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Effect of olive leaves extract on the antidiabetic effect of glyburide for possible herb-drug interaction

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ABSTRACT

The concomitant use of olive leaves (OL) and glyburide (GLB) is a possible therapy for diabetic patients. However, there is no report about the effect of OL on the antidiabetic effect of GLB till now. In the current study, the possible interaction of olive leaves extract (OLE) with GLB was assessed to determine if there was any pharmacological benefit over GLB alone. Seven groups of male Sprague Dawley rats were used. Normal rats of the 1st group treated with 2 mL/kg of 3% Tween 80 (vehicle). The 2nd–5th groups were diabetic rats received vehicle, GLB (5 mg/kg), OLE low dose and OLE high dose respectively, while the 6th–7th groups administered combinations of GLB plus OLE low dose and GLB plus OLE high dose, respectively. All treatments were administered orally once daily for 8 weeks.

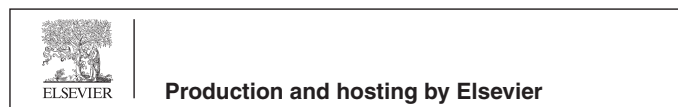
The use of GLB+OLE-500 obviously improved fasting blood glucose (FBG), insulin and glycated hemoglobin (HbA1c) in diabetic rats (95.5 ± 5.55 mg/dL, 6.8 ± 0.16 mg/dL and $6.1 \pm 0.29\%$, respectively) compared to those treated with GLB monotherapy (140.0 ± 6.36 mg/dL, 5.4 ± 0.19 mg/dL and $7.0 \pm 0.20\%$, respectively). The lipid profile [triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C)] was significantly improved in diabetic rats exposed to GLB+OLE-500 (35.6 ± 1.51 mg/dL, 48.5 ± 2.74 mg/dL, 25.1 ± 1.21 mg/dL and 17.0 ± 0.82 mg/dL, respectively) in comparison with diabetic group exposed to GLB alone (43.2 ± 2.15 mg/dL, 56.8 ± 2.14 mg/dL, 18.6 ± 0.96 mg/dL, 23.0 ± 1.26 mg/dL, respectively). Additionally, the benefit impacts of GLB+OLE-500 therapy on the antioxidant and lipid peroxidation parameters in the pancreatic tissues of diabetic rats were higher than those of GLB monotherapy. Moreover, GLB plus OLE-500 combination had the greatest effect on restoration of the insulin content of Beta (β) cells and reduction of the glucagon and somatostatin of Alpha (α) and Delta (δ) endocrine cells in the pancreatic islets among the different treatment. The current study suggests that OL and GLB combination could cause herb-drug interactions through modulation of insulin receptor (INR), glucose transporter 2 (Slc2a2) and peroxisome proliferator-activated receptor α (PPAR- α) genes expression in the liver of diabetic rats.

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1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia and impaired carbohydrates, lipids and proteins metabolism. It affects 382 million people, and this number could reach 592 million by 2035 (Flor and Campos, 2017). In DM either insufficient amounts of insulin is secreted by pancreatic islet cells of Langerhans or there is insulin resistance leading to an

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increase in blood glucose level (Chandran et al., 2016). In addition, DM is associated with increased oxidative stress. Oxidative stress causes cell dysfunction through multiple pathways, especially β -cells in pancreas (Pitocco et al., 2013).

Glyburide (glibenclamide), a sulphonylurea used as an oral hypoglycemic agent, is widely used for the treatment of type 2 DM. It is known to promote insulin secretion through inhibition of ATP-sensitive K^+ (K_{ATP}) channels in the pancreatic cells (Schmid-Antomarchi et al., 1987). The use of complementary therapies for treatment of diabetes is ever increasing and often remains unnoticed by a physician (Samala and Veeresham, 2016). Diabetic patients often consume herbal preparations along with routinely prescribed antidiabetic agents. Among these plants is the olive plant (*Olea europaea* L.) that belongs to the Oleaceae family. A study conducted by Candar et al. (2018) demonstrated that olive leaves were the most commonly preferred herbal products among diabetic patients. Its bioactive compounds such as oleuropein, verbascoside, rutin, tyrosol and hydroxytyrosol may be responsible for the biologic activities of this plant (Guex et al., 2019).

As people often take different herbs in combination with prescribed modern medication, there is a potential for interaction (Samala and Veeresham, 2016). Interactions between herbs and drugs may increase or decrease the pharmacological or toxicological effects of either components. Since there is a potential for the combined use of GLB and OLE by diabetic patients, the study is planned to evaluate the effect of OLE on glycemic regulation achieved by GLB in STZ induced diabetic rats.

2. Materials and methods

2.1. Plant material and extraction

Leaves of *Olea europaea* L. subsp. *cuspidata* (Wall. ex G. Don) Cif. F. Oleaceae was purchased from the local market in Riyadh city, Saudi Arabia. Plant identification and extraction conditions were described earlier (Soliman et al., 2019). The ground leaves (1000 g) were extracted to exhaustion by percolation at room temperature with 90% ethanol (15 L), and the extract was evaporated under reduced pressure to leave 160.82 g of the total extract.

2.2. LC-MS study of the extract

ESI-MS in both positive and negative ion acquisition mode was carried out on a XEVO TQD triple quadrupole mass spectrometer (Waters Corporation, Milford, MA01757, USA). LC performed on ACQUITY UPLC - BEH C18 1.7 μ m-2.1 \times 50 mm Column. Detailed conditions for the analyses were described elsewhere (Soliman et al., 2019).

2.3. Animals

Male Sprague Dawley rats weighing 250–270 g were used for animal experiments and maintained under standard conditions (22 ± 1 °C, $60 \pm 5\%$ humidity, and 12 h light/12 h dark cycle). Animals were fed a commercial pellet diet (Al-Marwa for Animals Feed Manufacturing, Egypt) and received water *ad libitum*. All animals were allowed to adapt to the laboratory environment for one week before experimentation. All procedures for the handling, use, and euthanasia of the animals were approved by the Institutional Ethical Committee of NRC (approval number: MREC-17-142), the Institutional Animal Care and Use Committee at Cairo University (approval number: CU-II-F-14-18), and following the guidelines of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.4. Induction of diabetes

Diabetes was induced by using STZ (45 mg/kg, b.w., i.p.) in citrate buffer (pH 4.5) to the overnight fasted Wistar rats (Soliman et al., 2019). After 72 h, blood samples were collected from rats by retro-orbital puncture, and the serum was analyzed for glucose levels. Animals with blood glucose level >200 mg/dL were considered as diabetic (Pournaghi et al., 2012) and were used for the study.

2.5. Experimental design

Rats were assigned to one of seven groups of six animals each:

- (i) Normal control group (NC): Non-diabetic rats received 1 mL of the vehicle (3% Tween 80).
- (ii) Diabetic control group (DC): STZ-diabetic rats received 1 mL of the vehicle (3% Tween 80).
- (iii) GLB group: STZ-diabetic rats treated with GLB (5 mg/kg).
- (iv) OLE-250 group: STZ-diabetic rats treated with OLE (250 mg/kg).
- (v) OLE-500 group: STZ-diabetic rats treated with OLE (500 mg/kg).
- (vi) GLB + OLE-250 group: STZ-diabetic rats treated with GLB (5 mg/kg) plus OLE (250 mg/kg).
- (vii) GLB+OLE-500 group: STZ-diabetic rats treated with GLB (5 mg/kg) plus OLE (500 mg/kg).

All treatments were administered orally, once daily using intra-gastric tube for a period of eight weeks.

Body weight and the blood levels of glucose and insulin were monitored in animals at four different time points: the beginning of the experiment (0-time), after 2, 4 and 8 weeks of treatments. Other parameters were estimated at the end of the experiment.

2.6. Estimation of serum biochemical parameters

Blood samples were withdrawn through the retro-orbital venous plexus under mild ketamine anesthesia from the overnight fasted animals into sampling tubes. Blood samples were centrifuged at 5000 rpm for 20 min to separate serum. The FBG levels in serum were estimated using the commercially available kits (Spinreact, Spain) while serum insulin levels were determined by using ELISA kits (Cobas, Belgium) according to the manufacturer's manual.

Serum levels of lipid profile [triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C)] were estimated according to the instructor manual of commercially available kits. Very low-density lipoprotein cholesterol (VLDL-C) was calculated according to the method of Friedewald et al. (1972): $VLDL-C = TG/5$. Atherogenic index (AI) was calculated using the formulae of Kayamori and Igarashi, 1994: $AI = (TC - HDL)/HDL$.

Markers of liver injury [(alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (γ -GT) and total bilirubin (BRN)] were estimated according to the instructor manual of commercially available kits. In addition, another blood sample was obtained from the retro-orbital region of each rat into tubes containing EDTA as anticoagulant and used for estimation of total hemoglobin (Hb) and glycosylated hemoglobin (HbA1c) using the commercially available kits (QCA, Spain).

2.7. Tissue collection

Following blood collection at the end of the experimental period, all animals were euthanized with an ip overdose of pentobarbital sodium. Pancreas were dissected immediately, washed in saline (0.9% NaCl), frozen in liquid nitrogen, and stored at -80°C until analysis.

2.8. Oxidative stress and lipid peroxidation markers in the pancreatic tissues

A portion of pancreas was weighted and washed with ice-cold saline immediately, and kept at -80°C until analysis. The pancreas tissues were homogenized separately in 0.1 M Tris-HCl (pH 7.4). Pancreas homogenates were centrifuged at 1700 rpm for 10 min and the supernatants were collected and maintained at -80°C until subsequent biochemical analysis. Activities of the antioxidant enzymes as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) and levels of reduced glutathione (GSH) and malondialdehyde (MDA) in pancreatic homogenates were estimated using the corresponding assay kits purchased from Biodiagnostic (Egypt) according to the standard procedures in the manufacturer's instructions.

