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Effect of ginkgo extract on eye microcirculation in patients with diabetes

Research Article

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Abstract: The prevalence of Diabetes mellitus has increased around the world in the last decade. Anyone with diabetes is at risk of diabetic eye complications. The aim of the study was to compare effects of standardized Ginkgo biloba (*Ginkgo biloba* L.) dry extract (Ex.Gb) with the placebo on the microcirculation lesions of the eye in randomized double-blind placebo-controlled trial. 44 patients with type 2 diabetes mellitus were randomized to Ex.Gb 160 mg per day or placebo, and were followed up for nine months. Dose of Ex.Gb was increased to 240 mg in next nine months. Total Antioxidant Status (TAS) of plasma was measured using the Trolox equivalent antioxidant capacity assay. Ophthalmologic examination was performed by the biomicroscopic method. Vascular, intravascular and perivascular alterations were evaluated, and total conjunctival index was calculated. Though the values of the total conjunctival index and its constituent decreased ($P<0.05$) during the study in Ex.Gb group, there were no significant differences between these parameters as compared with placebo group. Evaluation of plasma TAS showed gradually increment, although insignificant and very small, in Ex.Gb group. The significance of Ex.Gb on development of the alterations of eye microcirculation, especially in elder patients needs to be explored further.

Keywords: *Gingko Biloba extract • Antioxidant • Diabetic retinopathy • Total conjunctival index*

Abbreviations

ABTS – 2,2-Azino-di-(3-ethylbenzthiazoline sulphonate);
DM – Diabetes mellitus;
DR – Diabetic retinopathy;
Ex.Gb – standardized Ginkgo biloba (*Ginkgo biloba* L.) dry extract;
HbA1c – Glycated hemoglobin;
IVC – Index of the intravascular changes;
PVC – Index of the perivascular changes;
TAS – Total Antioxidant Status;
TCI – Total conjunctival index;
VC – Index of the vascular changes.

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1. Introduction

The prevalence of Diabetes mellitus (DM) has been accelerating at an alarming rate in the last decade. More than 366 million people live with diabetes worldwide and this number is expected to rise to 552 million by the year 2030, if no urgent action is taken [1]. Currently, more than 52.8 million of the European adult population has diabetes and another 19 million people are living with undiagnosed diabetes [2]. DM is a metabolic disorder of multiple etiology characterized by uncontrolled concentrations of glucose in the blood with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of DM include long-term damage, dysfunction and failure of various organs.

Diabetic retinopathy (DR) is the most common diabetic eye complication and the leading cause of visual impairment and acquired blindness in working adults (20-65 years) [3]. It is a duration-dependent disease that develops in stages; the incidence of retinopathy is rarely detected in the first few years of diabetes, but the incidence increases to 74% by 10 years or more [4,5]. The prevalence of DR is increasing due to prolonged survival of diabetic patients primarily because DR is diagnosed or treated too late. DR is the result of microvascular retinal changes. However, the first signs of the alteration of the eye microcirculation in patients with DM, are diagnosed in the conjunctiva vessels. As far back as 1973, Moricke has proposed a descriptive evaluation of conjunctival vessels adjust to the mathematical analysis by introducing a points system and calculating total conjunctival index (TCI) [6]. TCI is the amount of vascular, intravascular and perivascular changes expressed as scores. Initial or single alterations are evaluated by one point, far advanced or multiple alterations by two points [7]. Qualitative and quantitative changes in microcirculation of the conjunctiva depends on the stage and form of diabetic retinopathies. Values of changes of the TCI and its constituents are on an increase with aggravation of a pathological process in the fundus of the eye [8].

Although the abnormally high glucose concentration plays an important role in development of diabetic complications, the increased evidence from clinical research show that oxidative stress is associated with the pathogenesis of DM too [9-11]. Oxidative stress influences on the pathogenesis of insulin resistance and beta-cell dysfunction, i.e. two most relevant mechanism in the pathophysiology of type 2 diabetes [12] and lead to vascular damage [13,14]. The data of recent decade research on role of oxidative stress in development of DM complications stimulate to search for antioxidants to reduce the hyperglycemia–induced oxidative stress.

