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The lyophilized aqueous leaf extract of *Moringa oleifera* blunts streptozocin-induced diabetes in rats through upregulation of GLUT 4 signaling pathway and anti-oxidant effect

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ABSTRACT

The anti-diabetic property of aqueous leaf extract of *Moringa oleifera* was performed in streptozocin-induced diabetic rats using serum chemistry, histology and immunochemical parameters as indices of diabetes. The blood glucose level of the diabetic untreated group continues to increase while that of the treated group after 21 days decreased. While the animals in the diabetic untreated group experienced increase in the levels of markers of organ damage when compared to the control group (P values < 0.0001). ALT increased from 61.83±1.5 to 96.1±22.4, AST was 225.1±26.6 from 172.6±13.9, ALP 13.5±0.006 to 13.6±0.002, UREA 1.0±0.08 to 3.0±0.4, their reduction was observed in the extract-treated groups. ALT reduced from 96.1±22.4 to 73.70±9.7; AST from 225.1±26.6 to 184.4±18.2; ALP from 13.6±0.002 to 13.6±0.01; UREA from 3.0±0.4 to 2.0±0.4. Treatment with the extract significantly reduced markers of oxidative stress in the kidney [hydrogen peroxide (898.8±6.26 to 688.0±13.7), malondialdehyde (640±0.1 to 600±0.2) and protein carbonyl (548.4±1.5 to 458.1±1.6)]; heart [hydrogen peroxide (389.4±1.8 to 358.2±1.5), malondialdehyde (264.0±0.5 to 122.0±0.3), protein carbonyl (196.8±0.5 to 162.7±3.5)]; and liver [hydrogen peroxide (119.36±3.2 to 103.94±10.7), malondialdehyde (236.0±0.4 to 73.0±0.2), protein carbonyl (269.3±1.0 to 174.2±1.1) respectively. The levels of antioxidants were reduced in the diabetic untreated group but there was increase in the Moringa treated group. Glucose transporter 4 (GLUT 4) was down regulated in the diabetic untreated group while it was well expressed in the treated groups. The histology of pancreas and liver showed varied levels of infiltration of inflammatory cells, congestion and necrotic lesions, but these were mild in the treated groups. The result shows that the extract does have an anti-diabetic effect with the decrease in the levels of blood glucose and markers of oxidative stress as well as increase in the amount of antioxidants in the treated group

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when compared to the diabetic untreated group. More importantly, the extract caused up-regulation of GLUT 4, which is relevant in reversing insulin resistance in the same manner as pioglitazone, the standard antidiabetic agent used in this study.

Introduction

Diabetes mellitus [DM], a disease of metabolic disorder, characterized majorly by high blood glucose level due to insufficient endogenous insulin production or insulin secretion, is becoming a global phenomenon with respect to its high prevalence. Worldwide, Diabetes mellitus is a major cause of disability, included among the top ten killers [1]. Excessive release of free radicals through lipid peroxidation is usually associated with the metabolic disorder that occurs in diabetes mellitus due to change in the activity of several proteins [2], aggravated by a drastic drop in antioxidant immune mechanisms [3]. This disorder usually lead to oxidative stress, which is the main factor, associated with the severity and death in diabetes.

There are scarcities of resources in Africa to adequately treat and manage diabetes mellitus. Even in cases where the resources including anti-diabetic drugs are available to treat diabetic patient, β-cell function continue to decline making it difficult to attain adequate glycaemic control [4]. It is to be noted that to achieve glycaemic control, the use of two or more drugs for this purpose is commonly adopted in Nigeria [5]. However, there are cases of diabetic complications and hyperglycemic emergencies [6], therefore, the prescribed drugs may be up to four per day per patient [7]. The implication of this is that patients resort to chronic intake of these drugs, leading to side effects. These drugs are also expensive and the cost is borne by the patient themselves, who are in most cases cannot afford the cost, hence there many cases of non-adherence to prescribed drugs. Due to these aforementioned challenges, many patients turn to herbal medicines as alternative forms of therapy [5].

As a result of the setbacks of the antidiabetic drugs in circulation, there is therefore an urgent need to search for new antidiabetic drugs which should not only be more efficacious and less toxic than the current ones, but also of safer profile, easy to administer, cheap and available especially in rural areas. For these reasons, attention is therefore being focused on natural products from plants for the development of new therapies that are able to control diabetes mellitus [8].

Traditional use of several medicinal plants for thousands of years has been a consistent guide to numerous drug discoveries and developmental prototypes. In spite of advances in herbal drug research, including the various health benefits accruing from plant-based bioactive compounds more scientific research voyages are still sought to proffer curative solutions for some diseases, including Diabetes mellitus [9]. In the present study, we report the antidiabetic property of *Moringa oleifera* in streptozotocin- induced diabetic rats.

Moringa oleifera Lam is one of the best known, most widely distributed and naturalized species of a monogeneric family Moringaceae [10]. The Moringa tree is perennial, erect, slender, and medium sized with many arching branches. It has teardrop shaped round leaves, small white flowers and drumstick like fruits [11]. In different places, the Moringa plant bears various names such as drum stick (India), Nebeday (Senegal), Benoil tree (Haiti), Zogale, Kilba-Kabbi (Hausa), Fulani-Kabije as well as okwe oyibo or Agbaji by the Igbos all in Nigeria [12].

