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Ginkgo Biloba Extract 761 Reduces the Risk of Progression of Age-Related Macular Degeneration*

El extracto de Ginkgo biloba 761 reduce el riesgo de la progresión de la generación macular asociada a la edad

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ABSTRACT

Age-related macular degeneration (AMD) is the leading cause of blindness. Although its development is poorly understood, high concentrations of reactive oxygen species, maybe trigger it. The aim of this study was to analyze whether the antioxidant action of the dietary supplement Ginkgo biloba (EGb-761) can reduce the progression of AMD. A clinical trial was conducted in Mexico, with people over 55 years of age. AMD patients were treated with EGb-761: 50 mg/day (n = 26) or 100 mg/day (n = 22) for 3 months. A control group with 22 healthy people (without treatment) was included. Before and after treatment, functional and structural ocular tests were performed, and systemic oxidative stress (OS) was measured. Changes between baseline and after the treatment with both doses were significant ($P < 0.05$). Contrast sensitivity increased 16% and 17%, the number of metamorphopsias (central vision integrity) decreased 100% and 95%; central perimetry (scotoma) decreased 93% and 88%, and hyperpigmentation (autofluorescence) decreased 88% and 89%, respectively. For OS, malondialdehyde concentration decreased 7.6% and 8.5%, and reduced glutathione-levels increased 5.3% and 7.1%, respectively. We suggest giving low doses of Gb to older adults with early-stage AMD to reduce or avoid side effects.

Keywords: Ginkgo biloba; age-related macular degeneration; oxidative stress; reactive oxygen species; malondialdehyde; EGB-761.

* Artículo de investigación.

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RESUMEN
La degeneración macular relacionada con la edad (AMD) es la principal causa de ceguera. Su desarrollo es poco conocido, pero concentraciones altas de especies reactivas de oxígeno pueden desencadenarla. El objetivo del estudio fue analizar si la acción antioxidante del suplemento dietético ginkgo biloba (EGb-761) retrasa el desarrollo de la AMD. Se trata de un ensayo clínico hecho en México, con personas mayores de 55 años. Pacientes con AMD recibieron EGb-761: 50 mg/día (n = 26) o 100 mg/día (n = 22), durante 3 meses. Participó un grupo control de 22 personas sanas. Antes y después del tratamiento, se realizaron pruebas oculares funcionales y estructurales, y se midió el estrés oxidativo (OS). Se encontraron cambios significativos (P < 0,05) entre el inicio y después del tratamientos con ambas dosis. La sensibilidad al contraste aumentó 16 % y 17 %, las matamorfopsias (integridad de la visión central) 100 % y 95 %; la perimetría central (escotomas) disminuyó 93% y 88%; y la hiperpigmentación (autofluorescencia) disminuyó 88% y 89%, respectivamente. Para el OS, la concentración de malondialdehído disminuyó 7,6 % y 8,5 %, y los niveles de glutatión aumentaron 5,3 % y 7,1 %, respectivamente. El EGb-761 tuvo un efecto benéfico sobre la AMD. Se sugiere administrar dosis bajas de Gb a adultos mayores con AMD temprana, para reducir o evitar efectos secundarios.

Palabras clave: Ginkgo biloba; degeneración macular relacionada con la edad; estrés oxidativo; especies reactivas de oxígeno; malondialdehído; EGB-761.

INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of blindness in older people (over 50 years old). With an aging population, it was projected to 196 millions in 2020, increasing to 288 millions in 2040 (1). There is currently no cure available for AMD, and even palliative treatments are rare. Thus, this debilitating disease requires an urgent solution to supply quality of life to people and decrease treatment costs (2, 3). In such context, AMD is a chronic and progressive disorder characterized by changes within the macula, reflecting the aging process (4). This pathophysiological process affects the outer neural retina, the retinal pigment epithelium (RPE), the Brunch's membrane, and the choroid (5-8).

AMD is also characterized by a progressive loss of central vision attributable to degenerative and neovascular changes in the interface between the neural retina and the underlying choroid (9, 10). Also, the most debilitating form is exudative (wet) AMD, in which rapid and severe loss of vision occurs due to choroidal neovascularization. In contrast, non-exudative (dry) AMD is identified by a gradual loss of vision, which is associated with reticular pigmentary changes (drusen), and geographic atrophy of the pigmentary epithelium without neovascularization (11).

However, early AMD can be asymptomatic, despite the presence of drusen and changes in the RPE. As well, there may be a severe central visual-field loss in advanced AMD, but the peripheral visual acuity is usually preserved (12, 13).

