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The Effect of *Ginkgo biloba* and *Camellia sinensis* Extracts on Psychological State and Glycemic Control in Patients with Type 2 Diabetes Mellitus

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Interest in finding natural antioxidants for use in food or medical materials to prevent free radical imbalance has increased considerably over the past years. The aim of this research was to evaluate changes in glycemic control and psychological state of patients with type 2 diabetes mellitus (T2DM) after use of antioxidant plant preparations. Fifty-six patients with T2DM were randomly allocated to receive standardized *Ginkgo biloba* L. leaves dry extract, green tea dry extract, or placebo capsules. Diabetes glycemic control measured as glycated hemoglobin (HbA1c) level, antioxidant state and psychological data were evaluated at baseline, after 9 and 18 months of using either antioxidant preparations or placebo. The level of perceived stress lowered significantly after 9 months (p=0.038) and 18 months (p=0.030), and the psychological aspect of quality of life significantly improved after 18 months (p=0.019) of use of *G. biloba* extract. No significant differences were detected after using green tea extract. In patients using placebo, significant lowering of HbA1c level was observed after 18 months (p=0.017). In conclusion, antioxidant *G. biloba* leaf extract exhibited a mild effect on psychological state and a trend of improving glycemic control in patients with type 2 diabetes mellitus.

Keywords: Type 2 diabetes mellitus, Green tea (*Camellia sinensis* L.) extract, *Ginkgo biloba* L. extract, Emotional state, Quality of life, Perceived stress.

Oxygen is essential to human organisms, but the metabolism of oxygen generates free radicals which can induce oxidative damage to biomacromolecules [1]. Oxidative stress develops from an imbalance between oxidant production and antioxidant activity in cells and in plasma [2]. Free radicals and oxidative stress are reported to be involved in the occurrence of numerous diseases such as cancer, atherosclerosis, cardiovascular diseases, inflammatory diseases [3], and diabetes mellitus [4]. A number of studies have evidenced the pivotal role of oxidative stress in insulin resistance states such as metabolic syndrome, obesity, and type 2 diabetes mellitus (T2DM) [4-5]. Diabetic patients represent a population in whom oxidative stress is much higher than in the general population [6].

Research data supports an important association between increased risk of psychological and psychiatric impairments and chronic illness [7-8]. The presence of anxiety is reported to contribute to an additional time of hospitalization of T2DM patients [9]. Recent studies reveal the role of oxidative stress in anxiety-like behavior in rodents [10-12]. Induction of oxidative stress results in elevated anxiety and insulin resistance [10]. Masood *et al*. [13] reported that induction of oxidative stress in hypothalamus and amygdala occurs in parallel with anxiety in mice. The implications of comorbidity between psychical impairments and T2DM led to an investigation of the shared relationship underlying these conditions; oxidative stress is likely to be a contributing factor [10].

Antioxidants may be particularly important in combating the increased oxidative stress, as they diminish cumulative oxidative damage and can protect the body from the damage caused by free radicals [14]. The term antioxidant refers to any molecule capable of either stabilizing or deactivating free radicals. Humans have evolved highly complex antioxidant systems (enzymatic and nonenzymatic) which work synergistically, and in combination with each other to protect the cells and organ systems of the body against free radical damage. The antioxidants can be either endogenous or obtained exogenously as part of either diet or as supplements. Under conditions that promote oxidative stress, endogenous antioxidants may not be sufficient [15].

