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ANTIDIABETIC EFFECTS OF *MORINGA OLEIFERA ON* PANCREATIC ISLET CELL OF ADULT MALE WISTAR RATS

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ABSTRACT

Background: Diabetes mellitus is a complex metabolic disorder, which is life threatening as a result of an insufficient action of insulin or pancreatic β-cell dysfunction. This study was carried out to evaluate anti-diabetic effects of aqueous extract of Moringa seed on pancreatic islet cell in alloxan induced diabetic male Wistar rats. **Materials and methods:** Twenty- five (25) Adult Male Wistar rats were divided into five (5) groups (n=5). Administration was done via oral route over a period of two weeks. Group A: Control= normal saline, Group B diabetic model (120mg/kg of alloxan), Group C: 100mg/kg body weight of aqueous extract of *Moringa Oleifera* seed, Group D:diabetic model treated with 100mg/kg of aqueous extract of *Moringa Oleifera* seed and Group E: diabetic model treated with standard diabetic drug (5mg/kg Glibenclamide). Serum was analysed for lipid peroxidation enzyme-Malondialdehyde (MDA) and antioxidant enzymes Superoxide dismutase (SOD) activities while pancreas was processed for Haematoxylin and Eosin stain and Periodic Acid Schiffs stain for glycogen. **Results:** The results showed a significant decrease in final body weights of the experimental animals ($P<0.05$) compared to the control and their respectives initial body weights. Lipid peroxidation increased in group B as compared to A, C D and E. There was no significant difference in Mo and GLB reduction in peroxidation activity MDA as well as SOD levels. There was no distortion of the clustered Langerhans cells in the control and MO, GBL treated groups but severe distruption in Alloxan treated group. **Conclusions:** *Moringa Oleifera* has a potential cytoprotective effect on Islet of Langerhans against oxidative stress mediated diabetic disorder and can be used as a therapeutic in diabetic disease prevention and management.

KEYWORDS: *Moringa Oleifera*, Diabetic, Langerhans Cells, Glibenclamide, Malondialdehyde and Superoxide Dismutase.

INTRODUCTION

Diabetes mellitus (DM) is a deleterious public metabolic disorder and it is a major health challenge.[1,2] More than 150 million people is diagnosed globally and this figure is expected to increase to 300 million by 2025.[3] It is caused due to insufficient action of insulin (insulin resistance) and pancreatic β-cell dysfunction.[2,4] Pathogenesis of DM is characterized by hyperglycemia; altered metabolism of carbohydrates, and proteins linked with an increased risk of complications from vascular disease.[5-8] Diabetes mellitus is classified as Type I and II, Type I aetiology is via chronic inflammation that specifically targets and destroy the insulin-producing β-cells in the islets of Langerhans[9,10] and it is the most prevalent type[11], the Type II is a metabolic disorder that is characterized by insulin resistance and relative insulin deficiency leading to hyperglycemia.[12,13] Understanding the pathogenesis of DM is a possible lead to developing novel anti-diabetic agents with less side- effects, this has stimulated countless research involving herbal plants for a new drug discovery.[14] Most therapeutics drugs for DM are oral hypoglycemic agents and insulin injection with their limitations or side effects. There is need to discovery alternative therapy that is affordable and with better clinical or therapeutic outcome.[15]

In this regard, the World Health Organization, through its Expert Committee on Diabetes has considered the potential significance of some efficacious herbs in diabetes treatment and has recommended that traditional medicinal herbs be investigated.[16] Following this, many herbal medicines have been studied and are still being study for clear understanding of mechanism of antioxidant therapeutics in their phytochemical components.[14,17-20] Phytochemical screening of plant has shown that it contains a wide array of phytochemical

