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Curative and protective potentials of *Moringa oleifera* leaf decoction on the streptozotocin-induced diabetes mellitus in albino rats

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Abstract

The present study was designed to investigate the protective, and curative potentials of *Moringa oleifera* (MO) leaves powder against streptozotocin (STZ) induced type 1 diabetes mellitus (T1DM) in rats. Fifty adult Wistar male albino rats were randomized and divided into five equal groups: The normal control group, the Moringa group, The diabetic group, the therapeutic group, and the diabetic rats (3 days after STZ injection) were received-MO-for successive 8 weeks and the prophylactic group, the rats were received-MO-for 2 weeks before STZ induced diabetic rats and lasted for 8 weeks. The protective or treated oral administration of 1 ml freshly prepared aqueous leaf decoction of-MO-revealed a significant upregulation of the mRNA expression of PDX-1, Ngn3, VEGF, IGF-1, and GLUT-2. Additionally, it induced a significant downregulation of FBG level compared to that of the diabetic group, a significant reduction in MDA level and a significant elevation in the TAC level. Furthermore, the histopathological observations of pancreas, liver, and kidney tissues affirmed the improvement in treated and prophylactic groups compared to STZ-diabetic groups, and the improvement in the prophylactic group was more distinct than the treated group. MO-aqueous leaf extract can treat and protect against STZ-induced T1DM; via its antioxidant action (increase the TAC and decrease MDA). Thus, it has the potential for utilization as a prophylactic against diabetes.

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Introduction

Diabetes mellitus (DM) is a severe metabolic disorder. It is considered the most common endocrine disease, which is categorized as type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), or other types with different etiological factors. T1DM is an autoimmune disease associated with failure of the pancreas to produce enough insulin due to destruction of the insulin-producing beta-cells. T2DM, also termed non-insulin-dependent diabetes mellitus, marked by, insulin resistance, is a condition in which cells do not react appropriately to insulin. Gestational diabetes occurs when pregnant women, who has never had diabetes before, develop elevated blood sugar levels during pregnancy (1,2). The pancreatic beta-cells are damaged by immunological factors, such as cytokines and macrophages or T cells activated by autoimmune responses (3).

The American Diabetes Association defines diabetes as hyperglycemia resulting from failure of insulin secretion, action of the insulin receptor, or both, which provoke the development of diabetic complications such as neuropathy, retinopathy, nephropathy, and cardiovascular disease disorders (4,5). Induction of T1DM in rats by injection of STZ (a single dose of 65 mg/kg, i.p.) is characterized by hyperglycemia resulting from reduced secretion of endogenous pancreatic insulin due to the destruction of a large number of pancreatic beta-cells. T1DM patients require enduring insulin therapy to manage hyperglycemia. Without insulin, it is life-threatening due to the development of

diabetic ketoacidosis (DKA) (6). The main objective in the treatment of DM is to control and adjust the blood sugar levels to prevent its associated complications (7). Therefore, antidiabetic drugs can lower hyperglycemia; and thus, inhibit the loss of functional beta-cell (8) but, unfortunately, with significant side effects (9). Herbal medicines have long been used as an alternative medicine with a potent source of potential diabetes therapeutics with less side effects, and relatively low costs (10). Among these herbal medicines, the *Moringa oleifera* tree, known as the 'drumstick tree', is considered a gifted plant because of its numerous uses. The potential medicinal uses of-MO-leaves have been investigated in DM, as the leaves contain a significant quantity of flavonoids, alkaloidal constituents, sterols, phenolic, and triterpenoids that might be beneficial in the treatment and control of DM and its related complications (11). MO-leaves have shown outstanding hypoglycemic activity; because they can promote insulin release from pancreatic beta-cells. Interestingly, it also lowers blood glucose levels by direct interacting with anti-insulin antibodies (12).

The main objective of the present study is to investigate the therapeutic and protective potentials of aqueous decoction of *Moringa oleifera* leaves powder against streptozotocin (STZ)-induced type1 diabetes mellitus through the studying of the biochemical (BGL, TAC, and MDA), gene expression of the pancreas for regenerative markers (pdx1, NGn3, GLUT2, VEGF, and IGF-1), immunohistochemical investigation of insulin in the pancreas and histopathological changes of pancreas, liver, and kidney in rats.

Materials and methods

Ethical approve

All the authors of the present work ensure that all procedures of our experiment were performed under the Ethical Norms approved by the Institutional Animal Care and Use Committee (IACUC) of Zagazig University (ZU-IACUC committee approval number ZU-IACUC/2/F/74/2021).

Drugs and chemicals

Moringa oleifera greenish organic raw leaf powder (net wt. 454 gm) was obtained from Iyasa Holistics (Iyasa Organic-Moringa-Leaf Powder is sourced from NOP(USDA) certified farms in India. It was packaged in an FDA-registered facility. It is Vegetarian and Vegan, Non-GMO; Gluten, Soy, Wheat, and Corn free). The color of Streptozotocin (STZ) powder is white to light yellow; it was obtained from (Sigma-Aldrich, St. Louis, MO, USA), Kits of the total antioxidant capacity (TAC) and Malondialdehyde (MAD) were obtained from (Cell Biolabs, San Diego, CA, USA), and other used chemicals in the present study were obtained from (Sigma-Aldrich St. Louis, MO, USA).