2.9. Histopathological examination of pancreas

Pancreatic tissues from each group were harvested and fixed in 10% neutral buffered formalin and routinely processed for paraffin embedding to obtain $4\ \mu\text{m}$ sections according to the methods described by Bancroft and Gamble (2008).

2.10. Immunohistochemical analysis

The immunohistochemical analysis was done following the method described by Abdel-Rahman et al. (2019). After the tissue sections were deparaffinized, rehydrated and endogenous peroxidase blockage, the tissues sections were pretreated with 10 mM citrate buffer, pH 6.0 for 10 min in microwave oven for antigenic retrieval. The tissue sections were incubated with one of the following primary antibodies: mouse monoclonal anti-insulin 1:50 (18-0066; Zymed, San Francisco, CA), sheep polyclonal anti-glucagon antibody 1:100 (ab36232; Cambridge, UK) and rabbit polyclonal anti-somatostatin antibody 1:50 (ab108456; Cambridge, UK) for 4 h at 4°C in a humidified chamber. The tissue sections were incubated with a biotinylated goat anti mouse and rabbit antibody (Thermo scientific, USA) and rabbit anti-sheep antibody (ab6747; Cambridge, UK) for 10 min. Finally, streptavidin peroxidase (Thermo scientific, USA) was added to the sections then they were incubated with 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma) as chromagen for 5 min and counterstained with haematoxylin. Percentage of positive stained area was calculated as mean of 10 fields / slide. The morphometric analysis of the pancreatic islet cells composition was performed to determine the percent of insulin positive β -cells to the total islets of Langerhans area, glucagon positive α -cells to the total islets of Langerhans area, and the somatostatin positive δ -cells to the total islet area following the method described by Mu et al. (2006).

2.11. Quantitative real Time-RT-PCR

Liver samples of the different experimental groups were used for extraction of total RNA using Trizol reagent (Qiagen) and then reverse transcribed to cDNA using the cDNA synthesis kit (Thermo scientific). qPCR was performed using 1.5 μL of cDNA and SYBR Green Master Mix (Qiagen). Specific oligonucleotide primers (Table 1) were used for Slc2a2 gene (coding glucose transporter

2, GLUT2), peroxisome proliferator-activated receptor α (PPAR- α), and insulin receptor gene (INR) mRNA expression analysis. The qPCR was performed according to the manufacturer's manual. The used primers were tested for specificity by melting-curve analysis, agarose gel electrophoresis and using negative controls. The fold-change in mRNA expression was normalized to B. actin. Relative expression values were calculated using the $\Delta\Delta\text{CT}$ method.

2.12. Statistical analysis

Results were expressed as means \pm SE. Multiple group comparisons were performed by analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Difference was considered significant when $p \leq 0.05$, using Graph Pad prism[®] software (version 6.00 for Windows, San Diego, California, USA).

3. Results and discussion

3.1. LC-MS study

In the current study we used the same OLE characterized earlier. The major compound was identified as the anti-diabetic secoiridoid glycoside oleuropein (14%). The major phenolic compounds were identified as Ligustroside (3.29%), Luteolin-7-glucoside (2.15%), Verbacoside (1.2%) and Caffeic acid (1%). The triperpene oleanolic acid represents 7% and its methyl ether represents 2% of the total extract (Soliman et al., 2019).

3.2. Biological study

Glyburide is widely used in type 2 diabetes. It acts through stimulation of insulin release from the β -cells (Serrano-Martin et al., 2006). In recent years, some of the patients prefer to use conventional drugs with herbal medicine, concurrently. The leaves of the olive plant have been used for centuries in folk medicine to treat diabetes. In fact, concomitant use of drug and herbal medicine may produce some kind of interaction. In the current study, the possible interaction of herbal medicine with synthetic drug was assessed to determine if there was any pharmacological benefit over GLB alone.

3.2.1. Effect on body weight

Weight loss is a major characteristic of DM. In this study, DC rats gained significantly less weight during the course of the study as compared to non-diabetic rats (Table 2), which can be explained by insulin deficiency that can lead to protein catabolism in muscle tissue (Sundaram et al., 2013). At the end of 8 weeks of treatment, the body weight of the rats in the normal control, GLB, OLE-250, OLE-500, GLB + OLE-250 and GLB + OLE-500 treated groups, increased significantly by 52.41%, 26.63%, 28.70%, 32.59%, 30.91% and 41.07% respectively; compared with their weights at 0-time whereas the body weight of diabetic control group increased by 14.41% only.

Particularly, body weights of the diabetic rats received OLE at 500 mg/kg were higher than those treated with GLB alone all over the experimental period, suggesting that OLE may normalize energy metabolism in tissues. Interestingly, we noted that the GLB plus OLE-500 combination was more potent in diabetic rats for improving their body weight gain than the GLB monotherapy and GLB plus OLE-250 combination. GLB plus OLE-500 combination increased the body weight of diabetic rats to a value which is comparable to that of the normal group. The capability of the combination to protect the body from weight loss might be a result of its high ability to control high blood glucose levels.

Table 1
Oligonucleotide primers sequences used for RT-qPCR.

Gene	Forward primer (5' → 3')	Reverse primer (5' → 3')	Accession #
Slc2a2	TCTGTGCTGCTTGTGGAG	ACTGACGAAGAGGAAGATGG	XM_006232207.2
PPAR- α	TTCGGAAACTGCAGACCT	TTAGGAACTCTCGGGTGAT	NM_013196.1
INSR	TTTGTTCATGGATGGAGGCTA	CCTCATCTTGGGGTGAAGT	XM_006248753.2
B actin	GGTGGGTATGGGTCAG	ATGCCGTGTTCATG	NM_031144.3

Table 2
Effect of GLB, OLE and their combination on body weight of STZ-diabetic rats.

Treatment groups	Body weight (g)								
	0-time			2 weeks		4 weeks		8 weeks	
	M ± SE	M ± SE	%	M ± SE	%	M ± SE	%		
NC	257.4 ± 8.06	329.6 ± 9.15 ^{b,c}	28.05	361.8 ± 12.97 ^{b,c}	40.56	392.3 ± 12.26 ^{b,c}	52.41		
DC (STZ)	263.7 ± 2.08	270.3 ± 7.38 ^a	2.50	281.5 ± 6.60 ^{a,c}	6.75	301.7 ± 8.49 ^{a,c}	14.41		
GLB	260.6 ± 3.82	279.5 ± 7.00 ^a	7.25	304.3 ± 7.51 ^{a,b}	16.77	330.0 ± 9.47 ^{a,b}	26.63		
OLE-250	258.2 ± 4.41	280.8 ± 6.13 ^a	8.75	306.3 ± 7.58 ^{a,b}	18.63	332.3 ± 8.62 ^{a,b}	28.70		
OLE-500	264.5 ± 5.58	292.2 ± 7.51 ^a	10.47	323.3 ± 8.25 ^{a,b}	22.23	350.7 ± 9.67 ^{a,b}	32.59		
GLB + OLE-250	265.3 ± 4.65	290.4 ± 7.75 ^a	9.46	320.7 ± 6.49 ^{a,b}	20.88	347.3 ± 5.65 ^{a,b}	30.91		
GLB+OLE-500	263.2 ± 3.87	302.3 ± 7.12 ^{a,b,c}	14.86	346.7 ± 7.22 ^{b,c}	31.72	371.3 ± 11.58 ^{b,c}	41.07		

Values are expressed as mean ± SE (n = 6).

Multiple group comparisons were performed by analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test at $p \leq 0.05$.

^a $P \leq 0.05$, statistically significant from the normal control (NC) group.

^b $P \leq 0.05$, statistically significant from the diabetic control (DC) group.

^c $P \leq 0.05$, statistically significant from GLB group.

3.2.2. Effect on serum biochemical parameters

3.2.2.1. Effect on fasting blood glucose and insulin levels. The serum levels of glucose and insulin reflect the glycemic state of diabetic animals. Tables 3 and 4 illustrate the effects of eight weeks administration of GLB alone or in combination with either 250 or 500 mg/kg OLE on serum levels of FBG and insulin in diabetic rats, respectively. In this study, the DC rats showed high FBG and low insulin levels compared with NC rats during the experimental period. GLB is widely used in type 2 diabetes through its action on stimulating insulin release from the β -cells (Serrano-Martin et al., 2006). The results in this study supported this conclusion. Following 8 weeks treatment, GLB significantly reduced FBG levels (48.19%) and elevated insulin levels (50.00%) compared to basal values.

Additionally, several studies have established that OLE has a powerful antidiabetic effect (Wainstein et al., 2012, Al-Attar and Alsalami, 2019). As expected, OLE-250 and OLE-500 significantly reduced the elevated FBG (53.83 and 60.28%, respectively) and increased the reduced insulin levels 60.00 and 77.78%, respectively) in diabetic rats when they administered separately, compared to basal values. However, these levels were not returned to the normal state. The action mechanism for olive leaves in DM

can be explained by increased use of peripheral glucose and improved glucose-stimulated insulin secretion from the existing beta cells of pancreas (Sato et al., 2007). Also it has been reported that GLB acts by binding to the sulfonylurea receptor 1, a regulatory subunit of the ATP-sensitive potassium channels in the pancreatic beta cells (Sharma and Kar, 2014). Further, the efficacy of OLE-500 in controlling FBG and insulin levels is much higher than that of the GLB. The major constituents of OLE, which are accountable for the antidiabetic effect, are phenolic compounds, flavonoids, secoiridoids, and secoiridoid glycosides (Jerman et al., 2010). Various mechanisms have been reported in the literature to explain the possible antidiabetic effects of OLE. These mechanisms include the regeneration of β -cells with the increase of insulin secretion, inhibition of oligosaccharides and polysaccharides digestion, and the diminution of intestinal glucose absorption rate (Laaboudi et al., 2016).