In addition to advanced age, genetic risk factors include female gender and white race. Moreover, the non-genetic risk factors are cigarette smoking and a low dietary intake of zinc and carotenoids (5, 14-16). Also, the global prevalence of early, late and any AMD was calculated at 8.01%, with a higher prevalence in Europeans than in Asians (1, 17). Although no statistic shows the prevalence of AMD in Mexico, it is estimated to be 10%, based on the values of Latinos residing in Los Angeles (18).

AMD's pathophysiology is thus poorly understood, and the prevention or slowing of disease progression has been difficult to reach. Among molecular links in AMD pathogenesis is the oxidative stress in the retina, a structure that is particularly susceptible to damage by reactive oxygen species (ROS) (19, 20). In addition to oxidative

stress, the process of lipofuscin formation occurring mainly in the retina may also contribute to AMD development (21).

It has been proposed that antioxidants may prevent cellular damage in the retina by reacting with free radicals produced in light absorption. Therefore, dietary levels of antioxidant vitamins and minerals may reduce the risk of the progression of AMD (22). On this matter, the Ginkgo biloba extract (EGb-761), a standardized mixture of active flavonoids (quercetin, kaempferol and isorhamnetin), has shown antioxidant properties (23, 24). Thus, the flavonoids of Gb, scavengers of free radicals and ROS, inhibit lipid peroxidation of cell membranes and augment levels of endogenous antioxidants (25-28). In addition, some biochemical or pharmacological activities of Gb include an anti-inflammatory effect, a relaxing action on vascular walls, and an antagonistic effect on platelet-activating factors (26, 29). Therefore, Gb has been suggested to improve long-distance visual acuity in human patients suffering from senile macular degeneration (30, 31). Moreover, there are reports of studies using low doses of EGb 761 (40-80 mg) in animal models (32) and older patients with macular degeneration (33), which showed visual improvement, and they were also safe doses (34). However, Evans (35) has suggested that the beneficial effect of Gb on retarding the prevention of AMD is yet to be confirmed.

Therefore, we hypothesized that reducing oxidative eye damage with antioxidant compounds such as EGb-761 may reduce the risk of AMD progression. Thus, this study aimed to analyze whether the dietary supplement Ginkgo biloba extract 761 (EGb-761) can reduce the progression of AMD. We evaluated 2 doses of Gb to know which one would be beneficial for the elderly.

MATERIAL AND METHODS

A monocentric controlled clinical trial was carried out. Such study involved patients diagnosed with AMD treated at the Optometry and Ophthalmology Services in the Medicine Teaching Unit of the Autonomous University of Aguascalientes. Patients referred from the Ophthalmology Units of hospitals in Aguascalientes, Mexico, were also included. The study complied with the ethical principles stated in Helsinki's Declaration (64th General Assembly, Fortaleza, Brazil, October 2013). Also, the Bioethics Committee of the Autonomous University of Aguascalientes reviewed and approved the project (Number 314-14). Subsequently, the participants signed informed consent. A standardized commercial extract of Ginkgo biloba (EGb-761, Nutra Manufacturing-GNC, South Carolina-USA) was used (36). It's worth to mention that most EGb 761 extracts are standardized to 24% flavonoids and 6% terpenoids (37).

Inclusion criteria corresponded to people of indistinct gender, over 55 years of age, and diagnosed with early-stage AMD. Also, the presence and severity of various drusen features and typical age-related maculopathy lesions were determined by grading stereoscopic color fundus photographs with the Wisconsin Age-Related Maculopathy Classification System (23).

Exclusion criteria were having a psychiatric illness, history of severe gastrointestinal problems or defective absorption, allergy to any of the active ingredients of EGb-761, eye conditions (glaucoma, diabetes, hypertensive retinopathy, or history of laser therapy), and previous or current use of the studied supplement (Gb).

SUBJECTS

The study was blinded and conducted with 70 people (22 healthy participants and 48 with AMD).

In such context, AMD patients were randomly allocated into 2 groups to receive EGb-761: 50 mg/day (n = 26 or n = 52 eyes) or 100 mg/day (n = 22 or n = 44 eyes) (32-34) for 3 months (38-40). Also, 22 healthy people (n = 44 eyes) participated as the untreated control group that received no placebo. Thus, a total of 140 eyes were studied:

96 with dry AMD and 44 with healthy eyes. The sample of patients affected by AMD was elected for convenience to obtain early information about treatment effectiveness tendencies with Gb. Also, before the start of this study, we thought that if there were a rapid progression of visual impairment, it would be more difficult to show that treatment could slow the progression of vision decline. Therefore, the time intervals to review the progression of visual acuity loss will have to be short (37). Briefly said, because of that, time intervals for review of the progression of visual acuity loss would have to be short.

