Therapeutic Effects of Natural Antioxidant on Neurodegenerative Disease

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ABSTRACT

The free radical theory of aging hypothesizes that oxygen-derived free radicals are responsible for age-related damage at the cellular and tissue levels. In a normal situation, a balanced equilibrium exists among oxidants, antioxidants and biomolecules. Excess generation of free radicals may overwhelm natural cellular antioxidant defenses, leading to oxidation and further contributing to cellular functional impairment. The identification of free radical reactions as promoters of the neurodegenerative process implies that interventions aimed at limiting or inhibiting them should be able to reduce the rate of formation of degenerative changes with a consequent reduction in the aging rate and disease pathogenesis. Although the human diet is the main source of antioxidants, medicinal plants have received increasing attention in this context. Because antioxidant therapy is vital for the elimination of free radicals and ROS prevent the propagation of tissue damage and neuronal degeneration in the face of oxidative stress, diverse compounds and a broad variety of chemical structures have been investigated as therapeutic agents for acute central nervous system lesions. Indeed, there are currently many research groups working on this theme with the objective of discovering more potent and effective compounds. Here, we provide an overview of the current knowledge of the use of several medicinal plants as antioxidant agents to reduce the cellular damage produced by neurodegenerative diseases, focusing on basic and clinical evidence.

Keywords: Antioxidant, flavonoids, medicinal plants, neurodegenerative disease oxidative stress

INTRODUCTION

Neurodegenerative diseases are characterized by a slow and progressive loss of neurons and axons in the central nervous system, which is the primary pathological feature of acute and chronic neurodegenerative conditions such as Alzheimer’s disease (AD; Capone et al. 2009), and Parkinson’s disease (PD), neurotropic viral infections, stroke, paraneoplastic disorders, traumatic brain injury, epilepsy, multiple sclerosis and Huntington’s disease. Although different cell groups are affected in each disease, they likely share common pathways involving complex molecular processes leading to degeneration (Borlogan et al. 1996; Navarro et al. 2008). In mammalian brain the accumulation of dysfunctional mitochondrial DNA with decreased rates of electron transfer in complexes I and IV and of ATP production is associated with the accumulation of oxidation products of phospholipids and proteins, and these characteristics appear as determining factors in brain degeneration (Halliwell 2006; Boveris and Navarro 2008). Neurons are particularly at risk to oxidation products because many major antioxidant defense mechanisms, such as GSH, Nrf-2, and metallothionein, seem to be localized to astrocytes. On the other hand excessive ROS production is associated with activation of the Ca2+-dependent enzymes including proteases, phospholipases, and nucleases and alterations of signaling in addition to mitochondrial dysfunction producing neuronal apoptosis (Mattson 2007). Increase in oxidative products, such as HNE for lipid peroxidation, 3-N for protein carbonyl and protein nitrotyrosine adducts, and 8-OHdG for DNA damage, associated with neurodegenerative diseases support the notion that oxidative stress is a common ele-
ment in the progression of these diseases (Simonian and Coyle 1996; Halliwell 2006).

Oxidative stress is also a significant factor associated with the decline of function in the aging brain. With the disproportional increase in aging population in the next decade, there has been a considerable increase in neurodegenerative diseases which has increased attention to develop nutritional therapies to combat these age-related oxidative processes. Considerable attention is being paid to botanicals in vegetables, fruits, grains, roots, flowers, seeds, tea and red wine. Other nutritional interventions, such as dietary restriction and a Mediterranean diet, have also captured considerable attention, in particular among older population and subjects with mild cognitive impairments (Trushina and McMurray 2007; Burgener et al. 2008).

Compounds such as polyphenols are becoming recognized for their protective effects against inflammatory diseases, cancers, cardiovascular and neurodegenerative diseases. Although the mechanisms whereby these compounds display beneficial effects remain elusive, there is increasing evidence to support their anti-oxidative, anti-inflammatory, anti-apoptotic and metal-chelating properties (Ndiaye et al. 2005). Besides these polyphenolic compounds, there is increasing evidence for NADPH oxidase as an important source of ROS in the central nervous system.

**Oxidative stress and mitochondrial dysfunction**

The free radical theory of aging and degeneration is based on the works of Gerschman (Gerschman et al. 1954; Harman 1972), which considered that degeneration is caused by the continuous inactivation of biologically essential macromolecules due to chemical modifications produced in reactions mediated by oxygen free radicals. When the free radical theory of aging and degeneration, lacked the precision of the subcellular location of the oxidative reactions mediated by free radicals, focused on mitochondria, and as the mitochondrial theory of degeneration emerges (Vina et al. 2003; Harman 2006). Mitochondria were brought to attention in aging biology due to the central role of mitochondria in producing biochemical energy (ATP) to meet cellular requirements in aerobic cells and to the decline of basal metabolic rate and of physical performance that are characteristic of aging. Moreover, mitochondria are considered likely pacemakers of tissue aging due to their continuous production of reactive free radicals, focused on mitochondria, and as the mitochondrial theory of degeneration emerges (Vina et al. 2003; Harman 2006).

