Olive Leaf Extract Protect Diabetic Retinopathy in Diabetic Rats: Antioxidant and Advance Glycation End Products Pathway

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ABSTRACT

Diabetic retinopathy (DR), a major microvascular complication of diabetes mellitus (DM). Long-term hyperglycemia-induced irreversible damage in the retina, leading to DR. Olive leaf extract (OLE) has several health benefits. This research aimed to assess the protective effect of OLE against DR on streptozotocin (STZ)-induced DM in rats with underline antioxidant and protein glycation mechanisms. Diabetes induced in rats *via* injection with STZ intraperitoneal (i.p.). OLE (200 and 400 mg/kg/day) was given orally for 6 weeks and compared with Metformin (MT), as a reference drug. Change in body weight (BW), serum glucose and lipid profile levels were determined. Antioxidant status and advanced glycation end products (AGEs) biomarkers in retina tissue were measured. The changes in retina tissue in the different groups were examined under the microscope. Significant decrease in BW and increases in glucose, lipids, retina oxidative stress and AGEs levels compared with the control group. Administration of OLE (400 mg/kg) and MT reversed these parameters significantly compared with DM rats. In histology retina tissue showing focal abnormal vascularization of the ganglion layer with vacuolated cells with congested blood capillaries in different layers of the retina especially the ganglion layer, while OLE(400 mg/kg) and MT prevent most of these changes. Therefore, OLE displays a major role as an antihyperglycemic and antihyperlipidemic, also it possesses curative role against DR, through its antioxidant and inhibition of AGEs in DM rats.

Key Words:*Olive leaf extract, diabetic retinopathy, rat s, antioxidant, protein glycation.*

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INTRODUCTION

Diabetes mellitus (DM), an endocrine metabolic disorder, has very complex metabolic and autoimmune causes. [1] It is a predominant health concern that causes substantial mortality, morbidity and health complications [2]. DM is common all over the world, nevertheless it is more widespread (particularly Type 2) in the more developed countries [3]. Long-term hyperglycemia-induced several complications as heart conditions, stroke, cardiovascular or vascular disease, atherosclerosis, kidney disease, eye diseases, nerve damage, impaired thinking, infections and wounds, cancer, and musculoskeletal disorders[1]. Chronic hyperglycemia could disturb oxidative status in the body [4,5]. Diabetic retinopathy (DR), a major microvascular complication of DM. Long-term hyperglycemia-induced irreversible damage in the retina, leading to DR [6].

Olive leaves extract have potent antioxidant and scavenging power among the different parts of the olive [7]. It has been extensively utilized in traditional therapies in Mediterranean and European society as extracts, powder, and herbal tea. It encompasses numerous possibly bioactive compounds, which have antioxidant, hypolipidemic, and hypoglycemic properties [8-10]. De la Puerta *et al*. [11] and Impellizzeri *et al*. [12]

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reported that oleuropein, an active component in OLE, has both the ability to scavenge free radicals and induce an increase in the antioxidant status in DM rats.

This study aims to assess the effect of OLE in reducing DM complications, this was explored by investigating the ability of OLE to prevent AGEs formation, as well as the development and progression of oxidative stress in DM rats. It also compared the effects of OLE with metformin (MT) as a reference drug.

MATERIAL AND METHODS

Chemicals, Drug, and Kits

Streptozotocin, in white powder form, purchased from Sigma, USA. Metformin, in tablets form, obtained from KAU Hospital. Diethyl ether, saline (0.9% w/v sodium chloride), phosphate buffer saline (PBS) were purchased from Alfa Aesar Chemical Co, USA. Other chemicals were purchased from Pharmaceutical Solution Industry, Jeddah and Sigma-Aldrich (St. Louis, MO) Chemical Co. Ethylenediaminetetraacetic acid (EDTA) tubes were purchased from Al-Saggaf Trading Est., Jeddah, KSA. Kits for determinations of glucose-TV040CE004, total cholesterol (TC) -TV001CE002, triglyceride (TAG)-TV072CE002, high-density lipoprotein cholesterol (HDL-C)TV023CE002 purchased from Centronic Chemicals Co, Germany. Enzyme-linked immunosorbent assay ELISA kits for the determination of nitric oxide (NO)-E0703Ra, malondialdehyde (MDA)-E0156Ra, superoxide dismutase (SOD)-E0168Ra and advanced glycation end products (AGEs)-E0606Ra were purchased from Bioassay Technology Laboratory, China.

Experiment Animals

Forty male rats (200 ± 20 g) obtained from the Animal experimental unit of King Fahd Medical Research Center, KAU. All animals allowed to one-week adjustment in an animal house in standard laboratory conditions and fed a standard nutritionally balanced diet and free drinking water.

Plant Material

Olive leaf (*Olea europaea L.*) extract (OLE) in liquid form purchased from COMVITA Ltd, New Zealand. Every 5 ml consist of 22 mg of oleuropein.

Induction of DM

The rats after fasting for 12 hours, were intraperitoneal(i.p.) injected with STZ (65 mg/kg), which was freshly prepared in a 0.1 mol/L citrate buffer (pH 4.5) [13], then rats supplied with sucrose solution (5%) for 48 h [14]. Fasting blood glucose (FBG) was determined after 72 h, only rats with FBG ≥ 250 mg/dl were considered DM.

Study Protocol

Rats (n=40) divided into 5 groups (8 each):

Group 1: Con; rats receive a single i.p. the dose of 0.1 mmol/L citrate buffer.

Group 2:DM; rats were i.p. injected with STZ

Group 3:DM+ MT rats treated with MT (600 mg/kg) [15].

Group 4:DM+OLE(200 mg/kg), diabetic rats treated with OLE(200 mg/kg)[16].

Group5:DM+OLE(400 mg/kg)treated with OLE high dose(400 mg/ kg)

Biological Evaluation

During the period of the experiment (6 weeks) the bodyweight weekly recorded to monitor changes and to adjust the dose of OLE accordingly. Bodyweight gain percent (BWG %) was calculated.

Samples Collection

After the end of the experiment blood samples were centrifuged at 3000 r.p.m for 15 minutes for serum separation. Retina samples collected for histopathological and biochemical studies.

Serum Glucose and Lipid Biomarkers Analysis

Serum glucose [17], total cholesterol (TC) [18], triglycerides (TAG) [19], and high-density lipoprotein (HDL-C) [20] were determined using enzymatic colorimetric kits.

Determination of Retina Oxidative Stress Biomarkers and Advanced Glycation End Products

Retina NO, MDA, SOD, and AGEs were determined by double-antibody sandwich enzyme-linked immunosorbent assay ELISA kits.

