Neuroprotective Potential of *Ginkgo biloba* in Retinal Diseases

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

received March 22, 2019
revised May 22, 2019
accepted June 2, 2019

Bibliography
DOI https://doi.org/10.1055/a-0947-5712
Published online July 2, 2019 | Planta Med 2019; 85: 1292–1303 © Georg Thieme Verlag KG Stuttgart · New York | ISSN 0032-0943

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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 they regulate redox homeostasis and reduce oxidative stress. This is important for 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 information 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.

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, and 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]. ROS are involved in the progression of retina disorders, including aging, apoptosis, and post-ischaemic cellular injuries [5]. Glaucoma [6, 7], DR [8–10], ARMD [11, 12], and ischaemic retinal injuries [13] are examples of ROS and retina disease relationships. In addition, excessive ROS generation and the consequent induction of oxidative stress are some factors that trigger the cellular response to RD, and are also a major cytotoxic factor for photoreceptor apoptosis [14]. Indeed, antioxidants protect against oxidative stress and prevent ROS production, and they should act as neuroprotectants with therapeutic options for retinal diseases.

ABBREVIATIONS

ARMD	age-related macular degeneration
BD	Behcet's disease
CNV	choroidal neovascularisation
DR	diabetic retinopathy
GBEs	*Ginkgo biloba* extracts
IOP	intraocular pressure
NO	nitric oxide
NOX	NADPH oxidase
NTG	normal-tension glaucoma
PAF	platelet activating factor
POAG	primary open-angle glaucoma
PUFAs	polyunsaturated fatty acids
RCTs	randomised controlled trials
RD	retinal detachment
ROMs	reactive oxygen metabolites
ROS	reactive oxygen species
RP	retinitis pigmentosa
RPE	retinal pigment epithelium
TM	trabecular meshwork
VEGF	vascular endothelial growth factor

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 GBEs 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 and 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 acid-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, and 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 GBEs protect neurons against necrosis and apoptosis induced by ROS, Ca^{2+} overload, NO, and 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]. GBEs maintain ATP content thanks to mitochondrial respiration protection and oxidative phosphorylation preservation. The neuroprotective capacity of ginkgo leaves should allow for the treatment and prevention of ocular pathologies such as glaucoma, DR, and 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, GBEs 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 damage caused by oxidative processes: ARMD, DR, glaucoma, ischaemic retinal disease, retinal disease produced by light oxidation, and 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 GBEs. The search strategy was conducted using a systematic and standardised review of curated databases such as PubMed, MEDLINE, and SciFinder in 2000–2019. Papers published in 1985–2000 were used when they were necessary to explain concepts and processes. The search was based on the following key words: *Ginkgo biloba*, Ginkgoaceae, neurodegeneration, retinal diseases, age-related macular degeneration, diabetic retinopathy, glaucoma, and ischaemic retinal disease. Other key words were 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]. The macula, a small portion of the central area of the retina containing the maximum density of photoreceptors, is the part of the eye that provides sharp central vision, and is responsible for the most detailed vision that we need for objects that lie straight ahead. 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 than 50% by the year 2020 [29, 30–32]. 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 a damaged macula. Late ARMD is classified into two general subgroups: dry ARMD and wet ARMD. Dry (atrophic, non-exuda-