compounds with antioxidant properties, the basis of their anti-diabetic potential in DM.[21,22] Also, DM being a metabolic disorder associated with oxidative tissue damage, herbs with strong antioxidant potent can mob off free radical generation or activates endogenous secretion of glutathione[19,20,23] to arrest the pathogenesis of DM. Recently numerous traditional medicinal plants were tested for their anti-diabetic potential for example *Moringa Oleifera* (MO) (Miracle tree)[24,25], which is the most widely cultivated species of a monogeneric family, the *Moringaceae* and the most nutrient rich plant on the earth.[26] All parts of the Moringa tree are edible and have long been consumed by humans.[25,27,28] Phytochemical screening of this plant has shown that it contains a wide array of phytochemical compounds according to,[29,30] its ethanolic leaf extract contains flavonoids, tannins, anthraquinones, cardiac glycosides, alkaloids, triterpenoids, saponins, and reducing sugars while its seed has all the aforementioned with the exception of anthraquinones.[31] In addition the leaves is rich in beta carotene, protein, amino acid, vitamin C, calcium and potassium.[32,33] Most of these phytochemicals (e.g., glycosides, alkaloids, terpenoids and flavonoids) have been found to possess anti-diabetic properties.[34] It has been documented that MO seed and leave demonstrates hypocholesterolaemic activity in Wistar rats and rabbits.[28,33,35] MO plant parts have shown various medicinal properties such as antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities.[28,33,36] DM in animal model is induced using alloxan that selectively destroys the insulin-producing pancreatic beta –islets.[37] This study was intended to evaluate the protective and ameliorative potential of 100mg/kg bw M. oleifera aqueous seed extract on 120mg/kg bw alloxan induced diabetic Adult male Wistar rats

METHODOLOGY

Experimental animals' procurement and Care:

Twenty male Wistar rats (*Rattus norvegicus*), six weeks old, weighing between 150 -200 g were used for this study. They were obtained from the Animal Facility of the Department of Anatomy, Bingham University, Karu, Nasarawa State, Nigeria. They were cared for according to ethics in "*Guide for the Care and use of Laboratory Animals*.[38] They were kept in well ventilated rat metallic cages, in a standard laboratory condition (12 h light: 12 h dark cycle; temperature- 37.5˚C, humidity of 50%) and given pelleted rat feed (UAC, Vital Feeds, Jos, Nigeria) and water *ad libitum*. They were allowed to acclimatize for two weeks before experimentation.

Plant of Study *Moringa oleifera* seed and preparation of Aqueous Extract:

Fresh seeds of *M. oleifera* obtained from Bingham University, Karu, Nasarawa State, Nigeria, were harvested from their pods. The *M. oleifera* plant pod and seeds were authenticated by a botanist in the Department of Biological Sciences, Bingham University. The fresh seeds were trimmed, washed and air-dried in an open space. Thereafter, the clean dried white seeds of *M. oleifera* were pulverized into a fine homogeneous powder using a blender to obtain a 50 g of *M. oleifera* powder, out of which 25g was weighed and macerated in 1000 ml of distilled water for 72 h According to Agoreyo et al[39] and Obembe and Raji,[40] methods. The solution was stirred using a Vortex every six hours. Aqueous extraction method was done according to Idris et al.[41] report that water is a universal solvent in the extraction of phytochemicals from the seed because of its ease extraction and non-toxicity. The powdered seeds mixture was filtered and the filtrate was then concentrated to dryness over a hot water bath at 40°C. Freshly prepared *M. oleifera extract* was stored in a glass container and kept in the refrigerator prior to use. 100 mg/kg bw aqueous seed extract of *M. oleifera* was given orally to experimental animals according to Al-Maliki and Rabey.[42]

Figure 1: Picture of the *Moringa oleifera.*

Diabetic Rat Model: Alloxan induced

Alloxan monohydrate (Sigma-Aldrich®, St. Louis, USA). Diabetes was induced with alloxan monohydrate in saline (0.9% NaCl) at a dose of 120 mg/kg b.w. intraperitoneal injection.[43,44] Alloxan induces diabetes by damaging β-cells of the islets of Langerhans of pancreas the insulin secreting cells leading to hyperglycaemia.[45,46] According to Ajibola *et al.*,[37] Alloxan administered to experimental animals caused a reduction in plasma insulin concentration as a result of necrosis in the Insulin secreting Beta cells of experimental diabetic animal model. Experiments have reported that an hour following alloxan administration, there was an increase in blood glucose level, a decrease in insulin concentration and necrosis of the pancreatic beta cells.[37,47] In this study, DM animal model were fasted for 12 h, then given a single dose of intra-peritoneal injection of freshly prepared alloxan solution using normal saline according to Jarald et al.,[43] and Naseer and Muhammad,[48] methods. Diabetic condition was confirmed 24 h after alloxan injection. Not all the rats were diabetic at 24 h, but they were all found to be diabetic after 72 h similar to reports obtained from Ene et al.[49] DM condition was confirmed using a

glucometer by taking blood from the tail vein and animals with fasting blood glucose (FBG) higher than 220mg/dL was considered diabetic and included in the study.[48]

Standard Anti-diabetic drug

Glibenclamide (manufactured by Nigerian-German Chemicals Plc, Km 38, Lagos-Abeokuta Expressway, Sango-Otta, Ogun State) was used as a standard anti-diabetic drug. Glibenclamide at 5 mg/kg b.w. was used as a reference drug.[43,48]

Experimental design and Animal grouping

Group A: Control group given normal saline for 14 days, feed and water *ad libitum*.

