales et al. 2014). Based on the type of transmission, certain amino acids have the role of neurotransmitters with a stimulating or suppressing function. Amino acids and their derivatives stimulate transmission of the neural signal between the individual neurons, normalize excitation and inhibition processes (the basis of brain function), and also activate "dormant" backup neurons (Alexander 2004; Katzung 1989). Amino acids activate growth and differentiation of new nerve cells in the event of a nerve cell deficit caused by cell

S. Rajagopal, Ph.D. (✉) • S.R. Sangam • S. Singh • V.R. Joginapally
Department of Zoology, Nizam College, Hyderabad, India
e-mail: senthilanal@yahoo.com

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death, resulting from hypoxia and ischemia, cerebral hemorrhage, or toxic damage. They slow down the process of apoptosis, a programmed cell death (Rajagopal 2013; Rajagopal et al. 2014). Through these effects, amino acids are essential in the treatment of the following various neurological disorders:

1. Emphasis (language)
2. Dysgraphia (writing)
3. Dysarthria (speech)
4. Agnosia (identifying things or people)
5. Amnesia (memory)
6. Spinal cord disorders
7. Peripheral neuropathy and other peripheral nervous system disorders
8. Cranial nerve disorder such as trigeminal neuralgia
9. Autonomic nervous system disorders such as dysautonomia and multiple system atrophy
10. Seizure disorders such as epilepsy
11. Movement disorders of the central and peripheral nervous system such as Parkinson's disease, essential tremor, amyotrophic lateral sclerosis, Tourette's syndrome, multiple sclerosis, and various types of peripheral neuropathy
12. Sleep disorders such as narcolepsy
13. Migraines and other types of headache such as cluster headache and tension
14. Central neuropathy (Friedhoff et al. 1995; Friedhoff and Van Winkle 1962)

The role of amino acids in the brain function has been generally known for many years, while development in amino acid combinations of several components enabled the increase of their effectiveness (Bémeur et al. 2010; Suryawan et al. 1998). Formation and regeneration of motor and intellectual functions have been reported, and a considerable improvement of memory, speech, concentration, and perception has been observed. Supplementation of amino acid compounds facilitates elimination of the consequences of stress and chronic fatigue syndrome (Fernstrom 1990; Lipton and Rosenberg 1994; Growdon 1988). In healthy individuals, it increases, to a certain extent, intellectual effectiveness and concentration of perception and improves the ability to obtain information, as well as memory. In senior individuals, amino acids slow down the process of natural loss of the neural tissue (Rahman 2007; Ames and Shigenaga 1992).

The literature studies have shown the role of dietary protein intake on cognitive functioning as well as cognitive decline. Also, more research is needed to come to definitive conclusions and specific recommendations regarding protein intake or intake of specific amino acids for maintaining optimal cognitive functioning (van de Rest et al. 2013). Lower protein intakes are associated with a higher frailty risk (Beasley et al. 2010), and physical frailty is a predictor of cognitive decline and Alzheimer's diseases (Buchman et al. 2007; Auyeung et al. 2011). An association between protein intake and cognitive functioning may specifically be due to certain amino acids that are common constituents of protein-rich foods.

Several amino acids act as precursors of important neurotransmitters and neuromodulators, such as catecholamines (phenylalanine and tyrosine), histamine (histidine), nitric oxide (arginine), and serotonin (tryptophan). According to experimental results, plasma concentrations of amino acids may be a regulating factor of neurotransmitter biosynthesis in the brain (Lieberman 1999). Elevated phenylalanine concentrations may lead to brain damage through several mechanisms, including competition with other large neutral amino acids (LNAAs) (including tryptophan) for transport by the same carrier at the brain-blood barrier (BBB) (Hargreaves and Pardridge 1988) and decreased brain protein synthesis. Arginine, in particular, is a precursor for polyamines and nitric oxide (Blantz et al. 2000), both of which are important modulators of neuronal physiology and are thought to be involved in AD pathogenesis (Morrison et al. 1998). The CSF levels of alanine, glycine, leucine, and phenylalanine were found to be elevated in schizophrenic patients (Reveley et al. 1987). Consequently, when studying the turnover and transport of tyrosine, the turnover of alanine must be investigated from the start, i.e., plasma to end, i.e., CSF. In general, a CSF level of amino acids seems to be less reliable since they must be transported over four membranes to reach the brain. Three of them have a limited transport capacity due to competition. Furthermore, the levels of amino acids in plasma and CSF are not correlated (Bjerkenstedt et al. 1985).

The Role of Micronutrients in Neurological Diseases

Glutathione

The cytoprotective effects of glutathione are well known, and it plays an important role in neuroprotection from neurotoxicity. This antioxidant is used up faster in brain tissue in the presence of choline deficiency. The exciting news is that many mental, neurological disorders can be enhanced with diet and especially nutriceuticals. Some neurological diseases, disorders, or conditions can benefit from increased glutathione and/or other essential vitamins, minerals, and phytochemicals (Bavarsad et al. 2014; Adair et al. 2001).

Alpha-Lipoic Acid

This nutrient protects against the neuronal injury that occurs in the presence of toxic proteins found in brain tissue of Alzheimer's patients. Research clearly indicates that lipoic acid is a potent neuroprotective antioxidant, which strengthens memory and stimulates nerve growth (Mijnhout et al. 2010; Gonzalez-Perez et al. 2002).

B Vitamins

Folate, vitamin B6, and B12 are important in methylation processes. Deficiencies in one of these vitamins can raise a homocysteine level, which is linked to increased Alzheimer's risk. Vitamin B1 protects against mitochondrial dysfunction that causes dementia. B12 improves frontal lobe functions such as language, especially in the elderly (Golan et al. 2013).

Carnitine

The amino acid carnitine has potent antioxidant properties. Its role in the transport of fatty acids to the mitochondria explains its beneficial effects on fatigue, which include both physical and mental fatigue. Several trials have demonstrated a consistent improvement in memory, focus, and cognition with carnitine supplementation (Macleod and Appleton 2007; Shinawi et al. 1998).

Choline

Choline is the precursor molecule for the neurotransmitter acetylcholine, which is intimately involved in memory, another member of the B complex. Choline deficiency can induce mitochondrial dysfunction in the brain that clinically presents as cognitive impairment (Zeisel 2006; Zeisel and da Costa 2009).

Copper

Intracellular copper deficiency increases the formation of amyloid deposits in the brain. Specifically, copper accumulates in amyloid plaques while remaining deficiencies in neighboring brain cells, indicating that copper deficiency is a plausible cause of Alzheimer's (Desai and Kaler 2008; Madsen and Gitlin 2007).

Inositol

Inositol regulates cell membrane transport, thus explaining its key interaction with several hormones and regulatory functions. Research suggests it can protect against the formation of abnormally folded toxic proteins seen in Alzheimer's patients. Inositol treatment also has beneficial effects on depression and anxiety (Kevin et al. 2007; Kevin 2010).

Oleic Acid

This fatty acid is found primarily in olive oil and is the precursor to oleamide, which interacts with several neurotransmitters and has demonstrated antidepressant-like properties. Oleic acid also facilitates absorption of vitamin A into cells (Amtul et al. 2011).

Vitamin C

The highest levels of vitamin C in the body present in nerve endings, next to the adrenal glands. High intakes of vitamin C are associated with lower risk of Alzheimer's disease (Brau et al. 1984; Adams et al. 1991).

Vitamin E

In addition to the anti-oxidative properties, vitamin E reduces death to cells in the hippocampus and protects the brain from glutamate toxicity. High dietary intake of vitamin E may lower Alzheimer's risk (Adams et al. 1991; Farr et al. 2003).

Role of Important Amino Acids on Neurological Diseases

Serine

This amino acid is the major component of phosphatidylserine, an integral part of cell membranes in the brain. Phosphatidylserine increases the release of several neurotransmitters, including dopamine, serotonin, acetylcholine, and epinephrine, thus improving the rate at which mental processes occur, without the hyperactivity or compulsive behavior that often occurs with drugs that stimulate a single neurotransmitter (de Koning et al. 1999; de Koning and Klomp 2004; Madeira et al. 2015).

Glycine

An increasing local synaptic concentration of glycine presents a viable approach to potentiate NMDA receptor function. Enhancing NMDA receptor function directly via GluN2 glutamate agonists is not an option, as these agents can lead to excessive neuronal activation resulting in epileptic seizure and excitotoxicity (Wojciech and Parsons 1998); however, potentiation can be achieved via stimulation of the GluN1 glycine_B binding site without inducing glutamatergic over-excitation. Indeed, clinical

proof-of-concept studies have shown that high doses of glycine 60 g/day administered as an add-on to atypical antipsychotics led to a modest improvement of multiple schizophrenic symptom domains, including negative and cognitive symptoms (Heresco-Levy et al. 1999). However, long-term use of glycine was deemed impractical due to the large doses required resulting from poor pharmacokinetics (PK) and CNS penetrance. Nonetheless, these encouraging clinical results provided an impetus to discover alternative approaches for increasing synaptic glycine levels at the NMDA receptor (Wilcox et al. 1996; Altamura et al. 1995; Araki et al. 1995). Glycine is required by the body for the maintenance of the central nervous system and studies have shown that glycine can be beneficial in cases of chronic spasticity, including multiple sclerosis, and its inhibitory action can help to prevent epileptic seizures (Sattler and Rothstein 2006; Saunders et al. 2008). Glycine has also been used in treating manic psychological states and has a calming effect on the brain (Shaheen and Karen 2008; Leiderman et al. 1996; Javitt et al. 2001).

Tryptophan and Tyrosine

Tryptophan and tyrosine are unique among amino acids in being precursors to brain neurotransmitters, the synthesis and release of which are sensitive to relatively small, physiologic changes in precursor concentrations (Fernstrom 1983). The brain concentrations of both amino acids are readily modified by the ingestion of either amino acids, as well as other amino acids that share a competitive transporter for uptake into the brain from the circulation (Katri et al. 2011; Karhunen et al. 2008). By modifying serotonin and catecholamine synthesis and release, tryptophan, tyrosine, and its transport competitors can influence central nervous system function. Such effects can be produced either by ingesting amino acids or a variety of dietary proteins. The literatures revealed that these relationships, and how they have been and are being used to apply amino acid supplements and dietary proteins to the modification of mood, cognition, and physical performance (Fernstrom 1983, 2013). There are wide differences among studies in the amount of a protein or amino acid mixture administered, and little consideration is given to the formulation of LNAA mixtures. It would be useful to know how much protein or branched-chain amino acids (BCAA) are needed to elicit a particular effect (Rajagopal 2013; Rajagopal et al. 2014).

A broader, physiologic question is why serotonin neurons are at all susceptible to food, most notably protein intake. So far, no other transmitter has demonstrated such sensitivity to dietary precursor supply. One possibility, in relation to protein ingestion, is that the brain may receive information regarding dietary protein quality via serotonin neurons, though the correlation between dietary protein quality and the influence of protein ingestion on brain tryptophan and serotonin is not perfect (Fernstrom 1983). But it may be premature to dismiss this possibility, since the relevant databases are quite small. If there is a functionally important link between tryptophan and LNAA intake as constituents of dietary protein, brain serotonin, and perception of protein quality, it might have been most useful when humans were hunter-gatherers, often subsisting on near or below requirement intakes of proteins, with intermittent access to protein (Fernstrom 2013).

Tryptophan is found in nearly all protein-containing foods and is the precursor of the neurotransmitter serotonin. Tyrosine is one of the conditionally essential amino acids and is the precursor of the catecholamine neurotransmitters dopamine (DA), norepinephrine, and epinephrine (Fernstrom and Fernstrom 2007). Unlike almost all other neurotransmitter biosynthetic pathways, concentrations of brain tryptophan as well as tyrosine are readily modified by dietary intake. This relation is observed when either tryptophan or tyrosine is administered directly (Ashcroft et al. 1965) or by consumption of protein-containing foods or other amino acids that share the competitive transporter for uptake into the brain from the blood (Fernstrom and Fernstrom 1987).

The elderly people may be particularly vulnerable to inadequate amounts of protein, because their reduced reservoir in the form of skeletal muscle mass leads to a reduced whole body protein synthesis, which in turn may lead to decreased capacity to adapt to decreased dietary protein intake and reduced availability of neurotransmitter precursors (Young 1990). The effect of total protein on cognitive functioning is probably attributed to specific amino acids that are common constituents of protein-rich foods, such as tyrosine and tryptophan. Tryptophan loading then moves serotonin toward the optimal level and improves performance, whereas serotonin levels in healthy subjects move beyond the optimal level and have no or a detrimental effect on performance. To date, it has been difficult to show this in humans. Also, for the other amino acids and total protein intake, it would be useful to know how much is needed to elicit a particular effect. Furthermore, it is not known how often the treatment should be given to maintain the observed effect (Fernstrom 1983, 2013; Fernstrom and Fernstrom 1987).

In addition, especially for tyrosine, it is important to know whether the effect persists and if the treatment is provided chronically, since neurons can become unresponsive to additional tyrosine. If their firing frequency slows, tolerance may develop (Strauss et al. 2007; Bijarnia et al. 2008). Moreover, the availability of the specific precursor amino acids for brain neurotransmitters is highly dependent on each other, because the amino acids are competing with the same carrier to enter the BBB. To increase and maximize availability of tyrosine or tryptophan, keeping the right ration between the amino acid of interest and its competing amino acids has to be taken into account in supplementation studies (Glick et al. 1982; Blundell et al. 1995).

Sources of Aromatic Amino Acids

Humans and other mammals are incapable of synthesizing tryptophan or phenylalanine de novo and must ultimately obtain these essential amino acids by consuming proteins. The liver is able to make tyrosine from phenylalanine through the action of phenylalanine hydroxylase (PAH), hence mammals normally obtain tyrosine from an exogenous source, dietary protein, and endogenous synthesis (which provides about 15–20 % of the tyrosine in human plasma) (Barazzoni et al. 1998). Tryptophan is usually the least abundant amino acid in most dietary proteins, constituting, for example, only 1–15 % of the amino acids in casein, ovalbumin, and most meats

(Orr and Watt 1968); however, a few proteins – notably α-lactalbumin, a minor milk protein which is 6 % tryptophan – contain substantially more. Phenylalanine and tyrosine generally account for 3–4 % of the amino acids in most dietary proteins. All three aromatic amino acids are primarily metabolized in the liver by tryptophan dioxygenase (TDO), PAH, and tyrosine aminotransferase (TAT), respectively. Hence, only a portion of each actually enters the systemic circulation after a meal. The three aromatic amino acids can also be released from reservoirs in tissue or circulating proteins; however, this contribution is minor except in starvation (Felger and Miller 2012; Anisman and Merali 2003; Banks and Erickson 2010).

Glutamate and Asparagine

Both act as neurotransmitters in the brain (Butterworth et al. 1980; Meldrum 2000).

Ketogenic Diet

The ketogenic diet has been in clinical use for over 80 years, primarily for the symptomatic treatment of epilepsy. There is evidence from uncontrolled clinical trials and studies in animal models that the ketogenic diet can provide symptomatic and disease-modifying activity in a broad range of neurodegenerative disorders and may also be neuroprotective in TBI and stroke. These observations are supported by studies in animal models and isolated cells that show that ketone bodies, especially beta-hydroxybutyrate, confer neuroprotection against diverse types of cellular injury (Gasior et al. 2006). Although the mechanism by which the diet protects against seizures is unknown, there is evidence that it causes effects on intermediary metabolism that influence the dynamics of the major inhibitory and excitatory neurotransmitter systems in the brain (Hartman et al. 2007). During consumption of the ketogenic diet, marked alterations in brain energy metabolism occur, with ketone bodies partly replacing glucose as fuel. It is plausible that neuroprotection can result from enhanced neuronal energy reserves, which improve the ability of neurons to resist metabolic challenges and possibly through other actions including antioxidant and anti-inflammatory effects. As the underlying mechanisms become better understood, it will be possible to develop alternative strategies that produce similar or even improved therapeutic effects without the need for exposure to the ketogenic diet (Freeman et al. 2006).

Dietary Restriction

The mean and maximum life spans of many different organisms, including yeast, roundworms, rodents, and monkeys, can be increased by up to 50 % simply by reducing their food intake (Ashrafi et al. 2000). The incidence of age-related

cancers, cardiovascular disease, and deficits in immune function is decreased in rodents maintained on such dietary restriction (DR) feeding regimens (Weindruch and Sohal 1997). Data from clinical and epidemiological studies in humans support the antiaging and disease prevention effects of DR. Thus, a low-calorie diet decreases the risk of the most prominent age-related diseases in humans, including cardiovascular disease, diabetes, and cancers (Brochu et al. 2000). Recent findings reviewed and strongly suggest that DR can delay age-related functional deficits in the brain and may reduce the risk of major neurodegenerative disorders including AD, PD, and HD. DR may also increase resistance of neurons to acute insults such as stroke and severe epileptic seizures (Levi 1999).

DR has beneficial effects in animal models of PD and HD. The vulnerability of midbrain dopaminergic neurons to MPTP toxicity was decreased in mice maintained on dietary restriction, with more dopaminergic neurons surviving exposure to MPTP; the motor function of the mice was also improved in the restricted mice (Duan and Mattson 1999). The neuroprotective effects of DR in animal models of several different neurodegenerative disorders suggest that low-calorie diets may prove beneficial in reducing the incidence and/or severity of the corresponding human neurodegenerative disorders (Fig. 1).

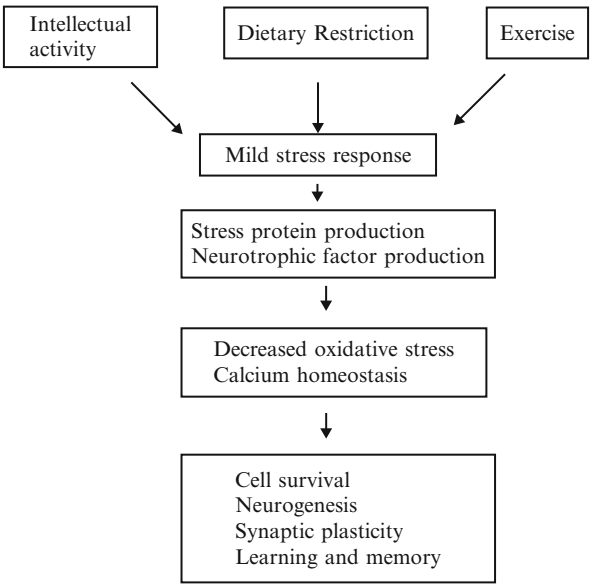

Fig. 1 Model of the mechanisms whereby dietary restriction, intellectual activity, and exercise promote neuronal survival and plasticity. Dietary restriction, activity in neuronal circuits, and physical exercise each induce a mild cellular stress response as a result of energetic factors (e.g., reduced glucose availability in dietary restriction and increased energy demand during intellectual and physical activity). Neurons respond to these stresses by activating signaling pathways that induce the expression of genes encoding proteins, such as growth factors and protein chaperones that promote neuronal survival and plasticity (neurogenesis, neurite outgrowth, and synaptic plasticity) (Mattson et al. 2002)

Cellular and Molecular Mechanisms Underlying the Neural Effects of Dietary Restriction

DR increases life span and reduces risk of many different age-related diseases including cardiovascular disease, diabetes, and cancers, and it might be expected that it modifies shared biochemical cascades that lead to cell dysfunction and disease. In the case of neurodegenerative disorders, it is clear that while different genetic and environmental factors may initiate the neurodegenerative process in different disorders, a shared biochemical cascade ensues. Increased oxidative stress, perturbed cellular calcium homeostasis, and impaired energy metabolism occurs in every neurodegenerative disorder studied to date (Mattson 1997a, b).

Conclusion

With respect to amino acids, the neurotransmitter concentrations are consistent with the subjects that are vulnerable. All together, the visible pictures from the study of the neurodegenerative disorders appear awfully complex, with many doubts and gaps to fill. The protein intake studies are very limited and the studies have already been done quite long ago. The further research can be done in the field of protein and amino acids in relation to neurodegenerative disorders.

Compliance with Ethics Requirements The authors declare that they have no conflicts of interest.