Histological Examination

The retina tissues after the routine procedure and stain for cellular detail examined under a light microscope.

Statistical

All data represented as (mean ± SE) using SPSS version 24 for windows.

RESULTS

Effect of OLE on Body Weight Gain in DM Rats

There was no significant difference in initial body weight (IBW) between all groups. In DM there was a significant reduction ($P<0.001$) in final BW (FBW) and body weight gain percent (BWG%) with percent change from Con (-36.76%, - 122.58 % in FBW and BWG%, respectively). Significant differences between treated and untreated DM on FBW and BWG% ($P<0.001$) were found. Also,

there was significant (P<0.01) differences between OLE (200 mg/kg)+DM and other treated DM groups with either MT or OLE (400 mg/kg) on BWG %Table (1).

Table 1: Effect of OLE on BW in STZ-induced diabetic rats

Experimental groups	IBW(g)	FBW(g)	BWG%
Con	205.00 ± 3.90	298.50 ± 7.31	45.62 ± 2.27
DM	210.00 ± 5.18	188.75 ± 5.15 [a]	- 10.3 ± 1.47 [a]
DM+MT	200.13 ± 4.60	234.38 ± 5.86 [b]	16.71 ±1.40 [b]
DM+OLE (200 mg/kg)	212.13 ± 3.57	231.25 ± 6.61 [b]	9.33 ± 1.40 [b c]
DM+OLE (400 mg/kg)	197.50 ± 3.85	236.55 ± 6.30 [b]	16.47 ±1.59 [b d]

Significance was considered at ($p < 0.05$). Data are represented as mean ± SE (n = 8). [a] Significant versus Con, [b] Significant versus DM group, [c] Significant MT, [d] Significant DM + OLE (200 mg/kg).

Effect of OLE on Serum Glucose and Lipid Levels in DM Rats

There were significant hyperglycemia and hyperlipidemia in DM untreated group compared with the Con group at P<0.001. There were significant decreases in serum glucose, TC and TAG levels with a significant increase in HDL-C level in DM treated groups with MT, OLE (200 mg/kg) and OLE (400 mg/kg) compared with DM group (P<0.001). Oral administration of OLE (200 mg/kg) and OLE (400 mg/kg) to DM rats showed significant improvement in lipid profiles as compared with the DM+MT group. Interestingly high dose of OLE (400 mg/kg) showed significant improvement (P<0.05) in serum glucose and lipid levels compared with a low dose of OLE (200 mg/kg) Table (2).

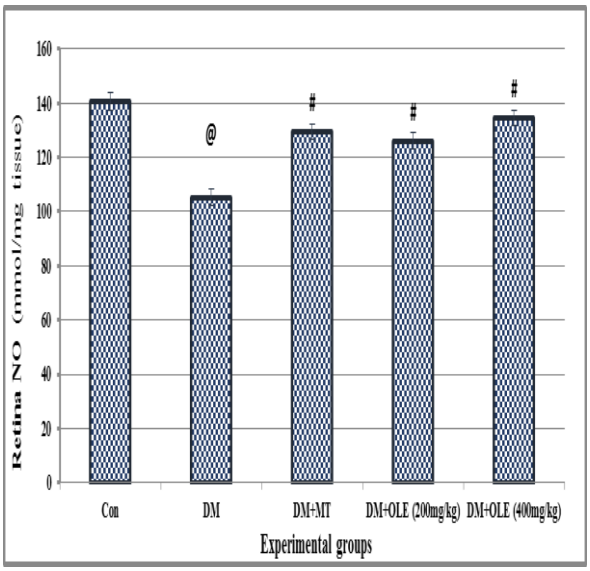
Effect of OLE on Retina Antioxidant Biomarkers

The results revealed that DM resulted in significant (P < 0.001) reduction in retina NO and SOD with significant (P < 0.001) elevation in retina MDA level in DM group as compared with the Con group, with change percentage from the Con group (- 33.40%, - 68.21% and 27.27% in NO, SOD, and MDA, respectively). Regarding the levels of retina NO, SOD and MDA in DM untreated group compared with the treated group with MT, OLE (200 mg/kg) and OLE (400 mg/kg) the data showed that there were significant differences between the DM untreated group compared with the treated DM groups in NO, SOD, and MDA (P<0.001). While there were no significant differences between DM treated with OLE (200 mg/kg) and OLE (400 mg/kg) as compared with DM+MT group Figures (1-3).\

Table 2: Effect of OLE on serum glucose and lipid levelsin STZ-induced diabetic rats

Experimental groups	Glucose (mg/dl)	TC (mg/dl)	TAG (mg/dl)	HDL-C (mg/dl)
Con	91.25 ± 4.45	65.88 ± 0.95	79.13 ± 1.69	48.20 ± 1.14
DM	389.88 ±6.37 [a]	90.25 ± 1.35 [a]	116.00 ± 1.76 [a]	28.66 ± 0.75 [a]
DM+MT	99.00 ± 6.26 [b]	76.75 ± 0.10 [b]	100.25 ± 1.28 [b]	35.38 ± 0.86 [b]
DM+OLE (200mg/kg)	228.38±13.84 [b c]	69.83 ± 1.49 [b c]	90.89 ± 2.35 [b c]	42.41 ± 1.51 [b c]
DM+OLE (400 mg/kg)	193.38±11.97 [b c d]	66.03± 1.00 [b c d]	85.36 ± 1.28 [b c d]	46.40 ± 0.97 [b c d]

Significance was considered at $p < 0.05$. Data are represented as mean ± SE (n = 8). [a] Significant versus Con, [b] Significant versus DM group, [c] Significant MT, [d] Significant DM + OLE (200 mg/kg).



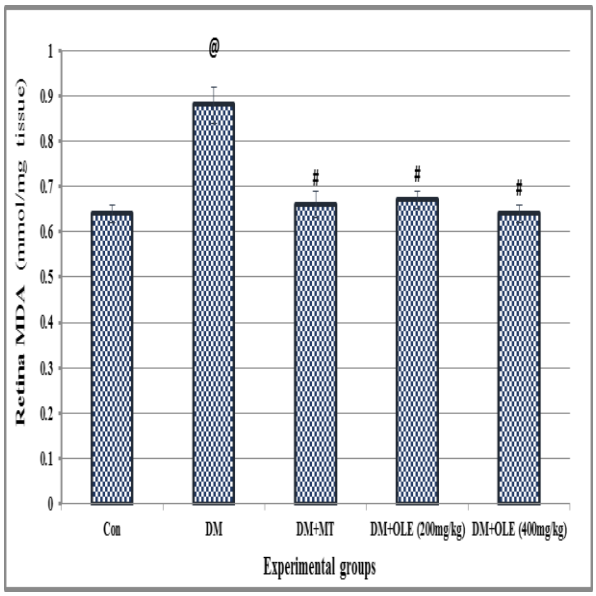
Significance was considered at p <0.05. Data are expressed as mean ± SE / 8 rats. @ Significant versus Con, #Significant versus DM group, $ Significant MT, &Significant DM + OLE (200 mg/kg) .