Extract preparation

The aqueous extract from MO-leaves powder was prepared at dose of 200 mg/kg B.wt. The aqueous extract of MO-was prepared by mixing MO-greenish organic raw leaf powder (1 gm) with boiling water (10 ml) for 15 minutes. Twice filtration of the mixture through aseptic filter paper of 2 µm pore into an aseptic tube, and then the filtrate was left to cool. The stock solution of the aqueous extract (100 mg/ml) was freshly prepared daily before oral animal supplementation (13).

Lab animals and Experimental protocol

Fifty normoglycemic sexually mature Wistar male albino rats 80-90 days old weighing 260±30 gm. was obtained from the Unit of Laboratory Animal, Faculty of Veterinary Medicine, Zagazig University, Egypt. One week prior to the experiment, ten rats were housed per cage under (temperature 23±2 ◦C; humidity 50 %; 12 h light/ dark period) with free access to food and water. After the acclimatization period to the laboratory environment, the rats were divided randomly into five groups (10 rats in each) as follows; Group 1: normal control group, rats received food and water only. Group 2: Moringa-group, rats were received (1 ml freshly prepared aqueous extract of MO/ rat) orally via stomach tube daily for eight weeks supplemented with diet (13). Group 3: Diabetic group, T1DM was induced in overnight fasted rats by STZ (freshly prepared, 60 mg/kg BW in 0.1 mol/L citrate buffer (pH 4.5), single-dose, injected intraperitoneally) (14). In order to prevent hypoglycemia, water with 2% glucose and feed were available for rats for about the next 16 hrs (15). Group 4: Therapeutic group, diabetic rats were received (1 ml freshly prepared aqueous extract of-MO/ rat) orally via stomach tube daily for eight weeks (13). Group 5: Prophylactic group, normoglycemic rats were received (1 ml freshly prepared aqueous extract of-MO/ rat) orally via stomach tube daily for seven days before STZ injection and then for eight weeks after STZ injection. Throughout the experiment, the rats were closely monitored for any clinical signs or deaths that occurred during this experiment.

Biochemical analysis

Blood samples were taken from the tail vein of the all rats. To confirm diabetes induction, the fasting blood glucose (FBG) levels were measured. Rats that showed FBG of 250 mg/dL or higher were recognized as diabetic and were involved in our study. The FBG was monitored using the RIGHT TEST Wiz Plus blood glucose monitoring system, BIONEM CORP. Switzerland. A colorimetric kit (Cell Biolabs, San Diego, CA, USA) was used to estimate the total antioxidant capacity (TAC) (16). Measurement of MDA level is the most commonly used method indicative of lipid peroxidation (17). Also, the most applied method for evaluating lipid peroxides is the estimation of thiobarbituric acid reactive substances (TBARS). Under high temperature

and acidic environments, the reaction of MDA and TBA forms the MDA-TBA adducts, which are measured calorimetrically at a wavelength of 530-540 nm. The estimated values were presented in MDA (Mmol/g tissue) (18).

mRNA expression of pancreatic tissue using real-time PCR analysis

Homogenized tissue of the pancreas (30 mg) was used for total RNA extraction using Trizol (Invitrogen; Thermo Fisher Scientific, Inc.). A full spectrum NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA) was used to evaluate the quality and the concentration, and a cDNA Synthesis Kit of HiSenScript™ RH (iNtRON Biotechnology Co., South Korea) was used for the synthesis of the cDNA. In a RotorGene Q 2 plex (Qiagen, Germany), the RT-PCR was performed using 5x HOT FIRE Pol EvaGreen qPCR Mix Plus (Solis BioDyne, Tartu, Estonia) following the instructions of the manufacturer. The cycling conditions of PCR included an initial denaturation (95 ◦C/12 min) followed by denaturation (40 cycles /95 ◦C /20 s), annealing (60 ◦C /30 s), and extension (72 ◦C /30 s). Sangon Biotech (Beijing, China) was used to synthesize the oligonucleotide-specific primers listed in table (1). Following PCR amplification, and analysis of the melting curve was performed. Gapdh (known housekeeping gene) was used to normalize the level of the target gene expression. According to the 2-ΔΔCt method, results are given as fold-changes compared to the normal control group (19). The primers of PCR are illustrated in table 1.