Group B: Diabetic Animal Model (120mg/kg single Alloxan intraperitoneal injection) and they were fasted overnight (12 -14 h) before and after alloxan treatment. According to Federiuk et al., 2004; Onyagbodor and Aprioku, 2017 method. Blood glucose level was measured at the beginning and twice weekly for 2 weeks.

Group C: *M. oleifera* group was given 100 mg/kg bw *M. oleifera* aqueous seed extract orally for 14 days, feed and water *ad libitum.*

Group D: *M. oleifera* treatment Diabetic Model- Animals were pretreated with 120mg/kg single Alloxan intraperitoneal injection 24 hours before given 100 mg/kg bw *M. oleifera* for 14 days, feed and water *ad libitum.*

Group E: Glibenclamide treatment Diabetic Model- Animals were pretreated with 120mg/kg single Alloxan intraperitoneal injection 24 hours before given 5 mg/kg body weight of Glibenclamide for 14 days, feed and water *ad libitum.*

Serum/ Blood glucose concentration was monitored at the beginning and twice weekly after alloxan administration[50] method using a acu-check glucometer. The treatment went on daily for fourteen days and the experimental animals were allowed to have access to food.

Animal Sacrifice and pancreatic tissue collection

Final weight was taken using a top loading digital scale (NV2101 model, OHAUS Corporation USA) following last dose or treatment. Animals were euthanized via cervical dislocation. Abdominal incision was made to expose the pancreas which was carefully dissected and excised Weight wet taken using digital scale. Pancreatic tissue were fixed in a 5ml specimen bottle containing 10% formol saline ready for histological tissue processing according to Bancroft and Gamble methods.

Pancreatic Tissue histopathological analysis using Hand E and Masson trichrome stain

The organs were dehydrated in graded concentrations of ethanol. The tissues were cleared in xylene before they were transferred into two changes of molten paraffin wax for 1h each in an oven at 60° for infiltration. They were subsequently embedded and serial sections cut using rotary microtome (LEICA) at 5 µm. The slides were cleared with xylene and passed through absolute alcohol (2 changes); 70% alcohol and then to water for 5min. the slides were then stained with haematoxylin and eosin (H and E). The slides were mounted using DPX and allowed to dry on hot plate for 2min.

Pancreatic tissue Collection for quantitative enzyme analysis: Pancreatic tissue were preserved in 5% sucrose solution, homogenised (using an automated homogenizer) and an aliquot centrifuge at 5, 000 rpm for 10 minutes for tissue spectrophotometry analysis. Pancreatic cells Supernatant aliquots were stored at -20C for enzyme analysis. Qunatitiave enzyme analysis for superoxide dismutase (SOD) and Malondialdehyde (MDA) was done using biochemical enyme kit via Spectrophotometric analysis methods.

Superoxide dismutase (SOD) Quantitative spectrophotometric analysis procedure

SOD analysis. SOD was determined in terms of its ability to catalyze the disproportionation of alkaline aqueous solution. The disproportionation was directly studied in a spectrophotometer, For SOD assay, the tissue homogenate 1:4 for pancreatic tissues was prepared in ice-cold 0·25 M sucrose solution containing 5% The crude homogenate was centrifuged at 34,880 *g* for 30 min and the supernatant was used. Sucrose (0·25 M) did not interfere with the SOD assay. The amount of tissue homogenate needed for SOD assay by the pyrogallol method was 2–5 μl in the case of pancreas.