Cells which use oxygen, to obtain of metabolic energy in ATP form produce, in addition to oxidation, molecular species whose cytotoxic potential must strictly be controlled. This control is performed by molecules such as antioxidants. A portion of the oxygen that we breathe is reduced by an alternative cytochrome oxidase pathway and gives rise to reactive species, such as superoxide, hydrogen peroxide, and hydroxyl radicals. Based on Cerda et al. (2010).

![Fig. 1 Monovalent reduction of molecular oxygen](image)

![Fig. 2 Synchronous activity of antioxidant enzymes in the metabolism of the radicals hydrogen superoxide and peroxide and the inhibition of the formation of hydroxyl radicals](image)

The reactivity of the ROS allows them to interact with a diverse array of macromolecules, such as lipids, proteins and nucleic acids, to modify their structure and function (Fig. 2). This oxidative stress is a major risk factor for the initiation and progression of many neurological disorders (Gysem et al. 2001; Bhatia 2002) via the high production of reactive free radicals secondary to either an overproduction of reactive species or a failure of cell buffering mechanisms that normally limit their accumulation. Oxidative damage to proteins, lipids, and nucleic acids has been found in the CNS of patients with degenerative diseases. Although mitochondria are capable of generating ROS, the rate of ROS production under physiological conditions is very low and proportional to the rate of mitochondrial oxygen utilization. However, this equilibrium can be altered in response to various pathological insults, such as hypoxia, reperfusion, changes in pH and ionic strength, and toxic compounds (Chaudière 1994). Excessiye ROS production is associated with the activation of Ca2+-dependent enzymes, including proteases, phospholipases, and nucleases and alterations of signaling pathways that lead to mitochondrial dysfunction and neuronal apoptosis (Mattson 2007; Bredesen 2008). An increase in oxidative products, such as 4-HNE (causes lipid peroxidation), 3-NT (causes protein carbonyl and nitrotyrosine adducts), and 8-OHGdG (causes DNA damage), associated with neurodegenerative diseases supports the notion that oxidative stress is a common element in the progression of these diseases (Fig. 3) (Simonian and Coyle 1996; Halliwell 1997).

Cells have evolved effective molecular mechanisms to resist the adverse effects of oxidative stress, including a range of antioxidants. An antioxidant is a molecule able to prevent and/or avoid the oxidation of another molecule, either by interacting with and stabilizing the reactive species or by transforming these reactive species into a more stable configuration/reducing their reactivity (Fig. 3) (Halliwell and Gutteridge 2007).

The homeostatic function between free radical production and antioxidant defenses is of great importance, as it maintains the reactive species below their cytotoxic thresholds. Because the formation of ROS is the result of oxygen consumption, ROS can exert important regulatory physiological effects under physiological or controlled conditions. ROS are mainly produced in mitochondria, which utilize most of the O2 consumed for substrate metabolism and ATP production, reducing O2 to water. ROS, produced under normal aerobic metabolism, are essential for cell signaling and for bacterial defense (Halliwell and Gutteridge 2007; Cerda et al. 2010).
Antioxidants and neurodegenerative diseases. Domínguez et al.

Overall, biological antioxidants can be divided into two groups of molecules: those with complex structures and high molecular weight (i.e., antioxidant enzymes) and those of smaller size and molecular weight, which includes vitamins (e.g., E and C), glutathione (GSH), uric acid, carotenoids, phenolic compounds, creatine and lipoic acid (Sies 1986; Bors and Michel 1999). Antioxidant protection requires the synchronized action of three enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-px), to be effective. These enzymes reduce the reactive species superoxide and hydrogen peroxide molecules by transforming them into more stable molecules and preventing the formation of additional ROS (e.g., the hydroxyl radical (OH); (Fig. 2; Blankberg 2003).

Therefore, reducing oxidative stress appears to be a rational choice for the prevention and reduction of the rate of progression of many neurological disorders. Moreover, the CNS contains excitatory amino acids and dopamine that generate ROS during their metabolism. The impairment of mitochondrial function contributes to the generation of free radicals and oxidative stress, which can lead to mitochondrial DNA mutations. These related processes converge into a common pathway leading to apoptosis. Therefore, ongoing efforts are focused on the development of potent antioxidants and energy-yielding compounds. The crucial properties of an antioxidant include the ability to cross the blood-brain barrier following systemic administration, the removal of O2, the scavenging to prevent ROS formation or their precursors, and the up-regulation of endogenous antioxidant defenses.