Interestingly, the hypoglycemic potential of the drug and herb improved when they were co-administered. Of the six diabetic-treatment groups, the GLB plus OLE-500 combination was found to be the most effective in decreasing FBG and elevating insulin levels throughout the experimental period. Results obtained from

Table 3
Effect of GLB, OLE and their combination on FBG level in STZ-diabetic rats.

Treatment groups	FBG (mg/dL)								
	0-time			2 weeks		4 weeks		8 weeks	
	M ± SE	M ± SE	%	M ± SE	%	M ± SE	%		
NC	78.8 ± 3.08 ^{b,c}	76.2 ± 3.64 ^{b,c}	-3.30	81.4 ± 4.88 ^{b,c}	3.30	81.7 ± 3.71 ^{b,c}	3.68		
DC (STZ)	282.0 ± 7.81 ^a	287.7 ± 11.96 ^{a,c}	2.02	289.3 ± 12.53 ^{a,c}	2.59	276.8 ± 10.13 ^{a,c}	-1.84		
GLB	270.2 ± 9.71 ^a	169.0 ± 6.92 ^{a,b}	-37.45	159.0 ± 8.38 ^{a,b}	-41.15	140.0 ± 6.36 ^{a,b}	-48.19		
OLE-250	278.3 ± 7.88 ^a	148.8 ± 6.70 ^{a,b}	-46.53	139.3 ± 4.89 ^{a,b}	-49.95	128.5 ± 6.50 ^{a,b}	-53.83		
OLE-500	276.2 ± 11.60 ^a	130.2 ± 4.65 ^{a,b,c}	-52.86	119.8 ± 4.93 ^{a,b,c}	-56.63	109.7 ± 5.48 ^{a,b,c}	-60.28		
GLB + OLE-250	266.0 ± 9.80 ^a	132.2 ± 4.05 ^{a,b,c}	-50.30	121.0 ± 5.50 ^{a,b,c}	-54.51	108.2 ± 5.01 ^{a,b,c}	-59.32		
GLB+OLE-500	278.8 ± 4.12 ^a	119.5 ± 4.51 ^{a,b,c}	-57.14	97.8 ± 5.57 ^{b,c}	-18.16	95.5 ± 5.55 ^{b,c}	-65.75		

Values are expressed as mean ± SE (n = 6).

Multiple group comparisons were performed by analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test at $p \leq 0.05$.

^a $P \leq 0.05$, statistically significant from the normal control (NC) group.

^b $P \leq 0.05$, statistically significant from the diabetic control (DC) group.

^c $P \leq 0.05$, statistically significant from GLB group.

Table 4
Effect of GLB, OLE and their combination on serum levels of insulin in STZ-diabetic rats.

Treatment groups	Insuline (mg/dL)							
	0-time		2 weeks		4 weeks		8 weeks	
	M ± SE	%	M ± SE	%	M ± SE	%	M ± SE	%
NC	7.4 ± 0.30 ^{b,c}	4.05	7.7 ± 0.10 ^{b,c}	4.05	7.6 ± 0.29 ^{b,c}	2.70	7.5 ± 0.16 ^{b,c}	1.35
DC (STZ)	3.7 ± 0.14 ^a	-2.70	3.6 ± 0.18 ^{a,c}	-2.70	3.5 ± 0.13 ^{a,c}	-5.41	3.6 ± 0.18 ^{a,c}	-2.70
GLB	3.6 ± 0.15 ^a	36.11	4.9 ± 0.11 ^{a,b}	36.11	5.2 ± 0.17 ^{a,b}	44.44	5.4 ± 0.19 ^{a,b}	50.00
OLE-250	3.5 ± 0.14 ^a	54.29	5.4 ± 0.29 ^{a,b}	54.29	5.5 ± 0.29 ^{a,b}	57.14	5.6 ± 0.20 ^{a,b}	60.00
OLE-500	3.6 ± 0.15 ^a	69.44	6.1 ± 0.15 ^{a,b,c}	69.44	6.3 ± 0.08 ^{a,b,c}	75.00	6.4 ± 0.09 ^{a,b,c}	77.78
GLB + OLE-250	3.7 ± 0.18 ^a	62.16	6.0 ± 0.16 ^{a,b,c}	62.16	6.4 ± 0.16 ^{a,b,c}	72.97	6.4 ± 0.07 ^{a,b,c}	72.97
GLB+OLE-500	3.6 ± 0.25 ^a	80.56	6.5 ± 0.29 ^{a,b,c}	80.56	6.7 ± 0.10 ^{b,c}	3.08	6.8 ± 0.16 ^{b,c}	88.89

Values are expressed as mean ± SE (n = 6).

Multiple group comparisons were performed by analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test at $p \leq 0.05$.

^a $P \leq 0.05$, statistically significant from the normal control (NC) group.

^b $P \leq 0.05$, statistically significant from the diabetic control (DC) group.

^c $P \leq 0.05$, statistically significant from GLB group.

this experiment reveal that, GLB and OLE show antidiabetic activity in decreasing order of GLB+OLE-500 > OLE-500 > GLB + OLE-250 > OLE-250 > GLB. At the 4th and 8th weeks, the rats treated with GLB+OLE-500 had normal FBG and insulin levels and there were no significant differences between them. Interestingly, the beneficial effect on glycemic control observed for GLB plus OLE-500 combination occurs without an increased risk of hypoglycaemia.

The synergistic effects on glucose lowering potential observing in this study might be due to the similar hypoglycemic mechanisms of OLE and GLB. Based on these data, it can be concluded that OLE and GLB had synergistic effects in hypoglycemic activity.

3.2.2.2. Effect on Hb and HbA1c levels. In diabetes, high blood glucose levels react with Hb to form glycated hemoglobin (HbA1c). Consequently, the total Hb level is diminished in diabetics. HbA1c is formed extensively and irreversibly over a period of time. Therefore, HbA1c levels are strongly correlate with blood glucose levels and this is the most widely used marker for monitoring glycemic control (Lorenzo-Medina et al., 2014).

Effects of GLB, OLE and their combination on Hb and HbA1c levels are shown in (Table 5). In our study, untreated diabetic rats showed significant increase ($p < 0.05$) in HbA1c level and decrease in blood Hb level when compared to NC levels indicating poor glycemic control. According to the glucose-lowering effect, OLE-250 and OLE-500 monotherapies and GLB plus OLE-250 combination significantly reduced HbA1c levels (6.9 ± 0.25 , 6.6 ± 0.28 and $6.5 \pm 0.31\%$, respectively) compared to DC rats ($11.7 \pm 0.29\%$) but did not significantly improve the rate of glycosylation compared with GLB treatment alone ($7.0 \pm 0.20\%$).

Table 5
Effect of GLB, OLE and their combination on blood levels of total Hb and HbA1c in STZ-diabetic rats.

Treatment Groups	Total hemoglobin (mg/dL)	HbA1c (%)
NC	14.5 ± 0.44 ^{b,c}	4.6 ± 0.21 ^{b,c}
DC (STZ)	10.3 ± 0.28 ^{a,c}	11.7 ± 0.29 ^{a,c}
GLB	12.2 ± 0.31 ^{a,b}	7.0 ± 0.20 ^{a,b}
OLE-250	12.5 ± 0.30 ^{a,b}	6.9 ± 0.25 ^{a,b}
OLE-500	13.0 ± 0.36 ^{a,b}	6.6 ± 0.28 ^{a,b}
GLB + OLE-250	13.2 ± 0.33 ^{a,b}	6.5 ± 0.31 ^{a,b}
GLB+OLE-500	12.6 ± 0.35 ^{b,c}	6.1 ± 0.29 ^{b,c}

Values are expressed as mean ± SE (n = 6).

Multiple group comparisons were performed by analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test at $p \leq 0.05$.

^a $P \leq 0.05$, statistically significant from the normal control (NC) group.

^b $P \leq 0.05$, statistically significant from the diabetic control (DC) group.

^c $P \leq 0.05$, statistically significant from GLB group.

Blood Hb and HbA1c levels were effectively controlled in the rats that received GLB plus OLE-500 combination more than that seen in animals treated with GLB alone. Both, Hb and HbA1c levels were reversed to normal range (13.6 ± 0.35 mg/dL and $6.1 \pm 0.29\%$, respectively) following 8 weeks administration of GLB plus OLE-500 combination. For most diabetic individuals, HbA1c values lower than 7% are considered an indication of good glycemic control (ADA, 2014). The present results reveal that, GLB, OLE and their combinations protect against hemoglobin glycation in decreasing order of GLB+OLE-500 > GLB + OLE-250 > OLE-500 > OLE-250 > GLB. This effect might be due to the effective control of hyperglycemia by GLB plus OLE-500 combination. This also indicates that the combination might be very effective for long-term control of DM and preventing further complications in diabetic individuals.

3.2.2.3. Effect on serum lipid profile. Hyperlipidemia is associated with diabetes. A well-known fact, that inadequate control of glucose in DM results in disturbance in the serum lipid profile (Shah and Khan, 2014). The present study showed significant ($P \leq 0.05$) increase in the serum levels of TG (51.8 ± 2.10 mg/dL), TC (68.3 ± 2.61 mg/dL) and LDL-C (28.7 ± 1.11 mg/dL) in STZ-induced diabetic rats compared to NC rats (32.6 ± 1.58 mg/dL, 45.7 ± 1.75 mg/dL and 16.2 ± 0.69 mg/dL, respectively). HDL-C was significantly reduced in DC rats when compared to NC group (Table 6). However, treatment of diabetic rats with GLB reversed all the changes in their lipid profile that might be related to its ability to reduce sugar level but the lipid profile remained significantly disturbed compared with NC animals. Similar lipid profiles were observed in diabetic rats exposed to OLE-250, OLE-500 and GLB plus OLE-250 treatments. The direct relationship of glycemic control with dyslipidemia has also been confirmed by Mohammadi et al., (2009).