Interest in finding natural antioxidants for use in food or medical materials to prevent free radical imbalance has increased considerably over the past years [16-17]. Compounds in medicinal plants and natural dietary agents have received considerable attention for their potential use for amelioration of oxidative stress [18-20]. The major components of interest are polyphenols (the major ones being flavonoids), which are responsible for the antioxidant and other health benefits in many plants [21]. Either polyphenols or polyphenol rich diets provide significant protection against the development and progression of many chronic pathological conditions, including cancer, cardio-vascular problems and diabetes [22-23]. *Ginkgo biloba* and green tea extracts are natural antioxidants containing large amount of polyphenolic compounds. Green tea extract is rich in antioxidant polyphenolic flavonoids (approximately 75 %) [24]. A major class of green tea flavonoids is the catechins (major one epigallocatechin-3-gallate), which play a key role as antioxidants in prevention and treatment of many diseases [25-26]. *G. biloba* leaf extract contains several active antioxidant constituents including 20-27% flavonoids (major of them isorhamnetin, quercetin, kaempferol, and proanthocyanidins),

5-7% terpenoids (major of them ginkgolides A, B, C, M, and J, and bilobalide) and 5-10% organic acids [27-28]. *G. biloba* and green tea polyphenols can be direct antioxidants by scavenging reactive oxygen species or chelating transition metals [24,29]. Alternatively, they may act indirectly by increasing the activity of antioxidant enzymes [30-31]. These extracts reduce oxidative stress and may have beneficial effects on DM and its complications [32].

With this background, the aim of the study was to evaluate possible changes in diabetes glycemic control and psychological state of patients with type 2 diabetes mellitus after use of preparations of plants with antioxidant activity.

The age of participants was 57.0±9.8 years, the duration of T2DM was 8.1±6.8 years, total antioxidant status was 1.6±0.1 mmol/L, and malondialdehyde + 4-hydroxy-2-nonenal (MDA+HNE) level was 6.9±3.9 μM. Oral medicament therapy was prescribed to 11 (18.2%) researched T2DM patients, insulin to 29 (52.7%) patients, and oral medicaments + insulin to 16 (29.1%) patients.

Of all 56 participants, 21 (37.5%) were male and 35 (62.5%) were female. Evaluating education of the participants, 10 (17.9%) had university education, and 46 (82.1%) had secondary or lower education. Evaluating employment, 20 (35.7%) were working, 4 (7.1%) were unemployed, 16 (28.6%) were retired, and 16 (28.6%) were disabled. Evaluating marital state, 6 (11.1%) were single, 33 (57.4%) were either married or cohabiting, 7 (13.0%) were divorced and 10 (18.5%) were widowed. Evaluating smoking, 4 (7.1%) were current smokers, 44 (78.6%) were not smoking, and 8 (14.3%) were former smokers. No significant differences between groups in education, employment, marital state and smoking status were found.

Table 1: Differences in glycemic control, antioxidant state and psychological parameters in patients with type 2 diabetes mellitus who used *Ginkgo biloba* extract, green tea extract and placebo at baseline.

	Participants who used *Ginkgo biloba* (n=25)	Participants who used green tea (n=17)	Participants who used placebo (n=14)	*P*
Age, years	57.0±9.8	57.2±8.4	56.8±11.9	NS
Duration of T2DM, years	7.9±6.5	9.0±7.9	7.1±6.0	NS
HbA1c, %	8.1±1.6	7.8±1.4	8.1±2.0	NS
Total antioxidant status, mmol/L	1.6±0.2	1.7±0.1	1.6±0.1	NS
MDA + HNE, μM	7.1±2.7	6.2±4.0	7.4±5.5	NS
Perceived stress	12.5±5.4	10.6±7.4	16.6±10.4	NS
QOL, physical	14.4±1.8	14.0±2.2	12.9±2.5	NS
QOL, psychological	13.6±1.5	14.0±1.9	13.0±2.7	NS
QOL, social	14.4±1.4	14.5±1.9	14.2±1.9	NS
QOL, environmental	15.2±2.3	15.1±2.0	14.0±2.0	NS
POMS, tension-anxiety	5.8±2.5	3.5±2.1	4.3±3.1	NS
POMS, depression-dejection	9.3±6.2	2.2±2.1	6.0±3.5	NS
POMS, anger-hostility	10.5±5.4	6.0±5.0	5.0±2.0	NS
POMS, vigor-activity	14.5±3.4	16.0±6.1	14.0±3.5	NS
POMS, fatigue-inertia	8.7±3.4	5.5±2.6	5.7±2.3	NS
POMS, confusion bewilderment	1.7±2.9	0.5±2.1	2.3±3.5	NS
DDS, emotional burden	21.9±18.0	18.1±20.6	25.2±20.9	NS
DDS, physician-related	15.6±20.8	16.2±29.8	20.0±31.6	NS
DDS, regiment-related	30.7±20.0	30.5±15.5	34.1±16.2	NS
DDS, interpersonal	15.9±23.6	7.0±19.2	4.2±11.8	NS