Table 1: PCR used primers

	Forward primer (5′-3′)	Reverse primer (5′-3′)	Size	Accession no.
Gapdh (Rat)	GGCACAGTCAAGGCTGAGAATG	ATGGTGGTGAAGACGCCAGTA	143	NM_017008.4
IGF1	AAGCCTACAAAGTCAGCTCG	GGTCTTGTTTCCTGCACTTC	166	NM_001082477.2
Ngn3	TCCAGACGCAATTTACTCCAG	CTAGTTCTCCGGGCTCAAAAG	136	NM_021700.1
PDX1	CCCGAGCTTCTGAAAACTTTG	CTTTTCATTGTCCTCAGTTGGG	121	NM_022852.3
VEGF	GATCCAGTACCCGAGCAGTCA	TCTCCTTTCTTTTTGGTCTGCAT	83	NM_053549.1
GLUT-2	GGTGGCACTGATGCTACACT	CTCCACAAGCAGCACAGAGA	169	NM_012879.2

Histopathological examination and lesion scoring of the pancreas, liver, and kidneys

When the experiment was finished, all rats were euthanized by diethyl ether, necropsied, and representative tissue specimens from the pancreases, livers, and kidneys were sampled (20). The tissue specimens were fixed in neutral buffered formalin solution (10%) for 72 hours. Then, they were washed under running tap water, dehydrated in ethyl alcohol with ascending concentrations, cleared in Histo-Choice® (Sigma-Aldrich, St. Louis, USA) clearing agent, impregnated, and embedded in paraffin wax, sectioned at 5 µm thickness, stained with hematoxylin and eosin stains (21) and finally examined microscopically. Next, a numerical multiparametric lesion scoring was accomplished, and the results were presented as percentages (means ± SEM). Briefly, a single slide per organ per rat was carried out then five randomly chosen, nonoverlapped HPF (40×) were captured (50 images per organ per group) using AmScope digital camera (AmScope, USA) connected to Olympus light microscope (Japan). Then, these images were analyzed for grading the histopathological alterations. The pancreatic tissue sections were evaluated for the frequencies of hemorrhages, congestions, necrosis, edema, and infiltration of inflammatory cells in the exocrine portion. The tested hepatic histological alterations were evaluated for the numbers of hepatocytes showing single-cell necrosis, steatosis, vacuolar and hydropic degenerations and the frequencies of pyknosis, infiltration of the inflammatory cell, and hemorrhages per images using ImageJ (the image analysis software, version 1.51v; Research Services Branch, NIH, Bethesda, MD, USA). The considered renal histopathological alterations were evaluated for the numbers of renal tubules with vacuolated epithelium, renal tubules with cast formation, renal tubules with necrotic epithelium, and dilated tubules in relation to the tubule's total numbers /images, and the frequencies of glomerular necrosis, collapse, congestion, the interstitial edema, hemorrhage and infiltration of the inflammatory cells per images.

Immunohistochemical investigation of the insulin in islets of the pancreas

A successive slide from the formalin-fixed, paraffin-embedded pancreatic tissue specimens per animal were prepared and stained for insulin using the rabbit recombinant multiclonal anti-insulin primary antibody [RM1019] (ab282459, abcam, Inc.) at 1/10000 (0.055 ug/ml) dilution following the avidin-biotin-peroxidase complex technique (22). The slides were incubated with 3,3′-Diaminobenzidine (DAB) to visualize the antigen-anybody complexes, and finally, the nuclei were countered and stained by Mayer's hematoxylin stain. Five nonoverlapped, low-power microscopic fields (10 objectives) per animal were captured because these images contain Langerhans' islets to quantify the area fractions the pancreatic islets. Next, the percentages of positively stained brown DAB area fractions in relation to the images' total areas were calculated using the ImageJ

software via the color deconvolution plugin, and the results were presented as percentages (means ± SEM).

Statistical analysis

Using the software of GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, United States), the statistical analysis was performed. All the analyzed data were expressed as mean ± SEM. A one-way ANOVA test followed by a post hoc Tukey test was used for statistical comparisons. The results were considered significance as * P<0.05, ** P<0.01, *** P<0.001, and **** P<0.0001.

Results

Biochemical analysis

The rats of groups 1 and 2 revealed a normal BGL throughout the experiment, while the rats of group 3 (STZ rats) had increased BGL from the third day after TIDM induction until the end of the experiment. The final BGL of the diabetic group (just before euthanasia) had a significant (P<0.001) elevation when compared with that of the normal control rats. Interestingly, oral administration of MO-in group 4 (post-TIDM induction) and group 5 (pre- and post-TIDM induction) had a significant (P<0.001) downregulation of FBG level in comparison with that of the diabetic group (Figure 1a). Rats with T1DM exhibited a significant (P<0.001) increase in MDA level compared to that of the normal control group, which was significantly reversed by the oral administration pre and/or post-diabetes induction (Figure 1b). The TAC level in the diabetic rats was significantly (P<0.001) lowered in contrast with the normal control one. Interestingly, MO treated-groups, either pre and /or post-diabetes onset, revealed a significant (P<0.001) increase in the TAC level (Figure 1c).

