Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 49, Number 3, p. 270-282, September 2021
DOI: 10.21608/zvjz.2021.86000.1149

RESEARCH ARTICLE

The influence of Ginkgo Biloba on hepatic gene expression of (PGC1-α, PPAR-α and GLUT-2), liver, kidney functions, hematological and lipid profile in type I diabetic rats

Ashraf Awad, Iman E. El Araby, and R. Albaiomy

Animal Wealth Development Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt.

***Corresponding author e-mail: mlight85@gmail.com**

Article History: Received: 13/07/2021 Received in revised form: 28/07/2021 Accepted: 17/08/2021

Abstract

Ginkgo biloba is one of a well-known medicinal plant worldwide, where is prescribed in working on various diseases, such as diabetes mellitus type I and II, peripheral vascular abnormalities, and neurodegenerative illnesses like Alzheimer's disease . The purpose of this study was to know how oral *Ginkgo biloba* administration affected the liver, kidneys, hematological parameters, lipid profile, and hepatic gene expression in type I diabetic rats. Seventy-five male adult Sprague Dawely rats were allocated into five equal groups 15 rats each, 1st group; control group, 2nd group; diabetic group, 3rd group; *Ginkgo biloba* group, 4th group; Diabetic treated group received single oral dose of *Ginkgo biloba* after diabetic induction as a treatment and 5th group; rats were administered daily single oral dose of *Ginkgo biloba* as a protective dose from the 1st day of the study prior to diabetes induction and continued after the diabetes induction till the end of the work. The results of study revealed that *Ginkgo biloba* actions in modulating of hepatic key genes *GLUT-2* **(**glucose transporter-2), *PPAR-α* **(**Peroxisome proliferator-activated receptor alpha), and *PGC1-α* **(**The peroxisome proliferator-activated receptor gamma coactivator-1). Also, *Ginkgo biloba* possesses antihyperlipidemic activities, supports liver and kidney functions and improves glucose metabolism, storage and utilization in liver by enhancing its key genes. based on the outcomes of current study *Ginkgo biloba* is an effective food supplement or a supportive treatment for type I diabetes mellitus..

Keywords: Hepatic key genes, Diabetes mellitus, *Ginkgo biloba*, Gene expression.

Introduction

In recent ages, significant public health diseases existed that threatening human life. One of these basic diseases is diabetes mellitus [1]. Diabetes mellitus is considered set of metabolic changes known as "chronic metabolic syndrome," which is associated with an elevated rate of morbidity and mortality. This disease is caused by limited insulin secretion from pancreatic β-cells as a consequence to β-cell damage, as well as a malfunction in insulin performance leading to disorders in the metabolism of carbohydrate, protein, fat [2] and hyperglycemia with glucosuria, polydipsia, and polyuria [3].

The cause of primary idiopathic diabetes may be hereditary or environmental. Diabetes mellitus can be juvenile-onset or mature-onset. The former is insulin-dependent, as it is linked to the pancreas' ability to release insulin being diminished or nonexistent. The non-insulin dependent is characterized by relatively inefficient insulin in spite of normal excretion. [4].

The popular name *Ginkgo* is a Japanese word for the tree; however, the species name *biloba* alludes to the two different lobes that are characteristic of the tree's leaves. It is a matchless plant due to its respective classification in the plant

kingdom, and it is considered a living fossil as one of the oldest known seed plants [5].

The active antioxidant constituents in *G. biloba* leaf extract contain 20-27 percent flavonoids (the majority are isorhamnetin, quercetin, kaempferol, and proanthocyanidins), 5-7 % terpenoids (the majority are ginkgolides A, B, C, M, J, and bilobalide) and 5-10% organic acids [6]. *G. biloba* has direct antioxidant properties by scavenging reactive oxygen radicals and chelating transition metals [7]. *G. biloba* treatment, on the other hand, can modulate fatty acid metabolism and enhance non-saturated fatty acid levels in the blood [8].

The basic hepatic liver transporter, glucose transporter 2 (*GLUT-2*), is a high-capacity transporter found on hepatocyte sinusoidal membranes, the basolateral membrane of intestinal epithelial cells, renal proximal tubule cells, and pancreatic beta-cells. Under insulin control, the hepatic glucose transporter *GLUT2* plays a key function in promoting bidirectional glucose transport across the plasma membrane of hepatocytes [9].

The key receptor peroxisome proliferator-activated receptor Alpha (*PPAR-α*) has emerged as an effective insulin sensitizer. There are three PPAR isotypes: α, δ and γ , all of which have a role in controlling the expression of genes involved in lipid storage, glucose metabolism, morphogenesis, and the inflammatory reaction [10].

A transcription factor *PGC-1α* (peroxisome proliferator-activated receptor-gamma coactivator-1 alpha), is a specific gene that influences lipid metabolism and long-chain fatty acid oxidation by upregulating the expression of numerous genes in the tricarboxylic acid cycle and mitochondrial fatty acid oxidation pathway [11].

This study aimed to estimate the *ginkgo biloba* antihyperlipidemic activities and its supportive role in glucose metabolism, utilization, and storage in liver by enhancing its key genes.

Materials and Methods

Plant and chemicals

Ginkgo Biloba was obtained from coated tablets manufactured by MARCYRL Pharmaceutical Industries. Each tablet was adjusted to contain 21.6-26.4% ginkgo flavonoid glycosides and 5.4-6.6% terpene lactones (ginkgolides, bilobalide). Streptozotocin (STZ) was obtained from (Sigma Chemical Co. St. Louis, Mo, USA).

Experimental animals

Present study was carried out on 75 male adult Sprague Dawely rats (150-200) body weight. Animals were kept under hygienic conditions in metal cages with hard wood shavings as bedding and maintained on light/darkness cycle 12/12 hours and given feed and water ad-libitum throughout the experimental period. This study was accepted and completed by the approval of the Zagzaig University Committee (Animal Care and Welfare Committee). The approval Number is **ZU-IACUC/2/F/83/ 2021**.

Experimental Design

The study lasted 60 days and consisted of five groups Each group was occupied by 15 rats.

1st Group (CG):

The group was represented as a negative control or non-diabetic one ,and were given a standard diet.

2nd Group (DG):

The rats of this group were considered as diabetic control or positive control by once I/P injection of STZ (Sigma, USA) at the dose of 60mg/kg at 30th day of the study [12].

3rd Group (GB):

This group involved rats that given *ginkgo biloba* (MARCYRL Pharmaceutical Industries) at the dose of 200mg/kg BW once daily by oral from the 1st day till the end of study for 60 days.

