ANTIOXIDANT PROPERTIES OF A *GINKGO BILOBA* LEAF EXTRACT (EGb 761) IN ANIMAL MODELS OF ALZHEIMER’S AND PARKINSON’S DISEASES

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ABSTRACT: Alzheimer’s and Parkinson’s diseases are the most common neurodegenerative disorders in aging. Oxidative stress is a key mechanism of cell death in these pathologies and is considered as a therapeutic target. The available treatments with synthetic drugs produce undesired side effects. Therefore, the use of antioxidant agents from natural resources such as EGb761 is an alternative. EGb761 is a well-characterized extract obtained from *Ginkgo biloba* leaves after following a standardized procedure. EGb761 has a neuroprotective action that is related to its antioxidant/free radical scavenger properties. Therefore, its potential use has been suggested for the treatment of both diseases. This review discusses the antioxidant efficacy of EGb761 in preclinical studies related to these neurodegenerative diseases. This also includes diverse aspects of EGb761 such as chemical constituents, pharmacokinetics, and side effects. Preclinical studies performed both in vitro and in vivo of Alzheimer’s and Parkinson’s disease, shows that EGb761 produces neuroprotective/antioxidant effect. The use of EGb761 as an antioxidant agent in clinical trials has not been explored directly but indirect information support its use to both neurodegenerative diseases. Further clinical trials are needed to explore the potential clinical use of EGb761 in the treatment of patients suffering from these health conditions.

KEY WORDS: EGb 761; Natural extract; Neuroprotection; Neurodegenerative diseases

INTRODUCTION

The most prevalent neurodegenerative diseases worldwide associated with aging are Alzheimer’s disease (AD) and Parkinson’s disease (PD), which are increasing due to an increment in life expectancy. Therefore, the health care of this population is of concern. These pathologies are characterized by the progressive loss of neuronal cell populations in specific brain regions. The cause of this mechanism of cell damage is strongly associated with oxidative stress, which could lead to dysfunction or cell death and contribute to disease pathogenesis (Li et al., 2013). Oxidative stress is a key mechanism in the pathogenesis of these diseases because from this, other mechanisms converge, leading to bidirectional feedback exacerbating neuronal damage.

Oxidative stress results from imbalance between pro-oxidant/antioxidant homeostasis producing the toxic reactive oxygen species (ROS) (Halliwell, 2007). The therapeutic alternative is the use of antioxidants as target therapies for prevention and treatment. Although the use of antioxidants has positive results in preclinical studies, a major challenge is to transfer this to clinical trials. There are few clinical studies using antioxidants and some of them report no beneficial. In neurodegenerative diseases, for example, the effects of vitamin E in PD and AD have been evaluated in clinical trials. Patients suffering from AD showed a positive effect on their health condition (Li et al., 2012), but no beneficial effects were seen in PD patients (Kamat et al., 2008). Therefore, it is necessary to explore other alternative antioxidants. In particular, the *Ginkgo biloba* (*G. biloba*) extract named EGb 761 has neuroprotective effects...
that are related to its antioxidant properties. It is a well-defined natural extract, with well-known pharmacological and toxicological properties, as well as clinical and safety efficacy (DeFeudis, 1998; Diamond and Bailey, 2013). EGb 761 has been used in the treatment and prevention of neuropathologies associated with aging. Therefore, in this review we describe the use of G. biloba in ancient times that support the development of EGb 761 as a phytomedicine, to be used as a potential alternative in two important neurodegenerative diseases, AD and PD. In addition, its chemical composition, pharmacokinetics, side effects and antioxidant properties are described.

**DEFINITION AND USES OF EGb 761**

The G. biloba tree (synonymous: Salisburia adiantifolia, Salisburia macrophylla, and Pterophylla salisburiensis) probably existed 180 million years ago and is native to eastern China (Zhejiang province) (DeFeudis, 1998). It is often referred to as a “living-fossil” because no evidence in the fossil record of the order Ginkgoales, other than G. biloba, is known after the Pliocene.

G. biloba has been used in traditional Chinese medicine for more than 600 years (DeFeudis, 1998). It was employed to treat diverse diseases such as asthma, bronchitis, kidney and bladder disorders (Mahadevan and Park, 2008) as well as a treatment for senility. In recent times, G. biloba has been used as a dietary supplement. However, research has focused on one particular G. biloba extract obtained from the dried leaves following a detailed procedure. This standardized extract was obtained for pharmaceutical purposes and developed in Germany in 1965, but the first commercial extract for human use was registered in 1974 in France and was named EGb 761 (DeFeudis, 1998). EGb 761 is approved for the treatment of cerebral and peripheral vascular insufficiency states, age-related cognitive impairment, and neurosensory problems (vertigo and tinnitus) (DeFeudis, 1998).

**CHEMICAL CONSTITUENTS OF THE G. BILoba EXTRACT EGb 761**

G. biloba leaf composition can change due to diverse circumstances (for example, cultivation, time of harvest, climate, and procedure of extraction). Therefore, it is necessary to control for the compounds of diverse extracts using standardized methods.

The chemical composition of EGb 761 has been well described and is obtained using a standardized procedure (DeFeudis, 1998). EGb 761 has two main pharmacological bioactive compounds, flavonoids glycosides (24%), terpene trilactones (6%) and other chemical compounds of EGb 761 include non-flavonol glycosides (~20%), carboxylic acids (~13%), proanthocyanidins (~7%), alkylphenols (~≤ 5 ppm) (DeFeudis, 1998; Mahadevan and Park, 2008).

It is prepared from G. biloba leaves in a stepwise process, and during this manufacturing procedure some compounds are enriched (terpenes and flavonoids) while others are removed. To have better control over monitoring G. biloba content, the HPLC-fingerprinting method has been suggested (Sun and Liu, 2007). The characteristics of EGb 761’s main compounds are described below:

**FIGURE 1. Mechanisms of oxidative stress in Alzheimer’s disease.** There are proposed mechanisms to promote oxidative stress in Alzheimer’s disease such as Aβ accumulation, inflammation, mitochondrial abnormalities, and hyperphosphorylated Tau. Oxidative stress exerted could cause cell damage, neurodegeneration and finally Alzheimer’s disease. However, EGb 761 is proposed to have mainly antioxidant action through the blockage of oxidative stress reducing the cell damage. EGb 761 = extract of Ginkgo biloba 761; Apo E = apolipoprotein E; Aβ = amyloid β-peptide.

