Planta Medica Thieme

Neuroprotective potential of Ginkgo biloba in retinal diseases

Journal:	*Planta Medica*
Manuscript ID	PLAMED-2019-03-0258-REV.R1
Manuscript Type:	Reviews
Date Submitted by the Author:	n/a
Complete List of Authors:	Martínez-Solís, Isabel; Universidad CEU Cardenal Herrera, Farmacia; Universidad CEU Cardenal Herrera, Biomedical Sciences Institute; Universitat de Valencia, Botanical Garden Acero, Nuria; Universidad CEU San Pablo, CC Farmacéuticas y de la Salud Bosch-Morell, Francisco; Universidad CEU Cardenal Herrera, Biomedical Sciences Institute; Universidad CEU Cardenal Herrera, Department of Biomedical Sciences, Faculty of Health Sciences Castillo, Encarna; Universidad CEU Cardenal Herrera, Department of Pharmacy, Faculty of Health Sciences González-Rosende, Maria Eugenia; Universidad CEU Cardenal Herrera, Department of Pharmacy, Faculty of Health Sciences Dolores, Muñoz - Mingarro; Univ. CEU San Pablo, Chemistry Ortega, Teresa; Pharmacy College, Pharmacology; Sanahuja, Maria Amparo; Universidad CEU Cardenal Herrera, Department of Pharmacy, Faculty of Health Sciences Villagrasa, Victoria; Universidad CEU Cardenal Herrera, Department of Pharmacy, Faculty of Health Sciences
Keywords:	neurodegeneration, Ginkgo biloba, retinal diseases, age-related macular degeneration, diabetic retinopathy, glaucoma, ischaemic retinal disease

Neuroprotective potential of *Ginkgo biloba* in retinal diseases

Isabel Martínez-Solís[1,2,3], Nuria Acero[4], Francisco Bosch-Morell[1,5], Encarna Castillo[2], María Eugenia González-Rosende[2], Dolores Muñoz-Mingarro[6], Teresa Ortega[7], María Amparo Sanahuja[2], Victoria Villagrasa[2]

1. Biomedical Sciences Institute. Universidad Cardenal Herrera-CEU, CEU Universities. Alfara del Patriarca, Valencia (Spain).
2. Department of Pharmacy, Faculty of Health Sciences. Universidad Cardenal Herrera-CEU, CEU Universities. Alfara del Patriarca, Valencia (Spain).
3. Botanical Garden. University of Valencia, Valencia (Spain).
4. Department of Pharmaceutical and Health Sciences, Faculty of Pharmacy. Universidad San Pablo-CEU, CEU Universities, Madrid (Spain).
5. Department of Biomedical Sciences, Faculty of Health Sciences. Universidad Cardenal Herrera-CEU, CEU Universities. Alfara del Patriarca, Valencia (Spain).
6. Department of Chemistry and Biochemistry, Faculty of Pharmacy. Universidad San Pablo-CEU, CEU Universities, Madrid (Spain).
7. Department of Pharmacology, Pharmacognosy and Botany. Universidad Complutense de Madrid, Madrid (Spain).

Correspondence: Prof. Isabel Martínez-Solís. Department of Pharmacy, Faculty of Health Sciences. Universidad Cardenal Herrera-CEU, CEU Universities. C/ Ramón y Cajal s/n. 46115 Alfara del Patriarca, Valencia (Spain). Telf. +34 96 136 90 00 | Ext. 64329. E-mail: isolis@uchceu.es

Abstract

Like other tissues of the central nervous system, the retina is susceptible to damage by oxidative processes that result in several neurodegenerative disease such as age-related macular degeneration, diabetic retinopathy, glaucoma, ischaemic retinal disease, retinal disease produced by light oxidation and detached retina, among other diseases. The use of antioxidant substances is a solution to some health problems caused by oxidative stress, because regulate redox homeostasis and reduce oxidative stress. This is important for the neurodegeneration linked to oxidation processes. In line with this, *Ginkgo biloba* is a medicinal plant with excellent antioxidant properties whose effects have been demonstrated in several degenerative processes, including retinal diseases associated with neurodegeneration. This review describes the current literature on the role of ginkgo in retinal diseases associated with neurodegeneration. The background leads to the conclusion that *G. biloba* extracts might be a good option to improve certain neurodegenerative retinal diseases but more research is needed to determine the safety and efficacy of *G. biloba* in these retinal degenerative processes.

Key words: *Ginkgo biloba*, Ginkgoaceae, neurodegeneration, retinal diseases, age-related macular degeneration, diabetic retinopathy, glaucoma, ischaemic retinal disease

Abbreviations

ARMD: age-related macular degeneration

BD: Behcet's disease

CNV: choroidal neovascularisation

DR: diabetic retinopathy

GBE: *Ginkgo biloba* extracts

NTG: normal-tension glaucoma

IOP: intraocular pressure

NO: nitric oxide

NOX: NADPH oxidase

POAG: primary open-angle glaucoma

PUFAs: polyunsaturated fatty acids

RCT: randomised controlled trials

RD: retinal detachment

ROS: reactive oxygen species

RP: retinitis pigmentosa

RPE: retinal pigment epithelium

TM: trabecular meshwork

VEGF: vascular endothelial growth factors

Introduction

The degeneration of retinal ganglion cells is involved in several optic neuropathies. These cells are neuronal retinal cells that project their axons to the brain by forming the optic nerve [1]. The long axons of these cells make them more vulnerable to hypoxia, exposure to free radicals, mechanical compression or photo-oxidative damage [2]. Some retinal diseases are directly related to oxidative stress, especially in aging eyes [3]. Like other neuronal cells, retinal ganglion cells have poor antioxidant capacities and insufficient nucleic acid repair mechanisms (mainly in mitochondria) [4]. Reactive oxygen species (ROS) are involved in the progression of retina disorders, including aging, apoptosis and post-ischaemic cellular injuries [5]. Glaucoma [6, 7], diabetic retinopathy (DR) [8-10], age-related macular degeneration (ARMD) [11, 12], ischaemic retinal injuries [13] are examples of ROS and retina disease relationship. In addition, excessive ROS generation and the consequent induction of oxidative stress are some factors that trigger the cellular response to retinal detachment (RD), and are also a major cytotoxic factor for photoreceptor apoptosis [14]. Indeed antioxidants protect against oxidative stress, prevent ROS production and they should act as neuroprotectants with therapeutic options for retinal diseases.

Ginkgo biloba L. (Ginkgoaceae), commonly known as ginkgo, is a living fossil as it is one of the most ancient living trees that has existed for more than 250 million years. The therapeutic benefits of ginkgo leaf extracts have long since been well-known, proven by the fact that they were used in traditional Chinese medicine 5000 years ago [15]. Dr. Willmar Schwabe introduced ginkgo leaf extract as medication in 1965 [16]. Many types of *G. biloba* extracts (GBE) can be found on the market nowadays, but preclinical and clinical studies have been performed mainly using EGb 761 and LI 1370 [17], especially the former. EGb 761 is one of the best characterised herbal extracts containing two major groups of active principles: flavonoids (24 %) and terpene lactones (6%). Quercetin, kaempferol, iso-rhamnetin, myricetin, laricitrin, mearnsetin and apigenin glycosides have been identified as the main flavonoids, together with biflavonoids like ginkgetin, isoginkgetin [18]. Ginkgolides A, B, C and J (diterpene lactones) and bilobalide (sesquiterpene lactones) are the most important components of the terpene fraction [19]. Another important aspect of this extract is that ginkgolic acids appear in concentrations below 0.0005%. Ginkgolic acids exert allergenic and genotoxic effects, with neurotoxic results. It has been proven that ginkgolic acids-induced death is mediated by both apoptosis and necrosis [20]. In fact due to low concentrations of this type of compounds in EGb 761, toxic effects in humans are not expected. EGb 761 and other ginkgo leaf extracts have been widely used to treat and prevent several disorders like Alzheimer´s disease, dementia, multiple sclerosis, asthma, vertigo, fatigue, tinnitus or circulatory problems [21-23]. The neuroprotective effect of this plant has been demonstrated both *in vitro* and *in vivo*. The

terpenoid and flavonoid fractions of GBE protect neurons against necrosis and apoptosis induced by ROS, Ca^{2+} overload, nitric oxide (NO), or β-amyloid induced toxicity [16]. The antioxidant capacity of the extract can be explained by its ability to act as a free radical scavenger of nearly all ROS types, and can inhibit lipid peroxidation. Furthermore, antioxidant properties of ginkgo are particularly interesting given the ability of its active principles, mainly the flavonoid fraction, to act at the mitochondrial level unlike other antioxidants [17]. GBE maintains ATP content thanks to mitochondrial respiration protection, and also to oxidative phosphorylation preservation. The neuroprotective capacity of ginkgo leaves should allow the treatment and prevention of ocular pathologies such as glaucoma, DR or ARMD [2]. The vasoregulator effects of ginkgo through the catecholaminergic system and the release of endothelial factors, and its capacity to increase microcirculation [24], are also interesting points for this propose [25]. Finally, GBE seem safe and well tolerated as adverse reactions to ginkgo treatment are rare and mild [26].

This review concentrates on the therapeutic potential of ginkgo to treat retinal diseases associated with neurodegeneration. In particular, it focuses on such damage caused by oxidative processes: ARMD, DR, glaucoma, ischaemic retinal disease, retinal disease produced by light oxidation, RD, among other diseases. The aim of the current review is to integrate information of the possible ways to protect and the therapeutic role of GBE. The search strategy is conducted using a systematic and standardized review in curated databases such as PubMed, MEDLINE and SciFinder in 2000-2019. Papers published in 1985-2000 are used when they are necessary to explain concepts and processes. The search is based on the main key words: *Ginkgo biloba*, Ginkgoaceae, neurodegeneration, retinal diseases, age-related macular degeneration, diabetic retinopathy, glaucoma, ischaemic retinal disease. Other key words are used for secondary search (oxidative processes, neurodegenerative disease, antioxidant substances).