4th Group (D+GB):

As a treatment group**,** rats of this group were diabetic at 30th day of the study with once I/P injection of STZ at the dose of 60mg/kg BW then applied by *ginkgo biloba* at the dose of 200mg/kg b.wt once daily by oral till the end of study [13].

5th Group (GB+D+GB):

As a protective group, rats of this group were given *ginkgo biloba* at the dose of 200mg/kg b.wt once daily by oral from the 1st day of study, then at 30th day of the study diabetes was induced by STZ 60 mg/ kg BW with once I/P injection of STZ 60mg/kg BW then *Ginkgo biloba* were applied 200mg/kg b.wt once daily till the end of study [14].

The study lasted 60 days. On the 30th day, the induction of diabetes was applied to groups (DG), (D+GB) and (GB+D+GB) by once intra- peritoneally injection of STZ (Sigma, USA) at the dose of 60mg/kg. Samples of fasting blood were obtained from tip of tail of rats after 3 days of STZ injection overnight and glucose levels was measured (ACCU check, Germany). Rats which had fasting blood glucose levels more than 250 mg/dl were included in the work as diabetic rats. Treatment with *Ginkgo biloba* [21.6-26.4% ginkgo flavonoid glycosides and 5.4-6.6% terpene lactones (ginkgolides, bilobalide)] started at dose of 200mg/kg BW once daily by oral and the blood samples were measured weekly by (ACCU check, Germany) till the end of study.

Samples

Blood sampling, Hematological and biochemical analysis

Five ml fasting state samples of blood were obtained aseptically via cardiac puncture of all rats at the end of the 60th days in a sterile vacationer tube containing Na_2EDTA as an anticoagulant for Haematological examination (RBCs, WBCs, Platlets and HB). In other sterile tube without anticoagulant, blood sample was obtained for separation of serum for biochemical analysis (FSG, AST, ALT, Urea, creatinine, albumin, globulin, total protein, triglyceride, HDL-C, LDL-C and VLDL-C) [15].

Tissue samples and hepatic genes mRNA expressions

Hepatic tissues were collected freshly and stored at -80 °C using Trizol. RNA extraction process occurred at (Laboratory of biotechnology, Faculty of Veterinary Medicine, Zagazig University) using Trizol (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. Quantification of total RNA was measured at 260 nm. . The A260/A280 ratio was analyzed using the NanoDrop® ND–1000 Spectrophotometer (NanoDrop Technologies; Wilmington, Delaware, United States) for 1.5 µl of the RNA. Total (1 µg) RNA was transformed to cDNA through the process of revers transcription using a High-Capacity cDNA Reverse Transcription Kit cDNA Kit; (Applied Biosystems™, USA) in a 20 µl reaction volume. Quantitative PCR was performed using TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX) (Cat. # P725 or P750) (Enzynomics, Korea). Primers were prepared as the manufacturer instructions and kept at -20°c until used for subsequent PCR analysis .Primers Sequence as listed in (Table 1) was selected by the primer3 website. By using GADPH, the mRNA levels were normalized as a housekeeping gene and by using the ΔΔCT method, relative quantification was performed.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to examine the values of all groups in the expression analysis. Results were expressed as means ± SE (standard error) of the mean and $P \leq 0.05$ were considered a significant in all tests, unless stated otherwise. Differences were considered to be significant at the level of ($P \leq 0.05$).

Table 1. Primers' sequences and expected product sizes used in the work.

Gene		Primer sequence (5'à3')	Expected product size (bp)	acession No.
GAPDH	*GAPDH Forward*	CAACTCCCTCAAGATTGTCAGCAA	118 bp	NM_017008
	GAPDH Reverse	GGCATGGACTGTGGTCATGA		
PGC1-α	*PGC1-α Forward*	TTCAGGAGCTGGATGGCTTG	70 bp	NM_031347.1
	PGC1-α Reverse	GGGCAGCACACTCTATGTCA		
PPAR- α	*PPAR-α Forward*	GTCCTCTGGTTGTCCCCTTG	176 bp	NM_013196.2
	PPAR-α Reverse	GTCAGTTCACAGGGAAGGCA		
Glut-2	*Glut-2 Forward*	GGTGTTCCTCTGGATGACCG	183 bp	NM_012879.2
	Glut-2 Reverse	CATTCCGCCTACTGCAAAGC		

GAPDH: Glyceraldehyde-3- phosphate dehydrogenase, *PGC1-α*: The peroxisome proliferator-activated receptor gamma coactivator-1 alpha, *PPAR- α:* Peroxisome proliferator-activated receptor alpha, *Glut-2*: glucose transporter-2.

Results

Blood and fasting serum glucose concentration

The results of the fasting blood glucose levels, were measured weekly after induction of diabetes by STZ from week 5 to week 8, revealed that type1 diabetes induced a significant ($P < 0.001$) increase in the mean values compared to control group. The results of 5th week and 8th week in DG were (412±16.52 mg/dl), (454.62±13.9 mg/dl) respectively compared to the control group (104.67±3.71 mg/dl), (114.67±3.71 mg/dl) respectively. However, orally administration of *G.biloba* either diabetic or non-diabetic groups (GB), (D+GB) and (GB+D+GB) significantly $P< 0.01$ reverted the mentioned parameters towards the normal average of physiological level. The results of the 5th and 8th weeks, GB group were 117.67±7.97 mg/dl,and 112±5.13 mg/dl) respectively. The D+GB group revealed 263.67±4.91 mg/dl,and 201.67±31.36 mg/dl correspondingly , moreover the GB+D+GB group had (265±3.46 mg/dl,and239.67±1.77 mg/dl levels respectively. Besides, the results of fasting serum glucose concentration in the DG at the end of study is significant **(***P*< 0.00001) higher (271.33±3.75 mg/dl) than CG (109.33± 2.60 mg/dl). However, *G.biloba* groups either diabetic or non-diabetic groups (GB), (D+BG) and (GB+D+GB) showed significant decrease (*P*< 0.00001) in FSG concentration (119.33±2.03 mg/dl), (235.33±3.75 mg/dl), (208.67±7.69 mg/dl) respectively compared to diabetic one (DG).