**FIGURE 2. Oxidative stress is promoted by diverse mechanisms in Parkinson’s disease.** Evidence suggests that dysfunction of dopamine metabolism, mitochondrial dysfunction, neuroinflammation, and metal ion dyshomeostasis produce oxidative stress leading to neuronal death, neurodegeneration, and Parkinson’s disease. Antioxidant therapy has been proposed to produce neuroprotection, EGb 761 is an antioxidant that can induce benefit by blocking oxidative stress. EGb 761 = extract of Ginkgo biloba 761.
**Flavonol glycosides**

EGb 761 contains 24% of flavonol glycosides. Flavonoids are compounds of low molecular weight and include a large group of polyphenols such as flavones, flavonol glycosides, acylated flavonol glycosides, bioflavonoids, flavan-3-ols, and proanthocyanidins, which are present in plants, fruits, and vegetables. Flavonol glycosides are the most abundant compounds present in *G. biloba* leaves and several of them have been identified as derivatives of the aglycones (quercetin, kaempferol and isorhamnetin) which themselves are in low concentration (van Beck and Montoro, 2009). Flavonoids have diverse actions such as antioxidant/free radical scavengers, enzyme inhibitors, and cation chelators.

**Terpene trilactones**

These compounds are only present in *G. biloba* which is the only natural product with a t-butyl group in its structure. Terpene trilactones are present in EGb 761 extract (6%) which includes 3.1% of ginkgolides (A,B,C, and M) and 2.9% of bilobalides. Ginkgolides are diterpenes consisting of six 5-membered carbocyclic rings, three lactones and a tetrahydrofuran (van Beek and Montoro, 2009). Bilobalides are sesquiterpenes with absence of tetrahydrofuran ring. Terpene trilactones are highly stable. It has been reported that terpenoids in EGb 761 extract have antiplatelet properties (Mahadevan and Park, 2008).

**Carboxylic acids**

This group of compounds can be classified in non-phenolic and phenolic acids (van Beek and Montoro, 2009). Among these, ascorbic acid, D-glucaric acid, quinic acid and shikimic acid are included in EGb 761.

**Proanthocyanidins**

These compounds have beneficial properties and occur in large amounts in EGb 761 extract (7%) (DeFeudis, 1998; van Beek and Montoro, 2009).

**Alkylphenols**

In particular, ginkgolic acids, cardanol, α-hydroxycardanol, urushiols, isourushiols, cardols are the main alkylphenols present in *G. biloba*. These constituents have strong allergens that can cause allergic actions (DeFeudis, 1998). They also have cytotoxic, mutagenic, genotoxic and neurotoxic properties (DeFeudis, 1998). Therefore, they are considered undesirable and are removed from EGb 761. In particular, it is recommended that ginkgolic acids be reduced to 5 ppm to prevent side effects (DeFeudis, 1998).

**PHARMACOKINETICS OF EGb 761**

EGb 761 is able to cross the blood-brain barrier. However, its pharmacokinetic is difficult to analyze given that its different compounds can interact at various targets by different mechanisms to exert beneficial effects. The main compounds, flavonol glycosides and terpene trilactones, have been of importance in the study of EGb 761 pharmacokinetics both in humans and experimental animals. The administration of whole extract or individual constituents exhibit half-lives of -2-6 h with activity levels at -1.5-4 h (DeFeudis, 1998). For example, diverse compounds of the flavonoid fraction are absorbed in the small intestine (DeFeudis, 1998; Diamond and Bailey, 2013) and metabolized in humans, with the maximum concentrations between -2-3 h and with a half-life of elimination of -2-4 h. Terpenoid compounds are also absorbed and show an elimination half-life of -2-6 h. However, particular differences have been shown between the diverse compounds of the flavonoid and terpenoid fraction.

**FIGURE 3. Oxidative stress plays an important role in the pathogenesis of Alzheimer’s disease and Parkinson’s disease.**

Antioxidants of natural resources such as the well-characterized EGb 761 are an alternative to treat these diseases.

**SIDE EFFECTS OF EGb 761**

EGb 761 presents a low rate of side effects in humans. These include: gastrointestinal disturbances (2.6%), headache (0.9%), sleep problems/dizziness (0.4%), skin reactions (0.3%), and nausea (DeFeudis, 1998; Mahadevan and Park, 2008). In addition, risk of bleeding has been reported after using *G. biloba* extracts but not specifically EGb 761 (Pedroso et al., 2011). Therefore, it is important to have standardized extracts to analyze side effects and reduce health risk.

As EGb 761 compounds promote blood circulation, caution should be shown when using this *G. biloba* extract in patients with bleeding disorders as well as those under anticoagulant
medication (for example, aspirin, warfarin). However, it is important to emphasize that medication with EGb 761 together with anticoagulants has not been shown to increase the risk of bleeding (Bone, 2008). Subjects taking drugs that affect platelet function and/or coagulation (for example aspirin and ibuprofen) and under self-medication with different G. biloba extracts, other than EGb 761, have been reported to show bleeding (spontaneous hyphaema, intracerebral haemorrhage) (Bone, 2008; Meisel et al., 2003). However, this information is controversial because studies performed specifically administrating EGb 761 to healthy humans did not result in alteration of platelet function or coagulation. Therefore, it has been suggested that bleeding episodes are not related to the pharmacological properties of EGb 761.

The therapeutic dose of EGb 761 is between 50 and 100 mg/kg/day although 10 mg/kg shows protection in some animal models (Rojas et al., 2001). The clinical use in humans is 120 or 240 mg daily (Diamond and Bailey, 2013) where beneficial effects in the treatment of neurological pathologies is only seen after four weeks.

**OXIDATIVE STRESS**

Oxidative stress occurs due to an imbalance between oxidant production and antioxidant systems. Enhancement of oxidative stress occurs during normal aging, but this process is exacerbated in neurodegenerative disorders such as Alzheimer’s disease and Parkinson’s disease (Jomova et al., 2010).

There are two main biological reactive oxidants referred to as reactive oxygen species (ROS) and the reactive nitrogen species (RNS), which include among others, superoxide radicals, hydrogen peroxide (H₂O₂), hydroxyl radicals, nitric oxide, and peroxinitrite. Most of them are free radicals.