Another mechanism involved in chronic, neurodegenerative, and acute CNS conditions (such as stroke and traumatic injury) is inflammation. Levels of pro-inflammatory cytokines including tumor necrosis factor-alpha, interleukin-1 beta, IL-2 and IL-6 were found to be increased in postmortem brains of patients with PD and AD and in spinal cords of amyotrophic lateral sclerosis patients (Szelenyi 2001). This observation, together with the presence of reactive inflammatory cells, especially microglia and other immune-associated proteins, in affected CNS areas, provided the basis of association of inflammation in the pathogenesis of neurodegenerative diseases (Fig. 3). Yet, it is still unclear whether the inflammatory reaction represents an attempt to repair neurons or further contributes to their injury. It is also possible that the increased immune reactivity causes increased vulnerability of neuronal cells to potential neurotoxic factor.

In this context, it is important to note that key differences between inflammatory processes within the CNS (neuroinflammation) and the periphery exist, partially due to the natural compartmentalization of the brain by the blood-brain barrier. As a result of these differences, classical anti-inflammatory agents have not played a major role in the management of CNS inflammatory conditions. However, some compounds derived from plants may have a potential effect on inflammation in the CNS by operating via different mechanisms of action.

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Oxidative stress and neurodegenerative disorders

1. Alzheimer’s disease (AD)

AD is the most common form of dementia, affecting more than 4 million people in the U.S. and 15 to 20 million others worldwide. Neuropathologically, AD is characterized by the accumulation of beta-amyloid (βA) protein to form plaques and tau phosphorylation resulting in tangle formation. AD is primarily an idiopathic disease with the exception of some rare (5%) early onset autosomal-dominant familial cases (Rocchi et al. 2003). Aside from genetic factors, epigenetic and environmental factors play an important role in the onset of sporadic AD. Cardiovascular abnormalities, such as hypertension, diabetes, mini-strokes, and atherosclerosis, are also factors precipitating an increased risk for AD. Dementia is best correlated to synaptic and neuronal loss, rather than directly to pathological burden, and so much interest has been focused on understanding the pathways that lead firstly to the formation of pathology, and then from pathology to synaptic damage, loss and then neuronal death.

Alzheimer brains have low levels of acetylcholine (Ach), which can arise from the accumulation of βA protein fragments that form hard plaques that can in turn interfere with the ability of Ach to effect synaptic transmission and initiate inflammatory processes that produce ROS. There is evidence that βA peptides contribute (at least partially) to the oxidative mechanism. These peptides (39-43 amino acids) are released from the amyloid precursor protein through βA and γ-secretases and, upon release, can aggregate into an oligomeric form. Oligomeric βA causes oxidative damage to neurons and glial cells and initiates changes in synaptic plasticity, events occurring long before their deposition and formation of the amyloid plaques (Selkoe 2003). Studies suggest that βA open channels in cell membranes, permitting calcium ions (Ca++) to enter the cell and triggering several processes leading to mitochondrial dysfunction, inflammation, and cell death (Butterfield et al. 2002; McKeel et al. 2004; Lipton 2007; Caponne et al. 2009). Inflammation often results from persistent oxidative stress, but other determinants include βA, protease inhibitors, pentraxins, inflammatory cytokines, and prosanglin- analogs of cyclooxygenases. Unhealthy neurons contain low levels of N-acetyl-aspartate (NAA), which may also be an issue. Exposure to pollutants can make the BBB permeable to toxins, thus causing oxidative stress, inflammation, and βA accumulation (Calderon-Garcidueñas et al. 2003, 2008). Another possible cause of cell death in AD is a chemical change in a protein tau that keeps microtubules stable. This causes a neuron’s microtubules to pair with other tubules producing tau (neurofibrillary) tangles that result in tubule disintegration and block neurotransmitters, leading to cell death. Indeed, there is great interest in the search for an effective therapy to combat oxidative damage in AD.

2. Parkinson’s disease (PD)

PD affects approximately 1% of the population over the age of 50. The clinical manifestations of PD include tremors, bradykinesia, muscle rigidity, and akinesia, and patho- logical landmarks include a progressive loss of dopaminergic neurons in the substantia nigra (Cardoso et al. 2005). Despite numerous hypotheses and speculations of the etiology of PD, oxidative stress remains the leading theory (Miller et al. 2008). The familial and sporadic forms of PD are indiscernible and share similar biochemical features of a deficit of brain dopamine and a reduction in dopamine transmission within the basal ganglia. Microscopically, there is a degeneration of dopaminergic cells and the presence of Lewy bodies in mesencephalic neurons of the substantia nigra, which project to the body striatum (nigrostriatal pathway). The extent of neuronal loss not only focuses on the dopaminergic system but also affects other classical neurotransmitter systems, such as the cholinergic (acetylcholine) and catecholaminergic nuclei. Therefore, the motor symptoms of PD are related to the dopaminergic systems, and the non-motor manifestations are not related to dopaminergic systems.