Materials and methods

A lyophilized aqueous leaf extract of *Moringa oleifera* was obtained from Sheda Science and Technology Complex (SHESTCO) Federal Ministry of Science and Technology laboratory in Abuja, the Federal Capital Territory (FCT), Nigeria. To prepare the lyophilized water extracts, 100 g of the dried leaf of *Moringa oleifera* was ground into a fine powder using domestic blender and was then added to 250 mL of distilled water. A magnetic stirrer stirred this mixture for 1 day at the temperature of 25 °C, and the extract was filtered with filter paper (Whatman No.1). The filtrates were frozen and lyophilized in a lyophilizer (Labconco, Freezone 1 L) at 5 mmHg at−50 °C. The lyophilized powder was stored at −30 °C until it was used for analysis. The voucher number of the plant is NIPRD/H/7081.

Experimental animals

Ethical approval was sought and obtained from the Animal Care and Use for Research Ethics Committee (ACUREC), University of Ibadan, Nigeria (Ref No UI-ACUREC/App/03/2017/011) before commencement of animal study. The procedures used in this study are in line with internationally accepted principles for the use of animals.

Twenty-five (25) laboratory male albino rats with weight between 150 g and 250 g were used for this study. The animals were purchased from and kept at the experimental animal house of the Department of Veterinary Pharmacology and

Toxicology, University of Ibadan throughout the period of this study, where they were housed in rat cages, and were fed with standard livestock pellets produced by Ladokun and Son Livestock Feed, Nigeria Limited. Each animal was left alone in a clean polyethylene cage under hygienic conditions in a well-ventilated house under the natural light conditions throughout the experiment and acclimatized for one week prior to start of the experiment.

Drugs

Pioglitazone HCL and Streptozotocin (STZ)

These were obtained from Sigma Biosciences, Egypt. STZ was freshly prepared in 0.1 mol/1 citrate buffer, pH 4.5, immediately before use and was injected at a dose of 70 mg/ kg, i.p.

Chemicals and reagents

Biuret reagent, Greiss reagent and other chemicals used in this study are of analytical grade, obtained from the British Drug Houses (Poole, Dorset, UK).

HPLC fingerprint of the aqueous leaf extract of Moringa oleifera

The HPLC fingerprint of the aqueous leaf extract of *Moringa oleifera* was carried out using CECIL CE4200 system equipped with gradient pump and empowered software for data acquisition and processing. The analysis was carried out for 20 μl of the solution in water in the gradient mode using ODS C-18 (5 μm particle size) column (150 mm X 4.6 mm; phenomex). Acetonitrile (A) and water with 0.5% (v/v) formic acid (B) were used in gradient elution program: from 0 to 7 min, 20%; from 8 to 13 min, 40% A; from 14 to 19 min, 60% A, and from 20 to 36 min 100% A. The mobile phase flow rate was set to 1.0 mL/min and total run time of 36 min. The chromatographs were recorded with UV/Visible detector at specific wavelength of 254 nm.

Experimental design

Fifteen diabetic animals were randomly allocated into three (3) groups of five animals per group and ten non-diabetic rats were allocated into two (2) groups of five animals each. A control group that received distilled water alone, and the group that received Moringa extract alone.

Group A: negative normal control group; non diabetic rats received no medication but given distilled water 2 ml/kg body weight, orally for 14 days once a day.

Group B: Positive diabetic control group; Rats were induced with 70 mg/kg of Streptozotocin (STZ) and did not receive medication.

Group C: Non-diabetic treated group: Rats were not given STZ but treated with Moringa extract in a dose of 100 mg/kg body weight orally for 14 days.

Group D: Diabetic treated group: Rats were induced with STZ and treated with Moringa extract in a dose of 100 mg/kg body weight/day orally for 14 days.

Group E: Diabetic treated group: Rats were induced with STZ and treated with Pioglitazone at 25 mg/kg body weight orally once daily for 14 days.

Induction of experimental diabetes

The rats were weighed and were fasted for 16 hours, but water was available ad libitum. Thereafter the blood samples of the rats were taken in order to check their fasting blood glucose (FBG) using Accu Check Glucometer. Thereafter diabetes was induced in rats by intraperitoneal (i.p) single injection of freshly prepared streptozotocin (STZ) at a dose of 70 mg/kg, dissolved in di-sodium citrate buffer (pH 4.5) in a dose volume of 1 ml/kg [13]. Rats were then given access to feed and 2% glucose water was also made available for about 16 h in order to prevent hypoglycaemia [14]. After this the rats had access to normal water. After 72h of STZ injection, diabetes was confirmed in rats showing blood glucose level greater than 200 mg/dl, [14]. Animals with blood glucose levels greater than 200 mg/dl were considered for further study and grouped as B, D and E.

Animals with elevated blood glucose were selected for treatment with Moringa extract at a dose of 100 mg/kg and Pioglitazone at a dose of 25 mg/kg orally for a period of fourteen (14) days. On the fifteenth day, blood was collected from each of the animal via the retro-orbital vein and the animals were then euthanized by cervical dislocation. The organs of interest were harvested and preserved in formalin for histopathology and immunohistochemistry while some parts of the kidney, liver and heart were kept frozen at -80C for biochemical assays.

Preparation of serum and homogenization of tissues for biochemical assays

With the aid of clean heparinized tubes blood samples were collected from retro-orbital venous plexus of the animals into dry clean heparinized tubes. These samples were then centrifuged at 4000 revolutions per minutes (rpm) for 15 min after which plasma was collected and stored in the freezer at 4 °C till the time of analysis.

Homogenization

Kidney, heart and liver tissues were quickly excised and washed in ice-cold normal saline solution after which they were blotted with filter paper and weighed. They were then chopped into bits and homogenized using the homogenizing buffer (0.1M phosphate buffer, pH 7.4) using a Teflon homogenizer. The resulting homogenate was centrifuged at 10,000 rpm for 10 min with a cold centrifuge at 4 °C to obtain post mitochondrial fraction (PMF). The supernatant was collected and used for biochemical analyses.