Interestingly the GLB plus OLE-500 combination had a superior effect on regulating serum lipid than GLB monotherapy. Following oral administration of GLB plus OLE-500 combination, lipid profile levels reverted back to those seen in normal rats which may be indicative of the antidiabetic role played by the combination therapy. This effect might be owed to increased insulin secretion from pancreatic β -cells that further stimulate fatty acid synthesis and incorporation of fatty acids into TG in the liver and adipose tissue as well. Reductions in serum lipids, particularly of the TG and LDL-C to normal levels are considered as beneficial for the long-term prognosis of diabetic patients (Chattopadhyay and Bandyopadhyay, 2005). Since the combination of OLE-500 with GLB produced further improvement in the lipid profile than that produced by GLB or OLE per se, it is suggested that OLE may be

Table 6
Effect of GLB, OLE and their combination on lipid profile in blood of STZ-diabetic rats.

Treatment groups	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL (mg/dL)	AI
NC	32.6 ± 1.58 ^{b,c}	45.7 ± 1.75 ^{b,c}	27.6 ± 1.51 ^{b,c}	16.2 ± 0.69 ^{b,c}	6.5 ± 0.26 ^{b,c}	0.66 ± 0.39 ^{b,c}
DC (STZ)	51.8 ± 2.10 ^{a,c}	68.3 ± 2.61 ^{a,c}	15.9 ± 0.31 ^{a,c}	28.7 ± 1.11 ^{a,c}	10.4 ± 0.45 ^{a,c}	3.3 ± 0.28 ^{a,c}
GLB	43.2 ± 2.15 ^{a,b}	56.8 ± 2.14 ^{a,b}	18.6 ± 0.96 ^{a,b}	23.0 ± 1.26 ^{a,b}	8.6 ± 0.39 ^{a,b}	2.05 ± 0.17 ^{a,b}
OLE-250	41.8 ± 1.57 ^{a,b}	55.4 ± 1.99 ^{a,b}	20.5 ± 1.27 ^{a,b}	22.3 ± 0.83 ^{a,b}	8.4 ± 0.33 ^{a,b}	1.70 ± 0.05 ^{a,b}
OLE-500	38.5 ± 1.47 ^{a,b}	51.7 ± 2.05 ^{a,b}	23.1 ± 1.84 ^{a,b}	19.8 ± 0.77 ^{a,b}	7.7 ± 0.31 ^{a,b}	1.24 ± 0.06 ^{b,c}
GLB + OLE-250	39.5 ± 1.39 ^{a,b}	53.2 ± 1.93 ^{a,b}	21.6 ± 1.47 ^{a,b}	20.5 ± 0.84 ^{a,b}	7.9 ± 0.42 ^{a,b}	1.46 ± 0.04 ^{b,c}
GLB+OLE-500	35.6 ± 1.51 ^{b,c}	48.5 ± 2.74 ^{b,c}	25.1 ± 1.21 ^{b,c}	17.0 ± 0.82 ^{b,c}	7.1 ± 0.40 ^{b,c}	0.93 ± 0.06 ^{b,c}

Values are expressed as mean ± SE (n = 6).

Multiple group comparisons were performed by analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test at $p \leq 0.05$.

^a $P \leq 0.05$, statistically significant from the normal control (NC) group.

^b $P \leq 0.05$, statistically significant from the diabetic control (DC) group.

^c $P \leq 0.05$, statistically significant from GLB group.

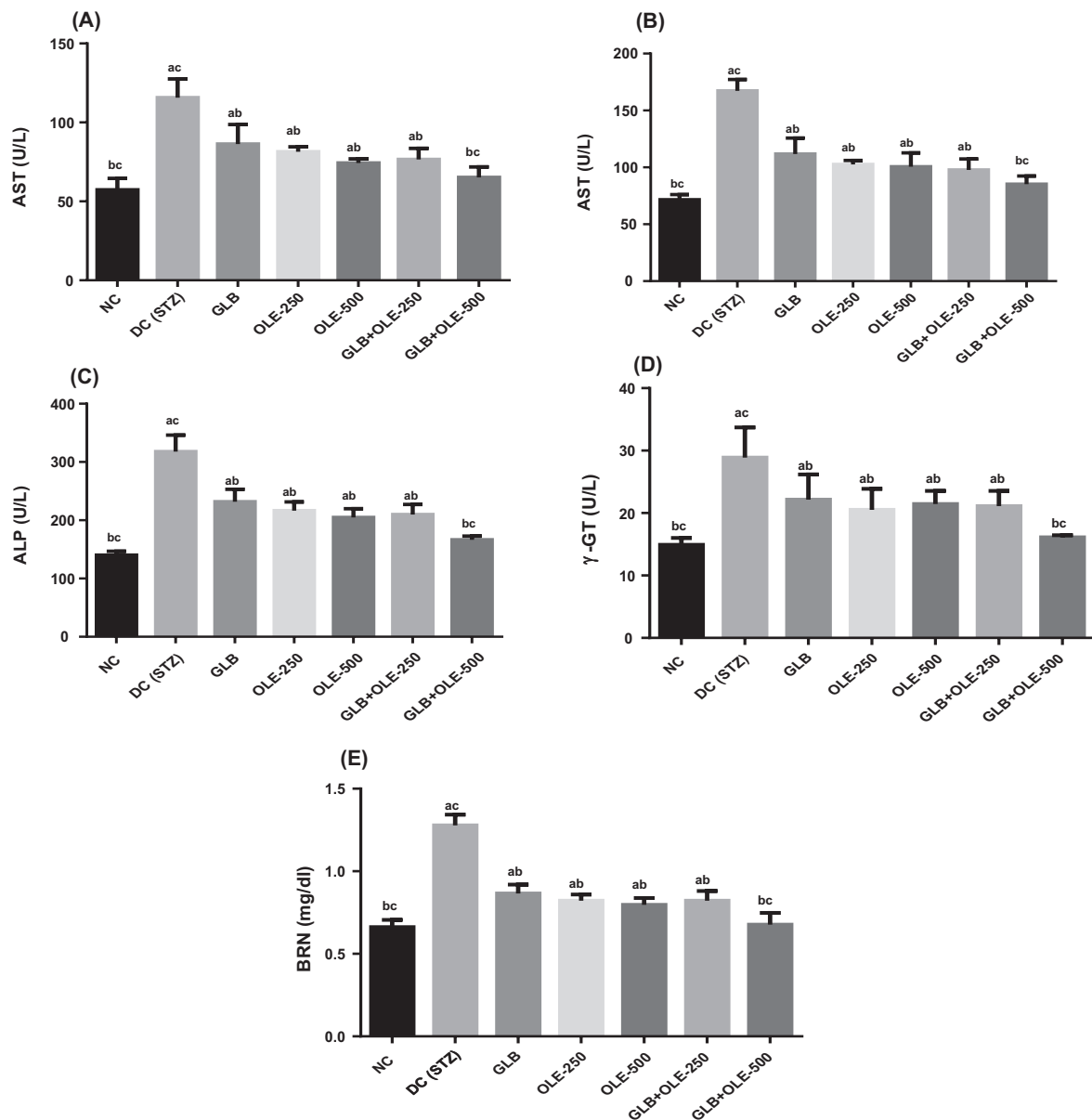


Fig. 1. Effect of GLB, OLE and their combination on liver function biomarkers in blood of STZ-diabetic rats. Values are expressed as mean ± SE (n = 6). ^a $P \leq 0.05$, statistically significant from the normal control (NC) group. ^b $P \leq 0.05$, statistically significant from the diabetic control (DC) group. ^c $P \leq 0.05$, statistically significant from GLB group. Multiple group comparisons were performed by analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test at $p \leq 0.05$.

acting by some different mechanism than that of GLB on lipid metabolic pathways. The beneficial effect of OLE on lipid profile of diabetic rats might be due to the presence of olive polyphenols.

The beneficial effects of polyphenols of the olive leaves on the lipid profile such as decrease of TG, TC, and LDL-C have been reported (Vogel et al., 2015).