In physiological situations, the mitochondria account for the highest consumption of oxygen, which results in the increased production of superoxide radicals that are reduced to ROS. Antioxidant enzymes such as SOD2 decrease ROS levels to a minimum, but when there are defects in the mitochondria (PD), this balance is disrupted (Zhou 2008). Experimental data indicate that PD is associated to two interdependent conditions of brain mitochondria: mitochondrial dysfunction and mitochondrial oxidative damage. Several studies have shown mitochondrial dysfunction and reduced activity of mitochondrial complex I in substantia nigra (Dexter et al. 1994; Schapira 2008) and in frontal cortex (Navarro et al. 2009) in PD patients. Moreover, similar mitochondrial complex I dysfunctions were reported in skeletal muscle and platelets of PD patients (Mann et al. 1992). This has been supported from studies in which the genes of the mitochondrial respiratory pathway were selectively manipulated. As a result of oxidative damage to phospholipids and polyunsaturated free fatty acids (PUFAs), the double lipid membranes in cells may be affected in PD by a decreased concentration of substantia nigra and an increased concentration of malondialdehyde, a product of lipid oxidation. Other evidence of the oxidation of lipids in this disease is an increase in 4-HNE, a product of the lipophilic per-oxidation of membrane-bound arachidonic acid. Similarly, variants of synuclein (the mutant and the natural form of amyloid fibrils similar to those seen in Lewy bodies) and oligomers that are not fibrillar (called protofibrils), have been proposed to be toxic forms of synuclein. Additionally, products such as 8-oxo-dG are increased in postmortem samples of the substantia nigra from PD brains.

As neuroinflammation is also seen in PD, inflammation-based experimental models have been developed, using, for example, lipopolysaccharide as a stimulus to activate TLR-mediated innate responses. Progressive features have been demonstrated in these models, particularly in the MPTP model, which leads to microglial activation as a prominent and persistent feature. That the substantia nigra is most often affected possibly correlates with the high number of microglia in this area. One factor that could contribute to microglial activation is overexpression of human α-synuclein in a transgenic model. In addition, while effector CD4+ T cells can be neurodestructive in the MPTP model, infiltration of CD4+ T-regulatory cells appears to be neuroprotective in this context (Harvey et al. 2008; Brochard et al. 2009; Reynolds et al. 2009).

Stroke

Stroke is the third leading cause of death and the foremost cause of disability in aging adults. Two types of stroke can occur, hemorrhagic stroke, and the more common, ischemic stroke. In hemorrhagic stroke, rupture of an artery results in uncontrolled bleeding to the affected area of the brain. In ischemic stroke, there is a blockage of blood flow to the brain due to the formation of a blood clot. This deprivation of oxygenated blood results in the formation of the ischemic core where cells die rather quickly and irreversibly due to necrosis. The onset of lipolysis, protein degradation, and the breakdown of ion homeostasis are some of the events responsible for the rapid death of these cells (Brouns and De Deyn 2009). The pathological manifestations in stroke are diverse and depend on the severity, duration, and localization of the ischemic damage. Many animal models have been developed in which blood flow is focally ischemic followed by reperfusion either globally, permanently or transiently, and completely or incompletely interrupted.

Many studies have indicated that the increase in oxidative stress contributes to lipid damage, protein alterations, and DNA damage. Ironically, the return of blood flow to the infarcted area of the brain causes harm along with its bene-
fits due to the increase in oxygen availability and the increase in oxidative stress that reperfusion causes. In these situations, lactic acid accumulates in the affected neurons promoting prooxidant effects by increasing the H+ concentration within the cells and generating more ROS (Allen 2009). The primary source of ROS is O2•−, which is generated by leakage from complex III of the electron transport chain of mal functioning mitochondria.

When the unaffected brain and the ischemic core lies a region where the struggle between the life and death of neurons ensues. This region of the brain is known as the penumbra. It is here that the brain is composed of damaged and malfunctioning, yet salvageable, tissue. Cells in this region are susceptible to a programmed form of cell death known as apoptosis. These cells can remain viable for several days following the onset of stroke (Schaller and Graf 2004). Here in the penumbra region is where a host of events related to oxidative stress take place. Ironically enough, reperfusion acts as a double-edged sword. While reperfusion is essential to save the cells affected by ischemia, it also brings along with it its own threat. When reperfusion occurs, there is a large and rapid influx of oxygenated blood to the infarct region. While this delivers the necessary blood, it also brings with it the elements necessary for producing ROS that contribute to the oxidative stress placed upon the already damaged brain tissue.

Previous studies with neurons in culture have demonstrated a role for ionotropic glutamate receptors, particularly the NMDA subtype, in triggering massive Ca2+ influx and, in turn, the activation of Ca2+-dependent enzymes that trigger mitochondrial dysfunction and apoptotic cell death. Although mitochondrial dysfunction produces ROS that cause neuronal apoptosis in cerebral ischemia (Chan 2001, 2004), recent studies also provide evidence for the involvement of ROS from NADPH oxidase (Wang et al. 2006, 2009). To combat the deleterious effects of oxidative stress associated with ischemic/reperfusion, a number of studies have attempted to upregulate antioxidant enzymes (e.g., SODs, CAT and GSH-px) (Saito et al. 2005).