Hematological measurements

As in Figure 1, results showed in diabetic gp. DG that there was a significant decrease ($p<0.05$) in Hb concentration, platelets and WBCs (12.53±0.49 g/dl), (444.33±63.50 10^3/µl) and (12.09±0.71 10^3/µl) respectively compared with the control group CG (14.4±0.23 g/dl), (741± 56.0 10^3/µl) and (18.8 ± 1.84 10^3/µl) respectively, showing features of anemia and leukopenia. Meanwhile, *ginkgo biloba* treated groups either diabetic or non-diabetic in GB, D+BG and GB+D+GB showed significant increase ($P < 0.05$) in HB (14.25±0.20 g/dl), (13.27±0.12 g/dl), (14.63±0.64 g/dl) respectively ,platelets (630±22.52 10^3/µl), (651±66.84 10^3/µl), (606.67 ±44.38 10^3/µl) respectively and WBCs levels (14.89 ± 0.36 10^3/µl), (18.22 ± 1.39 10^3/µl), (18.17±0.92 10^3/µl) respectively compared to diabetic one. Otherwise, RBCs count, the results showed that there were no significant differences among groups, $P= 0.448$ ($P > 0.05$).

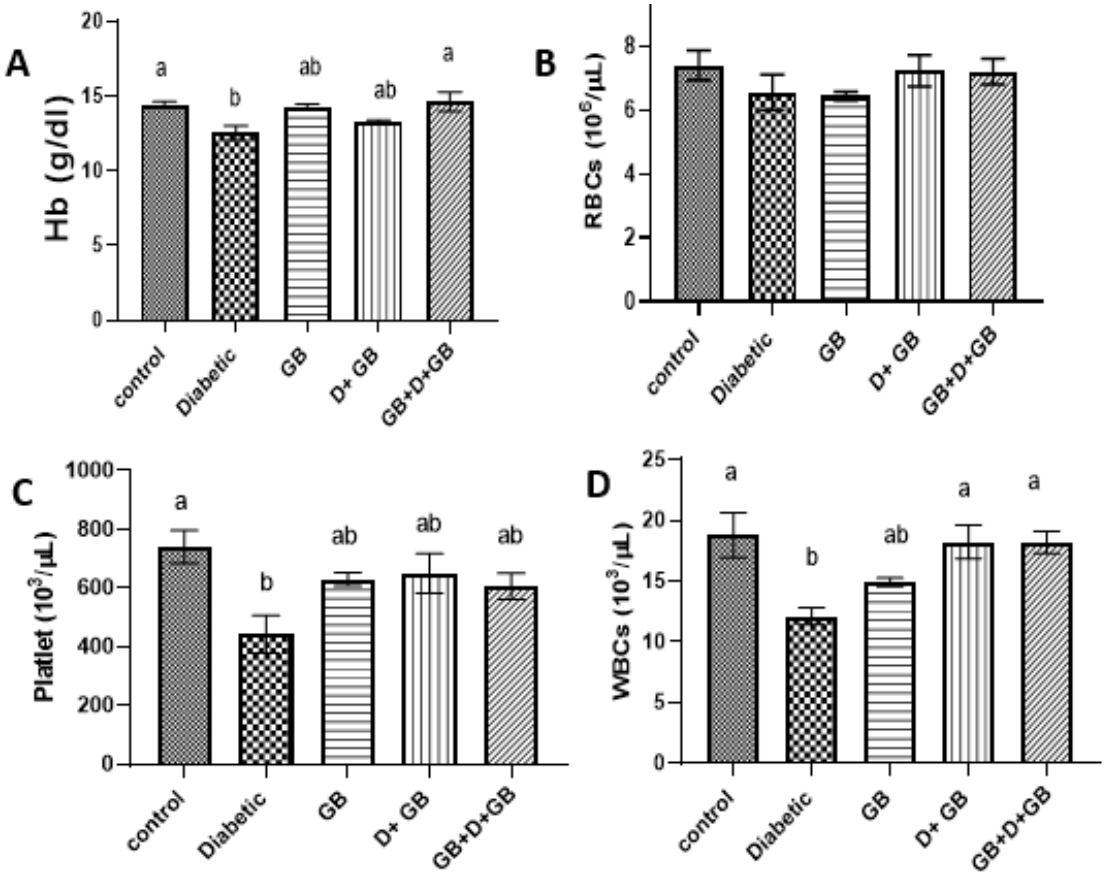

Figure 1: Effect of oral administration of *ginkgo biloba* (21.6-26.4% ginkgo flavonoid glycosides and 5.4-6.6% terpene lactones) 200 mg/kg BW at 60 days pre and post diabetic induction on the mean value of ; **A.** Hemoglobin (HB gm/dL), **B.** Red blood cells (RBCs 10^6/ µL), **C**. Platelets (10^3/ µL) and **D.** White blood cells (WBCs 10^3/ µL), in type (I) STZ-induced diabetic rats. All values are mean ± S.E.M; n=8. Control: control group; diabetic: diabetic group; GB: *Ginkgo biloba* group; D+GB: Diabetic+ *Ginkgo biloba* group; GB+D+GB: *Ginkgo biloba*+ Diabetic+ *Ginkgo biloba* group.

Biochemical parameters of the serum

Serum concentrations of AST, ALT, creatinine and urea in different groups were measured .In the diabetic group (DG), serum AST and ALT were significantly increased ($p \leq 0.001$) compared to control group. However, rats were administered orally with *ginkgo biloba* in groups (GB), (D+GB) and (GB+D+GB) showed significant decrease ($p \leq 0.001$) in AST and ALT compared to diabetic one. Concerning to urea and creatinine, there were significant increase ($p<0.00001$) and ($p < 0.05$) respectively in DG compared to CG. Meanwhile *ginkgo biloba* treated groups either diabetic or non-diabetic (GB), (D+ BG) and (GB+D+GB) showed significant decrease in urea and creatinine ($p<0.00001$) and ($p<0.05$) respectively compared to (DG) (Table 2).

Serum protein profile

Data revealed as in Figure 2 that diabetic gp. (DG) revealed a significant decrease ($p<0.05$) in albumin (4.253±0.578 gm/dl) compared to control group (CG) (4.563± 0.108 gm/dl). Meanwhile, rats administered orally with *ginkgo biloba* in groups (GB), (D+GB) and (GB+D+GB) had a significant increase ($p<0.05$) in the level of albumin (4.553±0.060 gm/dl), (4.326±0.037 gm/dl) and (4.63±0.040 gm/dl) respectively compared to (DG) with no significant changes between groups in total protein, globulin and A/G ratio, P = 0.129, 0.076 and 0.251 ($P>0.05$) respectively.

