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Ameliorative effect of Arabic gum Acacia and mori extracts in streptozotocin-induced diabetic rats: implications of Cas-3 and TGF-β

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Abstract. – OBJECTIVE: Arabic gum Acacia (AG) is rich in fiber which improves lipid metabolism besides its antioxidant effect. *Folium mori* (FM) is a widely used herb due to its immunomodulatory, antimicrobial, and antioxidant activity. In the current study, we explore the antidiabetic, anti-inflammatory, as well as antioxidant activities of AG and FM in Streptozotocin (STZ), induced diabetic rats.

MATERIALS AND METHODS: STZ diabetic rats were orally administrated with metformin and/or a combination of AG and FM for 4 weeks. Glycemic levels, Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), cholesterol, triglycerides, urea, and creatinine were determined. Malondialdehyde (MDA), glutathione peroxidase (GPx), and Superoxide dismutase (SOD) were also evaluated. Gene expression and profile as well as immunohistopathological were also evaluated.

RESULTS: The results elicited no toxicological profile of both AG and FM. Plasma glucose level was decreased starting from 1st week to 4th week; besides, there was an improvement in glycated hemoglobin, insulin, and fructosamine. Liver and kidney damage markers were decreased in both AG and FM-treated rats. A significant increase in the antioxidant defense system and a decrease in oxidative stress markers were also observed. Gene expression analysis in brain tissues revealed a significant decrease in Interleukin beta 1 (IL-β1), Caspase 3 (Cas-3), and Transforming growth factor beta (TGF-β).

CONCLUSIONS: Oral treatment of metformin with AG and FM in STZ-injected rats could ameliorate protective pathways and can be one of the promising oral anti-diabetic herbal agents.

Key Words:
Arabic gum, Folium mori, Streptozotocin, Diabetes, Metformin.

Introduction

Diabetes mellitus (DM) is an incurable chronic disease that results in the development of severe long-term complications if not carefully monitored and regulated. These complications mainly affect the eyes, kidneys, cardiovascular and nervous systems[1]. The incidence of diabetes rises globally causing many serious complications[2,3] mentioned that persistent and chronic hyperglycemia elaborates free radicals and reactive oxygen species (ROS) causing increased oxidative stress. The oxidative stress caused by free radicals and ROS increases lipid peroxidation, DNA damage, protein breakdown, and anti-oxidative defense system depletion[4]. The central and peripheral nerve systems appear to be the sites of many diabetes-related diseases[5]. The hippocampus, for example, is affected by the oxidative damage generated by diabetes[6].

Nutritional management is essential for patients with diabetes due to the undesirable effects of oral hypoglycemic agents. Insulin resistance and the development of diabetes are influenced by obesity and weight increase[7]. The elevated levels of triglycerides have been related to elevated levels of free fatty acids. This in turn regulates pathways responsible for linking insulin receptors with glucose transporters and impairing the normal function of beta cells[8,9]. Natural products are the most promising lead candidates and thus play a vital role in future drug development. In addition to their clinical use, natural medicinal products with immunostimulant and stress-relieving properties are frequently employed as feed supplements[9].

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Arabic gum (AG) is known as a non-absorbable polysaccharide with a very low glycemic index, it is extracted from trees of Acacia Senegal[10]. No adverse effects were recorded after AG use even at very high doses[11]. It prevents glucose absorption, and therefore, reduces plasma glucose by elevating the insulin secretion[12]. AG has a high fiber content which reportedly improves lipid metabolism in experimental animal studies. Furthermore, it has an antioxidant effect and improves capillary function[13].

Folium mori (FM) is widely used due to its antimicrobial and antioxidant properties in the treatment of many diseases[14]. It is also used for its anti-diabetic, immunomodulatory, antimicrobial, antioxidant, and anticancer properties[15-17]. Despite indications that the FM extract can dramatically reduce the symptoms and effects of type 2 diabetes mellitus (T2DM), such as dyslipidemia but it is still controversial[18]. The extract's activities can be enhanced synergistically if used in combination with other supplements[19].

Hence this study was conducted to determine the potential clinical anti-diabetic effects of a mixed herbal extract (MHE), FM extract, and AG using biochemical, gene analysis, and immunohistopathological examination.

Materials and Methods

Drugs and Herbs

STZ was used for induction of diabetes in rats, it was purchased from Sigma Company (Sigma, St. Louis, Mo, USA), and metformin hydrochloride was used for pharmacological validation of the AG and MF and was obtained from Merck Company (Darmstadt, Germany). AG was obtained from a local market as an instant gum powder granule. It was administered by gastric gavage after applying the toxicity test, and FM was obtained from a local market.

Preparation of Folium Mori Extract

FM aqueous extract was prepared by adding 200 g of distilled water and heated at 80°C. After the extraction, the extract was concentrated with a rotary evaporator[19]. It was administered through gastric gavage after applying the toxicity test.

Animals

One hundred and five, 8 weeks old male Wister rats, weighing ±325 g. Animals were obtained from Helwan animal house and kept in a well-ventilated room under standard laboratory conditions with a 12-hour light-dark cycle. The rats were given a standard rodent diet (62% starch, 23% protein, 4% fat, 7% cellulose, and salt mixture). The study protocol was approved in advance by the Medical Research Ethics Committee for animal research studies at the Faculty of Veterinary Medicine, Mansoura University (Cod No.: R\86). All conceivable attempts were made to minimize animal suffering according to Kilkenny et al[20].

Acute Toxicity Test

Acute toxicity test of FM and AG (LD50) was determined using 45 rats. The rats were equally divided into three groups, G1 (AG Group), G2 (FM group), and the third group G3 given only a vehicle (10% DMSO) as a control. Every group was subdivided into three subgroups, where five rats were allocated in each subgroup and dosed with 2, 4, and 8 g/kg body weights of AG and FM using gastric gavage. Animals were monitored for 24 hours to determine mortality. Herbal products showed no signs of toxicity or mortality in rats even at the highest dose. Therefore, both herbal products were continuously administered orally with a dose of 8 g/kg through gastric gavage for 4 weeks.

Animal Grouping

Sixty rats were divided randomly into four equal groups as follows:

Group 1 (G1): negative control group given only vehicle (10% DMSO).