While the extent of damage and repair mechanisms varies, the immune response provoked plays a crucial role in mediating neuronal damage. Experimental stroke is biphasic, generally involving the activation of leucocytes and the development of neurodegeneration. Recent studies have suggested that, in particular, the production of IL-23 and IL-17 by T cells entering the brain contributes to the neurological deficits that arise (Shichita et al. 2009).

Epilepsy

While defining epilepsy as a neurodegenerative disease remains controversial, there is sufficient evidence indicating that seizures and status epilepticus (SE) mainly produce irreversible neuronal damage. Epilepsy affects approximately 0.8% of the population. In the majority of patients, the seizures have a focal onset. In newly diagnosed patients with a clinically localizable seizure onset, approximately 30% of the seizures are non-structural (Shin et al. 2002). In most of these patients, the development of the disorder is an ongoing process: 1) an initial brain-damaging insult (e.g., genetic malformation, head trauma, stroke, infection, or SE), a latency period (epileptogenesis), and 3) the recurrence of spontaneous seizures (epilepsy). In a subpopulation of patients, epilepsy and the associated cognitive impairment worsen over time (Pitkänen and Sutula 2002). Neurological changes triggered by injury or insult in the adult brain include acute and delayed neuronal death, gliosis, axon and dendritic plasticity, neurogenesis, angiogenesis, a reorganization of the extracellular matrix, and a molecular reorganization of receptors and channels. These alterations continue in parallel or sequentially during epileptogenesis, which is a major challenge for the design of antiepileptic treatments. More recently, evidence for a more general involvement of mitochondria also in sporadic forms of epilepsy has been accumulated (Kunz et al. 2004; Kann and Kovács 2007). This might be related to the fact that mitochondria are intimately involved in pathways leading to neuronal cell death seen in experimental and human epilepsy. Accumulating evidence indicates that free radicals, oxidative stress and mitochondrial dysfunction are important factors in the general pathogenesis of epilepsy (Kann and Kovács 2007; Jarrett et al. 2008; Kudin et al. 2009; Waldbaum and Patel 2010). Therefore, it is reasonable to assume a considerable pathogenic role of mitochondrial dysfunction in the process of epileptogenesis and seizure generation.

Therefore, one rational component of an antiepileptogenic treatment regimen is neuro-protection, which is already one of the most attractive targets in CNS drug development. However, the question remains: does preventing neuronal death prevent other consequences? There is a clear distinction between preventing neuronal death and preventing the later development of epilepsy. Indeed, some endogenous neuro-protective pathways may be pro-epileptogenic by encouraging axonal reorganization and potentiating synaptic transmission (Sweatt 2004). Preventing calcium accumulation by inhibiting N methyl aspartate receptor (NMDA) should prevent downstream consequences. Indeed, NMDA receptor antagonists appear to prevent not only neuronal death but also the subsequent cognitive effects and epileptogenesis (Rice et al. 1998). However, NMDA receptor antagonism is not always sufficient to prevent the development of epilepsy, even when it has prevented neuronal damage (Brandt et al. 2003).

These examples show that oxidative stress and inflammation play a pivotal role in neurodegenerative diseases (Halliwell 2006). Thus, the implementation of radical scavengers, transition metal (e.g., iron and copper) chelators, quenchers of singlet and triplet oxygen, inhibitors of peroxidation and inflammation, and the non-vitamin natural antioxidant polyphenols may be appropriate therapeutic options. Because synthetic antioxidants could be potentially toxic and anti-inflammatory drugs have severe side effects, research has instead focused on natural antioxidants and anti-inflammatory products obtained from plants (Wang et al. 2009), which may offer new alternatives to the limited therapeutic options that currently exist for the treatment of neurological diseases and/or their symptoms.

Natural compounds with antioxidant effects

The use of plant-derived supplements for improving health is gaining popularity because most people consider these natural products to be safer and to produce less side effects than synthetic drugs (Raskin et al. 2002). Today, one in three Americans use herbal supplements.

I. Medicinal plants

Medicinal plants contain different biologically active substances, such as polyphenols, tocopherols, alkaloids, tannins, carotenoids, and terpenoids these compounds have potent antioxidant activity which can be useful. Flavonoids and polyphenolic acids exhibit various beneficial pharmacological properties, such as antioxidant, vasoprotective, anti-carcinogenic, anti-neoplastic, anti-viral, anti-inflammatory, and anti-allergic effects, and anti-proliferative activity on tumor cells (Cai et al. 2004; Bhatnagar et al. 2005; Beevi 2010).