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Plant-Derived Natural Products for Parkinson's Disease Therapy

T. Sengupta, J. Vinayagam, R. Singh, P. Jaisankar, and K.P. Mohanakumar

Abstract Plant-derived natural products have made their own niche in the treatment of neurological diseases since time immemorial. Parkinson's disease (PD), the second most prevalent neurodegenerative disorder, has no cure and the treatment available currently is symptomatic. This chapter thoughtfully and objectively assesses the scientific basis that supports the increasing use of these plant-derived natural products for the treatment of this chronic and progressive disorder. Proper considerations are made on the chemical nature, sources, preclinical tests and their validity, and mechanisms of behavioural or biochemical recovery observed following treatment with various plants derived natural products relevant to PD therapy. The scientific basis underlying the neuroprotective effect of 6 Ayurvedic herbs/formulations, 12 Chinese medicinal herbs/formulations, 33 other plants, and 5 plant-derived molecules have been judiciously examined emphasizing behavioral, cellular, or biochemical aspects of neuroprotection observed in the cellular or animal models of the disease. The molecular mechanisms triggered by these natural products to promote cell survivability and to reduce the risk of cellular degeneration have also been brought to light in this study. The study helped to reveal certain limitations in the scenario: lack of preclinical studies in all cases barring two; heavy dependence on in vitro test systems; singular animal or cellular model to establish any therapeutic

T. Sengupta • R. Singh
Division of Cell Biology & Physiology, Indian Institute of Chemical Biology (CSIR, Govt of India), 4, Raja S.C. Mullick Road, Jadavpur, Kolkata 700 032, India

J. Vinayagam • P. Jaisankar
Division of Chemistry, Indian Institute of Chemical Biology (CSIR, Govt of India), 4, Raja S.C. Mullick Road, Kolkata 700 032, India

K.P. Mohanakumar (✉)
Division of Cell Biology & Physiology, Indian Institute of Chemical Biology (CSIR, Govt of India), 4, Raja S.C. Mullick Road, Jadavpur, Kolkata 700 032, India

Inter University Centre for Biomedical Research & Super Specialty Hospital, Mahatma Gandhi University Campus at Thalappady, Rubber Board PO, Kottayam 686009, Kerala, India
e-mail: mohankumar@iicb.res.in; kpmohanakumar@yahoo.com; iucbr@mgu.ac.in

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potential of drugs. This strongly warrants further studies so as to reproduce and confirm these reported effects. However, the current literature offers scientific credence to traditionally used plant-derived natural products for the treatment of PD.

Keywords Traditional medicine • *Ayurveda* • Behavioral recovery • Striatal dopamine levels

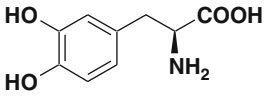
Introduction

Natural products are small molecules found in diverse natural sources, and they collectively possess a coveted position in the treatment of nearly all human ill-health conditions, including neurological diseases (Butler 2008). They are said to be the single most successful source for drug leads, as documented in literature (see Harvey 2008), and their prospects in drug research, discovery, and development are enormous (Newman and Cragg 2007). As per an estimate, one-third of all small-molecule drugs and three-fourths of the approved antibacterial drugs are derived from natural products (Newman and Cragg 2007). The importance of plant-derived natural products for the treatment of neurological diseases is illustrated by the fact that many of the earliest drugs used for the treatment of neurological disorders were derived from plants that include opiate and tropane alkaloids, galantamine, anticholinesterases like physostigmine, Huperzine A, to name a few. Saklani and Kutty (2008) reported that out of a total of 26 plant-based drugs launched during 2000–2006, 7 are for neurological diseases that included 3 for Parkinson's disease (PD). They observed that in the year 2006, as many as 13 plant-derived drugs for the treatment of pain and neurological ailments were on various phases of clinical trials (Saklani and Kutty 2008).

PD is the second most prevalent degenerative disorder of the central nervous system (CNS). The cardinal manifestations of this disorder are tremor, rigidity, akinesia, bradykinesia, and postural instability. Epidemiologically it is estimated that about 1–2 % and 3–5 % respectively of the population who are over 65 and 85 years suffer from PD (Fahn 2003). Although the disease affects motor functions primarily, it slowly gives rise to a wide array of non-motor complications as it progresses. Although the disease was reported in Western medical literature only in the nineteenth century by James Parkinson (Gourie-Devi et al. 1991), the description of the dysfunction and prescription for management of the disease can be found in ancient medical systems of India and China, where natural product based herbal therapy has been used. This vouches for the use of natural products since ancient times, and continues to hold great promise for the treatment of this debilitating disorder. The present study reviews plant-derived natural products, their chemical nature, test mode and mechanisms of neuroprotection relevant to the treatment of PD to identify existing lacunae in scientific evidences and to suggest future research needs in this area that may set the trend and direction of future research on the subject.

An Overview of the Pathophysiology and Treatment of PD

PD is characterized by about 80 % loss of the neurotransmitter dopamine (DA) in the *corpus striatum* region of the brain, resulting from more than 50 % loss of dopaminergic (DA-ergic) neurons in the substantia nigra pars compacta (SNpc) region of the brain (Toulouse and Sullivan 2008). One of the major pathological hallmarks of the disease is the presence of intra-neuronal protein aggregates known as Lewy bodies (Samii et al. 2004), suggested to be resulting from cellular inability to clear abnormal proteins (Toulouse and Sullivan 2008). There is no single factor that has been uncovered as the cause of this progressive neurodegenerative disease, but numerous mechanisms that have been proposed include oxidative stress, excitotoxicity, apoptosis, protein aggregation, proteasomal defects, mitochondrial dysfunction, genetic predisposition, and environmental factors.



L-DOPA

Despite five decades of efforts, no efficient treatment has been developed for this disorder. Present therapy mainly depends on symptomatic recovery, but many drugs currently used have severe side effects and their therapeutic benefits wear off with long-term use. The major therapeutic approach has been to either elevate the levels of DA by administering its precursor, L-3,4-dihydroxyphenylalanine (L-DOPA) along with a peripherally acting aromatic amino acid decarboxylase inhibitor, or by inhibiting monoamine oxidase (MAO) and/or catechol-*O*-methyl transferase (COMT) activities, both of which metabolically degrade DA. Post-synaptically acting DA receptor agonists are effective in treating the primary motor symptoms of PD, but their use is limited by cognitive side effects on prolonged use. The latest approach is neuroprotective strategies that aim at preserving the surviving neurons, and are administered in conjunction with symptomatic palliative medications.

Methodology

In order to collect relevant information for attaining the abovementioned goal a systematic review has been conducted using the PubMed (www.pubmed.com) search engine for accessing the MEDLINE database. All relevant English language articles published between 1970 and June 2015 were searched using the terms "natural product AND Parkinson's disease," "herb AND Parkinson's disease," "botanical AND Parkinson's disease," "plant AND Parkinson's disease," and "extract AND Parkinson's disease." Searches using "plant name AND Parkinson's disease" have also been made for any additional cross-references. This review includes products

derived from plant sources apart from a single fungus *Ganoderma lucidum* but traditional medicinal concoctions or energy drink (see Datla et al. 2004) whose exact compositions are not known, are excluded. All plants identified were confirmed using "The International Plant Names Index" (www.ipni.org) and cross-checked using Wikipedia (www.wikipedia.org), the online encyclopedia.

Animal and Cellular Models of PD

There are several animal and cellular models for PD which are extensively used, the former for obtaining information on the neuroanatomical and behavioral aspects of the disease while the latter for analyzing the mechanism of cell death including the molecular bases of the affliction. Some of the important animal and cellular models that are extensively used for carrying out preclinical tests of plant-derived natural products for PD are as follows:

The best-characterized animal model of PD has been developed by using the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Bloem et al. 1990). Remarkable clinical symptoms similar to sporadic PD in humans result after injection of MPTP (Langston et al. 1983). After its systemic administration, MPTP crosses the blood–brain barrier and is metabolized in astrocytes to its active metabolite, 1-methyl-4-phenyl-pyridinium ion (MPP^+) by MAO-B (Vila and Przedborski 2003). MPP^+ is selectively taken up by DA-ergic neurons due to its affinity for the DA transporters resulting in selective toxicity to DA-ergic neurons (Javitch et al. 1985). Infusion of MPP^+ into the median forebrain bundle (MFB) or striatum or SNpc in rats caused the significant loss of DA-ergic neurons in the SNpc with ensuring changes in the behavioral, neurochemical, and biochemical aspects of the lesion (Banerjee et al. 2006; Sindhu et al. 2006).

The neurotoxin 6-hydroxydopamine (6-OHDA) was the first agent used to model PD about half a century ago (Ungerstedt 1968). Because 6-OHDA cannot cross blood–brain barrier in the adult brain, it is stereotaxically administered to DA-ergic nuclei. 6-OHDA-induced pathology closely mimics those found in postmortem PD (Mendez and Finn 1975). The 6-OHDA model of the PD is based on the generation of free radicals as well as potent reversible inhibition of mitochondrial electron transport chain (ETC) complex-I and -IV (Glinka and Youdim 1995).

Rotenone is a naturally occurring compound derived from plants, which has been used as an insecticide in vegetable gardens, and to destroy or sample fish populations in lakes or reservoirs. Rotenone is known to be a high-affinity specific inhibitor of ETC Complex-I enzyme of the inner mitochondrial membrane that is involved in oxidative phosphorylation (Betarbet et al. 2000). More than 20 years ago Heikkila et al. (1985) have demonstrated that MFB infusion of rotenone caused damage to the dopaminergic neurons of the nigrostriatal pathway. Several years after, Ferrante et al. (1997) reported that systemic administration of rotenone produced a unique pattern of CNS damage by sparing SNpc but damaging both nucleus caudate putamen (NCP) and globus pallidus.

The *Rauwolfia* alkaloid, "reserpine" is a catecholamine-depleting agent that blocks vesicular storage of monoamines (Bernheimer et al. 1973). Systemic administration

of reserpine diminished the ability to perform spontaneous movements in rabbits due to the depletion of brain catecholamine levels. L-DOPA receptor agonists alleviated the reserpine-induced akinetic state (Utley and Carlsson 1965). Limitations of this model are that reserpine-induced changes are transient and do not result in any pathological changes in SNpc DA-ergic neurons.

Maneb (manganese ethylene-bis-dithiocarbamate) is an agricultural fungicide formerly used for the control of a wide variety of fungal diseases in fruits, vegetables, and ornamental plants. Exposure to a combination of maneb and the herbicide paraquat in mice led to increased SNpc neuronal pathology (Costello et al. 2009). Meco et al. (1994) reported permanent parkinsonism in a man with chronic exposure to the maneb. It has been shown that maneb has an inhibitory effect on locomotor activity and aggressiveness. Furthermore, when given an acute administration of this fungicide in experimental animal, it causes a depressant-like effect on the central nervous system, with the partial involvement of DA-ergic systems in the mediation of these effects (Morato et al. 1989).

Paraquat is a herbicide, which is structurally related to MPP^+ and is known to produce PD in experimental animals. Exposure to paraquat confers increased risk to PD (Liou et al. 1997). The toxicity of paraquat appears to be mediated through excessive reactive oxygen species (ROS) generation (Day et al. 1999).

The lipopolysaccharide (LPS) model is a comparatively recent addition to the animal models of PD and involves the intra-cranial or systemic infusion of LPS in an animal. The model has been shown to replicate fairly well the basic neuropathological features of PD. Acute intranigral infusion of LPS produces progressive loss of dopamine in the striatum, it is regionally specific to DA, and gravity of damage is DA-dependent (Kim et al. 2000). LPS infusion has been shown to induce microgliosis (Iczkiewicz et al. 2005), to disrupt blood–brain barrier (Tomás-Camardiel et al. 2004), increase pro-inflammatory cytokines (Dutta et al. 2008), to cause caspase-3 activation and calcium disturbances, and to inhibit state III and state V respiration by inhibiting mitochondrial ETC complexes I and II (Hunter et al. 2007).

Haloperidol is an antipsychotic drug that was developed in 1958. Haloperidol produces the extrapyramidal parkinsonian symptoms manifested in various behavioral abnormalities such as akinesia, catalepsy, and reduced swimming ability in experimental animals. Restoration of these motor dysfunctions can be used as preclinical model to study PD (Luthra et al. 2009).

Various reports suggest the causative mechanisms of PD to involve oxidative stress and inflammation, abnormal protein folding and "toxic" aggregation. Authenticity of many such factors could not be established further, due to inaccessibility of targeted living neuronal populations from PD patients, and lack of reliance on postmortem brain samples. The cell culture techniques allow replicating few of the characteristics, by deriving patient-specific cell lines from individuals with sporadic forms of PD and also those with known disease-causing mutations. There are cell lines with neuronal lineage, which have the potential to serve as a human cellular model for PD when differentiated into DA-ergic neurons. The cell culture models have got advantages over animal models since they can be human genome based, and thereby allow the direct investigation of pathophysiological characteristics in far less time, less labor intensive and such techniques can be developed for high throughput screening of therapeutic compounds.

SH-SY5Y (ATCC # CRL-2266) represents a neuroblastoma cell line, which can be differentiated into neurons. The other cell lines, which possess similar properties, include SK-N-MC (ATCC # HTB-10), SK-N-SH (ATCC # HTB-11), and SK-N-BE (ATCC # CRL-2271) cell lines. Various neurotoxins like MPP^+ (Kalivendi et al. 2003), rotenone (Watabe and Nakaki 2004), paraquat (Yang and Tiffany-Castiglioni 2008), 6-OHDA (Gomez-Lazaro et al. 2008) have been tested in SH-SY5Y cells for apoptosis, protein aggregation studies, etc. The PC12 (ATCC # CRL-1721) cell line is derived from a pheochromocytoma of the rat adrenal medulla. When exposed to nerve growth factor in vitro, these neoplastic cells undergo the last major steps of neuronal differentiation in order to acquire the neuronal phenotype (Levi et al. 1988).

Plant-Derived Natural Products with Antiparkinsonian Potential

Ayurvedic Herbs

Ayurveda, the Indian traditional system of medicine uses herbs, spices, and minerals for the treatment of diseases (see Table 1). Charaka Samhitha, the classical *Ayurveda* text mentions the use of four plants, viz. *Mucuna pruriens*, *Hyoscyamus niger*, *Withania somnifera*, and *Sida cordifolia* for the treatment of PD.

***Mucuna pruriens* (L.) DC. (Family *Leguminosae*)**

The tropical legume *M. pruriens* is unusual in its characteristics, and is very uniquely studied for the treatment of PD by several laboratories. A multicentric, preclinical trial reported for the first time its validity for the treatment of the disease, wherein 60 PD patients were treated for 12 weeks with a herbal formulation (HP-200) commercially available from the tropical legume (Anonymous 1995). The study found statistically significant reductions in Hoehn and Yahr (H&Y) scale and improvements in United Parkinson's Disease Rating Scale (UPDRS) scores from baseline level.

In a prospective clinical study Nagashayana et al. (2000) have demonstrated the efficacy of an *Ayurveda* treatment, consisting of a mixture in 200 ml cow's milk of powdered *Mucuna pruriens* (4.5 g) and *Hyoscyamus niger* (0.75 g) seeds and *Withania somnifera* (14.5 g) and *Sida cordifolia* (14.5 g) roots, in 18 clinically diagnosed parkinsonian patients. Symptomatically, the patient group that underwent both cleansing and palliative therapy as per *Ayurveda* principles exhibited better response in tremor, bradykinesia, stiffness and cramps as compared to the group that underwent only palliative therapy. Activities of daily living (ADL) and motor examination rating were found to improve in patients of both the groups.

Table 1 Ayurvedic herbs

Plants/source	Plant parts/preparations	Test mode	Properties	References
Mucuna pruriens	Seed extract	Clinical study in 6-OHDA, MPTP and paraquat models	Positive in clinical trials Antioxidant Metal chelator DA neurons not protected in MPTP model	Dhanasekaran et al. (2008) Kasture et al. (2009) Katzenschlager et al. (2004) Lieu et al. (2010) Manyam et al. (2004a, b) Nagashayana et al. (2000) Tharakan et al. (2007) Yadav et al. (2013)
Hyoscyamus niger	Seed extract	Clinical study MPTP model	Anticholinergic Ca^{2+} channel blocker	Gilani et al. (2008) Nagashayana et al. (2000) Sengupta et al. (2011)
Sida cordifolia	Root extract	Clinical study Rotenone model		Khurana and Gajbhiye (2013) Nagashayana et al. (2000)
Withania somnifera	Root extract	Clinical study MPTP and 6-OHDA models Haloperidol induced catalepsy in mice maneb–paraquat model	Antioxidant	Ahmad et al. (2005a) Kumar and Kulkarni (2006) Nagashayana et al. (2000) Nair et al. (2008) Rajasankar et al. (2009) Sankar et al. (2007)
Nardostachys jatamansi	Ethanolic extract of the roots	6-OHDA model	Improves locomotor activity; attenuates DA loss; reduces lipid peroxidation; increases glutathione content; increases D_2 receptor binding; increases TH expression; increases antioxidant enzyme activities	Ahmad et al. (2006a, b)
BR-16A	An Ayurvedic medication containing 20 different plants	Haloperidol and reserpine-induced catalepsy in mice	Protects against haloperidol and reserpine induced catalepsy	Kumar and Kulkarni (2006)

(continued)

Table 1 (continued)

Plants/source	Plant parts/preparations	Test mode	Properties	References
Centella asiatica	Whole plant extract	MPTP model	Antioxidant	Haleagrahara and Ponnusamy (2010)
Bacopa monnieri	Standardized extract	paraquat model 6-OHDA model Mutant model	Antioxidant Protects mitochondria Prevents alpha-synuclein aggregation	Hosamani and Muralidhara (2010) Hosamani et al. (2014) Jadiya et al. (2011) Shobana et al. (2012)
Stereospermum suaveolens	Whole plant extract	6-OHDA model	Antioxidant	Shalavadi et al. (2012)
Tinospora cordifolia	Whole plant extract	6-OHDA model	Protects mitochondrial complex-I Reduces iron asymmetry in SN	Kosaraju et al. (2014)

Given are herbs that are used in the traditional Indian medicine, *Ayurveda*. *6-OHDA* 6-hydroxydopamine, *DA* dopamine, *L-DOPA* L-2,4-dihydroxyphenylalanine, *MPTP* 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, *SN* substantia nigra, *TH* tyrosine hydroxylase

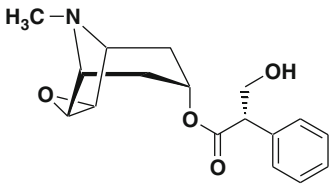
Katzenschlager et al. (2004), in a clinical study involving 8 patients, have shown that *M. pruriens* preparation possesses better clinical effects and pharmacokinetic properties in comparison to conventional L-DOPA administered along with a peripheral decarboxylase inhibitor and was without any effect on the severity of dyskinesia or peripheral adverse effects of DA. This pilot study suggested that *M. pruriens* formulation possesses potential advantages over commercially available L-DOPA formulations in terms of a rapid onset of action with a slightly longer duration of therapeutic response.

Although Nagashayana et al. (2000) have reported significant L-DOPA content in the milk sample containing powdered herbs (200 mg per dose, derived primarily from *M. pruriens*) and established this molecule as a major contributor to the efficacy of this Ayurvedic therapy, subsequent studies have thrown light on factors other than L-DOPA that might contribute to the usefulness of this treatment. Manyam et al. (2004a, b) have shown that long-term oral administration of *M. pruriens* endocarp preparation is more efficacious compared to L-DOPA in experimental animal models of PD. They have further demonstrated that the treatment did not exhibit any adverse effect on monoamine neurotransmitters in nigrostriatal tract unlike caused by the synthetically prepared precursor molecule. A recent study carried out in 6-OHDA model of the disease showed *M. pruriens* extract to be more potent in causing behavioral recovery in comparison to equivalent dose of L-DOPA (Kasture et al. 2009). Moreover, the dyskinetic potential of the extract has also been found to be less than L-DOPA, although the same study failed to show any protection in the MPTP model of parkinsonism.

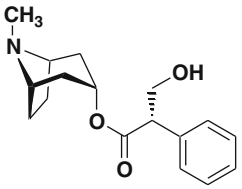
The advantages of *M. pruriens* therapy are attributed to several ingredients contained therein in addition to L-DOPA such as NADH and coenzyme Q_{10} (Manyam et al. 2004b). Additionally, *M. pruriens* administration has also been shown to increase brain complex-I activity without altering MAO activity in vitro, to significantly restore endogenous L-DOPA, DA, norepinephrine and serotonin levels in SN (Manyam et al. 2004a). Aqueous extract of *M. pruriens* seed cotyledon powder could chelate divalent copper ions and protect both plasmid and genomic DNA from Cu^{2+}-induced DNA strand scission and damage (Tharakan et al. 2007), and significantly inhibit oxidation of lipids and deoxyribose sugar by its antioxidant and metal chelating potential (Dhanasekaran et al. 2008). A recent study has pointed to the antioxidant potential of this extract in protecting against paraquat induced PD model as well (Yadav et al. 2013). Moreover, it has been shown that long-term *M. pruriens* treatment affords lesser risk of dyskinesia as compared to equivalent dose of L-DOPA (Lieu et al. 2010). These basic studies suggested that prolonged administration of *M. pruriens* is pharmacologically safer in comparison to the standard drug, L-DOPA. Therefore, the need of the hour is to isolate the active ingredients other than L-DOPA from *M. pruriens*, which may hold good promise for developing it into an effective therapy for PD.