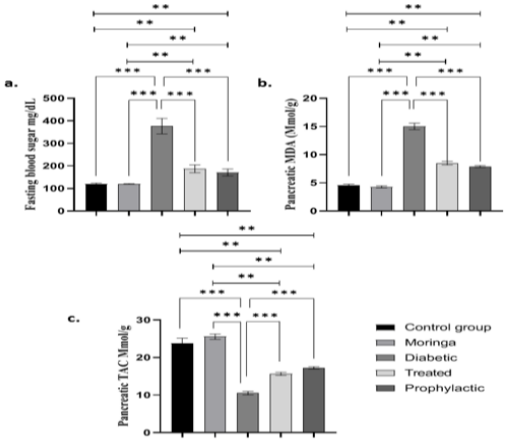

Figure 1: Effect of administration of aqueous extract of MO leaves powder either pre and /or post STZ injection in rats on a. fasting blood sugar, b. MDA and c. TAC. Values of the analyzed data presented as mean±SEM of each group (10 rats) * P<0.05, ** P<0.01, *** P<0.001, and **** P<0.0001.

mRNA expression of pancreatic tissue on the regenerative markers

The result of the current study showed a significant (P<0.001) downregulation in the relative expression of the mRNA of pancreatic (PDX1 and Ngn3) and pancreatic growth factors (VEGF and IGF1) in addition to GLUT-2 of the diabetic group than the control one. On the other side, MO-treated-groups, either pre and /or post-diabetes onset, revealed a significant (P<0.001) upregulation of the mRNA expression level of the markers mentioned above (Figures 2 a-e).

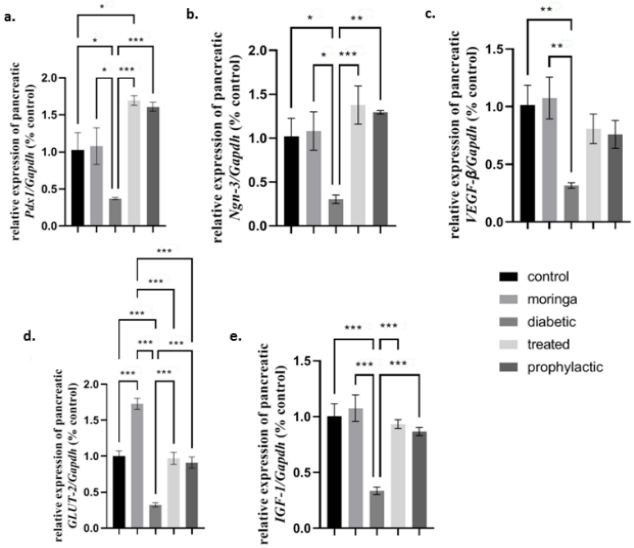

Figure 2: Effect of administration of aqueous extract of MO leaves powder either pre and /or post STZ injection in rats on regenerative markers of pancreas. a. Pdx1, b. Ngn3, c. VEGF, d. GLUT-2 and e. IGF-1. Values of the analyzed data presented as mean±SEM of each group (10 rats) * P<0.05, ** P<0.01, *** P<0.001, and **** P<0.0001.

Histopathological and immunohistochemical findings

The pancreases of the normal control and MO-rats showed normal histological architecture with no pathological findings (Figure 3 a and b). In contrast, the diabetic rats manifested various circulatory, degenerative and necrotic alterations, including vacuolation, pyknosis, and necrosis of beta-cells with shrinkage of the islets of Langerhans (Figure 3c), besides; vascular congestions, minute hemorrhages, edema, and inflammatory cell infiltration of the exocrine pancreas. Supplementation with MO, post STZ-injection in the therapeutic group, markedly diminished the STZ-induced beta-cell necrosis (Figure 3d). In contrast, the pancreatic tissue section of prophylactic rats showed mild beta-cell necrosis (Figure 3e).

Immunohistochemically, marked reductions in the DAB brown area fractions of beta-cells were seen in the diabetic rats compared to the normal control and MO-rats (Figure 3

f-h). Supplementation with MO-significantly maintained the DAB brown area fractions in the therapeutic group compared to the diabetic group (Figure 3 i and j). Interestingly, the prophylactic group significantly maintained the positively stained brown area fractions compared to the therapeutic group.

The hepatic tissue sections of the normal control and MO-rats revealed no histopathological alterations with normal histological pictures (Figure 4 a and b), while those of the diabetic rats exhibited a vast array of morphological changes. This group's most hepatic tissue section suffered vacuolar and hydropic degenerations, hepatic lipidosis (micro and macro vesiculation), and congestions of the portal blood vessels, central veins, and hepatic sinusoidal.

Still more, single-cell necrosis, focal necrotic foci, and inflammatory cell infiltrate were evident (Figure 4c). The hepatoprotective effects of MO-were notable as a highly sharp decrease in the occurrences and extents of the diabetic-related hepatopathy morphological alterations were seen in almost all the hepatic tissue sections of the therapeutic rats. The mainly frequent alterations in this group were vacuolar degeneration, vascular congestion, and multifocal minute mononuclear cell aggregates (Figure 4d). At the same time, the prophylactic hepatoprotective effects of MO-were significantly marked compared with the therapeutic group and represented mainly by multifocal mononuclear cell infiltration with almost normal hepatic parenchyma (Figure 4e).