The scavenging properties of antioxidant compounds (e.g., phenolic acids and flavonoid) are often associated with their ability to form stable radicals, which are reactive and can be removed by several mechanisms (Bors and Michiel 1999; Wolfe et al. 2008).

Polyphenols

are natural substances that are present in some liquid (e.g., olive oil, red wine, and tea) obtained from plants, fruits, and vegetables (Butterfield et al. 2002; Sun et al. 2008). Phenolic compounds may exist in free, esterified and glycosidic forms and are powerful chain-breaking antioxidants (Choi et al. 2002). Numerous studies in the past 10
The abundant phenolic hydroxyl groups on the aromatic compounds further divided into several categories (including flowers and fruits) and anthocyanins, colorless compounds (which are the major active oxygen species that cause lipid peroxidation when reacting with the substrate but rapidly with another lipid peroxyl radical (Choi et al. 2002; Owuor and Kong 2002).

**Flavonoids** are the largest group of polyphenols, a group that is mainly divided into anthocyanins (which are glycosylated derivatives of anthocyanidin present in colorful flowers and fruits) and anthoxanthins, colorless compounds further divided into several categories (including flavones, isoflavones, falvanols flavans, and flavonols) (Fig. 3) (Martínez et al. 2002).

Flavonoids consist of an aromatic ring that is condensed to a heterocyclic ring and attached to a second aromatic ring. The abundant phenolic hydroxyl groups on the aromatic ring confer the antioxidant activity, and the 3-OH is essential for the iron chelating activity of these compounds (Fig. 4) (Salazar-Aranda et al. 2008; Galleano et al. 2010).

Special interest has been assigned to the therapeutic role of antioxidants in neurodegenerative diseases, such as PD and AD (Halliwell 2006), where oxidative damage to neuronal biomolecules and an increased accumulation of iron in specific brain areas are major pathological aspects (Cardoso et al. 2005). Although the etiology of both disorders and their respective dopaminergic and cholinergic neuronal degeneration remain elusive, the chemical pathology of PD shows many similarities to AD, including an increase in iron concentration, the release of cytchrome c, alpha-synuclein aggregation, oxidative stress, a loss of tissue GSH, a reduction in mitochondrial complex I activity, and an increase in lipid peroxidation (Coyle and Puttfarcken 1993; Dauer and Przedborski 2003).

Although the specific mechanisms by which green tea polyphenols exert their neuro-protective action are not clearly defined, recent evidence indicates that aside from their antioxidant and iron chelating properties, polyphenols have a profound effect on cell survival/death genes and signal transduction (Aruoma 2003). The revelation of novel molecular targets possibly implicated in their neuro-protective action include calcium homeostasis (Dajas et al. 2003), the extracellular mitogen-activated protein kinases (Schoenen et al. 2002), protein kinases C, antioxidant enzymes, antioxidant regulatory element survival genes, and the amyloid precursor protein processing pathway (Samoylenko et al. 2010). Therefore, green tea polyphenols are now being considered as therapeutic agents in well-controlled epidemiological studies aimed at altering brain aging processes to serve as possible neuro-protective agents in progressive neurodegenerative disorders (Weinreb et al. 2004).

Because PD is caused by a loss of neurons from the substantia nigra of the brain and (once damaged) these neurons stop producing dopamine and compromise the brain’s ability to control movement, this pathology can be controlled by antioxidants as adjuvants with dopamine agonists or monoamine oxidase (MAO) inhibitors. Banisteriopsis caapi, which contains the MAO inhibitors ß-carbolines, harmine, and harmaline as active constituents responsible for anti-depressant activity, provides protection against neuro-degeneration and has potential therapeutic value for the treatment of PD (Sánchez 1991).

Although the use of Hypericum perforatum (St. John’s wort) has been recognized in the treatment of mild to moderate depression and has been better tolerated than conventional antidepressants, recent studies have shown that its use has a neuro-protective effect and an increased capacity for learning and memory (Kumar 2006). Moreover, *H. perforatum* has demonstrated a clear inhibitory effect on the neuronal uptake of several neurotransmitters, such as serotonin, noradrenaline, dopamine, gamma-aminobutyric acid (GABA), and l-glutamate (Müller 2003). In contrast, all other antidepressants are either specific to one system or show overlapping inhibitory effects on a maximum of two systems. These results and similar data from other studies investigating the effects of plant extracts may be explained by the fact that the effects in the CNS are not only due to a single active constituent or group of constituents but by many constituents/ molecular groups of the constituents, reflecting possible synergistic actions on neurological activity.