A standardized extract of Ginkgo biloba (EGb-761), Nutra Manufacturing-GNC, South Carolina-USA, was used. We did not include a placebo group, because other studies have shown that patients in the placebo group had a higher probability of advancing macular damage than those receiving antioxidants (41).

Also, the diagnosis is mainly clinical, but fluorescein angiography (FA) and optical coherence tomography (OCT) are diagnostic tests that have proven to be valuable tools in the characterization and treatment of the follow-up of this disease.

It's worth to mention that all tests have limitations, but the combination of structural and functional variables significantly improves their power to detect changes. In addition, we included measuring the levels of antioxidant enzymes to corroborate that the treatment had reduced free radicals. Structural and functional ocular parameters were measured in all subjects as well (from the control and Gb-treated groups) at baseline, and 3 months after treatment with GB.

FUNCTIONAL VARIABLES

a. Visual acuity was measured by contrast sensitivity (CS) (the ability of the optical system to determine textures of different intensities). After, the Hamilton-Veale Contrast Sensitivity Test was done with an exam recognized by the Food and Drug Administration (FDA-USA) as a precise method to evaluate vision clinical trials. The CS data were obtained using the commercially available Pelli-Robson chart.

b. The central vision's integrity was measured with the Amsler grid, a card with grids, and a central fixing point. Such method was used to detect retinal abnormalities in the central visual field (20°). Each frame measures 5 mm and occupies an angle of 1 degree in the distance visual field of 30 cm. In such scenario, the person was asked to look at the center of the card, and was asked questions, as if they perceived deformity of the picture lines or the presence of spaces that interrupt the grid. This study was made in a monocular way.

c. Computerized central perimetry was performed with an Optocol PTS Canon 1000 test that detects and measures the size and intensity of alterations in the visual field, such as scotomas, depressions, contractions, or reductions of sensitivity. As a result, several partial or total scotomas were observed.

STRUCTURAL VARIABLES

a. Autofluorescence photographs were obtained with a TRC-NW8F camera. In such context, the fundus test by autofluorescence is a diagnostic imaging procedure (non-invasive) that allows the evaluation of the present distribution of lipofuscin in the retinal pigment epithelium (RPE). These photographs capture the fluorescence of fundus elements. Thus, an increase in the fluorescence value represents the accumulation of lipofuscin and indicates that the cells of the RPE have begun to fail.

b. Retinal imaging was obtained by optical coherence tomography (OCT), with the Optovue equipment, allowing a diagnostic method that provides a high-resolution image of ocular microstructures through longitudinal and transversal cuts.

SYSTEMIC OXIDATIVE STRESS

a. Blood samples (10 mL) were obtained from the participants at the study's beginning and end. The samples were analyzed to measure malondialdehyde (MDA) levels according to the method reported by Jentzsch (42). This method is based on the reaction of MDA with 2-thiobarbituric acid (TBA) to form very stable MDA-TBA chromogenic adducts that can be quantified by visible absorption spectrophotometry (532-535 nm) (Spectrophotometer DMS-80).

b. The determination of reduced glutathione (GSH) in blood was performed by the method of Cohn and Lyle (43). This method is based on the reaction of GSH with fluorescent reagent o-phthalaldehyde (OPT), forming a fluorescent OPT-GSH complex. The fluorescence intensity for this complex is directly proportional to the concentration of GSH in the sample. The fluorescence emission was measured at 420 nm with the WINLAB program of a Perkin-Elmer Luminescence Spectrometer Model LS 50-B.

STATISTICAL ANALYSES

Data were expressed as mean (± SD) and were analyzed by the student's t-test; for qualitative values, and to determine if the variables are associated or not, a chi-square test was employed. The SPSS statistical package (Version 17) was used for data evaluation. Significant differences were considered when $p < 0.05$.

RESULTS

The participants' age was 64.6 ± 5.33 years (mean ± SD), and of these, 54.3% were female, and 45.7%, male. It is important to note that 39 right eyes and 57 left eyes were affected by AMD.

FUNCTIONAL STUDIES

After Gb-treatment, the functional variables of AMD patients approached the mean values of the control group. Moreover, both Gb-treatments (50 and 100 mg/day, for 3 months) produced significant changes among the final values of functional variables and the AMD baseline values: a) the contrast sensitivity increased significantly ($p < 0.01$), 15.9% and 16.8%, respectively (figure 1-A); as to b) central vision integrity, the number of metamorphopsias decreased significantly ($p < 0.001$) 100% and 95%, respectively (figure 1-B); and c) the central perimetry (scotoma) also decreased significantly ($p < 0.001$) 93% and 88%, respectively (figure 1-C).