The kidneys of the normal control and MO-rats revealed no histopathological alterations with normal histological pictures (Figures 4 f and g). The kidneys of the diabetic rats revealed a wide array of nephropathic changes, including the renal glomeruli (collapse, necrosis, and congestion), the renal tubules (vacuolations, pyknosis, necrosis, dilatation, and cast formation), and the interstitium (congestions, minute hemorrhages, and inflammatory cell infiltrations (Figure 4h). Supplementation with MO-moderately diminished the severities of the diabetic nephropathy associated changes, but mild nephropathic alterations such as vascular congestions, glomerular collapse, and tubular vacuolations appeared in most sections of therapeutic rats (Figure 4i). The prophylactic rats showed a minor severity of the diabetic renal lesions compared to the MO-therapeutic-group (Figure 4j).

Lesion scoring

Quantitative lesion scoring and immunohistochemical expression of insulin in the pancreases of rats in response to STZ and MO-treatments listed in table 2. Also, quantitative lesion scoring of the liver, kidneys and brain of rats in response to STZ and MO-treatments listed in table 3.

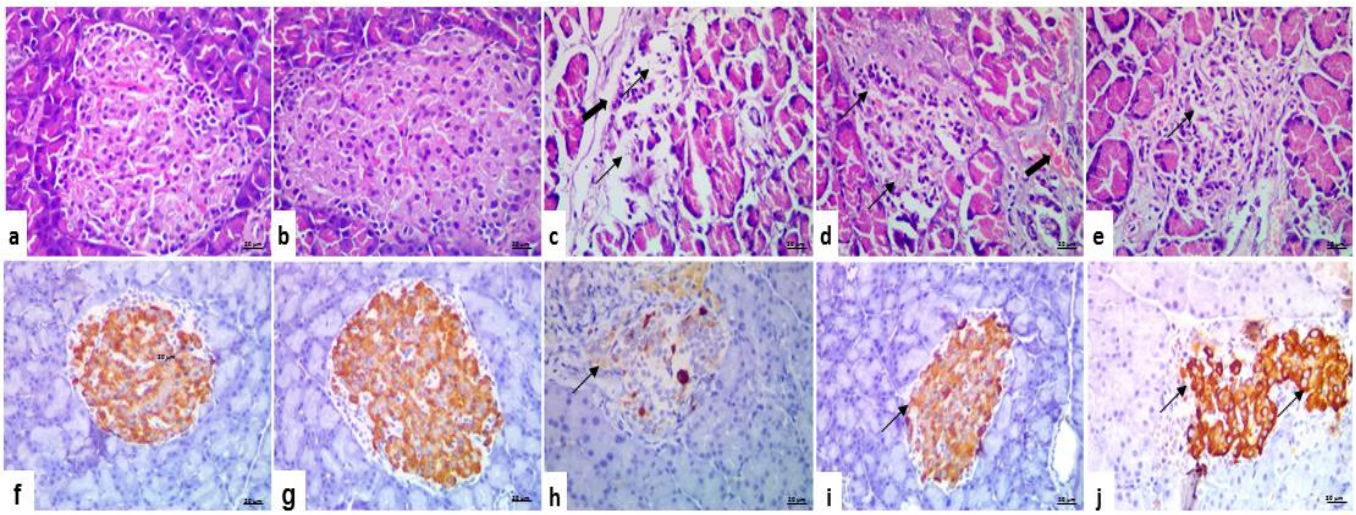

Figure 3: a-e Representative photomicrograph of H&E-stained pancreatic sections of normal control rats showing normal histological picture (a) and MO-non diabetic rats (b), beta-cell necrosis (arrows) with shrinkage of the islets (thick arrow) in diabetic (STZ) rats (c), moderate beta-cell necrosis (arrows) with vascular congestion (thick arrow) in MO-therapeutic rats (d), mild beta-cell necrosis (arrow) in MO-prophylactic rats (e). f-j Representative photomicrograph of the insulin IHC stained pancreatic tissue sections of normal control rats showing normal brown coloration of the beta cells (f), and MO-non diabetic rats (g), very weak insulin expression (arrow) in diabetic (STZ) rats (h), regeneration of large percent of immune positively stained beta cells (arrow) in MO-therapeutic rats (i), regeneration of most immune positively stained beta cells (arrows) in MO-prophylactic rats (j).