Group 2 (G2): diabetic group received STZ at a dose (60 mg/kg) once dissolved in citrate buffer (0.05 M, pH: 4.5) according to Makino and Kamata[21].

Group 3 (G3): treated with Metformin through gastric gavage dissolved in distilled water at a dose of 150 mg/kg body weight/day for 4 weeks starting two days after STZ administration according to Majithiya and Balaraman[22].

Group 4 (G4): treated with AG at dose 8 g/kg through gastric gavage and the aqueous extract of FM at dose 8 g/kg through gastric gavage for 4 weeks starting two days after STZ administration.

Wound Healing Ability Study

Three days after STZ injection (Day 0), rats were anesthetized using intramuscular administration of ketamine hydrochloride (75 mg/kg) and xylazine (10 mg/kg). The dorsal surface of rats was shaved, and a wound of 1 cm diameter was marked using transparent tracing paper then

a full-thickness excision was induced along the marking. 100 µL of saline was added to the wound daily in G1 and G2 groups and 100 µL metformin + 100 µL of both AG and FM was added to the wound along with the model protocol. The dimensions of the wound were measured at time intervals. The percentage wound area contraction was calculated using the following formula according to Ahmad et al[23].

% Wound closure = [(Wound area on day 0-Wound area on day n)/Wound area at day 0] *100.

Preparation of Blood Samples

After 4 weeks, all rats fasted overnight, blood from the tail vein was collected into heparinized tubes, and then centrifuged at 3,000 rpm for 15 min. The serum was separated and stored at -80°C to undergo further analysis. Rats were decapitated, but brain, liver, and kidneys were kept. Half of the tissues were put in formalin 10% for histopathological and immunohistochemistry examination. The other half was used to prepare tissue homogenates in phosphate-buffered saline (pH 7.4) using an MPW-120 homogenizer (Med instruments, Warsaw, Poland). The homogenate was centrifuged using a cooling centrifuge (Sigma Laborzentrifugen, GmbH, Harz, Germany) at 5,000 rpm for 10 min at 4°C; after removal of the cell debris, the supernatant was stored at -80°C until further analysis of inflammatory cytokines, oxidative and apoptotic biomarkers, and RNA extraction.

Determination of Glycemic Index

Insulin level was measured in plasma using ultrasensitive insulin ELISA according to the manufacturer's indications. The homeostasis model assessment (HOMA) was used to calculate insulin sensitivity using fasting glucose (G0) and insulin (I0) concentrations using the following formula: (G0 X I0)/22.5[24]. The quantitative insulin sensitivity check index (QUICKI) was determined by the following calculation: 1/(log (fasting glucose) + log (fasting insulin)[25]. The percentage of glycosylated hemoglobin (HbG%) in total blood was determined by ionic capture (IMX System, Abbott, IN, USA) according to the method described by Lowry et al[26]. Fasting blood glucose (FBG) was monitored weekly for all groups by Accu-check-go glucometer (Roche, Harz, Germany) using blood from the tail veins. The presence of blood glucose levels greater than 200 mg/dl was a criterion for diabetes.

Biochemical Analysis

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined calorimetrically using the method outlined by Reitman and Frankel[27]. Serum cholesterol and triglyceride were estimated according to Naito[28]. Serum urea concentration was determined using the enzymatic calorimetric method indicated by Coulomb and Farreau[29]. Serum creatinine level was analyzed using a calorimetric kinetic method as detailed by Husdan and Rapoport[30]. Serum fructosamine level was estimated *via* colorimetric assay according to methods described by Montilla et al[31].

Determination of Oxidative Stress Markers

Malondialdehyde (MDA) was measured by spectrophotometer according to the method of Ohkawa et al[32]. The determination of glutathione peroxidase (GPx) was determined according to the protocol of Paglia and Valentine[33]. Superoxide dismutase (SOD) activity was determined according to the method of Nishikimi et al[34]. Determination of catalase activity was determined according to Aebi[35].

Gene Expression Analysis

For RNA extraction, TriZol reagent (Invitrogen, Waltham, MA, USA) was used following the manufacturer's instructions. An equivalent of 1 mg of RNA was transferred to cDNA using the cDNA synthesis Kit (High-Capacity cDNA Reverse Transcription Kit; Applied biosystem, Thermo Fisher Scientific, Waltham, MA, USA) according to the instruction manual. QRT-PCR analysis was conducted using TOPreal qPCR 2x sybr green premix (Intron-Biotechnology, LiliF Diagnostics, South Korea) in a 96-well PikoReal PCR system (ThermoFisher Scientific, Waltham, MA, USA). Gene expression analysis was conducted to determine the expression levels of apoptotic factors and pro-inflammatory markers listed in (Table I)

Histopathological Examination

Liver, kidney, and brain tissues were examined for histopathological changes through processing in paraffin embedding sections, where de-paraffinization, dehydration, and staining with hematoxylin and eosin according to the procedures of Bancroft and Gamble[36].

Immunocytochemistry Study

Fixation of the brain for the immunocytochemical procedure was described before by Zandbergen et al[37]. Dewaxed brain sections were fixed

Table I. Primer used for gene expression analysis.

Gene	Sequence	Reference
IL-1β	F: TGCCACCTTTTGACAGTGATG R: AAGCTGGATGCTCTCATCAGG	53
CAS-3	F: GAATGTCAGCTCGCAATGGTAC R: AGTAGTCGCCTCTGAAGAAACTAG	53
β-actin	F: CACTGTCGAGTCGCGTCC R: CGCAGCGATATCGTCATCCA.	54
TGF-β1	F: ACCAAGGAGACGGAATAC, R: ACAGCAGTTCTTCTCTGT	54

with 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4), and 0.05% triton-X at RT for 30 min then the tissues were rinsed in graded sucrose solutions and frozen. Sagittal sections of 12 mm were cut in a cryostat and mounted on glass slides then immune-stained with the mouse monoclonal antibody against calbindin D28k (1:500; IgG antibody (1:1,000; Invitrogen Waltham, MA, USA). (Sigma-Aldrich, St. Louis, MO, USA), then the cells were photographed with an AxioCam digital camera (Zeiss, Berlin, Germany) on a Zeiss Axio Imager microscope (63× objective).