Figure 1: Effect of OLE on retina NO content in STZ-induced diabetic rats

Effect of OLE on Retina AGEs

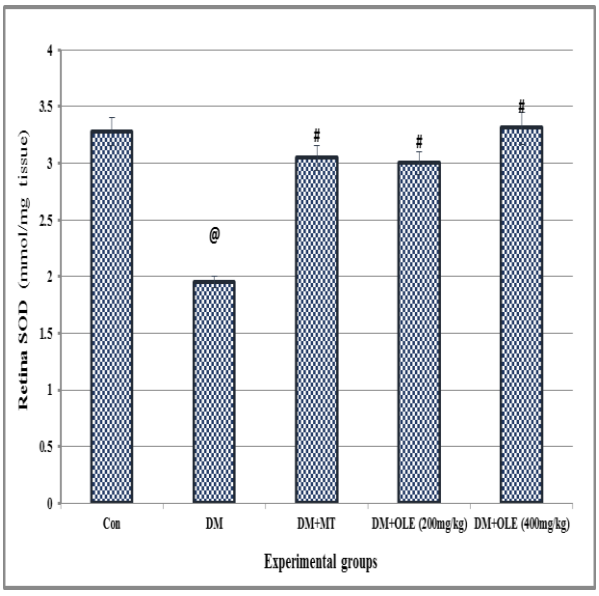
The DM resulted in significant (*P*< 0.001) elevation in retina AGEs concentration compared with the Con group with mean value (193.30 ± 4.07 *vs.* 290.62 ± 7.48 in Con and DM groups, respectively). While the retina AGEs concentration in DM untreated group compared with treated groups with MT, OLE (200 mg/kg) and OLE (400mg/kg) the data showed that there was a significant difference (*P* <0.001) between DM untreated group compared with all treated DM groups. Interestingly, there

was no significant difference in retina AGEs between all treated DM groups either with MT, OLE (200 mg/kg) or OLE (400mg/kg) (Figure 4).



Significance was considered at p <0.05. Data are expressed as mean ± SE / 8 rats. @ Significant versus Con, #Significant versus DM group, $ Significant MT, &Significant DM + OLE (200 mg/kg).

Figure 2: Effect of OLE on retina MDA content in STZ-induced diabetic rats

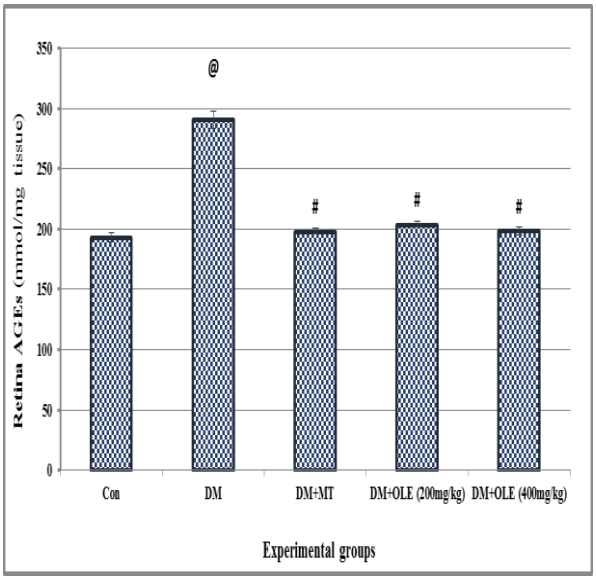


Significance was considered at p <0.05. Data are expressed as mean ± SE / 8 rats. @ Significant versus Con, # Significant versus DM group, $ Significant MT, &Significant DM + OLE (200 mg/kg).

Figure 3: Effect of OLE on retina SOD content in STZ-induced diabetic rats

Histopathological Results

A Photomicrograph of the retina section from Con rats showing the full thickness of the eyewall from the inner to the outer layer, with normal appearance of the different layers of the retina ganglion layer, bipolar cell layer and layer of rod and cones (Fig. 5.A). The typical cell layers with a narrow zone of the inner plexiform layer and the ganglionic layer of the retina (Fig. 6.A). In DM rats, the retina section showing focal abnormal vascularization of the ganglion layer with vacuolated cells (Fig. 5.B). The congested blood in capillaries in different layers of the retina especially the ganglion layer. The ganglion layer has vacuolated ganglion cells with pyknotic nuclei with focal areas of the retina detachment (Fig. 6.B). In DM rats treated with MT, the retina section shows a decrease in the congested blood capillaries in different layers of the retina (Fig. 5.C). The vacuolated ganglion cell layer has decreased with the thickened wall of the scleral blood vessels (Fig. 6.C). In DM rats treated with OLE (200mg/kg), the retina section showing light vacuolated ganglion cells in the ganglion layer, with few congested retina blood capillaries in the bipolar layer of the retina (Fig. 5.D and Fig. 6.D). In DM rats treated with OLE(400mg/kg) the retina section showing the apparent increase in the retina thickness with few focal areas of retina separation. A decrease in the congested blood capillaries in different layers of the retina (Fig. 5.E). An apparent increase in the retina thickness with foci of the retina separation (Fig. 6.E).



Significance was considered at p <0.05. Data are expressed as mean ± SE / 8 rats. @ Significant versus Con, #Significant versus DM group, $Significant MT, &Significant DM + OLE (200 mg/kg).