Serum markers of renal and hepatic damage

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, urea and albumin activity were estimated using the Randox kit (Randox, USA). Detailed procedures for the above measurements were performed according to the kit manufacturer's instructions.

Determination of activities of markers of oxidative stress and antioxidant enzymes

To determine the activities of markers of oxidative stress in the tissue homogenates, lipid peroxide was estimated by measuring malondialdehyde (MDA), while protein carbonyl and the activity of hydrogen peroxide were determined according to the method of Varshney and Rale [15] and Woff [16] respectively. Enzymatic and non-enzymatic antioxidants in the tissue homogenates were also assayed for in order to determine their levels of activities.

Immunohistochemistry of GLUT4, in the liver and pancreas

Immunohistochemistry of paraffin embedded tissue of the liver and pancreas was performed in line with the procedure described by Hsu et al [17]. At the end of the procedure, GLUT4 expression were viewed on each slide with 400 × magnifications with the aid of a photo microscope (Olympus) as well as a digital camera (Toupcam®, Touptek Photonics, Zhejiang, China).

Histopathology of heart, liver, pancreas and kidney tissues

The kidneys, liver and pancreas were kept in 10% neutral formalin solution. These organs that were processed and thereafter embedded in paraffin wax were and sectioned with the aid of a microtome. The stained sections using haematoxylin and eosin were viewed with the aid of a light microscope. The sections were evaluated for pathological alterations in kidneys and liver [18].

Statistical analysis

Results obtained from this study were analysed statistically using mean ± SD, ANOVA and student's test at 95% level of significance.

Results

Body weight changes

Table 1 shows the effect of *Moringa oleifera* on body weight (kg) depicted as percentage weight gain or loss in diabetic rats that were induced with streptozotocin. The weekly changes in body weight of animals from the various groups showed that the control and non-diabetic treated groups have a 25.38% and 19.89% increase in body weight respectively when compared to their initial weight. The diabetic non-treated group has −29.44% loss in body weight when compared to their weight at week 0. The Moringa and Pioglitazone treated groups showed a −32.60% and -19.68% decline in their weight respectively when compared to their initial weight with the Moringa treated group having the highest percentage decrease in body weight.

Table 1
Effect lyophilized aqueous leaf extract of *Moringa oleifera* on body weight changes and blood glucose levels in streptozotocin-induced diabetic rats (g).

This shows the effect of *Moringa oleifera* on body weight (g) depicted as percentage weight gain or loss as well changes in blood glucose levels in diabetic rats that were induced with streptozotocin

GROUP	INITIAL	FINAL	% GAIN /LOSS	
BODY WEIGHT				
A	146.0 ± 18.5	183.0 ±17.9	25.34	
B	214.0 ±39.1	151.0 ± 39.1	−29.44	
C	176.0 ± 4.2	211.0 ± 5.5	19.89	
D	181.0 ±24.1	122.0 ± 15.0	−32.60	
E	188.0 ± 54.0	151.0 ± 42.1	−19.68	
Glucose Values				
	DAY 0	DAY 1	DAY 7	DAY 14
A	103.0 ± 12.8	102.5 ± 6.5	101.4 ± 8.7	87.6 ± 5.8
B	85.2 ± 14.8[a]	295.0 ± 53.7	424.0 ± 68.7[a]	301.0±214.3[a]
C	90.2± 8.9	367.3 ± 9.2	102.0 ± 7.5[b]	81.2 ± 1.3[b]
D	94.4 ± 9.6	450.0± 34.6[b]	388.0 ± 7.1[a]	391.5 ± 9.2[a]
E	105.8 ± 3.3[b]	399.7±59.5[b]	151.0 ± 42.1[a,b]	398.3 ± 59.0[a]

Values presented as mean ± S.D.

Table 2
Effects lyophilized aqueous leaf extract of *Moringa oleifera* on serum markers of renal and hepatic damage.

This shows the effect of *Moringa oleifera* on the serum markers of renal and hepatic damage in diabetic rats that were induced with streptozotocin.

	A (Control group)	B (Diabetic not treated)	C (Non-diabetic treated)	D (Diabetic treated with 100mg/kg Moringa)	E (Diabetic treated with 25mg/kg Pioglitazone)
ALT	61.83±1.5	96.1±22.4[a]	64.0 ± 2.4[b]	73.70 ± 9.7[a,b]	78.9 ±14.1[b]
AST	172.6 ±13.9	225.1±26.6[a]	170.0±5.4[b]	184.4±18.2[b]	189.3±19.2[b]
ALP	13.5 ±0.006	13.6±0.002[a]	13.6±0.006[a,b]	13.6±0.01[a,b]	13.6±0.001[a]
ALBUMIN	1.3±0.2	0.8 ± 0.20[a]	1.6 ±0.3[b]	1.1±0.2[b]	1.1±0.04[a,b]
UREA	1.0±0.08	3.0±0.4[a]	0.9 ±0.2[b]	2.0±0.4[a,b]	1.9±0.20[a,b]
Creatinine	0.8 ± 0.06	0.7 ± 0.1[a]	0.7 ± 0.04[a]	0.7 ± 0.1	0.70 ± 0.08[a]

Values presented as mean ± S.D. Alphabets indicate significant difference across groups at α<0.0001. ALT (Alanine aminotransferase), AST (Aspartate aminotransferase). Superscript [a] indicates significant difference (P<0.0001) when groups B, C, D and E were compared with Group A, and superscript [b] indicates significant difference ($P < 0.0001$) when Groups C, D and E were compared with Group B.