Malondialdehyde (MDA) Quantitative spectrophotometric analysis procedure

Malondialdehyde levels in tissue were measured according to the protocol outline by Stocks and Domandy[51] as shown below. 0.1ml of homogenate was pipette into a plastic test tube. 1ml of 20% Trichloroacetic was added to it. The mixture was mixed and centrifuged at 2000g for 5mins. 0.5ml of the supernatant was pipette into a pyrex test tube. 0.05ml of 10.0umol/L of 1,1,3.3 – Tetramethoxylpropane was pipette into another pyrex test tube (std). 0.5ml of trichloroacetic acid solution and were stopped tightly. The tubes were heated in a water bath at 100^{o}C for 20minutes. All tubes were cooled in water. The spectrophotometer was blanked using the reagent blank at 532nm. Absorbance of tests and standard were read

$$\text{Tissue Malondialdehyde} = \frac{(\text{Absolute of test} \times 10)}{\text{Absolute of standard}} \times 10\text{umol/l}$$

Malondialdehyde; (MDA) by the thiobarbituric acid assay.[52]

Determination of MDA, GSH and SOD levels in heart, liver, spleen and kidney tissues of normal and diabetic rats without and with ECE treatments were performed by following instructions of their corresponding commercial kits. Results of these three measurements were expressed

as µmol/mg protein, µmol/g protein and kNU/g protein, respectively.

Statistical analysis

The results are expressed as mean ± SEM. Statistical analysis was done using SPSS version 17.0 via ANOVA software. Comparison between the control and diabetic control group was made with unpaired Students *t* test. Comparison between test groups and diabetic control was made with one way analysis of variance (ANOVA) followed by student T-test. Significance was considered at $p<0.05$.

Slides analysis and photomicrography

Photomicrographs were analysed using light microscope (Olympus, Japan). Using eyepiece lens of x 10. Each slide was viewed and photomicrography taken with attached colour digital camera using objective lens of x40.

RESULTS

Changes in Body weight in Diabetic rat Model and MO treated rats

There was a significant decrease in final body weights of the Group B (DM Model) when compared with Control (A) at $p<0.05$. Group C (MO) treated had s significant increase in final body weight when compared with A, B, D and E (Fig. 2) at $P<0.05$. However, Group D had a reduction in body weight gain when compared with B ($P<0.05$). Our study shows that Group E had a significant reduction in final body weight when compared with D, A and B at $p<0.05$.

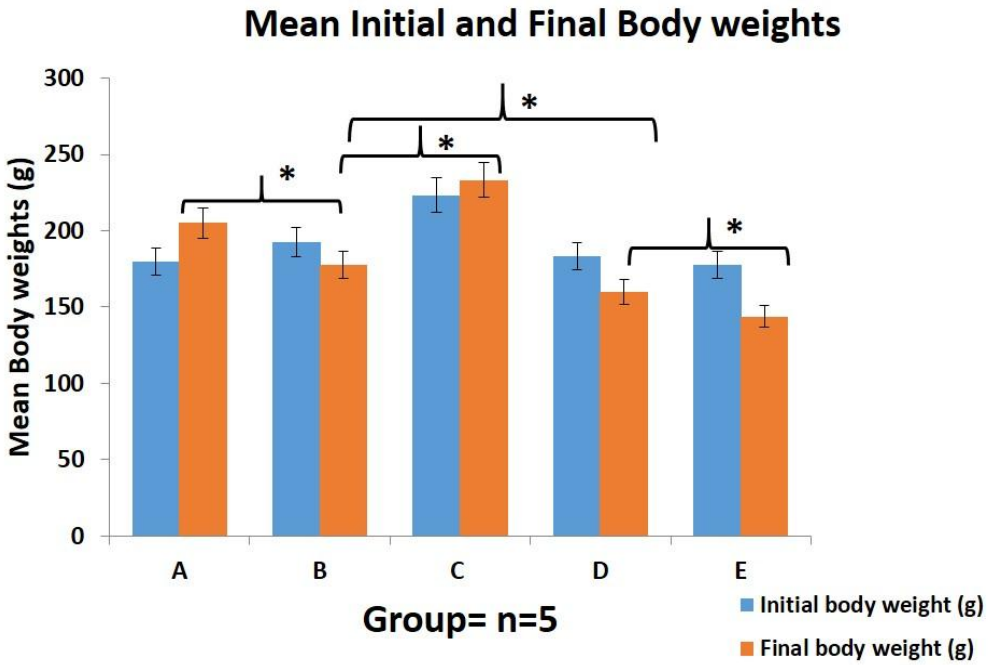

Figure 2: Graphical representation of Mean Initial and Final body weights of experimental animal of study. Data expressed as Mean ± Standard Error of Mean (SEM). Statistics was done using one way analysis of variance (ANOVA) followed by student T-test. Statistical Significance (*) was considered at p<0.05. Legend:- Group A: Control group, Group B: Diabetic Animal Model (120mg/kg single Alloxan intraperitoneal injection), Group C: Aqueous seed extract of *M. oleifera* (100 mg/kg bw) Group D: *M. oleifera* treatment Diabetic Model (120mg/kg Alloxan + 100 mg/kg bw Aqueous seed extract of *M. oleifera,* Group E: Glibenclamide treatment Diabetic Model- Animals were pretreated with 120mg/kg single Alloxan intraperitoneal injection 24 hours before given 5 mg/kg body weight of Glibenclamide for 14 days, feed and water *ad libitum*.