HbA1c – glycated hemoglobin, MDA+HNE - malondialdehyde + 4-hydroxy-2-nonenal, QOL – Quality of Life, POMS – Profile of Mood States, DDS – Diabetes Distress Scale.

Results of baseline characteristics in groups and between-group comparisons at baseline are shown in Table 1. At baseline,between-group analysis showed no significant differences in psychological, antioxidant and glycemic control aspects.

Table 2: Differences in glycemic control, antioxidant state and psychological parameters in patients with type 2 diabetes mellitus who used *Ginkgo biloba* extract, green tea extract and placebo after 9 months.

	Participants who used *Ginkgo biloba* (n=25)	Participants who used green tea (n=17)	Participants who used placebo (n=14)	*P*
HbA1c, %	7.7±1.3	7.5±1.3	7.5±1.5	NS
Total antioxidant status, mmol/L	1.7±0.1	1.6±0.5	1.6±0.1	NS
MDA + HNE, μM	6.2±2.8	5.7±3.3	6.8±2.6	NS
Perceived stress	9.2±6.9	8.8±8.0	10.7±6.0	NS
QOL, physical	14.5±1.8	14.2±2.5	13.8±1.8	NS
QOL, psychological	14.3±1.7	14.3±1.6	14.4±1.7	NS
QOL, social	14.0±1.4	13.7±1.5	14.0±1.2	NS
QOL, environmental	15.2±1.2	15.0±1.2	15.3±1.3	NS
POMS, tension-anxiety	4.7±3.5	4.1±3.5	6.0±3.4	NS
POMS, depression-dejection	6.9±5.6	5.4±4.9	7.1±4.6	NS
POMS, anger-hostility	6.4±4.3	7.0±2.7	8.0±3.2	NS
POMS, vigor-activity	14.5±5.2	14.6±5.6	17.4±5.1	NS
POMS, fatigue-inertia	7.1±4.0	5.2±3.7	6.2±3.3	NS
POMS, confusion bewilderment	1.5±3.6	1.6±2.3	2.6±3.6	NS
DDS, emotional burden	19.2±20.3	20.3±20.3	21.8±17.5	NS
DDS, physician-related	11.0±20.4	7.7±16.9	6.8±22.6	NS
DDS, regiment-related	35.0±11.4	31.3±14.7	35.6±11.7	NS
DDS, interpersonal	20.0±19.0	6,1±17.1	5.0±12.4	0.006

HbA1c – glycated hemoglobin, MDA+HNE - malondialdehyde + 4-hydroxy-2-nonenal, QOL – Quality of Life, POMS – Profile of Mood States, DDS – Diabetes Distress Scale.

Table 3: Differences in glycemic control, antioxidant state and psychological parameters in patients with type 2 diabetes mellitus who used *Ginkgo biloba* extract, green tea extract and placebo after 18 months.