Blood glucose measurement

Blood glucose was measured in different groups over the two weeks period. After induction of hyperglycaemia using Streptozotocin, all animals in the untreated diabetic, Moringa treated and pioglitazone treated diabetic groups remained hyperglycemic (FBG >200 mg/dL). After one week of treatment, the value of blood glucose in the untreated diabetic group still increased by 43.72% when compared to its initial value after the induction. Although the rats in the Moringa and pioglitazone treated groups were still diabetic, the value of the blood glucose in these groups reduced by 13.28% and 12.00% respectively when compared to their initial value after induction. After second week of treatment, although the induced groups still remained hyperglycemic, the blood glucose value in the Moringa and pioglitazone treated groups showed a 35.22% and 25.37% reduction in the blood glucose value when compared to their value at induction (Table 1).

The activities of serum hepatic biomarker enzymes

As shown in Table 2, diabetes induction in the untreated diabetic group significantly increased serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), urea, albumin and creatinine indicating severe renal damage and hepatotoxicity. Treatment with *Moringa oleifera* after diabetes induction however, showed a significant reduction in the activities of these serum markers of renal and hepatic damage except in the case of creatinine.

Markers of oxidative stress in tissue homogenates

The results of treating diabetic rats with *Moringa* for 2 weeks on markers of oxidative stress in the kidney, heart and liver tissue homogenate are given in Table 3. The mean values of MDA, protein carbonyl, and hydrogen peroxide were observed to increase in the positive control group significantly (at $\alpha < 0.05$) when compared with that of the negative control group in all the tissue homogenates. Treating diabetic rats with 100 mg/kg body weight of *Moringa* extract significantly (at $\alpha < 0.05$) reduced the levels of all the makers of oxidative stress in the kidney, heart and liver homogenate.

Table 3
Effects of lyophilized aqueous leaf extract of M*oringa oleifera* on markers of oxidative stress in the kidney, heart and liver of STZ-induced diabetic rats.

This shows the effect of *Moringa oleifera* on markers of oxidative stress in kidney, heart and liver of diabetic rats that were induced with streptozotocin.

	A (Control group)	B (Diabetic not treated)	C (Non-diabetic treated)	D (Diabetic treated with 100 mg/kg Moringa)	E (Diabetic treated with 25 mg/kg Pioglitazone)
Kidney					
H_2O_2	53.31 ± 4.4	89.88 ± 6.3 [a]	67.48 ± 0.7 [a,b]	68.8 ± 13.7 [a,b]	72.41 ± 6.3 [a,b]
MDA	0.51 ± 0.1	0.64 ± 0.07	0.37 ± 0.03 [a,b]	0.60 ± 0.2 [b]	0.60 ± 0.1
PC	13.89 ± 1.5	18.28 ± 1.5 [a]	10.97 ± 1.3 [a,b]	15.27 ± 1.6 [b]	15.54 ± 2.9 [b]
Heart					
H_2O_2	34.77 ± 0.9	38.94 ±1.8 [a]	32.06 ± 0.6 [a,b]	35.81 ± 1.5 [a,b]	32.68 ± 0.2 [a,b]
MDA	0.81 ± 0.02	2.64 ± 0.55	0.97 ± 0.25 [a,b]	1.22 ± 0.34 [b]	1.30 ± 0.17
PC	13.2 ± 0.47	19.68 ± 0.49 [a]	11.6 ± 1.08 [a,b]	16.27 ± 3.4 [b]	16.63 ± 2.01 [b]
Liver					
H_2O_2	93.5 ± 8.8	119.36 ± 3.2 [a]	70.3 ± 11.6 [a,b]	103.9 ± 10.7 [b]	110.8 ± 11.7 [a]
MDA	0.58 ± 0.1	2. 36 ± 0.4 [a]	0.36 ± 0.06 [b]	0.60 ± 0.2 [b]	0.60 ± 0.1[a,b]
PC	14.82 ± 1.0	26.93 ± 1.0 [a]	15.70 ± 0.7 [b]	17.42 ± 0.7 [a,b]	18.70 ± 2.8 [a,b]

Values presented as mean ± S.D. H_2O_2 (Hydrogen Peroxide), MDA (Malondialdehyde; nmol/mg protein) PC (Protein Carbonyl; nmol/mg protein). Alphabet (a) indicates significant difference (α <0.0001) when groups B, C, D and E were compared with Group A, and alphabet (b) indicates significant difference (α <0.0001) when Groups C, D and E were compared with Group B.

Enzymatic and non-enzymatic antioxidants in renal, cardiac and liver tissue homogenates

The effects of treating diabetic rats with the extract of *Moringa oleifera* for 2 and the subsequent response as it relates to its antioxidant effects in the kidney, heart and liver tissue homogenates are given in supplementary tables 1, 2 and 3. The parameters measured here are the enzymatic and non-enzymatic antioxidants such as superoxide dismutase (SOD), glutathione-s-transferase (GST), glutathione peroxidase (GPx), reduced glutathione (GSH), non-protein and protein thiol (NPT and PT).

Supplementary Table 1 (ST1) showed the results of renal enzymatic and non-enzymatic antioxidants where SOD of group B (untreated diabetic) showed higher level of significance compared to the other groups (control and diabetic treated). GST results showed that all the groups except that of C (non-diabetic treated) showed significant higher level than group B. In the case of GPx, all the groups showed significant lower level compared to group B, the toxicant group. The table also showed the result of renal non-enzymatic antioxidants where GSH, NPT and PT of all the groups showed significant higher level compared to group B.