Age-Related Macular Degeneration

ARMD is a multifactorial neurodegenerative ocular disease characterised by progressive macula lesions that result in irreversible central vision loss [27-29]. Currently, it represents the leading cause of visual impairment and acquired irreversible blindness in people aged over 60 years in developed countries. ARMD has a high prevalence worldwide. More than 170 million people suffer from ARMD worldwide and this number is expected to rise because of aging populations. Thus, the overall prevalence of advanced ARMD is estimated to increase by more 50% by the year 2020 [29, 31, 32]. ARMD often produces very few symptoms in its early stages, but in

later stages, it causes loss of the central, straight-ahead vision needed for activities like reading and driving. According to a classification system defined in the National Eye Institute's Age-Related Eye Disease Study (ARED) [29], ARMD can be categorised into three disease progression stages. People with early ARMD do not normally suffer from vision loss. Intermediate ARMD may cause some vision loss, but most people will not experience any symptoms. People with late ARMD suffer vision loss from damaged macula. Late ARMD is classified into two general subgroups: dry ARMD and wet ARMD. Dry (atrophic, non exudative) ARMD is characterised by drusen accumulating around the RPE with a gradual breakdown of not only the light-sensitive cells in the macula that convey visual information to the brain, but also of the supporting tissue beneath the macula. These changes cause vision loss, leading to a choroid and retinal atrophy (geographic atrophy). In wet (neovascular or exudative) ARMD, abnormal blood vessels grow underneath the retina. These vessels can leak fluid and blood, which may lead to the macula swelling and to damage. Approximately 85-90% of the macular degeneration cases are the dry type, while the wet type represents 10-15% of the overall prevalence [29, 33]. Several risk factors for ARMD to develop and progress have been established, including aging, genetic factors, smoking, nutrition, degree of pigmentation, arterial hypertension and UV rays [27, 30, 34]. Different studies provide consistent evidence to suggest a crucial role for retinal pigment epithelial cell damage and death behind the causes of ARMD. Although the underlying mechanisms of retinal degeneration are not fully understood, inflammation and oxidative stress responses are involved as central players in photoreceptor death and vision loss [12, 35, 36]. The abundance of polyunsaturated fatty acids in the retina, which are selective substrates for peroxidation, along with ROS generation, cause damage to photoreceptors and RPE via the lipid peroxidation process [37]. Moreover, aging cells are particularly vulnerable because the natural antioxidant capacity decreases and the overall efficiency of reparative systems against cell damage become impaired. Several research works report that excessive *N*-retinylidene-*N*-retinylethanolamine (A2E) accumulation in RPE is implicated in the pathogenesis of ARMD [38]. There is also compelling evidence to indicate the association of vascular endothelial growth factors (VEGF) to choroidal neovascularisation (CNV) processes in wet ARMD [28, 39]. Despite the advances made in understanding ARMD and the recent introduction of new treatments for some forms of ARMD, these new intravitreal applications of anti-vascular endothelial growth factors are not able to reverse the central visual loss due to the disease, and presently there is no available treatments for the majority of affected people [28, 30, 33, 40, 41]. With limited treatment options, and given the relatively high prevalence and the increased incidence of ARMD as population age, several studies have explored the potential benefits of nutrients and other supplements that can delay disease progression to minimise visual loss [42-45]. Oxidative stress is considered a crucial event for retinal tissue damage and is implicated as a contributing factor in the pathogenesis of ARMD.

Consequently, experimental and clinical studies have investigated the use of antioxidants and other micronutrient supplements as potential strategies to delay the progression of ARMD and visual loss [35, 36, 46, 47]. Thus, the potential protective properties of antioxidant and anti-inflammatory medicinal plants and phytochemicals on degenerative ocular diseases have been investigated [48-50]. The main herbal medicines recommended to treat ARMD include xanthophylls lutein, zeaxanthin and ginkgo [42, 48, 51, 52]. The literature also describes the photo-protective effect on age-related ocular diseases of berries, bilberry (*Vaccinium myrtillus* L.), cranberry (*Vaccinium oxycoccos* L.), blackcurrant (*Ribes nigrum* L.), wolfberry (*Lycium barbarum* L.), grapes (*Vitis vinifera* L.), stigmas as saffron (*Crocus sativus* L.), and roots and rhizomes as turmeric (*Curcuma longa* L.), or Dan Shen (*Salvia miltiorrhiza* Bunge) [36, 48, 50, 53-59]. Ginkgo has become an increasingly well-known medicinal plant worldwide, and is used to treat peripheral vascular disease and cerebral insufficiency [16, 22-24]. Experimental and clinical studies have revealed the potential benefits of ginkgo for a wide range of pathological conditions, including hepatoprotective, photoprotective effects, DNA repair mechanism, and antioxidant and anti-inflammatory activities [11, 12, 19, 60-63]. It has been reported that ginkgo effects are related to its free radical quenching properties, reduction of platelet aggregation and improved blood flow, mainly due to the antioxidant and radical scavenging properties of ginkgo flavonoids and terpenoids, and to the potent and selective platelet activating factor (PAF) antagonism of ginkgolides [48, 50, 59, 61]. Flavonoids seem to prevent or reduce cell membrane lipid peroxidation and reduce damage to lipid membranes [61-63]. Different *in vitro* and *in vivo* experimental investigations have shown that EGb 761 can inhibit or reduce functional retinal impairments and protect retinal tissue from oxidative stress [11, 64-66]. The EGb 761 effect on retinal microcirculation has also been demonstrated: increased microcircular blood velocity, flow, and volume [67]. Consequently, the use of ginkgo extract has aroused emerging interest to treat patients with age macular degeneration because vascular factors and oxidative damage are thought to be two potential mechanisms in the pathology of ARMD [43, 63]. Ginkgo extract also has minimal adverse effects within the daily dose range [26, 52, 61]. It should be noted, however, that there is some concern about ginkgo extract possibly increase the risk of bleeding because one of its components, ginkgolide B, is a potent PAF inhibitor. The literature reports only one case in which regular ginkgo use is associated with vitreous haemorrhage in a 78-year-old woman with ARMD [68]. Recently, several preliminary studies have investigated the efficacy of GBE in ARMD patients. The literature includes a systematic review published in 2013 [52] that reports two randomised controlled trials (RCT) using GBE with positive effects on vision in patients with ARMD. In the Lebuisson RCT [69], a double-blind trial was conducted to compare EGb761 (80 mg twice daily, 160 mg) with a placebo in 10 out-patients aged over 55 attending an eye clinic. Treatment lasted 6 months. Drug effectiveness was assessed on the fundoscopy results and on visual acuity and visual field measurements. In

spite of the small population sample, a statistically significant improvement in long-distance visual acuity was observed. In a controlled double-blind trial with 99 patients aged 59 years or more, Fies [70] compared the therapeutic efficacy of EGb 761 over a 6-month treatment period with either 240 mg/day (group I: 50 patients) or 60 mg/day (group II: 49 patients). The study participants' vision markedly improved in both the treatment groups only after 4 weeks, with more pronounced improvements in group I. According to Evans [52] and Sin [59], both RCT showed positive effects of ginkgo on vision, but these trials were small with a short observation period to provide concluding evidence. In addition, Barlett and Eperjesi [42] suggested that Lebuisson beneficial findings should be viewed cautiously as the assessment of outcome was not masked. Dubey *et al*. [63] considered that the question as to whether ARMD patients should take EGb 761 has not yet been fully answered and further studies to establish the efficacy of ginkgo are required. To conclude, although there are many positive outcomes for preventive, or even therapeutic, uses of GBE for ARMD, its efficacy remains controversial and there is still no compelling scientific evidence. Further research into the potential clinical role should be conducted.

Retinal detachment

RD is a serious event that can result in vision loss. Retinal photoreceptors receive oxygen and metabolic support from the choroid. If the retina separates from the choroid, photoreceptor cells die and cause damage that can lead to blindness. Basically, there are three types of RD, the most frequent being rhegmatogenous (RRD) that develops due to retinal rupture and by fluid passing from the vitreous cavity to the potential space below the retina, which leads to the retina separating from the underlying choroid. This requires surgical treatment [71]. Although RD can appear in any eye, certain eyes are predisposed to develop it, and the risk factors to be considered are: axial myopia, post-cataract surgery, ocular trauma, RD in an eye, family history of RD and various retinal disorders, including ARMD [72] and DR [73], among others. Despite the high prevalence of these conditions, there are currently few or no treatments available. Retinal treatment is successfully treated in 80-99% of the cases, retinal death due to RD has not treatment. Therefore, preventive intervention may be the most effective course of action against these age-related eye diseases. It should be noted that all these retina diseases are associated with aging and their aetiology shares some mechanisms of action. These pathways include oxidative stress, inflammation and apoptotic factors, which provide information on potentially targetable areas [74]. Currently, one of the therapeutic approaches does not focus so much on the causes of diseases, but on ways to prevent cell death, such as administration of anti-