	Participants who used *Ginkgo biloba* (n=25)	Participants who used green tea (n=17)	Participants who used placebo (n=14)	*P*
HbA1c, %	7.6±1.3	7.2±1.5	7.2±1.4	NS
Total antioxidant status, mmol/L	1.6±0.2	1.7±0.1	1.6±0.1	NS
MDA + HNE, μM	5.8±2.3	6.6±2.6	5.8±2.3	NS
Perceived stress	7.4±8.0	11.1±8.6	16.1±13.8	NS
QOL, physical	13.5±1.7	12.1±4.5	12.8±1.8	NS
QOL, psychological	14.4±1.4	12.5±4.8	13.5±2.0	NS
QOL, social	14.3±1.9	11.9±8.5	14.5±1.2	NS
QOL, environmental	15.6±0.8	13.3±5.5	14.8±1.9	NS
POMS, tension-anxiety	5.1±5.3	4.1±4.3	5.7±4.0	NS
POMS, depression-dejection	7.8±4.7	6.1±5.4	10.2±7.8	NS
POMS, anger-hostility	7.1±5.5	6.9±5.0	10.5±5.2	NS
POMS, vigor-activity	13.7±3.4	13.9±4.1	14.5±2.4	NS
POMS, fatigue-inertia	5.9±3.9	6.2±4.2	7.5±3.4	NS
POMS, confusion bewilderment	2.2±3.4	1.9±3.3	3.2±4.2	NS
DDS, emotional burden	30.8±24.2	13.2±16.1	21.1±26.3	NS
DDS, physician-related	16.4±23.8	10.0±18.4	15.0±22.9	NS
DDS, regiment-related	33.7±16.9	31.2±13.2	37.7±17.1	NS
DDS, interpersonal	20.9±32.8	10.7±19.7	21.9±36.9	NS

HbA1c – glycated haemoglobin, MDA+HNE - malondialdehyde + 4-hydroxy-2-nonenal, QOL – Quality of Life, POMS – Profile of Mood States, DDS – Diabetes Distress Scale.

Between-group analysis after 9 and 18 months showed only minimal differences. After 9 months the only significant difference in between-group analysis was detected in the interpersonal domain of diabetes distress scale (Table 2). After 18 months, no significant difference between groups was found (Table 3).

Comparison of HbA1c, antioxidant state and psychological data at baseline, and after 9 and 18 months of using antioxidant preparations of *G. biloba* are presented in Table 4, of *Camellia sinensis* in Table 5 and of placebo in Table 6.

Some significant differences after 9 and 18 months of use of *G. biloba* extract were observed. The level of perceived stress significantly lowered after 9 and 18 months and the psychological domain of quality of life significantly improved after 18 months of use.

Table 4: Differences in glycemic control, antioxidant state and psychological parameters in patients with type 2 diabetes mellitus who used *Ginkgo biloba* extract for 9 and 18 months (n=29).

	At baseline	9 months	18 months	*P* between 0 and 9 months	*P* between 0 and 18 months
HbA1c, %	8.1±1.6	7.7±1.3	7.6±1.3	NS	NS
Total antioxidant status, mmol/L	1.6±0.2	1.7±0.1	1.6±0.2	NS	NS
MDA + HNE, μM	7.1±2.7	6.2±2.8	5.8±2.3	NS	NS
Perceived stress	12.5±5.4	9.2±6.9	7.4±8.0	0.038	0.030
QOL, physical	14.4±1.8	14.5±1.8	13.5±1.7	NS	NS
QOL, psychological	13.6±1.5	14.3±1.7	14.4±1.4	NS	0.019
QOL, social	14.4±1.4	14.0±1.4	14.3±1.9	NS	NS
QOL, environmental	15.2±2.3	15.2±1.2	15.6±0.8	NS	NS
POMS, tension-anxiety	5.8±2.5	4.7±3.5	5.1±5.3	NS	NS
POMS, depression-dejection	9.3±6.2	6.9±5.6	7.8±4.7	NS	NS
POMS, anger-hostility	10.5±5.4	6.4±4.3	7.1±5.5	NS	NS
POMS, vigor-activity	14.5±3.4	14.5±5.2	13.17±3.4	NS	NS
POMS, fatigue-inertia	8.7±3.4	7.1±4.0	5.9±3.9	NS	NS
POMS, confusion bewilderment	1.7±2.9	1.5±3.6	2.2±3.4	NS	NS
DDS, emotional burden	21.9±18.0	19.2±20.3	30.8±24.2	NS	NS
DDS, physician-related	15.6±20.8	11.0±20.4	16.4±23.8	NS	NS
DDS, regiment-related	30.7±20.0	35.0±11.4	33.7±16.9	NS	NS
DDS, interpersonal	15.9±23.6	20.0±19.0	20.9±32.8	NS	NS

HbA1c – glycated hemoglobin, MDA+HNE - malondialdehyde + 4-hydroxy-2-nonenal, QOL – Quality of Life, POMS – Profile of Mood States, DDS – Diabetes Distress Scale.