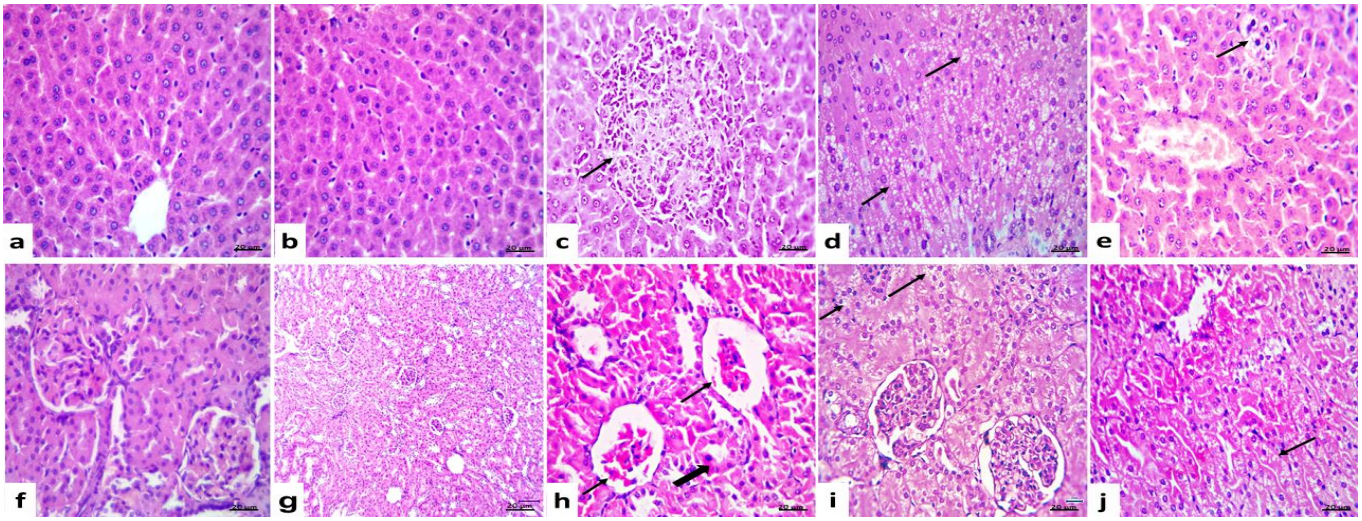

Figure 4: a-e Representative photomicrograph of H&E-stained hepatic sections of normal control rats showing normal histological picture (a) and MO-non diabetic rats (b), single-cell necrosis with acute cellular swelling and focal necrosis infiltrated with mononuclear cells (arrow) in diabetic (STZ) rats (c), fatty change (micro-vesiculation with few macro-vesiculation) (arrows) in MO-therapeutic rats (d), few necrotic hepatocytes with almost normal hepatic parenchyma (arrow) in MO-prophylactic rats (e). f-j Representative photomicrograph of H&E-stained renal sections of normal control rats showing normal histological picture (f) and MO- non diabetic rats (g), glomerular necrosis with widened Bowman's space (arrows), and tubular necrosis (thick arrow) in diabetic (STZ) rats (h), fatty change (micro-vesiculation with few macro-vesiculation) (arrows) in MO-therapeutic rats (i), fatty change of renal tubular epithelium (arrow) in MO-prophylactic rats (j).

Table 2: lesion scoring and immunohistochemical expression of insulin in the pancreases

		Control	MO	Diabetic	Treated	prophylactic
Area	Insulin area fraction	2.79±0.23a	2.87±0.22a	1.00±0.11c	1.74±0.14b	1.94±0.15b
Frequencies	Congestion	0.00a	0.00a	11.00±2.77c	6.0±2.21b	5.00±1.67b
	Hemorrhage	0.00a	0.00a	4.00±1.63a	2.2±1.47a	2.00±1.33a
	Edema	0.00a	0.00a	2.00±2.00a	1.0±1.00a	0.00 a
	Necrosis of exocrine tissue	0.00a	0.00a	8.00±2.49c	5.0±1.66b	4.00±1.63b
	Beta cell necrosis	0.00a	0.00a	100±00c	35.0±6.19b	31.00±6.23b

Values are means ± SEM for 10 samples /group analyzed using a one-way ANOVA followed by a post hoc Tukey test. Means within the same row (in each parameter) carrying different superscripts (a,b,c) are significantly different at $P<0.05$.

Discussion

In our present work, the histopathological examination of the pancreatic, hepatic and renal sections of the diabetic rats of revealed an extensive pathological change compared to the normal structure of the control rats. The pancreatic tissue revealed degenerative changes and necrosis of beta-cells with shrinkage of the islets of Langerhans, besides vascular congestions, minute hemorrhages, edema, and inflammatory cell infiltration of the exocrine pancreas. Immunohistochemical results showed marked reductions in the DAB brown area fractions (positively stained beta-cells) compared to the normal control and MO- rats. The results were inconsistent with (23), who showed that the size and number of pancreatic islets were decreased in diabetic rats. (24) noticed that; the pancreas of diabetic rats revealed a notable decrease of beta-cells with a sharp decrease in the number of insulin-positive stained cells by the immunoreactivity for anti-insulin antibodies. Moreover, most hepatic tissue sections of diabetic rats suffered acute cellular swelling, hepatic micro and macro steatosis, necrosis, and congestions of the hepatic blood vessels. Still more, focal replacement of necrotic hepatocytes by inflammatory cells; mainly lymphocytes were evident. These results are in harmony with (25). Furthermore, the kidneys of the diabetic rats revealed hyaline cast in the lumens of some renal tubules, multitubular coagulative necrosis, atrophy of glomerular capillaries, and interstitial congestions, minute hemorrhages, and inflammatory cell infiltrations. The results mentioned above are partially like those observed by (11).

