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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
Abbreviations: AD, Alzheimer's disease, **Ba**, beta amyloid, **HNE**, 4- hydroxynonenal, **NO**, nitric oxide, **NMDA**, *N*-methyl-D-aspartic acid, **3NT**, 3-nitrotyrosine, **8-OHdG**, 8-hydroxy-deoxyguanosine, **PD**, Parkinson's disease, **ROS**, reactive oxygen species, $\mathbf{O_2^{\cdot}}$, superoxide anion radical, **XO**, xanthine oxidase

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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, paraneoplasic 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 brain mitochondria 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 metallothienin, seem to be localized to astrocytes. On the other hand excessive ROS production is associated with activation of the Ca^{2+}-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-NT 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-

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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 focused on 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 poliphenols 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 Gerschaman (Gerschaman *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 the free radicals superoxide ($O_2^{\cdot}$) and nitric oxide (NO), to the mitochondrial sensitivity to free radical mediated oxidative damage and to the accumulation of phospholipid, protein, and DNA oxidation products in aged animals (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 partially reduced forms of molecular oxygen (i.e., reactive oxygen species or ROS) that are responsible for the phenomenon of oxidative stress (**Fig. 1**; Halliwell and Gutteridge 2007).

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 (Gilgun *et al.* 2001; Barja 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

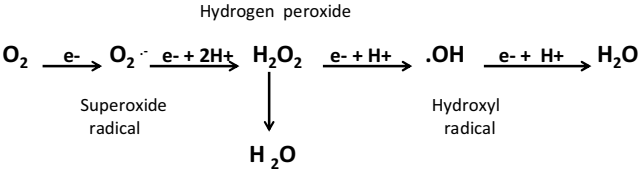


Fig. 1 Monovalent reduction of molecular oxygen. Oxygen is reduced by an alternative cytochrome oxidase pathway by a sequential monovalent mechanism. This mechanism captures electrons of progressive forms and gives rise to reactive species, such as superoxide, hydrogen peroxide, and hydroxyl radicals. Based on Cerda *et al*. (2010).

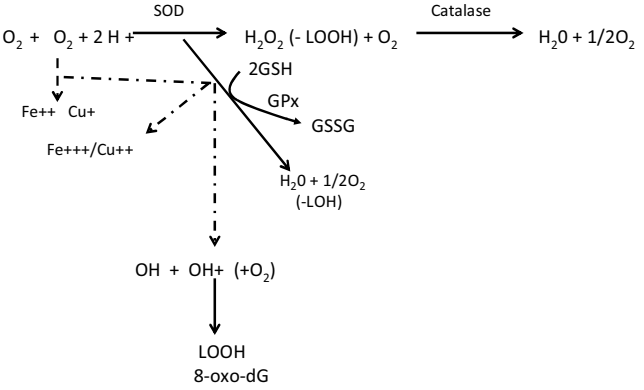


Fig. 2 Synchronous activity of antioxidant enzymes in the metabolism of the radicals hydrogen superoxide and peroxide and the inhibition of the formation of hydroxyl radicals. The performance of SOD, in addition to the activities of CAT and GSH-px, in a synchronous form (continuous lines of trenches) the most effective of the three enzymes listed for the reduction of the concentrations of superoxide and hydrogen peroxide. The main objective of this defensive mechanism is to avoid the interaction between both peroxides and hydrogen with transition metals (dashed lines), thereby preventing the formation of hydroxyl radicals through Haber-Weiss and Fenton reactions. Based on Cerda *et al*. (2010).

various pathological insults, such as hypoxia, reperfusion, changes in pH and ionic strength, and toxic compounds (Chaudiére 1994). Excessive ROS production is associated with the activation of Ca^{2+}-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-OHdG (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 O_2 consumed for substrate metabolism and ATP production, reducing O_2 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).