Table 5: Differences in glycemic control, antioxidant state and psychological parameters in patients with type 2 diabetes mellitus who used green tea extract for 9 and 18 months (n=17).

	At baseline	9 months	18 months	*P* between 0 and 9 months	*P* between 0 and 18 months
HbA1c, %	7.8±1.4	7.5±1.3	7.2±1.5	NS	NS
Total antioxidant status, mmol/l	1.7±0.1	1.6±0.5	1.7±0.1	NS	NS
MDA + HNE, μM	6.2±4.0	5.7±3.3	6.6±2.6	NS	NS
Perceived stress	10.6±7.4	8.8±8.0	11.1±8.6	NS	NS
QOL, physical	14.0±2.2	14.2±2.5	12.1±4.5	NS	NS
QOL, psychological	14.0±1.9	14.3±1.6	12.5±4.8	NS	NS
QOL, social	14.5±1.9	13.7±1.5	11.9±8.5	NS	NS
QOL, environmental	15.1±2.0	15.0±1.2	13.3±5.5	NS	NS
POMS, tension-anxiety	3.5±2.1	4.1±3.5	4.1±4.3	NS	NS
POMS, depression-dejection	2.2±2.1	5.4±4.9	6.1±5.4	NS	NS
POMS, anger-hostility	6.0±5.0	7.0±2.7	6.9±5.0	NS	NS
POMS, vigour-activity	16.0±6.1	14.6±5.6	13.9±4.1	NS	NS
POMS, fatigue-inertia	5.5±2.6	5.2±3.7	6.2±4.2	NS	NS
POMS, confusion bewilderment	0.5±2.1	1.6±2.3	1.9±3.3	NS	NS
DDS, emotional burden	18.1±20.6	20.3±20.3	13.2±16.1	NS	NS
DDS, physician-related	16.2±29.8	7.7±16.9	10.0±18.4	0.046	NS
DDS, regiment-related	30.5±15.5	31.3±14.7	31.2±13.2	NS	NS
DDS, interpersonal	7.0±19.2	6.1±17.1	10.7±19.7	NS	NS

HbA1c – glycated haemoglobin, MDA+HNE - malondialdehyde + 4-hydroxy-2-nonenal, QOL – Quality of Life, POMS – Profile of Mood States, DDS – Diabetes Distress Scale.

No statistically significant differences were detected in HbA1c level, antioxidant state and psychological data after 9 and 18 months of using green tea extract. Physician-related distress in DDS significantly decreased after 9 months of use of this preparation, but after 18 months it increased again.

Table 6: Differences in glycemic control, antioxidant state and psychological parameters in patients with type 2 diabetes mellitus who used placebo for 9 and 18 months (n=14).