apoptotic, anti-inflammatory and neurotrophic compounds. In fact the use of antioxidant compounds, antiapoptotic, anti-inflammatory and viability factors can slow down retina neurodegeneration by delaying retinal cell death [75]. In recent years, many epidemiological and clinical studies have been conducted and demonstrate the beneficial effects of plant-derived compounds in eye diseases. Many natural compounds exhibit strong antioxidant, anti-inflammatory and antiapoptotic properties, such as flavonoids and terpenes, which are the main active ingredients in ginkgo leaves. In fact GBE have relevant properties, such as protection against free radical damage and lipid peroxidation [48, 50, 59, 61-63, 74]. Marcocci *et al*. [76] pointed out the protective capacity that GBE show against NO in studies into mammalian cells. By preventing loss of ganglion cells in the retina and optic nerve atrophy, these GBE properties can protect the optic nerve from degeneration and, thus, prevent blindness in patients with glaucoma, RD and retinitis pigmentosa (RP) [77]. Ma *et al*. [78] conducted studies with Sprague-Dawley rats by injecting GBE, followed by the crushing of the optic nerve. The animals that received GBE extract by intraperitoneal injection prior to optic nerve crushing showed a significantly higher survival rate of ganglion cells in the retina than the controls. Since the development of EGb 761 in the sixties [79], this extract has been the most widely studied extract in clinical research, whose effect has been investigated in a wide range of disorders and diseases. Numerous studies show that EGb 761 is a relevant antioxidant extract that can provide effective protection against oxidative stress [80, 81]. Therefore, the therapeutic potential of EGb 761 can, at least in part, be attributed to its antioxidant action. In another study with an experimental model of tractional RD, Baudouin *et al*. [82] confirmed the efficacy of EGb 761 to prevent retinal retraction. These authors investigated the effects of this extract administered orally for 1 month after inducing vitreoretinopathy. The untreated animals developed an inflammatory reaction, and extensive intravitreal and preretinal membranes, which led to tractional RD. In treated animals, EGb 761 prevented the inflammatory reaction, reduced vitreoretinal proliferation and decreased the frequency of RD. Studies conducted with Kunming mice show that EGb 761 inhibits the apoptosis of photoreceptor cells and increases cell survival after damaging or intense light exposure [83]. EGb761 has also been indicated to prevent RD-related inflammation after inducing vitreoretinopathy and, thus, reduced the occurrence of RD [77]. Paasche *et al*. [84] studied age-related changes of mitochondria in Müller (retinal glial) cells from guinea pigs fed with, or not, externally applied EGb 761, an established radical scavenger. The obtained results suggested that many structural and functional parameters of the mitochondrial aging of Müller cells were affected by oxidative damage, and that externally applied radical scavengers could protect organelles from the damaging actions of free radicals. Treatment with EGb 761 increased the intrinsic glutathione content of aged guinea pig Müller cells, and the protective effect of the radical elimination of the drug was mediated both directly and indirectly. In the article “Neuroprotection for Retinal Detachment”, Huckfeldt and Vavvas

[85] reviewed the main pathways of cell death activated by RD and progress in the development of neuroprotective strategies. They pointed out that, although current results are promising, functional aspects in the clinical setting have not yet been tested. The physiological responses observed after RD are complicated and effective neuroprotection may require a combinatorial approach, such as surgical treatment and complementary drug therapy, to address inflammatory and proliferative causal responses. Similarly, Murakami *et al*. [86] conducted a study on photoreceptor cell death and RD, and considered that a better understanding of the molecular mechanisms related to the death of these cells would allow new therapies to be developed to prevent vision deficits due to loss of photoreceptor cells. According to Li *et al*. [87], currently no effective therapy is clinically available to protect photoreceptor cells. Therefore, the development of neuroprotective reagents for photoreceptors could contribute to long-term visual stability for postoperative RD patients. These authors used an experimental RD model and provided evidence that oxidative stress and inflammation are activated in the retina after RD, and photoreceptors are an important source of ROS production by regulating NADPH oxidase (NOX). Their results pointed out that adrenergic receptors were novel therapeutic targets for neuroprotective substances to prevent the death of photoreceptors induced by RD.

Diabetic Retinopathy

DR is the commonest microvascular complication of diabetes mellitus (DM) and one of the leading causes of blindness worldwide [48]. One third of the world's diabetic population presents different DR stages [88]. DR is a complex event; oxidative stress and inflammation are the main pathways to affect the pathogenesis of DR, which is characterised by neuronal and pericyte cell loss, the formation of acellular-occluded capillaries and microaneurysms that result in capillary non-perfusion and hypoxia, increased leukostasis and vascular basement membrane thickening. These alterations trigger microvascular complication by leading to the breakdown of the blood-retina barrier, exudates, haemorrhages and profound ischaemia of the retina leading to neovascularisation [89]. Injuries induced by diabetes and hyperglycaemia are related with increased arginase activity and low levels of bioavailable NO, and overactive arginase may contribute to DR by reducing NO and increasing oxidative stress due to arginase activity. Furthermore, the serum of diabetic patients increases the levels of reactive oxygen metabolites (ROM) compared with healthy subjects, and it has been demonstrated that ROM increases rapidly in serum as DR progresses [89]. The main treatment for DR is intensive glycemic control. Another important treatment is laser photocoagulation, but it is not effective in many patients. In patients with diabetic macular oedema, this being the mayor complication of DR,

intravitreal steroids injections are used (including triamcinolone, that it is not very common nowadays, and dexamethasone implant, more common). The intravitreal administration of VEGF has been developed in recent years. [90]. The prevention of the vascular complications of diabetes is now an important public health priority worldwide. Currently the treatments associated with antidiabetic drugs mostly intend to regulate vascular changes, inflammation, and to reduce oxidative stress. In this context, GBE have drawn attention given their antioxidant and platelet anti-aggregation properties and other effects, such as dilated blood vessels and improved abnormal blood rheology [89, 91]. According to recent studies, using ginkgo is a good option to prevent the progress of DR and to reduce its prevalence. Zhao *et al*. [91] demonstrated that administering ginkgo leaf tablets contributes to reduce the oxidative stress, which plays an important role in DR. Previously in their randomised double-blind placebo-controlled study with 140 patients that lasted 3 three years, Zhao *et al*. [92] showed that administering ginkgo leaf tablets with Liuwei dihuang pills can reduce the risk of DR and its prevalence by 66% (25% from the control group *versus* 8.5% from the treatment group). Zhao *et al.* [93] conducted a study by performing blood testing among diabetic patients to conclude that GBElower the apoptosis rate of retinal vascular endothelial cells. Other research works done in rats [89] link GBE with the prevention of an increase in VEGF and TFN-α, proinflammatory mediators. In other diabetic animal models, GBE have been seen to reduce NO-induced oxidative stress, besides decreasing its production. This attenuates the apoptosis of retinal ganglion cells and photoreceptor cells. GBE have anti-inflammatory effects, and one of the possible mechanisms is the ability to down-regulate the expression of the platelets activating factor (PAF). GBE can also significantly reduce the transcriptional expressions of hypoxia inducible factor, HIF-1α, and VEGF in retinal pigment epithelial cells under hypoxic conditions [88]. Interleukin regulation appears to play a key role in the anti-inflammatory effect of GBE [94]. According to the background, GBE improve retinal health by increasing the retinal capillary blood flow in patients with DR. Correct GBE use lowers the incidence and progression of this sight-threatening complication in diabetic patients. GBE could be a potential candidate for use in the prevention and treatment of DR.

Glaucoma

Glaucoma can be defined as a series of ocular diseases characterised by progressive reduction of visual field due to secondary optic nerve damage. It is mainly a consequence of increased intraocular pressure (IOP) that causes optic nerve injuries, starting with retinal ganglion cells apoptosis [74]. This disease causes progressive and irreversible damage, and it remains

asymptomatic and it is not usually detected until it is severe and has progressed to advanced stages. Glaucoma is a leading cause of vision loss in people aged 60 and more. However, it can be prevented if treatment begins in early disease stages [95]. There are several types of glaucoma, the commonest of which is primary open-angle glaucoma (POAG). Among other causes, it is provoked by the slow clogging of drainage canals, which raises intraocular pressure. Glaucoma is linked to changes in NO metabolism, oxidative damage and vascular disorders. The main POAG-related damage is believed to be the degeneration of the sclero-corneal trabecular meshwork (TM). Several studies report reducede field of vision and increased IOP associated with the oxidative damage of TM cells' DNA in patients glaucoma, plus an imbalance in the redox status being related to the high expression of endothelial-leukocyte adhesion molecule in TM [50]. Nitrosative stress, together with oxidative stress caused by ROS of endogenous and exogenous sources, can induce cell damage and death and, accordingly, ophthalmic diseases [42, 50, 96]. Current glaucoma treatments are based mainly on the arrest of disease progression, but they do not restore vision loss [97]. Previous studies report that several antioxidants, such as vitamins C and E, omega-3 and omega-6 fatty acids, and GBE, can help to regulate IOP and protect retina from oxidative or nitrosative stress [96]. However, further research is needed to find new and more effective therapies to prevent the irreversible blindness caused by glaucoma. Ginkgo scientific research started given the great interest that the antioxidant and neuroprotective properties of ginkgo generated, which can be used against glaucoma. Thus GBE have demonstrated to play an important role in the improvement of several degenerative eye diseases, including glaucoma. GBE can improve the vision of patients with normal-tension glaucoma (NTG) and other ophthalmic vascular diseases [98]. These beneficial effects can be explained by antioxidant and vascular protector properties, which result in improved ocular blood flow after being administered. Despite extensive information, more studies are needed to provide conclusive evidence [99]. Terpenes and flavonoids are the main compounds responsible for the antioxidant ability of GBE used against glaucoma [100]. To conclude about GBE and glaucoma, and based on the background, GBE as a neuroprotector can prevent retinal ganglion cells damage. These extracts can also be used to prevent and treat other retinal neurodegenerative diseases. The use of GBE is proposed to improve oxidative stress in NTG patients and to increase visual acuity, but further studies are needed.