Ginkgo biloba (*Ginkgo biloba* L.) leaves extract (Ex.Gb) contain several active antioxidant constituents including 20-27% flavonoids, 5-7% terpenoids, and 5-10% organic acids. The major flavonoids are primarily derived from the flavonol rutin and include isorhamnetin, quercetin, kaempferol, and proanthocyanidins. The primary terpenoids are ginkgolides A, B, C, M, and J, and bilobalide [15,16]. Although the mechanism of action of ginkgo leaf is only partially understood, one of the theories is that ginkgo leaf might work by protecting tissues from oxidative damage. Ginkgo leaf flavonoids have antioxidant and free radical scavenging properties [17-19]. The flavonoids seem to prevent or reduce cell membrane lipid peroxidation [19,20], and decrease oxidative damage to erythrocytes [21]. Flavonoids of Ginkgo also protect retinal tissue from oxidative stress [22-24]. Protecting tissues from oxidative damage might prevent progression of tissue degeneration in patients with DM. There is some evidence that taking ginkgo leaf extract orally for six months can significantly improve measures of color vision in patients with early DR [25]. And there is no doubt that Ex.Gb effect on retinal microcirculation: increase microcircular blood velocity, flow, and volume in healthy individuals [26,27]. However, there is no evidence of Ex.Gb effects on the microcirculation lesion of the eye in type 2 diabetic patients.

The aim of this study was to estimate the effects of *Ginkgo biloba* L. leaves dry extract on prevention and suppression of the microvascular alterations of the eye in patients with type 2 diabetes. This study is a part of international “Eureka“ project No.E! 3695 „Creation of the methodology for effects of natural antioxidants on the development of the Diabetes mellitus complications“.

2. Materials and methods

This randomized double blind placebo-controlled study was conducted in Endocrinological Clinic, Hospital of Lithuanian University of Health Sciences Kaunas Clinics, Lithuania. The experimental protocol used in this study was approved by the Lithuanian Bioethics Committee, State Data Protection Inspectorate. Written, informed and voluntary consent was obtained from all subjects. All subjects were outpatients of the Endocrinological Clinic, Hospital of Lithuanian University of Health Sciences Kaunas Clinics, Lithuania. Subjects diagnosed with type 2 diabetes mellitus (treated with Insulinum, Metforminum or combination of both), aged from

35 to 80 years old and followed up for diabetic retinopathy were enrolled in this study. The exclusion criteria include glycated hemoglobin (HbA1c) > 13%, body mass index > 45 kg/m^2, history of uncontrolled hypertension, other significant medical problems (major cardiovascular, hepatic, and other endocrine diseases), hypersensitivity to the test drug, and not being able to comply with the study protocol. The subjects were not deprived of taking their regular prescribed medications, but were advised to abstain from other dietary supplements rich in antioxidants. All patients were randomly allocated to receive either standardized Ginkgo biloba dry extract or placebo capsules.

Ophthalmological parameters and biochemical measurements were evaluated at the baseline. The baseline measurements were repeated after 9 and 18 months of receiving preparations. During the first nine months patients received Ex.Gb or placebo capsules twice a day (160 mg of Ex.Gb), in the second nine months – three times a day (240 mg of Ex.Gb). Placebo capsules were made from microcrystalline cellulose, a material indifferent to disease (Joint-stock company "Sanitas", Veiveriu str. 134B, LT-46352 Kaunas, Lithuania; sanitas@sanitasgroup.com). Capsule of the active product contains 80 mg of standardized dry extract of Ginkgo biloba leaves, adjusted to 19,2 mg Ginkgo flavone glycosides and 4,8 mg terpene lactones (ginkgolides, bilobalide) (Joint-stock company "Aconitum", Inovaciju str. 4, LT-54469 Kaunas district, Lithuania; aconitum@aconitum.lt).

Ophthalmologic examination was performed by the biomicroscopic method. The changes of vessel walls (vascular changes) and bloodstream, intravascular and perivascular alterations were evaluated. Expression of each pathological symptom is defined in a score. The maximum amount of change can be 51 points [7]. TCI and its constituents (Index of the vascular changes (VC), Index of the intravascular changes (IVC) and Index of the perivascular changes (PVC)) were calculated. Values of changes of TCI and its constituents were used to evaluate abnormalities in bulbar conjunctiva vessels and microcirculation.

In parallel with ophthalmological examination the plasma total antioxidant status was measured too. This was performed in Laboratory of Biochemistry, Riga Stradins University, Latvia. The quantitative determination of Total Antioxidant Status was performed automatic spectrophotometrically according to the manufacturer's instructions for analyser RX Daytona (Randox Laboratories, Ltd., 55 Diamond Road, Crumlin, UK). Assey priciple: 2,2-Azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS) is incubated with peroxidase (metmyoglobin) and hydrogene peroxide, to produce the radical cation ABTS. This has a relatively stable blue-green colour, which is measured at 600 nm. Antioxidants in the added sample cause supression of this colour production to a degree which ir proportional to their concentration [28].

Data were analyzed using the computer software packages SPSS for Windows 20.0 and GraphPad 3.00. Data are expressed as mean ± S.E. There were used nonparametric tests because of small number of variables. The comparisons between the two groups (placebo and Ex.Gb) were made by Mann-Whitney U test at each time point. The comparisons between three different measurements (at baseline, after 9 and 18 months) were made using Friedman test and Two-way ANOVA. The results were considered statistically significant at $p < 0.05$.