The cardiac enzymatic antioxidants such as SOD recorded was significantly higher for group B, the toxicant group, when compared to the control and also non-significantly higher than the values observed in the other groups (Supplementary Table 2 i.e. ST2). The result of GPx is similar to that of SOD. The results of GST on the other hand showed that all the groups recorded significant higher level compared to group B, the toxicant group. Also the cardiac non-enzymatic antioxidants were evaluated. The results showed that the parameters measured that is GSH, NPT and PT of all the groups showed significant higher levels compared to that of group B.

Supplementary Table 3 (ST3) on the other hand showed the mean values of liver enzymatic antioxidants evaluated in this study. Here the results of SOD and GPx of group B animals showed significant higher levels compared to the other groups while the reverse is the case for GST where all the groups showed significant higher levels when compared with that of group B, the toxicant group. Also in this table the results of liver non-enzymatic antioxidants where all the groups showed significant higher level when compared to the group B animals.

Histopathology of the pancreas

In Fig. 1, the negative control group plate shows normal exocrine acini with zymogen granules (blue arrows), the intralobular and interlobular ducts are essentially normal and in some cases contain pancreatic secretion. The islets appear normal in varying sizes (green arrows). In the case of the positive control group, plates show vacuolation of the exocrine acini with zymogen granules (blue arrows), there is a focal area intralobular duct with tributaries of smaller duct (slender arrows). No islet was seen in the specimen. The non-diabetic treated group showed normal exocrine acini with zymogen granules (blue arrows), the intralobular and interlobular ducts are essentially normal and in some cases contain pancreatic secretion (slender arrows). The islets appear normal in varying sizes (green arrows). Plates of the Moringa treated diabetic rats showed normal exocrine acini with zymogen granules (blue arrows), the intralobular and interlobular ducts are essentially normal and in some cases contain pancreatic secretion (slender arrows). The islets appear normal in varying sizes (green arrows). However, there is disseminated congestion (black arrows). In the pioglitazone treated group, the plate shows normal exocrine acini with zymogen granules (blue arrows), the intralobular and interlobular ducts are essentially normal and in some cases contain pancreatic secretion (slender arrows). The islets appear normal in varying sizes (green arrows).

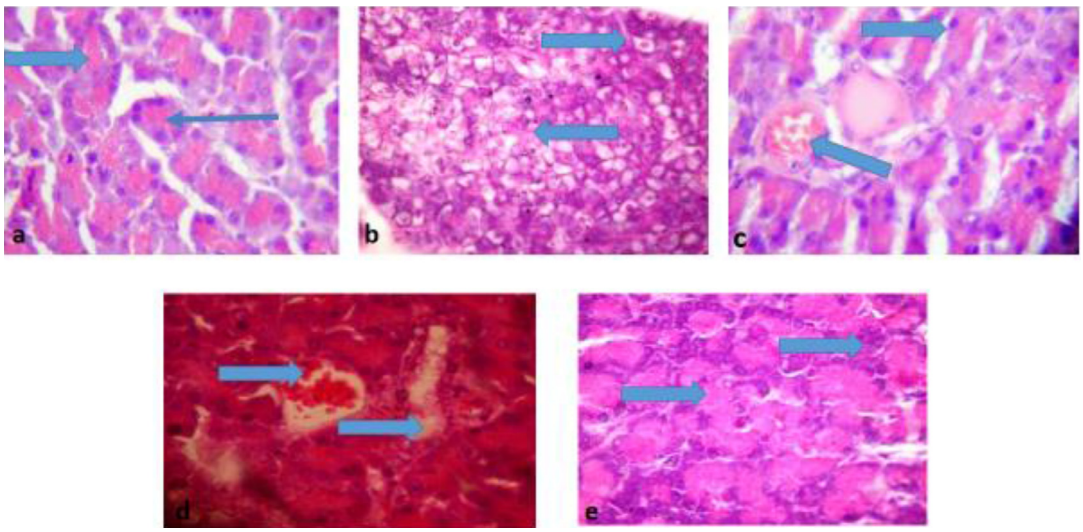

Fig. 1. Histological sections of the pancreas for groups A (a), B (b), C (c), D (d) and E (e). Plate (a) showed normal exocrine acini with zymogen granules were seen, the intralobular and interlobular ducts are essentially normal and in some cases contain pancreatic secretion. The islets appear normal in varying sizes. (b) Plates show vacuolation of the exocrine acini with zymogen granules, there is a focal area intralobular duct with tributaries of smaller duct. No islet was seen in the specimen. Plate (c) showed normal exocrine acini with zymogen granules, the intralobular and interlobular ducts are essentially normal and in some cases contain pancreatic secretion, (d) showed normal exocrine acini with zymogen granules, the intralobular and interlobular ducts are essentially normal and in some cases contain pancreatic secretion. Plate (e) showed normal exocrine acini with zymogen granules, the intralobular and interlobular ducts are essentially normal and in some cases contain pancreatic secretion.

Histopathology results

Pathology of the liver

Plate of the negative control group shows mild disseminated infiltration of zone 2 by inflammatory cells while that of the diabetic not treated group (B) shows moderate disseminated congestion. Mild disseminated infiltration of zone 2 by inflammatory cells was observed in the Moringa non-diabetic group. In the Moringa treated diabetic group, plate showed marked disseminated congestion involving the veins and sinusoid and moderated disseminated infiltration of zone 2 by inflammatory cells while that of Pioglitazone treated group showed moderate disseminated congestion (Fig. 2).