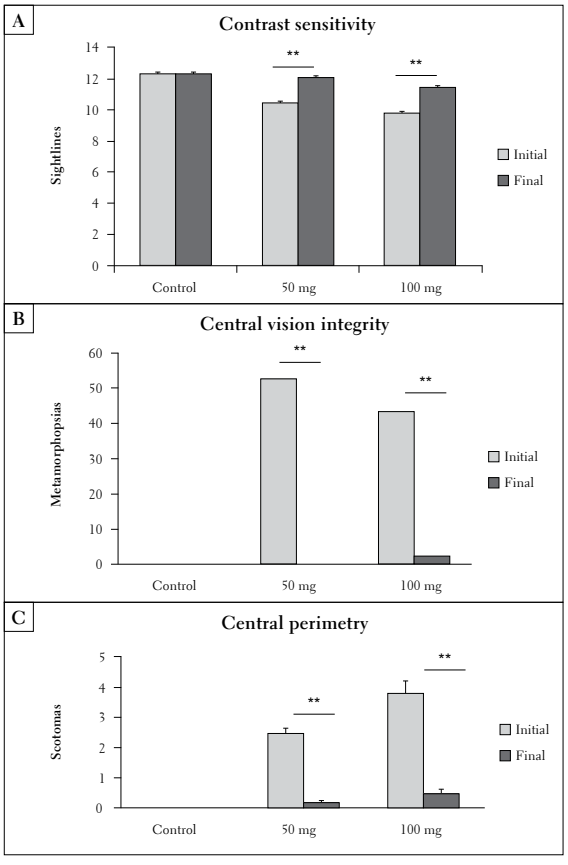

FIGURE 1. **Functional changes**

Note: A) Contrast sensitivity for the Gb-treated groups (50 mg and 100 mg) showed a statistically significant improvement. In the control group, there were no significant changes. B) Central vision integrity. Both groups treated with Gb (50 mg and 100 mg) showed a significant decrease in metamorphopsias. C) Central perimetry. The changes in computerized perimetry were represented by the number of defects or scotomas in the studied population. In both Gb-treated groups, a significant improvement was observed. The mean values (± SE) were shown for Gb-treated groups. (**) $p = 0.001$.

Source: own work

AMD STRUCTURAL STUDIES

Before Gb-treatments, the autofluorescence photographs showed increased hyperpigmentation in AMD patients, compared to those of the control group ($p < 0.001$) (figures 2B-2C). Besides, hyperpigmentation decreased significantly ($p < 0.001$) after both Gb-treatments (88.4% and 89%, respectively), compared to AMD baseline values (figure 3A). On the other hand, the figure (2D, E) shows an OCT of a patient with dry macular degeneration (right eye). Although the images with an automated record of the fund allow obtaining reproducible images in a series of the same area of the macula, no algorithm allows detecting drusen changes over time. Regarding OCT, no significant changes were observed in the mean values of the different groups (figure 3B).

SYSTEMIC OXIDATIVE STRESS

The systemic oxidative stress was unchanged in the control group. However, after Gb-treatments, AMD patients' MDA and GSH plasma levels were approximate to the control group mean values. Furthermore, both Gb-treatments (50 and 100 mg/day, for 3 months) produced significant changes among the final values of oxidative stress variables and the AMD baseline values. In fact: a) the plasma MDA levels decreased significantly ($P < 0.01$), 7.6% and 8.5%, respectively (figure 4A); and b) the plasma GSH levels increased significantly ($p < 0.05$), 5.3% and 7.1%, respectively (figure 4B).