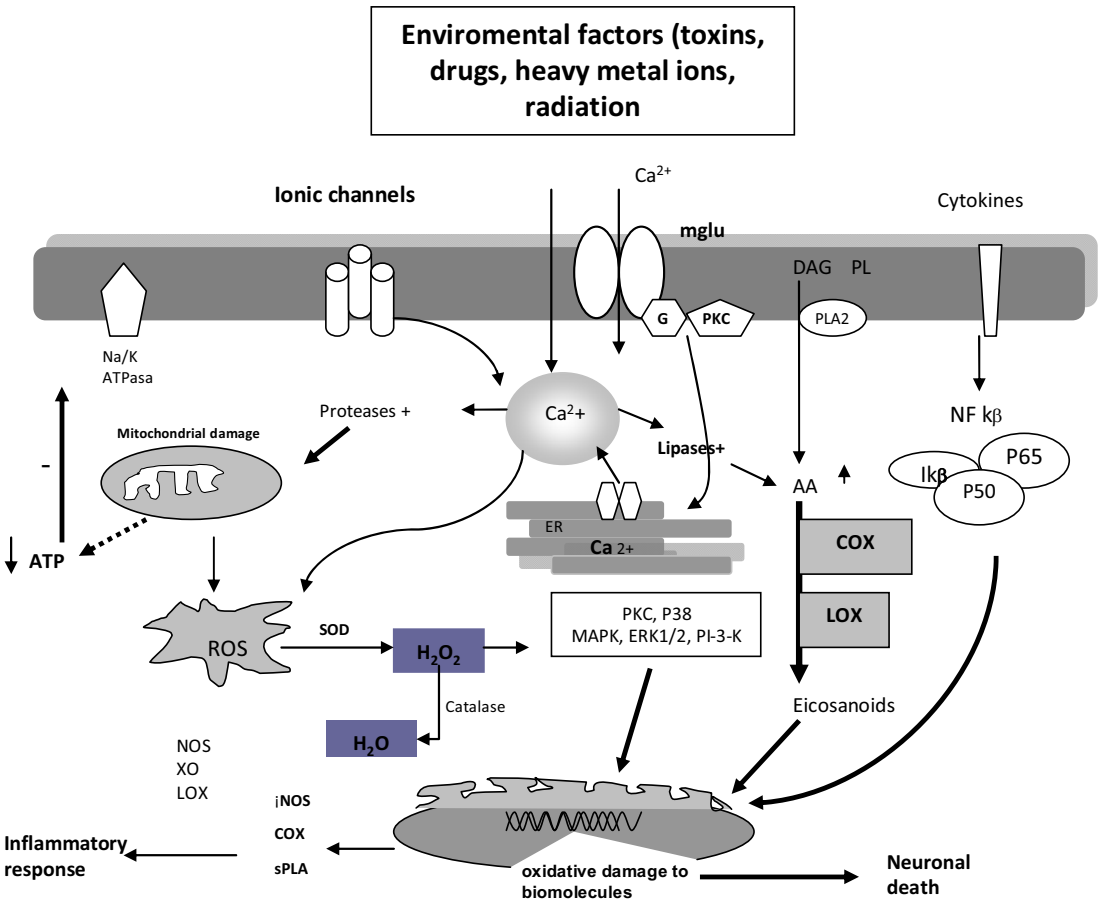


Fig. 3 Signaling pathways associated with oxidative stress. The initial mitochondrial metabolic failure (ATP) leads to disruption of ionic pump functioning at the membrane level and massive neurotransmitter and glutamate release which in turn increase the Ca^{2+} entry. There is an activation of enzymes (XO, NOS, COX, LOX kinases). Reactive oxygen and nitrogen species production (such as O2-, NO and H_2O_2) is part of this process, generating lipid peroxidation and nuclear DNA damage. AA = arachidonic acid; COX = cyclooxygenase; DAG = diacylglycerol; ER = endoplasmic reticulum; G = G protein; LOX = lipoxygenase; mglu = metabotropic glutamate receptors. NO = nitric oxide; NOS = nitric oxide synthase; PKC = protein kinase C; PL = phospholipids; PLA2 = phospholipase A2; XO = xanthine oxidase. Modified from Sun *et al*. (2008), Dajas *et al*. (2003) and Lorigados *et al.* (2010).

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 O_2, 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, 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.

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 suggests that βA open channels in cell membranes, permitting calcium ions (Ca^{2+}) to enter the cell and triggering several processes leading to mitochondrial dysfunction, inflammation, and cell death (Butterfieldt *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 prostanglandin-generating 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 pathological 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 indistinguishable and share the common 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 (as is assumed in 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 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 peroxidation 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 O_2, which is generated by leakage from complex III of the electron transport chain of mal functioning mitochondria.

In the area between 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 Ca^{2+} influx and, in turn, the activation of Ca^{2+}-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 begin in the temporal lobe (Manford *et al.* 1992). 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 (Pitaknen and Sutula 2002). Neurobiological changes triggered by an epileptogenic 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 antiepileptogenic 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 anti-epileptogenic 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

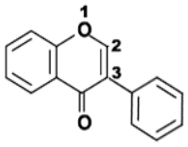
The use of plant-derived supplements for improving health is gaining popularity because most people consider these natural products to be safer and to produce less side effects than synthetic drugs (Raskin *et al.* 2002). Today, one in three Americans use herbal supplements.