	At baseline	9 months	18 months	*P* between 0 and 9 months	*P* between 0 and 18 months
HbA1c, %	8.1±2.0	7.5±1.5	7.2±1.4	NS	0.017
Total antioxidant status, mmol/L	1.6±0.1	1.6±0.1	1.6±0.1	NS	NS
MDA + HNE, μM	7.4±5.5	6.8±2.6	5.8±2.3	NS	NS
Perceived stress	16.6±10.4	10.7±6.0	16.1±13.8	NS	NS
QOL, physical	12.9±2.5	13.8±1.8	12.8±1.8	NS	NS
QOL, psychological	13.0±2.7	14.4±1.7	13.5±2.0	NS	NS
QOL, social	14.2±1.9	14.0±1.2	14.5±1.2	NS	NS
QOL, environmental	14.0±2.0	15.3±1.3	14.8±1.9	NS	NS
POMS, tension-anxiety	4.3±3.1	6.0±3.4	5.7±4.0	NS	NS
POMS, depression-dejection	6.0±3.5	7.1±4.6	10.2±7.8	NS	NS
POMS, anger-hostility	5.0±2.0	8.0±3.2	10.5±5.2	NS	NS
POMS, vigor-activity	14.0±3.5	17.4±5.1	14.5±2.4	NS	NS
POMS, fatigue-inertia	5.7±2.3	6.2±3.3	7.5±3.4	NS	NS
POMS, confusion bewilderment	2.3±3.5	2.6±3.6	3.2±4.2	NS	NS
DDS, emotional burden	25.2±20.9	21.8±17.5	21.1±26.3	NS	NS
DDS, physician-related	20.0±31.6	6.8±22.6	15.0±22.9	NS	NS
DDS, regiment-related	34.1±16.2	35.6±11.7	37.7±17.1	NS	NS
DDS, interpersonal	4.2±11.8	5.0±12.4	21.9±36.9	NS	NS

HbA1c – glycated hemoglobin, MDA+HNE - malondialdehyde + 4-hydroxy-2-nonenal, QOL – Quality of Life, POMS – Profile of Mood States, DDS – Diabetes Distress Scale.

In patients who used placebo, significant lowering of HbA1c level was observed after 18 months, showing improved diabetes glycemic control.

Some significant correlations between HbA1c level, antioxidant state and psychological factors were detected after using *G. biloba*, green tea extracts and placebo preparations (Table 7). In the group of patients who used *G. biloba* extract, a positive correlation between HbA1c level and MDA+HNS level, as well as strong negative correlation between DDS regiment-related distress and POMS anger-hostility score were detected. In the group of patients who used green tea extract antioxidant preparation, negative strong correlations between MDA+HNS level and POMS tension-anxiety, POMS depression-dejection, and DDS regiment-related distress were found. In the group of patients who used placebo, strong negative correlation between Total Antioxidant Status and DDS regiment-related distress was observed.

Table 7: Detected significant correlations between glycemic control, antioxidant state and psychological parameters in patients with type 2 diabetes mellitus who used antioxidant preparations.

Group according to used antioxidant preparation	Significant correlations between		r	*p*
Ginkgo biloba extract	MDA+HNE	HbA1c level	0.497	0.019
	DDS regiment-related distress	POMS anger-hostility	- 0.911	0.011
Green tea extract	MDA+HNE	POMS tension-anxiety	- 0.745	0.021
	MDA+HNE	POMS depression-dejection	- 0.744	0.022
	MDA+HNE	DDS regiment-related distress	- 0.659	0.038
Placebo	Total antioxidant status	DDS, physician-related distress	- 0.795	0.033

HbA1c – glycated hemoglobin, MDA+HNE - malondialdehyde + 4-hydroxy-2-nonenal, , QOL – Quality of Life, POMS – Profile of Mood States, DDS – Diabetes Distress Scale.

Our study showed that the antioxidant extract of *G. biloba* leaves, but not *C. sinensis* extract exhibited a mild effect on psychological

state (decreased perceived psychological stress and improved psychological aspect of quality of life) in patients with T2DM. Nevertheless, the antioxidant state measured as Total Antioxidant Status and MDA+HNE level did not change significantly after 9 and 18 months of treatment, though MDA+HNE level decreased a little. As the antioxidant state in the patients with T2DM may have been significantly impaired for many years, it is possible that a longer duration of using antioxidant preparations may have had greater impact on changing the antioxidant state.