tive) 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. Such damage may be rapid and severe, unlike the more gradual course of dry ARMD. Approximately 85–90% of 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]. Despite its pathogenesis remaining obscure, 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 PUFAs 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 the RPE is implicated in the pathogenesis of ARMD [38]. There is also compelling evidence to indicate the association of VEGF to CNV processes in wet ARMD [28, 39]. Although currently available treatments can slow down the vision loss rate in neovascular ARMD, they do not significantly improve vision. Presently, the therapeutic mainstays used to treat wet ARMD include verteporfin (Visudyne) photodynamic therapy and intravitreal anti-VEGF agents [28, 33]. 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-VEGF 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 the limited treatment options available, and given the relatively high prevalence and increased incidences of ARMD as populations 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 of berries [bilberry (*Vaccinium myrtillus* L.), cranberry (*Vaccinium oxycoccos* L.), blackcurrant (*Ribes nigrum* L.), wolfberry (*Lycium barbarum* L.), grapes (*Vitis vinifera* L.)], stigmas [such as saffron (*Crocus sativus* L.)], and roots and rhizomes [such as turmeric (*Curcuma longa* L.) and Dan Shen (*Salvia miltiorrhiza* Bunge) [36, 48, 50, 53–59] on age-related ocular diseases. 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 PAF antagonism of ginkgolides [48, 50, 58, 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 studies 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, with increased microcircular blood velocity, flow, and volume [67]. Consequently, the use of ginkgo extracts in treating patients with age macular degeneration has aroused emerging interest because vascular factors and oxidative damage are thought to be two potential mechanisms in the pathology of ARMD [43, 63]. Ginkgo extracts also have 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 increasing 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 GBEs in patients. The literature includes a systematic review published in 2013 [52] that reports two RCTs using GBEs with positive effects on vision in patients with ARMD. In the Lebuisson et al. RCT [69], a double-blind trial was conducted to compare EGb761 (80 mg twice daily, 160 mg) with a placebo in 10 outpatients aged over 55 years attending an eye clinic. Treatment lasted for 6 months. Drug effectiveness was assessed on fundoscopy results and on visual acuity and visual field measurements. Despite 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 and Dienel [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 treatment groups only after 4 weeks, with more pronounced improvements in group I. According to Evans [52] and Sin et al. [59], both RCTs showed positive effects of ginkgo on vision, but these trials were too small with a short observation period to provide concluding evidence. In addition, Bartlett and Eperjesi [42] suggested that Lebuisson's beneficial findings should be viewed cautiously as the assessment of the 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 GBEs 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

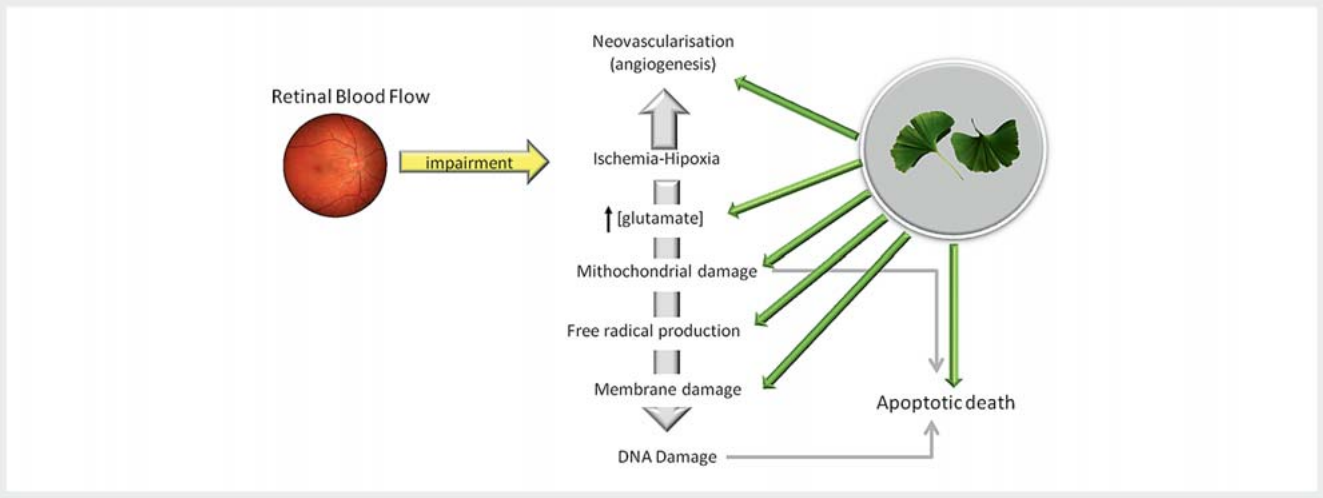
RD is a serious event that can result in vision loss. Retinal photoreceptors receive oxygen and metabolic support from the choroid. If the retina separates from the choroid, photoreceptor cells die and cause damage that can lead to blindness. Basically, there are three types of RD, the most frequent 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 successful in 80–99% of cases, but retinal death due to RD has no 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 antiapoptotic, anti-inflammatory, or neurotrophic compounds. In fact, the use of antioxidant compounds or antiapoptotic, anti-inflammatory, or 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 demonstrated 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, GBEs 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 GBEs show against NO in studies on mammalian cells. By preventing the 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 RP [77]. Ma et al. [78] conducted studies with Sprague-Dawley rats by injecting a GBE, followed by the crushing of the optic nerve. The animals that received the GBE by intraperitoneal injection prior to the 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 1960s [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, EGb 761 has powerful properties to eliminate free radicals and offers protection against oxidative stress. The therapeutic potential of EGb 761 can, at least in part, be attributed to this 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 showed 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 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 most common microvascular complication of diabetes mellitus, 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 complications 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 ROMs compared with healthy subjects, and it has been demonstrated that ROMs increase rapidly in serum as DR progresses [89]. The main treatment for DR is intensive glycaemic control. Another important treatment is laser photocoagulation, but it is not effective in many patients. In patients with diabetic macular oedema, this being the major complication of DR, intravitreal steroid injections are used (including triamcinolone, which it is not very common nowadays, and dexamethasone implants, which is 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, GBEs 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 reducing 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 vs. 8.5% from the treatment group). Zhao et al. [93] conducted a study by performing blood testing among diabetic patients to conclude that GBEs lower the apoptosis rate of retinal vascular endothelial cells. Other research works done in rats [89] link GBEs with the prevention of an increase in VEGF and TFN-α, proinflammatory mediators. In other diabetic animal models, GBEs have been seen to reduce NO-induced oxidative stress, besides decreasing its production. This attenuates the apoptosis of retinal ganglion cells and photoreceptor cells. GBEs have anti-inflammatory effects, and one of the possible mechanisms is the ability to downregulate the expression of PAF. GBEs 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 GBEs [94]. According to their background, GBEs 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. GBEs could be potential candidates for use in the prevention and treatment of DR.