In traditional Chinese medicine, *Huperzia serrata* is mainly used as an anti-inflammatory and analgesic, but it has also been used to correct memory loss. Huperzine A, a lycopodium alkaloid isolated from the moss *H. serrata*, shows an ability to inhibit acetylcholinesterase (AChE) in vitro and in vivo. In a clinical trial, huperzine A significantly improved memory and behavior in AD patients. Moreover, it was less toxic than the synthetic AChE inhibitors donepezil and tacrine. Additionally, huperzine A may have potential in the attenuation of memory deficits and neuronal damage that occur after ischemia, therefore providing a benefit in the treatment of cerebrovascular types of dementia (Howes et al. 2003).

The dried root of Scutellaria baicalensis has also been widely used in China to treat depression. Its antidepressant action appears to result from the inhibition of MAO-A and MAO-B. In this context, MAO-A is more important to the metabolism of the major neurotransmitter monoamines, such as noradrenaline, dopamine, and 5-hydroxytryptamine (Zhu et al. 2006).

The neuropharmacological effects of Magnolia dealbata Zucc, used in traditional Mexican medicine as a tranquilizer and to treat epilepsy have been tested in CNS disorders (e.g., spinal cord injury) and found to increase functional motor recovery in experimental animals and in epilepsy, delay the onset of phenytoin tetrazolium (PTZ) induced myoclonus and clonus, and reduce the occurrence of tonic seizures and mortality (Martínez et al. 2006).

Prolyl oligopeptidase is associated with schizophrenia,
bipolar affective disorders, and other related neuropsychiatric disorders and may have important clinical implications. The flavonoid baicalin, isolated as the active component of the root of S. baicalensis, has an important prolyl oligopeptidase inhibitory activity. This new pro-drug has a long history of safe administration in humans, making it an attractive base from which to develop new treatments for neuropsychiatric diseases (Tarragó et al. 2008).

Determination of the molecular mechanisms responsible for the neurological bioactivity of medicinal plants are unknown, as are the constituents responsible for their bioactivity. However, these plants have clear potential as attractive targets for future studies to understand their molecular mechanisms of action, identify the active constituents, and uncover new alternatives to our limited therapeutic arsenal for the treatment of the majority of neurodegenerative diseases, especially for those therapies with side effects that limit their effectiveness.

Pharmacology of natural antioxidants

Medicinal plants with CNS activity have an extensive history in Mexico. The following is a review of the pharmacology of Mexican plants investigated for their antioxidant activity and/or evaluated as a treatment for brain disorders. This review also highlights the identification and evaluation of the bioactive compounds.

Free radicals or oxidative injury now appear to be the fundamental mechanism underlying a number of human neurological (and other) disorders, which may be reverted and prevented by the presence of antioxidant constituents in the body. However, the strategy most often used to evaluate the antioxidant properties of plants is the use of dichlorofluorescin, which is a compound that is easily oxidized to the fluorescent compound dichlorofluorescein in human cells. A decrease in cellular fluorescence compared to control cells indicates the antioxidant capacity of the tested compounds (Mermelstein 2008).

The DPPH test (Wagner and Bladt 1996) may be the most frequently used method in the research of antioxidant properties of plant extracts (Wolfe and Liu 2007; Mermelstein 2008; Wolfe and Liu 2008). This test provides information on the reactivity of test compounds with a stable free radical. Due to its odd electron, DPPH gives a strong absorption band at 517-523 nm in visible spectroscopy (i.e., a deep violet color). As the electron is paired in the presence of a free radical scavenger, the absorption decreases. The resulting decolorization is stoichiometric with respect to the number of electrons taken up.

Mexican plants and antioxidant activity evaluation

Mexican plants and antioxidant activity evaluation

Mexico has an extensive variety of plants; it is the fourth richest country worldwide in this respect, with 25,000 species registered. Further, it is hypothesized that nearly 30,000 additional plant species have yet to be described (Adame and Adame 2000). Plants from northeastern Mexico have great medicinal relevance for many diseases in this region. The antioxidant properties of extracts prepared from 17 wild plants belonging to different genera were tested (Torres et al. 2006; Salazar-Aranda et al. 2009). From these species, only 8 displayed significant in vitro free radical (DPPH) scavenging activity between 10.5 and 35.2 μg/mL in comparison to quercetin as the positive control. EC50 for quercetin was 3.0 μg/mL (8.9 μM) (Salazar-Aranda et al. 2009) similar to that reported by Torres et al. (2006). Extracts from the roots and bark were more effective than stems or leaves but less than flowers as follows: Ceanothus coeruleus > Chrysactinia Mexicana > Cyperus alternifolius > Schinus molle > Colubrina greggi > Phyla nodiflora > Heliotropium angiospermum > Cordia boissieri (Salazar-Aranda et al. 2009).