However, ROS is the most important group produced in living systems, and which are necessary and beneficial for normal physiological function. They can protect from infections by destroying pathogens at inflammation sites (Valko et al., 2007) acting as secondary messengers in the regulation of cardiac and vascular cell functioning (Valko et al., 2007) and they are involved in intracellular regulation of calcium concentration, in protein phosphorylation and/or dephosphorylation, and transcription factor activation. But ROS overproduction may cause damage to important macromolecules in living systems such as to DNA, proteins, and lipids (Jomova et al., 2010; Valko et al., 2007).

The ROS group of biological importance includes H₂O₂, superoxide radicals, and hydroxyl radicals. The superoxide and hydroxyl radicals are products of oxygen during metabolism mainly in mitochondria (Popescu and Nichol, 2011; Beal, 2005). Superoxide is not reactive by itself but it can be converted to H₂O₂ in a reaction catalyzed by superoxide dismutase. Finally, H₂O₂ is decomposed to hydroxyl radicals in the presence of iron (Fe).

In particular, the mammalian brain has a high level of metals such as copper (Cu), zinc (Zn) and (Fe) ions as compared to other tissues. These metals are required in metabolic processes as well as in the metal-dependent enzyme (Popescu and Nichol, 2011). However, reaction of Cu and Fe with molecular oxygen produces ROS. The high concentration of these metals is intrinsic in mitochondria, and in the presence of a microenvironment rich in oxygen generates H₂O₂. This reaction in mitochondria makes this organelle more susceptible to ROS production inside the cell (Popescu and Nichol, 2011; Beal, 2005). Mitochondrial dysfunction occurs during the normal process of aging as well as in neurodegeneration, probably due to an enhancement of ROS production (Beal, 2005). On the other hand, Cu and Fe, for example, have catalytic functions in important antioxidant enzymes such as superoxide dismutase. The most important ROS in biological systems is the highly reactive superoxide radical which is produced primarily in mitochondria (Popescu and Nichol, 2011; Beal, 2005). This superoxide radical can be converted by superoxide dismutase (SOD) enzyme to H₂O₂ and this can react in the presence of the reduced metals, Cu²⁺ and Fe²⁺, to generate the highly reactive hydroxyl radicals. It is important to note that H₂O₂ crosses biological membranes very easily to produce oxidative damage, a process associated with neurodegeneration.

ROS can alter the normal status of DNA nucleotides affecting their sequence, producing mutation, or modifying gene expression (Beal, 2005). Also ROS can produce protein oxidation, generating loss of sulphhydril groups and alterations of amino acids leading to nonfunctional proteins (Stadman and Levine, 2003). Lipid peroxidation (LP) caused by ROS induces damage to membranes of the cellular organelles and cell membrane (Valko et al., 2007).

In addition, nitric oxide overproduction can contribute to neuronal damage through a mechanism referred to as “nitrosative stress” (Gu et al., 2010). Nitric oxide is synthesized from L-arginine by nitric oxide synthase (NOS) isoenzymes such as neuronal NOS (nNOS), and endothelial NOS (eNOS), which are constitutive enzymes, and activated by enhancement of intracellular Ca²⁺. Another isoenzyme, inducible NOS (iNOS), is activated by endotoxic or pro-inflammatory cytokines (Zhou and Zhu, 2009). Therefore, inflammation or neuronal excitation that increase intracellular Ca²⁺ may enhance the production of nitric oxide. Superoxide radicals plus nitric oxide produce RNS, including peroxynitrite. The SOD enzyme usually competes with nitric oxide for superoxide radicals. However, where SOD is insufficient or there is overproduction of nitric oxide, the reactive peroxynitrite is formed. Therefore, nitric oxide becomes toxic when high concentrations are produced (Gu et al., 2010). However, nitric oxide has also an important role, for instance, in neurotransmission, control of mitochondrial respiration, and vascular tone, and antimicrobial activities (Knot and Bossy-Wetzel, 2010).

In addition, the brain is highly vulnerable to oxidative stress as well as related damages produced by this mechanism. This is due to a) the high brain oxygen consumption (Jomova et al., 2010), which is mainly used to produce ATP; b) low levels of endogenous antioxidants (Jomova et al., 2010; Beal, 2005);
c) affinity to accumulate metal ions particularly Fe and Cu and
d) high levels of polyunsaturated fatty acids (PUFAs) such as arachidonic acid (AA) and docosahexanoic acid (DHA). These PUFAs, the major component of the neural cell membrane, are released after brain damage. Enzymatic metabolism of AA produces proinflammatory mediators, prostaglandins, leukotrienes, and thromboxanes (Phillis et al., 2010). On the other hand, non-enzymatic metabolism of AA generates isoprostanes and 4-hydroxynonenal (4-HNE). These mediators may increase ROS production resulting in oxidative damage by LP (Phillis et al., 2010), which can lead to loss of lipid asymmetry, and to apoptosis (Volinsky and Kinnunen, 2013).

ANTIOXIDANT SYSTEMS

Physiological levels of ROS can be under control due to diverse cellular antioxidant mechanisms classified as enzymatic and non-enzymatic pathways (Gandhi and Abramov, 2012). The primary antioxidant enzymes in the brain include Cu/Zn-superoxide dismutase (SOD-1), Mn-superoxide dismutase (SOD-2), and catalase (CAT) and glutathione peroxidase (GSH-Px) (Gandhi and Abramov, 2012).

SOD enzyme has been implicated in diverse brain disorders in the elderly population. In particular, SOD-1 (cytosolic enzyme) and SOD-2 (mitochondrial enzyme) catalyze the conversion of reactive superoxide radicals to O_2 and H_2O_2 (Liochev and Fridovich, 2007) which are then decomposed to H_2O and oxygen by CAT and GSH-Px preventing hydroxyl radicals formation. However, H_2O_2 is very deleterious when reacting with free Cu^{+} or Fe^{2+} ions through Fenton reaction to produce the extremely reactive hydroxyl radical. CAT uses Fe or manganese as a cofactor and is mainly located in peroxisomes of brain cells. GSH-Px is in the cytosol and the mitochondria.