Glaucoma

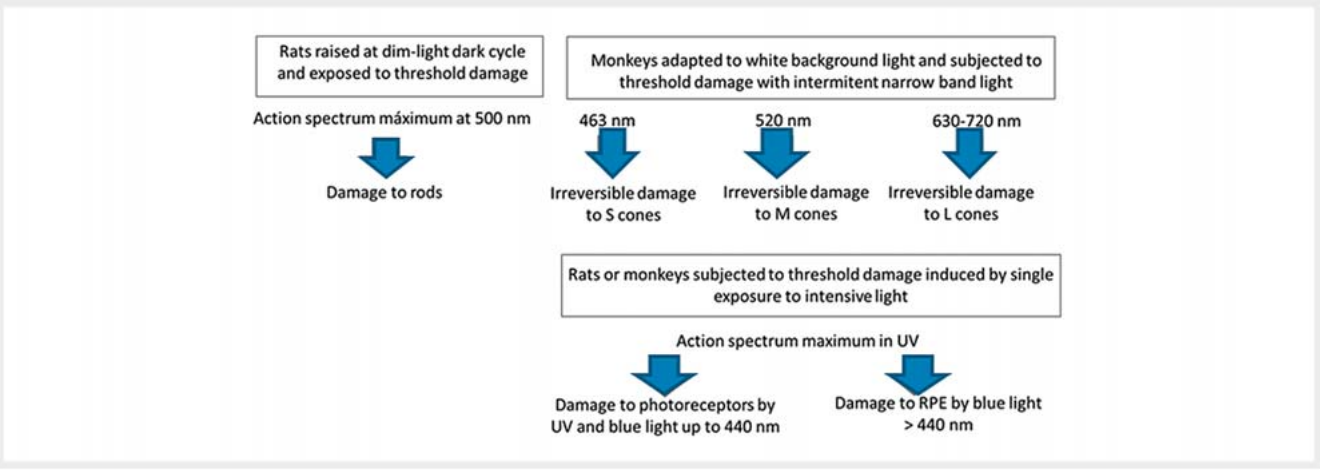
Glaucoma can be defined as a series of ocular diseases characterised by a progressive reduction of the visual field due to secondary optic nerve damage. It is mainly a consequence of IOP that causes optic nerve injuries, starting with retinal ganglion cell apoptosis [74]. This disease causes progressive and irreversible damage, and it remains asymptomatic and 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 years and more. However, it can be prevented if treatment begins in the early disease stages [95]. There are several types of glaucoma, the most common of which is 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 sclerocorneal TM. Several studies report a reduced field of vision and increased IOP associated with the oxidative damage of TM cells' DNA in the patients' glaucoma, plus an imbalance in the redox status being related to the high expression of endothelial-leukocyte adhesion molecules 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 GBEs, can help to regulate IOP and protect the 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 began due to the great interest that the antioxidant and neuroprotective properties of ginkgo generated, which can be used against glaucoma. Thus, GBEs have demonstrated that they play an important role in the improvement of several degenerative eye diseases, including glaucoma. GBEs can improve the vision of patients with 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 GBEs used against glaucoma [100]. In conclusion regarding GBEs and glaucoma, based on their background, GBE as a neuroprotector can prevent retinal ganglion cell damage. These extracts can also be used to prevent and treat other retinal neurodegenerative diseases. The use of GBEs is proposed to improve oxidative stress in NTG patients and to increase visual acuity, but further studies are needed.