3. Results

There were a total of 91 patients involved in the project. 44 patients participated in this study, and 2 of them ended participation after several months for personal reasons. Mean age were 60.9 ± 2.2 and 61.4 ± 2.6 years in the Ex.Gb and placebo groups, respectively. Mean duration of DM 10.5 ± 1.6 years in the Ex.Gb group and 11.5 ± 1.9 years in placebo group. Male preponderance in both groups was observed. Mean body weight and BMI at baseline and after 9 and 18 months were similar in both groups. Data of the level of HbA1c is given in Table 1.

The biomicroscopic study of the conjunctival vessels demonstrated microcirculation disturbances in all patients at the beginning of study. Values of changes of the total conjunctival index and its constituents at baseline were enlarged two to three times in comparison with limits. Although the decrease of the TCI and its constituent values was observed in Ex.Gb group, there were no statistically significant differences between Ex.Gb and placebo groups (Table 2).

Table 1. Mean level of HbA1c (%) at baseline, after 9 and 18 months. There were no significant differences in the level of HbA1c at all measurements in both groups.

Group	At baseline		After 9 months		After 18 months	
Ex.Gb	7.17±0.51	p = 0.52	7.44±0.26	p = 0.83	7.27±0.45	p = 0.28
Placebo	8.02±0.41		7.59±0.31		7.67±0.36	

Table 2. Mean of the total conjunctival index and its constituents at baseline, after 9 and 18 months in both groups.

		Baseline		9 month		18 month	
TAS (mmol/L)	Ex.Gb	1.59±0.038	p = 0.51	1.65±0.038	p = 0.92	1.67±0.042	p = 0.21
	Placebo	1.55±0.040		1.68±0.040		1.57±0.041	

When data were analyzed separately for each of the two groups, significant differences were observed between three different measurements in Ex.Gb group. VC decreased by 1.2 point after 9 months (p = 0.04) and by 0.94 point after 18 months (p = 0.14) as compared with baseline. PVC decreased by 0.45 point after 9 months (p = 0.11) and by 0.7 point after 18 months (p = 0.02) as compared with baseline. Meanwhile, the changes of VC and PVC in placebo group were not statistically significant. Evaluation of IVC didn't shows significant changes in either Ex.Gb, nor in placebo groups. While, as compared data at baseline and after 18 months, IVC decreased by 0.15 point in Ex.Gb group and increased by 0.12 point in placebo group. However, these differences were not statistically significant. The changes of TCI at different time points of the study in both groups are shown in Figure 1.

The values of TAS were normal in the both groups as compared to the reference values (between 1.30 and 1.77 mmol/L) given by RANDOX. Evaluation of plasma TAS didn't show statistically significant differences between Ex.Gb and placebo groups (Table 3). There were, however, some changes of the dynamics between three different measurements in each group. The changes of TAS at different time points of the study in both groups are shown in Figure 2.

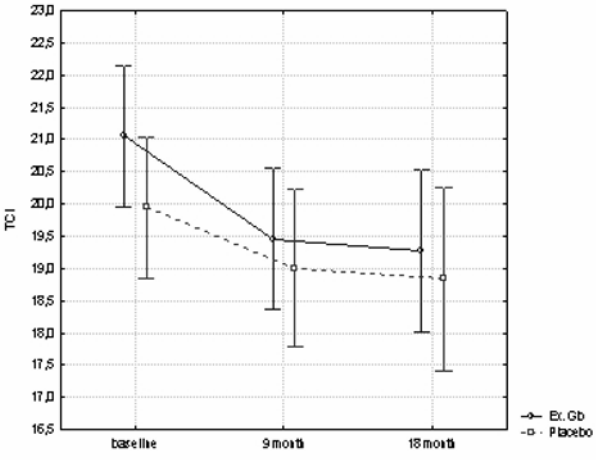

Figure 1. Changes of the total conjunctival index at baseline, after 9 and 18 months in both groups TCI significantly decrease by 1,6 point after 9 months (p = 0.043) and by 1.78 point after 18 months (p = 0.037) as compared with baseline in Ex.Gb group. While the changes of TCI in placebo group were not significant: 0.95 point after 9 month (p = 0.254) and 1.12 point after 18 months (p = 0.219) as compared with baseline.