Figure 4: Effect of OLE on retina AGEs content in STZ-induced diabetic rats

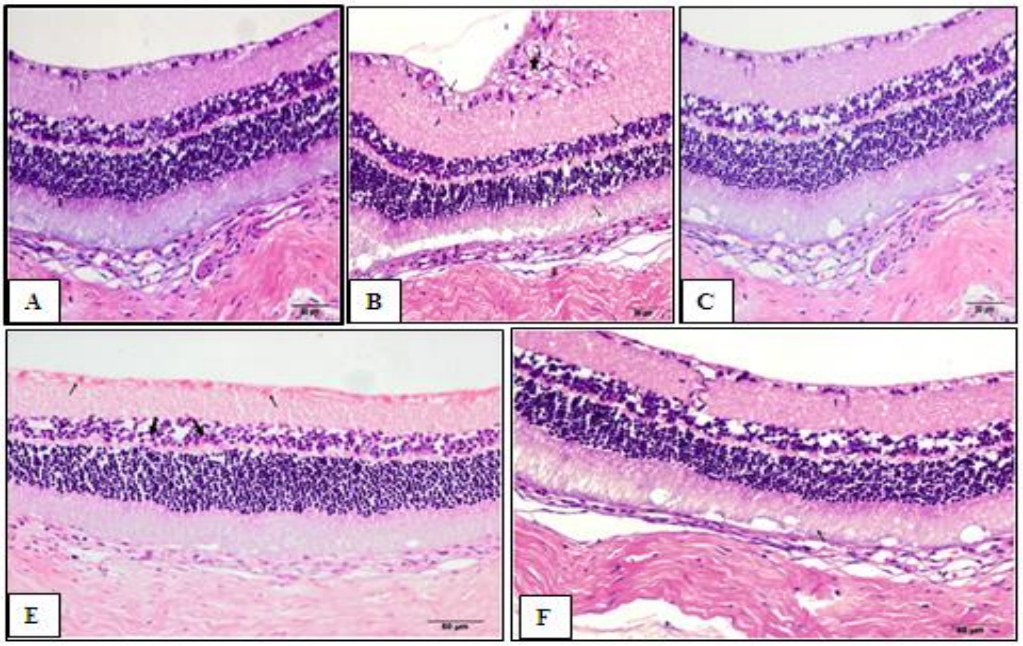


Figure 5: Photomicrograph illustrating the effect of OLE on retina sections in DM rats. A photomicrograph of a retina section from the Con rats showing the full thickness of the eyewall from the inner to the outer layer. Notice the different layers of the retina ganglion layer (G), Bipolar cell layer (B) and the layer of rod and cones (R) (Fig A). A retina section from the DM rats showing focal abnormal vascularization of the ganglion layer (*). Notice vacuolated cells (thin arrows) (Fig B). In DM rats treated with MT, a retina section showing a decrease in the congested blood capillaries in different layers of the retina (Fig C). A retina section from the DM rats treated with OLE (200mg/kg) showing slight vacuolated ganglion cells in the ganglion layer (thin arrows). Notice the few congested retina blood capillaries in the bipolar layer of the retina (thick arrows) (Fig D). A retina section from DMrats treated with OLE (400mg/kg) showing the apparent increase in the retina thickness with few focal areas of retina separation (thin arrow). Notice the decrease in the congested blood capillaries in different layers of the retina (Fig E).

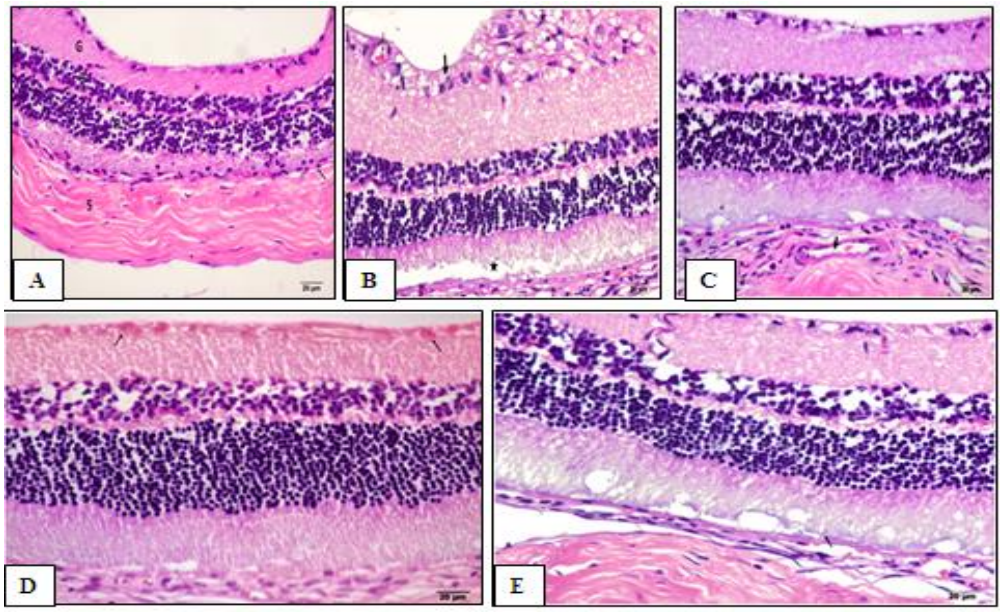


Figure 6: A higher magnification of photomicrograph illustrating the effect of OLE on retina sections in DM rats. Higher magnification of a retina section from the Con rats showing the thin layer of the choroid (→) and the surrounding sclera (S). Notice the typical cell layers with a narrow zone of the inner plexiform layers and the ganglionic layer of the retina (Fig. A). A retina section from the DM rats showing the congested blood capillaries in different layers of the retina especially the ganglion layer (thin arrows). The ganglion layer has vacuolated ganglion cells with pyknotic nuclei (thick arrow). Notice focal areas of the retina detachment (*) (Fig. B). A retina section from DM rats treated with MT showing a decrease in the congested blood capillaries in different layers of the retina. The vacuolated ganglion cell layer has decreased (thin arrows). Notice the thickened wall of the scleral blood vessels (thick arrow) (Fig. C). A retina section from the DM rats treated with OLE (200mg/kg) showing few vacuolated ganglion cells in the ganglion layer (thin arrows) (Fig. D). A retina section from DM rats treated with OLE (400mg/kg) showing an apparent increase in the retina thickness with few foci of the retina separation (thin arrow) (Fig. E).

DISCUSSION

The chronic hyperglycemia of DM is associated with long-run dysfunction, damage, and failure of diverse organs, especially the eyes, nerves, kidneys, heart, and blood vessels [21]. Oxidative stress is an important cause of DM complications. The OLE has a potent antioxidant, hypoglycemic and hypolipidemic activities. Therefore, this study aims to find out the mechanism of OLE in reducing DR through explored the ability of OLE to prevent AGEs formation, as well as the development and progression of oxidative stress in DM rats.