Hyoscyamus niger L. (Family *Solanaceae*)

H. niger has been used in the traditional medical systems of various cultures such as Indian, Chinese, Roman and Byzantine for the treatment of a wide range of diseases. There is no mention of its independent use for the treatment of parkinsonism in human patients or in any animal model in the literature. However, its usefulness in conjunction with three other Ayurvedic herbs has been validated in a clinical study (Nagashayana et al. 2000). While the L-DOPA content of *H. niger* is negligible (Nagashayana et al. 2000), it is rich in tropane alkaloids, hyoscine and hyoscyamine, which are well known for their anticholinergic effects (Brown and Taylor 2001). As DA is depleted in the striatum in PD, there is a relative excess of the neurotransmitter acetylcholine in this region of the brain, which is said to be one of the causes of various motor abnormalities associated with PD. Therefore, anticholinergics are used in the treatment of PD, particularly tremor (Rezak 2007). Another study showed that methanolic extract of *H. niger* seed possessed Ca^{2+} channel blocking property apart from muscarinic receptor antagonistic action (Gilani et al. 2008). All these properties of *H. niger* probably add to the therapeutic potential of the Ayurvedic preparation, which is mainly derived from the anticholinergic effects of the molecules contained therein. However, extensive investigation into the therapeutic efficacy of *H. niger* is warranted, since we have recently found out that aqueous methanolic extracts of the seeds could significantly attenuate MPTP-induced DA depletion and several of the behavioural dysfunctions (Sengupta et al. 2011).



Hyocyne or Scopolamine



Hyoscyamine

Withania somnifera L. (Dunal) (Family *Solanaceae*)

W. somnifera is unique in its properties as a tonic and metabolic rejuvenator. It is the Indian ginseng, and its extract has been reported to dose-dependently attenuate various parameters of neurotoxicity caused by 6-OHDA lesioning such as, behavioral abnormality, increased lipid peroxidation, decreased contents in DA and reduced-glutathione (GSH) contents, alterations in the activities of glutathione-S-transferase (GT), glutathione reductase (GR), glutathione peroxidase (GP), superoxide dismutase (SOD), and catalase, reduced DA-ergic D2 receptor binding and loss in tyrosine hydroxylase (TH) expression (Ahmad et al. 2005a). Such antioxidant, and pro-DA-ergic properties of *W. somnifera* root (Sankar et al. 2007) or leaf (Rajasankar et al. 2009) extract have been implicated in its neuroprotective effects in the MPTP model of PD. *W. somnifera* extract has also been found to reverse haloperidol- or reserpine-induced catalepsy in albino mice (Kumar and Kulkarni 2006; Nair et al. 2008) and maneb–paraquat combination-induced PD model (Prakash et al. 2013).

BR-16A, a polyherbal medication derived from *Ayurveda*, contains over 20 different ingredients with the exact formulation differing between pediatric and adult formulations. BR-16A or *W. somnifera* L. has been shown to render protection against haloperidol or reserpine-induced catalepsy and provide hope that BR-16A could be used in preventing the drug-induced extrapyramidal side effects, and therefore may offer a new therapeutic approach to the treatment of PD (Kumar and Kulkarni 2006).

Sida cordifolia L. (Family *Malvaceae*)

The herb *S. cordifolia*, used as an Ayurvedic medicine for the treatment of various common ailments such as asthma, nasal congestion, and rheumatoid arthritis, has been found to have CNS depressive activity (Franco et al. 2005). Similar to *H. niger* there is no mention of its independent use for the treatment of human PD patients or any report on studies in animal model of the disease in the literature, baring one. However, its usefulness in conjunction with three other Ayurvedic herbs has been validated in a clinical study as discussed before (Nagashayana et al. 2000). In a recent study the ameliorative effect of aqueous extract of *S. cordifiloia* and its subfractions on various PD pathological markers have been demonstrated in a chronic rotenone-induced model of PD (Khurana and Gajbhiye 2013). In this study, treatment with the crude extract and its aqueous fraction produced protection from behavioral and oxidative stress related damages and DA loss caused by prolonged rotenone exposure. Less polar fractions (viz. hexane or chloroform) failed to show significant anti-PD effects (Khurana and Gajbhiye 2013).

Nardostachys jatamansi DC. (Family *Valerianaceae*)

Ethanolic extract of the Ayurvedic medicinal plant *N. jatamansi* roots can slow down the neuronal injury in a 6-OHDA-rat model of PD (Ahmad et al. 2006b). Treatment of the animals with the extract prior to 6-OHDA lesioning effectively reduced the neurotoxin-induced lipid peroxidation, increased GSH content, the activities of GT, GR, GP, SOD, and catalase, attenuated loss of catecholamines, increased DA-ergic D_2 receptor binding and TH-immunoreactivity. The increase in dopaminomimetics-induced rotations and deficits in locomotor activity and muscular coordination due to nigrostriatal degeneration were also dose-dependently restored by *N. jatamansi* extract.

Centella asiatica L. (Family *Apiaceae*)

This plant is widely used in the *Ayurveda* and other traditional systems of medicine. A study has demonstrated that *C. asiatica* extract can prevent MPTP-induced neurotoxicity in aged rats (Haleagrahara and Ponnusamy 2010). Supplementation with the extract led to a significant diminution in lipid hydroperoxides and protein-carbonyl levels in the striatum. On the other hand, levels of antioxidant enzymes such as SOD, GP and catalase and catalase were also significantly elevated by the extract (Haleagrahara and Ponnusamy 2010).

Bacopa monnieri L. (Family *Plantaginaceae*)

This plant is widely used in *Ayurveda* as a cognitive enhancer. In recent years some preclinical studies have brought out its use in treating PD. In the paraquat-induced PD model in *Drosophila* and mice, *B. monnieri* extract was found to have significant prophylactic action primarily through antioxidant properties and restoration of the mitochondrial ETC complexes activities (Hosamani and Muralidhara 2010; Hosamani et al., 2014). Similar antioxidant action of the alcoholic extract of the plant was shown to play a major role in its ability to protect 6-OHDA-lesioned rats against behavioral and biochemical abnormalities (Shobana et al. 2012). Interestingly in PINK1 mutant flies *B. monnieri* restored the climbing ability (Jansen et al. 2014). In a genetically induced PD model in *C. elegans*, *B. monnieri* treatment reduced α-synuclein aggregation and DA neurodegeneration (Jadiya et al. 2011). Alcoholic extract of *B. monnieri* has also been shown to protect against behavioral and biochemical lesions caused by 6-OHDA administration in rats (Shobana et al. 2012).

Stereospermum suaveolens DC (Family *Bignoniaceae*)

This plant is widely used in *Ayurveda*. Methanolic extract of this plant offers significant protective effect in the 6-OHDA model of PD in terms of behavior and reduction in oxidative stress (Shalavadi et al. 2012).

Tinospora cordifolia Willd. Miers (Family *Menispermaceae*)

This is a deciduous climbing herb found in India, China and some other parts of Asia. The plant is widely used in the Ayurvedic system of medicine. In a recent study its anti-parkinsonian properties have been demonstrated in 6-OHDA model of the disease (Kosaraju et al. 2014). Chronic dosing of ethanolic extract of the plant led to an increase in skeletal muscle coordination, striatal DA level and mitochondrial ETC complex-I activity in 6-OHDA infused animals (Kosaraju et al. 2014). Interestingly, the iron asymmetry ratio in the SN region of the brain was attenuated by treatment with this extract (Kosaraju et al. 2014).

Chinese Herbs

The most important characteristic feature of traditional Chinese medicine (TCM) is the use of multiple, crude herbal extracts along with acupuncture for the treatment of PD. Such treatment modalities existed in Eastern Asia since thousands of years and are even now extremely popular in countries such as China, Japan, and Korea. The scientific basis of TCM for the treatment of PD has been reviewed by various authors (Li et al. 2006a, b; Wang et al. 2008). Major formulations and herbs used in this category (Table 2) are assessed here for their scientific modalities of action.

Bak Foong

Bak Foong is a well known poly-herbal drug used in TCM and the formulation contains a mixture of *Panax ginseng* (Family *Araliaceae*), *Angelica sinensis* (Family *Apiaceae*), *Glycyrrhiza uralensis* (Family *Leguminosae*), and *Ligusticum chuanxiong* (Family *Apiaceae*). Bak Foong has demonstrated neuroprotective effects in both MPTP- and 6-OHDA-models of PD. It has been reported to significantly attenuate loss in DA transporter (DAT) and TH mRNA levels in the midbrain and striatum of C57BL/6 mice treated with MPTP and to possess anti-apoptotic properties (Liu et al. 2004, 2008a). Bak Foong prevented MPP^+-induced apoptosis and cell death in PC12 cells by reducing the expression of the pro-apoptotic gene, Bax (Liu et al. 2008a). Similarly 6-OHDA-induced cellular toxicity, in terms of reduction in cell viability, increased nitric oxide levels, activated caspase-3 and apoptosis, are prevented by Bak Foong in PC12 cells (Jia et al. 2005). Thus amongst TCM, Bak Foong has been well studied, and has unequivocal scientific validation for its antiparkinsonian benefits. *Panax ginseng* water extract can prevent apoptosis and oxidative stress in SH-SY5Y cells exposed to MPP^+ toxicity (Hu et al. 2011). A recent study has demonstrated that *P. ginseng* extract affords significant neuroprotection and behavioral recovery in a progressive model of PD produced by chronic exposure to β-sitosterol-β-D-glucoside (Van kampen et al. 2014). Panaxatriol saponins isolated from *Panax* sp. has been shown to be neuroprotective in the MPTP-model of the disease by modulating inflammation and mitochondria mediated apoptosis (Luo et al. 2011).

Table 2 Chinese herbs

Plants/source	Active substances/ compounds	Type of substances/ compounds	Test models	Properties	References
Bak Foong pill	Traditional Chinese medicine	Polyherbal drug containing *Panax ginseng*, *Angelica sinensis*, *Glycyrrhiza uralensis*, and *Ligusticum chuanxiong*	MPTP model of PD in mice and in PC 12 cell line 6-OHDA model in PC12 cell	Anti-apoptotic by reducing Bax gene expression. Increases TH and DAT m-RNA levels NCP and midbrain. Inhibited the activation of caspase-3 and reduced NO in the 6-OHDA model	Liu et al. (2012) Liu et al. (2004) Liu et al. (2008a) Jia et al. (2005)
Buddleia lindleyana	Pedicularioside A	Phenylethanoid glycoside	MPP^+-induced toxicity of rat mesencephalic neuron primary culture	Protects TH-positive neurons from toxicity, inhibits the expression of the caspase-3 gene, inhibits cleavage of PARP in cultures exposed to MPP^+	Li et al. (2008a, b)
Cistanche deserticola	Total glycosides		MPTP model MPP^+-induced toxicity in SH-SY5Y cells	Behavioral recovery in climbing test, attenuates the loss of TH-positive neurons, protection to SHSY5Y cells is mediated by downregulation of the expression of pro-apoptotic gene GADD153	Li et al. (2008a, b) Wang et al. (2007)
Cistanche salsa	Echinacoside	Phenylethanoid glycosides	6-OHDA model MPTP model	Behavioral recovery in terms of spontaneous movement and latency on the rotating rod. Increases striatal DA and its metabolites. Increases TH-positive neurons. Reduces pro-apoptotic caspase 3 and 8 in the cerebellar granule cells	Chen et al. (2007a) Geng et al. (2004) Geng et al. (2007)
Ganoderma lucidum	Spore oil		MPTP model	Reduces involuntary movements in the pole test. Increases striatal DA and DOPAC levels. Increases TH positive neurons in SN	Zhu et al. (2005)

Gingko biloba	Egb 761, a standardized extract of *G. biloba*	A well-defined mixture of compounds from the leaves of *Gingko biloba*	MPTP model in mice paraquat induced toxicity in PC12 cells 6-OHDA model in rats	In the MPTP model: blocks lipid peroxidation, reduces superoxide radical production, prevents apoptosis In the paraquat model: attenuates collapse of mitochondrial membrane potential, reduces apoptosis, reduces caspace-3 positive cells, increases TH and Bcl-2 positive cells In the 6-OHDA model: pretreatment with Egb 761 caused behavioral recovery in terms of contralateral forepaw adjusting steps, restored activities of glutathione dependent enzymes, SOD and catalase in the striatum, attenuated loss of DA, its metabolites and D_2 receptor in the striatum, inhibits MAO-B, and attenuates L-DOPA induced toxicity	Ahmad et al. (2005b) Cao et al. (2003) Kang et al. (2007) Kim et al. (2004) Rojas et al. (2008) Yang et al. (2001)
Salvia miltiorrhiza	Salvianolic acid B	Polyphenolic antioxidant	6-OHDA-induced toxicity in SH-SY5Y.	Antioxidant, reduces intracellular calcium, reduces caspase-3 activity, prevents cytochrome *C* translocation, increases Bcl-x/Bax ratio, activation of protein kinase C	Tian et al. (2008)

(continued)

Table 2 (continued)

Plants/source	Active substances/ compounds	Type of substances/ compounds	Test models	Properties	References
Scutellaria baicalensis	Baicalein	flavonoid	LPS-induced toxicity in primary midbrain neuron–glia cultures from E-14 rat embryos H_2O_2-induced toxicity in rat PC12 cells MPTP model in mice 6-OHDA-induced toxicity in SH-SY5Y and PC12 cells 6-OHDA-induced toxicity in rats	In LPS-induced toxicity: blocks microglial activation, inhibits NO and superoxide radicals from microglia, reduces excessive production of TNF-α in microglia In H_2O_2-induced toxicity: increases cell survivability, increases the activities of SOD and Na^+-K^+-ATPase, lowers malondealdehyde levels, lowers LDH release In the MPTP model: increases DA and serotonin in the striatum, increases DA-ergic cell count in the striatum, inhibits oxidative stress, prevents astroglial activation. In the 6-OHDA model in vitro: prevents cellular apoptosis in SHSY5Y cells, promotes neural growth in PC12 cells In the 6-OHDA model in vivo: attenuates muscular tremor, prevents loss of TH-positive neurons, attenuates astroglial response in SN	Cheng et al. (2008) Li et al. (2005a, b) Mu et al. (2009) Shang et al. (2006)

Stephania intermedia	Stepholidine		Clinical trial MPTP and MPP^+ induced animal models of PD	Possesses DA D1 agonistic action, preliminary clinical trial has demonstrated that stepholidine, in combination with low dose of bromocriptine, can alleviate the symptoms of PD, protects DA neurons from MPTP/MPP^+ toxicity	Li et al. (1999) Yang et al. (2007)
Toki-to	Traditional Japanese/ Chinese herbal remedy		MPTP-induced neurotoxicity in mice	Improves MPTP-induced bradykinesia in pole-test, Protects DA neurons in SN, attenuates MPTP induced reduction in TH and DAT m-RNA levels in SN, suppresses gene expression of serum- and glucocorticoid regulated kinase that drives PD pathogenesis	Sakai et al. (2007)
Tripterygium wilfordii	1. Triptolide 2. Celastrol, extracted from the root-bark	Triptolide—diterpene Celastrol, a triterpene extracted from the root and bark	Triptolide: LPS induced toxicity in primary mesencephalic neuron–glia mixed culture in MPP^+ induced toxicity in rats Cealstrol: MPTP-induced toxicity	Triptolide: attenuates LPS-induced decrease in DA uptake and loss of TH-positive neurons, blocks LPS-induced activation of microglia and excessive production of TNF-α and NO, blocks excessive production of IL-1β, increases the expression of NGF mRNA levels Celastrol: anti-inflammatory prevents astrogliosis, antioxidant, Attenuates DA loss, induces heat shock protein 70 within dopaminergic neurons, decreases TNF-α and nuclear factor kappa B	Cleren et al. (2005) Gao et al. (2008) Li et al., 2003 Xue et al. (2007)
Tussilago farfara	Tussilagone extracted from the flower bud	Sesquiterpene	LPS-activated microglia	Inhibits inducible NO; prostaglandin E_2; cyclooxygenase-2, therefore acts as an inhibitor to inflammation mediated by microglia	Lim et al. (2008)

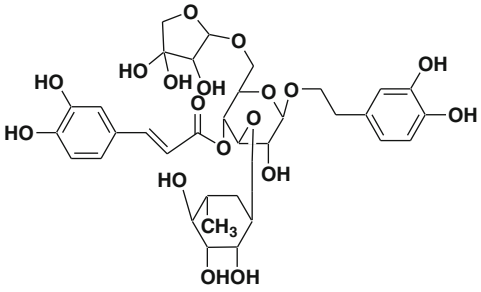
(continued)

Table 2 (continued)

Plants/source	Active substances/ compounds	Type of substances/ compounds	Test models	Properties	References
Cassia tora	Alaternin and nor-rubrofusarin glucose	Phenolic compounds	In vitro study	Spectrophotometric analysis has shown that these compounds can scavenge $ONOO^-$, and decrease $ONOO^-$-mediated tyrosine nitration	Park et al. (2004)
Eucommia ulmoides	Chlorogenic acid	Ester of caffeic acid and quinic acid	6-OHDA-induced toxicity in SH-SY5Y cells	Antioxidant, protects loss in mitochondrial function, blocks 6-OHDA-induced nuclear translocation of NF-κB	Kwon et al. (2014)
Gynostemma pentaphyllum	Gypenosides	Saponin	6-OHDA MPP^+ models	Protects DA, TH positive neurons, attenuates L-DOPA-induced dyskinesia	Choi et al. (2010) Shin et al. (2015)

Herbs/plants/preparations that are used in Chinese traditional medicine. *DAT* dopamine transporter, *DOPAC* 2,3-dihydroxyphenyl acetic acid, *IL* interleukin, *LPS* lipopolysaccharide, *MAO* monoamine oxidase, *MPP^+* 1-methyl-4-phenyl pyridinium ion, *NGF* nerve growth factor, *NO* nitric oxide, *PARP* poly (ADP-ribose) polymerase, *$ONOO^-$* peroxynitrite, *PD* Parkinson's disease, *SOD* superoxide dysmutase, *TNF* tumor necrosis factor. All the other abbreviations are as listed in the legends of Table 1.

***Buddleia lindleyana* Fort. (Family *Verbenaceae*)**

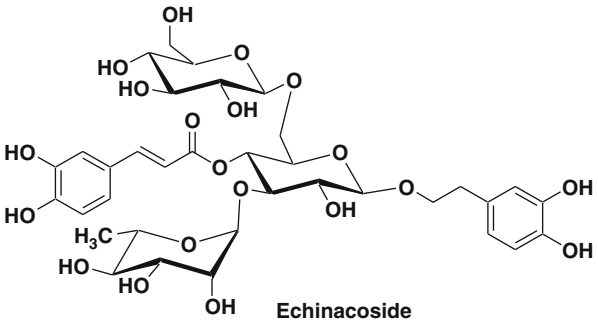


Pedicularioside A

One of the TCMs, *Buddleja lindleyana* is reported to contain Pedicularioside A, a phenylethanoid glycoside. This compound was found to be neuroprotective in MPP^+-induced death of rat mesencephalic neurons in primary cultures (Li et al. 2008a, b) as revealed by a marked increase in the number of TH-positive immunoreactive neurons in the culture. It also inhibits the MPP^+-mediated increased expression of the caspase-3 gene, and cleavage of poly (ADP-ribose) polymerase (PARP) in the neuronal cultures (Li et al. 2008a, b).

***Cistanche deserticola* Y.C. Ma (Family *Scrophulariaceae*)**

The bark of *Cistanche deserticola* tree is the primary source of the Chinese herbal medicine *Cistanche*. The total glycosides present in *Cistanche* have been shown to attenuate behavioral dysfunction and loss of TH-positive neurons in the SNpc area of the brain in MPTP-treated mice (Li et al. 2008a, b). *Cistanche* extract itself protects SH-SY5Y cells from MPP^+-insult by downregulation of the pro-apoptotic gene GADD153 expression, also known as chop (Wang et al. 2007).



Echinacoside

Cistanche salsa (C. A. Mey.) Beck (Family *Scrophulariaceae*)

Another Chinese medicinal herb belonging to the same genus is *C. salsa*. It contains a phenylethanoid glycoside, echinacoside. This compound has been shown to minimize striatal DA and its metabolites loss in both 6-OHDA- (Chen et al. 2007a) and MPTP- (Geng et al. 2004) models of PD. In the latter animal model, echinacoside caused a marked increase in the TH expression relative to mice treated with MPTP alone, along with the reduction in pro-apoptotic caspase-3 and caspase-8 activities in the cerebellar granule neurons (Geng et al. 2007). The use of non-DA-ergic neurons, such as cerebellar granule invalidates the relevance of this study.

Ganoderma lucidum (Curtis) P. Karst (Family *Ganodermataceae*)

Oil from the spores of the rare Chinese herb, *Ganoderma lucidum* was shown to possess neuroprotective effects on DA-ergic neurons from MPTP-induced neurotoxicity in mice (Zhu et al. 2005). Mice pretreated with the oil were found to present significantly less involuntary movement of the limbs in the pole-climbing test than the mice in MPTP-treated group. DA and DOPAC levels in the striatum also showed higher levels as compared with those in the MPTP alone treated group. The number of TH-positive neurons in SNpc of mice in *Ganoderma* spores oil + MPTP group was significantly greater than that in MPTP-alone treated group. This has also been validated with respect to TH protein expression (Zhu et al. 2005).