Changes in pancreatic weight in Diabetic rat Model and MO treated rats

Mean wet weight of Pancreas of Group A increased significantly as compared with B, C, D and E at $p<0.05$. However, the Diabetic model (B) showed a significant reduction in wet weight when compared with A, C, D and E @ $P<0.05$ (Fig 3). Group D (MO treated DM model) had a significant increase in mean organ weight when compared with B (DM model) at $P<0.05$. Group D-MO treated DM model increased Pancreas weight as compared with B. Although Group E showed an increase in pancreas wet weight D had a significant increase as compared to D at $p<0.05$.

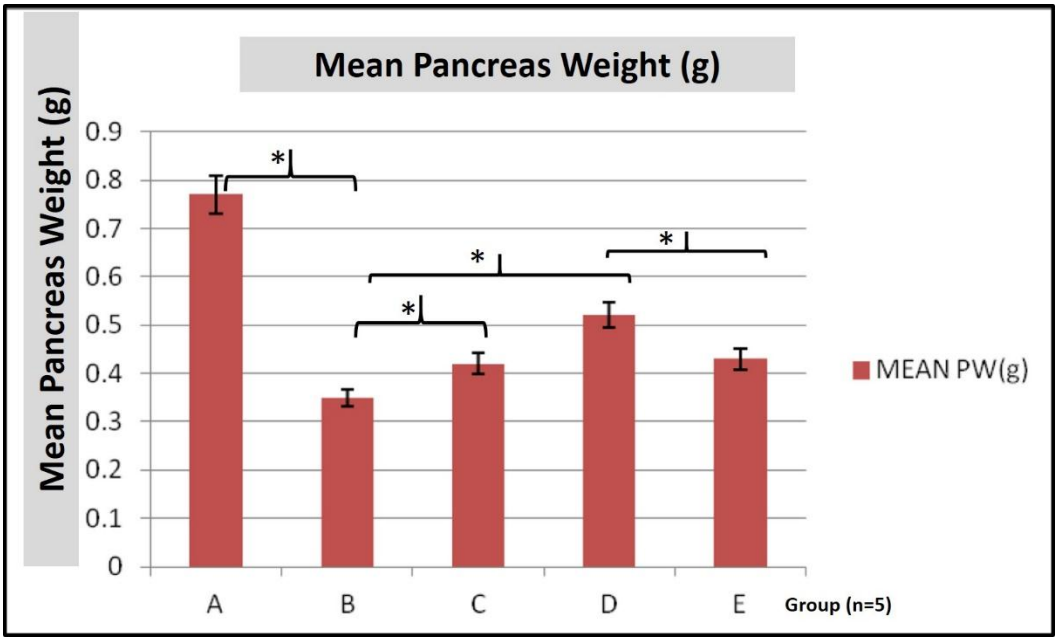

Figure 3: Graphical representation of Mean Pancreas weights of experimental animal of study. Data expressed as Mean ± Standard Error of Mean (SEM). Statistics was done using one way analysis of variance (ANOVA) followed by student T-test. Statistical Significance (*) was considered at p<0.05. Legend:- Group A: Control group, Group B: Diabetic Animal Model (120mg/kg single Alloxan intraperitoneal injection), Group C: Aqueous seed extract of *M. oleifera* (100 mg/kg bw) Group D: *M. oleifera* treatment Diabetic Model (120mg/kg Alloxan + 100 mg/kg bw Aqueous seed extract of *M. oleifera,* Group E: Glibenclamide treatment Diabetic Model- Animals were pretreated with 120mg/kg single Alloxan intraperitoneal injection 24 ours before given 5 mg/kg body weight of Glibenclamide for 14 days, feed and water *ad libitum*

Aqueous seed extract of MO elevates SOD Antioxidant activity in the Pancreas

In this study, antioxidant activity of MO was tested by assessing SOD antioxidant profile. It was deduced that Group B (DM model) had a significant reduction in SOD profile when compared with A, C, D and E at P<0.05. in Group C, SOD activity increased as compared with B. Groups D and E respectively showed an increase in SOD activity as compared to B and C (Fig 4) at p<0.05. *Moringa Oleifera* (MO) treated groups had significant increase in SOD activity in pancreatic tissue milieu when compared with Control (A) and Alloxan induced Diabetic animals.