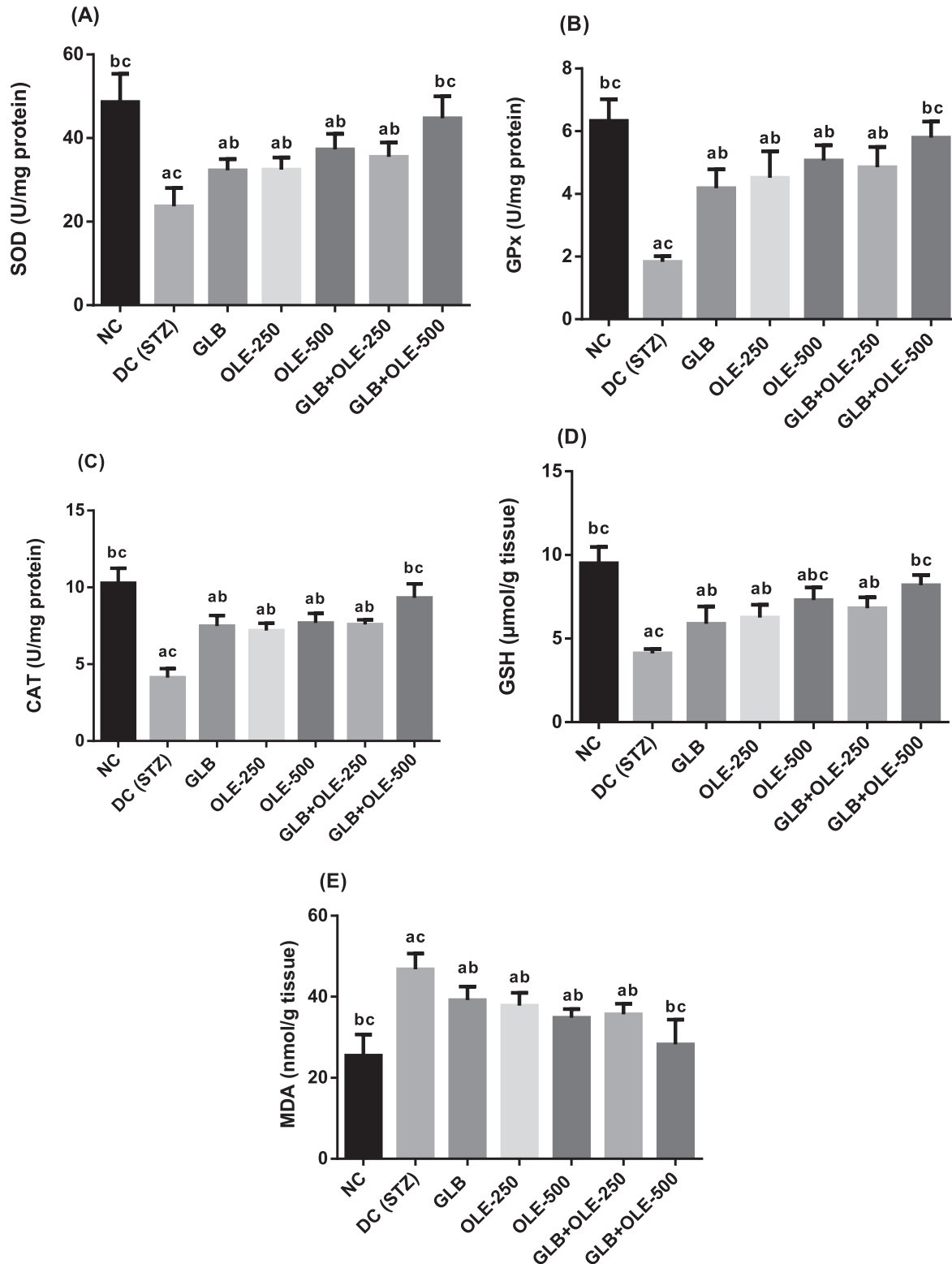


Fig. 2. Effect of GLB, OLE and their combination on oxidative stress and lipid peroxidation parameters in pancreatic tissues of STZ-diabetic rats. Values are expressed as mean \pm SE (n = 6). ^a $P \leq 0.05$, statistically significant from the normal control (NC) group. ^b $P \leq 0.05$, statistically significant from the diabetic control (DC) group. ^c $P \leq 0.05$, statistically significant from GLB group. Multiple group comparisons were performed by analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test at $p \leq 0.05$.

Atherogenic index (AI) showed pronounced ($p \leq 0.05$) elevation in DC rats (3.3 ± 0.28). OLE-500, GLB plus OLE-250 and GLB plus OLE-500 treated rats exhibited improved atherogenic index as comparable to GLB-treated rats. This improvement might be due

to the potent antioxidant effect of OLE that makes it a good alternative to reduce the risk of atherosclerosis and coronary heart disease and other free radical associated health problems (Ahmadvand et al., 2012).

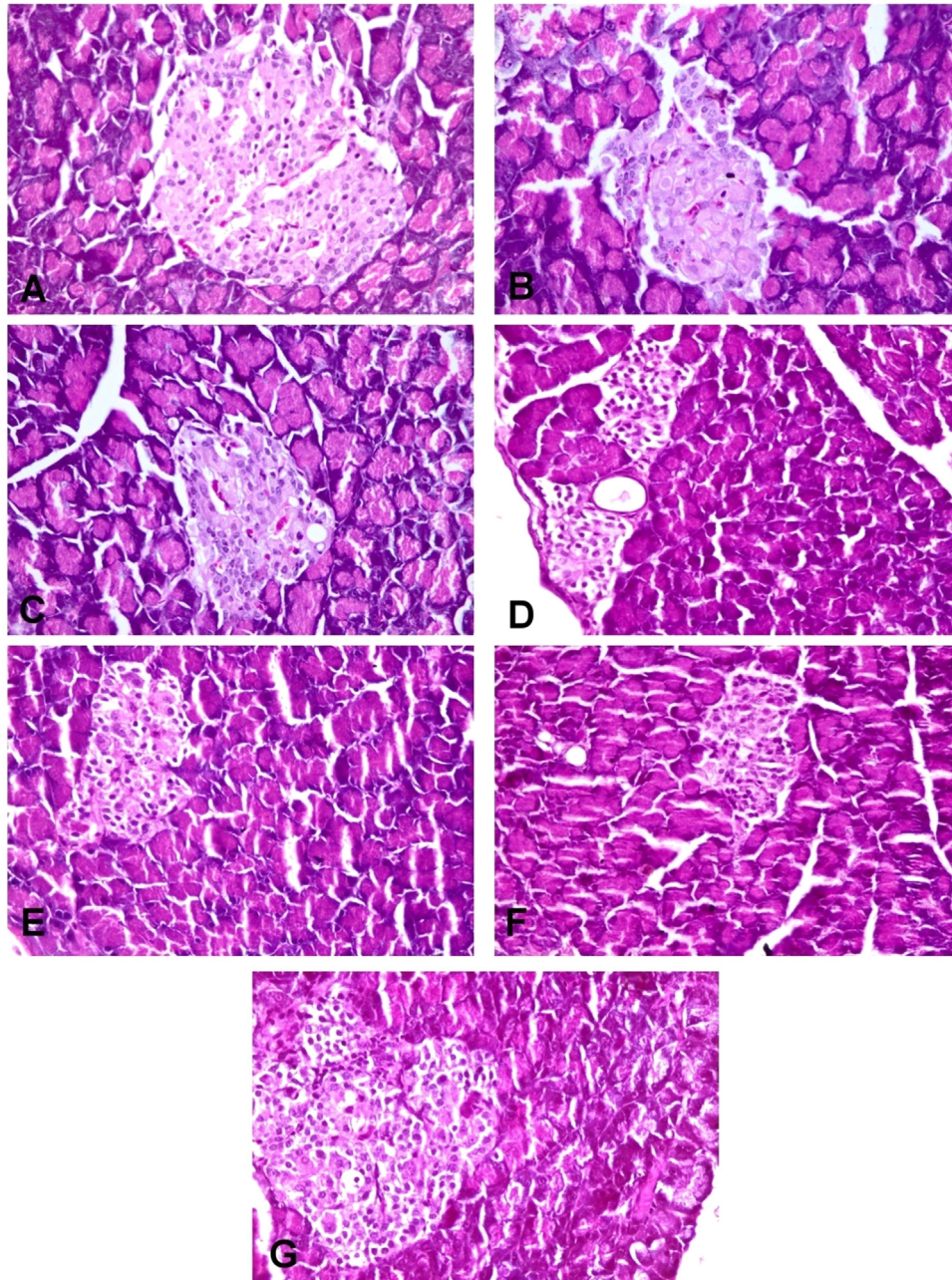


Fig. 3. The histopathological findings of the pancreas (H&E, X 400). (A) NC group showing normal histological architecture of the pancreas. (B) DC group showing marked reduction in the number, necrosis and apoptosis of β - cells and distortion of the islet. (C) GLB-treated group showing marked increase in the number of β - cells. (D&E) OLE-250 and OLE-500 groups showing moderate increase in the number of the β - cells. (F&G) GLB + OLE-250 and GLB+OLE-500groups showing marked increase in the centrally located β -cells with mild vacuolization of its cytoplasm.

3.2.2.4. Effect on liver function biomarkers. The liver diseases are more prevalent in the diabetic population. In the current study, the activities of the liver marker enzymes (AST, ALT, ALP and γ -GT) and level of BRN in serum of diabetic rats were significantly elevated in comparison with normal controls (Fig. 1). The increase in the levels of these liver markers in serum may be interpreted as a result of hepatocyte damage or changes in their membrane permeability indicating hepatocellular damage (Eldesoky et al., 2018). However, treatment of STZ-induced diabetes in rats with GLB, OLE-250, OLE-500 and GLB plus OLE-250 combination reduced AST, ALT, ALP and γ -GT activities and BRN levels but their levels still above the normal values. Treatment with GLB plus OLE-500 combination significantly reversed the increased levels of liver marker enzymes and BRN. The activities of those hepatotoxicity markers in

the combination-treated group remained almost the same as normal, indicating stimulation of insulin secretion into the circulation and a resulting hepatoprotective effect.