Table 2. The effect of *Ginkgo biloba* on biochemical parameters (ALT, AST, Creatinine and Urea) and Lipid profile (Total cholesterol, Triglycerides, HDL-C, LDL-C and VLDL-C) for 60 days in type (I) STZ-induced diabetic rats.

	ALT (µ/L)	AST (µ/L)	Creatinine mg/dL	Urea mg/dL	Cholesterol mg/dL	TG mg/dL	HDL-C mg/dL	LDL-C mg/dL	VLDL-C mg/dL
Control	53.00^{b}	183.5 ^{bc}	0.733 ^{b}	23.5^{b}	104.66^{bc}	86.00^{b}	52.00^{a}	70.5^{abc}	16.566^{b}
	±1.732	±3.175	±.049	±2.02073	±6.009	±6.658	±1.154	±4.330	±1.69
Diabetic	108.0^{a}	238.00^{a}	1.536^{a}	58.667^{a}	136.00^{a}	139.5^{a}	37.33^{b}	84.00^{a}	27.233^{a}
	±10.50	±9.237	±0.248	±4.09	±0.577	±10.037	±4.33	±0.577	±1.714
GB	56.00^{b}	175.00 ^{bc}	0.7300 ^{b}	23.00 ^{b}	94.00^{c}	79.00^{b}	44.00^{ab}	53.33^{bc}	17.133^{b}
	±3.464	±13.279	±0. 017	±577	±3.511	±6.429	±2.309	±3.756	±1.702
D+GB	68.00^{b}	162.33 ^{c}	0.7233 ^{b}	26.5^{b}	95.00^{c}	65.5^{b}	45.5^{ab}	49.5^{c}	13.4^{b}
	±4.358	±7.623	± 0.003	±.866	±8.082	±0.288	±2.020	±6.062	±0.115
GB+D+GB	73.00^{b}	206.33 ^{ab}	0.7400 ^{b}	31.00^{b}	124.00^{ab}	69.333^{b}	49.5^{a}	74.5^{ab}	18.00^{b}
	±5.033	±2.603	±0.051	±1.154	±1.154	±5.811	±0.866	±6.062	±0.346
P-value	0.0004	0.001	0.002	0.000002	0.0003	0.00009	0.015	0.002	0.0003

Means within the same column carrying different superscripts are sig. different at P < 0.05 based on Tukey's Honestly Significant Difference (Tukey's HSD) test. ALT: Alanine transaminase enzyme, AST: Aspartate transaminase enzyme, TG: Triglyceride, HDL-C: High density lipoprotein, LDL-C: Low density lipoprotein and (VLDL-C) Very low-density lipoprotein.

Serum lipid profile

The results showed a significance increase in serum cholesterol ($p<0.001$), triglyceride ($p<0.0001$), LDL-C ($p<0.05$) and VLDL-C ($p<0.001$) in STZ-diabetic rats (DG) and a significant decrease ($p<0.05$) in HDL-C level compared with control group (CG). However, rats administered orally with *ginkgo biloba* in groups (GB), (D+GB) and (GB+D+GB) showed a significant decrease in triglyceride ($p<0.0001$), cholesterol ($p<0.001$), LDL-C ($p<0.05$), VLDL-C ($p<0.001$) and a significant increase ($p<0.05$) in HDL-C level compared to (DG) (Table 2).

mRNA Expression of Hepatic genes

The study illustrated the hepatic key genes mRNA expression of *PGC1-α* (The peroxisome proliferator-activated receptor alpha coactivator-1), *PPAR-* α (Peroxisome proliferator-activated receptor alpha) and Glut-2 (glucose transporter-2).

The results of this current work showed a significant downregulation ($p<0.0001$) in *Glut-2, PPAR-α* and *PGC1-α* mRNA expression in diabetic group (DG) (0.33±0.01), (0.19±0.05) and (0.28±0.04) respectively compared with control group (CG) (1±0.05), (1.01±0.08) and (1±0.01) respectively. However, *Ginkgo biloba* treated groups either diabetic or non-diabetic (GB), (D+GB) and (GB+D+GB) showed a significant upregulation ($P<0.0001$) in mRNA expression of *Glut-2* **(**1.5±0.01**),** **(**4.19±0.55 **)** and **(**1.42±0.14 **)** respectively**,** *PPAR-α* **(**1.65±0.16), (5.11±0.35) and (1.39±0.07) respectively and *PGC1-α* **(**1.1±0.04), (3.83±0.09) and (1.48±0.17) respectively, compared with diabetic one Figure 3.