Statistical Analysis

The Statistical Package of Social Science (SPSS) version 16 (SPSS Inc., Chicago, IL, USA) was used to conduct the statistical analysis for this study. Results were represented as mean ± standard error (SE). The significant effects were considered at $p < 0.05$. For parametric data, analysis of variance (ANOVA) (f test) was used to compare different groups, followed by a least significant difference test to avoid a multiple comparison effect. For nonparametric data, Kruskal-Wallis ANOVA followed by a post hoc test (based on the Dunn procedure) was used to compare each pair of groups.

Results

Acute Toxicity Study

Acute toxicity tests revealed that there was no toxicity observed and the test compounds was found to be safe for the study to be carried out because of no signs of death.

Effect of Co-Administration of Herbal Drugs with Metformin on the Wound Healing Ability in Diabetic Rats

Diabetes caused a decrease in the percentage of wound healing (245.8%) in the STZ group compared with the normal group. Metformin increased the percentage of wound healing (180.4%) compared to the STZ group. Meanwhile, the diabetic rat that was treated with a combination of AG and the FM along with Metformin depicted a tremendous increase in the percentage of wound healing (140.2%) compared to the STZ group (Figure 1).

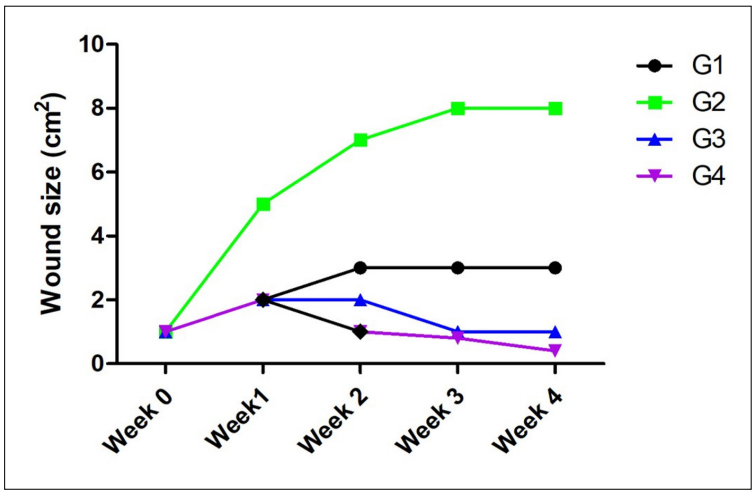

Figure 1. Effect of co-administration of herbal drugs with Metformin on the wound healing ability in diabetic rats.

Effect of Co-administration of Herbal Drugs with Metformin n Glycemic Index in Diabetic Rats

The present investigation revealed that STZ-challenged rats (G2) displayed a significant increment ($^{***}p$ <0.0001) in the fasting blood glucose levels (FBS) all over the experimental period in comparison to the control rats. Metformin treated group (G3) showed a significant decline ($^{**}p$ <0.001) in FBS levels from week 1 to week 4. Meanwhile, the diabetic rat that was treated with a combination of AG and the FM along with Metformin depicted a tremendous fall in FBS from week 0 to week 4 (Table II).

Serum insulin levels (µIU/ml) were found to be significantly decreased ($^{*}p$ <0.01) in herbal-drug treated animals (G4) in comparison to the diabetic group (G2). Likewise, after 4 weeks of treatment, insulin sensitivity parameters (HOMA and QUICKI) also showed a significant fall ($^{*}p$ <0.01) in group 4 (Figure 2).

A brand-new mathematical transformation of fasting blood sugar and insulin levels is called the quantitative insulin-sensitivity check index (QUICKI). In comparison to minimal-model estimates, QUICKI greatly improves the linear correlation between glucose clamp measurements of insulin sensitivity in obese and diabetic participants.

Table II. Fasting blood glucose levels (FBG) in different studied groups (mg/dl). The data is provided as mean ± SEM, (n = 6).

Week 0	Week 1	Week 2	Week 3	Week 4
105 ± 0.01[b]	110 ± 0.01[c]	115 ± 0.08[c]	117± 0.2[c]	125 ± 0.01[c]
490 ± 0.17[a]	450 ± 0.10[a]	390 ± 0.12[a]	420 ± 0. 4[a]	440 ± 0. 4[a]
490 ± 0.12[a]	300 ± 0.511[b]	270± 0.521[b]	200± 0.011[b]	179± 0.41[b]
390 ± 0.41[a]	250± 0.21[b]	220± 0.601[b]	189± 0.80[c]	135± 0.21[c]

[a]p < 0.001, [b]p < 0.01 and [c]p < 0.05; [a, b, c] correspond to the significance of values.