1. Medicinal plants

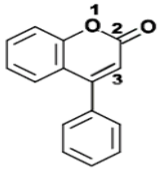
Medicinal plants contain different biologically active substances, such as polyphenols, tocopherols, alkaloids, tannins, carotenoids, and terpenoids these compounds have potent antioxidant which can be useful. Flavonoids and phenolic acids exhibit various beneficial pharmacological properties, such as antioxidant, vasoprotective, anti-carcinogenic, antineoplasic, 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 Michel 1999; Wolfe *et al*. 2008).

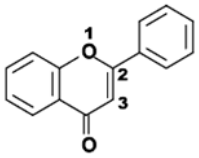
Polyphenols are natural substances that are present in some liquid (e.g., olive oil, red wine, and tea) obtained from plants, fruits, and vegetables (Butterfield *et al.* 2002; Sun *et al*. 2008). Phenolic compounds may exist in free, esterified and glycosidic forms and are powerful chain-breaking antioxidants (Choi *et al*. 2002). Numerous studies in the past 10



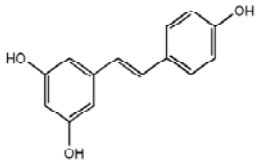
Chemical structure of 2-fenilcromen-4-one (2-phenyl-1 ,4-benzpyrone), the skeleton of flavonoids.



4-fenilcumarine (4-phenyl-1,2-benzopyrone), the chemical structure from which neoflavonoids are derived



Chemical structure of 3-fenilcromen-4-ona (3-fenil-1,4-benzopirone), the isoflavonoid skeleton



Chemical structure of resveratrol 5-[(E)-2-(4-ydroxyphenyl) ethenyl]benzene-1,3 diol

Fig. 4 Chemical structures of some flavonoids.

years have shown that polyphenols have *in vitro* and *in vivo* activity in the prevention or reduction of the deleterious effects of oxygen-derived free radicals associated with several chronic and stress-related human and animal diseases. Polyphenols scavenge lipid peroxyl radicals and thereby break the radical chain sequence via the same mechanism as their scavenging activity on hydroxyl radicals (which are the major active oxygen species that cause lipid oxidation and enormous biological damage):

a) Hydrogen atom transfer from the antioxidant to the lipid peroxyl radical results in the formation of a stable antioxidant radical and relatively stable *cis*- and *trans*-lipid hydroperoxide;
b) Deactivation of the lipid peroxyl radicals by single electron transfer; and
c) Chelation of transition metals to suppress the initiation of radical formation during the catalytic oxidation of lipids. The formed phenoxyl radical is relatively stable, and it reacts slowly 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 anthoxantins, 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 cytochrome *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 (Schoroeter *et al.* 2002), protein kinase 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 *β*-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 phentylene 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 an extract from 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).

Currently, many 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 from plants (Polterait 1997; Pietta 2000; Joseph *et al*. 2005; Wolfe and Liu 2007, 2008). Animal models and human studies are the best methods to determine the actual efficacy of antioxidants in the body. However, *in vitro* methods are the strategy most often used to evaluate the antioxidant properties of plants. According to Mermelstein (2008) the most widely used *in vitro* methods are oxygen radical absorbance capacity, total radical-trapping antioxidant parameter, Trolox equivalent antioxidant capacity, total oxyradical scavenging capacity, peroxyl radical scavenging capacity, ferric reducing/antioxidant power, the Folin-Ciocalteau total phenolic assay, and the 2,2-diphenylpicrylhydrazyl (DPPH) assay. Currently, a method proposed for the *in vivo* analysis of antioxidant properties 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

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 are known to be good sources of antioxidants, but their potency can vary depending on the species and growing conditions. Therefore, the antioxidant activity of these plants may vary considerably depending on where they are grown. The antioxidant capacity of many Mexican plants has been reported to be due to the presence of flavonoids. Flavonoids are natural substances that possess antioxidant and anti-radical properties (Nakayoma and Yamada 1995; Polterait 1997; Pietta 2000). Their antioxidant activity depends on the chemical structure and the position and number of substituents in the flavonoid nuclei and in the B ring (Pietta 2000). The presence of a double bond between C-2 and C-3, a d–OH on C-3, and an ortho-substitution on C-3' and C-4' in the B ring are indispensable for increasing antioxidant potency (Harborne 1984; Lien *et al.* 1999; Harborne and Williams 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. EC_{50} 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), *Juglans 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 l,l-diphenyl-2-picrylhydrazyl radical test by high-resolution liquid chromatography (HPLC) and spectrophotometry, 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 pilosin, cirsimartin, narigenin, kaempferol, isokaemferide, a derivative of catechin and a non-identified hexoside of quercetin were observed as possibly responsible (Valentao *et al*. 2002; González *et al.* 2007).