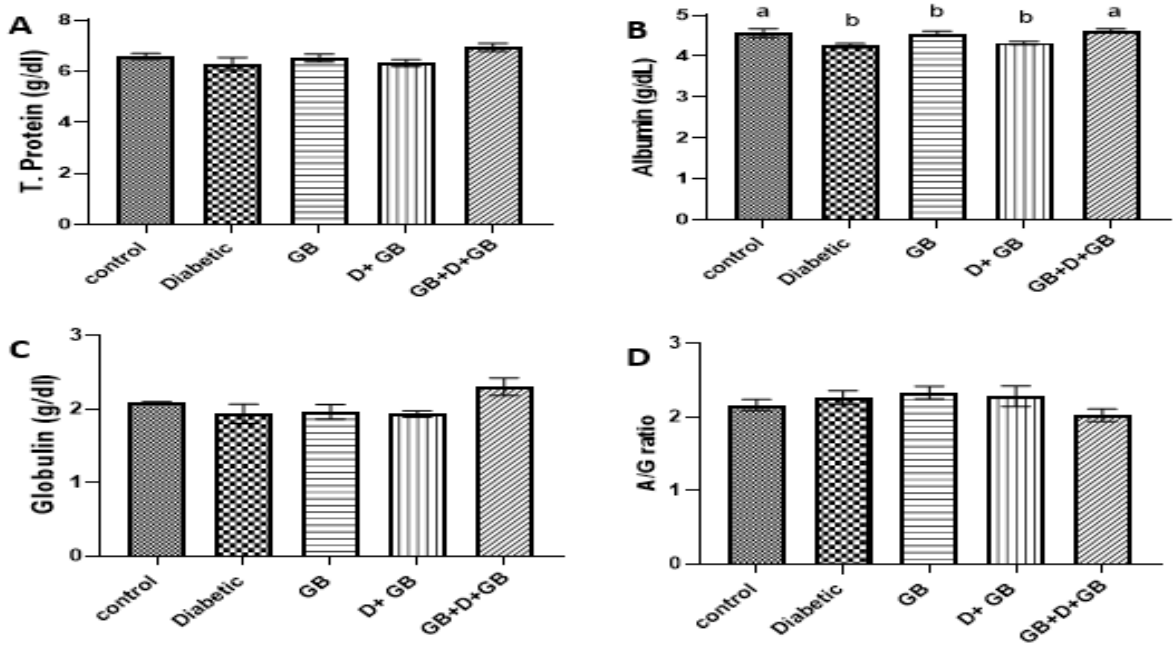

Figure 2: Effect of oral administration of *ginkgo biloba* (21.6-26.4% ginkgo flavonoid glycosides and 5.4-6.6% terpene lactones) 200 mg/kg BW at 60 days pre and post diabetic induction on the mean value of; A. Total protein (g/dL), B. Albumin (g/dL), C. Globulin (g/dL) and D. Albumin/ Globulin ratio (A/G) in type (I) STZ-induced diabetic rats. All values are mean ± S.E.M; n=8. control: control group; diabetic: diabetic group; GB: *Ginkgo biloba* group; D+GB: Diabetic+ *Ginkgo biloba* group; GB+D+GB: *Ginkgo biloba*+ Diabetic+ *Ginkgo biloba* group.

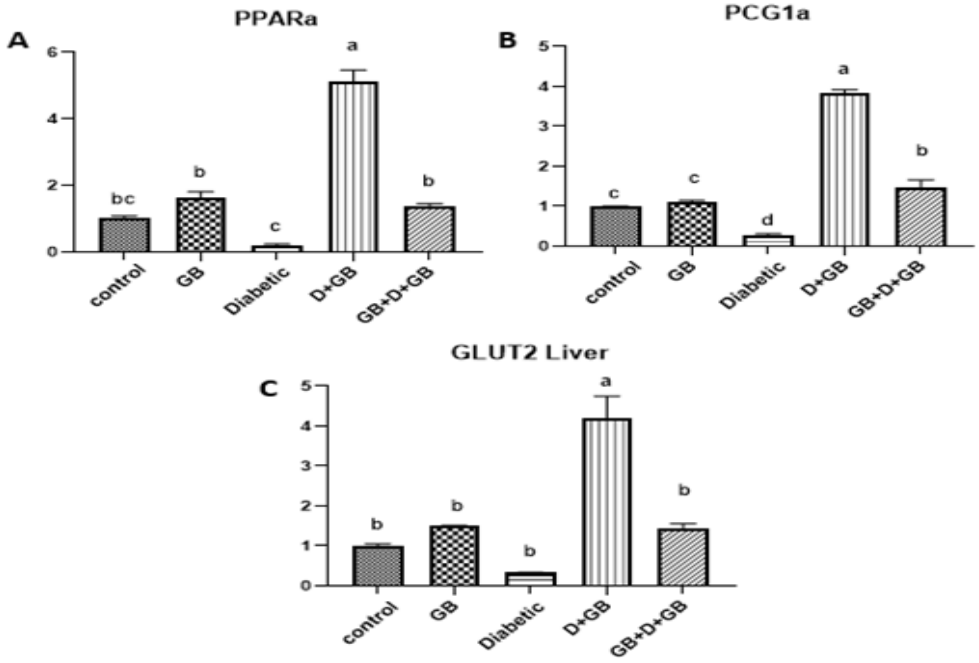

Figure 3: Effect of oral administration of *ginkgo biloba* (21.6-26.4% Ginko flavone glycosides and 5.4-6.6% terpene lactones) 200mg/kg BW at 60 days pre and post diabetic induction on the mean fold change; **A.** Hepatic peroxisome proliferator-activated receptor alpha (*PPAR- α*) relative *mRNA*/*Gapdh* (%control), **B.** Hepatic peroxisome proliferator-activated receptor gamma coactivator-1 alpha (*PGC1-α*) relative *mRNA*/*Gapdh* (% control) and **C.** Hepatic glucose transporter-2 (*GLUT-2*) relative *mRNA*/*Gapdh* (% control) in type (I) STZ-induced diabetic rats. All values are mean ± S.E.M ; n=8. Control: control group; GB: *Ginkgo biloba* group; diabetic: diabetic group; D+GB: Diabetic+ *Ginkgo biloba* group; GB+D+GB: *Ginkgo biloba*+ Diabetic+ *Ginkgo biloba* group.

Discussion

Diabetes is a global disease that will become increasingly frequent in the future decades, and it is a major global health issue [16]**.** This is a pathological disorder with oxidative stress as one of the primary causes [17]. *Ginkgo biloba* is a medicinal herb that contains flavonoids, antioxidants, and terpenes that suppress platelet activation factor [18]. Because of its neuroprotective, immunomodulatory, and anti-inflammatory properties, G. biloba leaf extract is also utilized to treat neurological and cardiovascular problems [19]. So, for G. biloba benefits and values, many authors reported a daily single dose of 120 mg of ginkgo leaf extracts in the clinical trials [20].

Concerning to Erythrogram and Hemoglobin, our results recorded significant reduction in platelets and Hb values in STZ-diabetic rats. (DG) compared with the control one. However, *Ginkgo biloba* administered groups. (GB), (D+GB) and (GB+D+GB) revealed a significant increase of HB compared with diabetic one. The observed anemia may be due to oxidative stress on bone marrow and polyuria resulted in excessive excretion of water-soluble vitamins [21]. These results agreed with [13] with using GBE 200mg/kg body weight orally once a day for a period 30 days. Also, [22] found that (EGb 761) helped in elevation of HB, erythrocyte count and made changes in hematological parameters and erythrocyte osmotic fragility.

Concerning Leukogram, decrease in the count of TLC (total leukocytic count) was obtained in STZ- diabetic rats in gp (DG) compared with control group (CG) showing features of leukopenia. Meanwhile *ginkgo biloba* treated groups either diabetic or non-diabetic (GB), (D+GB) and (GB+D+GB) showed significant increase close to control group (CG). Such results may be due to oxidative stress caused by STZ that caused bone marrow hypoperfusion and improper hematopoiesis due to oxidative stress on bone marrow caused by STZ and the results partially agreed with [23]. Also, the obtained results agreed with [24] who used *ginkgo biloba* extract treated at 50 mg/kg, 100mg/kg, and 200 mg/kg body weight for 15 days and showed a great improvement in leukogram picture due to its antioxidant and immunostimulant capacity.