Aqueous seed extract of MO reduces lipid membrane peroxidation in the pancreatic Islet cells as demonstrated by MDA profile

In this study, demonstrated the attenuation of lipid peroxidation by Aqueous MO seed extract (Fig 4). Group B (DM model) had a statistical significance elevation of MDA activity in the pancreatic tissue milieu, this was reversed by MO (seen in Groups C and D). MO had a statistical significant reduction in lipid peroxidation seen in Group D when compared with B.

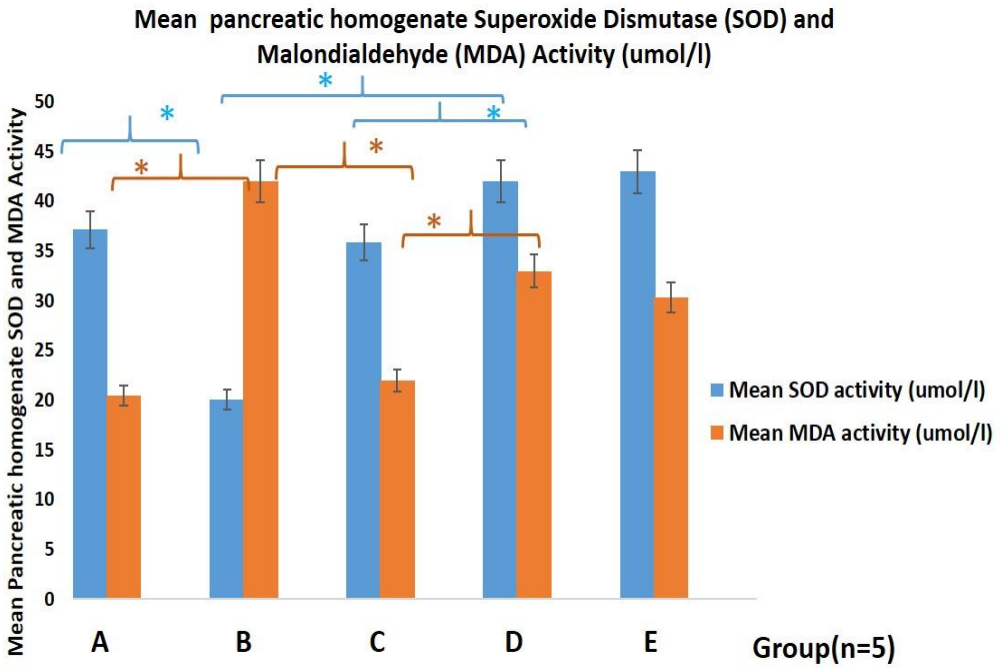

Figure 4: Graphocal represntation of Mean SOD antioxidant profile versus Mean MDA lipid peroxidation enzyme marker in the Homogenate of pancreas tissue of experimental aimals. Data expressed as Mean ± Standard Error of Mean (SEM). Statistics was done using one way analysis of variance (ANOVA) followed by student T-test. Statistical Significance (*) was considered at $p<0.05$. Legend:- Group A: Control group, Group B: Diabetic Animal Model (120mg/kg single Alloxan intraperitoneal injection), Group C: Aqueous seed extract of *M. oleifera* (100 mg/kg bw) Group D: *M. oleifera* treatment Diabetic Model (120mg/kg Alloxan + 100 mg/kg bw Aqueous seed extract of *M. oleifera*, Group E: Glibenclamide treatment Diabetic Model- Animals were pretreated with 120mg/kg single Alloxan intraperitoneal injection 24 hours before given 5 mg/kg body weight of Glibenclamide for 14 days, feed and water *ad libitum*.