The non-enzymatic antioxidant defense consists of molecules that react with ROS as scavengers or chelating agents resulting in free radical chain reactions. These molecules are composed of either metabolic or dietary sources-based antioxidants. Metabolic antioxidants include glutathione, lipoic acid, L-arginine, and transferrin, which are produced during metabolism in the cell. Dietary sources-based antioxidants are exogenous sources of antioxidants such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), carotenoids, flavonoids, and polyphenols. In particular, the central nervous system has a high concentration of vitamin C (Harrison and May, 2009).

EGb 761 AND ANTIOXIDANT EFFECTS

Oxidative stress has been described as a relevant mechanism in the pathogenesis of neurodegenerative diseases including Alzheimer's and Parkinson's diseases, and will be described below. Therefore, the use of antioxidants is an alternative therapy for these health conditions. It is suggested that diverse natural antioxidants have no severe side effects. However, several natural antioxidants are available as dietary supplements, and their safety and effect is not regulated and could produce serious interactions with prescribed medications for health conditions. Another important challenge is to know the pharmacokinetic (brain bioavailability and frequency of administration) and pharmacodynamics (therapeutic index and onset of action) of the antioxidant(s).

EGb 761 is a very well characterized phytomedicine that has diverse properties including its potent antioxidant effect. EGb 761 scavenges ROS, including hydroxyl radicals, peroxyl radicals, superoxide radicals, nitric oxide radicals, and H_2O_2 and ferryl ion species (Maitra et al., 1995; Ni et al., 1996; Ahlemeyer and Krieglstein, 2003). The effect of producing direct ROS attenuation by EGb 761 may also stabilize the cellular redox state by upregulation of antioxidant enzymes activity as well as their proteins (Ahlemeyer and Krieglstein, 2003). In this context, this extract is also able to enhance the antioxidant enzyme activities (SOD, GSH-Px, CAT and/or heme-oxygenase-1), which promotes its antioxidant effect (Mahadevan and Park, 2008). The flavonoid fraction of EGb 761 could have antioxidant effects acting directly on ROS (Smith and Luo, 2003) increasing expression of SOD and glutathione metabolites (Gohil and Packer, 2002). The bilobalide compounds of the terpenoid fraction also enhance antioxidant enzyme activities such as CAT and SOD (Mahadevan and Park, 2008) although there is a debate as to their antioxidant activity. The flavonoids in the extract are probably more efficient in producing a scavenging activity of hydroxyl radical than the terpenoid fraction (Zimmermann et al., 2002). In particular, flavonoids in the extract are scavengers of hydroxyl and superoxide free radicals (Gardes-Albert et al., 1990), and the terpenoid fraction, excluding ginkgolide A, are more efficient scavenging superoxide free radicals (Scholtysek et al., 1997). However, there is a controversy whether bilobalides and ginkgolides B, C and J have superoxide scavenging properties (Scholtysek et al., 1997; Pietri et al., 1997). This problem could be related to the experimental model used to produce oxidative stress producing diverse results. The basic structure of flavonoids includes an aromatic ring and a double bond, which produces directly hydroxyl radical scavenging (Zimmermann et al., 2002). As well as phenolic hydroxyl groups of this flavonoid fraction have the ability to chelate metal ions related to production of free radicals (Gohil and Packer, 2002), and avoiding hydroxyl radical formation (Zimmermann et al., 2002). It has been recognized that proanthocyanidins content in EGb 761 extract can inactivate antioxidant enzymes (GSH-Px, lactate dehydrogenase, and CAT) (Pietri et al., 1997). In addition, it has been reported that EGb 761 inhibits superoxide radical production in human postmortem brain tissue, although there was a reduction of SOD. (Gsell et al., 1995) Other oxidative stress markers showed that EGb 761 is able to produce neuroprotection against LP due to its interaction and penetration to lipid bilayer of membranes (Saia et al., 1995). EGb 761 reduces monoamine oxidase B (MAO-B) activity, which is also a source of ROS (Pardon et al., 2000), as well
as regulates expression of mitochondrial enzymes that can induce ROS production in mitochondria (Mahadevan and Park, 2008; Gohil and Packer, 2002). It is important to note that EGb 761 also produces enhancement of PUFAs, such as omega 3 and omega 6 species, which are known to improve membrane fluidity as well as act against oxidative damage (Drieu et al., 2000). Omega-3 fatty acids in the brain play an important role in neuronal membrane plasticity, neurogenesis, synaptogenesis, learning ability, and cognitive development (Crupi et al., 2013). With respect to regulation of gene expression, the preventive and therapeutic actions of EGb 761 probably involve modification of the expression of genes. The capacity of EGb 761 to affect gene expression is a novel mechanistic proposal of its biological activity (Gohil and Packer, 2002; Smith and Luo, 2004). Diverse studies using microarray assays support the neuroprotective effects of EGb 761. This suggest that EGb 761 may modulate the expression of genes involved in diverse biological processes including cell proliferation, differentiation, apoptosis, and the regulation of genes associated with resistance to stress and antioxidant protection (Smith and Luo, 2004).

EGb 761 could increase the resistance of neurons to oxidative stress-induced injury by the induction of the antioxidant enzyme heme oxygenase-1 (Shah et al., 2011). The upregulation of g-glutamyl-cysteiny1 synthetase by EGb 761 is also associated with the increased expression of the gene encoding for GSH-Px, during aging and in AD. In addition to its direct action as a NO-scavenger, the bioactive free radical, EGb 761 can decrease the expression of the gene encoding for iNOS. This property of EGb 761 is particularly interesting as NO is involved in neuroinflammation (Kotakadi et al., 2008). Further, EGb 761 modulates genes related to other antioxidant enzymes, for example, increasing SOD-1 mRNA, and decreasing glutathione reductase mRNA (Gohil and Packer, 2002; Smith and Luo, 2004).

These data suggest that EGb 761, in addition to its free radical scavenger property, is also able to modulate the transcription of several genes related to the oxidative stress response. This effect can increase cellular tolerance to oxidative stress and thereby could prevent or protect against oxidative damage occurring in some neurodegenerative diseases (Gohil and Packer, 2002).