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

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 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]. In regards to natural products, very few studies on 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 (▶ **Fig. 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, EGb761 administration significantly improves 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 case 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. In 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 GBEs on the biomarkers of ocular perfusion in patients with open-angle glaucoma 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 crossover 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 crossover study (N:45), the effects on blood flow of a complex preparation containing ginkgo and other antioxidant substances (vitamins, minerals, omega-3 from fish oil, PUFAs, *N*-acetylcysteine, bilberry fruit extract, coenzyme Q10, grape seed extract,

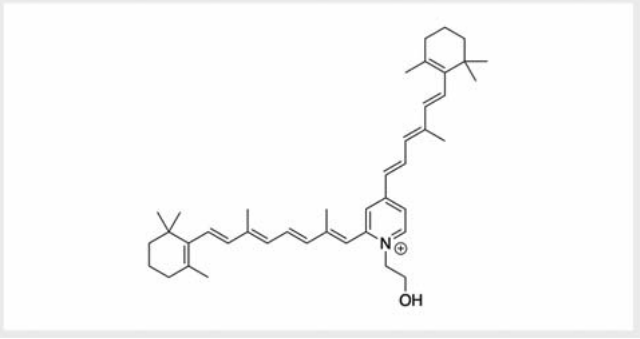


▶ **Fig. 2** Types of light-induced damage to the retina. Based on the article of Boulton et al. [121].

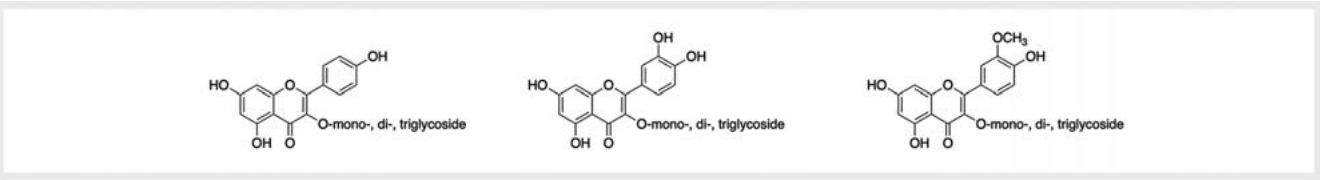
quercetin, flax seed oil, etc.) in patients with open-angle glaucoma were tested [118]. One month after treatment, a statistically significant increase in blood flow velocities in all the retrobulbar blood vessels was observed as compared to the placebo. Superior and inferior retinal capillary mean blood 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]. The 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 (▶ **Fig. 2**) [121]. A photosensitive derivative of visual pigment *N*-retinylidene-*N*-retinylethanolamine (▶ **Fig. 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 (▶ **Fig. 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, and 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



▶ **Fig. 3** *N*-retinylidene-*N*-retinylethanolamine.