Pathology of the kidney

Focal area of inflammation was observed on the plate of negative control group rats while the plates of the positive control group showed focal area of tubular desquamation and mild disseminated glomerular congestion with glomerulonephritis. The glomerular loops are partially obstructed by endothelial cells. Plates of the Moringa non-diabetic treated rats showed normal architecture of the kidney with mild disseminated glomerular congestion/messangialisation. The Moringa treated diabetic rats showed marked disseminated congestion and moderate to marked disseminated glomerular congestion while the pioglitazone treated rats showed moderate disseminated congestion, moderate disseminated glomerular congestion (green arrows) and focal area of infiltration by inflammatory cells (Fig. 3).

Glucose transporter 4

Supplementary Figures 1 and 2 (SF1 and 2) showed the effect of 2 weeks treatment of diabetic rats with *Moringa* on the expression of glucose transporter 4 (GLUT 4) in the liver and pancreas tissue homogenate respectively. The expression of GLUT 4 in the negative control group was high as against the low expression observed in the diabetic not treated group in both tissues. Treatment of the diabetic rats with 100 mg/kg *Moringa* leaf increased the expression of GLUT 4 when compared with the positive control group.

HPLC finger printing for the aqueous leaf extract

The HPLC fingerprint in this study showed the presence of gallic acid quercetin and other flavonoids (Supplementary Fig. 3 i.e. SF3).

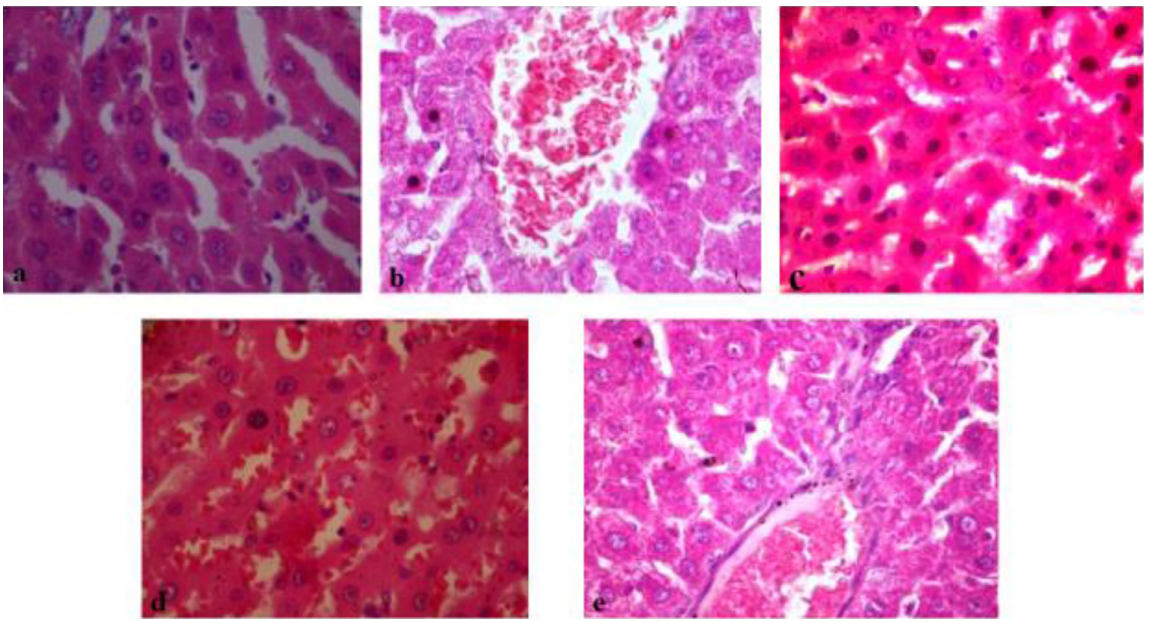

Fig. 2. Histological sections of the liver for groups A (a), B (b), C (c), D (d) and E (e). Plate (a) showed mild disseminated infiltration of zone 2 by inflammatory cells, (b) showed moderate disseminated congestion, (c) showed mild disseminated infiltration of zone 2 by inflammatory cells, (d) showed marked disseminated congestion involving the veins and sinusoid and moderated disseminated infiltration of zone 2 by inflammatory cells while (e) showed moderate disseminated congestion.

Discussion

A fundamental index of physiological or pathological state of a living organism is usually changes in weight. From this study, the sharp decrease in body weight of untreated diabetic group compared to normal control and experimental treated animals during experimental period is an indication of diabetes being a tissue wasting disease as result of its poor glycemic control and this usually fosters protein and fat mobilization [19].

Villarruel-López et al [20] showed that diabetic rats treated with the extract of *Moringa oleifera* experienced increase in body weight. Some other study also showed that the 50 and 100 mg of the seed powder of the plant when used to treat diabetic rats also caused significant change in the body weight [21]. The result of our study is thus similar to these previous studies. It may be inferred from these studies that the weight gained in the diabetic rats treated with the extract from Moringa plant may be due to the presence of phytochemicals such as essential amino acids and vitamins especially the fat soluble ones. It is also known that anti-oxidants, antimicrobial compounds such as coumarins, phenol, alkaloids, tannins etc. can also promote growth [22].

In this study, there is percentage weight loss in the diabetes-induced groups as against the significant weight gain in the control and Moringa non-diabetic treated groups. It should be noted that diabetes is a wasting disease with various complications arising from the condition. The generation of oxidative stress and free radicals could be responsible for the weight loss observed in diabetes-induced groups. Tissue destruction in hyperglycemic state is due to the release of oxygen radicals and ROS [23].