Recent evidence suggested that diabetic subjects have reduced antioxidant capacity which could favor antioxidant stress. A decline in important cellular antioxidant defense mechanisms significantly increases the susceptibility to oxidative stress. *In vivo* studies revealed that oxidative stress due to hyperglycemia occurs before late complications become clinically evident [33,34]. In the studies of humans and rodents, the supplementation with antioxidants, flavonoids among them, was found to induce changes that could be beneficial in reducing insulin resistance and protecting the vascular endothelium [35,36].

The main fraction with antioxidant effect in the preparation of *G. biloba* is that of flavonoids, which have antioxidant effects resulting from direct attenuation of reactive oxygen species by chelating pro-oxidant transitional metal ions, and also by promoting the expression of antioxidant proteins which, in turn, increases antioxidant metabolites such as glutathione [37,38]. In addition to its neurological and vascular protective effects *G. biloba* extract has been reported to reduce hyperglycemia, and increase glucose uptake and glycogen synthesis in rats. It was also shown that the glucose-lowering effect of *G. biloba* extract in rats was caused by the inhibition of alpha-amylase and glucosidase [39]. There have been some reports about improvement of glucose homeostasis after using *G. biloba* extract in humans [40,41]. A recent study proved that *G. biloba* extract induces insulin secretion and that this is mediated by increased intracellular calcium transients. Another group reported that *G. biloba* extract ingestion increases plasma insulin levels in response to oral glucose loading in subjects with T2DM. The data suggest that this anti-oxidant preparation enhances pancreatic beta cell function [40,41].

Recently some studies have reported the role of oxidative stress in anxiety-like behavior in rodents and revealed that induction of oxidative stress in hypothalamus and amygdala occurs in parallel with anxiety in mice [10,12-13]. Also, studies demonstrated abnormal mitochondrial function and oxidative stress in specific brain regions (hippocampus, hypothalamus, amygdala) in major depressive disorder [42]. Hippocampal function is important in verbal memory. Amygdala regulates emotional control and may enable long-term memory. Hypothalamus and its extended neurohypophysis may effect neuroendocrine regulation [42].

Patients with T2DM have reduced antioxidant capacity that increases their susceptibility to oxidative stress [33,34], and this may impact not only on metabolic and neuroendocrine regulation, but also on psychological state. So, antioxidants may have positive influence not only on metabolic and neuroendocrine, but also on psychological state of patients with T2DM. Our study provides some evidence of such an effect (though mild) of antioxidant preparation of *G. biloba*, but it needs further investigation.

Results of some studies support the contribution of oxidative stress to the multifactorial etiology of insulin resistance in the whole body and also to neuroendocrine regulation and psychological impairments [2,10,13,42]. Animal models and human studies support the utility of antioxidant treatments for reducing oxidative stress by clear improvements in glucose tolerance and insulin sensitivity [2,39-51]. The bigger problem has been the translation of the antioxidant intervention strategies to the effective treatment of insulin-resistance states in human subjects [2]. So, our study could be useful in providing direction for further development and implementation of effective antioxidant supplementation for diabetic patients.

There are several limitations to this study. The sample size was relatively small when stratified for the antioxidant preparation used. Besides, there were many variables being analyzed. The strength is that the study was carried out over 18 months and looking at substances which still need more scientific research.

In conclusion, the antioxidant *G. biloba* leaf extract exhibited a mild effect on psychological state and a trend in improving glycemic control in patients with type 2 diabetes mellitus. Nevertheless, it needs further investigation with bigger samples. The past decades have seen considerable changes in the opinion regarding the applications of herbal preparations, especially those with antioxidant effects. Herbal antioxidant medications can be good alternatives in supplementation trying to reach better glycemic control, psychological state, quality of life and well-being of diabetic patients, because as herbal medicaments are derived from plants, they are considered to be relatively safe and have rather few side effects.

Experimental

Study design: The randomized double blind placebo-controlled study of parallel design was performed in The Clinic of Endocrinology, Kaunas Clinical Hospital of Lithuanian University of Health Sciences, Lithuania. The study was approved by the Lithuanian Bioethics Committee (Protocol No. BE-2-5) and the State Data Protection Inspectorate. Written, informed and voluntary consent was obtained from all participants.