3.3. Effect on oxidative stress and lipid peroxidation markers in the pancreatic tissues

Oxidative stress can result from inadequate glycemic control with glucose levels above normality. Clinical evidence has shown that DM is closely related to oxidative stress, resulting in either increased production of free radicals or reduction in antioxidant defense systems (Susztak et al., 2006). To protect against free radicals, cells protect their vital functions by enzymatic or non-enzymatic mechanisms. These antioxidant enzymes include SOD,

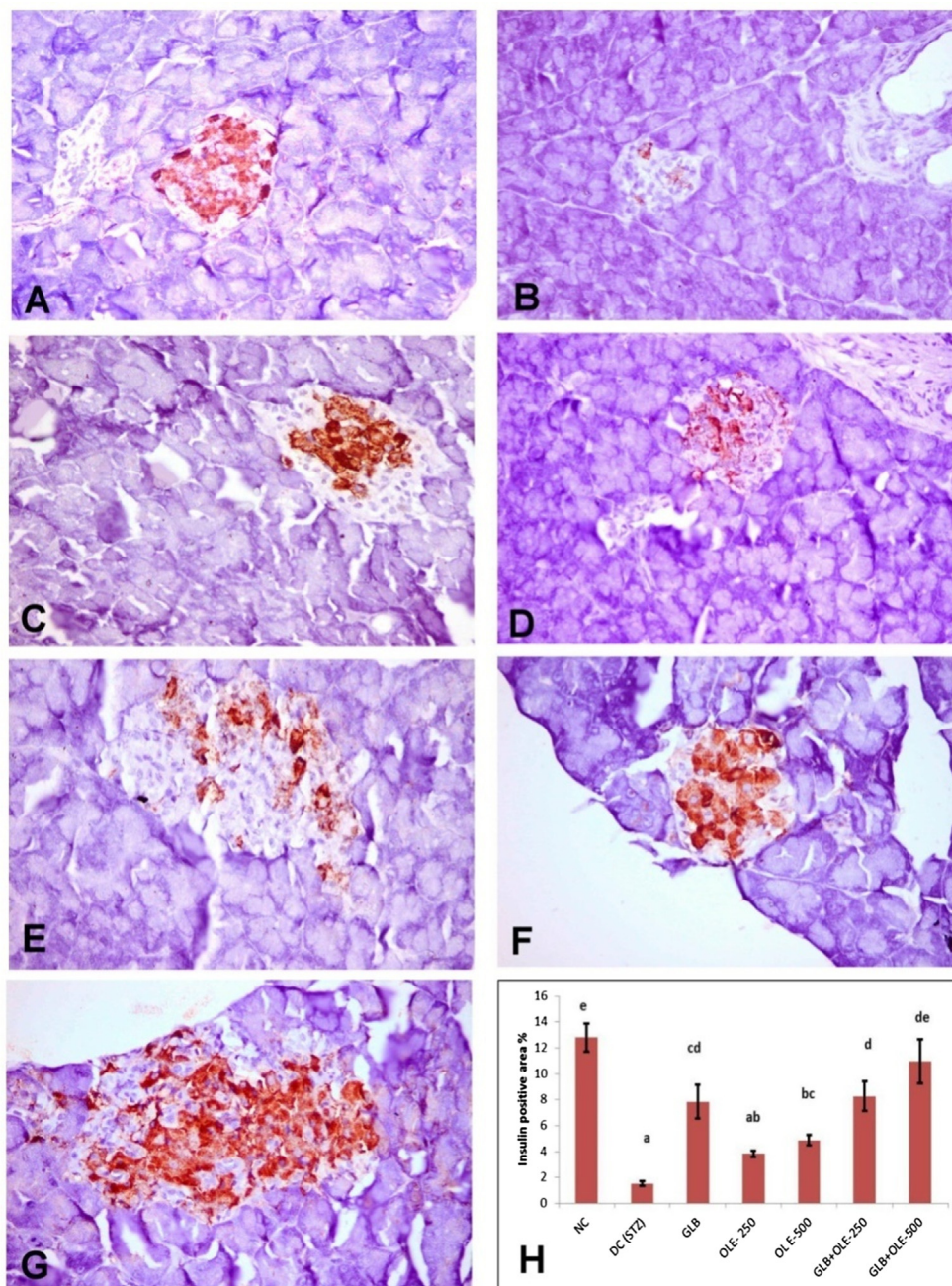


Fig. 4. Representative anti-insulin immunohistochemistry in the pancreas of the different experimental groups (X400). (A) NC group showing normal distribution of the insulin in centrally located β - cells. (B) DC group showing marked reduction in the insulin content of the β - cells. (C) GLB-treated group showing marked increase in the insulin content of β - cells. (D&E) OLE-250 and OLE-500 groups showing moderate increase in insulin content of the β - cells. (F&G) GLB + OLE-250 and GLB+OLE-500 groups showing marked increase in the insulin content of centrally located β - cells. (H) Bar chart represents the insulin immunopositivity expressed as area %. Values are expressed as the mean \pm S.E. Values with different superscripts are significantly different ($p \leq 0.05$).

GPx and CAT and are considered as the first line of defense against oxidative stress. GSH is non-enzymatic efficient antioxidant present in almost all living cells and is also considered as a biomarker of redox imbalance at cellular level.

Pancreatic β -cells are highly prone to oxidative stress and damage because they express low levels of antioxidant enzymes. STZ may damage pancreatic tissue via imposition of oxidative stress, which in turn can induce apoptosis in pancreatic cells (Manna et al., 2009). Fig. 2 illustrates the effect of GLB, OLE and their combinations on oxidative stress and lipid peroxidation markers in the pancreatic homogenates of rats. In the present study, SOD, GPx and

CAT activities and GSH level were significantly reduced in diabetic control rats compared to NC group.

An important pathogenetic mechanism of pancreatic β -cell damage during experimental STZ-induced diabetic animals is increased ROS production in pancreatic islets (González et al., 2002). Additionally, oxidative stress induction in the pancreas might be due to a hypoxic state as diabetes results in HbA1c formation that interferes with oxygen delivery at the pancreas. However, oral administration of GLB to STZ-induced diabetic rats resulted in increased levels of SOD, GPx, CAT and GSH in their pancreatic tissues. Research from the antioxidant studies suggested that GLB

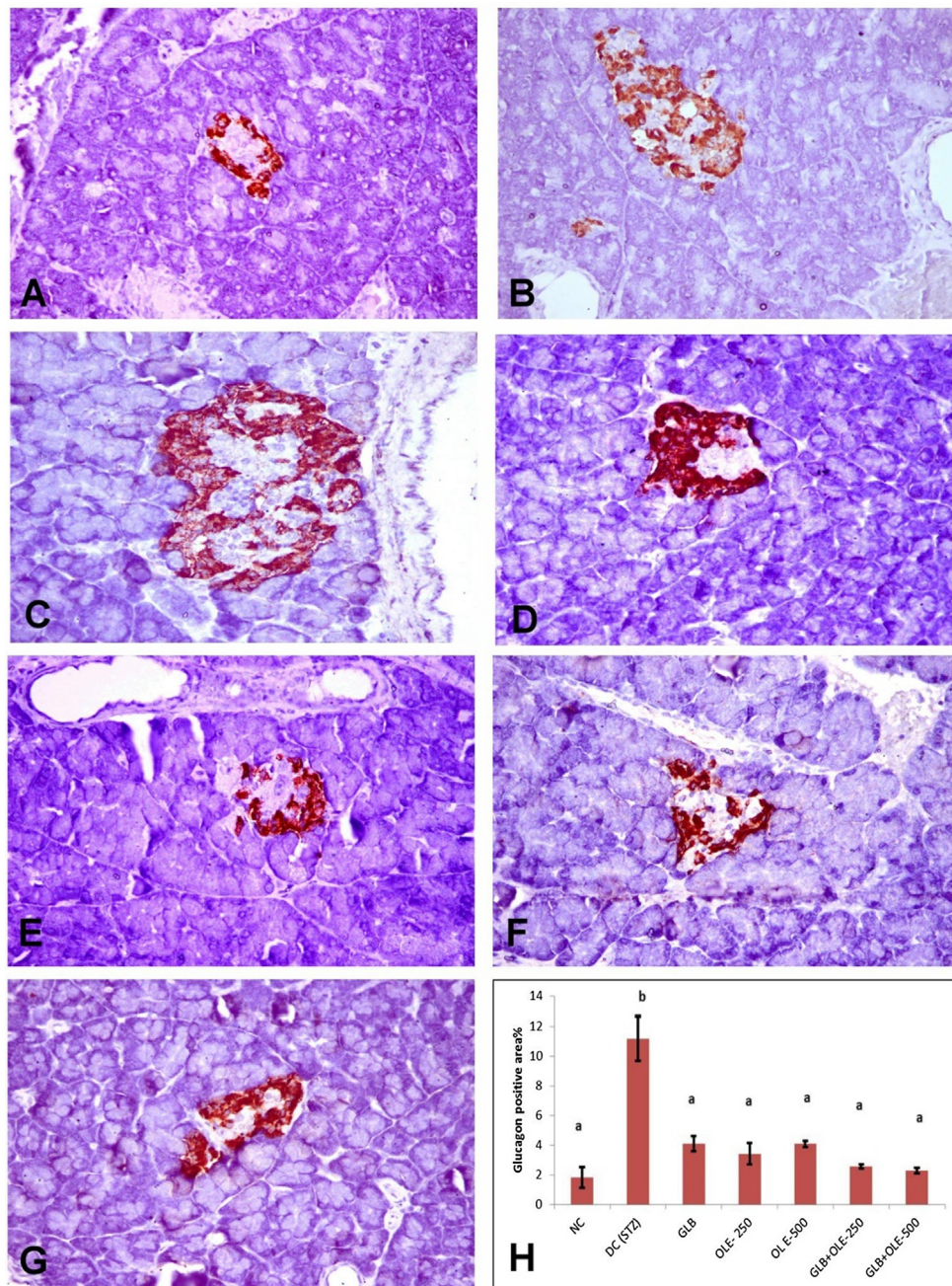


Fig. 5. Representative anti-gulcagon immunohistochemistry in the pancreas of the different experimental groups (X400). (A) NC group showing normal peripheral distribution of gulcagoncontent of α -cells. (B) DC group showing marked increase of the gulcagon content of the α -cells occupying both peripheral and central areas of the islet. (C) GLB-treated group. (D) OLE-250 group. (E) OLE-500group. (F) GLB + OLE-250 group. (G) GLB+OLE-500 group. (H) Bar chart represents the gulcagon immunopositivity expressed as area %. Values are expressed as the mean \pm S.E. Values are expressed as the mean \pm S.E. Values with different superscripts are significantly different ($p \leq 0.05$).

has the potential to counteract the ROS mediated oxidative stress (Rabbani et al., 2010). Similarly, OLE-250 and OLE-500 alleviated oxidative stress by inducing the activities of antioxidant enzymes and GSH levels that were reduced in the pancreas of diabetic rats. The protective effect of both doses of OLE and GLB plus OLE-250 combination against oxidative stress in the pancreatic tissues of diabetic rats was superior to that seen in the group of rats that received GLB monotherapy. These beneficial effects may be due to the antioxidant components of olive leaves, especially oleuropein (El and Karakaya, 2009). The literature indicates that most of the compounds present in olive leaves have antioxidant properties, preventing or reducing the deleterious effects of oxidative stress associated with diabetes (Hamden et al., 2009; Cvjetičanin

et al., 2010). The anti-oxidant efficacy of GLB plus OLE-500 combination was more beneficial in diabetic rats than treatment with either GLB or GLB plus OLE-250 combination. After oral administration of GLB plus OLE-500 combination to diabetic rats, the altered levels of SOD, GPx, CAT and GSH in their pancreatic tissues were brought back to normal. These results suggest that GLB and OLE-500 have a synergistic effect against oxidative stress in STZ diabetes.