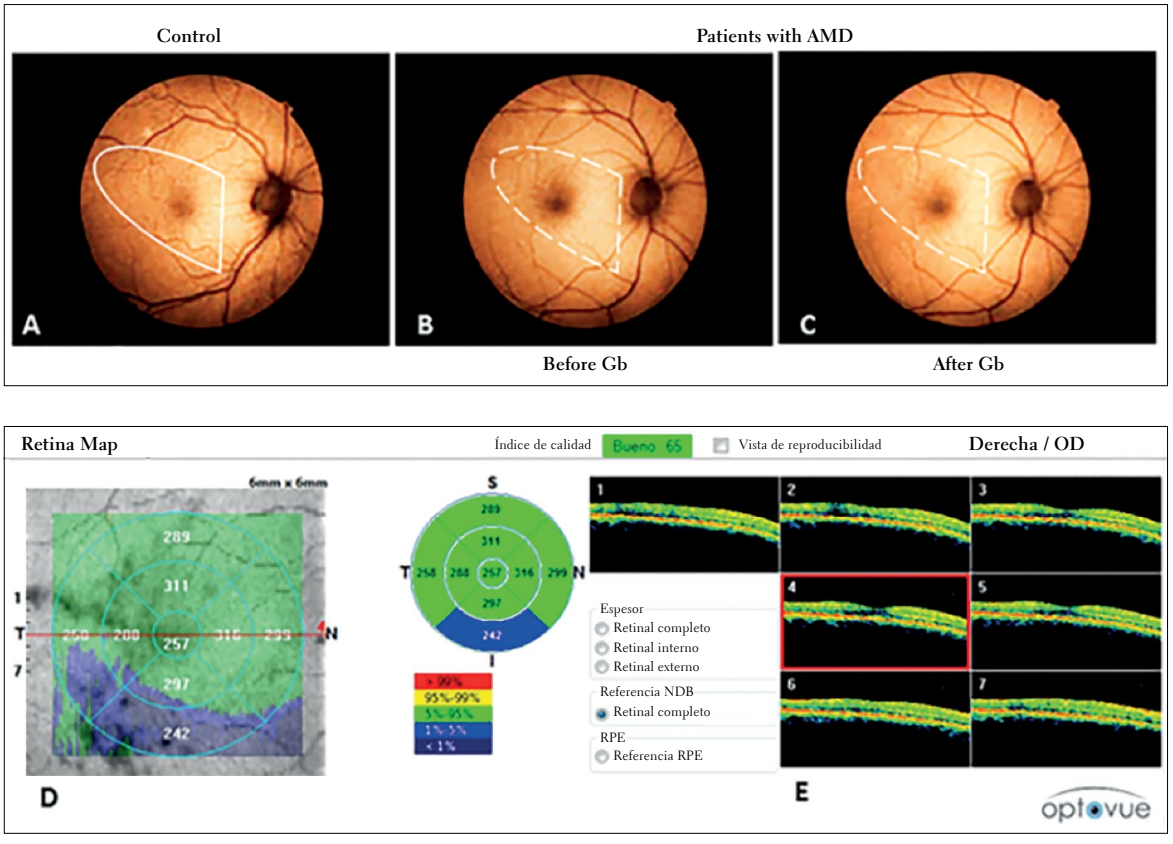

FIGURE 2. **Structural changes photographs**

Note: A) Autofluorescence photography showed low pigmentation in the control subject. B) In female patient with AMD before Gb treatment, the autofluorescence photographs showed hyperpigmentation. C) After GB treatment (50 mg per day), the same female patient showed a decrease in the macular area's pigmentation. The white line shows the pigmented area. D) Optical coherence tomography (OCT) map exemplifying automated output measures such as macular volume and central macular thickness measurements. Retinal thickness is automatically measured as the distance between the 2 borders. E) Retinal imaged by OCT provides visual information on the integrity of the retina (continuity) and values of the thickness of the macular area.

Source: own work

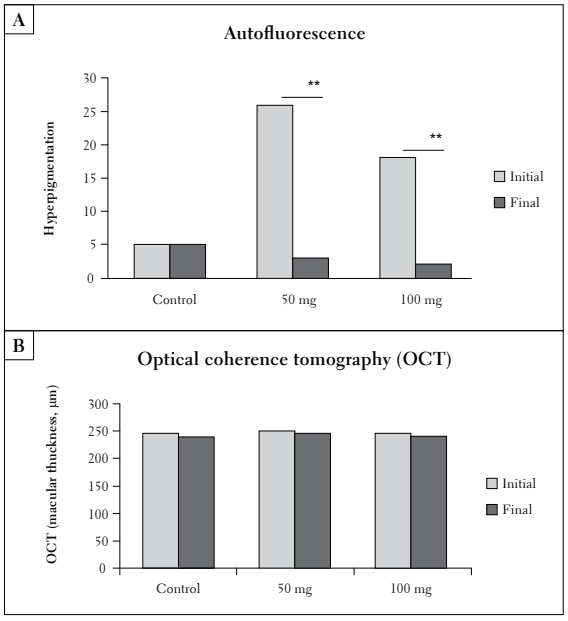

FIGURE 3. **Structural changes values**

Note: A) Autofluorescence. Changes in the final values of hyperpigmentation of the 3 studied groups. Both Gb-treated groups (50 mg and 100 mg) showed statistically significant reduction in hyperpigmentation. (**) p = 0.001. B) Optical coherence tomography (OCT). Macular thickness in the 3 studied groups showed a slight decrease in the post-treatment mean values, but the changes were not statistically significant. The observed decrease in the control group is normal due to aging.