Lipid metabolism is substantially influenced by uncontrolled hyperglycemia [25]. STZ-diabetic rats (DG) had higher blood cholesterol, triglycerides, and LDL-C levels than the control group (CG), but lower HDL-C levels. This could be due to changes in triglyceride-rich lipoprotein metabolism and insulin resistance, that both contribute to the development of diabetic dyslipidemia [26], or it could be due to an increase in hepatic lipase as a result of reduced lipogenesis and enhanced lipolysis in the hepatic tissue, which is the result of glucose underutilization [27]. However diabetic rats administered orally with *Ginkgo biloba* showed a significant decrease in serum cholesterol, triglycerides and LDL-C and a significant increase in HDL-C. These results agreed with [28] who revealed that The *G. biloba* regulates fatty acid profile by lowering the levels of LDL and TG and increasing the levels of HDL in blood.

Concerning Liver function enzymes , an elevation in serum ALT , AST were obtained in STZ- induced diabetic rats (DG) while decrease in albumin levels were existed, that might be due to the toxic impacts of STZ on liver by oxidative hepatic cell damage which led to increase liver enzymes in blood and decrease albumin synthesis [29]**.** Meanwhile, diabetic rats were administered orally with *Ginkgo biloba*, showed significant decrease in liver enzymes activities indicating to partial restoration of body enzymes activities .The results agreed with [30] who explained the effect of 90-day GKB extract (120 mg / capsule) on 80 diabetic patients and the results illustrated partial restoration of serum liver enzymes activities ALT,

AST, Albumin, and total protein to normal levels compared to no treated diabetic patients.

Diabetic nephropathy (DN), the most prevalent diabetic complication, is the most common cause of end-stage renal disease [31]. The obtained data suggested that diabetic gp. (DG) had higher serum creatinine and urea levels than the control group. However, diabetic rats administered *ginkgo biloba* orally in groups (GB), (D+GB), and (GB+D+ GB) showed a significant reduction in renal cell activity, indicating that *Ginkgo biloba* can restore renal cell function. The results agreed with [32] when GB therapy was utilized for 4 weeks in STZ-induced diabetic rats, the results showed that creatinine and urea were significantly higher in the diabetic group than in the control group. Urea, creatinine, and glucose levels were considerably reduced in all ratstreated with GB.

In terms of GLUT-2 mRNA expression in the liver, the results showed a significant downregulation in GLUT-2 mRNA expression in the diabetic group, but a significant upregulation in GLUT-2 mRNA expression in the *ginkgo biloba* treated groups, both diabetic and non-diabetic, reflecting the effect of *ginkgo biloba* in improving glucose transportation and metabolism. The findings were consistent with those of [33], who looked at the effects of Lupinus Albus (LA) seed extract (7mg/100g BW) on streptozotocin (STZ)-induced diabetic rats and found a slight increase in expression of biomarkers genes for pancreatic beta cells (β-cells) function, liver GLUT-2, and glucokinase when compared to STZ diabetic rats.

Regards to *PPAR-α* expression, the results showed marked downregulation in *PPAR- α* mRNA expression in 2nd diabetic group. However, the treated groups with *ginkgo biloba* showed upregulation in *PPAR- α* mRNA expression compared with diabetic one that indicated to the role of *ginkgo biloba* improvement of the glycogen content in the liver. The results agreed with [34] who illustrated in his study by using flavonoids 100 mg/kg b.w. made upregulation in flavonoids treated rats compared to diabetic one.

Results of this current investigation revealed a downregulation in mRNA expression of *PGC-1α* in diabetic group compared with control negative gp. Meanwhile, *ginkgo biloba* treated groups either diabetic or non-diabetic showed upregulation in *PGC-1α* mRNA expression compared with diabetic one indicated to the ability of *ginkgo biloba* to regulate metabolic hemostasis in liver. The results agreed to [35] that implied that in the diabetic rats, decreased ***PGC-1α*** expression was associated with increased mitochondrial ROS generation in the renal cortex, increased proteinuria, glomerular hypertrophy, and higher glomerular 8-OHdG (a biomarker for oxidative stress).

Conclusion

This study concluded that *ginkgo biloba* extracts might be a good therapeutic option for treating type-I diabetes mellitus and its complications because of its strong antihyperglycemic and antilipidemic effects, besides it controls serum liver enzymes, urea, and creatinine levels. Besides, it improves glucose metabolism in liver by modulating and enhancing the hepatic key genes.

Conflict of interest

The authors have no conflict of interest to declare.

References

[1] Yang, L. X.; Liu, T. H.; Huang, Z. T.; Li, J. E.; and Wu, L. L. (2011): Research progress on the mechanism of single-chinese medicinal herbs in treating diabetes mellitus. Chin. J. Integr. Med., 17(3):235-40. https:// doi. zorg/ 10.1007/s11655-010-0674-6

[2] Nayak, B. S. and Roberts, L. (2006): Relationship between inflammatory markers, metabolic and anthropometric variables in the Caribbean type 2 diabetic patients with and without microvascular complications. J. Inflamm., 3 (1):1-7. https://doi.org/10.1186/1476-9255-3-17

[3] Roden, M. (2016): Diabetes mellitus – Definition, Klassifikation und Diagnose. Wien. Klin. Wochenschr., 128(2):37-40. https://doi.org/ 10.1007/ s00508-015-0931-3

[4] Sanyoura, M.; Woudstra, C.; Halaby, G.; Baz, P.; Senée, V.; Guillausseau, P. J.; Zalloua, P., and Julier, C. (2014): A novel ALMS1 splice mutation in a non-obese juvenile-onset insulin-dependent syndromic diabetic patient. Eur. J. Hum. Genet., 22(1): 140–143. https://doi.org/10.1038/ejhg.2013.87

[5] Mohanta, T. K.; Occhipinti, A.; Zebelo, S.; Foti, M.; Fliegmann, J.; Bossi, S.; Maffei, M. E. and Bertea, C. M. (2012): Ginkgo biloba responds to herbivory by activating early signaling and direct defenses. PLoS ONE, 7(3): e32822. https://doi.org/ 10.1371/ journal.pone.0032822

[6] Wichtl, M. (2004): Herbal drugs and phytopharmaceuticals: a handbook for practice on a scientific basis 3rd Ed. Medpharm GmbH Scientific Publishers.