MDA is considered a good biomarker of the lipid peroxidation process. Results from previous studies showed that MDA levels in plasma and tissue are elevated in STZ-induced diabetic rats (Nakhaee et al., 2009). Consistent with this finding, the present study indicated that the exposure of rats to STZ resulted in a severe

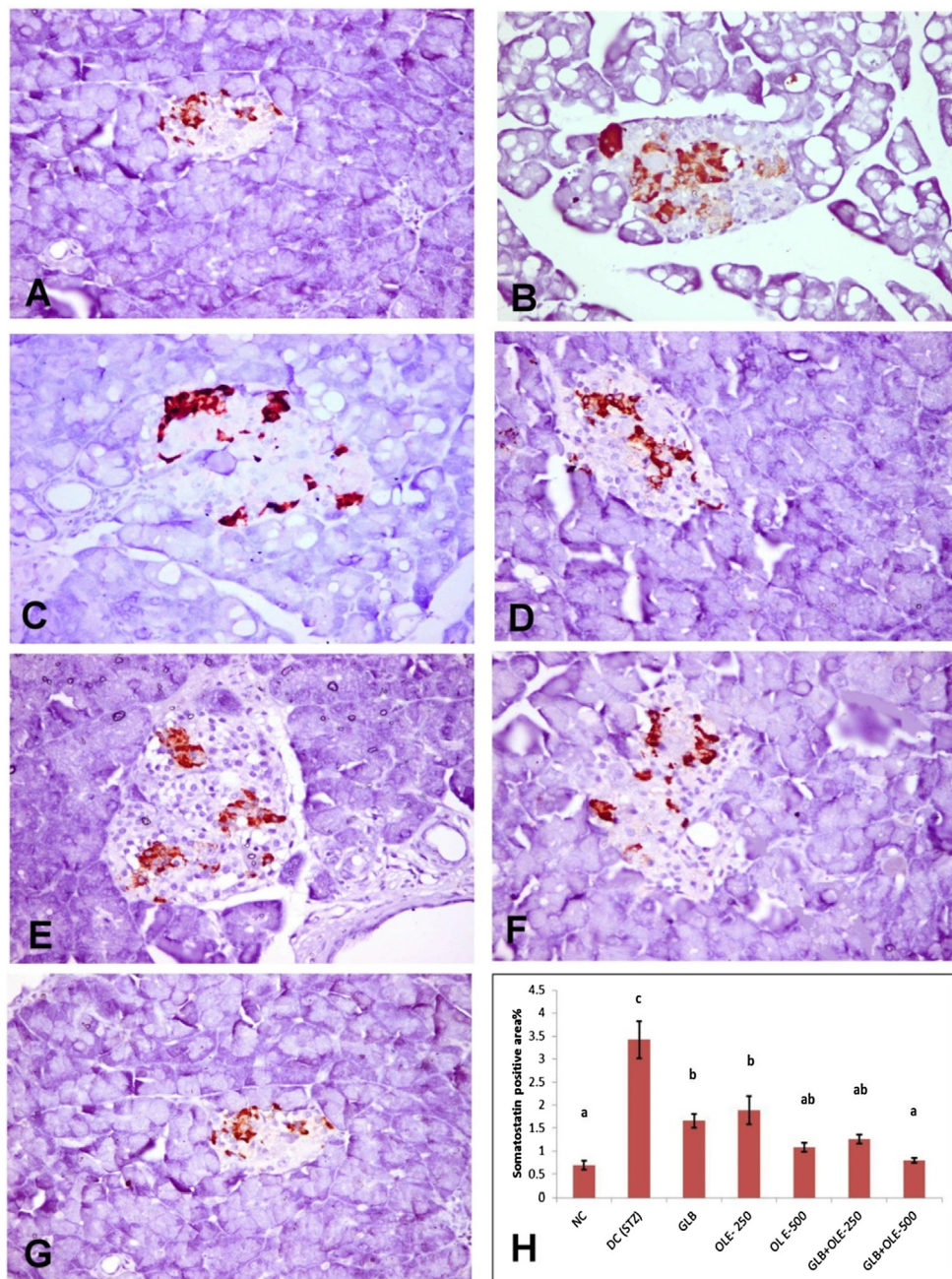


Fig. 6. Representative anti-somatostatin immunohistochemistry in the pancreas of the different experimental groups (X400). (A) NC group showing normal peripheral distribution of somatostatin content of δ -cells which forming incomplete ring in the islet. (B) DC group showing marked increase of somatostatin content of δ -cells occupying the peripheral and central area of the islet. (C) GLB-treated group. (D) OLE-250 group. (E) OLE-500 group. (F) GLB + OLE-250 group. (G) GLB+OLE-500 group. (H) Bar chart represents the somatostatin immunopositivity expressed as area %. Values are expressed as the mean \pm S.E. Values with different superscripts are significantly different ($p \leq 0.05$).

LPO in pancreatic tissues as evidenced by significant elevation in the pancreatic content of MDA compared with NC rats. The elevated pancreatic lipid peroxidation observed in the diabetic rats can be attributed to enhanced production of ROS, which leads to oxidative stress (Ilhan et al., 2001). Medication of diabetic rats with GLB, OLE-250 and OLE-500 monotherapies or GLB plus OLE-250 combination resulted in a significant reduction in the pancreatic contents of MDA compared to DC group ($p \leq 0.05$). Decreased level of MDA in diabetics is an indication of improvement in defense mechanisms of enzymatic and non-enzymatic antioxidants (Saddala et al., 2013). Additionally, eight-week GLB plus OLE-500 administration has successfully normalized the disordered level

of MDA in the pancreas of diabetic rats. These results suggest that GLB and OLE-500 have a synergistic effect on reducing lipid peroxidation in the pancreas of STZ diabetic rats.

3.4. Histopathological examination of pancreases

The pancreas of normal control group revealed a normal histological architecture of the pancreatic acini and islets of Langerhans (Fig. 3A). The islets consisted of a central core of (β) cells enclosed by a mantle of Alpha (α) and Delta (δ) endocrine cells in the periphery. In the diabetic group, there was marked reduction in the number of β -cells with increase in number of both α - and δ -endocrine

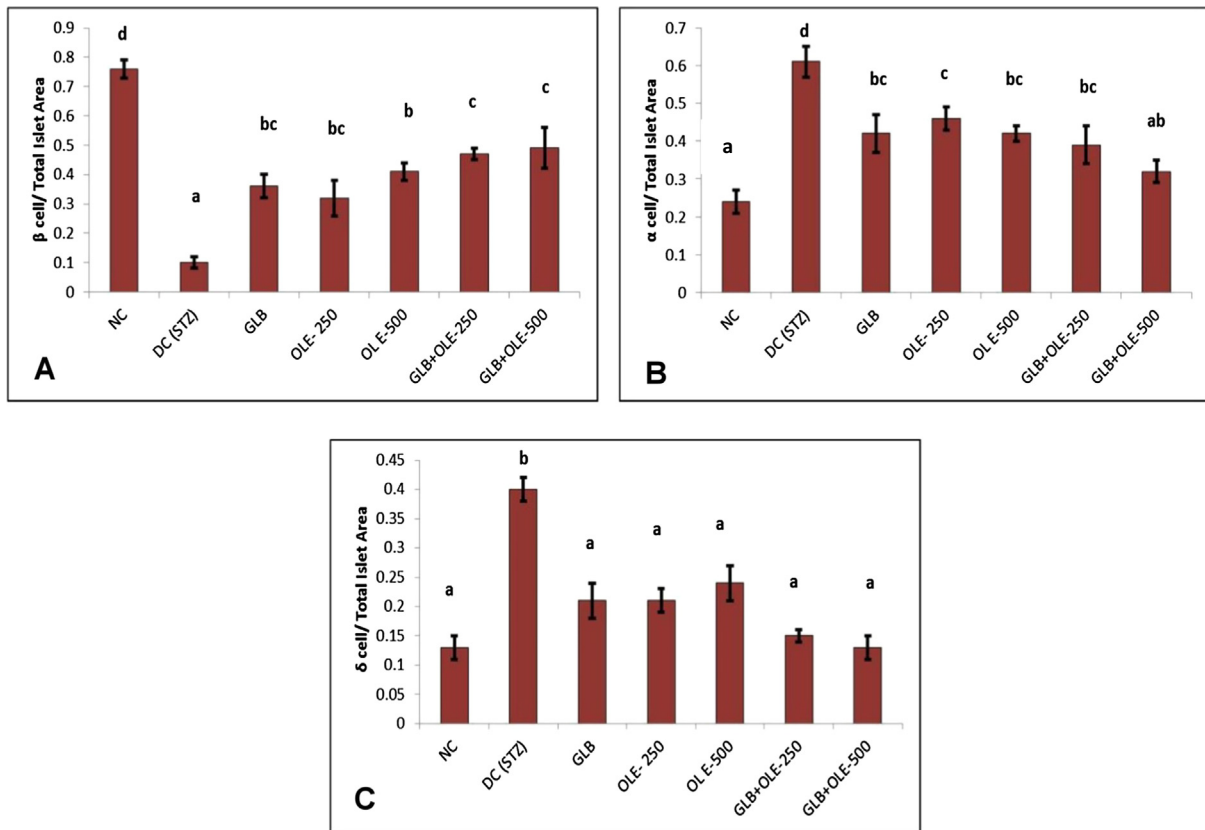


Fig. 7. Effect of OLE on the islet morphometry in STZ induced diabetic rats. (A) β -cell/total islet area; (B) α -cell/total islet area; (C) δ -cell/total islet area. Values are expressed as the mean \pm S.E. Values with different superscripts are significantly different ($p \leq 0.05$).

cells of the islets of Langerhans leading to disruption of their appearance (Fig. 1B). In addition to that, pancreatic duct exhibited dilation and papillary hyperplasia of their epithelial lining. STZ has abundant mechanisms to induce β -cell toxicity and subsequent decrease in insulin production, by free radicals production and nitric oxide donation (Nugent et al., 2008). All different treatments improved the histopathological lesions in the islet of Langerhans, particularly the β -cell loss (Fig. 3C,D,E,F,G). Therefore, olive extract had a strong effect on maintaining β -cell and insulin production in STZ-induced diabetic rats.