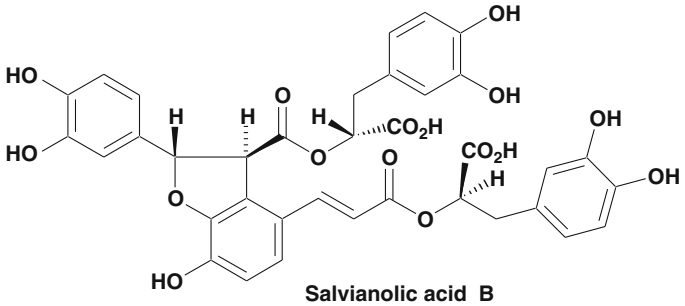
Ginkgo biloba L. (Family *Ginkgoaceae*)

EGb761 is a patented and well-defined mixture of active compounds extracted from the leaves of the Chinese medicinal tree *Ginkgo biloba* L. EGb761 has demonstrated potent anti-parkinsonian properties on several counts, based on both in vitro and in vivo evidences. PC12 cells injured by paraquat, a neurotoxin that has been shown to affect DA-ergic system to cause parkinsonian phenotypes in animals, has been demonstrated to be reversed by prior exposure of EGb761 (Kang et al. 2007). The results showed EGb761 exposure could reduce the paraquat-induced decrease of cell viability and the collapse of mitochondrial membrane potential of these cells. This in turn reduced the percentage of cells undergoing apoptosis in the culture by increasing Bcl-2 expression, and by inhibiting caspase-3 activity. Moreover, EGb761 pretreatment increased the number TH-immunopositive cells in paraquat treated PC12 culture (Kang et al. 2007).

Antioxidant potential along with its anti-apoptotic properties of EGb761 are shown to play major roles in its neuroprotective effects in MPTP- and MPP^+-induced animal models of PD (Yang et al. 2001). EGb761 possessed significant MAO inhibitory action, and therefore supplementation of this drug has been shown to inhibit both isoforms of MAO (MAO-A and MAO-B) in vivo in mice (Rojas et al. 2004). It has also been found to protect against nigrostriatal DA-ergic neurotoxicity in MPTP-induced parkinsonism in mice in terms of significantly defying the

neurotoxin-mediated increased lipid peroxidation, superoxide radical production, striatal DA loss, and impaired locomotion (Rojas et al. 2008). Interestingly, the protective effect of EGb761 against MPP^+-neurotoxicity has been claimed to be due to the regulation of copper ion homeostasis in the brain (Rojas et al. 2009).

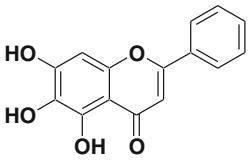
EGb761 possesses neuroprotective effects in 6-OHDA model of parkinsonism as well (Kim et al. 2004). In this study rats given a week of pretreatment with daily administrations of EGb761, and unilaterally infused with 6-OHDA in the striatum, exhibited lesser problems in contralateral forepaw adjusting steps, and less progressive deficit in general motor ability. Eight weeks after 6-OHDA lesion the number of contralateral forepaw adjusting steps was significantly higher in rats that were treated with high doses of EGb761 (100 mg/kg daily) than in those treated with low dose (50 mg/kg) or with the vehicle. DA neuronal loss in SNpc and depletion in striatal DA corresponded with behavioral deficit exhibited by these animals. EGb761 also dose-dependently restored the activities of GT, GP, GR, catalase and SOD in the striatum (Kim et al. 2004). The significant decrease in the levels of DA and its metabolites and increase in the number of DA-ergic D_2 receptors in the striatum and decrease in TH-positive neurons in SNpc were brought towards normalcy following EGb761 treatment in 6-OHDA-mediated hemiparkinsonian animals (Ahmad et al. 2005b). Interestingly, EGb761 has been found to reduce the toxic effects of L-DOPA treatment in the 6-OHDA model of PD (Cao et al. 2003). EGb761 has also been found to upregulate the expressions of PH and Nurr1 genes in the SN and striatum of MPTP-treated mice (Rojas et al. 2012).



Salvianolic acid B

Salvia miltiorrhiza Bunge (Family *Lamiaceae*)

Salvianolic acid B is an antioxidant derived from the Chinese herb, *S. miltiorrhiza*. Pretreatment of SH-SY5Y cells with salvianolic acid B significantly reduced 6-OHDA-induced generation of ROS and increment in intracellular calcium (Tian et al. 2008). 6-OHDA-induced apoptosis is also reversed by salvianolic acid B treatment. Neuroprotective mechanism included reduction in 6-OHDA-induced increase of caspase-3 activity, reduction in cytochrome *C* translocation into the cytosol from mitochondria, prevention of the decrease in the Bcl-2/Bax ratio, reduction in the activation of extracellular signal-regulated kinase and the activation of 6-OHDA-suppressed protein kinase C (Tian et al. 2008).

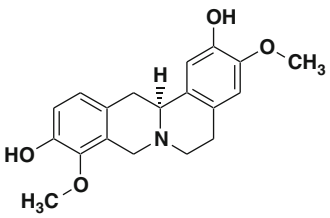


Baicalein

Scutellaria baicalensis Georgi (Family *Lamiaceae*)

Scutellaria baicalensis is a flowering herb widely used in TCM. The major flavonoid present in this herb is baicalein, which possesses anti-inflammatory and antioxidant properties. Baicalein can protect primary midbrain neuron–glia cultures from E-14 rat embryos from LPS-induced cellular toxicity (Li et al. 2005a, b). Pretreatment with baicalein concentration-dependently attenuated LPS-induced decrease in DA uptake and loss of TH-positive neurons. Morphological study showed that baicalein can block LPS-induced activation of microglia. Excessive production of TNF-α and free radicals such as NO and superoxide by LPS stimulation were also attenuated by baicalein in a concentration-dependent pattern.

The flavonoids present in the stem and leaves of *S. baicalensis* possess protective effects in H_2O_2-induced toxicity in rat PC12 cells (Shang et al. 2006). Pretreatment of the cells with this plant flavonoids notably elevated cell survival and activities of SOD and Na^+-K^+-ATPase, and lowered malondialdehyde (MDA) formation and LDH release following H_2O_2. Baicalein has also demonstrated neuroprotection in the MPTP- (Cheng et al. 2008) and 6-OHDA- (Mu et al. 2009) models of PD. In the MPTP-model baicalein was found to increase the levels of striatal DA and serotonin and the SNpc DA-ergic cell count, inhibited oxidative stress and prevented astroglial activation. In in vitro experiments baicalein could significantly ameliorate 6-OHDA-induced SH-SY5Y cellular apoptosis and also promote neurite outgrowth of PC12 cell (Lee et al. 2005; Mu et al. 2009). In in vivo experiments, baicalein significantly attenuated muscle tremor, but had no effect on apomorphine-induced stereotypic rotations. Moreover, baicalein treatment could also increase the number of TH-positive neurons of the midbrain. The neuroprotective action of baicalein was coincident with an attenuated astroglial response within SN region (Mu et al. 2009).



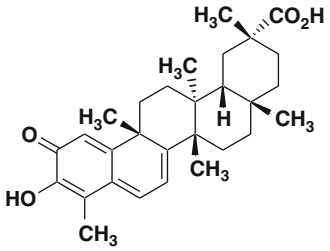
Stepholidine

Stephania intermedia H. S. Lo. (Family *Menispermaceae*)

Stepholidine, a natural product isolated from the Chinese herb *Stephania intermedia*, possesses D_1 receptor partial agonistic and D_2 antagonistic properties in the nigrostriatal and mesocorticolimbic DA-ergic pathways. Stepholidine was found to protect DA neurons from MPTP- and MPP^+-toxicity (Li et al. 1999). Preliminary clinical trials demonstrated that it in combination with low doses of bromocriptine, could alleviate the symptoms of PD in patients (Li et al. 1999). Similarly, stepholidine can relieve the motor symptoms of PD when co-administered with L-DOPA. Furthermore, this compound exhibited neuroprotective effects through an antioxidant mechanism and slowed down the progression of neuronal degeneration in the SNpc of PD patients and/or animal models (Yang et al. 2007). Thus, stepholidine may be able to exert symptomatic therapeutic effects and prevent further DA neuronal degeneration in PD.

Toki-to

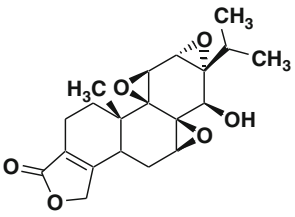
A traditional Japanese/Chinese herbal remedy Toki-to has demonstrated an anti-PD effect in the MPTP-model of PD (Sakai et al. 2007). Administration of Toki-to caused improvement in MPTP-induced bradykinesia in the pole climbing test, and recovered DA-ergic neuronal loss in SNpc. It also helped in reversing MPTP-induced changes in DAT and TH mRNA levels. DNA microarray analyses indicated that Toki-to suppressed gene expression of serum and glucocorticoid regulated kinase, a molecule believed to drive the pathogenesis of PD (Sakai et al. 2007).



Celastrol

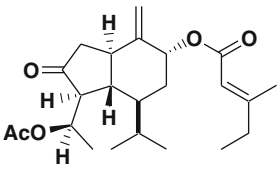
Tripterygium wilfordii Hook F. (Family *Celastraceae*)

Celastrol and triptolide, a triterpene and a diterpene respectively, are two of the major active components of the Chinese herb *Tripterygium wilfordii*. Both of these possess potent anti-inflammatory, antioxidant and immunosuppressive properties. Celastrol is found to be neuroprotective in the MPTP model (Cleren et al. 2005). Celastrol has been shown to significantly attenuate the loss of DA neurons following MPTP treatment. It inducted heat shock protein 70 within DA-ergic neurons, and decreased TNFα and NF-kB levels (Cleren et al. 2005).



Triptolide

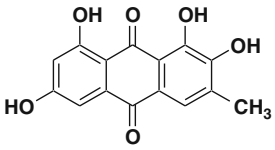
Triptolide effectively refurbished the LPS-induced decrease in DA uptake and loss of TH-immunoreactive neurons in primary mesencephalic neuron–glia mixed culture (Li et al. 2004a, b). It blocked LPS-induced activation of microglia and excessive TNFα, nitric oxide and interleukin-1β production of (Zhou et al. 2005). Triptolide application caused a significant increase in the content and release of NGF, and its mRNA expression (Xue et al. 2007). Triptolide significantly inhibited microglial activation, DA-ergic cell death; promoted axonal elongation and brain-derived neurotrophic factor mRNA expression, and improved behavioral performances in animals intranigrally infused with MPP^+ (Gao et al. 2008). Tripchlorolide treatment significantly increased the survival of DA-ergic neurons in SNpc along with prevention of striatal DA levels in rats, resulting in attenuated stereotypic rotational behavior caused by amphetamine in these animals (Li et al. 2003). Triptolide's ability to inhibit cyclooxygenase (COX)-2 and prostaglandin E2 (PGE_2) expression are implicated in its anti-PD property (Geng et al 2012).



Tussilagone

Tussilago farfara L. (Family *Asteraceae*)

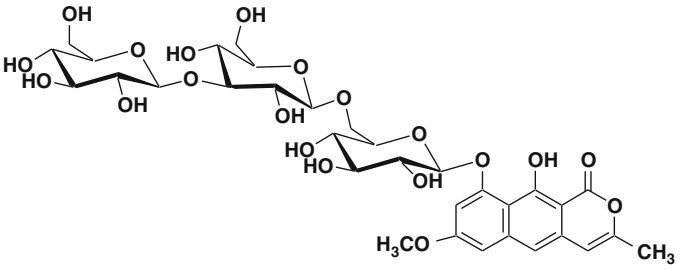
Tussilagone is a major bioactive compound isolated from the flower buds of the plant *Tussilago farfara* used in TCM. This compound possesses therapeutic potential in the treatment of neurodegenerative diseases such as PD, and this is believed to be due to its potent anti-inflammatory properties (Lim et al. 2008). Tussilagone showed a dose-dependent inhibition of nitric oxide and PGE_2 production in LPS-activated microglia by means of its suppressive effects on the expression of protein and mRNA of inducible nitric oxide synthase and COX-2.



Alaternin

Cassia tora L. (Family *Caesalpiniaceae*)

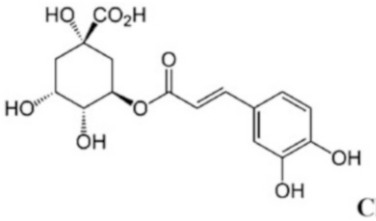
Peroxynitrite, formed from the reaction of superoxide and nitric oxide, is a potent pro-oxidant and pro-inflammatory molecule indicted as a causal factor in neurodegenerative diseases, such as Alzheimer's disease, PD, and atherosclerosis. Lack of endogenous enzymes that regulate peroxynitrite warranted the search for a strong peroxynitrite scavenger for use in these neurodegenerative diseases. Extract of *Cassia tora*, a well known oriental herb in traditional medicine, showed potent peroxynitrite scavenging activity, probably due to the presence of phenolic active components, alaternin and nor-rubrofusarin glucose. In vitro studies demonstrated decrease in the peroxynitrite-mediated nitration of tyrosine through electron donation by alaternin and nor-rubrofusarin glucose, and suggested the potential use of the herb as a suitable antiparkinsonian agent (Park et al. 2004).



Nor-rubrofusarin glucose

Eucommia ulmoides Oliv. Bark. (Family *Eucommiaceae*)

This tree is well known in TCM. Recently it has been reported that pretreatment with an extract of *E. ulmoides* protected SH-SY5Y cells from 6-OHDA-induced neurotoxicity (Kwon et al. 2014). The principal modes of action were found to be attenuation of oxidative stress and mitochondrial dysfunction. Interestingly, the extract could suppress phosphorylation of JNK, PI3K/Akt, and GSK-3β and even blocked 6-OHDA induced nuclear translocation of NF-κB. One of the active agents found in the extract was chlorogenic acid (Kwon et al. 2014).



Chlorogenic acid

***Gynostemma pentaphyllum* Thunb. (Family *Cucurbitaceae*)**

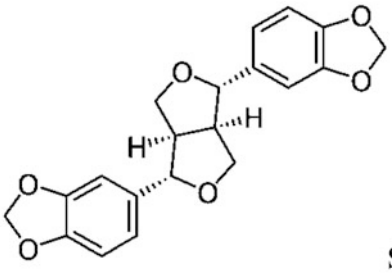
This plant is used in TCM. Extract of this plant has been shown to attenuate loss of DA, its metabolites in the striatum and TH-positive neurons in SNpc of 6-OHDA-treated rats (Choi et al. 2010). Subsequently, the antiparkinsonian properties of gypenosides, the saponin extract derived from *G. pentaphyllum* have been investigated. Gypenosides have been found to protect dopaminergic neurons from MPP^+-toxicity (Wang et al. 2010). Recently, gypenosides have also been found to reduce L-DOPA induced dyskinesia in 6-OHDA-lesioned animals (Shin et al. 2015).

Plants Essentially Not Listed in Any Traditional Medical System

Other than plants used traditionally in India and China/Japan, there are numerous sources of health-beneficial herbs/plants that are used by humans based on anecdotal evidences, which are provided below. A summary is provided in Table 3 for a quick assessment.

***Acanthopanax senticosus* Harms (Family *Araliaceae*)**

The aqueous extract of the bark of *Acanthopanax senticosus* and its lignin component sesamin are found to afford protection against rotenone-induced behavioral abnormalities, bradykinesia and catalepsy, TH- and glial cells-derived neurotrophic factor (GDNF)-positive neuronal loss (Fujikawa et al. 2005a). *A. senticosus* extract exhibited similar neuroprotective effects in MPTP- and MPP^+-models of PD too (Fujikawa et al. 2005b; An et al. 2010a, b; Liu et al. 2012). A recent proteomics study revealed, an extract of this plant can correct the abnormal expression of several biomarker proteins in a mutant alpha-synuclein overexpressing cell line model of PD (Li et al. 2014).



Sesamin

Table 3 Plants essentially not listed in any traditional medical system

Plant/source	Name of active molecule	Type of compound	Test model	Properties	References
Acanthopanax senticosus	Aqueous extract of stem bark, its lignin component—sesamin	Lignin	Rotenone model of PD, MPTP model of PD in rat	Reduces bradykinesia and catalepsy, prevented DA cell loss as seen by TH-immunoreactivity, corrects abnormal protein expression in PD model	An et al. (2010a, b) Fujikawa et al. (2005a, b) Liu et al. (2012)
Alpinia oxyphylla	Ethyl acetate extract yielded the active substance protocatechuic acid, chrysin	Phenolic compound	MPP^+- and rotenone- induced toxicity in PC12 cells	Anti-apoptotic, enhance activities of SOD and catalase, reduced H_2O_2 and sodium nitroprusside-induced cell death, prevents formation of ROS and loss in glutathione, prevents caspase-3 activation and downregulation of Bcl-2	An et al. (2006) Guan et al. (2006) Liu et al. (2008b) Zhang et al. (2015)
Amburana cearensis	Amburoside A	glucoside	6-OHDA in rat mesencephalic cell culture	Prevents nitrite formation, decreases lipid peroxidation	Leal et al. (2005)
Anemopaegma mirandum (a Brazilian tree)	Commercial plant extract		Rotenone-induced toxicity in SH-SY5Y neuroblastoma cells	Preserves cell and organelle (cytoplasmic and mitochondrial) integrity	Valverde et al. (2008)
Banisteriopsis caapi	Stem extract contains Harmine and Harmaline	Alkaloids of the beta carboline family	MAO activity in liver homogenate, DA release in rat brain slices	Stem extract and harmaline can inhibit MAO-A in liver homogenate; stem extract, harmanine, and harmaline can cause release of DA from rat brain slices	Lutes et al. (1988) Mehta et al. (2004) Milner et al. (1995) Schwarz et al. (2003)
Buddleja officinalis	Verbascoside	Phenylpropanoid glucoside	MPP^+-induced toxicity in PC12 cells	Anti-apoptotic, reduces extracellular H_2O_2 level, prevents activation of caspase-3, prevents collapse of mitochondrial membrane potential	Sheng et al. (2002)

(continued)

Table 3 (continued)

Plant/source	Name of active molecule	Type of compound	Test mode	Properties	References
Cammelia sinensis (tea plant)	Catechins Theaflavin, tea extracts	Polyphenols	6-OHDA-induced toxicity in rats, SH-SY5Y cells and PC 12 cells, MPTP and probenecid-induced PD model in mice	Tea drinking is associated with reduced the risk of PD due to the following general properties: antioxidant, anti-inflammatory, free radical scavenger, iron-chelating action, DA uptake inhibition, inhibition of COMT activity, reduction in protein kinase C *Black tea*: reduces NO levels, prevents formation of lipid peroxidation, reduces nitrite/nitrate content, reduces inducible nitric oxide synthase, reduces protein-bound 3-nitro-tyrosine level *Green tea*: suppresses the ROS-NO pathway, Prevents Ca^{2+} accumulation, inhibits NF-κB nuclear translocation and its activation	Anandhan et al. (2012) Chaturvedi et al. (2006) Dutta and Mohanakumar (2015) Guo et al. (2005, 2007) Hu et al. (2007) Kandinov et al. (2009) Levites et al. (2002b) Mandel et al. (2005, 2006, 2008) Pan et al. (2003)
Cannabis sativa	Δ^9-tetrahydrocannabinoid and cannabinol	Cannabinoid compounds	6-OHDA model	Prevents DA depletion, prevents loss of TH mRNA levels, a randomized double blind study on PD patients receiving oral cannabis failed to show any improvement	Carroll et al. (2004) Lastres-Becker et al. (2005)
Chaenomeles speciosa fruit extract	Flowering quince		MPTP and 6-OHDA models and in Chinese hamster ovary cells expressing dopamine transporter (DAT)	Potent DAT inhibitor, attenuates stereotypic rotational behavior in 6-OHDA treated rats, improves deficits in endurance performance in MPTP treated mice, reduces loss of TH-positive neurons in the SN in MPTP-treated mice	Zhao et al. (2008b)