OXIDATIVE STRESS IN NEURODEGENERATIVE DISEASES

Oxidative stress in Alzheimer’s disease

Alzheimer’s disease (AD) is a neurodegenerative disorder affecting ~10% of elderly adults (60-65 years old) worldwide and its prevalence probably will increase to 1 in 85 by 2050 (Prince et al., 2013). This disease is produced by neurodegeneration of two important brain regions such as hippocampus and neocortical regions producing impairment in cognitive abilities (Prince et al., 2013). AD has two important hallmarks, aggregation of extracellular amyloid β-peptide (Aβ)-rich senile plaques, which results from the proteolysis of the amyloid precursor protein (APP); and the accumulation of intracellular neurofibrillary tangles (NFT), which consist of an aggregated form of hyperphosphorylation of Tau protein.

Oxidative stress associated with enhancement of ROS and RNS production play an important role in the progression of AD (Chen and Zhong, 2014) because it promotes Aβ deposition and Tau hyperphosphorylation leading to synapse loss and neuronal death. Oxidative stress markers are increased in AD brain which include i) LP: elevated levels of malondialdehyde (MDA) and 4-HNE, two major products of LP have been reported in AD brain and AD animal models (Chen and Zhong, 2014; Zhao and Zhao, 2013); ii) protein oxidation: enhancement of protein carbonyl groups, an oxidative marker of proteins, has been reported in AD brain (Bitterfield and Kanski, 2001); iii) nucleic acid oxidation: 8-hydroxy-2′-deoxyguanosine (8-OHdG), a marker of oxidative stress to DNA, is increased in mitochondrial DNA from cortex of AD patients, as well as oxidative modification to RNA which is increased in AD brain (Numomura et al., 1999).

Reduced antioxidant defense is another important feature of AD, for example SOD-1 activity is reduced in postmortem AD brain as well as in AD animal models (Chen and Zhong, 2014; Zhao and Zhao, 2013). Animal models expressing a mutation which produces familial AD in humans causes enhancement of ROS production as well as reduction of SOD-1 activity (Leutner et al., 2000). It has been reported that reduction of SOD enzymes correlates to an increase of oxidative stress markers such as MDA in mild cognitive impairment and AD patients (Torres et al., 2011). However, controversial results showed that SOD levels are higher in AD patients (Doecke et al., 2011). This debate could be related to the biological sample use for the study, SOD activity and SOD protein, for example.

Glutathione (GSH) is a highly abundant cellular antioxidant but also can exist as oxidized glutathione disulfide (GSSG), ratio of GSH/GSSG is a marker of oxidative stress in vivo. A reduced GSH/GSSG ratio has been reported in erythrocytes of AD patients as compared to controls (Torres et al., 2011; Bermejo et al., 2008), which has been correlated with cognitive performance. For example, a reduced GSH/GSSG ratio in mild cognitive impairment patients (Bermejo et al., 2008) is also associated with an increase of LP markers (Torres et al., 2011) suggesting it as an early event in AD progression. However GSH-Px activity in AD patients is inconsistent, reporting enhancement (Torres et al., 2011) or reduction (Padurariu et al., 2010).

Oxidative stress in AD is promoted by diverse mechanisms including a) metal accumulation; b) mitochondrial dysfunction; c) inflammation; d) hyperphosphorylated Tau; e) Aβ accumulation; and f) apolipoprotein E (apo E). a) Metal accumulation: abnormal levels of Cu, Zn and Fe in hippocampus and amygdala have been reported in AD...
patients (Deibel et al., 1996). These metals could interact with Aβ to produce oxidative stress, for example when Aβ binds Cu or Fe, producing ROS. Therefore, metal chelators are able to reduce Aβ levels, resulting in the prevention of its aggregation (Greenough et al., 2013); b) mitochondrial dysfunction has been found in hippocampus of AD patients (Silva et al., 2012) and this organelle is the main site of ROS production (Beal, 2005; Gandhi and Abramov, 2012). Cytochrome oxidase is a key enzyme in the electron transport and its reduction produces enhancement of ROS and reduction of energy stores in AD (Chen and Zhong, 2014; Zhao and Zhao, 2013); c) inflammation, when it is produced in microglia and astrocytes generates ROS (Tuppo and Arias, 2005). Aβ attracts and activates microglia, creating clusters around Aβ deposits in the brain (Tuppo and Arias, 2005); d) hyperphosphorylated Tau: Tau has been implicated in neurodegeneration associated with oxidative stress in AD. For example, SOD overexpression or antioxidant treatments such as vitamin E reduces the neuronal death provoked by Tau (Dias-Santagata et al., 2007); e) Aβ accumulation: Aβ-induced oxidative stress is also another important hypothesis to explain the mechanisms of damage related to AD. For example, Aβ alters the electron transport chain (Wang et al., 2008) increasing ROS in Tg 2576 mice, prior to Aβ plaques formation (Manczak et al., 2006). Aβ has been related for its impact in oxidative phosphorylation and ROS production generating reduction in mitochondrial membrane potential, cytochrome c oxidase activity, and ATP production (Chen and Zhong, 2014; Zhao and Zhao, 2013). AD transgenic mouse models related to APP have been shown to increase ROS and nitric oxide production, oxidation of lipids and proteins which have been related to Aβ accumulation, suggesting that oxidative stress promotes Aβ (Manczak et al., 2006; Apelt et al., 2004). f) The apolipoprotein E (Apo E) has been linked to oxidative stress in AD. In particular, Apo E is the major cholesterol carrier in the brain that supports lipid transport, also playing an important role in neuroplasticity. It has been reported that Apo E has the risk to be attacked by free radicals and has free radical scavenger action, but this effect is isoform-dependent (Leininger-Muller et al., 1998). It is reported that E4 isoform is more sensitive to these mechanisms than E3 and E2 isoforms. In particular, E4 allele of Apo E is related to late-onset of sporadic and family forms of AD, but E2 allele is likely to produce protection. It has been suggested that Apo E produces neuronal protection against free radicals, however the Apo E isoforms 2 and 3 are more effective than isoform 4 (Miyata and Smith, 1996). LP in postmortem samples of AD patients showed that this event is dependent on Apo E genotype, and LP was higher when allele E4 was present (Ramassamy et al., 1998). These findings suggest that LP level of AD subjects is inversely proportional to Apo E content (Ramassamy et al., 1998), and that Apo E has a protective effect against oxidative stress.