Effect of OLE on the Biological Evaluation of DM

In the present experiment, the results indicated that the DM group recorded a significant reduction (P<0.001) in the FBW and BWG % compared with the Con group. These findings agree with [22-24]. Yang and Kang [25] reported that there was a noticeable loss of body weight compared with the Con group. The obtained results could be explained by injurious effects of STZ which caused alkylation of DNA and produced hyperglycemia and necrotic lesions [26]. In the present study, there was a significant difference between the DM group and DM treated with MT on FBW and BWG %. These results agree with Meng *et al*. [27] who explained this effect of MT by the improvement of metabolism in rats with T2DM.

The oral administration of OLE induced a significant increase in FBW and BWG % compared with the DM group. This agrees with Hedeab *et al*. [9], Jung *et al*. [28] and Guex *et al*. [29]. The obtained results could be explained by the action mechanism of OLE in DM through increase the use of peripheral glucose and improved glucose-stimulated insulin secretion, that can prevent protein catabolism in muscle tissue [30].

Effect of OLE on Glucose and Lipid Levels in DM

There were significant hyperglycemia and hyperlipidemia in the DM group compared with the Con group (P<0.001). These results agree with Prohp and Onoagbe [31], Masomeh *et al*. [32], Zayed *et al*. [33], Jayaraman *et al*. [34] and Guex *et al*. [29]. These results could be explained *via* STZ inhibits insulin secretion and causes a state of IDDM through alkylating potency [35], β-cell destruction and insulin resistance [36]. Insulin insufficiency induced stimulation of hepatic TAG synthesis leading to hypertriglyceridemia and elevated LDL and VLDL. As well as, increased fatty acids concentration thus producing acetyl CoA and cholesterol leads to hypercholesterolemia [37]. In this study, the DM group treated with MT revealed significant improvement in glucose and lipid levels compared with the DM group. These results agree with Jin *et al.* [38] and Horakova *et al*. [39]. The hypoglycemic effect of MT could be explained through the regulation of glucose homeostasis [40], as well as reduced hepatic glucose production [41].

Administration of DM rats OLE, in a dose-depend manner, induced hypoglycemic and hypolipidemic compared with DM group. These results agree with Abunab *et al*. [42], Abd El-Moneim *et al*. [43] and AlAttar and Alsalmi [44]. This hypoglycemic activity of OLE may result from two mechanisms potentiation of glucose-induced insulin release, and increase peripheral uptake of glucose [45]. Hydroxytyrosol, oleuropein and their Secoiridoids derivatives, the major phenolic compounds of OLE, enhance insulin secretion, activate some enzymes as hexokinase and pyruvate kinase, which implicated in glucose metabolism, and protect pancreatic cells from oxidative damage through their strong antioxidant activity[46-49]. The hypolipidemic activity of OLE due to its bio-functional components, which have anti-atherosclerotic activity *via* regulatory lipid metabolism [50-51].

Effect of OLE on Renal Antioxidant Biomarkers and AGEs in DM Rats

In the present study, DM resulted in a significant increase in retina oxidative stress and AGEs levels compared with the Con group. The obtained data agreement with Nasri *et al*. [52], Cai *et al*. [53] and Afify *et al*. [54]. Hyperglycemia induces free radicals and impairs the endogenous antioxidant defense system. This could be explained by auto-oxidation of glucose and decreased tissue concentrations of antioxidants [55, 56]. It has been suggested that the pathogenesis of early DR may involve reduce or diminished production of NO [57]. On the other hand, hyperglycemia stimulates the production of AGEs, activates protein kinase C, and enhances the polyol pathway leading to increased superoxide anion formation. Superoxide anion interacts with NO, forming the potent cytotoxin peroxynitrite, which attacks various biomolecules in the vascular endothelium, vascular smooth muscle, and myocardium, leading to oxidative damage. The pathogenetic role of nitrosative stress and peroxynitrite and downstream mechanisms including poly (ADP-ribose) polymerase (PARP) activation contribute to the development and progression of diabetic nephropathy, retinopathy, and neuropathy [58].

In the present study, the treatment of DM rats with OLE (200 and 400 mg/kg) showed significant improvement in the antioxidant status in a dose-dependent manner compared with the DM group. These results agree with Abd El-Rahman [59], Marta *et al*. [60] and Soliman *et al*. [61]. The antioxidant activity of OLE could be due to the bioactive compounds in OLE as flavonoids and flavones, which are a superior hydroxyl radical scavenger and inhibits peroxidation [8, 62]

Effect of OLE on Retina Histopathological Changes in DM

In the present study, the retina sections in the DM group showed focal abnormal vascularization of the ganglion layer, vacuolated cells, and congested blood capillaries in different layers of the retina especially the ganglion layer. In DM rats treated with MT, the retina section showed decreased in the congested blood capillaries in different layers of the retina, decreased the vacuolated ganglion cell layer with the thickened wall of the scleral blood vessels. These results agree with Yan Teng *et al*. [63] Nasiry *et al*. [64] and Singh *et al*. [65]. The obtained results could be explained *via* hyperglycemia accelerates the death of capillary cells and neurons in the inner retina by a process consistent with apoptosis[66]. Also, Bax, a mitochondrial membrane protein that mediates cell death, increases in the retina in diabetes and may contribute to the development of vascular complications in DR [67]. Moreover, hyperglycemia affected the cell survival and induced apoptotic changes within the inner layers of the retina including the inner plexiform layer and ganglion cell layer [68]. Also, Javidanpour *et al*. [69]found that the administration of STZ increased the expression of caspase-3, which plays a critical role in apoptosis. Apoptosis is a key mechanism of degenerative diseases, which is triggered by some factors such as hyperglycemia toxicity.

Hyperglycaemia a key metabolic abnormality observed in DM. Controlling glucose levels preventing the onset or delaying the progression of microvascular diseases. Inside the cell, a high level of glucose may increase the flux through glycolytic pathways, stimulating protein kinase C, activation of the polyol 1-poly (ADP-ribose) polymerase (PARP) and hexosamine pathways. Furthermore, it increases the production of ROS, also high glucose level increases the nonenzymatic glycation leads to a high level of advanced glycated end products. In diabetes control by insulin or anti-hyperglycemic therapy, the risk for development and progression of DR was significantly decreased thus delaying the progression of DR [70-72].

In this study, oral administration of DM with OLE attenuated the damage caused by STZ in the retina in a dose-dependent. These results agree with Li *et al*. [73], Jin *et al*. [74] and Correa *et al*. [75]. This improvement could be explained by a flavonoid, the antioxidant active components in OLE, which can down-regulation of Bax and the up-regulation of Bcl-2 [76, 77].

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REFERENCES

[1] Hajzadeh MA, Rajaei Z, Ghamami G, Tamiz A. The effect of *Salvia officinalis* leaf extract on blood glucose in streptozotocin-diabetic rats. Pharmacologyonline. 2011;1:213-20.