Coffea arabica	Kahweol extracted from the beans Eicosanoyl-5-hydroxytryptamide	Diterpene	6-OHDA-induced cellular toxicity in SH-SY5Y cells MPTP model in mice	Prevents generation of ROS, prevents caspase-3 activation, upregulates heme oxygenase-1 expression, induces PI3K and p38 activation, modulation of phospho-protein phosphatase activity	Hwang and Jeong (2008) Lee et al. (2013)
Coffea spp.	Caffeine		MPTP-, 6-OHDA- and rotenone-mediated models of PD; MPP^+-mediated toxicity in SH-SY5Y cells	Several epidemiological studies have shown that coffee consumption reduces the risk of PD. Caffeine: blocks MPTP induced DA neuron loss and blood–brain barrier dysfunction, adenosine A2A receptor blocker, prevents apoptosis by lowering of caspase-3 through the activation of PK13/Akt pathway, causes behavioral recovery and protects dopaminergic cells in 6-OHDA model	Aguiar et al. (2006) Chen et al. (2008b) Joghataie et al. (2004) Kalda et al. (2006) Nakaso et al. (2008) Ragonese et al. (2003), Hernan et al. (2002), Ascherio et al. (2001) Saaksjarvi et al. (2008) Schwarzschild et al. (2003) Sommer and Stacy (2008)
Curcuma longa	Carcumin Naringenin	Polyphenol	6-OHDA-model MPP^+-induced toxicity in PC12 cells LPS-induced neurotoxicity in primary rat neuron–glia culture MPTP model 6-OHDA-induced toxicity in rats and in SH-SY5Y cells	Protects TH-positive neurons from 6-OHDA insult, prevents apoptosis in MPP^+-induced toxicity in PC12 cells, acts as an antioxidant in MPP^+-induced toxicity in PC12 cells, inhibits α-synuclein aggregation In LPS model: prevents DA neuronal loss, prevents morphological changes in microglia, acts as a potent anti-inflammatory and anti-apoptotic agent In the MPTP-model affords neuroprotection by inhibiting MAO Protects brain mitochondria from peroxy-nitrite induced toxicity both in vitro/in vivo	Chen et al. (2006) Jagatha et al. (2008) Lou et al. (2014) Mythri et al. (2007) Ojha et al. (2012) Pandey et al. (2008) Rajeswari and Sabesan (2008) Yang et al. (2008) Zbarsky et al. (2005)

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Table 3 (continued)

Plant/source	Name of active molecule	Type of compound	Test mode	Properties	References
Delphinium denudatum		Extract	6-OHDA model of PD	Prevents lipid peroxidation and depletion of reduced glutathione in SN, attenuates the activities of SOD and catalase in striatum, recovers DA, its metabolites and dopaminergic D_2 receptors in striatum, increases TH-positive neurons in the lesioned side	Ahmad et al. (2006a)
Fraxinus seiboldiana	6,7-Di-*O*-glucopyranosyl-esculetin extracted from the bloom		DA induced toxicity in human SH-SY5Y cells	Antioxidant, enhances SOD, GSH levels Anti-apoptotic: regulates p53, Bax and Bcl-2 expression, protects mitochondrial membrane potential, Inhibits the release of Cyt-c and AIF, Inhibits expression of caspase 3	Zhao et al. (2008a)
Hypericum perforatum	Plant extract		H_2O_2-induced toxicity in PC 12 cells, MPTP model of PD	Protects PC 12 cells form H_2O_2-induced toxicity, potentiates neuroprotective effect of bromocriptine in MPTP-treated mice in terms of behavior, recover and prevention of striatal DA loss, prevents lipid peroxidation, MAO-B inhibition, reduction in astrocyte activation in striatum of MPTP-treated mice	Lu et al. (2004) Mohanasundari and Sabesan (2007)
Ilex paraguariensis	Hydroalcoholic extract of stem and leaf (rutin, caffeine, theo-phylline theo-bromine, quercetin)	Phenolic compounds	MPTP and reserpine induced PD models Rotenone induced toxicity in SH-SY5Y cells	Prevented hypolocomotion, potentiates the effect of apomorphine in preventing catatonia, decreases nitric oxide levels Scavenges free radicals, prevents the oxidation of deoxyribose	Milioli et al. (2007) Park et al. (2014)
Magonia glabrata	Quebrachitol extracted from the fruits	2-*O*-Methyl-L-inositol	6-OHDA-induced toxicity in mesencephalic cells	Prevents cell death, possesses antioxidant activity, prevents apoptosis and necrosis, preserves cellular morphology, protects TH^+ cells from 6-OHDA-induced cell death	Nobre et al. (2006)

Morus spp.	Oxyresveratrol Resveratrol	Polyhydroxylated stilbene	6-OHDA induced toxicity in SH-SY5Y cells; Resveratrol has been effective various in vivo and in vitro models of PD	Reduces release of LDH, reduces activity of caspase-3, acts as an antioxidant, attenuates phosphorylation of c-Jun and c-Jun N-terminal kinase, increases basal levels of SIRT1, an enzyme involved in cellular regulation and a sensor for toxic neuronal processes	Chao et al. (2008) Chung et al. (2003) Lorenz et al. (2003) Rasheed et al. (2015)
Myracrodruon urundeuva	Chalcone-enriched fraction from the ethyl acetate extract prepared from the stem bark		6-OHDA-induced toxicity in mesencephalic cells	Reduces 6-OHDA induced lipid peroxidation, reduces nitrite levels, prevents apoptosis and necrosis and preserves cellular morphology, protects both $TH^{+/-ve}$ cells from 6-OHDA-induced cell death	Nobre et al. (2009)
Nicotiana tabacum	Nicotine		6-OHDA model MPTP-induced chronic hemiparkinsonism in monkeys	More than 50 prospective cohort and case-control studies over the last half century indicate that smoking and other forms of tobacco use (e.g., nicotine gum) appear protective against PD in both males and females, in 6-OHDA treated animals nicotine can reduce contralateral rotations Nicotine has been found to have synergistic effects with L-DOPA in MPTP-induced chronic hemiparkinsonian monkeys and reduce L-DOPA-induced dyskinesias in primates, Tobacco smoke contains compound which is a MAO-B inhibitor	Allam et al. (2004) Clemens et al. (1995), Ebersbach et al. (1999) Clemens et al. (1995), Ebersbach et al. (1999) Domino et al. (1999) Ishikawa and Miyatake (1993) Khalil et al. (2006) Mitsuoka et al. (2002) Ritz et al. (2007) Thacker et al. (2007)

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Table 3 (continued)

Plant/source	Name of active molecule	Type of compound	Test mode	Properties	References
Paeonia lactiflora, *Paeonia veitchii*, *Paeonia suffruticosa*	Paeoniflorin	Dried root	MPTP model	Protects TH+ve neurons dose dependently Attenuates MPTP-induced proinflammatory gene upregulation, reduces microglial and astrocyte upregulation, neuroprotective effect due to A1 receptor upregulation, prevents mitochondria mediated apoptosis	Kim et al. (2014) Liu et al. (2006)
Plumbago scandens	Crude ethanolic extract and its acetate fraction		Tremorine-induced tremor model	Acetate fraction was more effective in suppressing tremor	Morais et al. (2004)
Polygonum multiflorum	*P. multiflorum* extract and its ethanol-soluble fraction		Combination of paraquat and maneb in male C57BL/6 mice	Reduces decline in DA loss, attenuates loss of spontaneous locomotor activity and motor incoordination, reduces loss of TH- neurons in the SN	Li et al. (2005a, b)
Psoralea corylifolia	Seed (Fructus Psoraleae) extract		MPP^+- induced toxicity in D8 cells Mice treated with reserpine	Strong inhibitor of DA transporter, mode of action similar to cocaine, possesses long-lasting stimulatory effects on intact and reserpinized mice	Zhao et al. (2007)
Rehmannia glutinosa	Catalpol, isolated from the root	Glucoside	MPTP-induced toxicity in mesencephalic neuron–astrocyte and astrocyte cultures MPTP mice model.	Reverses mitochondrial complex-I activity and membrane potential, reduces intracellular Ca^{2+} levels, prevents ROS accumulation, prevents mitochondrial permeability transition pore opening, possesses MAO-B inhibitory potential Prevents TH+ve neuronal loss and reverses DA turnover rates in vivo	Bi et al. (2008) Jiang et al., 2008

Rosmarinus officinalis	Carnosic acid Carnosol	Phenolic diterpenes	Carnosic acid was tested in dieldrin-induced neurotoxicity in dopaminergic cells (SN4741) Carnosol was tested in rotenone-induced neurotoxicity on cultured DA cells	Carnosic acid: inactivates caspace-3 and 12, prevents Jun-N terminal kinase phosphorylation, enhances brain derived neurotrophic factors, prevents apoptosis Mediates glutathione synthesis Carnosol: downregulates caspase-3, increases TH activity, increases Nurr1 and ERK 1/2	Chen et al. (2012) Kim et al. (2006) Park et al. (2008) Wu et al. (2015) Yan et al. (2009)
Scutellaria lateriflora	Baicalin	Flavonoid	MPTP model of PD	Prevents loss of TH+ve neurons in SN, and loss of striatal DA, increases brain GSH content	Chen et al. (2007b)
Solanum lycopersicum	Lycopene	Carotene pigment	MPTP model	Prevents DA loss	Suganuma et al. (2002)
Toxicodendron vernicifluum	Fustin	Isoflavonoid	6-OHDA toxicity in SK-N-SH, rotenone toxicity in SH-SY5Y	Reduces ROS, reduces intracellular calcium, acts as anti-apoptotic	Park et al. (2007) Sapkota et al. (2011)
Valeriana officinalis	Aqueous extract of the rhizomes		Rotenone-induced apoptosis in SH-SY5Y, rotenone-induced toxicity in *Drosophila*	Increases cell viability	de Oliveria et al. (2009) Sudati et al. (2013)
Vicia faba	L-DOPA		Clinical study	Substantial clinical improvement, plasma L-DOPA levels found to be substantially more in comparison to patients who had L-DOPA+carbidopa	Rabey et al. (1993)
Althaea officinalis			6-OHDA model	Prevents behavioral abnormality, reduces lipid peroxidation in brain, protects DA neurons in SN	Rezaei and Alirezaei (2014)

(continued)

Table 3 (continued)

Plant/source	Name of active molecule	Type of compound	Test mode	Properties	References
Crocus sativus			MPTP model in mice	Protects TH-positive neurons	Purushothuman et al. (2013)
Trigonella foenum-graecum	Trigonelline	Alkaloid	Clinical study 6-OHDA, MPTP models	Effective adjuvant therapy to L-DOPA in human patients, reduced motor dysfunction in animal models	Gaur et al. (2013) Nathan et al. (2014)
Uncaria tomentosa			6-OHDA toxicity in SH-SY5Y, Transgenic *C. elegans*	Protects mitochondrial membrane potential, antioxidant, prevents α-synuclein aggregation	Shi et al. (2013)
Olea europaea	Oleuropein Leaf extract		6-OHDA-induced toxicity in PC-12 cells	Antioxidant, anti-apoptotic	Pasban-Aliabadi et al. (2013)
Eucalyptus citriodora			*Drosophila* expressing human α-synuclein	Recovers behavioral abnormality, antioxidant	Siddique et al. (2013)
Garcinia indica			6-OHDA model	Recovers behavioral abnormality Recovers DA loss	Antala et al. (2012)
Acorus gramineus			LPS induced toxicity in BV-2 microglia MPTP model in mice	Anti-inflammatory	Jiang et al. (2012)
Spirulina			Rats injected with α-synuclein in the SN	Modulates microglial activation	Pabon et al. (2012)
Indigofera tictoria	SF-6, an active compound		α-synuclein, 6-OHDA, H_2O_2 toxicity in SH-SY5Y cells 6-OHDA model in rats	Recovers behavioral abnormality, antioxidant	Rajendra et al. (2012)

Cinnamomum sp.			*Drosophila* expressing A53T α-synuclein	Prevents behavioral abnormalities, prevents α-synuclein aggregation	Shaltiel-Karyo et al. (2012)
Selaginella delicatula			Rotenone induced model in Drosophila	Reduces behavioral abnormalities, antioxidant, protects mitochondrial function and integrity	Girish and Muralidhara (2012)
Sesamum indicum	Sesame seed oil		6-OHDA model in mice	Protects DA neurons, prevents DA loss Anti-inflammatory	Ahmad et al. (2012)
Rhus verniciflua			Rotenone-induced toxicity in SH-SY5Y	Antioxidant, anti-apoptotic, protects mitochondrial membrane potential	Kim et al. (2011)
Hibiscus asper			6-OHDA model	Antioxidant, anti-apoptotic, prevents DNA cleavage in 6-OHDA treated rats	Hritcu et al. (2011)
Chrysanthemum indicum			MPP^+-induced toxicity in SH-SY5Y, LPS-induced activation of microglial cells	Antioxidant, anti-apoptotic, prevents microglial activation	Kim et al. (2011)
Yeoldahanso-tang	Polyherbal mixture—ten herbs		MPP^+-induced toxicity in PC-12 cells, MPTP model	Protects PC-12 cell death, protects TH-positive neurons	Bae et al. (2011)
Paullinia cupana			Rotenone-induced toxicity in SH-SY5Y	Anti-apoptotic	de Oliveira e al. (2011)
Croton celtidifolius			Intranasal treatment of MPTP in rats	Attenuates behavioral abnormality, prevents inhibition of mitochondrial complex, protects TH-positive neurons	Moriera et al. (2010)
Pinus pinaster	Pycnogenol, a standardized extract		MPTP model	Attenuates behavioral abnormality, antioxidant, increases glutathione content, reduces thiobarbituric acid reactive substances	Khan et al. (2010)

(continued)

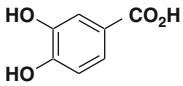
Table 3 (continued)

Plant/source	Name of active molecule	Type of compound	Test mode	Properties	References
Gastrodia elata			MPP^+-induced toxicity in SH-SY5Y cells	Antioxidant and anti-apoptotic	An et al. (2010a, b)
Thuja orientalis			6-OHDA-induced toxicity in SH-SY5Y	Antioxidant, anti-apoptotic, restores mitochondrial membrane potential	Ju et al. (2010)

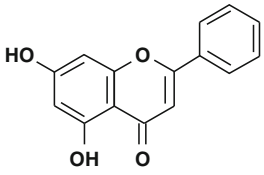
Plants found to have antiparkinsonian activity, which are not traditionally used. *AIF* apoptosis-inducing factor, *Cyt-c* cytochrome *c*, *LDH* lactate dehydrogenase, *Nurr1* nuclear receptor related protein 1, *ERK1/2* extracellular signal-regulated kinase 1 and 2. All the other abbreviations used are as explained in Tables 1 and 2

Alpinia oxyphylla Miq. (Family *Zingiberaceae*)

Protocatechuic acid, a phenolic compound isolated from the kernels of *A. oxyphylla* showed antioxidant neuroprotective effects in MPP^+- or rotenone-induced loss in mitochondrial membrane potential, formation of ROS, GSH depletion, activation of caspase-3 and downregulation of Bcl-2 (Guan et al. 2006; Liu et al 2008b). It stimulated cell proliferation, SOD and catalase activities and reduced apoptotic cell death in PC12 cells (An et al. 2006). A recent study demonstrated that a combination therapy of protocatechuic acid and another naturally occurring flavonoid, chrysin can exhibit enhanced neuroprotection in animal models of PD (Zhang et al. 2015). Interestingly, both protocatechuic acid and chrysin failed to reproduce the neuroprotective activity of ethanolic extract *A. oxyphylla* in 6-OHDA-induced parkinsonism in PC12 cells (Zhang et al. 2012). This study points to the need for identification of active ingredients in the plant (Zhang et al. 2012).



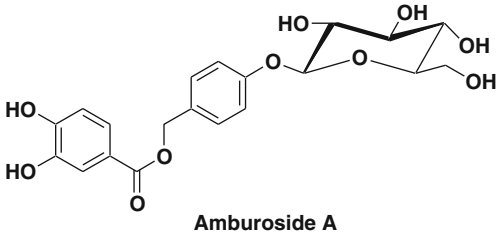
Protocatechuic acid



Chrysin

Amburana cearensis (Allemao) A.C.Sm (Family *Leguminosae*)

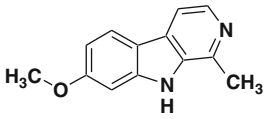
Pretreatment of rat mesencephalic cell culture with Amburoside A, a glucoside isolated from *A. cearensis*, protected against 6-OHDA-induced toxicity, probably by reducing the free radical nitrite formation and blocking cellular lipid peroxidation (Leal et al. 2005).



Amburoside A

Anemopaegma mirandum Mart ex. DC. (Family *Bignoniaceae*)

The commercial extract of *Anemopaegma mirandum*, a Brazilian tree, showed cytoprotective effects in human neuroblastomas SH-SY5Y cells against rotenone-induced apoptosis as revealed by increased cell survivability, condensation of the cytoplasm and nuclear fragmentation (Valverde et al. 2008). The extract also helped in preserving the integrity of cytoplasmic and mitochondrial membrane integrity.



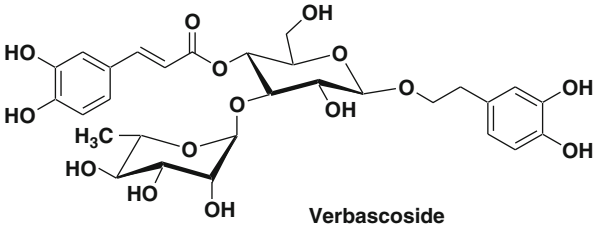
Harmine

Banisteriopsis caapi (Spruce ex Griseb.) Morton (Family *Malpighiaceae*)

B. caapi, a woody vine that grows in the Amazonian basin, is famous as an ingredient of a popular hallucinogenic drink. *B. caapi* stem extract contains two active ingredients, harmine and harmaline. The root extract and harmaline showed a concentration-dependent inhibition of MAO-A, but not of MAO-B activity. The extract, harmine and harmaline caused a significant increase in DA release from rat striatal slices, and the amount of harmine present alone could not account for the total activity of the extract. These results suggested a basis for the usefulness of *B. caapi* stem extract in the treatment of PD (Schwarz et al. 2003). Although the claim for the use of the plant is equivocal, tremorogenic action of harmaline in experimental animals is unequivocal in literature, and therefore is a serious concern that contradicts its use in the disease (Lutes et al. 1988; Milner et al. 1995; Mehta et al. 2003).

Buddleja officinalis Maxim. (Family *Buddlejaceae*)

A phenylpropanoid glucoside called verbascoside, which is a constituent of *B. officinalis* has been demonstrated to markedly attenuate MPP^+-induced apoptotic cell death, increase in extracellular H_2O_2 level, activation of caspase-3 and the collapse of mitochondrial membrane potential, indicating the usefulness of this compound as an antiparkinsonian agent (Sheng et al. 2002).



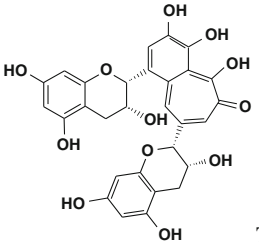
Verbascoside

Camellia sinensis Kuntze (Family *Theaceae*)

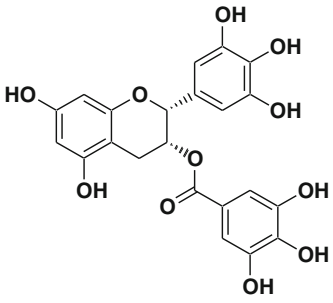
The leaves and leaf buds of *Camellia sinensis* are used to produce tea, and tea drinking is associated with lower risk of PD (Kandinov et al. 2009; Hu et al. 2007). Tea is rich in polyphenolic catechins, which have important properties relevant to

neuroprotection in PD. Their antioxidant, anti-inflammatory, free radical scavenging, iron-chelating, DA uptake inhibitory, COMT inhibitory, protein kinase C or extracellular signal-regulated kinases signaling activation and their ability to protect neuronal death in a wide array of cellular and animal models of neurological diseases are strong indicators for their value in the control of PD syndromes in humans (Pan et al. 2003; Mandel et al. 2008). Mandel et al. (2005, 2006, 2008) have demonstrated modulation of signal transduction pathways, upregulation of cell survival/death genes and mitochondrial function that contribute significantly to the induction of cell viability. Black tea is reported to exhibit recovery in behavioral and neurochemical properties in the 6-OHDA-induced model of PD (Chaturvedi et al. 2006; Guo et al. 2007). Green tea polyphenols are shown to dose-dependently prevent midbrain DA loss and striatal 6-OHDA-induced increases in ROS and nitric oxide production, lipid peroxidation, inducible nitric oxide synthase activity, and protein-bound 3-nitro-tyrosine levels (Guo et al. 2007).

Green tea polyphenols protected against 6-OHDA-induced toxicity in SH-SY5Y cells by suppressing the ROS-nitric oxide pathway and preventing Ca^{2+} accumulation in the cells (Guo et al. 2005). Introduction of green tea extract to PC12 or SH-SY5Y cells prior to application of 6-OHDA inhibited NF-kappaB nuclear translocation and iron binding activity induced by this DA-ergic toxin to protect against the neurotoxin-induced cell death (Levites et al. 2002a). Black tea polyphenol, theaflavin has been found to be neuroprotective in the MPTP and probenecid-induced model of the disease in mice (Anandhan et al. 2012).