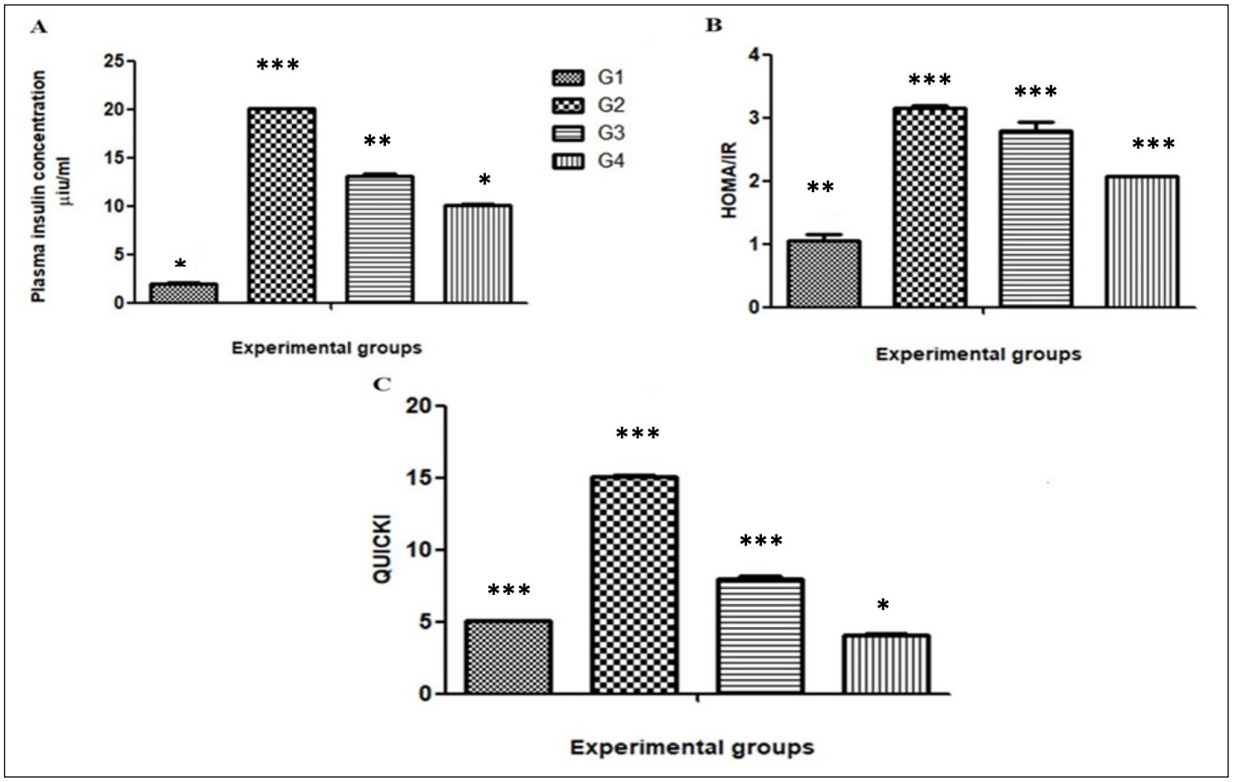

Figure 2. Diagram showing Plasma Insulin concentration (µIU/ml). **A**, Insulin sensitivity parameters {HOMA (**B**) and QUICKI (**C**)**}.** ***p<0.0001, **p<0.001, *p<0.01 were found to be statistically significant.

Effect of Co-administration of Herbal Drugs with Metformin on Serum Biochemical Analysis in Diabetic Rats

Serum concentrations of triglycerides, cholesterol, and fructosamine were found to be significantly ($^{***}p$ <0.0001) elevated in the diabetic rats G2. Treatment with the combination of tested herbal drugs with metformin for 4 weeks showed a significant reduction ($^{*}p$ <0.01) of triglycerides, cholesterol, and fructosamine levels with the values of (55±23, 38±2.1 mg/dl, and 0.5±0.61) mmol/l, respectively, as well as the Glycated hemoglobin (HbG %) was observed to have a lessened significantly ($^{*}p$ <0.01) comparing to diabetic non treated rats (Table III).

The concentrations of serum AST, ALT, urea, and creatinine were found to be significantly elevated ($^{***}p$ <0.0001) in a diabetic group of the control animals; however, rats in G 4 were shown to have a significant decline ($^{*}p$ <0.01) in AST, ALT, urea and creatinine levels (Table IV).

Antioxidant Activity and Markers of Oxidative Stress in Liver, Kidney, and Brain Homogenates in Diabetic Rats

In this study, Figure 3 illustrated that rat with STZ- induced diabetes G2 exhibited a significant (***p <0.001) elevation of MDA levels in the liver, kidney, and brain homogenates. The herbal medicated group in combination with metformin G4 showed a significant decline ($^{***}p$ <0.0001 and $^{***}p$ <0.001) in MDA concentrations. While GPx, CAT, and SOD were significantly elevated ($^{***}p$ <0.0001) compared to the diabetic group G2. However, the decrease in the CAT and SOD levels was found in diabetic rats G2, while the combination of AG and FM with metformin significantly restored the depleted enzyme levels to normalcy compared to the control animals (Figure 3).

Gene Expression Analysis of Apoptotic Factor and Proinflammatory Markers in Diabetic Rats

The expression of Il-1β revealed the highest magnitude in both G2 and G4 with a significant decrease in expression in G3 in comparison with both G2 and G4 (*p <0.05) that was showing the same magnitudes as G1 and G2. While G1and G2 showed the highest expression of TGF-β which was significantly decreased in both G3 and G4 (*p <0.05). There was a significant difference between both G3 and G4. The combination of metformin with AG and FM showed the lowest expression in Cas-3 and returned to the levels of the control group (G1), while G3 showed a significant decline when compared with G2 (*p <0.05) (Figure 4).

Histopathological Study

STZ- induced diabetic rats showed microsteatosis and cell death with hyaline degeneration of the vascular wall of portal blood, numerous bile ductulus, and mild lymphocytic infiltration in the liver; whereas, in the combination treatment of

Table III. Plasma triglyceride (mg/dl), cholesterol (mg/dl) fructosamine (mmol/l) and HbG % after 4 weeks of treatment in different studied groups. The data is provided as mean ± SEM, (n = 6).

Triglyceride	Cholesterol	Fructosamine	HbG %
40±6.9^{c}	36±2.8^{c}	0.45±0.07^{c}	4.59±0.03^{c}
213±58^{a}	52±1.1^{a}	2.1±0.22^{a}	20.12±0.07^{a}
123±08^{b}	42±2.1^{b}	0.91±0.02^{b}	15.55±0.01^{b}
55±23^{c}	38±2.1^{c}	0.5±0.61^{c}	8.32±0.17^{c}

$^{a}p < 0.001$, $^{b}p < 0.01$ and $^{c}p < 0.05$; $^{a, b, c}$ correspond to the significance of values.

Table IV. Levels of AST (IU/mL), ALT (IU/mL), Urea (mg/dl) and Creatinine (mg/dl) after 4 weeks of treatment in different studied groups. The data is provided as mean ± SEM, (n = 6).

Group	AST	ALT	Urea	Creatinine
G1	10.13±0.70^{c}	14.40±1.52^{c}	25.13±0.12^{c}	0.51± 0.09^{c}
G2	127.38± 14.19^{a}	78.40±1.65^{a}	99.17 ± 0.96^{a}	1.93± 0.96^{a}
G3	37.90± 4.06^{b}	33.00 ±19.03^{b}	44.19± 1.60^{b}	0.83± 0.07^{b}
G4	14.70± 0.52^{c}	26.97 ±0.97^{c}	29.15±0.10^{c}	0.61±0.01^{c}

$^{a}p < 0.001$, $^{b}p < 0.01$ and $^{c}p < 0.05$; $^{a, b, c}$ correspond to the significance of values.