[2] Aziz, N., Wal, A., Wal, P., Pal, R.S. Preparation and evaluation of the polyherbal powder: the nature's pharmacy for the treatment of diabetes mellitus and its complications. Pharmacophore.2019; 10(1):60-70.

[3] Ali, A., Aljhni, A.O., Bahumid, A.A., Shata, A.A., Abumansour, R.N., Alsufi, A.M., Al Zahrani, A., Khairi, A.R.M. Effect of type 1 diabetes mellitus on children. Pharmacophore.2018;9(6): 26-40.

[4] Kedziora-Kornatowska KZ, Luciak M, Paszkowski J. Lipid peroxidation and activities of antioxidant enzymes in the diabetic kidney: effect of treatment with angiotensin convertase inhibitors. IUBMB life. 2000;49(4):303-7.

[5] Baydas G, Canatan H, Turkoglu A. Comparative analysis of the protective effects of melatonin and vitamin E on streptozocin-induced diabetes mellitus. Journal of Pineal Research. 2002;32(4):225-30.

[6] Lai AK, Lo AC. Animal models of diabetic retinopathy: summary and comparison. Journal Diabetes Research. 2013;2013.

[7] Peralbo-Molina Á, de Castro MD. Potential of residues from the Mediterranean agriculture and agrifood industry. Trends in Food Science &Technology. 2013;32(1):16-24.

[8] Japón-Luján R, Luque-Rodríguez JM, De Castro ML. Dynamic ultrasound-assisted extraction of oleuropein and related biophenols from olive leaves. Journal of Chromatography A. 2006;1108(1):76-82.

[9] Mostafa-Hedeab G, Sati LM, Elnaggar HM, Elgatlawey ZO, Eltwab AA, Elsaghayer WA, Ali H. Ameliorating effect of olive leaf extract on cyclosporineinduced nephrotoxicity in rats. Iranian Journal of Kidney Diseases. 2015;9(5):361.

[10] Olmez E, Vural K, Gok S, Ozturk Z, Kayalar H, Ayhan S, Var A. Olive leaf extract improves the atherogenic lipid profile in rats fed a high cholesterol diet. Phytotherapy Research. 2015;29(10):1652-7.

[11] de la Puerta R, Domínguez ME, Ruíz-Gutíerrez V, Flavill JA, Hoult JR. Effects of virgin olive oil phenolics on scavenging of reactive nitrogen species and upon nitrergic neurotransmission. Life Sciences. 2001; 69(10):1213-22.

[12] Impellizzeri D, Esposito E, Mazzon E, Paterniti I, Di Paola R, Bramanti P, Morittu VM, Procopio A, Britti D, Cuzzocrea S. The effects of oleuropein aglycone, an olive oil compound, in a mouse model

of carrageenan-induced pleurisy. Clinical Nutrition. 2011;30(4):533-40.

[13] Lutz AJ, Pardridge WM. Insulin therapy normalizes GLUT1 glucose transporter mRNA but not immunoreactive transporter protein in streptozocin-diabetic rats. Metabolism. 1993;42(8):939-44.

[14] Peschke E, Ebelt H, Brömme HJ, Peschke D. Classicaland newdiabetogens—comparison of their effects on isolated rat pancreatic islets *in vitro*. Cellular and Molecular Life Sciences CMLS. 2000;57(1):158-64.

[15] Derkach K, Zakharova I, Zorina I, Bakhtyukov A, Romanova I, Bayunova L, Shpakov A. The evidence of metabolic-improving effect of metformin in Ay/a mice with genetically-induced melanocortin obesity and the contribution of hypothalamic mechanisms to this effect. PloSOne. 2019;14(3):e0213779.

[16] El-Amin M, Virk P, Elobeid MA, Almarhoon ZM, Hassan ZK, Omer SA, Merghani NM, Daghestani MH, Al-Olayan EM. Anti-diabetic effect of Murraya koenigii (L) and Olea europaea (L) leaf extracts on streptozotocin induced diabetic rats. Pak J Pharm Sci. 2013;26(2):359-65.

[17] Trinder P. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. Journal of Clinical Pathology. 1969;22(2):246.

[18] Allain CC, Poon LS, Chan CS, Richmond WF, Fu PC. Enzymatic determination of total serum cholesterol. Clinical chemistry. 1974;20(4):470-5.

[19] Jacobs NJ, VanDemark PJ. The purification and properties of the α-glycerophosphate-oxidizing enzyme of Streptococcus faecalis 10C1. Archives of Biochemistry and Biophysics. 1960;88(2):250-5.

[20] Demacker PN, Vos-Janssen HE, Hijmans AG, Van't Laar A, Jansen AP. Measurement of high-density lipoprotein cholesterol in serum: comparison of six isolation methods combined with enzymic cholesterol analysis. Clinical Chemistry. 1980;26(13):1780-6.

[21] ADA; American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010; 33(Suppl1); S62–9.

[22] Tahmasebpour N, Dehghan G, Feizi MA, Esmaeili HA. Variation in body weight and some hematological parameters in streptozotocin-induced diabetic rats, treated with *Teucrium orientale*. Pharmacology. 2013;3:32-6.

[23] El Kabbaoui M, Chda A, Azdad O, Mejrhit N, Aarab L, Bencheikh R, Tazi A. Evaluation of hypoglycemic and hypolipidemic activities of aqueous extract of cistus ladaniferus in streptozotocin-induced diabetic rats. Asian Pacific Journal of Tropical Biomedicine. 2016;6(12):1044-9.

[24] Yin P, Wang Y, Yang L, Sui J, Liu Y. Hypoglycemic effects in alloxan-induced diabetic rats of the phenolic extract from *Mongolian oak* cups enriched in ellagic acid, Kaempferol and their derivatives. Molecules. 2018;23(5):1046.

[25] Yang DK, Kang HS. Anti-diabetic effect of cotreatment with quercetin and resveratrol in streptozotocin-induced diabetic rats. Biomolecules &Therapeutics. 2018;26(2):130.

[26] Piyachaturawat P, Poprasit J, Glinsukon T, Wanichanon C. Gastric mucosal lesions in streptozotocin-diabetic rats. Cell Biology International Reports. 1988;12(1):53-63.

[27] Meng XM, Ma XX. tian Yl, Jiang Q, wang ll, Shi R, Ding l, Pang SG. Metformin improves the glucose and lipid metabolism *via* influencing the level of serum total bile acids in rats with streptozotocin-induced type 2 diabetes mellitus. European Review Medical Pharmacological Sciences. 2017;21:2232-7.