Theaflavin

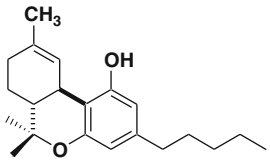


Epigallocatechin gallate

Catechin protected against oxidative stress mediated dopaminergic mesencephalic neuron from apoptosis (Mercer et al. 2005). The most important catechin polyphenol constituent of tea viz. epigallocatechin gallate (EGCG) has been found to exert remarkable neuroprotective/neurorescue activities in a wide variety of cellular and animal models of neurological disorders. Pretreatment of SH-SY5Y cells with EGCG significantly attenuated cell death and the decline in STAT3 induced by 6-OHDA (Wang et al. 2009). The neuroprotective mechanism of EGCG against oxidative stress-induced cell death includes stimulation of phosphokinase C and modulation of cell survival/cell cycle genes (Levites et al. 2002a). EGCG can also prevent 6-OHDA- (Nie et al. 2002) or paraquat- (Hou et al. 2008) induced apoptosis in PC12 cells. Pretreatment with EGCG was found to significantly attenuate MPP^+-induced DA cell loss in primary mesencephalic cell cultures (Hou et al. 2008).

In MPTP-treated C57BL/6 mice, EGCG at a concentration as low as 1 mg/kg, could provide significant protection against MPTP-induced TH-positive cell loss in SNpc (Li et al. 2006a, b). EGCG elevated striatal levels of antioxidant enzymes SOD and catalase (Levites et al. 2001). MPTP- (Li et al. 2006a, b) or LPS- (Li et al. 2004a, b) mediated microglial activation both in SH-SY5Y and primary rat mesencephalic culture was blocked by EGCG. Moreover, oral intake of both tea and EGCG has been found to decrease the expression of neuronal nitric oxide synthase activity (Choi et al. 2002). EGCG could also prevent iron accumulation and Lewy body formation in the SNpc region of MPTP-treated mice (Mandel et al. 2004). Conversely, EGCG caused potentiation of rotenone-induced toxicity in SH-SY5Y cells by enhancing oxidative stress and apoptosis (Chung et al. 2007). Interestingly, the suppression of the oxidative stress via the SIRT1/PGC-1α signaling pathway has been proposed to be one its mechanisms of action (Ye et al. 2012). In a recent study epigallocatechin gallate was given as a dietary supplement to *Drosophila* flies, expressing human α-synuclein. In this model of PD this natural product was effective in attenuating abnormalities in behavior, oxidative stress, and apoptosis (Siddique et al. 2014).

Tea and its constituents such as theaflavin, epigallocatechin, and epigallocatechin gallate are suggested to synergistically activate the therapeutic effects of traditionally used allopathic drugs, such as L-DOPA, L-deprenyl, bromocriptine by influencing dopamine metabolism (Dutta and Mohanakumar 2015). Such effects are evident not only in preclinical animal studies, but also in controlled clinical trials conducted in different human populations world over (Dutta and Mohanakumar 2015).



delta 9-tetrahydrocannabinoid

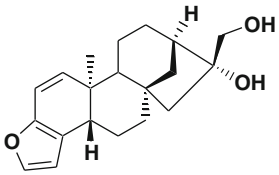
Cannabis sativa L. (Family *Cannabaceae*)

Marijuana is the common name of the plant *C. sativa*, which has been in use for centuries for both recreational and medicinal purposes. The plant contains at least 66 cannabinoid compounds of which Δ^9-tetrahydrocannabinoid (Δ^9-THC) is the primary psychoactive component that mediates its therapeutic effects (Glass 2001). Daily administration of Δ^9-THC for 2 weeks following unilateral 6-OHDA infusion produced a significant reduction in striatal DA depletion, loss in TH activity and its mRNA levels in the lesioned striatum, which was found to be sustainable for several weeks (Lastres-Becker et al. 2005).

Cannabinoil, another cannabinoid present in the same plant (with negligible affinity for cannabinoid CB_1 receptors) showed similar neuroprotective features (Lastres-Becker et al. 2005). Further in vitro data also suggested modulation of glial function in relation to the protective effects of cannabinoids (Lastres-Becker et al. 2005). However, a randomized double blind study on PD patients receiving oral cannabis failed to show any improvement in either the symptoms of the disease or the side effects associated with prolonged administration of L-DOPA (Carroll et al. 2004).

Chaenomeles speciosa Nakai (Family *Rosaceae*)

Flowering quince (*C. speciosa*) fruit extract is a potent DAT inhibitor and has shown neuroprotective potential in cellular and animal models of PD (Zhao et al. 2008b). In vitro it could inhibit DA uptake by Chinese hamster ovary (CHO) cells expressing DAT and by synaptosomes. The extract alleviated MPTP-induced toxicity in CHO cells, mitigated stereotypic rotational behavior in unilaterally 6-OHDA-infused rats and markedly reduced the loss of TH-positive neurons in the SNpc in MPTP-treated mice (Zhao et al. 2008b).

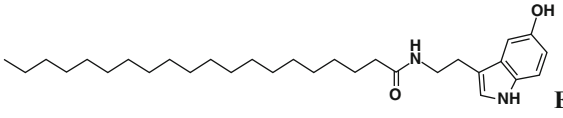


Kahweol

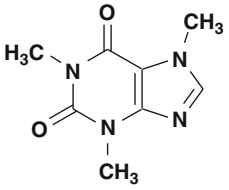
Coffea arabica L. (Family *Rubiaceae*)

Kahweol is a diterpene molecule found in the beans of the coffee plant, *C. arabica*. Pretreatment of SH-SY5Y cells with Kahweol had been revealed to significantly reduce 6-OHDA-induced cellular toxicity by preventing generation of ROS, caspase-3 activation and regulating heme oxygenase-1 expression via induction of the

transcription factors Nrf2, PI3K, and p38 (Hwang and Jeong 2008). Another component of coffee, which has come to light in recent times is eicosanoyl-5-hydroxytryptamide (EHT) (Lee et al. 2011; 2013). When supplemented through diet this compound was found to protect DA neurons from MPTP-mediated neurotoxicity (Lee et al. 2013). Results in cellular models of PD have revealed that the mechanisms of action of this compound include antioxidant, antiapoptotic and modulation of phosphoprotein phosphatase activity (Lee et al. 2013).



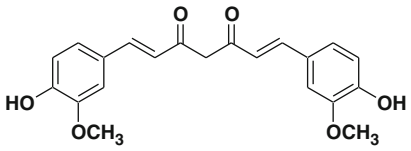
Eicosanoyl-5-hydroxytryptamide



Caffeine

Coffea sp. (Family *Rubiaceae*)

Several epidemiological studies suggest that coffee consumption reduces the risk of PD (Ascherio et al. 2001; Hernan et al. 2002; Ragonese et al. 2003; Saaksjarvi et al. 2008) and caffeine is considered to be the responsible agent (Ascherio et al. 2001). Caffeine has been shown to be effective as an adenosine A_{2A} receptor blocker, in the striatopallidum to functionally antagonize D_2 receptors in the region (Schwarzschild et al. 2003; Sommer and Stacy 2008). In experimental studies, caffeine blocked MPTP- or 6-OHDA-induced DA-ergic neuronal loss, blood–brain barrier dysfunction, and behavioral anomalies (Joghataie et al. 2004; Aguiar et al. 2006; Chen et al. 2008b). Caffeine could protect SH-SY5Y cells from apoptosis induced by MPP^+, 6-OHDA and rotenone by lowering of caspase-3 through the activation of PK13/Akt pathway (Nakaso et al. 2008). In recent times caffeine has emerged as a leading non-DA-ergic therapeutic target for the treatment of PD (Kalda et al. 2006), probably working through adenosinergic A_2 receptors (see Dutta and Mohanakumar 2015).

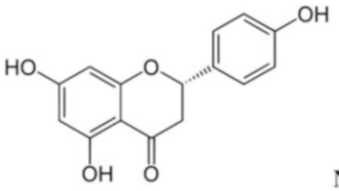


Curcumin

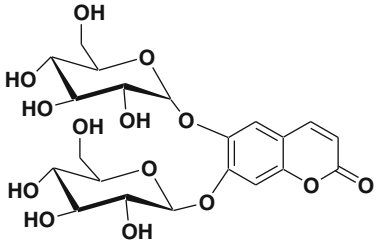
Curcuma longa L. (Family *Zingiberaceae*)

Curcuma longa contains the polyphenol curcumin, possesses antioxidant properties and has demonstrated neuroprotective effects. Literature reveals in 6-OHDA model, rats pretreated with curcumin or naringenin have shown clear protection of the number of TH-positive cells in the SN and DA levels in the striata (Zbarsky et al. 2005). Curcumin is demonstrated to be neuroprotective in MPTP model, and MAO-B inhibitory (Rajeswari and Sabesan 2008) and anti-inflammatory activities (Ojha et al. 2012) are suggested to be responsible for this effect.

In MPP^+-induced toxicity of PC12 cells, curcumin prevents apoptosis by inducing over expression of Bcl-2, blocking the loss of mitochondrial membrane potential, the overproduction of ROS and increases in nitric oxide synthase activity (Chen et al. 2006). Curcumin pretreatment protects brain mitochondria against peroxynitrite-induced loss in mitochondrial functions in vitro by direct detoxification and prevention of 3-nitrotyrosine formation and in vivo by elevation of total cellular GSH levels (Jagatha et al. 2008; Mythri et al. 2007). Curcumin inhibits α-synuclein aggregation (Pandey et al. 2008). In LPS-induced neurotoxicity in primary rat neuron–glia cultures curcumin provided neuroprotection (Yang et al. 2008). Moreover, production of many pro-inflammatory factors and their gene expressions were found to be decreased dramatically after curcumin treatment. Results have also revealed that curcumin treatment decreased LPS-induced activation of two transcription factors NF-κB and activator protein-1 (AP-1). A recent study has demonstrated that in 6-OHDA-mediated model of PD in SH-SY5Y cells and in mice naringenin provides neuroprotection by activating Nrf2/ARE signaling pathway (Lou et al. 2014).



Naringenin



6,7-di-*O*-glucopyranosyl-esculetin

Delphinium denudatum Wall. (Family *Ranunculaceae*)

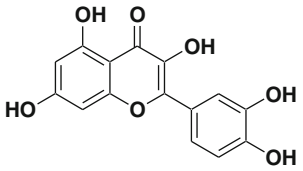
Pretreatment with *D. denudatum* extract is shown to prevent lipid peroxidation and depletion of GSH content in the SNpc area and increased SOD and catalase activities in the striatum of 6-OHDA rat model of parkinsonism (Ahmad et al. 2006a). 6-OHDA mediated decrease in the level of DA and its metabolites and increased D_2 receptors in the striatum were found to be reversed to control values by the extract. Moreover, an increase in the expression of TH in the ipsilateral striatum of the lesioned groups following treatment with *Delphinium* extract was also observed.

Fraxinus seiboldiana Blume (Family *Oleaceae*)

6,7-di-*O*-glucopyranosyl-esculetin, extracted from *F. sieboldiana* bloom, has been found to protect SH-SY5Y neuroblastoma cells from neurotoxicity caused by 0.1–10 μM DA (Zhao et al. 2008a). This protective effect of 6,7-di-*O*-glucopyranosyl-esculetin derive from its anti-oxidative properties such as reduction of ROS, decrease in the release of cytochrome *c* from mitochondria, blockade of apoptosis-inducing factor, enhancement of SOD activity and GSH levels, and upregulation of p53, Bax, and Bcl-2 expression (Zhao et al. 2008a).

Hypericum perforatum L. (Family *Clusiaceae*)

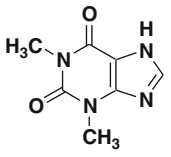
Hypericum perforatum is a widely used medicinal herb indigenous to Europe. The standard extract of *H. perforatum* is found to protect against H_2O_2-induced toxicity in PC12 cells (Lu et al. 2004). Mohanasundari and Sabesan (2006) reported that administration of *H. perforatum* extract in combination with bromocriptine prior to MPTP produced a pronounced neuroprotective effect in mice in terms of behavioral recovery (rotarod test, hang test, and forepaw stride length), attenuation of DA and DOPAC loss and prevention of lipid peroxidation. In a follow-up study the same laboratory has demonstrated the extract's MAO-B inhibitory activity (Mohanasundari and Sabesan 2007), suggesting the possibility that the extract blocked the conversion of MPTP to its neurotoxic metabolite, MPP^+ in the brain.



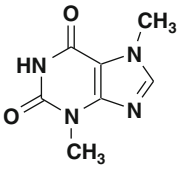
Quercetin

Ilex paraguariensis A. St. Hil. (Family *Aquifoliaceae*)

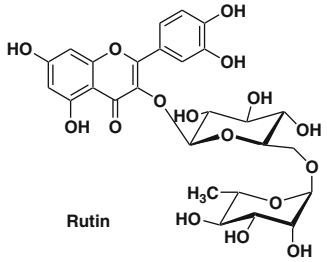
Ilex paraguariensis is a medicinal plant widely cultivated in South America and is with various reputed medicinal properties that can be attributed to the abundant phenolic constituents of the leaves; caffeine, theophylline and theobromine, besides the flavonoids, quercetin and rutin. The hydroalcoholic extract of the stem and leaf of the plant can prevent MPTP-induced hypolocomotion and reserpine-induced catalepsy in mice (Milioli et al. 2007). Biochemical studies showed that hydroalcoholic extract of the plant could cause a significant decrease in the nitric oxide levels, and exhibited free radical scavenging ability preventing deoxyribose oxidation. Another recent study demonstrated that rutin can protect SH-SY5Y cells from rotenone-mediated toxicity (Park et al. 2014).



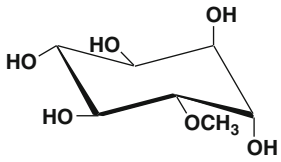
Theophylline



Theobromine



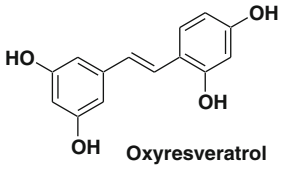
Rutin



Quebrachitol

Magonia glabrata St. Hill (Family *Sapindaceae*)

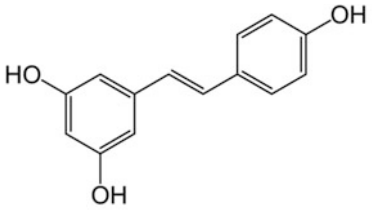
Quebrachitol (2-*O*-methyl-L-inositol), extracted from the fruits of *M. glabrata* is a bioactive constituent and has similar physiological effects as inositol. Quebrachitol is reported to prevent the cytotoxic effect of 6-OHDA on cultured rat fetal mesencephalic cells concentration-dependently (Nobre et al. 2006). It offered a protection against cell death induced by 6-OHDA by reduced formation of nitrite-nitrate and thiobarbituric acid-reactive substances, attenuated apoptotic or necrotic characteristics in TH-positive mesencephalic neurons (Nobre et al. 2006).



Oxyresveratrol

Morus sp. L. (Family *Moraceae*)

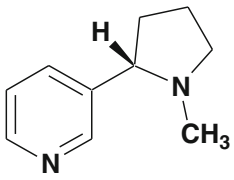
Oxyresveratrol, a polyhydroxylated stilbene, is present in mulberry (*Morus* sp.) is a known antioxidant (Lorenz et al. 2003) and an anti-inflammatory agent (Chung et al. 2003). Both pre- and post-application of oxyresveratrol in human neuroblastoma SH-SY5Y cultures significantly reduced the release of lactic acid, caspase-3 activity, and the generation of intracellular ROS triggered by 6-OHDA (Chao et al. 2008). Oxyresveratrol attenuated 6-OHDA-induced phosphorylation of the transcription factors c-Jun and c-Jun N-terminal kinase involved in neurodegeneration. Interestingly, oxyresveratrol can increase the basal levels of sirtuin (silent mating type information regulation 2 homolog, SIRT1), an enzyme involved in cellular regulation and a sensor for toxic neuronal processes, which may disclose new pathways accounting for the neuroprotective effects of oxyresveratrol in PD (Chao et al. 2008). Resveratrol is another stilbene present in this and other plants. Several studies exist about the antiparkinsonian properties of this compound. It has been found to lessen oxidative stress, mitochondrial dysfunction, aberrant apoptosis, and defective autophagy (Rasheed et al. 2015).



Reseveratrol

Myracrodruon urundeuva M. Allemao (Family *Anacardiaceae*)

The chalcone-enriched fraction isolated from the ethyl acetate extract prepared from the bark of *M. urundeuva,* a Brazilian medicinal plant presented neuroprotective action in 6-OHDA-induced neuronal cell death, in rat mesencephalic cells (Nobre et al. 2009). This fraction helped in reducing the concentration of thiobarbituric acid reactive substances, nitric oxide formation and oxygen radical production, apoptosis and necrosis and increased the number of cells presenting a normal morphology.



Nicotine

Nicotiana tabacum L. (Family *Solanaceae*)

Nicotine is a biologically active alkaloid present in *Nicotiana tabacum* and some other plants of the *Solanaceae* family. Although many evidences suggest that nicotine may be useful in the therapeutic management of PD, some conflicting reports also exist. One of the first reports investigated the effect of smoking combined with the nicotine gum in six individuals with PD; smoking was found to reduce tremor, rigidity, bradykinesia and gait disturbances albeit transiently (Ishikawa and Miyatake 1993). The finding in this study that nicotine gum alone exhibited much lesser effect (Ishikawa and Miyatake 1993) is confirmed later in two elderly patients treated with the nicotine gum (Fagerstrom et al. 1994). Studies on the effects of acute nicotine exposure have, however, yielded variable results, some reporting worsening of symptoms (Clemens et al. 1995; Ebersbach et al. 1999), and others, improvement (Mitsuoka et al. 2002). More than 50 prospective cohort and case-control studies over the last half-century indicate that smoking and other forms of tobacco use appear to be protective in PD in both males and females (Allam et al. 2004; Ritz et al. 2007; Thacker et al. 2007).

Experimental studies supported nicotine's beneficial effects in PD models. In 6-OHDA treated rats nicotine could reduce apomorphine-induced contralateral rotations (Meshul et al. 2007). Nicotine has been found to have synergistic effects with L-DOPA methyl ester in MPTP-induced chronic hemiparkinsonian monkeys (Domino et al. 1999). Nicotine has also been shown to reduce L-DOPA-induced dyskinesia in nonhuman primates (Quik et al. 2007). Tobacco smoke has also been found to contain a selective MAO-B inhibitor farnesylacetone (Khalil et al. 2006), suggesting contribution of such factors in the therapeutic benefits observed in PD patients following tobacco use (Allam et al. 2004; Ritz et al. 2007; Thacker et al. 2007). The compound 2,3,6-trimethyl-1,4-naphthoquinone is known to be present in tobacco and tobacco smoke (Castagnoli et al. 2001). A MAO-A and -B inhibitor, this compound protected against the MPTP-mediated depletion of striatal DA levels in C57BL/6 mouse (Castagnoli et al. 2001).

Paeonia lactiflora Pallas. and *Paeonia veitchii* Lynch. (Family *Rananculaceae*)

Paeoniflorin, the principal bioactive component of the dried roots of either of the *Paeonia* species was found to be neuroprotective in the MPTP model of PD (Liu et al. 2006). Paeoniflorin treatment subsequent to MPTP insult is shown to protect TH-positive neurons in SNpc, and significantly attenuate MPTP-induced microglial and astrocytic activation and pro-inflammatory gene upregulation, effects which have been shown to be mediated via adenosine A1 receptors (Liu et al. 2006). Recently, it is demonstrated that *Paeonia suffruticosa* root bark extract affords behavioral and dopaminergic neuroprotection in MPTP model of PD (Kim et al. 2014). The principal site of action is reported to be mitochondria. The extract was found to increase expression of phosphorylated Akt, ND9, mitochondrial

transcription factor A, and H2AX in the SNpc. Mitochondria-mediated apoptosis was also inhibited, via the regulation of B-cell lymphoma family proteins and the inhibition of cytochrome *C* release and caspase-3 activation (Kim et al. 2014).

Plumbago scandens L. (Family *Plumbaginaceae*)

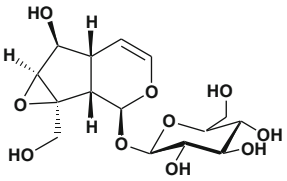
Crude ethanolic extract and total acetate fraction of *P. scandens* are shown to reduce tremorine-induced tremor in animals (Morais et al. 2004). The dose used being unusually high (in g/kg), questions the validity of such studies.

Polygonum multiflorum Thub. (Family *Polygonaceae*)

Polygonum multiflorum extract, but not its ethanol-soluble fraction prevented degeneration of nigrostriatal DA-ergic neurons caused by a combination of the pesticide paraquat and the fungicide maneb administration in male C57BL/6 mice (Li et al. 2005a, b). Mice were treated with the extract once daily for 6 weeks during which period these animals received paraquat and maneb twice a week. They showed attenuation in the DA-mediated effects such as reduced spontaneous locomotor activity, motor incoordination, and decline in DA level in the striatum and TH-positive neurons in SNpc.