Source: own work

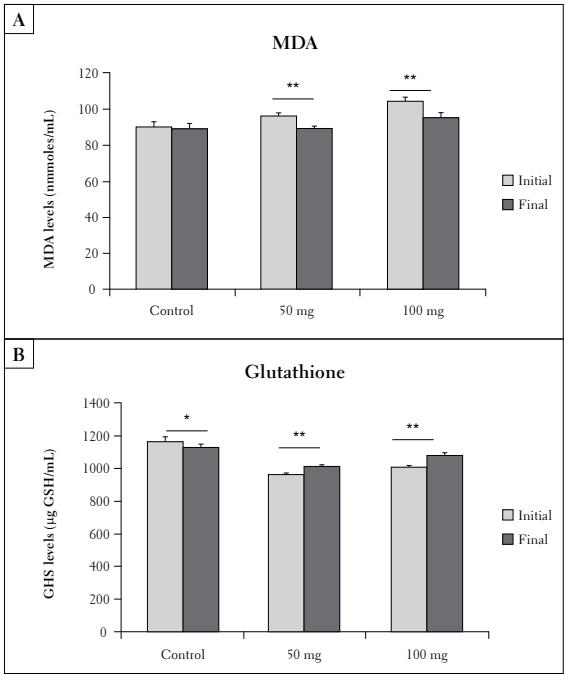

FIGURE 4. **Systemic oxidative stress**

Note: A) Both Gb-treated groups (50 mg and 100 mg) showed a statistically significant decrease in the blood MDA levels. B) Both Gb-treated groups (50 mg and 100 mg) showed a significant increase in the plasma GHS levels. The mean values (± SE) were shown for Gb-treated groups. (**) p = 0.001.

Source: own work

In such context, table 1 summarizes the changes in all visual and oxidative stress tests. When we compare the values before and 3 months after treatment with EGb-76 in the 3 study groups, it is easier to visualize that the 3 functionality parameters showed an increase in the post-treatment value in both doses (50 and 100 mg) of the Gb extract (data expressed in percentage). In structural tests, fluorescence increased in the AMD-treated groups and showed no changes in the control group. Regarding oxidative stress markers, the patients with AMD showed high MAD levels, and the treatment with the Gb extract reduced a low percentage in both groups (8.1, 9.3%). On the other hand, the control group without treatment did not show any change. Baseline levels after 3 months were similar.

While, regarding oxidative stress markers, patients with AMD showed elevated levels of MAD, and treatment with the Gb extract reduced levels by a low percentage in both groups.

DISCUSSION

Oxidative stress has long been hypothesized to play a substantial role in the development of AMD. Concerning oxidative damage, lipofuscin granules have been shown to produce singlet oxygen, superoxide radical, hydrogen peroxide, and increased lipid peroxidation (19, 44, 45). In such context, the retina is particularly susceptible to oxidative stress because of its high consumption of oxygen, large amounts of polyunsaturated fatty acids concentrated in the photoreceptors, and continual exposure to visible light (46, 47). Also, the formation of reactive oxygen species (ROS) causes the oxidation of docosahexaenoic acid (DHA), which is one of the major pathways for cellular damage or degeneration of the photoreceptors in AMD (3, 10, 48).

Considering that oxidative damage takes place in the retina, several studies have shown an age-related increase. Particularly in 1) 8-oxo-7,8-dihydro-2´-deoxyguanosine (8-oxodG) in the retina (49), 2) mitochondrial DNA (mtDNA) damage

Table 1. **Changes in the percentage of the study parameters in the three groups**

Parameters	Control group	p	Group 1 (50 mg)	p	Group 2 (100 mg)	p
Functionality:						
Contrast sensitivity	↑ 0.16%	0.323	↑ 15.9%	0.001	↑ 14.3%	0.001
Central computerized campimetry	Without changes	NS	↑ 93%	0.001	↑ 88%	0.001
Amsler grid	Without changes	NS	↑ 100%	0.001	↑ 95%	0.001
Structural:						
Autofluorescence	Without changes	NS	↑ 46%	0.001	↑ 61%	0.001
Central thickness in OC	↓ 2%	0.09	↓ 1.22%	0.06	↓ 1.6%	0.06
Oxidative stress:						
MDA	↓ 0.9%	0.23	↓ 8.1%	0.001	↓ 9.3%	0.001
GHS	↓ 3.06%	0.17	↑ 5.2%	0.001	↑ 7.07%	0.001

Source: own work

(19), 3) structural modifications of drusen proteins such as carboxyethylpyrrole protein adducts (50, 51), 4) advanced lipid peroxidation and glycosylation end products (AGEs)-carbohydrate posttranslational protein modifications that may initiate an inflammatory response (52), and 5) 4-hydroxynonenal (4HNE) and malondialdehyde (MDA), which induce lysosomal dysfunction and lipofuscinogenesis in the RPE (53).