Ischaemic retinal disease

Ischaemic retinopathy is a pathological state caused by the partial or total suppression of ocular blood supply. It is a common cause of visual impairment, and sometimes of blindness. The

dysregulation of ocular vessels can also contribute to the progression of ocular diseases like glaucoma (vascular theory of glaucoma pathogenesis) [101]. Ischaemic retinopathy has been related to atherosclerotic disease by causing obstruction of vessels (carotid artery occlusive disease), central retinal artery occlusion or branch retinal artery occlusion, retinal vein occlusion, diabetes, etc. Its consequences can determine neurotoxic effects due to the sensitivity of retinal cells to ischaemic injury. Compensation mechanisms are initiated by forming new abnormal blood vessels in the retina. As a result, retinal ischaemia can lead to morphological and functional changes, and produces pain and visual loss depending on the part of the retina that is affected [13]. In cellular terms, neuronal depolarisation increases intracellular calcium concentrations, and oxidative stress is produced as a consequence of the lack of blood flow-dependent energy. Glutamatergic stimulation may also increase. However, given the retina's special characteristics, it is more resistant to the damage induced by reduced blood flow than the brain. Animal models have been used to study retinal ischaemia and different treatments have been proposed to avoid it [102]. As regards natural products, very few studies into ginkgo and retinal ischaemia have been reported [103-108]. *In vitro* and *in vivo* experiments show the ability of ginkgo, its extracts and its components to improve blood flow in different vascular territories, and to prevent oxidative damage induced at the cellular level [109, 110]. Ginkgo properties as a vasodilator and a free radical scavenger have been confirmed, as have its activities as a membrane stabiliser and a regulator of metabolism (Figure 1). The EGb761 extract is able to act as a NO scavenger by further inhibiting iNOS mRNA expression [111]. Moreover, the effects on mechanisms of angiogenesis have been tested [15]. Some clinical trials performed with EGb761 indicate an effect of increasing ocular blood flow in glaucoma patients with an improved visual field [112, 113]. Among its components, flavonoids can be partially responsible for this activity. These phenolic compounds can also counteract the negative effects caused by ischaemic-dependent oxidative stress to the extent that they are effective as free radical scavengers and are able to inhibit lipid peroxidation [109, 114]. Studies into structure-activity relations performed with flavonoids show a positive relationship between the presence of three free hydroxyl (OH) phenolic groups and increased ocular blood flow in rabbit eyes [115]. In healthy subjects, EGb761administration significantly improves the ocular blood flow [112]. The vasodilatory activity mediated by the release of the relaxing factors of the vascular endothelium and prostacyclin (PGI2) due to the presence of flavonoids, and the antiplatelet activity mediated by diterpenes, might justify these effects. Vascular ocular alterations may be linked to NTG development. Park *et al.* [116] studied the effects of EGb761 on 30 NTG patients with a randomised double-masked, placebo-controlled clinical trial. The cases group included 15 patients treated orally with 80 mg of ginkgo extract, the equivalent to 19.2 mg of flavonoids, twice daily for 4 weeks. The control group patients (N:15) received a placebo. After treatment with EGb761, a statistically significant increase in peripapillary blood flow was recorded.

Volume and velocity also significantly increased. No side effects were recorded in any patient. The few patients recruited, the trial duration and variations in the vascular effects depending on specific retinal territories were the main limitations of this study. The effects of GBE on the biomarkers of ocular perfusion in patients with open-angle glaucoma (OAG) have also been evaluated. Wimpissinger *et al*. [117] found no effects on retinal blood flow in healthy subjects (N:15) in a randomised double-masked, placebo-controlled, two-way cross-over study after a single dose treatment (240 mg of the EGb761 extract corresponding to 57.6 mg of flavonoids and 14.4 mg of terpenlactones). In a randomised double blinded, placebo-controlled cross-over study (N:45), the effects of a complex preparation containing ginkgo and other antioxidant substances (vitamins, minerals, omega-3 from fish oil, polyunsaturated fatty acids (PUFA), *N*-acetylcysteine, bilberry fruit extract, coenzyme Q10, grape seed extract, quercetin, flax seed oil, etc.) on blood flow in patients with open-angle glaucoma were tested [118]. One month after treatment, a statistically significant increase in blood flow velocities in all the retrobulbar blood vessels was observed as compared to the placebo. Superior and inferior retinal capillary mean blood flows also increased. The patients treated with the complex combination showed reduced vascular resistance in the central retinal and nasal short posterior ciliary arteries *versus* the placebo group. The high composition complexity of the employed preparation limited the direct relationship between the ginkgo extract and the observed beneficial effects on retinal blood flow.

Retinal disease produced by light oxidation

Light has a phototoxic effect on ocular tissues, and more specifically on the retina. Retinal damage depends on light intensity, its wavelength and exposure time. While acute exposure damage causes eyelid burns, photokeratitis and solar retinopathy, different epidemiological studies have found an association between long-term sun exposure and cataracts, ARMD (the leading cause of vision loss in the Western world in people aged 65 and over), climatic droplet keratopathy and pterygium [119]. Retina and RPE are tissues exposed to high photo-oxidative stress levels due to raised concentrations of available oxygen, and also to the presence of a number of endogenous photosensitisers, such as vitamin A derivatives, lipofuscin, melanin, flavins and porphyrins. The outer retina contains high concentrations of polyunsaturated fatty acids, including the most unsaturated fatty acid in the human body, docosahexaenoic acid with six double bonds, which are extremely susceptible to peroxidation. Light exposure increases photoreceptor degeneration and diminishes the cell density of certain retinal layers (inner and outer nuclear layers and the ganglion cell layer). This cell loss occurs by the apoptosis mediated

by alterations in gene expression [120]. Photochemical damage to the retina can be classified as different types (Figure 2)[121]. A photosensitive derivative of visual pigment *N*-retinylidene-*N*-retinylethanolamine (Figure 3) may be involved in this phototoxicity. Many studies have demonstrated that antioxidant compounds are able to prevent light-induced retinal damage. Among other natural antioxidants, EGb 761 stands out. *In vitro* studies have shown the EGb761 effect on some important protein expression levels, such as cathepsin B, heat shock protein, and cytochrome c reductase, and have pointed out that multiple pathways can be involved in light-induced damage and the extract protective effect [37]. Daily oral EGb 761 administration partially inhibits the apoptosis of photoreceptor cells, which results in increased photoreceptor cell survival. This extract also preserves retinal morphometry and provides functional protection [122]. Flavonoid glycosides are the main compounds responsible for the antioxidant capacity of the ginkgo extract (Figure 4). These compounds are able to scavenge O_2 radicals, hydroxyl radicals, lipid peroxides and iron ions [119]. In addition to ginkgo flavons, the extract also contains other substances of less interest, such as organic acids. Thus EGb 761 possesses both hydrophilic and lipophilic characteristics, unlike other antioxidants such as ascorbic acid, glutathione or uric acid. Intraperitoneal EGb 761 administration (100 mg/kg) lasting between 1 week before and 2 weeks after light exposure increases retinal antioxidant capacity and can partially inhibit photoreceptors apoptosis [123]. Grosche *et al.* [124] demonstrated that EGb 761 administration does not prevent Müller cells hypertrophy, but inhibits the expression of pathological marker molecules.

Others

GBE can be used in processes that are the primary cause of the aforementioned retina pathologies, or are secondary causes that lead to disease developing. In this context, EGb 761 can prevent retinal pigmented epithelial degeneration because it is a scavenger of NO and potentially protects RPE cells from its antiproliferative action [11]. The same extract also has a positive effect on inflammatory ritinitis according to an experimental model of autoimmune uveoretinitis induced in rats [125]. Some studies demonstrate that ginkgo can be useful for treating Behcet's disease (BD), which is a multisystem disorder including ophthalmic manifestations such as iritis or uveitis. BD is linked to oxidative processes. For this reason, the use of pharmacological agents with antioxidant properties is considered increasingly important. Indeed EGb 761 is one of the proposed pharmacological agents because it is a free radical scavenger and its use strengthens the organism’s antioxidant defence system and greatly contributes to clinical improvement in BD [126]. Other authors have also investigated the EGb

761 effect on uveitis using animal models. They concluded that the ginkgo extract decreases the uveitis anti-inflammatory process, but also notably suggest the need for more research about EGb 761 properties and effects to gain a better understanding of the mechanism and to confirm activities like blocking iNOS protein expression and the anti-inflammatory effect on eyes [127, 128].

RP is another retina disease related to oxidative processes. This disorder leads to reduced vision and particularly affects night vision, and can lead to blindness in severe cases because of alterations that the retinal rod and cone cells undergo. GBE act on preventing loss of retinal ganglion cells and optic nerve atrophy by protecting the optic nerve from degeneration. Thus these extracts may prevent blindness in patients with RP. Other studies suggest that GBE conserve mitochondrial metabolism and ATP production in tissues by inhibiting morphological distortion and oxidative damage from mitochondrial aging. Others have demonstrated the ability of GBE for scavenging NO and, hence, their protector effect against NO reactivity [74].

Discussion, future perspectives and conclusions

Retinal diseases are related to oxidative stress, and flavonoids, terpenes, and other antioxidant components of ginkgo extracts play a particular important role in their treatment and prevention. Together with antioxidant properties, anti-inflammatory activities are also of interest. These ginkgo extract capacities can be attributed to the synergistic activity of different extract components [129]. Two mechanisms of action have been suggested in order to explain GBE antioxidant capacity. Components of the extracts are able to scavenge different free radicals, including ROS such as hydroxyl radicals ($OH\cdot$) [130], superoxide radicals (O_2^-), peroxyl radicals ($ROO\cdot$) or hydrogen peroxide (H_2O_2), among others, and also reactive nitrogen species, as nitric oxide ($NO\cdot$) and ferryl ion species [131]. This activity is mainly related to flavonoids, while terpenes increase cell enzymatic antioxidant mechanisms. Bilobalide and ginkgolides increase superoxide dismutase, glutathione peroxidase, catalase and heme-oxigenase-1 activities, which promotes GBE antioxidant effect [132]. In addition, it has been reported that EGb 761 is able to regulate Mn and Cu homeostasis in the brain, metals that act as cofactors of antioxidant enzimes [133, 134]. The NO scavenging capacity of GBE can inhibit iNOS expression. In this sense, the inhibition of NF-κB mediated by GBE should contribute to the downregulation of iNOS and other inflammatory mediators such as TNF-α, IL-6, IL-2, IL-8 and ICAM-1, corroborating ginkgo dual role as antioxidant and anti-inflammatory agent [135]. The abundant polyunsaturated fatty acids (PUFAs) in the retina could be protected against peroxidation by ginkgo extracts, as has been previously demonstrated in rat liver microsomes

[136]. Drieu *et al.* (2000) [137] reported that EGb 761 produces an increase in circulating and cellular PUFA amounts, including arachidonic, docosahexaenoic acids and eicosapentaenoic acid. This effect should be mediated acting on synthesis and/or catabolism of PUFAs. These results pointed out that in addition to antioxidant capacity of ginkgo components, the increase in PUFAs can explain a most durable effect of EGb 761. PUFAs may be target of oxidative damage preserving other important molecules. On the other hand, ginkgo extract avoids peroxidation of membrane PUFAs preserving membranes fluidity, and keeping their integrity. Furthermore, docosahexaenoic acid that was increased by two fold in rats after EGb 761 treatment plays an essential role in visual function, regulating via rhodopsin the photo-signal transduction, and protecting RPE from oxidative stress [138].