**EGb 761 and AD**

The role of oxidative stress in AD pathogenesis suggests that the therapeutic use of free radical scavengers and antioxidants could be of potential benefit in the treatment of the disease (Figure 1). It is supported because it has been suggested that diverse natural antioxidants and/or free radical scavengers have no major side effects (e.g. vitamin E, vitamin C, curcumin, carnitines, EGb 761) and have been used in preclinical and clinical studies of AD with promising results. Therefore the use of antioxidants as an alternative in slowing down the progression of AD point out that oxidative damage might play a role in the cognitive and functional reduction seen in AD. However, several antioxidants, including vitamin C and E, are a therapeutic alternative to reduce symptoms, but not neurodegeneration.

EGb 761 exerts neuroprotective/antioxidant effect both in vitro and in vivo models of AD. The following findings support EGb 761’s beneficial action. This defined natural extract reduces LP in brains of AD subjects (Ramassamy et al., 1998). It prevents toxicity, aggregation, and diffusible ligands of Aβ in vitro, a hallmark of AD that produces oxidative stress (Yao et al., 2001). The extract is also able to attenuate the levels of H2O2-related ROS in transgenic nematodes (Caenorhabditis elegans) expressing human Aβ and neuroblastoma N2a cells expressing Aβ, both of which are AD models (Smith and Luo, 2003).

It has also been suggested that mitochondrial dysfunction plays an important role in pathological mechanisms of AD (Silva et al., 2012). The mitochondrial respiratory chain is the main source of ROS and a target for antioxidant/protective effects in this organelle. For example, EGb 761 prevented mitochondrial dysfunction induced by Aβ, probably by reducing ROS production (Shi et al., 2009). It also restores mitochondrial function and reduces ROS production in cells overexpressing APP (Rhein et al., 2010). In addition, it is reported that EGb 761 prevents neuronal death and dysfunction induced by Aβ deposits (Bastianetto and Quiron, 2002) and induces α-secretase activity, possibly by reducing Aβ formation (Colciaghi et al., 2004). Apo E, is a major risk factor for developing AD. It has a free radical scavenger action and plays an important role during neurodegeneration in AD. EGb 761 induces ApoE and prevents its oxidation in an isoform-dependent way, this effect is more clearly seen with Apo E4 (Ramassamy et al., 1998; Ramassamy et al., 1999).

Other preclinical studies support the benefit of EGb 761 in AD models. For example, the Tg2576 transgenic mouse overexpressing human APP (isoform 695, “Swedish mutation”), is able to develop memory deficits and plaques (Hsiao et al., 1996). However, treatment with EGb 761 attenuated learning and memory deficits before plaques formation (eight months of age) and when plaques are well established at 14 months of age (Stackman et al., 2003). Additionally, treatment until 16 months with EGb 761 in these transgenic mice reduced human APP levels (Augustin et al., 2009). These findings are relevant since it is suggested that oxidative stress may contribute to the Aβ-induced learning and memory deficits in mice and this might occur in AD patients. In line with these
findings EGB 761 also protects the hippocampus, a key brain region in memory and affected in AD (Rocher et al., 2011). Additionally, the natural extract facilitates synaptic plasticity in the hippocampal CA1 region that is related to spatial learning and memory (Wang et al., 2006).

The antioxidant effect of EGB 761 in AD patients has not been directly measured. However, biological markers mainly in preclinical studies, as described above, suggest its role as an antioxidant agent. It also seems that EGB 761 interferes with pathomechanisms relevant to dementia in AD which are oxidative stress-related mechanisms, such as Aβ aggregation, Apo E induction, and mitochondrial dysfunction. Indeed, oxidative stress is promoted by Aβ accumulation and this could contribute to learning and memory deficits as seen in AD patients. The administration of EGB 761 reduces learning and memory deficits before Aβ plaques formation, suggesting that this mechanism could function in AD patients treated with EGB 761, who showed improvement in cognition parameters. In particular, the inhibition of Aβ-induced neurotoxicity as well as the prevention of cell death produced by EGB 761 can control the neurodegeneration (Bastianetto and Quirion, 2002). EGB 761 is also able to upregulate the expression of Cyclic AMP response element-binding protein (CREB) which is altered in AD (Xu et al., 2007).

Dementia is a feature of AD and characterized by progressive deterioration in cognitive ability. Cognitive impairment symptoms in dementia affect notably the memory, executive function, attention, and the formation of new memories, for example. The use of EGB 761 in clinical studies, in particular with dementia, has shown improvement of dementia symptoms when compared to placebo-controlled trials (Maurer et al., 1997; Le Bars et al., 2000; Kanowski and Hoerr, 2003). These studies were performed with different doses of EGB 761 (120 mg and 240 mg) as well as different treatment times (12-52 weeks).

It is suggested that EGB 761 (240 mg/day) used to treat mild to moderate dementia in the named GINDEM-NP study group and the GOTADAY study improved cognitive function (Napryevenko and Borzenko, 2007; Ihl et al., 2011). In addition, other studies have confirmed that EGB 761 at a daily dose of 240 mg is safe and effective in the treatment of dementia (Schneider et al., 2005; Kasper and Schubert, 2009; Weinmann et al., 2010; Ihl, 2012).

Meta-analysis studies showed that EGB 761 reduced progression of dementia when evaluating clinical dementia ratings and improvement in attention and memory (Kasper and Schubert, 2009; Weinmann et al., 2010; Ihl, 2012). However, previous studies produced controversial results on the clinical efficacy of EGB 761 in dementia. These differences are related, in part, to the dose of EGB 761 used such as 120 mg or 160 mg in patients with dementia who did not show improvement in cognition abilities (Schneider et al., 2005; Kasper and Schubert, 2009; Weinmann et al., 2010; Ihl, 2012; van Dongen et al., 2003). At the present time support for the clinical use of EGB 761 is due to its safety, tolerability, and efficacy at 240 mg/day as reported in many studies (Schneider et al., 2005; Kasper and Schubert, 2009; Weinmann et al., 2010; Ihl, 2012).

These inconsistent results could be related to the age, the degree of cognitive impairment, differences in dose administration, pharmaceutical form, ethnicity, environmental factors, genetic background, as well as patients with particular characteristics (Sarris et al., 2012). Elderly people often use various medicines at the same time and EGB 761 could interact with other drugs, reducing or increasing its therapeutic properties. For example, vitamin D shows different therapeutic effects according to gender, ethnicity, hormone levels and xenobiotic-metabolizing enzymes genotypes (Correale et al., 2010).