The blood glucose levels of rats that were received Streptozotocin increased above the normal reference value. Rats that were administered 70 mg/kg of Streptozotocin showed significant hyperglycaemia. The blood glucose was observed to increase by 43.7% by day 7 in the diabetic untreated group while the diabetic treated groups had a reduction in their blood glucose level by day 7. Olayaki et al [21] observed that oral administration of extract of *M. oleifera* significantly reduces blood glucose concentration and inhibits weight loss in alloxan-induced diabetic rats. This is consistent with the results of this study.

There is a significant ($p < 0.05$) decrease in the level of enzymatic and non-enzymatic antioxidants in the heart, kidney and liver tissue when compared to the control group. This observation has nothing to do with decrease in the rate at which anti-oxidants are synthesized but rather possibly due to unaltered or accelerated rates of the synthesis of glutathione. This depends largely on the blood glucose levels. The increased utilization of glutathione in diabetes may be responsible for its depletion [24]. However, treatment with *Moringa oleifera extract* reduced the enzymatic anti-oxidants and almost brought these to normalcy. The therapeutic effect of *Moringa oleifera* extract may have to do with its phytoactive constituents present in the plant [25,26]. As a matter of fact, Ghiridhari et al [25] has shown that *Moringa oleifera* possess anti-oxidant activity due mainly to the presence of flavonoids and phenols and these chemicals are free radical scavengers. Studies have also shown that this plant when used in the treatment of diabetes enables diabetic patients to experience better glucose

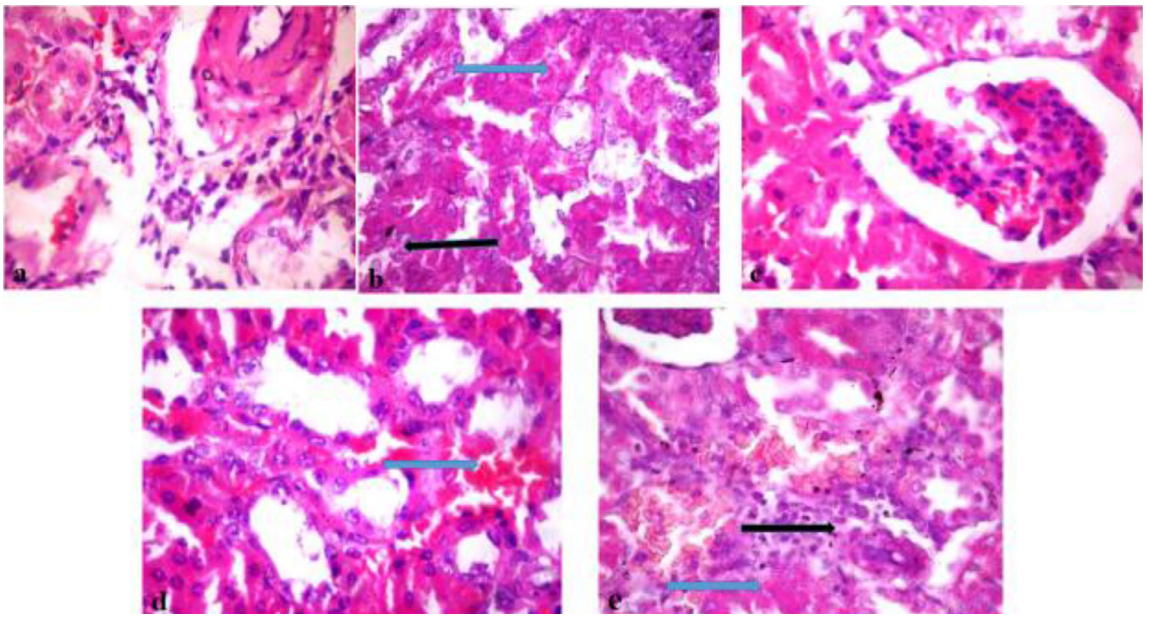

Fig. 3. Histological sections of the kidney for groups A (a), B (b), C (c), D (d) and E (e). (a) showed focal area of inflammation, (b) showed focal area of tubular desquamation and mild disseminated glomerular congestion with glomerulonephritis. The glomerular loops are partially obstructed by endothelial cells. (c) showed normal architecture of the kidney with mild disseminated glomerular congestion/messangialisation, (d) showed marked disseminated congestion and moderate to marked disseminated glomerular congestion while (e) showed moderate disseminated congestion, moderate disseminated glomerular congestion and focal area of infiltration by inflammatory cells.

tolerance [25,26]. Phytochemical studies have actually shown that glucosinolates (glucomoringin), flavonoids (quercetin and kaempferol), and phenolic acids (chlorogenic acid) are three classes of phytochemicals present in Moringa [27] and these three phytochemicals exhibit anticancer, hypotensive, anti-inflammatory, anti-oxidant, hypoglycaemic, and antidyslipidemic effects [28].

In this study, it was observed that SOD level increased in the tissues in both the diabetic treated group and the pioglitazone and Moringa treated groups, which is in contrast to the report of Al-Malki and El- Rabey [29]. The observed uniform increase in the level of SOD could be explained as compensatory mechanism.

Markers of oxidative stress assayed for in this experiment include H_2O_2, MDA, PC in the tissue homogenate. Streptozocin (STZ) is known to donate nitric oxide (NO) which on its own has the ability to mediate pancreatic islet destruction through its ability to damage the DNA [30]. Apart from this, STZ mechanism of action has to do with the generation of reactive oxygen species (ROS) from the mitochondria. It also has the potential to increase the activity of xanthine oxidase [31]. Oxidative stress in STZ-induced diabetic animals is due to glucose auto-oxidation, protein glycation, formation of the advanced glycation products and the polyol pathway that generates free radicals [13]. This study showed an increase in the markers of oxidative stress in the tissues of untreated diabetic group when compared to the control group. The levels of the markers in the Moringa and pioglitazone treated groups were observed to be lower than that of the toxicant group.