[28] Jung YC, Kim HW, Min BK, Cho JY, Son HJ, Lee JY, Kim JY, Kwon SB, Li Q, Lee HW. Inhibitory effect of olive leaf extract on obesity in high-fatdiet-induced mice *in vivo*. 2019;33(3):707-15.

[29] Guex CG, Reginato FZ, de Jesus PR, Brondani JC, Lopes GH, de Freitas Bauermann L. Antidiabetic effects of Olea europaea L. leaves in diabetic rats induced by high-fat diet and low-dose streptozotocin. Journal of Ethnopharmacology. 2019;235:1-7.

[30] Sato H, Genet C, Strehle A, Thomas C, Lobstein A, Wagner A, Mioskowski C, Auwerx J, Saladin R. Anti-hyperglycemic activity of a TGR5 agonist isolated from Olea europaea. Biochemical and Biophysical Research Communications. 2007;362(4):793-8.

[31] Prohp TP, Onoagbe IO. Plasma electrolyte concentrations in normal and streptozotocin induced diabetic rats treated with extracts of *Triplochiton scleroxylon* K. Schum. America Journal of Research Communication. 2014;2(5):154-74.

[32] Farsani MK, Amraie E, Kavian P, Keshvari M. Effects of aqueous extract of alfalfa on hyperglycemia and dyslipidemia in alloxan-induced diabetic Wistar rats. Interventional Medicine and Applied Science. 2016;8(3):103-8.

[33] Zayed AE, Saleh A, Gomaa A, Abd-Elkareem M, Anwar MM, Hassanein K, Elsherbiny MM, Kotb AM. Protective effect of *Ginkgo biloba* and magnetized water on nephropathy in induced type 2 diabetes in rat. Oxidative Medicine and Cellular Longevity. 2018;2018.

[34] Jayaraman R, Subramani S, Abdullah SH, Udaiyar M. Antihyperglycemic effect of hesperetin, a citrus flavonoid, extenuates hyperglycemia and exploring the potential role in antioxidant and antihyperlipidemic in streptozotocin-induced diabetic rats. Biomedicine & Pharmacotherapy. 2018;97:98-106.

[35] Lenzen S. The mechanisms of alloxan-and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216-26.

[36] Kusakabe T, Tanioka H, Ebihara K, Hirata M, Miyamoto L, Miyanaga F, Hige H, Aotani D, Fujisawa T, Masuzaki H, Hosoda K. Beneficial effects of leptin on glycaemic and lipid control in a mouse model of type 2 diabetes with increased adiposity induced by streptozotocin and a high-fat diet. Diabetologia. 2009;52(4):675-83.

[37] He L, Hao L, Fu X, Huang M, Li R. Severe hypertriglyceridemia and hypercholesterolemia accelerating renal injury: a novel model of type 1 diabetic hamsters induced by short-term high-fat/high-cholesterol diet and low-dose streptozotocin. BMC Nephrology. 2015;16(1):51.

[38] Jin J, Lim SW, Jin L, Yu JH, Kim HS, Chung BH, Yang CW. Effects of metformin on hyperglycemia in an experimental model of tacrolimus-and sirolimus-induced diabetic rats. Korean Journal of Internal Medicine. 2017;32(2):314.

[39] Horakova O, Kroupova P, Bardova K, Buresova J, Janovska P, Kopecky J, Rossmeisl M. Metformin acutely lowers blood glucose levels by inhibition of intestinal glucose transport. Scientific Reports. 2019;9(1):6156.

[40] Galuska D. Zierath J, Thorne A, Sonnenfeld T, Wallberg-Henriksson H. Metformin increases insulin-stimulated glucose transport in insulin-resistant human skeletal muscle. Diabetes Metab. 1991;17:159-63.

[41] Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA. Prigaro 441 BJ, Wood JL, Bhanot S, MacDonald MJ, Jurczak MJ, Camporez JP, Lee HY, Cline 442 GW, Samuel VT, Kibbey RG & Shulman GI. Metformin suppresses gluconeogenesis by 443 inhibiting mitochondrial glycerophosphate dehydrogenase. Nature. 2014;510:542-6.

[42] Abunab H, Dator WL, Hawamdeh S. Effect of olive leaf extract on glucose levels in diabetes-induced rats: A systematic review and meta-analysis. Journal of Diabetes. 2017;9(10):947-57.

[43] Afify AE, El-Beltagi HS, Fayed SA, El-Ansary AE. Beneficial and potent effect of olive leaves extract on hyperglycemic state, kidney and liver function in STZ-induced type 2 diabetes mellitus. Fresen Environ Bull. 2018 1;27:3733-9.

[44] Al-Attar AM, Alsalmi FA. Effect of Olea europaea leaves extract on streptozotocin induced diabetes in male albino rats. Saudi Journal of Biological Sciences. 2019;26(1):118-28.

[45] Gonzalez M, Zarzuelo A, Gamez MJ, Utrilla MP, Jimenez J, Osuna I. Hypoglycemic activity of olive leaf. Planta Medica. 1992;58(06):513-5.

[46] Hamden K, Allouche N, Damak M, Elfeki A. Hypoglycemic and antioxidant effects of phenolic extracts and purified hydroxytyrosol from olive mill waste in vitro and in rats. Chemico-biological Interactions. 2009 14;180(3):421-32.

[47] Nivitabishekam SN, Asad M, Prasad VS. Pharmacodynamic interaction of Momordica charantia with rosiglitazone in rats. Chemico-Biological Interactions. 2009;177(3):247-53.

[48] Silvestre RA, Egido EM, Hernández R, Marco J. Tungstate stimulates insulin release and inhibits somatostatin output in the perfused rat pancreas. European Journal of Pharmacology. 2005;519(1-2):127-34.

[49] Laaboudi WA, Ghanam JA, Ghoumari OU, Sounni FA, Merzouki MO, Benlemlih MO. Hypoglycemic and hypolipidemic effects of phenolic olive tree extract in streptozotocin diabetic rats. International Journal of Pharmacy and Pharmaceutical Sciences. 2016;8(12):287-91.

[50] Jemai H, Bouaziz M, Fki I, El Feki A, Sayadi S. Hypolipidimic and antioxidant activities of oleuropein and its hydrolysis derivative-rich extracts from Chemlali olive leaves. Chemico-Biological Interactions. 2008 Nov 25;176(2-3):88-98.