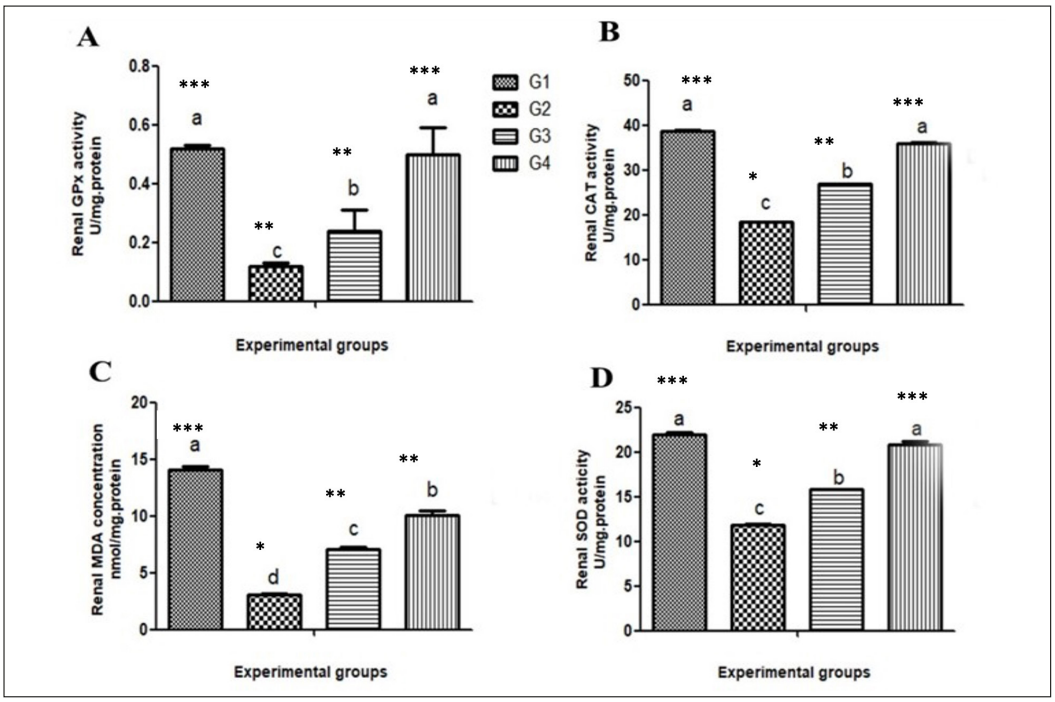

Figure 3. **A**, Diagram shows the renal GPX activity which was expressed in (U/mg) in different groups (G1, G2, G3 and G4). **B**, Diagram shows the renal CAT activity which was expressed in (U/mg) in different groups (G1, G2, G3 and G4). **C**, Diagram shows the renal MDA concentration which was expressed in (nmol/mg) in different groups (G1, G2, G3 and G4). **D**, Diagram shows the renal SOD activity which was expressed in (U/mg) in different groups (G1, G2, G3 and G4). ***p<0.0001, **p<0.001, *p<0.01 were found to be statistically significant.

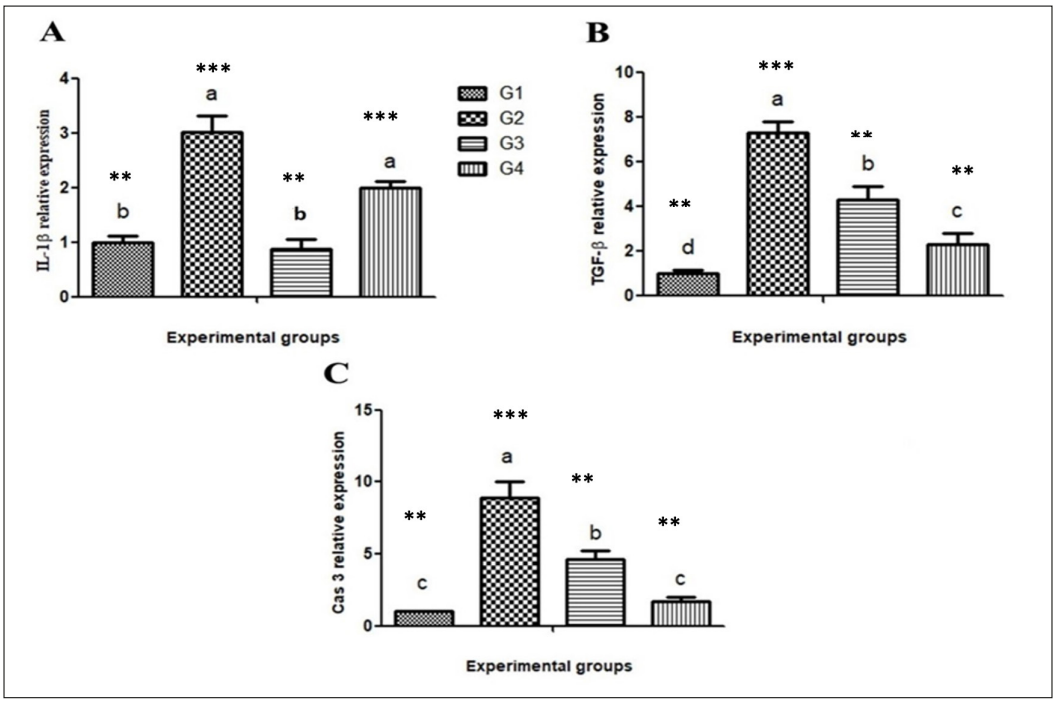

Figure 4. Diagram showing fold changes of IL-1b (**A**), TGF-b (**B**) and CAS-3 (**C**) expression levels in brain tissue. ***p<0.0001, **p<0.001, *p<0.01 were found to be statistically significant.

herb-drug the histopathological examination of the liver showed a pronounced amelioration of the diabetic lesions, and the majority of the hepatic parenchyma was normal (Figure 5a), Moreover, there were necrotic tubular epithelia, distorted and congested glomeruli and blood vessels in the kidney of diabetic animals G2, while in the treated group G4 the kidney sections showed a remarkable enhancement with mild renal changes (Figure 5b). In the brain section of STZ-induced diabetic rats, congestion in the meningeal and cerebral blood vessels could be observed this was accompanied by intense pyknotic (necrotic) neurons and edema in the maximum part of cerebral cortex layers, meanwhile, brain histology revealed a great improvement and also restored the morphologic picture to the normal state in the treated group G4 (Figure 6).