Table 3: Quantitative lesion scoring in the liver, kidneys and brain of rats

Organ	Lesion	Control	MO	Diabetic	Treated	prophylactic
Liver	Vacuolar degeneration	0.00 a	0.00 a	19.40±4.50 c	10.61±0.88 b	9.90±0.81 b
	Steatosis	0.00 a	0.00 a	3.63±0.89 c	1.89±0.48 b	1.68±0.44 b
	Pyknosis	0.00 a	0.00 a	3.42±0.46 c	1.38±0.43 b	1.24±0.39 b
	Single-cell necrosis	0.00 a	0.00 a	4.88±0.30 c	2.43±0.38 b	1.97±0.37 b
	Inflammatory infiltrate	0.00 a	0.00 a	12.00±3.27 c	8.00±3.26 b	6.00±3.06 b
	Hemorrhage	0.00 a	0.00 a	8.00±3.27 a	5.00±2.69 a	4.00±2.67 a
Kidney	Glomerular congestion	0.00 a	0.00 a	8.00±3.27 a	6.00±2.67 a	5.00±2.24 a
	Glomerular collapse	0.00 a	0.00 a	7.00±3.00 a	5.00±2.69 a	4.00±2.67 a
	glomerular necrosis	0.00 a	0.00 a	4.00±1.63 a	3.00±1.53 a	2.00±1.33 a
	vacuolated epithelium	0.00 a	0.00 a	19.40±1.32 c	10.22±1.38 b	9.82±1.32 b
	necrotic epithelium	0.00 a	0.00 a	3.35±0.54 c	0.91±0.32 b	0.68±0.25 b
	cast formation	0.00 a	0.00 a	1.84±0.44 a	0.87±0.31 a	0.80±0.29 a
	Dilated tubules	0.00 a	0.00 a	0.35±0.24 a	0.19±0.19 a	0.12±0.12 a
	Interstitial congestion	0.00 a	0.00 a	17.00±5.59 c	4.00±2.21 b	3.00±1.53 b
	Interstitial hemorrhage	0.00 a	0.00 a	2.00±1.33 a	1.00±1.00 a	1.00±1.00 a
	Interstitial Edema	0.00 a	0.00 a	3.00±1.53 a	2.50±1.70 a	2.00±1.33 a
	Interstitial leukocytic	0.00 a	0.00 a	9.00±2.77 a	5.00±2.24 a	4.00±1.63 a

Values are means ± SEM for 10 samples /group analyzed using a one-way ANOVA followed by a post hoc Tukey test. Means within the same row (in each parameter) carrying different superscripts (a,b,c) are significantly different at $P<0.05$.

The histopathological changes observed in STZ rats may be credited to hyperglycemia, consistent with previous findings (26). Hyperglycemia is a crucial contributor to the generation of free radicals, which induces oxidative stress and leads to vascular disorder, cellular proteins, membrane lipids, and nucleic acid damage (27,28). On the other hand, our histopathological findings are compatible with the biochemical results in which a significant ($P<0.001$) reduction in the TAC level and a significant ($P<0.001$) elevation in the level of MDA in diabetic rats. In addition, the result of the current study showed a significant ($P<0.001$) downregulation in the relative expression of the mRNA of PDX-1, Ngn3, VEGF, IGF, and GLUT-2 of the diabetic group than the control one. Pdx1 and Ngn3 co-expression firmly indicated pancreatic beta-cell regeneration in T1DM in the murine model (29). Pdx1 is a transcription factor exhibited in specific pancreatic cell types (30), and plays a crucial role in the function and survival of pancreatic beta-cells (31). Moreover, GLUT2, interestingly, is the critical regulator of insulin production by pancreatic beta-cells (32,33).

In contrast, the histopathological examination in the current work verified the improvement in therapeutic and prophylactic groups compared to the diabetic group, and the improvement in the prophylactic group was more distinct than the therapeutic group. The pancreatic tissue section of the MO-therapeutic rats revealed partial recovery of the normal histoarchitecture except for moderate beta-cell necrosis and vascular congestion.

The insulin IHC-stained pancreatic tissue of the MO-therapeutic rats showed regeneration of a large percent of immune positively stained beta-cells. The pancreatic tissue of the prophylactic rats demonstrated reversal of these pathological destructions as apparent by intact islets of Langerhans with an average beta-cells population. The insulin IHC stained pancreatic tissue showed powerful insulin expression. Per our results, after histopathological evaluation of the pancreas from diabetic rats, MO-treatment has revealed significant damage reversed in the islet cells histoarchitecture (34).

Moreover, the hepatic tissue of the MO-therapeutic rats showed a mild reduction in the lesions of the diabetic rats. The most frequent lesions in this group were micro-steatosis with few macro-steatosis. In contrast, the hepatic section of prophylactic rats showed a significant decline in the diabetic-induced hepatopathy morphological alterations. The most frequent alterations in this group were multifocal minute mononuclear cell aggregates. These results are similar to this study (35).

The kidneys of MO-therapeutic rats showed a moderate diminution in the severities of the diabetic nephropathy associated changes. Furthermore, the kidneys of prophylactic rats showed a significant decline in the diabetic-induced nephropathic morphological alterations occurrences and extents. In partial accordance with our results of (11), noted that Moringa-seeds powder (50 or 100mg /kg body) treatment in the diabetic rats restored the normal renal histoarchitecture.

The current investigation demonstrates that MO-aqueous leaves decoction has substantial antioxidant activities; serving as a scavenger for free radicals and protecting the examined tissues from damage in diabetic rats. The antioxidant activity can be investigated from our results which showed the ability of MO-aqueous leaves extract to

increase the TAC and decrease MDA in treated diabetic rats. Our investigations are partially similar to the study (36,37).