Psoralea corylifolia L. (Family *Leguminosae*)

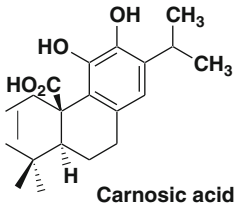
Petroleum ether extract of the seeds of *Psoralea corylifolia* L. was found to possess a significant mitigating effect on MPP^+-induced injury to D8 cells, by counteracting DA uptake inhibition by DAT in D8 cells, SK-N-SH cell line and synaptosomes of rat brains (Zhao et al. 2007). A single compound isolated from *Psoralea corylifolia* L., δ-(3),2-hydroxybakuchiol is found to protect SK-N-SH cells from MPP^+ insult primarily by strong DAT inhibitory activity. In the MPTP model too behavioral and cellular protection is afforded by this molecule due to its monoamine transporters inhibitory potential (Zhao et al. 2009).



Catalpol

Rehmannia glutinosa Libosch (Family *Scrophulariaceae*)

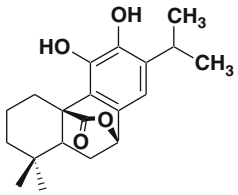
Catalpol, a glucoside isolated from the root of *R. glutinosa*, showed neuroprotective effects in MPTP-induced toxicity in mesencephalic astrocytes cultures and in neuron–astrocyte cocultures (Bi et al. 2008). Results showed that treatment with catalpol (0.5 mM) 1 h prior to MPTP, significantly reversed the neurotoxin-induced mitochondrial complex I inhibition, loss of mitochondrial membrane potential, increase in intracellular Ca^{2+} levels, ROS accumulation and mitochondrial permeability transition pore opening in mesencephalic neuron–astrocyte cultures. Since catalpol exhibited MAO-B activity inhibition, it suggested that failure of conversion of MPTP into its neurotoxic metabolite, MPP^+ caused such effects. In C57/BL/6 mice catalpol prevented MPTP-induced TH-positive neuron loss and reversed DA turnover rates in the nigrostriatal pathway (Jiang et al. 2008).



Carnosic acid

Rosmarinus officinalis L. (Family *Lamiaceae*)

Carnosic acid and carnosol are phenolic diterpenes present in *R. officinalis* L. (Rosemary) a woody perennial herb belonging to the family *Lamiaceae*. Both these are considered to contribute to the plant's potent antioxidant properties (Kim et al. 2006; Yan et al. 2009). Carnosic acid has demonstrated protective effects on dieldrin-induced SN4741 DA-ergic neuronal cell death in cultures by inhibiting the pro-apoptotic caspases 3 and 12 activation, Jun N-terminal kinase phosphorylation and upregulating brain-derived neurotrophic factor (Park et al. 2008). A recent study involving the anti-parkinsonian properties of carnosic acid in 6-OHDA induced neurotoxicity has pointed to the antioxidant and antiapoptotic properties of this molecule (Wu et al. 2015) and its ability to mediate glutathione synthesis (Chen et al. 2012).



Carnosol

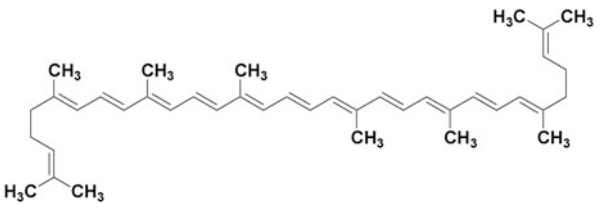
Carnosol on the other hand showed beneficial effects in rotenone-induced neurotoxicity in cultured DA-ergic cells (Kim et al. 2006). Carnosol caused increased cell viability through downregulation of caspase-3. It significantly enhanced TH protein levels, nuclear receptor related protein 1 (Nurr1), a transcription factor essential for the development, survival, and functional maintenance of midbrain DA-ergic neurons and extracellular signal-regulated kinase 1 and 2 (ERK 1/2), involved in the neuroprotection of DA neurons. These results suggest that carnosic acid and carnosol in combination would have synergistic protective effects on dying DA-ergic neurons, and therefore have potential as a possible novel means to treat PD.

Scutellaria lateriflora L. (Family *Lamiaceae*)

This North American perennial herb contains the flavonoid baicalin, which is neuroprotective in the MPTP model of PD (Chen et al. 2007b). Baicalin administration by gastric perfusion could prevent the loss of TH positive neurons in SNpc and the decrease of DA content of striatum in parkinsonian C57/BL/6 mouse, and significantly raise the content of GSH in the brain, but MPTP-induced motor dysfunction was not improved by the short-time medication.

Solanum lycopersicum L. (Family *Solanaceae*) (Tomato)

Tomato powder is rich in lycopene, a carotene pigment, and is neuroprotective in the MPTP model of parkinsonism (Suganuma et al. 2002). A 4-week ingestion of the experimental diet containing 20% (w/w) lyophilized tomato powder before MPTP treatment is found to prevent MPTP-induced decrease in the striatal DA level.



Lycopene

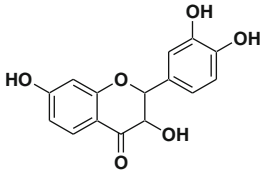
Althaea officinalis L. (Family *Malvaceae*)

This is a perennial herb indigenous to Africa. A recent study reported that an extract of this plant could ameliorate parkinsonian symptoms in 6-OHDA treated rats (Rezaei and Alirezaei 2014). Administration of the extract started 6 days prior to 6-OHDA infusion and continued 3 days following intracranial administration of the

neurotoxin. It was found that *A. officinalis* extract attenuated rotational stereotypy, reduced brain lipid peroxidation level and protected DA neurons in the SN region (Rezaei and Alirezaei 2014).

Toxicodendron vernicifluum (Stokes) F. A. Barkley formerly *verniciflua* Stokes (Family *Anacardiaceae*)

An extract of this plant was found to protect SH-SY5Y cells from rotenone-mediated toxicity (Sapkota et al. 2011). Fustin, a flavonoid isolated from *T. vernicifluum* showed neuroprotective effects on 6-OHDA-induced neuronal death in neuronal catecholaminergic cell line, SK-N-SH (Park et al. 2007). Fustin suppressed 6-OHDA-induced cell death in a concentration-dependent manner and blocked the neurotoxin-mediated increases in ROS, Ca^{2+}, Bax–Bcl-2 ratio, caspase-3 activity, and p38 phosphorylation.



Fustin

Valeriana officinalis L. & Maillefer (Family *Valerianaceae*)

The aqueous extract of the rhizomes of *Valeriana officinalis* is found to possess moderate cytoprotective effects on rotenone-induced apoptosis in human SH-SY5Y neuroblastoma cells (de Oliveria et al. 2009). Lately it is demonstrated that this plant's extract can also protect *Drosophila* flies from rotenone-induced toxicity by enhancing the expression of antioxidant enzymes like SOD and catalase (Sudati et al. 2013).

Vicia faba L. (Family *Fabaceae*)

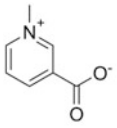
L-DOPA is identified in the seedlings, pods, and beans of *Vicia faba*. In a prospective clinical study, five healthy volunteers and six PD patients (mean age, 63.5 years; mean disease duration, 13 years; stage III, Hoehn-Yahr scale) ate 250 g cooked *Vicia faba* seeds after 12 h off medication. Plasma showed significantly high levels of L-DOPA following the *Vicia faba* food and a substantial clinical improvement was noted in all patients, three of them exhibiting dyskinesia. The clinical profile in the PD patients were comparable to those found after 125 mg L-DOPA + 12.5 mg carbidopa ingested on another day (Rabey et al. 1993).

***Crocus sativus* L. (Family *Iridaceae*)**

The spice saffron is produced from the flowers of this plant. Recently it has been demonstrated that pretreatment of mice with saffron led to significant protection of TH^+-neurons in the SNpc region of MPTP-treated mice (Purushothuman et al. 2013).

***Trigonella foenum-graecum* L. (Family *Fabaceae*)**

In a recent clinical study a standardized extract of this plant was used as a nutritional adjuvant to L-DOPA in PD patients (Nathan et al. 2014). Six months of treatment in 50 patients with matching placebo led to favourable outcomes. In terms of efficacy outcomes it was found that UPDRS and H&Y scores were lower compared to placebo pointing to the usefulness of this extract as an adjuvant therapy in PD management (Nathan et al. 2014). In an earlier study a single administration of standardized extract of this plant was found to affect motor symptoms in 6-OHDA- and MPTP-induced models of the disease (Gaur et al. 2013). The principal ingredient of this extract was found to be trigonelline (Gaur et al. 2013).



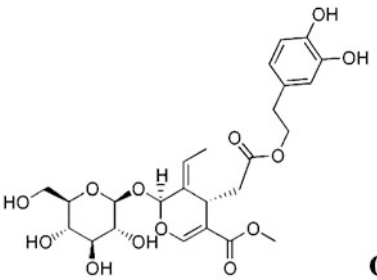
Trigonelline

***Uncaria tomentosa* Wild. (Family *Rubiaceae*)**

This is a woody vine widely used in folk medicine. A recent study has brought to light its anti-PD potential (Shi et al. 2013). The study with standardized aqueous extract of *U. tomentosa* showed dose-dependent protection of SH-SY5Y cells from 6-OHDA-induced damage and restoration of the lost mitochondrial membrane potential. In transgenic *C. elegans* PD model this extract could significantly reduce alpha-synuclein aggregation. The extract has also been shown to scavenge several types of free radicals. All this point to the therapeutic potential of *U. tomentosa* in PD (Shi et al. 2013).

***Olea europaea* L. (Family *Oleaceae*)**

In a recent study olive leaf extract and its main phenolic component oleuropein have been shown to possess protective properties in the 6-OHDA-induced PD model in PC12 cells. Both the agents were found to exhibit significant antioxidant and anti-apoptotic effects in this model pointing to their therapeutic potential (Pasban-Aliabadi et al. 2013).



Oleuropein

***Eucalyptus citriodora* Hook. (Family *Myrtaceae*)**

In a recent study the leaf extract of this tree was supplemented with diet and administered in *Drosophila* flies expressing human α-synuclein (Siddique et al. 2013). The extract was found to dose-dependently delay the loss in climbing ability and brain oxidative stress in these flies (Siddique et al. 2013).

***Garcinia indica* Choisy (Family *Clusiaceae*)**

Methanolic extract of this plant was shown significant protective effect in the 6-OHDA model in terms of behavior and protection against striatal DA loss in rats (Antala et al. 2012).

***Acorus gramineus* Sol. (Family *Acoraceae*)**

An extract of this plant is shown to be effective against both LPS-induced cellular toxicity in BV-2 microglial cells and MPTP-induced animal model of PD (Jiang et al. 2012). The principal mode of action is suggested to be through activating anti-inflammatory pathways through regulating NF-κB, mitogen-activated protein kinases and TRIF-dependent signaling pathways (Jiang et al. 2012).

Spirulina

This cyanobacterium is widely acclaimed to be a useful dietary supplement. It has been demonstrated that rats were protected against neurotoxicity following intranigral α-synuclein, when the animals were fed with diets supplemented with spirulina (Pabon et al. 2012). This effect is found to be caused by spirulina's ability to modulate microglial activation by increasing expression of fractalkine receptor (CX3CR1) on microglia (Pabon et al. 2012).

Indigofera tictoria L. (Family *Fabaceae*)

This plant is widely used in traditional systems of Indian medicine for brain related disorders. An active compound SF-6 has been isolated from this plant, which has exhibited anti-PD properties in in vivo and in vitro conditions (Rajendra et al. 2012). In the 6-OHDA model of the disease this compound was found to attenuate apomorphine-induced stereotypic rotation and to correct behavioral abnormalities in rotarod, Y-maze and passive avoidance tasks (Rajendra et al. 2012). By scavenging free radicals the compound was also found to protect SH-SY5Y cells from toxicity caused by α-synuclein, 6-OHDA or H_2O_2 (Rajendra et al. 2012).

Cinnamomum sp.

Cinnamon extract demonstrated anti-PD effect in *Drosophila* expressing mutant A53T α-synuclein in the nervous system (Shaltiel-Karyo et al. 2012). The extract was able to attenuate behavioral abnormalities and α-synuclein aggregation in the diseased flies (Shaltiel-Karyo et al. 2012).

Selaginella delicatula Alston (Family *Selaginellaceae*)

Aqueous extract of this plant has exhibited neuroprotective efficacy in rotenone-induced PD in *Drosophila* (Girish and Muralidhara 2012). Rotenone induced lethality, motor dysfunction; oxidative stress, mitochondrial dysfunction and neurotoxicity were prevented by diet enriched with this extract. Interestingly the extract normalized the activity of NADH-cytochrome *c* reductase and succinate dehydrogenase enzymes suggesting its ability to protect mitochondrial integrity (Girish and Muralidhara 2012).

Sesame Seed Oil

This is a common edible oil obtained from the plant *Sesamum indicum* L. (Family *Pedaliaceae*). A recent study has demonstrated the neuroprotective capability of this substance in the 6-OHDA-induced model of PD in mice (Ahmad et al. 2012). In the study mice were exposed to diet containing sesame seed oil following which 6-OHDA was infused into the right striatum. In the oil treated group TH expression, DA level and content of various antioxidant enzymes were significantly more. Another interesting finding of the study was that sesame seed oil could inhibit NADPH oxidase dependent inflammatory pathway to afford protection against 6-OHDA toxicity (Ahmad et al. 2012).

Rhus verniciflua Stokes (Family *Anacardiaceae*)

Leaf extract of this plant has been found to protect SH-SY5Y cells from rotenone-induced toxicity (Kim et al. 2011). Key findings of this study were that the extract could suppress rotenone induced generation of ROS, apoptosis and contain damage to mitochondrial membrane potential (Kim et al. 2011).

***Hibiscus asper* Hook. (Family *Malvaceae*)**

Methanolic extract of this plant has potent anti-apoptotic and antioxidant properties in the 6-OHDA model (Hritcu et al. 2011). Chronic administration of the methanolic extract also caused an absence of characteristic DNA cleavage patterns in the 6-OHDA-treated rats (Hritcu et al. 2011).

***Chrysanthemum indicum* L. (Family *Asteraceae*)**

An extract of this plant protected SH-SY5Y cells from MPP^+-induced toxicity by preventing oxidative stress and apoptosis (Kim et al. 2011). Interestingly when BV-2 microglial cells were activated using LPS, the extract could also prevent this effect (Kim et al. 2011).

Yeoldahanso-tang

This is a Korean polyherbal formulation containing ten herbs namely, *Pueraria lobata* (Willd.) Ohwi (Family *Fabaceae*), *Angelica tenuissima* Nakai (Family *Apiaceae*), *Scutellaria baicalensis* Georgi (Family *Lamiaceae*), *Platycodon grandiflorum* (Jacq) (Family *Campanulaceae*), *Angelicae dahurica* (Family *Apiaceae*), *Cimicifuga heracleifolia* Kom (Family *Ranunculaceae*), *Raphanus sativa* L. (Family *Brassicaceae*), *Polygala tenuifolia* (Willd.) (Family *Polygalaceae*), *Acorus gramineus* Soland. (Family *Acoraceae*), and *Dimocarpus longan* Lour. (Family *Sapindaceae*) at a ratio of 6:4:2:1:2:2:2:4:6:6 by dry weight. This formulation is found to protect PC12 cells from MPP^+-toxicity by modulating autophagy (Bae et al. 2011). In MPTP-induced parkinsonism, the formulation was found to protect TH^+ neurons in SNpc (Bae et al. 2011).

***Paullinia cupana* L. (Family *Sapindaceae*)**

A dimethylsulfoxide extract of this plant is reported to protect SH-SY5Y cells from rotenone-induced toxicity (de Oliveira et al. 2011). Chromatin condensation and nuclear fragmentation in these cells were found to be significantly reduced by the extract (de Oliveira et al. 2011).

***Croton celtidifolius* Baill. (Family *Euphorbiaceae*)**

This tree is widely available in the Atlantic coast of south Brazil. Proanthocyanin rich extract from the bark of this tree demonstrated significant neuroprotective properties in intranasal MPTP-treated rats (Moreira et al. 2010). The extract prevented both mitochondrial complex-I inhibition in olfactory bulb and the striatum, and

decrease in TH expression in olfactory bulb and SNpc. Behavioral abnormalities caused by MPTP such as reduction in locomotion and depression were also attenuated by this extract (Moreira et al. 2010).

Pinus pinaster Aiton (Family *Pinaceae*)

Pycnogenol is a standardized extract obtained from the bark of *P. pinaster*. Pretreatment with pycnogenol was found to reduce behavioral and biochemical abnormalities caused by MPTP (Khan et al. 2010). Activity of antioxidant enzymes and glutathione content were restored, and the level of thiobarbituric acid reactive substances was found to be reduced by pycnogenol in MPTP-lesioned animals (Khan et al. 2010).

Gastrodia elata Blume (Family *Orchidaceae*)

This plant is widely used in oriental folk medicine. In SH-SY5Y cells the extract of *G. elata* is stated to afford protection against MPP^+-mediated cytotoxicity by preventing oxidative stress and apoptosis (An et al. 2010a, b).

Thuja orientalis L. (Family *Cupressaceae*)

This plant is widely used in traditional medicine. A leaf extract of this plant is shown to be effective in protecting SH-SY5Y cells from 6-OHDA-induced toxicity (Ju et al. 2010). The main pathways involved were found to be prevention of oxidative stress, restoration of the lost mitochondrial membrane potential and downregulation of the apoptotic pathway (Ju et al. 2010).

Some Major Active Molecules of Plant Origin

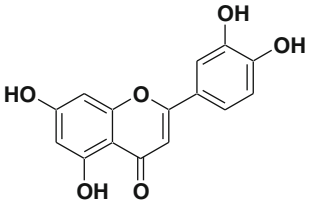
Nutraceuticals and brain health is getting tremendous attention these days. Two recent issues of *Neurochemistry International* (Williams et al. 2015a; 2016a) published a collection of papers that dealt with several molecules or extracts of plant origin that had significant effects on brain health (see also Williams et al. 2015b; 2016b). Table 4 lists the plant sources and the active ingredients that provide such beneficial effects to CNS function.

Flavonoids

Short-term supplementation with plant extracts rich in flavonoids has been found to protect nigrostriatal DA-ergic neurons in 6-OHDA rat model of PD. Pretreatment of animals with extracts of tangerine peel (rich in polymethoxylated flavones), cocoa-2

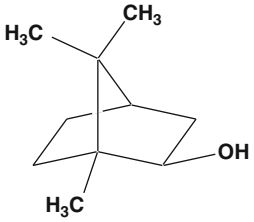
(rich in procyanidins), and red clover (rich in isoflavones) significantly attenuated the 6-OHDA-induced DA-ergic neuronal loss in SNpc and DA and its metabolites in the striata (Datla et al. 2007).

Another flavonoid luteolin, found in foods of plant origin, belongs to the flavone subclass of flavonoids, and significantly inhibited LPS-induced activation of microglia and excessive production of TNF-α, nitric oxide and superoxide anions, and decrease in DA uptake and loss of TH-positive neurons in mesencephalic neuron–glia cultures and microglia-enriched cultures (Chen et al. 2008a). Food supplemented with flavonoids present in dietary blueberry extract significantly increased the survival of implanted DA neurons and ameliorated rotational behavior asymmetries as compared to transplanted animals consuming a standard diet in a hemiparkinsonian model of PD (McGuire et al. 2006). Baicalein in μM concentrations could inhibit the formation of α-synuclein fibrils, strengthening the therapeutic potential of this flavonoid (Zhu et al. 2004).



Luteolin

Quercetin, another member of the flavonoids family, is one of the most prominent dietary antioxidants present ubiquitously in almost all plants, and food supplements arising out of it. Quercetin through its COMT and MAO enzyme-inhibiting properties helped to potentiate the anticatatonic effect of L-DOPA plus carbidopa treatment (Singh et al. 2003). However, in an analogous study quercetin failed to provide any protection against 6-OHDA-induced neurotoxicity either in vivo or in vitro in SH-SY5Y neuroblastoma cell culture (Kaariainen et al. 2008). Quercetin exhibited both neuroprotective and neurotoxic effects in 6-OHDA-induced toxicity in SH-SY5Y cells, where early protective and late toxic phases were observed (Ossola et al. 2008). This derogatory effect in high doses has been reproduced in other neuronal populations too (Chakraborty et al. 2015). Quercetin helps to upregulate mitochondrial ETC complex-I activity and function to protect against rotenone-induced neurodegeneration in rats (Karuppagounder et al. 2013).