Immunohistochemistry Study

Sections of rat immune-stained with the mouse monoclonal antibody against calbindin D28k. from control negative (G I) showed that no immunoreactive cells were observed, while in diabetic non- treated rats (G II) there was a huge

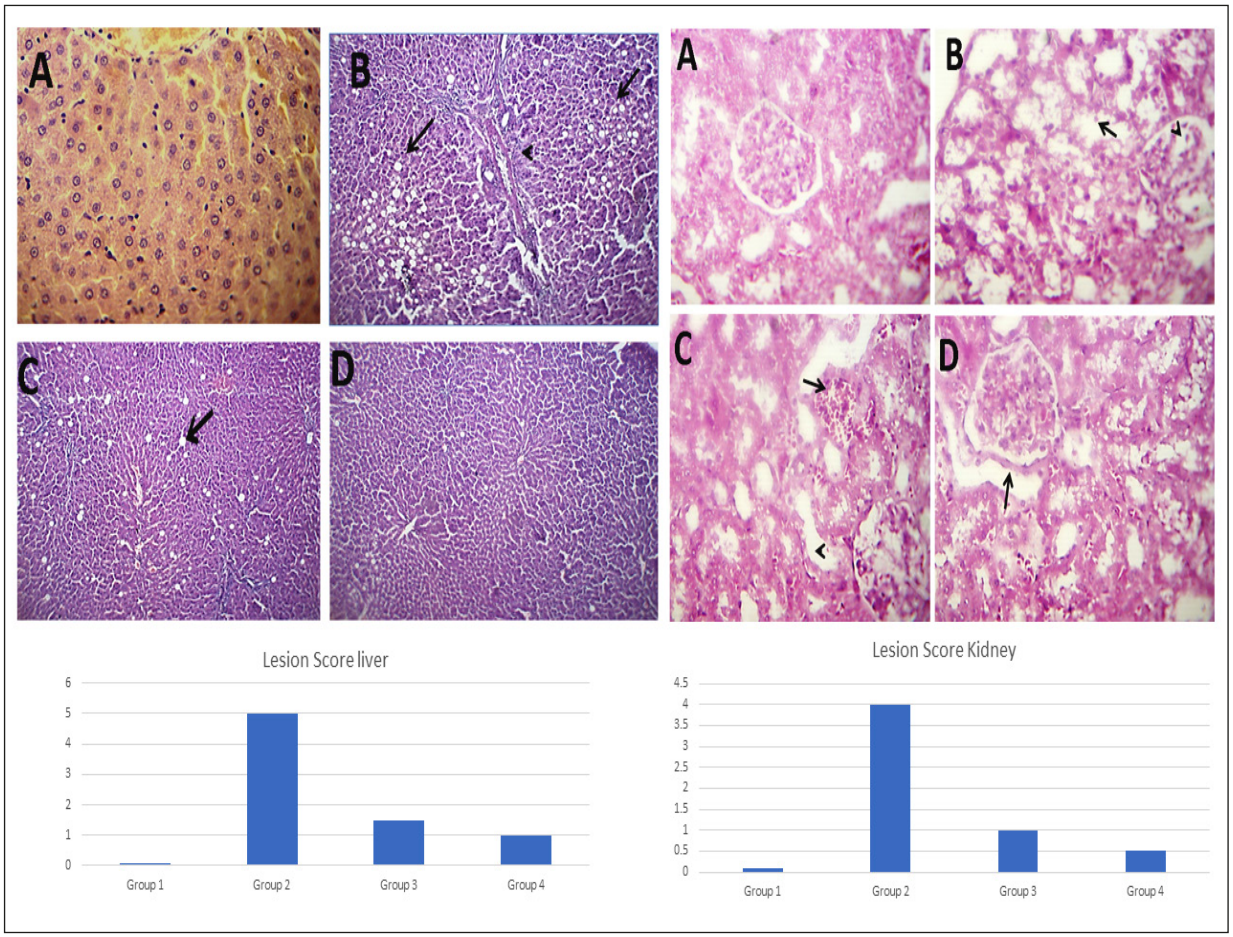

Figure 5. a, Microscopic pictures of hematoxylin and eosin-stained hepatic sections of all animal groups (A, B, C and D) with their lesion score. G1 (**A**, Control) group shows normal hepatic parenchyma (H&EX400). G2 (**B**, Diabetic) group shows intense microsteatosis (arrow) in the majority of the hepatic cells with hyalinized vascular wall (arrowhead) in the portal area (H&EX400). G3 (**C**, Metformin) shows scattered microsteatosis (arrow) in some hepatic cells (H&E X400). G4 (**D**, Metformin, folium and Arabic gum) shows microsteatosis in a few hepatic cells and the majority of the hepatic parenchyma normal (H&E X400). **b**, Microscopic pictures of hematoxylin and eosin-stained renal sections of all animal groups A, B, C and D with their lesion score, G1 (**A**, control) group shows normal renal parenchyma (H&EX400). G2 (**B**, Diabetic) group shows necrotic renal tubule (arrow) and distorted glomerular tuft (arrowhead). (H&EX400). G3 (**C**, Metformin) shows congested blood vessels (arrow) and mild degenerative changes in some renal tubules (arrowhead) (H&EX400). G4 (**D**, Metformin, *folium* and Arabic gum) shows mild reversible degenerative changes in some renal tubules (H&EX400).