[51] Wang L, Geng C, Jiang L, Gong D, Liu D, Yoshimura H, Zhong L. The anti-atherosclerotic effect of olive leaf extract is related to suppressed inflammatory response in rabbits with experimental atherosclerosis. European Journal of Nutrition. 2008;47(5):235-43.

[52] Nasri R, Abdelhedi O, Jemil I, Daoued I, Hamden K, Kallel C, Elfeki A, Lamri-Senhadji M, Boualga A, Nasri M, Karra-Châabouni M. Ameliorating effects of goby fish protein hydrolysates on high-fat-high-fructose diet-induced hyperglycemia, oxidative stress and deterioration of kidney function in rats. Chemico-biological Interactions. 2015;242:71-80.

[53] Cai Y, Zhang X, Xu X, Yu Y. Effects of puerarin on the retina and STAT3 expression in diabetic rats. Experimental and Therapeutic Medicine. 2017;14(6):5480-4.

[54] Afify AM, El-Beltagi HS, Fayed SA, El-Ansary AE. *In vivo* correlation of olive leaves extract on some

oxidative stress markers in streptozotocin-induced diabetes mellitus in rats. Grasas y Aceites. 2018;69(1):243.

[55] Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress− activated signaling pathways mediators of insulin resistance and β-cell dysfunction?. Diabetes. 2003;52(1):1-8.

[56] Johnson JS, Harns AK, Rychly DJ, Ergel A. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical pratice. Card Diabetol. 2005;4:5-11.

[57] Kim J, Oh YS, Shinn SH. Troglitazone reverses the inhibition of nitric oxide production by high glucose in cultured bovine retinal pericytes. Experimental Eye Research. 2005;81(1):65-70.

[58] Pacher P, Obrosova IG, Mabley JG, Szabó C. Role of nitrosative stress and peroxynitrite in the pathogenesis of diabetic complications. Emerging new therapeutical strategies. Current Medicinal Chemistry. 2005;12(3):267-75.

[59] HSM AE. The effect of olive leaf extract and α-tocopherol on nephroprotective activity in rats. Journal Nutrition Food Science. 2016;6(479):2.

[60] Navarro M, Morales FJ, Ramos S. Olive leaf extract concentrated in hydroxytyrosol attenuates protein carbonylation and the formation of advanced glycation end products in a hepatic cell line (HepG2). Food &function. 2017;8(3):944-53.

[61] Soliman GA, Saeedan AS, Abdel-Rahman RF, Ogaly HA, Abd-Elsalam RM, Abdel-Kader MS. Olive leaves extract attenuates type II diabetes mellitus-induced testicular damage in rats: Molecular and biochemical study. Saudi Pharmaceutical Journal. 2019;27(3):326-40.

[62] Arora A, Nair MG, Strasburg GM. Structure–activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. Free Radical Biology and Medicine. 1998;24(9):1355-63.

[63] Teng Y, Cui H, Yang M, Song H, Zhang Q, Su Y, Zheng J. Protective effect of puerarin on diabetic retinopathy in rats. Molecular Biology Reports. 2009;36(5):1129.

[64] Nasiry D, Khalatbary AR, Ahmadvand H. Therapeutic potential of Juglans regia L. leaf extract against diabetic retinopathy in rat. Iranian Journal of Basic Medical Sciences. 2017;20(11):1275.

[65] Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. Diabetologia. 2001;44(2):129-46.

[66] Podesta F, Romeo G, Liu WH, Krajewski S, Reed JC, Gerhardinger C, Lorenzi M. Bax is increased in the retina of diabetic subjects and is associated with pericyte apoptosis *in vivo* and *in vitro*. The American Journal of Pathology. 2000;156(3):1025-32.

[67] Sharpe JC, Arnoult D, Youle RJ. Control of mitochondrial permeability by Bcl-2 family members. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2004;1644(2-3):107-13.

[68] Li YH, Zhuo YH, Lin LÜ, Chen LY, Huang XH, Zhang JL, Li SY, Wang XG. Caspase-dependent retinal ganglion cell apoptosis in the rat model of acute diabetes. Chinese Medical Journal. 2008;121(24):2566-71.

[69] Javidanpour S, Tabtabaei SR, Siahpoosh A, Morovati H, Shahriari A. Comparison of the effects of fresh leaf and peel extracts of walnut (Juglans regia L.) on blood glucose and β-cells of streptozotocin-induced diabetic rats. InVeterinary Research Forum 2012 (Vol. 3, No. 4, p. 251). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

[70] Stitt A, Gardiner TA, Anderson NL, Canning P, Frizzell N, Duffy N, Boyle C, Januszewski AS, Chachich M, Baynes JW, Thorpe SR. The AGE inhibitor pyridoxamine inhibits development of retinopathy in experimental diabetes. Diabetes. 2002;51(9):2826-32.

[71] Goh SY, Cooper ME. The role of advanced glycation end products in progression and complications of diabetes. The Journal of Clinical Endocrinology & Metabolism. 2008;93(4):1143-52.

[72] Ola MS, Nawaz MI, Siddiquei MM, Al-Amro S, El-Asrar AM. Recent advances in understanding the biochemical and molecular mechanism of diabetic retinopathy. Journal of Diabetes and its Complications. 2012;26(1):56-64.

[73] Li D, Yang F, Cheng H, Liu C, Sun M, Wu K, Ai M. Protective effects of total flavonoids from Flos Puerariae on retinal neuronal damage in diabetic mice. Molecular Vision. 2013;19:1999-2010.

[74] Jin CJ, Yu SH, Wang XM, Woo SJ, Park HJ, Lee HC, Choi SH, Kim KM, Kim JH, Park KS, Jang HC. The effect of lithospermic acid, an antioxidant, on development of diabetic retinopathy in spontaneously obese diabetic rats. PLoS One. 2014;9(6):e98232.

[75] González-Correa JA, Rodríguez-Pérez MD, Márquez-Estrada L, López-Villodres JA, Reyes JJ, Rodriguez-Gutierrez G, Fernández-Bolaños J, De La Cruz JP. Neuroprotective effect of hydroxytyrosol in experimental diabetic retinopathy: Relationship with cardiovascular biomarkers. Journal of Agricultural and Food Chemistry. 2018;66(3):637-44.

[76] Lee SH, Jeon YJ. Anti-diabetic effects of brown algae derived phlorotannins, marine polyphenols

through diverse mechanisms. Fitoterapia. 2013;86:129-36.

[77] Yu Q, Li X, Cao X. Cardioprotective Effects of Phenylethanoid Glycoside-rich extract from cistanche deserticola in ischemia-reperfusion–induced myocardial infarction in rats. Annals of Vascular Surgery. 2016;34:234-42.