Lipid peroxidation is known to be associated with hyperglycaemia [32]. When β cells of the pancreas are destroyed, which is experienced in hyperglycaemic, the body tissues switch over to the use of fatty acids and acetyl-CoA for energy utilization [32]. The levels of oxidative stress markers in the untreated hyperglycaemic animals experienced an increase in contrast to the treated groups. The ability of some constituents of Moringa leaf extract such as oleic acid enhanced the release of insulin [33] and prevention of lipid peroxidation helped in the early attainment of hypoglycaemia in the Moringa treated group. The above reason can explain the low MDA levels seen in the tissues of Moringa treated group and this decrease was significantly different from control and untreated hyperglycaemic animals.

Liver is an important organ in maintaining normal glucose concentration in body, which functions in insulin disposal and produces inflammatory cytokines. Increased levels of serum ALP, ALT, AST are symptoms of liver damage where the enzymes leak into the bloodstream from cytosol upon hepatic cellular damage [34]. The markers assayed for in this study include ALT, AST, ALP, urea, creatinine and albumin. In this study, there was a significant ($P < 0.05$) increase in the serum level of hepatic and renal enzymes in the untreated diabetic group when compared to the control group proving that hepatotoxicity and renal toxicity do occur in diabetes. Oral administration of *M. oleifera* (MO) was able to prevent the oxidative stress related hepatic and renal damage through antioxidant supplementation.

There was a significant reduction in the serum level of these enzymes in the Moringa and Pioglitazone treated groups when compared to the toxicant group. The reversal of elevated serum intracellular enzyme levels by MO extract after the induction of diabetes may be attributed to its cell membrane stabilizing ability hence preventing enzymes leakages as earlier

postulated [35]. High concentrations of these liver enzymes in the diabetic untreated group showed the level of liver damage caused by diabetes. This might be due to the highly antioxidant activity of liver and this is in accordance with the study of Al-Malki and El Rabey [29]. Conversely, other researchers reported that ALT and AST decreased in diabetic rats [36].

The result in this study showed a reduction in the level of total protein (albumin) in serum of untreated diabetic rats; this may be due to hepatocellular damage, oxidative stress which increase the amount of ROS radicals, impaired liver function and also may be due to the decrease capacity of the hepatocytes to synthesize protein [37].

The expression of GLUT 4 appears to correlate with the level of circulating insulin. There is therefore a reduced expression of glucose transporter in group B, which is the untreated diabetic group when compared to the expression observed in the negative control group. This is in accordance to the report of Olson [38] who stated that the regulation of expression of GLUT4 gene is based on glucose level, which invariably is in consonant with the physiologic state of the organism. Therefore, in altered glucose homeostasis, *GLUT4* gene expression will be observed and this is observed for example in severe insulin deficiency and starvation where *GLUT4* mRNA expression is reduced [39].

Transcription of Glucose transporter-4 (GLUT 4) gene is known to be enhanced in the presence of peroxisome proliferating receptor-γ (PPAR-γ). Peroxisome proliferating receptor-γ enhances the transcription of several insulin-responsive genes that are involved in the regulation of glucose and lipid metabolism especially in the liver, muscle, and adipose tissue [40]. Pioglitazone, a diabetes drug (thiazolidinedione-type) reduces insulin resistance in the liver and peripheral tissues. This drug also increases the expense of insulin-dependent glucose, hence decreases the withdrawal of glucose from the liver. This PPAR-γ agonist also reduces quantity of glucose in the bloodstream. One other property of pioglitazone is to help improve glycaemic control leading to the lowering of circulating HbA1C and insulin levels in type 2 DM patients. Pioglitazone has potent anti-hyperglycemic action by reducing insulin resistance. Because pioglitazone probably acts on PPAR-α, it also lowers serum triglyceride level and raises HDL level without much change in LDL level. It has also been used to treat non-alcoholic steatohepatitis (fatty liver). In diabetes patients, Pioglitazone is known to improve sensitivity to insulin, glycaemic control, hypertension, dyslipidaemia and microalbuminuria [41].

The HPLC fingerprint in this study showed the presence of gallic acid quercetin and other flavonoids (Supplementary Figure 3 i.e. SF3). This is similar to the earlier work on antidiabetic study of *Moringa oleifera* by Khan et al [42] indicating that these **phytoconstituents and other** flavonoids as well as phenolic metabolites present may be responsible for its antidiabetic activity.

Conclusion

Since *Moringa oleifera* also exhibited similar traits as pioglitazone in upregulating the expression of GLUT 4, it may be safe to state that this plant extract reduces insulin in the liver and peripheral tissues; increased the expense of insulin-dependent glucose, decreased withdrawal of glucose from the liver and reduces quantity of glucose in the bloodstream.

Declaration of Competing Interest

Authors declare that there is no conflict of interest.

CRediT authorship contribution statement

Adeolu Alex Adedapo: Conceptualization, Resources, Writing - review & editing, Funding acquisition. **Iyanuoluwa Omolola Ogunmiluyi:** Methodology, Writing - original draft. **Olufunke Olubunmi Falayi:** Methodology, Writing - original draft. **Blessing Seun Ogunpolu:** Methodology, Writing - original draft. **Ademola Adetokunbo Oyagbemi:** Conceptualization, Resources, Funding acquisition. **Temidayo Olutayo Omobowale:** Conceptualization. **Oluwafemi Omoniyi Oguntibeju:** Resources, Writing - review & editing, Funding acquisition.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2020.e00619.

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