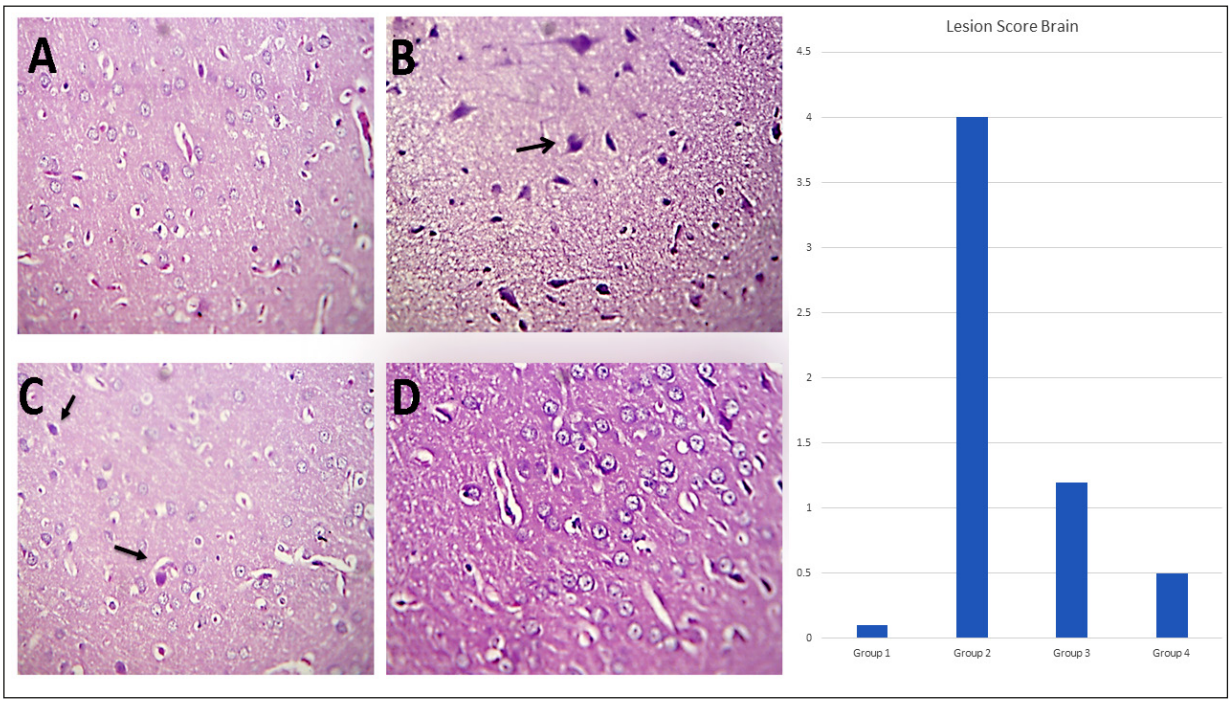

Figure 6. Microscopic pictures of hematoxylin and eosin-stained brain sections of all animal groups A, B, C and D with their lesion score. G1 (**A**, Control) group shows normal brain tissue (H&EX400). G2 (**B**, Diabetic) group shows numerous necrotic neurons and perineuronal (arrow) in the brain cortex (H&EX400). G3 (**C**, Metformin) shows some degenerated neurons in the cerebral cortex (H&EX400). G4 (**D**, Metformin, *folium* and Arabic gum) shows apparent normal brain tissue (H&EX400).

number of immunoreactive cells, Meanwhile, metformin-treated rats (G III) showed moderate labeled cells, while the number of immunoreactive cells was decreased in an herbal medicated group (G IV) which were represented in the Figure 7 with alphabets A, B, C and D.

Discussion

Globally, diabetes is considered a metabolic disorder that needs meticulous management throughout its life span. Persistent elevation in blood glucose levels encourages a diabetic patient to exacerbate complications. A strong relationship between oxidative stress and diabetes can be observed, as the release of free radicals during oxidative stress also leads to the development of many complications. However, there is less evidence and reports of drugs that conquer good control of blood glucose levels and related parameters along with the complications[51]. Many research scientists have focused on herbal medicines to combat such complications, keeping aside chemical entities as chemical drugs confess several adverse effects. Thus, they may preferably try to consume additional supplements in the form of food or drug which satisfies both in terms of side effects and therapeutic value.

In the pre-clinical models, STZ was used to bring about a state of hyperglycemia acts *via* uncontrolled production of reactive oxygen species (ROS). A sequence of episodes is produced leading to the production of superoxide radicals, hydrogen peroxide, and hydroxyl radicals which in turn leads to oxidative pancreatic B cell destruction[38.] After treatment with the two tested herbs along with metformin, the fasting blood glucose level dropped significantly along with the serum insulin concentration. For measuring insulin resistance and -cell function, the homeostasis model assessment (HOMA), which is based on plasma levels of fasting glucose and insulin, has received widespread validation. In large-scale epidemiologic studies, the HOMA model is preferred over the sophisticated "gold standard" approaches because it only calls for a single measurement of insulin and glucose in the basal state, as opposed to those methods' customarily requiring dynamic data *via* pricy and invasive procedures[52].