GBE have been used for the treatment of cerebral and peripheral vascular insufficiency due to their vasorelaxant properties [139] as well as their capacity to regenerate injured peripheral nervous system [140]. An increase in angiogenesis has been reported by Zhu *et al*. [141] as a consequence of VEGF significant rise in rats treated with EGb 761 after acellular nerve allografts. GBE have also shown a promotion of VEGF expression in ischemic brain tissue after subarachinoid hemorrhage in rat model [142]. However, there are contradictory results regarding to ginkgo effect over this growth factor. Oh *et al*. [143] reported that GBE reduce hypoxia-inducible factor-1α and VEGF expression in cultured RPE cells under chemical hypoxia. Juarez *et al*. [144] found similar results in a murine model. The vasodilating properties of GBE makes choroidal circulation has large-caliber vessels, high flow rate, providing cells more oxygen, and attenuating some retinal diseases in a non-invasively way. However, more studies are needed in order to clarify inconsistent data before GBE can be used in therapeutic of diseases as wet macular degeneration.

The experimental and clinical data indicate that gingko leaf extract is well tolerated and is apparently safe in humans. Most studies demonstrate its potential therapeutic role and its efficacy in degenerative diseases of the retina, such as AMD, DR, glaucoma, ischaemic retinal disease, retinal disease produced by light oxidation and RD. The specific mechanism by which GBE induce effects is still to be fully elucidated. Nevertheless, beneficial gingko effects can be explained by its antioxidant, anti-inflammatory and vascular protector properties. In conclusion, GBE could be an effective phytochemical therapeutic target, but further studies are necessary.

REFERENCES

[1] Carelli V, La Morgia C, Ross-Cisneros FN, Sadun AA. Optic neuropathies: the tip of the neurodegeneration iceberg. Hum Mol Genet 2017; 26 (R2): 139-150

[2] Schmidt KG, Bergert H, Funk RHW. Neurodegenerative diseases of the retina and potential for protection and recovery. Curr Neuropharmacol 2008; 6: 164-178

[3] Chhunchha B, Singh P, Singh DP, Kubo E. Ginkgolic acid rescues lens epithelial cells from injury caused by redox regulated-aberrant sumoylation signaling by reviving Prdx6 and Sp1 expression and activities. Int J Mol Sci 2018; 19: 3520

[4] Kortuem K, Geiger LK, Levin LA. Differential susceptibility of retinal ganglion cells to reactive oxygen species. Invest Ophthalmol Vis Sci 2000; 41: 3176-3182

[5] Rohowetz LJ, Kraus JG, Koulen P. Reactive oxygen species-mediated damage of retinal neurons: drug development targets for therapies of chronic neurodegeneration of the retina, Int J Mol Sci 2018; 19: 3362

[6] Vasudevan SK, Gupta V, Crowston JG. Neuroprotection in glaucoma. Indian J Ophtalmol 2011; 59: S102-S113

[7] He Y, Leung KW, Zhang YH, Duan S, Zhong XF, Jiang RZ, Peng Z, Tombran-Tink J, Ge J. Mitochondrial complex I defect induces ROS release and degeneration in trabecular meshwork cells of POAG patients: protection by antioxidants. Invest Ophthalmol Vis Sci 2008; 49: 1447-1458

[8] Li C, Miao X, Li F, Wang S, Liu Q, Wang Y, Sun J Oxidative stress-related mechanisms and antioxidant therapy in diabetic retinopathy. Oxid Med Cell Longev 2017; 2010: 9702820

[9] Castilho AF, Aveleira CA, Leal EC, Simões NF, Fernandes CR, Meirinhos RI, Baptista FI, Ambrósio AF. Heme oxigenase-1 protects retinal endotelial cells against high glucose and oxidative/nitrosative stress-induced toxicity. PLoS ONE 2012; 7: e42428

[10] Whiteside CI. Cellular mechanisms and treatment of diabetes vascular complications converge on reactive oxygen species. Curr Hypertens Rep 2005; 7: 148-154

[11] Droy-Lefaix MT. Effect of the antioxidant action of *Ginkgo biloba* extract (EGb 761) on aging and oxidative stress. Age 1997; 20: 141-149

[12] Datta S, Cano M, Ebrahimi K, Wanh L, Handa JT. The impact of oxidative stress and inflammation on the RPE degeneration on non-neovascular AMD. Prog Retin Eye Res 2017; 60: 201-218

[13] Osborne NN, Casson RJ, Wood JP, Chidlow G, Graham M, Melena J. Retinal ischemia: mechanisms of damage and potential therapeutic strategies. Prog Retin Eye Res 2004; 23(1): 91-147

[14] Roh MI, Murakami Y, Thanos A, Vavvas DG, Miller JW. Edaravone, an ROS scavenger, ameliorates photoreceptor cell death after experimental retinal detachment. Invest Ophthalmol Vis Sci 2011; 52(6): 3825-3831

[15] Montes P, Ruiz-Sánchez E, Rojas C, Rojas P. *Ginkgo biloba* extract 761: a review of basic studies and potential clinical use in psychiatric disorders. CNS Neurol Disord Drug Targets 2015; 14: 132-149

[16] Yin B, Xu Y, Wei R, Luo B. *Ginkgo biloba* on focal cerebral ischemia: a systematic review and meta-analysis. Am J Chin Med 2014; 42(4): 769-783

[17] Cybulska-Heinrich AK, Mozaffarieh M, Flammer J. *Ginkgo biloba*: an adjuvant therapy for progressive normal and high tension glaucoma. Mol Vis 2012; 18: 390-402

[18] Beck S, Stengel J. Mass spectrometric imaging of flavonoid glycosides and biflavonoids in *Ginkgo biloba* L. Phytochemistry 2016; 130: 201-206

[19] Ahlemeyer B, Krieglstein J. Neuroprotective effects of *Ginkgo biloba* extracts. CMLS Cell Mol Life Sci 2003; 60: 1779-1792

[20] Ahlemeyer B, Selke D, Schaper C, Klumpp S, Krieglstein J. Ginkgolic acids induce neuronal death and activate protein phosphatase type-2C. Eur J Pharmacol 2001; 430: 1-7

[21] Mojaverrostami S, Bojnordi MN, Ghasemi-Kasman M, Ebrahimzadeh MA, Hamidabadi HG. A review of herbal therapy in multiple sclerosis. Adv Pharm Bull 2018; 8: 575-590

[22] Bachinskaya N, Hoerr R, Ihl R. Alleviating neuropsychiatric symptoms in dementia: the effects of *Ginkgo biloba* extract EGb 761. Findings from a randomized controlled trial. Neuropsychiatr Dis Treat 2011; 7: 209-215

[23] Yuan Q, Wang CW, Shi J, Lin ZX. Effects of *Ginkgo biloba* on dementia: An overview of systematic reviews. J Ethnopharmacol 2017; 195: 1-9

[24] Wu Y, Li S, Cui W, Zu X, Du J, Wang F. *Ginkgo biloba* extract improves coronary blood flow in healthy elderly adults: role of endothelium-dependent vasodilation. Phytomedicine 2008; 15: 164–169

[25] Kubota Y, Tanaka N, Kagota S, Nakamura K, Kunitomo M, Umegaki K, Shinozuka K. Effects of *Ginkgo biloba* extract on blood pressure and vascular endothelial response by acetylcholine in spontaneously hypertensive rats. J Pharm Pharmacol 2006; 58: 243-249

[26] McKenna DJ, Jones K, Highes K. efficacy, safety, and use of *Ginkgo biloba* in clinical and preclinical applications. Altern Ther Health Med 2001; 7: 70-86

[27] García-Layana A, Cabrera-López F, García-Arumí J, Arias-Barquet L, Ruiz-Moreno J. Early and intermediate age-related macular degeneration: update and clinical review. Clin Interv Aging 2017; 12: 1579–1587

[28] Ratnapriya R, Chew EY. Age-related macular degeneration-clinical review and genetics update. Clin Genet 2013; 84: 160-166

[29] Jager RD, Mieler WF, Miller JW. Age-related macular degeneration. N Engl J Med 2008; 358: 2606-2617

[30] Lawrenson JG, Evans JR. Advice about diet and smoking for people with or at risk of age-related macular degeneration: a cross-sectional survey of eye care professionals in the UK. BMC Public Health 2013; 13: 564

[31] National Eye Institute (NEI) National Institutes of Health (NIH). Research today…vision tomorrow. Age-related macular degeneration (AMD). Available at: https://nei.nih.gov/eyedata/amd. Accessed December, 2018

[32] Wong WL, Su X, Li X, Cheung CMG, Klein R, Cheng C-Y, Wong TY. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet 2014; 2: e106-116

[33] National Eye Institute (NEI), National Institutes of Health (NIH). Research Today … Vision Tomorrow. Facts about age-related macular degeneration. Available at: https://nei.nih.gov/health/maculardegen/armd_facts. Accessed December, 2018

[34] World Health Organization. Blindness and vision impairment prevention. Priority eye diseases. Available at: https://www.who.int/blindness/causes/priority/en/index7.html. Accessed December, 2018

[35] Athanasiou D, Aguila M, Bevilacqua D, Novoselov SS, Parfitt DA, Cheetham MEM. The cell stress machinery and retinal degeneration. FEBS Lett 2013; 587: 2008-2017

[36] Gorusupudi A, Nelson K, Bernstein PS. The eye-related eye disease 2 study: micronutrients in the treatment of macular degeneration. Adv Nutr 2017; 8: 40-53

[37] Zhou YY, Chen CZ, Su Y, Li L, Yi ZH, Qi H, Weng M, Xing YQ. Effect of EGb761 on light-damaged retinal pigment epithelial cells. Int J Ophtalmol 2014; 7: 8-13

[38] Yoon SM, Lee BL, Guo YR, Choung SY. Preventive effect of *Vaccinium uliginosum* L. extract and its fractions on age-related macular degeneration and its action mechanisms. Arch Pharm Res 2016; 39: 21-32

[39] Nagineni CN, Raju R, Nagineni KK, Kommineni VK, Cherukuri A, Kutty RK, Hooks JJ, Detrick B. Resveratrol suppresses expression of VEGF by human retinal pigment epithelial cells: potential nutraceutical for age-related macular degeneration. Aging Dis 2014; 5: 88-100