[7] Bridi, R.; Crossetti, F. P.; Steffen, V. M.; and Henriques, A. T. (2001): The antioxidant activity of standardized extract of Ginkgo biloba (EGb 761) in rats. Phytother Res, 15(5): 449–451. https://doi.org/10.1002/ptr.814

[8] Zhang, Q.; Wang, G. J.; Ji-ye, A.;Wu, D.; Zhu, L. L.; Ma, B.; and Du, Y. (2009): Application of GC/MS-based metabonomic profiling in studying the lipid-regulating effects of Ginkgo biloba extract on diet-induced hyperlipidemia in rats. Acta Pharmacol. Sin., 30(12): 1674–1687. https://doi.org/10.1038/aps.2009.173

[9] Weinstein, S. P.; O'boyle, E.; Fisher, M.; and Haber, R. S. (1994): Regulation of GLUT2 glucose transporter expression in liver by thyroid hormone: Evidence for hormonal regulation of the hepatic glucose transport system. Endocrinology, 135(2): 649–654. https://doi.org/10.1210/endo.135.2.8033812

[10] Janani, C.; and Ranjitha Kumari, B. D. (2015): PPAR gamma gene - A review. Diabetes Metab Syndr,9(1):46-50. Elsevier Ltd. https://doi.org/ 10.1016/j.dsx.2014.09.015

[11] Cheng, C. F.; Ku, H. C., and Lin, H. (2018): Pgc-1α as a pivotal factor in lipid and metabolic regulation. Int. J. Mol. Sci., 19(11):3447. MDPI AG. https://doi.org/10.3390/ijms19113447

[12] Paik, S.; Fleischer, N.; and Shin, S. (1980): Insulin-dependent diabetes mellitus induced by subdiabetogenic doses of streptozotocin: Obligatory role of cell-mediated autoimmune processes. Proc. Natl. Acad. Sci. U.S.A, 77(10): 6129–6133. https:// doi.org/10.1073/pnas.77.10.6129

[13] Cheng, D.; Liang, B.; and Li, Y. (2013): Antihyperglycemic effect of ginkgo biloba extract in streptozotocin-induced diabetes in rats. Biomed Res. Int2013, Article ID 162724. https://doi.org/10.1155/2013/162724

[14] Shankar, P. K.; Kumar, V. and Rao, N. (2005): Evaluation of Antidiabetic Activity of Ginkgo Biloba in Streptozotocin Induced Diabetic Rats. Iran. J. Pharmacol. Ther., 4(1):16-19. http://ijpt.iums.ac.ir

[15] Kumar, R. (2012): Correlation of Selenium and other antioxidants in diabetic patients with and without complications. Free Radicals and Antioxidants, 2(1):6–8. https:// doi. org/ 10.5530/ax.2012.2.3

[16] Wild, S.; Roglic, G.; Green, A.; Sicree, R.; and King, H. (2004): Global Prevalence of Diabetes: Estimates for

the year 2000 and projections for 2030. Diabetes Care, 27(5): 1047–1053. https://doi.org/10.2337/diacare.27.5.1047

[17] T Ebuehi, O. A.; Ajuluchukwu, A. E.; Afolabi, O. T. and Akinwande, A. I. (2010): Oxidative Stress in Alloxan-Induced Diabetes in Female and Male Rats. Advances in Medical and Dental Sciences, 3(3): 71–75.

[18] Maltas, E.; and Yildiz, S. (2012): Evaluation of Phytochemicals and Antioxidant Activity of Ginkgo biloba from Turkey. Pharmacologia, 3(4): 113–120. https://doi.org/10.5567/pharmacologia.2012.113.120

[19] Vellas, B.; Coley, N.; Ousset, P. J.; Berrut, G.; Dartigues, J. F.; Dubois, B.; Grandjean, H.; Pasquier, F.; Piette, F.; Robert, P.; Touchon, J.; Garnier, P.; Mathiex-Fortunet, H.; and Andrieu, S. (2012): Long-term use of standardised ginkgo biloba extract for the prevention of Alzheimer's disease (GuidAge): A randomised placebo-controlled trial. The Lancet Neurol, 11(10): 851–859. https://doi.org/ 10.1016/S1474-4422(12)70206-5

[20] Kudolo, G. B.; Wang, W.; Javors, M. and Blodgett, J. (2006): The effect of the ingestion of Ginkgo biloba extract (EGb 761) on the pharmacokinetics of metformin in non-diabetic and type 2 diabetic subjects-A double blind placebo-controlled, crossover study. Clin Nutr., 25(4): 606–616. https://doi.org/10.1016/j.clnu.2005.12.012

[21] Imai, E.; Sano, M.; Fukuwatari, T. and Shibata, K. (2012): Urinary excretion of water-soluble vitamins increases in streptozotocin-induced diabetic rats without decreases in liver or blood vitamin content. J. Nutr. Sci. Vitaminol., 58 (1):54-58 https:// doi.org/ 10.3177 /jnsv.58.54

[22] Abdulrazak, S.; Abdulkadir Nuhu, A.;and Yashim, Z. I. (2019): Effect of Ginkgo biloba Leaf Extract (EGb 761) on Changes in Haematological Parameters and Erythrocyte Osmotic Fragility in Hypotonic and Chlorpyrifos Exposed Rats. Iran J Vet Med, 13 (4): 332-341. https://doi.org/10.22059/IJVM.2019.279519.1004980

[23] Oikawa, A.; Siragusa, M.; Quaini, F.; Mangialardi, G.; Katare, R. G.; Caporali, A., Van Buul, J. D.; Van Alphen, F. P. J.; Graiani, G.; Spinetti, G.; Kraenkel, N.; Prezioso, L.; Emanueli, C.; and Madeddu, P. (2010): Diabetes mellitus induces bone marrow microangiopathy. Arterioscler Thromb Vasc Biol 30(3): 498–508. https://doi.org/10.1161/ATVBAHA.109.200154

[24] Raju, S.; Sivanesan, S. K.; Gudemalla, K.; Mundugaru, R.; and Swaminathan, M. (2019): Effect of ginkgo biloba extract on hematological and biochemical alterations in fluoride toxicity in wistar rats. Res J Pharm Technol 12(8): 3839-3846. https:// doi.org/ 10.5958/0974-360X.2019.00674.7

[25] Khan, H. A.; Sobki, S. H.; and Khan, S. A. (2007): Association between glycaemic control and serum lipids profile in type 2 diabetic patients: HbA1c predicts dyslipidaemia. Clin. Exp. Med., 7(1): 24–29. https:// doi.org/ 10.1007/ s10238-007-0121-3

[26] Krauss, R. M. (2004): Lipids and lipoproteins in patients with type 2 diabetes. In Diabetes Care, 27(6): 1496-1504. https:// doi.org/10.2337/ diacare.27.6.1496