The comparison between EGB 761 versus other antidementia drugs (memantine, galantamine) shows that this natural extract is as effective as other prescribed medication. Indeed a review of 30 manuscripts distinguished the significant effect of EGB 761 in particular related to cognitive function in AD (Montes et al., 2015). It is important to note that preclinical studies have shown that oxidative stress is related to memory deficits (Chen and Zhong, 2014). In general, the use of antioxidants from the pre-clinical stage to clinical settings have difficulties mainly regarding the pharmacokinetics and pharmacodynamics. Also life style and environmental factors differ in animal studies and AD patients. Furthermore, AD could coexist with other central nervous system pathologies, which could alter the drug efficacy and dose efficacy. It is also important that clinical trials start with the antioxidant therapy at the right time since normally this occurs at a very late stage. Therefore, EGB 761 is a promising nutraceutical agent to be explored as a potential antioxidant in AD (Figure 1). Further studies with clinical trials are needed to support its antioxidant/neuroprotective role.

**Oxidative stress in Parkinson’s disease**

“Parkinson’s disease (PD) is the second most common neurodegenerative disorder after AD, affecting approximately 2% of the population worldwide over the age of 65” (Willis, 2013). The disease is characterized by motor deficits such as bradykinesia, resting tremor, rigidity, and posture instability. The clinical signs appear after 50-70% of dopaminergic neurons of the substantia nigra are lost leading to reduced dopamine (DA) levels (~80%) in striatum, which is a brain region crucial in voluntary motor functions (Braak et al., 2003). In addition to the motor symptoms, non-motor symptoms such as cognitive impairment, depression, sleep alterations, and sensory deficits are present in PD patients (Torodova et al., 2014).

The causal factor(s) that produce degeneration and death of dopaminergic neurons of the nigrostriatal pathway in PD is unclear. The evidence supports the idea that oxidative stress induced by free radical production, and the generation of ROS plays a major role in neuronal loss in PD via diverse mechanisms (Dias et al., 2013).

Oxidative damage to proteins, lipids and nucleic acids has
been suggested in human postmortem brain from patients with PD (Dias et al., 2013; Jenner and Olanow, 1996; Yoritaka et al., 1996; Alam et al., 1997). Oxidative damage occurring in lipids of PD brains has shown significant enhancement of 4-HNE, MDA, lipid hydroperoxides which are LP products (Dias et al., 2013; Yoritaka et al., 1996). In addition, the concentration of PUFAs is reduced in PD brains (Dias et al., 2013). Furthermore, protein and DNA oxidation produced by ROS, has been reported after finding increased levels of protein carbonyls and 8-hydroxydeoxyguanosine, respectively, in postmortem samples from PD patients (Alam et al., 1997). Deletions in mitochondrial DNA, which could be produced by oxidative stress mechanisms, are found in PD brains (Reale et al., 2012).

The important antioxidant enzyme SOD-2, highly inducible in response to an excess of ROS, has an elevated activity in substantia nigra of PD patients (Radunović et al., 1997), with the possibility to respond to enhancement of ROS levels. The reduced glutathione (GSH), important antioxidant enzyme that catalyzes the reduction of ROS is reduced in PD brains (Dias et al., 2013). In addition, GSH facilitates ROS reduction when forming complexes, for example with GSH-Px (Smyne and Smyne, 2013).

The use of animal models of PD that reproduce motor aspects of the disease strongly supports that oxidative stress mechanism is related to dopaminergic neurons neurodegeneration. For example, 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, paraquat, and 6-hydroxydopamine (6-OHDA) are commonly used animal models of PD which produce oxidative stress (Abdulwahid and Ahmad, 2010).

The oxidative stress in PD can be promoted by diverse mechanisms including: a) dysfunction of DA metabolism, b) neuroinflammation, c) metal ion dyshomeostasis, d) mitochondrial dysfunction (Hwang, 2013). a) The loss of dopaminergic neurons in PD results in increased of DA metabolism. However the high oxidation of this neurotransmitter by MAO-B in PD involves ROS production and free radical formation. In particular, MAO-B activity is elevated in these patients (Dias et al., 2013) and MAO-B inhibitors produce neuroprotection in this disease (Teo and Ho, 2013). The products of DA metabolism through MABO-B activity, produce H$_2$O$_2$, a highly reactive molecule. As well as DA autoxidation, due to its unstable properties, produce DA quinones and free radicals. b) Neuroinflammation contributes to the pathophysiology of PD (More et al., 2013). In particular, substantia nigra pars compacta (SNpc) has a higher density of microglia than other brain regions. Microglia are phagocytic cells in the brain that are activated during injury or immune challenge (More et al., 2013). Activated microglia produce diverse proinflammatory mediators related to oxidative stress such as ROS and nitric oxide, plus of a variety of cytokines. Therefore, this mechanism can contribute to promoting neurodegeneration in PD (More et al., 2013). Activated microglia and presence of iNOS have been reported in substantia nigra of PD patients (Hirsch et al., 2003). Moreover, activated microglia have also been reported in diverse animal models of Parkinson's disease, using MPTP, rotenone or 6-OHDA (More et al., 2013; McNaught and Jenner, 1999). c) metal ion dyshomeostasis has been shown in PD patients after reporting enhancement of Fe and Zn, as well as a reduction of Cu (Jomova et al., 2010; Oakley et al., 2007). The metal ion imbalance could have the potential to increase ROS production via Haber-Weiss and Fenton reactions and exerting oxidative stress. In particular, the substantia nigra has the most elevated content of Fe in the brain, but in PD brain the Fe has a more elevated content in that region (Jomova et al., 2010). The parkinsonism can be exerted after infusion of Fe in the brain of rats (Sengstock et al., 1993), and metal chelator prevents neurodegeneration in PD models (Kaur et al., 2003). This suggests that Fe may play an important role in PD neurodegeneration. d) mitochondrial dysfunction: mitochondria produces ROS and are also a target of these oxygen species. Mitochondrial impairment is an important factor in cellular damage and neurodegeneration of PD patients (Reale et al., 2012). The mitochondrial electron transport chain is the major source of ROS producing superoxide radical that can be converted to H$_2$O$_2$, a potential compound to generate hydroxyl radical, through SOD enzyme. Mitochondrial complex I activity is reduced in platelets and in substantia nigra of PD patients (Hanagasi et al., 2005), and this may contribute to and result in neurodegeneration. Mitochondrial dysfunction in complex I has been reported in toxins used as a model of PD, for example MPTP and rotenone.