[40] Englander M, Kaiser PK. Combination therapy for the treatment of neovascular age-related macular degeneration. Curr Opin Ophthalmol. 2013; 24: 233-238

[41] Kaiser PK, Do DV. Ranibizumab for the treatment of neovascular AMD. Int J Clin Prac 2007; 61: 501-509

[42] Bartlett H, Eperjesi F. Nutrition and age-related ocular disease. Curr Top Nutraceutical Res 2005; 3: 231-242

[43] Bartlett H, Eperjesi F. An ideal ocular nutritional supplement? Ophthalmic Physiol Opt 2004, 24: 339-349

[44] Pinazo-Durán MD, Gómez-Ulla F, Arias L, Araiz J, Casaroli-Marano R, Gallego-Pinazo R, García-Medina JJ, López-Gálvez MI, Manzanas L, Salas A, Zapata M, Diaz-Llopis M,

García-Layana A. Do nutritional supplements have a role in age macular degeneration prevention? J Ophthalmol 2014; 2014: 901686

[45] Krishnadev N, Meleth AD, Chew E. Nutrition supplements for age-related macular degeneration. Curr Opin Ophthalmol 2010; 21: 184-189

[46] Evans JR, Lawrenson JG. Antioxidant vitamin and mineral supplements for slowing the progression of age-related macular degeneration. Cochrane Database Syst Rev 2017; 7: CD000254

[47] Evans JR, Lawrenson JG. A review of the evidence for dietary interventions in preventing or slowing the progression of age-related macular degeneration. Ophthalmic Physiol Opt 2014; 34: 390–396

[48] Wilkinson JT, Fraunfelder FW. Use of herbal medicines and nutritional supplements in ocular disorders: an evidence-based review. Drugs 2011; 71: 2421-2434

[49] Chu K, Pang C. Herbal molecules in eye diseases. Taiwan J Ophthalmol 2014; 4: 103-109

[50] Rhone M, Basu A. Phytochemicals and age-related eye diseases. Nutr Rev 2008; 66: 465-472

[51] Abdel-Aal ESM, Akhtar H, Zaheer K, Ali R. Dietary sources of lutein and zeaxanthin carotenoids and their role in eye health. Nutrients 2013; 5: 1169–1185

[52] Evans JR. *Ginkgo biloba* extract for age-related macular degeneration. Cochrane Database Syst Rev 2013; 1: CD001775

[53] Abu-Amero KK, Kondkar AA, Chalam KV. Resveratrol and ophthalmic diseases. Nutrients 2016; 8: 200

[54] Ishihara T, Kaidzu S, Kimura H, Koyama Y, Matsuoka Y, Ohira A. Protective effect of highly polymeric A-type proanthocyanidins from seed shells of japanese horse chestnut (*Aesculus turbinata* Blume) against light-induced oxidative damage in rat retina. Nutrients 2018; 10: 593

[55] Chang CH, Chiu HF, Han YC, Chen IH, Shen YC, Venkatakrishnan K, Wang CK. Photoprotective effects of cranberry juice and its various fractions against blue light-induced impairment in human retinal pigment epithelial cells. Pharm Biol 2017; 55: 571-580

[56] Osada H, Okamoto T, Kawashima H, Toda E, Miyake S, Nagai N, Kobayashi S, Tsubota K, Ozawa Y. Neuroprotective effect of bilberry extract in a murine model of photo-stressed retina PLoS ONE 2017; 12: e0178627

[57] Park SI, Lee EH, Kim SR, Jang YP. Anti-apoptotic effects of *Curcuma longa* L. extract and its curcuminoids against blue light-induced cytotoxicity in A2E-laden human retinal pigment epithelial cells. J Pharm Pharmacol 2017; 69: 334-340

[58] Pescosolido N, Giannotti R, Plateroti AM, Pascarella A, Nebbioso M. Curcumin: therapeutical potential in ophthalmology. Planta Med 2014; 80: 249-254

[59] Sin HP, Liu DT, Lam DS. Lifestyle modification, nutritional and vitamins supplements for age-related macular degeneration. Acta Ophthalmol 2013; 91: 6-11

[60] Mohanta TK, Tamboli Y, Zubaidha PK. Phytochemical and medicinal importance of *Ginkgo biloba* L. Nat Prod Res 2014; 28: 746-752

[61] Diamond BJ, Shiflett SC, Feiwel N, Matheis RJ, Noskin O, Richards JA, Schoenberger NE. *Ginkgo biloba* Extract: Mechanisms and clinical indications. Arch Phys Med Rehabil 2000; 81: 668-678

[62] Singh M, Mathur G, Jain CK, Mathur A. Phyto-pharmacological potential of *Ginkgo biloba*: A review. J Pharm Res 2012; 5: 5028-5030

[63] Dubey AK, Shankar PR, Upadhyaya D, Deshpande VY. *Ginkgo biloba*- an appraisal. Kathmandu Univ Med J (KUMJ) 2003; 2: 225-229

[64] Clostre F. *Ginkgo biloba* extract (EGb 761). State of knowledge in the dawn of the year 2000. Ann Pharm Fr 1999; 57: 1S8-88

[65] Droy-Lefaix MT, Cluzel J, Menerath JM, Bonhomme B, Doly M. Antioxidant effect of a *Ginkgo biloba* extract (EGb 761) on the retina. Int J Tissue React 1995; 17: 93-100

[66] Pritz-Hohmeier S, Chao TI, Krenzlin J, Reichenbach A. Effect of *in vivo* application of the *Ginkgo biloba* extract EGb 761 (Rökan) on the susceptibility of mammalian retinal cells to proteolytic enzymes. Ophthalmic Res 1994; 26: 80-86

[67] Spadiene A, Savickiene N, Jurgeviciene N, Zalinkevicius R, Norkus A, Ostrauskas R, Skesters A. Silova A, Rodovicius H, Francaite-Daugeliene M. Effect of ginkgo extract on eye microcirculation in patients with diabetes. Cent Eur J Med 2013; 8: 736-741

[68] MacVie OP, Harney BA.Vitreous haemorrhage associated with *Gingko biloba* use in a patient with age related macular disease. Br J Ophthalmol 2005; 89: 1368-1387

[69] Lebuisson DA, Leroy L, Rigal G. Treatment of senile macular degeneration with *Ginkgo biloba* extract. A preliminary double-blind drug vs. placebo study. Presse Med 1986; 15: 1556-1558

[70] Fies P, Dienel A. Ginkgo extract in impaired vision-treatment with special extract EGb 761 of impaired vision due to dry senile macular degeneration. Wien Med Wochenschr 2002; 152: 423-426

[71] Jalali S. Retinal detachment. Comm Eye Health 2003; 16: 25-26

[72] Dunaief JL, Dentchev T, Ying GS, Milam AH. The role of apoptosis in age-related macular degeneration. Arch Ophthalmol 2002; 120: 1435-1442

[73] Barber AJ, Lieth, E Khin SA, Antonetti D, Buchanan AG, Gardner TW. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. J Clin Invest 1998; 102: 783-791

[74] Huynh TP, Mann SN, Mandal NA. Botanical compounds: effects on major eye diseases. Evid Based Complement Alternat Med 2013; 2013: 549174

[75] Fernández-Sánchez L, Lax P, Noailles A, Angulo A, Maneu V, Cuenca N. Natural compounds from saffron and bear bile prevent vision loss and retinal degeneration. Molecules 2015, 20: 13875-13893

[76] Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. Biochem Biophys Res Commun 1994; 201: 748-755

[77] Maclennan KM, Darlington CL, Smith PF. The CNS effects of *Ginkgo biloba* extracts and ginkgolide B. Prog Neurobiol 2002; 67: 235-257

[78] Ma K, Xu L, Zhang H, Zhang S, Pu M, Jonas B. The effect of *Ginkgo biloba* on the rat retinal ganglion cell survival in the optic nerve crush mode. Acta Ophthalmol 2010; 88: 553-557

[79] Falsini B, Piccardi M, Minnella A, Savastano C, Capoluongo E, Fadda A, Balestrazzi E, Maccarone R, Bisti S. Influence of saffron supplementation on retinal flicker sensitivity in early age related macular degeneration. Invest Ophthalmol Vis Sci 2010; 51: 6118-6124

[80] Ramassamy C, Clostre F, Christen Y, Costentin J. Prevention by a *Ginkgo biloba* extract (GBE 761) of the dopaminergic neurotoxicity of MPTP. J Pharm Pharmacol 1990; 42: 785-789

[81] Sastre J, Millán A, de la Asunción JG, Plá R, Juan G, Pallardo FV, O'Connor E. Martin JA, Droy-Lefaix MT, Viña J. A *Ginkgo biloba* extract (EGb-761) prevents mitochondrial aging by protecting against oxidative stress. Free Radic Biol Med 1998: 24: 298-330

[82] Baudouin C, Ettaiche M, Imbert F, Droy-Lefaix MT, Gastaud P, Lapalus P. Inhibition of preretinal proliferation by free radical scavengers in an experimental model of tractional retinal detachment. Exp Eye Res 1994; 59: 697-706

[83] Qiu QH, Z. G. Xie ZG, X. Xu X, S. X. Liang SX, Gao Y. EGb 761 on retinal light injury in rats. Chin Med J 2012; 125: 2306-2309

[84] Paasche G, Gärtner U, Germer A, Grosche J, Reichenbach A. Mitochondria of retinal Müller (glial) cells: the effects of aging and of application of free radical scavengers. Ophthalmic Res 2000; 32: 229-236

[85] Huckfeldt RM, Vavvas DG. Neuroprotection for retinal detachment. Int Ophthalmol Clin 2013; 53 (49): 105-117

[86] Murakami Y, Notomi S, Hisatomi T, Nakazawa T, Ishibashi T, Miller JW, Vavvas DG. Photoreceptor cell death and rescue in retinal detachment and degenerations. Prog Retin Eye Res 2013; 37: 114-140