Pollen from the anthers of the flowers of *Zea mays* L. (Poaceae), *Tagetes* sp. (Compositae), *Amaranthus hybridus* L. (Amarantaceae), *Solanum rostratum* Dun. (Solanaceae), *Bidens odorata* Cav. (Compositae), and *Ranunculus 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. Pollen from *A. hybridus* L. had the lowest antiradical activity (EC_{50} = 14 µg/ml). Extracts from *Z. mays* L., *R. peitolaris* HBK, and *B. odorata* Cav. had intermediate levels of activity (EC_{50} = 10.3, 9.9 and 9.3 µg/ml, respectively) without significant differences between them, despite large differences in flavonol content. Pollen 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: *Licaria arborea* Seem (Chysobalanaceae), *Bunchosia canesens* (Malphigiaceae), *Syderoxylon capiri* (Sapotaceae), *Annona squamosa* L. (Annonaceae), *Piper leucophyllum C.D.C.* (Piperaceae), *Swietenia humidis* Zucc. (Meliaceae), *Rupechtia fusca* (Polygonaceae), *Bursera grandifolia* Engl. (Burseraceae), *Pseudobombax ellipticum* HB&K (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 taken orally and administered repeatededly to control seizures. The flowers of *Magnolia grandiflora*, "flower of the heart", *Mexican Talauma* "yolloxóchitl" (Martínez 1959; de la Cruz 1964; Lozoya 1998) and *Ruta chalepensis* L. (Aguilar and Tortoriello 1996) have traditionally been used to treat epilepsy.

Magnolia dealbata Zucc. (Magnoliaceae) is a plant with a limited distribution in six populations of the cloud forests located in the south-central region of Mexico (Hernández 1980). In mice, *M. dealbata* produces a significant and dose-dependent reduction in anxiety. The major components responsible for this activity are two neolignans, magnolol and honokiol (Martínez *et al.* 2006), and these compounds are considered the active phenolic compounds responsible for the central depressant action (i.e., eliciting muscle relaxation, sedation, sleep induction and anesthesia) of *M. dealbata* in mice (Watanabe *et al.* 1975, 1983). These compounds have antioxidant activity preventing lipid oxidation that is weaker than that of α-tocopherol but stronger than caffeic acid > *p*-ethylphenol > guaiacolas, as measured by the thiobarbituric acid assay (Ogata *et al.* 1997). Regarding the effects of *R. chalepensis*, this species replicated the anticonvulsant effect of diazepam (1 mg/kg), delaying the presence of seizures and reducing tonic convulsions and mortality in animal models (González *et al.* 2006).

Plants such as *Annona squamosa* and *Sapium macrocarpum* display two-fold greater antioxidant activity than the commercial butylated hydroxyanisole antioxidant (Ruiz *et al.* 2008). Species from the Annona genus are a good resource for therapies for CNS diseases. *Annona diversifolia* Saff. (Annonaceae) is an indigenous Mexico tree that has many local names, such as ilama ("old" in the Nahuatl language), ilama zapote, ilamazapotl, izlama, hilama, papausa, papauce, and zapote de vieja (Ruíz and Morett 1997). The fruits of this plant are used for food, and the leaves are popularly used for their antiepileptic properties. The neuropharmacological profile of an ethanol extract of *A. diversifolia* demonstrated a significant and dose-dependent delay in the onset of PTZ-induced seizures and reduced mortality in mice (González *et al.* 1998). This effect was also previously reported for *Annona muricata* L. (N'Gouemo *et al* 1997).

CONCLUDING REMARKS

Despite the complex and diverse genetic and epi-genetic factors underlying the manifestations of different neurodegenerative diseases, there are strong reasons to believe that oxidative stress is a common factor that plays a central role in the pathogenesis of these diseases. Indeed, many pathological conditions are associated with ROS production from mitochondria. Various phenolic compounds and their isomers derived from medicinal plants have potent antioxidant effects. For example, the assessments of the Mexican plants described in this review suggest that flavonoids are widely distributed, secondary metabolites with antioxidant and antiradical properties, from which we can derive potential new treatments for neurological disorders. It is important that basic and clinical researchers focus on gathering scientific evidence to reinforce the application of both plants and their potential active metabolites for the treatment of neurological diseases. These studies support the use of plant-derived phenolic supplements in promoting general health and the prevention of age-related diseases in humans.

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