As mentioned previously, the treatment options for AMD people have been severely limited (54, 55). Currently, no proven therapies for atrophic disease are available. However, early-stage to late-stage illness progression can be slowed with high-dose zinc and antioxidant vitamin supplements (16). Thus, targeting pathways that reduce oxidative damage and ROS generation offers valuable therapeutic strategies. However, for Evans (56), it is not clear that Ginkgo biloba brings benefits, but two small trials have suggested a possible benefit of Ginkgo biloba on vision people with AMD. Contrary to these results, a regular diet rich in flavonoids has recently been shown to prevent the progression of AMD (57), which agrees with our results.

Additionally, 2 small trials have suggested the possible benefit of Gingko biloba on vision. While, 1 trial compared gingko biloba to a placebo (80 mg twice daily) (30), and the other compared 2 different doses of the extract (240 mg per day and 60 mg per day) (38). Although both trials reported some positive effects of Ginkgo biloba on vision, these studies did not perform any vision improvement measurements or changes in oxidative stress.

In our study, the Gb-treatments (50 or 100 mg/day, for 3 months) for people affected by AMD improved all the functional and structural variables analyzed, except the retinal thickness (OCT). This improvement was accompanied by a decrease in the systemic oxidative stress in AMD patients after Gb-treatments. The MDA concentration, a biomarker of lipid peroxidation, decreased in the blood, and the GSH level increased in plasma.

Studies show also that after oral ingestion of radioactive Gb, glandular, neuronal, and eye tissues showed a high affinity for the labeled Gb (58). Other studies show that Gb increased ocular blood flow velocity after oral administration (59-61).

Also, as already mentioned, contrast sensitivity, central vision integrity, central perimetry, and retinal autofluorescence improved after both Gb-treatments, compared to AMD basal values. As well, contrast sensitivity is a valuable measure of visual function in patients with AMD, and provides essential information about visual disability (62, 63). In such context, the Amsler grid is useful to detect vision problems resulting from damage to the macula as the AMD.

According to Amsler's original work in patients with maculopathies, subjective symptoms often precede objective signs, making the grid suitable for detecting macular disease at an early stage (64, 65). Besides, the visual field defects in patients with AMD, usually begin as relative scotomas and may progress to become absolute in the center over time. Thus, central perimetry is also a useful tool for monitoring the visual field defects in patients with AMD (66). Autofluorescence imaging is an ideal tool for the *in vivo* examination of the normal and diseased macula. It offers information on AMD pathogenic mechanisms, such as the RPE cell dysfunction secondary to lipofuscin accumulation (67).

While, regarding oxidative stress, various studies have shown that circulating MDA levels are increased in AMD patients compared to those in the controls (39, 68, 69). Our results showed an increase in circulating MDA levels in AMD patients, but this increase was not significant compared to the control group. However, 3 months after Gb treatment, we observed a significant decrease in MDA levels in the groups with both treatment doses. As well, GSH participates in cellular defense against xenobiotics and naturally occurring harmful compounds, such as free radicals and hydroperoxide (70). Also, the GSH deficiency contributes to oxidative stress, which plays a crucial role in aging and the pathogenesis of many chronic diseases (71). Thus, it has been reported that GSH level decreases in erythrocytes and human plasma are associated with aging and AMD, diabetes, cystic fibrosis, and other disease processes (72-74). Due to the essential antioxidant roles of GSH, such declines in plasma or tissue could contribute to the development and/or progression of age-related diseases (74). On their own, our findings of decreased MDA and increased GSH levels in the blood produced by Gb-treatment on AMD patients show a likely association with reducing oxidative eye damage.

In this context, the underlying principle behind the therapeutic action of Gb on chronic diseases has focused on its antioxidant properties (26). The 2 proposed mechanisms of action are: 1) directly scavenging the ROS (such as hydroxyl, peroxyl, superoxide, and hydrogen peroxide); and 2) indirectly inhibiting the formation of free radicals (75, 76). In addition, it has also been reported that Gb has vascular protective properties (77) and reduces ischemic injury (78). Besides its direct attenuation of ROS, Gb may also stabilize the cellular redox state by up-regulation of the protein level and the activities of antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase, catalase, and heme-oxygenase-1 (29, 76, 79). Furthermore, the activity of GSH reductase and gamma-glutamylcysteine synthetase, 2 enzymes critical for the reduction and synthesis of GSH, are also enhanced by Gb (80-82).