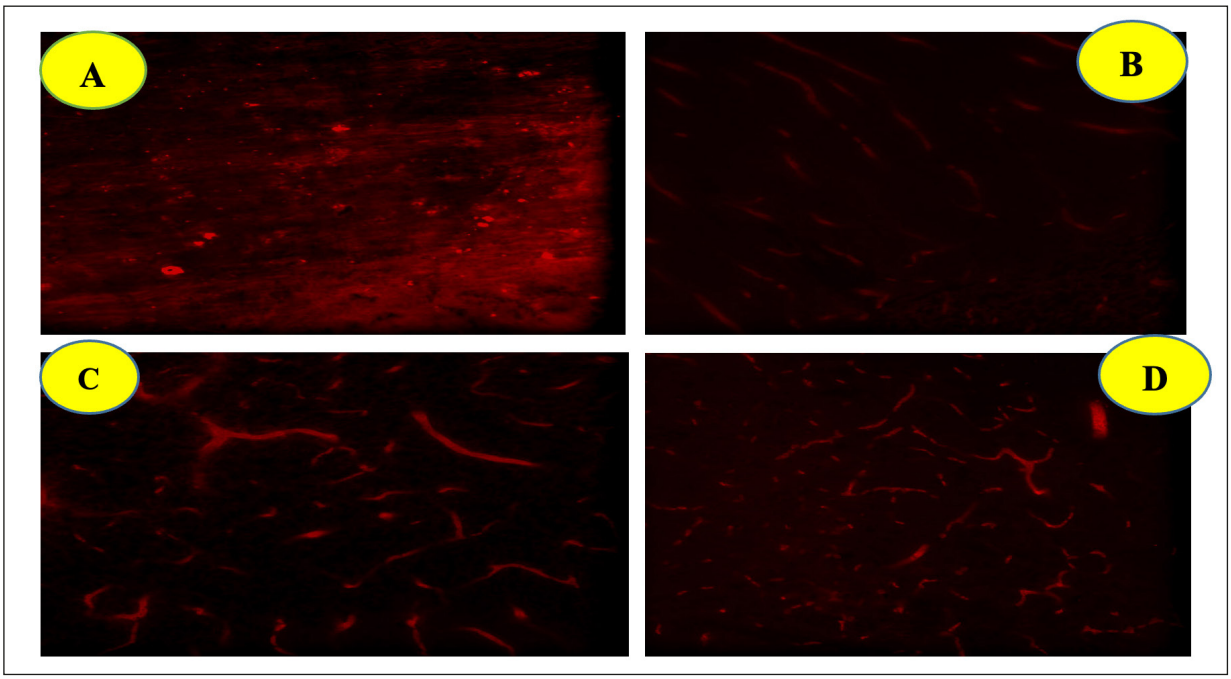

Figure 7. Sections of brain immune-stained with the mouse monoclonal antibody against calbindin D28k. From control negative (**A**), Diabetic (**B**), Metformin treated group (**C**), Herbal medicated group in combination with metformin (**D**) (H&E X400).

Serum Fructosamine and glycohemoglobin (HbG %) are used to perfect the blood glucose levels in diabetes. High levels of triglycerides and cholesterol is a condition observed in hyperlipidemia, one of the frequent complications noted in chemically induced diabetes and is related to an increased risk of vascular diseases[39]. In the present investigation, triglycerides and cholesterol levels increased significantly in STZ-induced diabetic rats, indicating that glucose is underutilized, and extra fat is recruited from the adipose tissue. However, the combined use of herb-drug showed a significant refinement in serum Fructosamine and glycohemoglobin (HbG%) indicating a short-term (fructosamine) and long-term (glycohemoglobin) diabetic control[40], also triglycerides and cholesterol were significantly reduced after herbal treatment possibly by activation of enzyme lipoprotein lipase and causing hydrolysis of triglycerides. This mechanism may be attributed to the secretion of serum insulin levels.

In diabetes, there is an overproduction of free radicals that cause tissue destruction, these radicals attack the cell membrane through peroxidation of unsaturated fatty acids[41]. Lipid peroxidation damages the membrane and disruption in function. The generation of free radicals is considered the foremost biochemical process in diabetes, which unveils a notable fall in the levels of antioxidant enzymes like GST, SOD, and CAT. During the lipid peroxidation process, these enzymes extinguish the free radicals and protect the biological system. A decline in the generation of lipid peroxides and improvement in the antioxidant status contributes to the battle against the complications of diabetes. In the present investigation, it has been observed that there was a significant rise in the enzymatic levels of GST, SOD, and CAT with subsequent dipping in the levels of MDA.

STZ-induced diabetes in rats can provoke the expression of pro-inflammatory markers and other apoptotic markers in tissues of rats especially IL-1β which was thought to be increased as a result of B cell dysfunction to exacerbate the existing condition[42]. Diabetes mellitus can also produce a significant overexpression in IL-1β and TNF-α expression in sciatic nerves[43]. Similarly, IL-1β was highly expressed in the retina and retinal blood vessels in high glucose concentration in rat microglial cells and astrocytes[44]. TGF-β is an effective marker for diabetic nephropathy through stimulation of components of extracellular matrix proteins and matrix metalloproteinase[45]. Hathaway et al declared that the lower expression of TGF-β can improve the condition of diabetic nephropathy and *vice versa* that highlighted

the mere exacerbating effects of TGF-β in worsening diabetic condition[46]. Therefore, drugs that target TGF-β signaling, such as silymarin, curcumin, and resveratrol, might have a promising tool as a therapeutic agent in curing diabetes[47,48]. Regarding apoptotic marker, Cas-3 expression showed a significant increase in the brain of diabetic rats in the current study which was confirmed by a research work conducted by Nagayach et al[49] in the 6th week after exposure to STZ intoxication. AG is characterized by its anti-inflammatory function which is found to attenuate pro-inflammatory cytokines expression in inflammatory conditions in ureters obstruction in male rats[49]. Similarly, FM is found to have a direct anti-inflammatory role through decreased production of IL-1β and IL-6, and TNF-α[50]. To evaluate the hepatic and renal protective activities of AG and FM, AST, ALT, urea, and creatinine were quantified. It was found that G4 showed excellent fall-off in AST, ALT, urea, and creatinine levels. The values were comparable with the standard G3. This was perceptible to possess hepato-protective function[51], while also improving the function of the kidney[53].

Finally, the therapeutic potential of the herbal drug in the treatment of diabetes was due to the absorption of glucose, thereby reducing plasma glucose by raising insulin secretion[12]. Also, it improved lipid metabolism in experimental rats with antioxidant activity[54]. FM extract also demonstrated significant antioxidant and antidiabetic properties which might be due to the presence of numerous chemical components, including flavonoids, alkaloids, and phytochemicals[55]. The combined therapeutic effect of both herbal drugs might be attributed to the synergistic outcome, leading to a beneficial and successful treatment to overcome diabetes and its complications[56].

Conclusions

Orally treatment of metformin with Arabic gum and *Folium mori* in Streptozotocin injected rats could ameliorate protective pathways and it can be one of the proposed dietary supplements for the treatment of diabetes.

Ethics Approval

The study protocol was approved in advance by the Medical Research Ethics Committee for animal research studies at the Faculty of Veterinary Medicine, Mansoura University (Cod No.: R\86). All conceivable attempts were made to minimize animal suffering.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contributions

R.A. El-Shafei designed the study, M.A. El-Adl performed the biochemical analysis, H.S. Ali has performed histopathology and immunohistopathology, Y. Nomier analyzed and interpreted the data and wrote the manu-script. All authors read and approved the final manuscript.

Availability of Data and Materials

The combined datasets are available from the corresponding author (Yousra Nomier) upon reasonable re-quest.

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