Moreover, in the current study, MO-extract showed a significant hypoglycemic activity compared to the diabetic group. This investigation is following (38). Our finding states another evidence for the antidiabetic activity of MO-extract via its ability to increase the insulin release as affirmed by the significant beta-cells structure improvement, with a significant insulin immunoreaction upregulation. Hence the MO-aqueous leaves extract revealed its important beneficial role via its hypoglycemic and antioxidant effects. The hypoglycemic activity of MO-contributed to its ability to lower blood sugar through the reaction with anti-insulin antibodies and also promote insulin release from beta-cells of the pancreas (12). In the present study, MO-treated groups, either pre and /or post diabetes onset, revealed a significant ($P<0.001$) upregulation in the relative expression of the mRNA of PDX-1, Ngn3, VEGF, IGF and GLUT-2, indicating the prophylactic and curative effects of MO-pancreatic beta-cells.

Beta-cells secreting insulin have a long lifetime. They can duplicate little during the healthy conditions of life. Interestingly, they reveal increased self-replication after beta-cell loss or increased metabolic need (39). There are two major theories of pancreatic beta-cells regeneration. The 1st is to avert the loss of beta-cells through suppressing apoptosis and necrosis of beta-cells and undifferentiation. The 2nd is promoting diabetes in the newborn (40).

Furthermore, (41) found that when nearly all of the beta-cells had been destroyed, the alfa-cell spontaneously changed into functioning beta-cells when mice were given insulin therapy to keep them alive. The alfa-cell considers a proper candidate for reprogramming to the phenotype of beta-cell. Moreover, alfa and beta-cells are very related functionally, with a similar role in glucose metabolization and hormone release, both types of cells exert ATP-regulated K^+ channels and glucokinase (42).

Conclusion

In conclusion, the histopathological findings of pancreatic, hepatic, and renal tissues and pancreatic IHC, biochemical, and molecular changes of diabetic rats were improved by *Moringa oleifera* aqueous leaves decoction via its antioxidant action. Furthermore, it has a significant role in pancreatic beta-cells regeneration where the insulin IHC-stained pancreatic tissue shows regeneration of a large percent of immune positively stained beta cells. Thus, MO-aqueous leaf extract has the potential to be used as prophylactic and antioxidant supplements against diabetes.

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Conflict of interest

All the authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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الإمكانات العلاجية والوقائية لمغلي أوراق المورينجا أوليفيرا على داء السكري المستحدث بالاستربتوزوتوسين في الجرذان البيضاء

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الخلاصة

صُممت الدراسة الحالية لتقييم الإمكانات الوقائية والعلاجية للمستخلص المائي لمغلي أوراق المورينجا أوليفيرا ضد مرض السكري من النوع الأول المستحدث بالاستربتوزوتوسين في الجرذان. تم استخدام خمسين جرذا من ذكور الجرذان البيضاء وتقسيمهم بشكل عشوائي إلى خمس مجموعات متساوية: المجموعة الضابطة، مجموعة المورينجا، مجموعة مرض السكري، المجموعة العلاجية: حيث الفئران المصابة بالسكري (٣ أيام بعد حقن الاستربتوزوتوسين) أخذت المورينجا اوليفيرا

لمدة ثمانية أسابيع متتالية، والمجموعة الوقائية: حيث الفئران الطبيعية أخذت المورينجا اوليفيرا لمدة أسبوعين قبل استحداث مرض السكري بحقن الاستربتوزوتوسين ثم استكملت المورينجا لمدة ثمانية أسابيع متتالية بعد حقن الاستربتوزوتوسين. وبنهاية التجربة قد أظهرت المجموعة العلاجية والوقائية التي تناولت ١مل من مغلي أوراق المورينجا عن وجود ارتفاع معنوي لتعبير الحمض النووي الريبوزي الرسول البنكرياسي في جينات عامل منشط الأنسولين ١ و نيوروجينين-٣ و عامل النمو البطاني الوعائي و عامل النمو شبيه الأنسولين ١ وناقل الجلوكوز ٢ بالإضافة إلى ذلك، فقد تسبب في انخفاض كبير في تنظيم مستوى السكر في الدم و مستوى بيروكسيد الدهون مقارنة بمستوى مجموعة مرض السكري بالإضافة إلى ارتفاع كبير في مستوى السعة الكلية المضادة للأكسدة. كما أظهرت الدراسة تحسن في التغيرات النسيجية المرضية لأنسجة البنكرياس والكبد والكلى عند مقارنتها بمجموعة مرض السكري، وكان التحسن في المجموعة الوقائية أكثر تميزا من المجموعة المعالجة. مستخلص المورينجا اوليفيرا يمكن أن يعالج ويحمي من مرض السكري من خلال تأثيره المضاد للأكسدة. وبالتالي؛ فإن المستخلص المائي لأوراق المورينجا أوليفيرا لديه القدرة على استخدامه كمكملات وقائية ومضادة للأكسدة ضد مرض السكري.