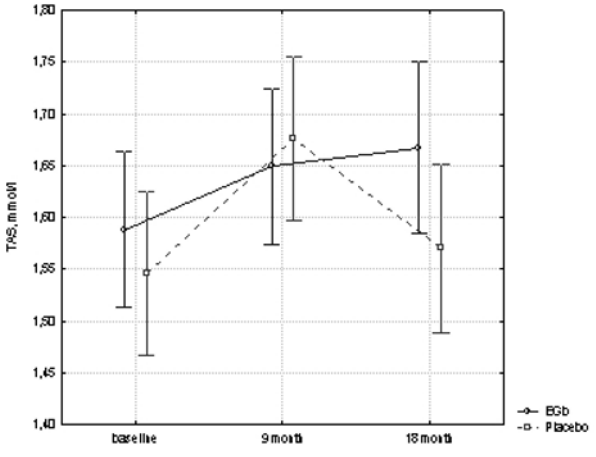

Figure 2. Changes of the total antioxidative status (mmol/L) at baseline, after 9 and 18 months in both groups.
Though the changes of TAS in both groups were not statistically significant (except the increment after 9 months (p = 0.023) in placebo group), the results shows that TAS gradually increased in Ex.Gb group during the whole time.

Table 3. Mean of oxidative stress parameters at baseline, after 9 and 18 months in both groups.

		Baseline		9 month		18 month	
VC	Ex.Gb	10.40±0.41	p = 0.30	9.20±0.41	p = 0.91	9.46±0.47	p = 0.14
	Placebo	9.40±0.41		9.18±0.46		8.67±0.54	
IVC	Ex.Gb	8.15±0.19	p = 0.60	8.20±0.19	p = 0.74	8.00±0.21	p = 0.28
	Placebo	8.3±0.19		8.13±0.21		8.42±0.24	
PVC	Ex.Gb	2.50±0.20	p = 0.49	2.05±0.20	p = 0.35	1.80±0.23	p = 0.94
	Placebo	2.30±0.20		1.69±0.22		1.75±0.25	
TCI	Ex.Gb	21.05±0.55	p = 0.37	19.45±0.55	p = 0.71	19.27±0.64	p = 0.52
	Placebo	19.95±0.55		19.00±0.62		18.83±0.71	

4. Discussion

Oxidative stress has become a focus of interest in much of the clinical research in recent decades. Diabetes and hyperglycemia can also lead to oxidative stress and formation of reactive oxygen species (free radicals), leading to vascular damage [12-14]. The excess of free radicals or an ineffective natural defense mechanism that would deactivate free radicals has been implicated as a contributing factor in many diseases [9-11]. DR is the most common microvascular eye complication of DM, resulting in blindness worldwide [4,5]. Not only the early diagnosis but also development of effective preventatives and treatments of DR are essential to save sight. The data of recent studies on role of oxidative stress in pathogenesis of DM and its complications support the concept that an antioxidant therapy may be of great interest to patients with type 2 DM. Natural (plant derivation) antioxidants are very important nutrition supplements which inhibit the formation of free radicals. The most studied of these is *Ginkgo biloba* L. leaves extract, which has several biological actions that combine to make it a potentially important agent in the prevention of diabetic eye complications.

Though, there wasn't significant change in TAS between the two groups, TAS did result in gradual incremental change in Ex.Gb group as compared between three measurements. There was significant increment of TAS after first 9 months in placebo group, but in the next nine months this again decreased to the initial amount. This may be due to the "placebo" effect at the beginning of the study, which was not shown in the next nine months.

We expected there won't be alteration in TCI during the study in Ex.Gb group, and increment of this index in placebo group. However, the findings demonstrated a statistically significant decrement of the values of changes of the TCI after 9 and 18 months in Ex.Gb group as compared with baseline. Though, the concentration of Ex.Gb wasn't of great significance. According to the results, the decrement of TCI depends on the reduction of PVC and VC. So, Ex.Gb the most influences on perivascular changes (perivascular intumescence, haemorrhages, accumulation of hemosiderin, lipid infiltration of conjunctiva) in bulbar conjunctiva vessels. There were slight reductions of vascular changes (diameter, sinuosity and microaneurysms of arterioles, venules and capillaries) in the eye too. The decrement of VC was observed immediately after receiving 160 mg of Ex.Gb per day for nine months. Meanwhile, the significant decrement of PVC was observed only after receiving 240 mg of Ex.Gb per day. Alternatively, this may be due to the longer usage. However, according to the data of current study, Ex.Gb has no effects on intravascular changes (rate and continuity of bloodstream) in the eye. Though, significant decrement of TCI was observed in Ex.Gb group, there were no statistically significant differences between these parameters in comparison with the placebo group. Supposedly, it was influenced by the older age of patients, already existing significant vascular, intravascular and perivascular changes in the conjunctiva blood vessels, and the duration of the study. Considering the results, statistical significance is probable by using Ex.Gb for a long-term or in patients with early microvascular eye changes. In this instance further exploration needs to be done. The finding that Ex.Gb might reduce the values of changes of the TCI makes it attractive for long-term usage by the elderly, even with more progressed microvascular eye changes.

5. Conclusions

The results of this study show that ingestion of *Ginkgo biloba* L. leaves extract determines significant changes in microcirculation in the conjunctiva blood vessels, and might enhance the TAS, but further exploration needs to be done to confirm results in different circumstances.

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