▶ **Fig. 4** EGb 761 main flavonoids.

et al. [124] demonstrated that EGb 761 administration does not prevent Müller cell hypertrophy, but inhibits the expression of pathological marker molecules.

Others

GBEs can be used in processes that are the primary cause of the aforementioned retina pathologies, or are secondary causes that lead to disease development. 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 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 the 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. GBEs act on preventing the 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 GBEs conserve mitochondrial metabolism and ATP production in tissues by inhibiting morphological distortion and oxidative damage from mitochondrial aging. Others have demonstrated the ability of GBEs for scavenging NO and, hence, their protector effect against NO reactivity [74].

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 GBEs antioxidant capacity. Components of the extracts are able to scavenge different free radicals, including ROS, such as hydroxyl radicals (OH·) [130], superoxide radicals (O_2^-), peroxyl radicals (ROO·), and hydrogen peroxide (H_2O_2), among others, and also reactive nitrogen species, such as nitric oxide (NO·) 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 GBEs 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 enzymes [133, 134]. The NO scavenging capacity of GBEs can inhibit iNOS expression. In this sense, the inhibition of NF-κB mediated by GBEs 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's dual role as an antioxidant and anti-inflammatory agent [135]. The abundant 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. [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 the synthesis and/or catabolism of PUFAs. These results pointed out that in addition to the antioxidant capacity of ginkgo components, the increase in PUFAs can explain a most durable effect of EGb 761. PUFAs may be the target of oxidative damage, preserving other important molecules. On the other hand, ginkgo extract avoids peroxidation of membrane PUFAs, preserving membrane fluidity and keeping their integrity. Furthermore, docosahexaenoic acid, which was increased twofold in rats after EGb 761 treatment, plays an essential role in visual function, regulating via rhodopsin the photosignal transduction, and protecting RPE from oxidative stress [138].

GBEs 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 an injured peripheral nervous system [140]. An increase in angiogenesis has been reported by Zhu et al. [141] as a consequence of VEGF, with a significant rise in rats treated with EGb 761 after acellular nerve allografts. GBEs have also shown, in a rat model, a promotion of VEGF expression in ischaemic brain tissue after a subarachinoid haemorrhage [142]. However, there are contradictory results regarding a ginkgo effect over this growth factor. Oh et al. [143] reported

that GBEs reduce hypoxia-inducible factor-1α and VEGF expression in cultured RPE cells under chemical hypoxia. Juárez et al. [144] found similar results in a murine model. The vasodilating properties of GBEs make choroidal circulation have large-calibre vessels and a high flow rate, providing cells more oxygen and attenuating some retinal diseases in a noninvasive way. However, more studies are needed in order to clarify inconsistent data before GBEs can be used as a therapeutic agent in diseases such as macular degeneration.

The experimental and clinical data obtained to date indicate that gingko leaf extract is well tolerated and is apparently safe for 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 diseases produced by light oxidation, and RD. The specific mechanism by which GBEs 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, GBEs could be an effective phytochemical therapeutic target, but further studies are necessary.

Authors' Contributions

All authors contributed to the design of the study, the selection of the retinal diseases, the establishment of the inclusion and exclusion criteria, the background selection, and the approach of our review study. All authors have assessed all comments and criticisms to write and review the article. Each author was responsible for a specific section. Isabel Martínez-Solís and Francisco Bosch-Morell together investigated the background of *G. biloba* use in cases of several retinal diseases such as Behcet's disease and retinitis pigmentosa. Nuria Acero was mainly responsible for the introduction and discussion sections. Encarna Castillo was mainly responsible for the section on ginkgo use when the retinal disease was produced by light oxidation. María Eugenia González-Rosende was mainly responsible for the section on ginkgo use in the event of ARMD. Dolores Muñoz-Mingarro was responsible for the section linked to the use of ginkgo for retinal detachment. Teresa Ortega investigated the use of *G. biloba* in patients that suffered from ischaemic retinal disease. María Amparo Sanahuja was responsible for the section related to the use of ginkgo for glaucoma. Finally, Victoria Villagrasa investigated ginkgo and diabetic retinopathy. The work was coordinated by Isabel Martínez-Solís.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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