Thus, EGb-761 has been tried out in experimental and clinical ocular pharmacology. Evans and Henshaw (83) analyzed 2 randomized trials in people affected by AMD to determine the effect of Gb on the progression of this disease. Both studies reported some positive results of Gb on vision. Still, this review's overall conclusion is that the beneficial effect of Gb on retarding the progression of AMD is yet to be confirmed. On this matter, our results support that EGb-761 may be a useful drug to reduce the velocity of AMD development, because both Gb-treatments (50 and 100 mg/day for 3 months), administered to AMD patients, improved the eye variables that were analyzed (3 functional and 1 structural). Other studies show that perimetry and microperimetry provide more information about AMD patients' visual functional changes (84).

It is worth noting that a better understanding of the characteristics of visual loss in AMD will help the clinician to quantify the beneficial effects of any therapeutic intervention for AMD patients.

Concerning adverse effects and toxicity of Gb doses to humans, other authors have summarized the efficacy and safety of Gb. They suggest that

an amount of 120 mg EGb-761 per day is acceptable (85). Moreover, it is essential to note that the mechanisms of biotransformation and excretion of xenobiotics are reduced in aged people (86, 87). Because of that, the doses of EGb-761 used in our study were low and not toxic for patients and volunteers (32-34).

However, reports of patients taking AREDS formulation presented side effects that included 1) skin yellowing due to high intake of β carotene, and 2) medical problems associated with the urinary tract due to zinc treatment (41). As we have discussed previously, there are nutritional recommendations for the treatment of AMD, and one of them is the intake of minerals and vitamins. Nonetheless, trouble can arise with the misuse of these products when the patient ingests vitamins or fat-soluble micronutrients in doses that can be toxic in the long term. Therefore, an extract with anti-inflammatory and antioxidant activity, with a defined concentration and usefulness in low concentrations becomes a more attractive treatment.

Therefore, the AMD patients received low doses of EGb-761 to reduce the risk of adverse effects such as blood coagulation and blood pressure alterations and/or pro-oxidant effects (76, 88, 89).

In table 1, no changes were observed in the control group (patients without AMD, and this group was only a time control, which showed no important changes over time in normal eyes). Similarly, the sensitivity contrast post-treatment was minimal in patients with AMD, since the eye does not quickly recover contrast sensitivity values. However, in the tomography test, the difference between before and after was greater in the control group.

Regarding GSH values, they were low in patients with AMD, but after treatment, an increase (5.2 and 7.07%) was observed in the groups treated with 50 and 100 mg, respectively. In contrast, the baseline value in the control group decreased (3.06%). This data is important, since it shows that part of the visual deterioration corresponds to aging in older people. While, there are no AMD management guidelines at the initial stages in Mexico, but some food supplements and vitamins are prescribed (mainly vitamin A). Due to AMD pathogenesis complexity, effective treatments are few, but Gb remains among the pharmacological approaches to reduce this disease's oxidative stress (90, 91). Therefore, with the results of this work, we can propose further research to combine Gb with other food supplements or medications. In terms of sample size, it would also be necessary to develop a broader subsequent randomized clinical trial.

One limitation of this study is that the extract is administered orally, and the final concentration that reaches the eye is reduced. While, the beneficial effect of GB on improving blood flow and reducing ROS in patients with AMRE is less noticeable. Nonetheless, administering higher doses can be toxic if done chronically. However, our study's strength is its clinical applicability in our community due to the need to manage AMD for prevention and treatment in the initial stage.

CONCLUSIONS

This study shows that low doses of EGb-761 (50 or 100 mg/day, for 3 months) have positive effects on vision and delay AMD's progress. Contrast sensitivity, central perimetry, central vision integrity and retinal autofluorescence (macula) improved after EGb-761 treatment in AMD patients. A decrease in systemic oxidative stress accompanied this improvement. We suggest that the dietary supplement EGb-761 offers beneficial effects for people affected by AMD.

One limitation of this study is that oxidative stress levels are measured in plasma, and it will not be possible to do so in the ophthalmic artery, which is very close to the patient's eyes. However, our study's strength is its clinical applicability in our community, due to the lack of management of AMD for prevention and treatment in the initial stage.

CONFLICT OF INTEREST

The authors declare no conflict of interest. Research funding does not come from private funds, and there is no financial relationship with the pharmaceutical industry that produces the drug.

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