[27] Raju, J.; Gupta, D.; Rao, A. R.; Yadava, P. K.and Baquer, N. Z. (2001): Trigonella foenum graecum (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. Mol. Cell. Biochem., 224 (1–2): 45–51. https://

doi. org/10.1023 /A:1011974630828

[28] Eisvand, F.; Razavi, B. M. and Hosseinzadeh, H. (2020): The effects of Ginkgo biloba on metabolic syndrome: A review. Phytother Res, 34(8):1798-811. https://doi.org/10. 1002/ptr.6646

[29] Kaneko, J.; Harvey, J. J.; and Bruss, M. L. (2008): Clinical Biochemistry of Domestic Animals. In Clinical Biochemistry of Domestic Animals. Elsevier Inc. https://doi.org/ 10. 1016/B978-0-12-370491-7.X0001-3

[30] Aziz, T. A.; Hussain, S. A.; Mahwi, T. O.; Ahmed, Z. A.; Rahman, H. S., and Rasedee, A. (2018): The efficacy and safety of Ginkgo biloba extract as an adjuvant in type 2 diabetes mellitus patients ineffectively managed with metformin: A double-blind, randomized, placebo-controlled trial. Drug Des Devel Ther, 12: 735–742. https://doi. org/10.2147/DDDT.S157113

[31] Zubiri, I.; Posada-Ayala, M.; Benito-Martin, A.; Maroto, A. S.; Martin-Lorenzo, M.; Cannata-Ortiz, P.; De La Cuesta, F.; Gonzalez-Calero, L.; Barderas, M. G.; Fernandez-Fernandez, B.; Ortiz, A., Vivanco, F.; and Alvarez-Llamas, G. (2015): Kidney tissue proteomics reveals regucalcin downregulation in response to diabetic nephropathy with reflection in urinary exosomes. Transl Res., 166(5): 474-484.e4. https://doi.org/10. 1016/j.trsl. 2015.05.007

[32] Zayed, A. E.; Saleh, A.; Gomaa, A. M. S.; Abd-Elkareem, M.; Anwar, M. M.; Hassanein, K. M. A.; Elsherbiny, M. M.; and Kotb, A. M. (2018): Protective effect of Ginkgo biloba and magnetized water on nephropathy in induced type 2 diabetes in rat. Oxid. Med. Cell. Longev., 2018: Article ID 1785614. https://doi.org/10.1155/2018/1785614

[33] Marghani, B.; Ateya, A.; Saleh, R.; and Eltaysh, R. (2019): Assessing of Antidiabetic and Ameliorative Effect of Lupin Seed Aqueous Extract on Hyperglycemia, Hyperlipidemia and Effect on pdx1, Nkx6.1, Insulin-1, GLUT-2 and Glucokinase Genes Expression in Streptozotocin-induced Diabetic Rats. J. Food Nutr. Res., 7(5): 333–341. https://doi.org/10.12691/jfnr-7-5-1

[34] Keshari, A. K.; Kumar, G.; Kushwaha, P. S.; Bhardwaj, M.; Kumar, P.; Rawat, A.; Kumar, D.; Prakash, A.; Ghosh, B., and Saha, S. (2016): Isolated flavonoids from Ficus racemosa stem bark possess antidiabetic, hypolipidemic and protective effects in albino Wistar rats. J Ethnopharmacol, 181, 252–262. https://doi.org/10.1016/j.jep.2016.02.0 04

[35] Guo, K.; Lu, J.; Huang, Y.; Wu, M.; Zhang, L.; Yu, H.; Zhang, M.; Bao, Y.; He, J. C.; Chen, H., and Jia, W. (2015): Protective role of PGC-1α in diabetic nephropathy is associated with the inhibition of ROS through mitochondrial dynamic remodeling. PLoS ONE, 10(4): e0125176.https://doi.org/10.1371/ journal. pone.0125176.

الملخص العربي

تأثير الجنكجو بيلوبا على التعبير الجيني الكبدي لـ (PGC1-α و PPAR-α و GLUT-2) ووظائف الكبد والكلى وأمراض الدم والشحوم في الفئران المصابة بداء السكري من النوع الأول

د. أشرف عوض ، د. إيمان السيد العربي ، د. رنا البيومي

قسم تنمية الثروة الحيوانية، كلية الطب البيطري ، جامعة الزقازيق ، الزقازيق ، مصر

أصبحت أوراق الجينكجو بيلوبا نباتًا طبيًا معروفًا في جميع أنحاء العالم ، حيث يتم وصفه في علاج مرض مختلف مثل مرض السكري واضطرابات الأوعية الدموية الطرفية والاضطرابات العصبية مثل مرض الزهايمر.. تم تصميم هذه الدراسة لمعرفة تأثير تناول الجينكو بيلوبا عن طريق الفم على وظائف الكبد والكلى والمعايير الدموية و الدهون وتعبير الجينات الكبدية في الفئران المصابة بداء السكري من النوع الأول .تم تخصيص خمسة وسبعين ذكورًا من جرذان Sprague Dawely البالغة في خمس مجموعات متساوية 15 جرذًا لكل مجموعة ، المجموعة الأولى ؛ المجموعة الضابطة ، المجموعة الثانية ؛ مجموعة مرضى السكري ، المجموعة الثالثة. مجموعة الجنكة بيلوبا ، المجموعة الرابعة ؛ تلقت المجموعة التي عولجت من مرض السكري جرعة فموية واحدة من الجنكة بيلوبا بعد تحريض مرضى السكري كعلاج والمجموعة الخامسة ؛ تم إعطاء الجرذان جرعة فموية واحدة يوميًا من الجنكة بيلوبا كجرعة وقائية من اليوم الأول للدراسة قبل تحريض مرض السكري واستمرت بعد تحريض مرض السكري حتى نهاية العمل. أظهرت نتائج الدراسة إجراءات الجنكة في تعديل جينات المفاتيح الكبدية GLUT-2 (ناقل الجلوكوز -2) ، PPAR-α (مستقبل ألفا المنشط بالبيروكسيسوم) ، و PGC1-α (مُنشط جاما المُنشط لمستقبلات بيروكسيسوم المنشط- 1). أيضًا ، تمتلك الجنكة أنشطة مضادة لفرط شحميات الدم ، وتدعم وظائف الكبد والكلى ، وتحسن استقلاب الجلوكوز وتخزينه واستخدامه في الكبد من خلال تعزيز جيناته الرئيسية. خلصت هذه الدراسة إلى أن التوصية باستخدام الجنكجو بيلوبا كمكمل غذائي أو علاج داعم لمرض السكري من النوع الأول.