A total of 250 patients diagnosed with type 2 diabetes mellitus, aged from 37 to 78 years and followed up for diabetic retinopathy, nephropathy or neuropathy were recruited for the study. Of them, 194 failed to use the antioxidant preparations in the prescribed way or refused to participate in the study after some time. For the research and statistical analysis we used only the data of 56 participants who managed to use the antioxidant preparations in the prescribed way for the period of the study, and who also came for the second (after 9 months) and third visits (after 18 months).

Glycemic control of diabetes was measured by the level of glycated hemoglobin (HbA1c). Antioxidant state was measured as Total Antioxidant Status and malondialdehyde + 4-hydroxy-2-nonenal (MDA+HNE) level. Psychological state and quality of life of the participants were also evaluated at the baseline and after 9 and 18 months of using *G. biloba* extract, green tea extract or placebo preparations. All psychological evaluations were performed by a medical psychologist with a university master's degree in health psychology.

Antioxidant preparations and biochemical measurements: All patients were randomly allocated to receive standardized *G. biloba* dry extract, green tea extract, or placebo capsules. For the first 9 months patients used one capsule twice a day, and for the second 9 months – 1 capsule 3 times a day. Placebo capsules were made from microcrystalline cellulose, a material indifferent to disease (Joint-stock company "Sanitas"). *G. biloba* dry extract capsules contain 80 mg of standardized dry extract of *G. biloba* leaves, adjusted to

19.2 mg Ginkgo flavone glycosides and 4.8 mg terpene lactones (ginkgolides, bilobalide) (Joint-stock company "Aconitum"). Green tea extract capsules contained 200 mg standardized extract of *C. sinensis* leaves, adjusted to 70 % polyphenols (Joint-stock company "Sanitas").

Morning fasting vein blood samples for evaluation of HbA1c level, MDA+HNE level and total antioxidant status were taken. Serum was separated and stored at -20ºC until analyzed. HbA1C level was measured on a Siemens DCA – 2000 Analyzer (USA) according to the manufacturer instructions and using its own kits. Total antioxidant status was measured by the method of Miller 443] on an automatic chemical analyzer "Dayton RX" based on spectrophotometer methods according to instructions provided by Randox Laboratories Ltd., UK and using its own kits. The concentration of MDA+HNE were evaluated using a "LPO Microplate Based Assay Kit" Cat.No.FR22 (Oxford Biomedical Research, USA) on a microplate absorbance reader (TECAN, Austria) by the method of Esterbauer and Cheeseman [34].

Psychological assessment: Perceived psychological stress was measured by the Perceived Stress Scale [45]. This measures the level to which situations in one's life over the past month are appraised as stressful. The scale is a 10-item measure with each item scored from 1 (never) to 4 (very often). A higher score represents a higher level of perceived stress.

Emotional state was evaluated by means of Profile of Mood States (POMS) [46], which measures six subscales: tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia and confusion-bewilderment. A higher score represents a higher level of a certain emotion.

Quality of life was assessed by WHO Brief Quality of Life Questionnaire (WHOQoL) [47]. It measures 4 domains: physical, psychological, social relationships and environmental. A higher score represents a better quality of life.

Diabetes-related distress was evaluated by Diabetes Distress Scale (DDS) [48]. DDS is a 17-item measure with each item scored from 1 (no distress) to 6 (serious distress) concerning diabetes-related distress experienced over the last month. It assesses 4 sub-scales: emotional burden, physician-related distress, regiment-related distress, and diabetes interpersonal distress. A higher score represents a higher level of experienced distress.

Statistical analysis: Analysis was performed using SPSS 17.0 software. The values are given as mean ± standard deviation. The differences between the means in groups were calculated using the Wilcoxon test. The Kruskal-Wallis test was used for the between-group analysis. Associations between somatic and psychological data were determined using the Spearman correlation coefficient. The limit of significance was defined as a two-sided *p*-value lower than 0.05.

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