[87] Li T, Yang S, She X, Yan Q, Zhang P, Zhu H, Wang F, Luo X, Sun X. Pharmacological interference of adrenergic receptor signaling preserve photoreceptors after retinal detachment through inhibition of oxidative stress and inflammation. Br J Pharmacol 2019; doi: 10.1111/bph.14565

[88] Behl T., Kotwani A. Chinese herbal drugs for the treatment of diabetic retinopathy. J Pharm Pharmacol 2017; 69: 223-235

[89] Bucolo C, Marrazzo G, Platania CBM, Drago F, Leggio M, Salomone S. Fortified extract of red berry, *Ginkgo biloba* and white bark in experimental early diabetic retinopathy. J Diabetes Res 2013; 2013: 432695

[90] Gupta N, Mansoor S, Sharma A, Sapkal A, Sheth J, Falatoonzadeh P, Kuppermann BD, Kenney MC. Diabetic retinopathy and VEGF. Open Ophthalmol J 2013; 7: 4-10

[91] Zhao Y, An X, Liu J, Liu S, Xu W, Yu X, Yu J. The improvement of oxidative stress by two proprietary herbal medicines in type 2 diabetes. Complement Ther Med 2018; 40: 120-125

[92] Zhao Y, Yu J, Liu J, An X. The role of Liuwei Dihuang pills and Ginkgo leaf tablets in treating diabetic complications. Evid Based Complement Alternat Med 2016; 2016: 7931314

[93] Zhao M, Wang XX, Wan. Effects of the *Ginkgo biloba* extract on the superoxide dismutase activity and apoptosis of endothelial progenitor cells from diabetic peripheral blood. Genet Mol Res 2014; 13: 220-227

[94] Nuhu AA. *Ginkgo biloba*: A "living fossil" with modern day phytomedicinal applications. J Appl Pharm Sci 2014; 4: 96-103

[95] IMO (Instituto de Microcirugía Ocular). Nd. Glaucoma IMO Available at: https://www.imo.es/es/glaucoma. Accessed November, 2018

[96] Pinazo-Duran MD, Shoaie-Nia K, Zanon-Moreno V, Sanz-Gonzalez SM, del Castillo JB, Garcia-Medina JJ. Strategies to reduce oxidative stress in glaucoma patients. Curr Neuropharmacol 2018; 16: 903-918

[97] Loskutova E, O'Brien C, Loskutov I, Loughman J. Nutritional supplementation in the treatment of glaucoma: A systematic review. Surv Ophthalmol. 2019; 64: 195-216

[98] Shim SH, Kim JM, Choi CY, Kim CY, Park KH. *Ginkgo biloba* extract and bilberry anthocyanins improve visual function in patients with normal tension glaucoma. J Med Food 2012; 15: 818-823

[99] Kang JM, Lin S. *Ginkgo biloba* and its potential role in glaucoma. Curr Opin Ophthalmol 2018; 29: 116-120

[100] González Aguaviva B. Monográfico del *Ginkgo biloba.* Med Natur 2011; 5: 93–99

[101] Chan KKW, Tang F, Tham CCY, Young AL, Cheung CY. Retinal vasculature in glaucoma: A review. BMJ Open Ophthalmol 2017; 1: e000032

[102] Lesk MR, Wajszilber M, Deschenes MC. The effects of systemic medications on ocular blood flow. Can J Ophthalmol 2008; 43: 351-355

[103] Kim SY, Kwak JS, Shin JP, Lee SH. The protection of the retina from ischemic injury by the free radical scavenger EGb 761 and zinc in the cat retina. Ophthalmologica 1998; 212: 268-274

[104] Menerath JM, Cluzel J, Droy-Lefaix MT, Doly M. Experimental electroretinographic exploration of retinal ischemia: preventive use of free radical scavengers and anti-PAF agents. J Ocul Pharmacol Ther 1997; 13: 81-88

[105] Droy-Lefaix MT, Menerath JM, Szabo-Tosaki E, Guillaumin D, Doly M. Protective effect of EGb 761 on ischemia-reperfusion damage in the rat retina. Transplant Proc 1995; 27: 2861-2862

[106] Szabo ME, Droy-Lefaix MT, Doly M, Braquet P. Free radical-mediated effects in reperfusion injury: a histologic study with superoxide dismutase and EGb 761 in rat retina. Ophthalmic Res 1991; 23: 225-234

[107] Szabo ME, Droy-Lefaix MT, Doly M. EGb 761 and the recovery of ion imbalance in ischemic reperfused diabetic rat retina. Ophthalmic Res 1995; 27: 102-109

[108] Szabo ME, Droy-Lefaix MT, Doly M. Direct measurement of free radicals in ischemic/reperfused diabetic rat retina. Clin Neurosci 1997; 4: 240-245

[109] Clostre F. Protective effects of a *Ginkgo biloba* extract (EGb 761) on ischemia-reperfusion injury. Therapie 2001; 56: 595-600

[110] Tian J, Liu Y, Chen K. *Ginkgo biloba* Extract in vascular protection: molecular mechanisms and clinical applications. Curr Vasc Pharmacol 2017; 15: 532-548

[111] Varga E, Bodi A, Ferdinandy P, Droy-Lefaix MT, Blasig IE, Tosaki A. The protective effect of EGb 761 in isolated ischemic/reperfused rat hearts: a link between cardiac function and nitric oxide production. J Cardiovasc Pharmacol 1999; 34: 711-717

[112] Chung HS, Harris A, Kristinsson JK, Ciulla TA, Kagemann C, Ritch R. *Ginkgo biloba* extract increases ocular blood flow velocity. J Ocul Pharmacol Ther 1999; 5: 233-240

[113] Chung SY, Cheng FC, Lee MS, Lin JY, Lin MC, Wang MF. *Ginkgo biloba* leaf extract (EGb761) combined with neuroprotective agents reduces the infarct volumes of gerbil ischemic brain. Am J Chin Med 2006; 34: 803-817

[114] Thiagarajan G, Chandani S, Harinarayana Rao S, Samuni AM, Chandrasekaran K, Balasubramanian D. Molecular and cellular assessment of *Ginkgo biloba* extract as a possible ophthalmic drug. Exp Eye Res 2002; 75: 421-430

[115] Park YH, Chiou GC. Structure-activity relationship (SAR) between some natural flavonoids and ocular blood flow in the rabbit. J Ocul Pharmacol Ther 2004; 20: 35-42

[116] Park JW, Kwon HJ, Chung WS, Kim CY, Seong GJ. Short-term effects of *Ginkgo biloba* extract on peripapillary retinal blood flow in normal tension glaucoma. Korean J Ophthalmol 2011; 25: 323-328

[117] Wimpissinger B, Berisha F, Garhoefer G, Polak K, Schmetterer L. Influence of *Ginkgo biloba* on ocular blood flow. Acta Ophthalmol Scand. 2007; 85: 445-449

[118] Harris A, Gross J, Moore N, Do T, Huang A, Gama W, Siesky B. The effects of antioxidants on ocular blood flow in patients with glaucoma. Acta Ophthalmol. 2018; 96: e237-e241

[119] Lerman S. Effects of sunlight on the eye. In: Ben Hur E, Rosenthal I, editors. Photomedicine. Boca Raton: CRC Press; 1985: 79–121

[120] Rahman K. Studies on free radicals, antioxidants, and co-factors. Clin Interv Aging 2007; 2: 219-226

[121] Boulton M, Rózanowska M, Rózanowski B. Retinal photodamage. J Photochem Photobiol B. 2001; 64: 144-61

[122] Ranchon I, Gorrand JM, Cluzel J, Droy-Lefaix MT, Doly M. Functional protection of photoreceptors from light-induced damage by dimethylthiourea and *Ginkgo biloba* extract. Invest Ophthalmol Vis Sci 1999; 40: 1191-1199

[123] Xie Z, Wu X, Gong Y, Song Y, Qiu Q, Li C. Intraperitoneal injection of *Ginkgo biloba* extract enhances antioxidation ability of retina and protects photoreceptors after light-induced retinal damage in rats. Curr Eye Res 2007; 32: 471-479

[124] Grosche J, Grimm D, Clemens N, Reichenbach A. Retinal light damage *vs.* normal aging of rats: altered morphology, intermediate filament expression, and nuclear organization of Müller (glial) cells. J Hirnforsch. 1997; 38: 459-470

[125] Bauduin C, Pisella PJ, Ettaiche M, Goldschild M, Becquet F, Gastaud P, Droy-Lefaix MT. Effects of EGb 761 and superoxide dismutase in an experimental model of retinopathy generated by initravitreal production of superoxide anion radical. Graefe's Arch Clin Exp Ophthalmol 1999; 237: 58-66

[126] Kose K, Dogan P, Ascioglu M, Ascioglu O. *In vitro* antioxidant effect of *Ginkgo biloba* extract (EGb 761) on lipoperoxidation induced by hydrogen peroxide in erythrocytes of Behcet's patients. Jpn J Pharmacol 1997; 75: 253-258

[127] Bilgihan A, Aricioğlu A, Bilgihan K, Onol M, Hasanreisoğlu B, Türközkan N. The effect of EGb 761 on retinal lipid peroxidation and glutathione peroxidase level in experimental lens induced uveitis. Int Ophthalmol 1994; 18: 21-24

[128] Ilieva I, Ohgami K, Shiratori K, Koyama Y, Yoshida K, Kase S, Kitamei H, Takemoto Y, Yazawa K, Ohno S. The effects of *Ginkgo biloba* extract on lipopolysaccharide-induced inflammation *in vitro* and *in viv*o. Exp Eye Res 2004; 79: 181-187

[129] Liu XP, Luan JJ, Goldring CE. Comparison of the antioxidant activity amongst Gingko biloba extract and its main components. Zhong Yao Cai 2009; 32: 736-740

[130] Wang J, Zheng M, Chen L, Liu Z, Zhang Y, Liu CM, Liu S. Rapid screening, separation, and detection of hydroxyl radical scavengers from total flavonoids of Ginkgo biloba leaves by chromatography combined with molecular devices. J Sep Sci 2016; 39: 4158-4165

[131] Rojas C, Rojas-Castañeda J, Ruiz-Sánchez E, Montes P, Rojas P. Antioxidant properties of a Ginkgo biloba leaf extract (EGb 761) in animal models of Alzheimer´s and Parkinson´s diseases. Curr Top Nutraceutical Res 2015; 3: 105-120

[132] Mahadevan S, Park Y. Multifaceted therapeutic benefits of Ginkgo biloba L.: chemistry, efficacy, safety, and uses. J Food Sci 2008; 73: R14-9.