Isoborneol

Table 4 Active molecules grossly from plant sources and components of nutraceuticals

Source	Type of compound/active substance	Test mode	Properties	References
Essential oils of numerous medicinal plants	Monoterpenoid alcohol/ isoborneol	6-OHDA induced toxicity in SHSY5Y cells	Reduces ROS, intracellular calcium, Bax–Bcl-2 ratio, and caspase-3 activity; acts as anti-apoptotic agent; prevents translocation of Cyto-*C*; decreases the activation of c-Jun N-terminal kinase; induces activation of PKC	Tian et al. (2007)
Tobacco (*Nicotiana tabacum*)	Quinones/2,3,6-trimethyl-1,4-naphthoquinone	MPTP model of PD	Inhibits MAO-A and MAO-B	Castagnoli et al. (2001)
Psoralea corylifolia	Delta (3),2-hydroxybakuchiol	MPP^+-induced toxicity in SK-N-SH cells and in MPTP model	Inhibits DAT and other monoamine transporters	Zhao et al. (2009)
Tea	Catechins Epigallocatechin gallate	6-OHDA induced toxicity in SH-SY5Y cells; 6-OHDA- and paraquat-induced toxicity in PC 12 cells; MPP^+-induced toxicity in primary mesencephalic cell cultures; MPTP model in mice *Drosophila* expressing human α-synuclein; LPS-induced microglial activation in SH-SY5Y and primary rat mesencephalic culture	Attenuates 6-OHDA induced death of SH-SY5Y cells and decline in STAT3; promotes neuronal cells proliferation and stimulates of PKC; attenuates MPP^+-induced TH-immunoreactive cell loss; provides protection against TH-positive cell loss in SN at a very low dose (1 mg/kg) in C57/BL6 mice; prevents iron accumulation and Lewy body formation in mouse SN region; prevents microglial activation. Oral intake of the compound decreases the expression of neuronal nitric oxide synthase; suppresses oxidative stress via the SIRT1/PGC-1α signaling pathway; antioxidant and anti-apoptotic	Choi et al. (2002) Chung et al. (2007) Hou et al. (2008) Levites et al. (2002a, b) Li et al. (2004a, b, 2006a, b) Dutta and Mohanakumar (2015) Siddique et al. (2014) Wang et al. (2009) Ye et al. (2012)
Citrus fruits	Flavanone glycoside, Hesperidin, Naringin	6-OHDA model MPP^+ model	Attenuates DA depletion Protects DA neurons by enhancing GDNF	Antunes et al. (2014) Leem et al. (2014)

Extracts of tangerine peel, cocoa-2, red clover, blueberry extract and many other vegetables, fruit, wine as well as countless food supplements	Flavonoids/polymethoxylated flavones, procyanidins, isoflavones, luteolin, quercetin, and baicalein	6-OHDA model; LPS-induced toxicity in primary mesencephalic neuron–glia cultures and microglia enriched cultures; 6-OHDA induced toxicity in SH-SY5Y cells; Drug-induced catalepsy in rats	Attenuates 6-OHDA induced oxidative stress, apoptosis and DA-cell loss Prevents LPS-induced decrease in DA uptake and loss of TH-positive neurons; inhibits LPS-induced activation of microglia and excessive production of TNF-α, NO and superoxide Inhibits the formation of α-synuclein fibrils Potentiates anticatatonic effect of L-DOPA plus carbidopa treatment by inhibition of COMT and MAO	Datla et al. (2007) Mercer et al. (2005) Chen et al. (2008a) McGuire et al. (2006) Zhu et al. (2004) Kaariainen et al., 2008 Ossola et al., 2008 Singh et al., 2003
Silybum marianum	Flavonoid—silibinin	MPP^+ model	Protects DA neurons; anti-inflammatory	Jung et al. (2014)
Blueberry, blackcurrant, grape seed, plum and prune	Anthocyanin and proanthocyanin (polyphenols)	Rotenone, LPS induced toxicity models	Protects mitochondria; anti-inflammatory	Strathearn et al. (2014)
n-Butylidene-phthalide	Isolated from the chloroform extract of *Angelica sinenis*	6-OHDA model in *C. elegans*	Protects DA neurons; prevents α-synuclein aggregation; prevents behavioral aberrations; anti-apoptotic; enhanced proteasomal activity; prolonged life span	Fu et al. (2014)
Zingiber officinale	6-Shogaol: 1-(4-hydroxy-methoxyphenyl)-4-decen-3-one isolated from ginger	MPTP model; MPP^+ toxicity in mesencephalic culture	Protects TH-positive neurons Inhibits microglia activated inflammatory pathway	Park et al. (2013)
Salvia miltiorrhiza	Danshensu: β-3,4-dihydroxyphenyl-lactic acid	6-OHDA toxicity in PC-12 cells	Enhances the expression of heme oxygenase 1 enzyme	Chong et al. (2013)
Several plants	Phenolic acid—gallic acid	6-OHDA model	Antioxidant; prevents memory loss	Mansouri et al. (2013)
Robinia pseudoacacia, *Turnera diffusa*, *Betula pendula*	Acacetin	MPTP model in mice; MPP^+ model in vitro	Attenuates behavioral abnormality; anti-inflammatory by inhibiting microglial activation; protects DA neurons	Kim et al. (2012)

(continued)

Table 4 (continued)

Source	Type of compound/active substance	Test mode	Properties	References
Polygala tenuifolia	Tenuigenin	LPS model in rats; 6-OHDA toxicity in SH-SY5Y cells	Recovers striatal DA; protects TH-positive neurons; prevents excessive cytokines production; protects mitochondrial membrane potential	Yuan et al. (2012)
Plant -derived antioxidant compound	Nordihydroguaiaretic acid	*Drosophila* expressing human α-synuclein	Prevents behavioral dysfunction	Siddique et al. (2012)
Clausena lansium	Flavonoid—Bu-7	Rotenone toxicity in PC-12 cells	Anti-apoptotic; Protects mitochondrial membrane potential and reduces protein phosphorylation	Li et al. (2011)
Tea and various fruits like grape and apple.	Flavonoid—Kaempferol	MPTP model	Prevents motor disability, striatal DA loss and loss of TH-positive neurons in SNpc. Increases antioxidant enzyme level and activity; reduces the content of malondialdehyde in the SNpc	Li and Pu (2011)
Roots of *Pueraria lobata*	Isoflavone, puerarin	6-OHDA model	Prevents striatal DA loss; prevents loss of TH-positive neurons in SNpc; anti-apoptotic; enhances GDNF levels in the striatum	Zhu et al. (2010)

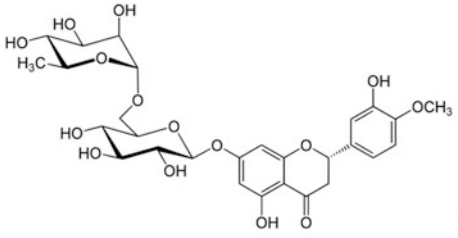
This list includes the molecules that are not listed in earlier tables, but are essentially components of neuronutraceuticals (see Williams et al. 2015b; 2016b). *COMT* catechol-*O*-methyl transferase, *PKC* phosphokinase C. Other abbreviations are as in Tables 1 and 2

Isoborneol

Isoborneol is a monoterpenoid alcohol present in the essential oils of numerous medicinal plants and is a known antioxidant. Isoborneol showed significant reduction in 6-OHDA-induced generation of ROS, accumulation of intracellular calcium, increases in caspase-3 activity, cytochrome *c* translocation into the cytosol from mitochondria, and apoptosis by decreasing the Bax–Bcl-2 ratio (Tian et al. 2007). It has also been observed that isoborneol decreased the activation of c-Jun N-terminal kinase and induced activation of protein kinase C, which is suppressed by 6-OHDA.

Hesperidin

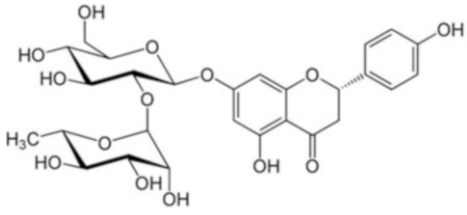
Hesperidin is a flavanone glycoside found in citrus fruits. In a recent study this molecule was effective in attenuating striatal DA depletion due to 6-OHDA insult (Antunes et al. 2014).



Hesperidin

Naringin

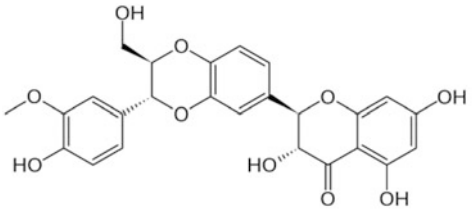
Naringin is another flavanone glycoside, which occurs naturally in citrus fruits especially in grapes. A recent study demonstrated that this molecule can prevent DA neuron loss due to MPP^+-insult and this effect is thought to be primarily mediated through enhancement of GDNF synthesis (Leem et al. 2014).



Naringin

Silibinin

Silibinin is a major flavonoid in the plant *Silybum marianum* (popularly known as milk thistle), which belongs to the family *Asteraceae*. A recent study has demonstrated that this compound possesses neuroprotective properties in the MPP^+-model of the disease (Jung et al. 2014). At moderate doses the compound could significantly attenuate MPP^+-induced neurodegeneration in SNpc, whereas it required a higher dose (100 mg/kg) to bring down the levels of proinflammatory molecules such as TNF-α, IL-1β and iNOS (Jung et al. 2014).



Silibinin

Anthocyanin and Proanthocyanin

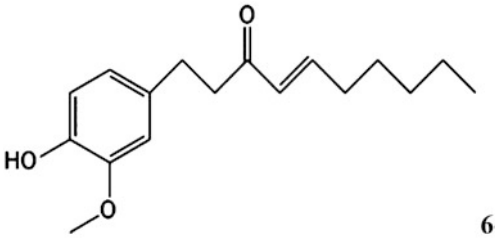
A recent study demonstrated that various plant extracts rich in anthocyanin and proanthocyanin possess neuroprotective ability against rotenone-induced toxicity in primary midbrain culture (Strathearn et al. 2014). An interesting feature of this study is that anthocyanin rich extracts from Blueberry and Blackcurrant but not Purple Basil could attenuate rotenone toxicity indicating that plant specific anthocyanin profile is important in offering neuroprotection. HPLC analysis showed that the major anthocyanins in Blueberry and Blackcurrant were malvidin, delphinidin, and cyanidin glycosides. Proanthocyanin was obtained from extracts of grape seed, plum, and prune. Among these grape seed extract had the most potent neuroprotective effect. A significant feature of this study is that anthocyanin and proanthocyanin rich extracts are more neuroprotective, activating distinct pathways compared to those containing other types of polyphenols. The major mechanism of action was found to involve amelioration of both rotenone-induced mitochondrial dysfunction and LPS-mediated microglial activation (Strathearn et al. 2014).

n-Butylidenephthalide

This is a natural product isolated from the chloroform extract of *Angelica sinenis*. In a recent study this compound demonstrated neuroprotective properties in a *Caenorhabditis elegans* model of PD (Fu et al. 2014). 6-OHDA insult led to DA neuron loss, α-synuclein accumulation and behavioral abnormalities in *C. elegans*. All these symptoms were attenuated by n-butylidenephthalide treatment. The compound was not only found to be anti-apoptotic and able to enhance proteasomal activity but it also prolonged life span in 6-OHDA treated animals (Fu et al. 2014).

6-Shogaol or 1-(4-hydroxy-methoxyphenyl)-4-decen-on

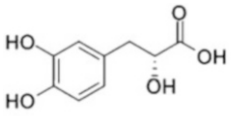
This is a pungent compound isolated from ginger (*Zingiber officinale* Roscoe, Family Zingiberaceae), which has demonstrated anti-PD properties both in vivo and in vitro (Park et al. 2013). Prior treatment of MPTP-treated mice with 6-Shogaol led to correcting the neurotoxin-induced behavioral abnormalities and also protected TH neurons from death. The main mechanism of action was found to be inhibition of microglia mediated inflammatory pathway (Park et al. 2013). By deactivating the same pathway this compound was found to protect TH-positive mesencephalic culture cells from MPP^+ -induced toxicity (Park et al. 2013).



6-Shogaol

Danshensu (β-3,4-dihydroxyphenyl-lactic acid)

This compound is isolated from the roots of *Salvia miltiorrhiza* (Family *Lamiaceae*), a plant widely used in TCM. A recent study has demonstrated that Danshensu can protect PC12 cells from 6-OHDA-mediated toxicity (Chong et al. 2013). The principal mode of action was found to be enhancement of the expression of heme oxygenase-1, which in turn protected the cell from 6-OHDA-induced oxidative damage (Chong et al. 2013).

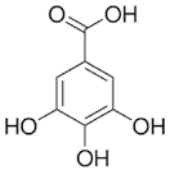


Danshensu

Gallic Acid

This is a phenolic acid found in several plants. It has been demonstrated that this compound can prevent memory loss and cerebral oxidative stress in the 6-OHDA-induced animal model of PD (Mansouri et al. 2013). 6-OHDA treatment led to a reduction in memory as revealed in the passive avoidance test and increased the levels of malondialdehyde in hippocampus and striatum. Oral administration of

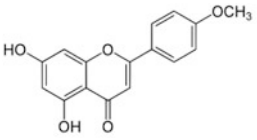
gallic acid significantly attenuated these effects, thus pointing to its potent anti-PD action (Mansouri et al. 2013).



Gallic acid

Acacetin

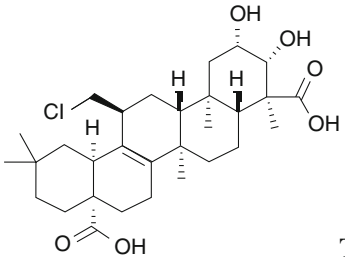
This is a natural constituent of several plants such as *Robinia pseudoacacia*, *Turnera diffusa*, and *Betula pendula.* In the MPTP- and MPP^+-induced in vivo and in vitro models this compound has exhibited significant anti-PD properties (Kim et al. 2012). In MPTP-treated animals acacetin could attenuate behavioral dysfunctions in the pole climbing test and prevented degeneration of DA neurons. Both in vitro and in vivo studies point to the anti-inflammatory role of this compound via inhibition of microglial activation (Kim et al. 2012).



Acacetin (5,7-dihydroxy-4'-methoxyflavone)

Tenuigenin

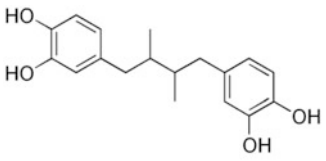
This is an active component of the herb *Polygala tenuifolia* Willd. (Family *Polygalaceae*). This compound has been found to protect DA neurons in rats infused with LPS in the striatum (Yuan et al. 2012). By preventing excessive cytokines production this compound could lead to behavioral and DA recovery, and protect TH-positive neurons in this model of PD (Yuan et al. 2012). The compound has also been found to protect SH-SY5Y cells from 6-OHDA-induced toxicity including protecting mitochondrial membrane potential (Liang et al. 2011). Interestingly, *P. tenuifolia* extract as a whole has been found to attenuate DA neuronal loss in MPTP-treated animals and protect PC-12 cells from 6-OHDA toxicity (Choi et al. 2011).



Tenuigenin

Nordihydroguaiaretic Acid

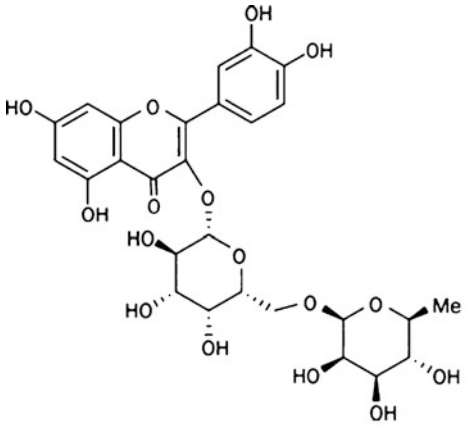
This is a plant-derived antioxidant compound. This compound has been shown to mitigate PD like symptoms in *Drosophila* expressing human α-synuclein (Siddique et al. 2012). Diet supplemented with this compound led to a dose-dependent recovery in the loss of climbing ability in *Drosophila* with neuron expressing human α-synuclein (Siddique et al. 2012).



Nordihydroguaiaretic acid

Bu-7

This is a flavonoid obtained from the plant *Clausena lansium* Lour. (Family *Rutaceae*). This compound has shown protection against rotenone-induced injury in PC12 cells (Li et al. 2011). The compound was found to be successful in preventing rotenone-induced apoptosis, mitochondrial membrane potential loss and protein phosphorylation.

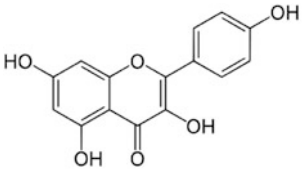


Chemical Structure for Bu-7

Kaempferol

This a flavonoid present in tea and various fruits like grape and apple. It is known to possess strong antioxidant and anti-inflammatory properties. A solitary study has revealed its neuroprotective potential in the MPTP-model of PD in mice (Li and Pu 2011). When kaempferol was administered prior to MPTP it prevented motor

disability and DA loss in the brain. It increased the levels of antioxidant enzymes and reduced the content of malondialdehyde in SN. Interestingly, it could prevent the loss of TH-positive neurons caused by MPTP-toxicity.



Kaempferol

Puerarin

This is an isoflavone obtained from the roots of *Pueraria lobata* (Family *Fabaceae*). This compound showed potency to protect TH-positive neurons of the SNpc and to restore DA and its metabolites levels in the brains of 6-OHDA lesioned animals (Zhu et al. 2010). Puerarin has also been found to prevent apoptosis and to upregulate GDNF in the striatum of rats (Zhu et al. 2010).

Conclusions

Several plant-derived natural products have the potential to be used as drugs for the treatment of PD. The principal modes of neuroprotection are antioxidant property, prevention of apoptosis, inhibition of DA-transporter function, prevention of microglial activation, anti-inflammation, decrease in nitric oxide synthesis, monoamine oxidase inhibition, and enhancement of trophic factors, including BDNF and GDNF. Some additional features are demonstrated by some plant products, e.g., modulation of endogenous transition metal homeostasis, upregulation of heme oxygenase, inhibition of prostaglandin production, regulation of cyclooxygenase-2 activity and blockage of adenosine A_{2A} receptors. It is to be reiterated here that clinical study on these natural products is glaringly lacking, barring a few (*Ayurveda, Stephania intermedia,* tea polyphenols, and *Vicia faba*), which warrants an imminent need for such studies to ascertain the anti-PD potential of at least some more of these.

One of the major concerns is the lack of unequivocal evidences to support the neuroprotective effect of most of the plant extracts or active components. Alarmingly, many under this category have supports from single studies, and that too in in vitro systems. Some of the model systems used are inappropriate, insufficient and lack specificity of purpose and provide only information on the metabolic hindrances of the neurotoxic action of the compound or extracts tested, but not the neuroprotective effects. Yet another grey area is the lack of evidence for the blood–brain barrier permeability of “active constituents” of the extracts or single isolated compounds.

The neurorescue effects vs. neuroprotective actions are interchangeably used in literature, which adds to the confusion. In the cellular models, a heavy dependency on neuroblastoma cell lines needs to be critically viewed, and the obvious prudent suggestion is to use neuronally differentiated cell lines that have copious DA containing neurons.

At this juncture it is strongly suggested to employ valid cellular model(s) as preliminary screens, and thereafter conduct investigations in at least two reliable animal models of the disease, preferably the MPTP and rotenone models of the disease, the former having shown to cause the disease in humans, and the latter to develop cytoplasmic inclusion body pathology (Lewy bodies) in animals. Some plants like *Gingko biloba*, *Mucuna pruriens*, and *Scutellaria baicalensis* look promising, mainly due to their traditional use as ani-PD drugs for several decades, and due to its scientifically proven multiple pathways of neuroprotection, e.g., antiapoptotic, antioxidant, MAO inhibition, and neuroprotective potential in multiple models of the disease. Evidently these actions of these herbal extracts or compounds could be effectively used to get synergistic effects of the existing symptomatic drugs, such as L-DOPA (see Williams et al. 2015b), and probably would help in reducing the dose of these drugs to be used in patients (see Naskar et al. 2015).

Although introduction of L-DOPA has ameliorated the suffering of many PD patients in the last three decades, the side effects and therapeutic variability have necessitated the search for novel drugs. The most important aspect of these natural products is that they possess multiple pharmacological actions relevant to PD therapy, not only attenuating behavioral abnormalities but also preventing the demise of DA-ergic cells. Given the wide plethora of data currently available on the neurochemical mechanisms behind the activity of many of these traditional medicinal entities, the need of the hour is to apply the principle of rational drug design so that natural molecules can be subjected to "fine-tuning" by chemical derivatization and synthesis of analogues. Another important criterion that can be looked into is the formulation of effective combinations or cocktails of these natural products so that maximum therapeutic potential is achieved.

Compliance with Ethics Requirements The authors declare that they have no conflicts of interest.

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ERRATUM

The Benefits of Natural Products for Neurodegenerative Diseases

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