3.5. Immunohistochemical analysis of insulin, glucagon and somatostatin protein expression and islet morphology

The normal control group showed positive β -cells with a diffuse distribution of the insulin content which occupied most of the islets of Langerhans (Fig. 4A). Glucagon was localized in α -cells and somatostatin was localized in δ -cells which are located in the periphery of the pancreatic islet (Figs. 5, 6A). Diabetic control group exhibited a significant reduction in the insulin content of β -cells (Fig. 4B) with significant elevation in both glucagon (Fig. 5B) and somatostatin (Fig. 6B) contents in the pancreatic islets. In the various treated groups, the insulin content (Fig. 4H) of the β -cells was significantly elevated in the core of the islet, with a marked reduction in the glucagon and somatostatin contents in the α -cells and δ -cells in the islet, respectively, particularly in the core, as shown in Figs. 5 and 6.

The morphometric analysis of the pancreatic islets was summarized in Fig. 7. The β -cell/total islet area was markedly reduced in diabetic control group comparing to normal group. In addition, α -cell/total islet area and δ -cell/total islet area were significantly

elevated than normal control group. At different treatment, there were marked increase in β -cell/total islet area and marked decline α -cell/total islet area and δ -cell/total islet area comparing to STZ treated group. STZ induced β -cells toxicity and β -cells loss resulting in marked reduction of their insulin content and subsequent elevation of glucagon and somatostatin contents in the α -cells and δ -cell. All treatment had a significant effect in restoration of insulin content of the β -cells. However, GLB plus OLE-500 was the most efficient treatment that restored the insulin content of the β -cells among the other groups.

3.6. Changes in hepatic gene expression

In the liver tissue, the metabolic action of insulin occurs through the phosphatidylinositol 3 kinase (PI3K) pathway, which initiated by insulin binding to IR, activation of IR by autophosphorylation, phosphorylation of selective tyrosine residues on insulin receptor substrate 2e 1 an (IRS-1, IRS-S) that allow their binding to PI3K, and finally activation of protein kinase B (PKB). PKB activation is crucial for induction of various processes, including glucose transport, glycogen synthesis and lipogenesis (Werner et al., 2004; Elmudhun et al., 2013).

Hepatic mRNA expression of selected genes related to glucose uptake and metabolism in the different experimental groups are summarized in Fig. 8. DC group presented significant reduction in hepatic mRNA levels of Slc2a2, PPAR- α and INSR genes relative to the normal control ($p \leq 0.05$), while the different treatment regimens counteracted this reduction for all the studied genes. Although GLB, OLE 500 and OLE 250 were capable of reversing the decreases Slc2a2 expression level in the liver of STZ-diabetic rats, the combination treated groups; GLB plus OLE-250 and GLB

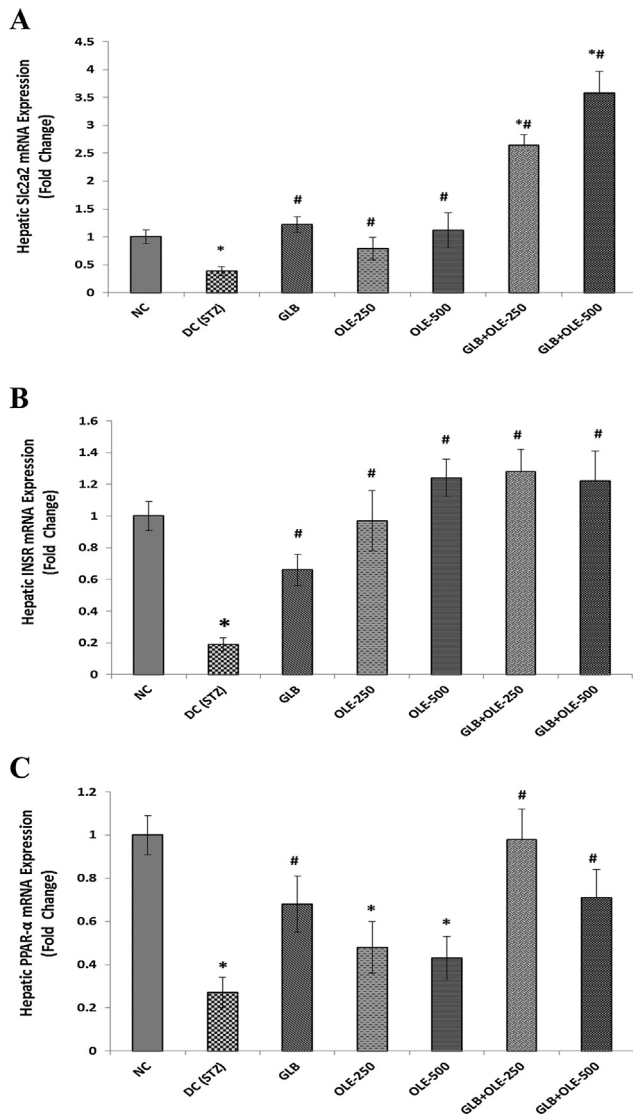


Fig. 8. Effect of OLE and/or GLB on some diabetes-related genes expression in the liver of STZ-diabetic rats. Effect of GLB, OLE-250, OLE-500, GLB + OLE-250 and GLB + OLE-500 on hepatic mRNA expression of Slc2a2 (A), PPAR- α (B) and INSR (C) in STZ diabetic rats. Treatments were gavaged orally for eight weeks. All values are presented as mean \pm S.E of three to five independent experiments as compared with normal control (*) or diabetic control (#) at $p \leq 0.05$.

plus OLE-500 showed marked upregulation of Slc2a2 by 2.64 and 3.58 folds of the normal level, respectively (Fig. 8 A). These findings demonstrate that the hypoglycemic mechanism of GLB plus OLE combination may be attributed to the inhibition of hepatic glucose uptake via the insulin-independent pathway.

The major constituent of OL, gallic acid, has been reported to up-regulate the expression of proteins related to insulin signal transduction, including IR, insulin receptor substrate 1 (IRS 1), Akt/protein kinase B, phosphatidylinositol-3 kinase, and GLUT-2 (Huang et al, 2016). Also, Yeh et al. (2017) reported that OL might be able to improve insulin sensitivity via regulation of inflammatory signaling. A similar expression pattern was observed for PPAR- α transcription factor that play a key role in lipid homeostasis by regulating the transcription of genes involved in lipid metabolism. The activation of PPAR- α results in the sequestration of lipids into the liver for oxidation (Ismail et al., 2015). The GLB-treated group showed increased expression levels of PPAR- α , whereas animals of the OLE-250 and OLE-500 groups showed a

slight increases (or a trend to increased) in PPAR- α expression, reaching values not different from the DC group. However, rats treated with the combinations displayed PPAR- α expression levels near that of the NC (Fig. 8 B).

As far as insulin signaling is concerned, the expression level of INSR was significantly decreased in the DC group with respect to the NC group ($p \leq 0.05$). Notably, GLB, OLE-250 and OLE-500, individually or their combinations restored INSR expression to the control levels (Fig. 8 B). These results come in accordance with previous studies (Al-Attar and Alsalmi, 2019; Liu et al, 2014) who showed that olive leaf extract efficiently restored the expression levels of insulin IRS1 and IR in diabetic rats. These findings indicate that OLE may attenuate insulin resistance and lower blood glucose levels by improving insulin signal transduction. Moreover, the enhanced expression of IRS-1 by OL administration associated with an increased insulin activity (Liu et al, 2014).

4. Conclusion

In conclusion, this study indicates that OLE at 500 mg/kg enhances the antidiabetic effect of glyburide in diabetic rats, as shown by the reduction in the blood levels of glucose and HbA1c and the improvement in the pancreatic histopathological parameters. The antidiabetic effect of GLB plus OLE-500 combination is likely to be mediated through its antioxidant effect and its capability to restore the insulin content of Beta (β) cells and to reduce the glucagon and somatostatin of Alpha (α) and Delta (δ) endocrine cells in the pancreatic islets. Moreover, the current study suggests that GLB plus OLE-500 combination could cause herb-drug interaction through modulation of insulin receptor (INR), glucose transporter 2 (Slc2a2) and peroxisome proliferator-activated receptor α (PPAR- α) genes expression in the liver of diabetic rats. The use of such combination appreciably modulates hepatic glucose sensitivity and metabolism. Hence, glyburide doses may require special attention if used along with OLE preparations to offer the patients better control of the disease.

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Declaration of Competing Interest

The authors declare that; there is no conflict of interest.

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