[133] Rojas P, Montes S, Serrano-García N, Rojas-Castañeda J. Effect of EGb 761 supplementation on the content of copper in mouse brain in an animal model of Parkinson's disease. Nutrition 2009; 25: 482-485

[134] Rojas C, Montes S, Rojas O. EGb 761 supplementation increases manganese content in mouse brain in an animal model of Parkinson's disease. Curr Top Nutraceutical Res 2014; 12: 57-64

[135] Kaur S, Sharma N, Nehru B. Anti-inflammatory effects of Ginkgo biloba extract against trimethyltin-induced hippocampal neuronal injury. Inflammopharmacology 2018; 26: 87-104

[136] Dumont E, D'Arbigny P, Nouvelot A. Protection of polyunsaturated fatty acids against iron-dependent lipid peroxidation by a Ginkgo biloba extract (EGb 761). Methods Find Exp Clin Pharmacol 1995;17: 83-88

[137] Drieu K, Vranckx R, BenassayadC, Haourigi M, HassidJ, Yoa RG, Rapin JR, Nunez EA. Effect of the extract of Ginkgo biloba (EGb 761) on the circulating and cellular profiles of polyunsaturated fatty acids: correlation with the anti-oxidant properties of the extract. Prostaglandins, Leukot Essent Fatty Acids 2000; 63: 293-300

[138] Tachikawa M, Akanuma S-I, Imai T, Okayasu S, Tomohiro T, Hatanaka Y, Hoso K-I. Multiple Cellular transport and binding processes of unesterified docosahexaenoic acid in

outer blood–retinal barrier retinal pigment epithelial cells. Biol Pharm Bull 2018; 41: 1384–1392

[139] Ibarra M, Moreno L, Vera R, Cogolludo A, Duarte J, Tamargo J, Perez-Vizcaino F. Effects of the flavonoid quercetin and its methylated metabolite isorhamnetin in isolated arteries from spontaneously hypertensive rats. Planta Med 2003; 69: 995-1000

[140] Lin H, Wang H, Chen D, Gu Y. A dose-effect relationship of Ginkgo biloba extract to nerve regeneration in a rat model. Microsurgery 2007; 27: 673-677

[141] Zhu Z, Zhou X, He B, Dai T, Zheng C, Yang C, Zhu S, Zhu J, Zhu Q, Liu X. Ginkgo biloba extract (EGb 761) promotes peripheral nerve regeneration and neovascularization after acellular nerve allografts in a rat model. Cell Mol Neurobiol 2015; 35: 273-282

[142] Sun BL, Hu DM, Yuan H, Ye WJ, Wang XC, Xia ZL, Zhang SM, Wang LX. Extract of Ginkgo biloba promotes the expression of VEGF following subarachnoid hemorrhage in rats. Int J Neurosci 2009; 119: 995-1005

[143] Oh JH, Oh J, Togloom A, Kim SW, Huh K. Effects of Ginkgo biloba extract on cultured human retinal pigment epithelial cells under chemical hypoxia. Curr Eye Res 2013; 38: 1072-1082

[144] Juárez CP, Muiño JC, Guglielmone H, Sambuelli R, Echenique JR, Hernández M, Luna JD. Experimental retinopathy of prematurity: angiostatic inhibition by nimodipine, ginkgo-biloba, and dipyridamole, and response to different growth factors. Eur J Ophthalmol 2000; 10: 51-9

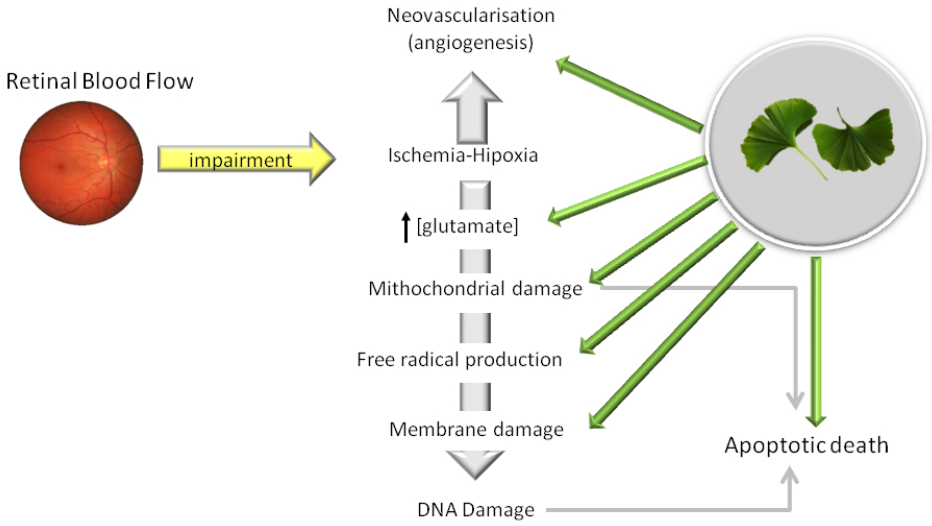
FIGURE LEGENDS:

Figure 1. Mechanisms of action of ginkgo in ischaemic retinal disease. Based on the article: Osborne *et al* [13].

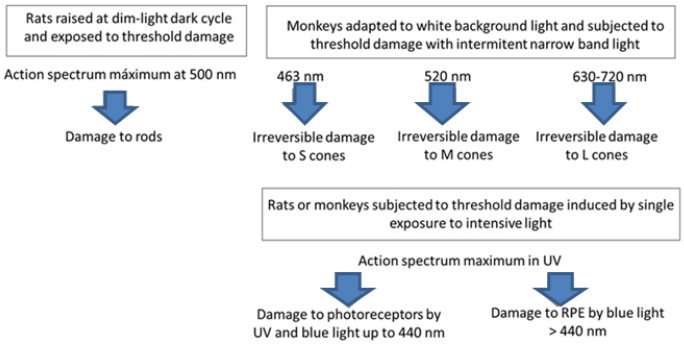
Figure 2. Types of light-induced damage to the retina. Based on the article: Boulton *et al*. [121].

Figure 3. N-retinylidene-N-retinylethanolamine

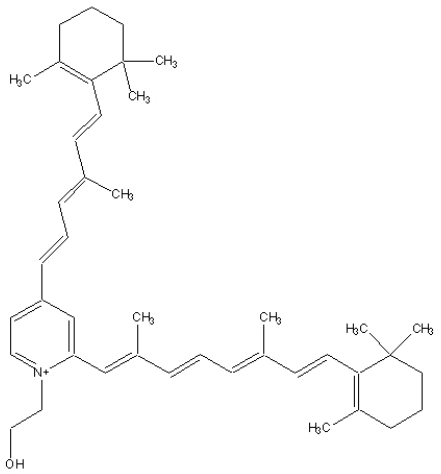
Figure 4. EGb 761 main flavonoids.



254x190mm (96 x 96 DPI)

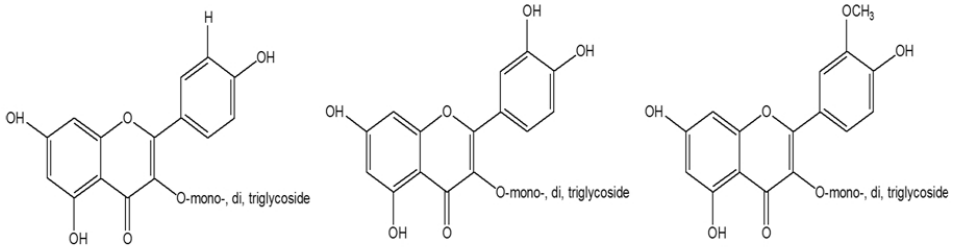


254x190mm (96 x 96 DPI)



N-retinylidene-N-retinylethanolamine

254x190mm (96 x 96 DPI)



Kaempherol derivatives Quercetin derivatives Isorhamnetin derivatives

254x190mm (96 x 96 DPI)

CEU Universidad Cardenal Herrera

Letter to the Editor

Dra Isabel Martínez Solís
Dpto Farmacia
Facultad de Farmacia
Universidad Cardenal Herrera CEU
+34 96 136 90 00 | Ext. 64329
isolis@uchceu.es

Dear Editor, Professor José L. Ríos,

Firstly, we would like to thank *Planta Medica* for this opportunity to publish our work in this prestigious journal. It is very important for us because *Planta Medica* is one of the most influential publications in the phytotherapy field.

All reviewers' comments and suggestions have been considered in the revised manuscript. We believe that the paper has been significantly improved after introducing these corrections. We would like to thank the reviewers for their interest and arduous work.

Please if any further corrections are needed, let us know and we would be pleased to correct it again.

Sincerely yours and awaiting for your response.

The authors

Universidad Cardenal Herrera-CEU, CEU Universities

Alfara del Patriarca, Valencia, Spaini

Monday, March 22, 2019

Dear Editors:

Please find enclosed a manuscript entitled: “Neuroprotective potential of *Ginkgo biloba* in retinal diseases" which we are submitting for exclusive consideration of publication as an article in *Planta Medica*.

Editors invited us for publishing a review article in the neuroprotection field. In this sense, we raise the effect of *Ginkgo biloba* on neurodegenerative diseases in the retina because apoptotic nerve cell death is implicated in the pathogenesis of several neurodegenerative conditions that affect retinas. Like other tissues of the central nervous system, the retina is susceptible to damage by oxidative processes that result in age-related macular degeneration, diabetic retinopathy, glaucoma, ischaemic retinal disease, retinal disease produced by light oxidation and detached retina, among other diseases. The main purpose of this review paper is to provide a concise, accurate information to the subject matter about the neuroprotective effect of *Ginkgo biloba* on retina neurodegenerative diseases, and inform the reader critical of the latest developments.

Thank you for your consideration of our work! Please address all correspondence concerning this manuscript to the corresponding author.

Sincerely,

The authors