**EGB 761 and PD**

L-3,4-dihydroxyphenylalanine (L-DOPA) routinely treats PD. However, this medication promotes oxidative stress due to autooxidation products as well as the enhancement of DA content and turnover (Müller, 2011). In particular, this drug produces some severe adverse effects, which prompts the need to search for new therapeutic drugs to combat the oxidative stress using antioxidant agents.

The antioxidant therapies have been evaluated in preclinical models or clinical studies with controversial results. The human clinical trials conducted specifically with the antioxidant vitamin E in PD are the most studied. The well-known DATATOP (Deprenyl and Tocopherol Antioxidant Therapy of Parkinsonism) study showed no benefits after treatment with vitamin E to patients suffering from early PD, that where confirmed in other clinical trials (Miklya et al., 2003). This could be due to vitamin E having limited transport across the blood-brain barrier (Gilgun-Sherki et al., 2001). Coenzyme Q10 trials have shown moderate results in PD but controversy is related to its antioxidant effect (Weber and Ernst, 2006). Therefore, it is relevant to explore other potential direct antioxidants, which interfere with formed free radicals and can cross the blood-brain barrier. EGb 761 is a strong possibility for these aspects because of its free radical scavenger properties; it enhances the antioxidant enzymes activities, and has compounds of low molecular weight which allows it to
cross the blood-brain barrier (DeFeudis and Drieu, 2000).

The antioxidant effect of EGb 761 has been explored in some models of PD such as 6-OHDA and MPTP which produce oxidative stress (Rojas et al., 2008; Ahmad et al., 2005). The use of EGb (crude preparation), containing the main compounds of EGb 761 (terpenoid and flavonoids), in 6-OHDA, an animal model of PD, showed that diverse oxidative stress indexes are reduced after EGb administration. These indexes show that this G. biloba preparation produces a reduction of LP and restoring GSH content in a dose-dependent manner in 6-OHDA neurotoxicity. EGb also restores the activities of antioxidant enzymes such as glutathione-S-transferase (GST), glutathione reductase (GR), GSH-Px, CAT, and SOD (Ahmad et al., 2005) in a dose-dependent pattern in this model of PD. Other authors have also reported that EGb (flavonoids 30% and terpenes 8% as major compounds) reduced LP (a marker of oxidative stress) and enhancement of SOD after oxidative stress induced by MPTP neurotoxicity (Yang et al., 2001). The neuroprotective effect specifically of EGb 761 in MPTP neurotoxicity has also been related to preventing oxidative stress (Rojas et al., 2008). A particular study reported that EGb 761 administration started after the last MPTP administration blocked LP and reduced the superoxide radical production (Rojas et al., 2008). As well as EGb 761 pretreatment followed by 1-methyl-4-phenylpyridinium (MPP+), the active metabolite of MPTP, also blocked LP (Rojas et al., 2001). MAO activity is an important enzyme in DA metabolism as well as producing ROS leading to oxidative stress. The EGb 761 neuroprotective effect is related to reducing MAO activity in the MPP+/MPTP neurotoxicity (Rojas et al., 2004; Wu and Zhu, 1999).

Metals play an important role in brain function, for example, manganese and Cu are required as cofactors of important antioxidant enzymes including SOD, that catalyzes ROS metabolism (Flynn and Melov, 2013). It is also reported that changes in these metals have been related to PD (Jomova et al., 2010). The reduction of these metals in MPTP/MPP neurotoxicity was attenuated after EGb 761 administration (Rojas et al., 2009; Rojas et al., 2014). It is suggested that EGb 761 is able to regulate manganese and Cu homeostasis in the brain after MPTP/MPP neurotoxicity.

However, there are no reports of clinical trials that explore the neuroprotective and/or antioxidant action of EGb 761 in PD. A case report of a patient suffering from PD showing the typical impairment of motor abilities, the typical medication named Sinemet (carbidopa-L-DOPA), had only little symptomatic relief, while tremors were reduced by 80-90% after taking EGb 761 and multivitamin-multimineral supplement (containing beta, carotene, vitamins C, D, E, B1, B2 B3, B12, folic acid, calcium, selenium, copper, manganese, and other compounds). It is important to note that this G. biloba extract has antioxidant and free radical scavenger properties, and L-DOPA is able to generate ROS inducing oxidative stress. Therefore, it is suggested that improvement of tremors in this PD patient was related to the antioxidant action of EGb 761 and/or multivitamin-multimineral supplement (Figure 2). However, clinical trials are needed to confirm these findings.

CONCLUSIONS

The use of phytomedicines and phytonutrients to treat diverse health conditions is expanding worldwide (WHO, 2004). However, the concept that herbal remedies are safe without of side effects is untrue. Herbal products are able to produce serious adverse effects leading to serious health conditions (Ernst, 2002). In most countries that herbal products are introduced without safety test, toxicological analysis, quality standards and are available on the market without prescription (Chan, 2003).

In recent years, science and technology have improved to produce a phytomedicine with quality, efficacy, and safety. One of the best examples is the EGb 761 extract, a phytomedicine that has a very well known composition and pharmacokinetics, low side effects, and has been introduced in medical practice to treat diverse neuropathologies. It should be noted that greater side effects have been reported for some non-standardized extracts of G. biloba, but these are totally different to EGb 761.

On the other hand, current treatments for AD or PD are mainly directed at attenuating the particular symptoms of each disease. Nonetheless, the similarities in neuronal damage mechanisms in both diseases, such as oxidative stress, suggest the use of potent antioxidants such as the EGb 761 to prevent or decrease the progression of these diseases (Figure 3). Therefore, EGb 761 alone could be a potential treatment in common to both neurodegenerative diseases, or used in combination with specific treatments.

The clinical trials that have been performed with EGb 761 are mainly on AD and there is only one case report for PD. In these clinical studies no indexes of oxidative stress were measured. Further clinical trials are needed to explore the potential clinical use of EGb 761 in the treatment of patients suffering from these health conditions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

References


Antioxidant effect of EGB 761


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