Investigating other plants from this region, the antioxidant properties of extracts other species (e.g., Turnera diffusa Wild. (Turneraceae), Cucurbita foetidissima Kunth (Cucurbitaceae), Flourensia cernua D.C. (Asteraceae), Selaginella pilifera A. Braun (Selaginellaceae), Juglandis mollis Engelm. (Juglandaceae) and Centaurea americana Nutt. (Asteraceae alt. Compositae) prepared as methanol extracts were also evaluated by means of different assays. These assays included the 1,1-diphenyl-2-picrylhydrazyl radical test by high-resolution liquid chromatography (HPLC) and spectro-photometry, the inhibition of XO activity, and total phenolics content. Five plants showed high scavenging potential; their total phenolics content was also high. Further, the extracts from four plants inhibited the activity of XO. Two of the most promising plants, T. diffusa and J. mollis, did not show cytotoxicity and were recommended for the treatment and prevention of degenerative illness due to their antioxidant potential (Salazar et al. 2008).

Mexico is the main exporter of Mexican oregano (Lippia graveolens), accounting for 35-40% of the international market. The high demand for Mexican oregano is due to the quality of essential oil contained in the leaf. However, a study of the antioxidant activity of the organic and aqueous extracts of oregano evaluated by the radical DPPH assay detected that the antioxidant activity was due to the presence of flavonoids, of which pinocembrin, kaempferol, isokaempferide, a derivative of catechin and a non-identified hexoside of quercetin were observed as possibly responsible (Valentao et al. 2002; González et al. 2007).

Pollon from the anthers of the flowers of Zea mays L. (Poaceae), Tagetes sp. (Compositae), Amaranthus hybridus L. (Amarantaceae), Solanum rostratum Dun. (Solanaceae), L. odorata Cav. (Amarantaceae), and Rorjeria petiolaris HBK (Ranunculaceae) collected from La Parrilla Durango, Mexico and prepared as hydroalcoholic extracts were evaluated for antioxidant activity and their correlation with phenol composition. Pollon from A. hybridus had the lowest antiradical activity (EC50 = 14 μg/mL). Extracts from Z. mays L., R. petiolaris HBK, and L. odorata Cav. had intermediate levels of activity (EC50 = 10.3, 9.9 and 9.3 respectively) with the hydroalcoholic extract of R. petiolaris having a significantly different EC50 values, despite large differences in flavonol content. Pollon from S. rostratum Dun. showed a high level of antiradical activity (8.4 μg/mL), whereas extracts from Tagetes sp. had the highest antiradical activity (6.8 μg/mL). These results provide evidence that compositions of the flavonol and phenolic acids, rather than their concentrations, may be a determining factor in the antiradical activities of these plants (Almaraz et al. 2004).

Sixty-six extracts prepared as hexane, acetone and...
methanol extracts from 22 species of plants collected in the state of Morelos in southern Mexico were studied for scavenging and antioxidant activities using DPHD and the β-carotene bleaching method. The latter method consists of measuring the ability of extracts to minimize the coupled oxidation of β-carotene and linoleic acid in an emulsified solution, which loses its orange color when reacting with the radicals. In this study, only nine of the plant extracts prepared with methanol displayed major antioxidant activity, and a clear relationship between the total phenolic content of the extracts and their antioxidant activity was found. Phenolic content decreased as follows: Larcia arborea Seem (Chrysobalanaceae), Bunchosia canesens (Malpighiaceae), Sydoroxylylin capiri (Sapotaceae), Annona squamosa L. (Annonaceae), Piper leucomphylum C.D.C. (Piperaceae), Swietenia humidz Zuc. (Meliaceae), Rupchita fusa (Polygonaceae), Bursaria grandifolia Engl. (Borseraceae), Pseudobombax ellipticum HBK & (Bombaceae) and Comocladia engleriana (Anacardiaceae). The methanolic extract of L. arborea exhibited the highest total phenolic content value (Ruiz-Terán et al. 2008).

Mexican plants with effects on CNS

In traditional Mexican medicine, plant preparations are Mexican plants with effects on CNS (Polygonaceae), S. capiri (Psidieaceae), Seem (Chysobalanaceae) and a limited distribution in six populations of the cloud forests of Claudia engleriana (Anacardiaceae). In traditional Mexican medicine, plant preparations are considered the active phenolic compounds responsible for this activity are two neolignans, magnolol and a clear relationship between the total phenolic content of medicinal and food plant extracts. Mutation Research/Reviews in Mutation Research 544 (2-3), 203-215

Barja G (2002) Rate of generation of oxidative stress-related damage and animal longevity. Free Radical Biology and Medicine 33 (9), 1167-1172


(7), 1035-1042
Pathological concept as a basis for clinical therapy.
Redox Signaling 5 (5), 549-556
Annals of the New York Academy of Sciences 1147 (1), 93-104
Annual Review of Pharmacology and Toxicology 36, 83-106
Tarragó T, Kichik N, Claussen B, Prades R, Teisidó M, Giralt E (2008) Baicalein, a prodrug able to reach the CNS, is a prolyl oligopeptidase inhibitor. Bioorganic and Medicinal Chemistry 15, 7554-7564