Aqueous seed extract of MO protects Islet Cells of Langerhans (Beta Insulin Secreting cells) from oxidative tissue damaged mediated by Alloxan induced necrosis (H and E stain) and loss basement membrane integrity (Masson trichrome stain)

We demonstrated the histological appearance of the Islet cell using H and E stain (Fig 5). It shows that Control A has well clustered Islet Cells, absence of necrosis and vacuolation within it and its surrounding exocrine pancreatic cells (Fig 5A) as compared to B- treated with Alloxan showing gross disruption of the Islet cells, presence of vacuolation within it and its surrounding Exocrine cells (Fig 5B). MO treated (C) had normal arrangement similar to the control. However, Groups D and E displayed various degree of repair from Alloxan assault in the presence of MO and Glibenclamide (Fig 5D and 5E).

We demonstrated masson trichrome highlighting greenish colouration of collagen, which was poorly demonstrated in group B (Fig 6B) when compared to A, C, D and E as seen in Fig 6 showing well highlighted collagen between the Islet cells and exocrine cells.

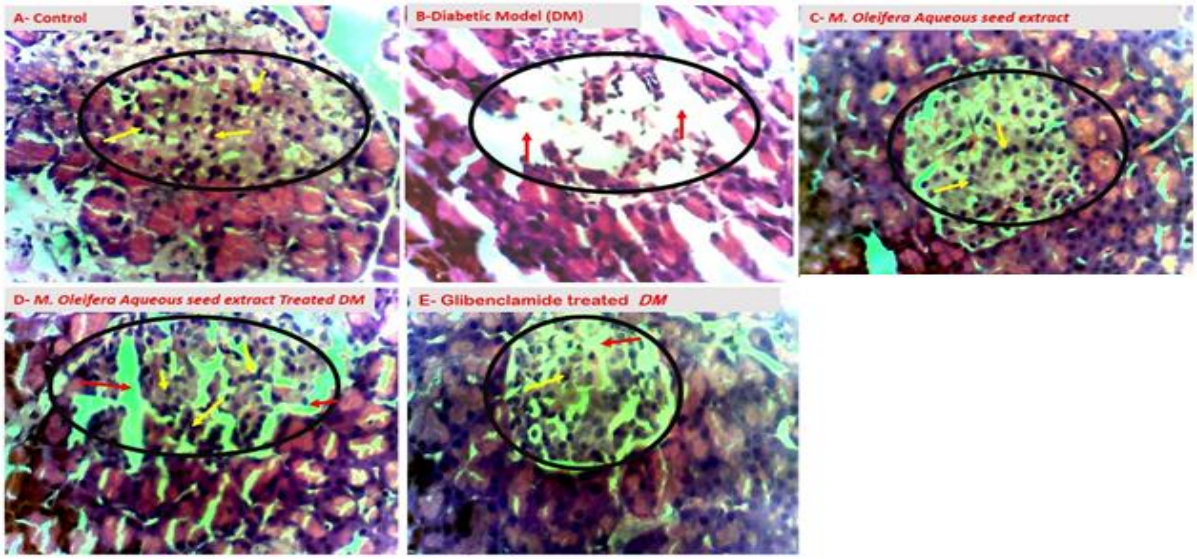

Figure 5: Photomicrograph of a cross section of the pancrease of Adult Male Wistar rats. Control group A, Shows well clustered Islet Langerhans cells surrounded by the exocrine acinar cells. Legend:- Group A: Control group, Group B: Diabetic Animal Model (120mg/kg single Alloxan intraperitoneal injection), Group C: Aqueous seed extract of *M. oleifera* (100 mg/kg bw) Group D: *M. oleifera* treatment Diabetic Model (120mg/kg Alloxan + 100 mg/kg bw Aqueous seed extract of *M. oleifera,* Group E: Glibenclamide treatment Diabetic Model- Animals were pretreated with 120mg/kg single Alloxan intraperitoneal injection 24 hours before given 5 mg/kg body weight of Glibenclamide for 14 days, feed and water *ad libitum. Black Circle: Islet Cell of Langerhans, Yellow arrows: Nuleus of the Beta Cells and Red arrows: Vacuolation within the Islet cells.* Histological displayed using H and E stain at Mag x 400 Light Microsocpy. Scalebar x50 µm a) Pancreas of rat from the negative control group showing no histopathological changes, (b) pancreas of rat from the diabetic positive control (G2) group showing necrosis and vacuolations of pancreatic acini and Langerhans islets cells (arrow), (c) pancreas of diabetic rat treated with 100 mg/kg b.w. MO showing mild histopathological repair and (d) pancreas of diabetic rat treated with Glibenclamide) showing repair of the Islet cells (H&E ×400) Scale bar x50 µm.

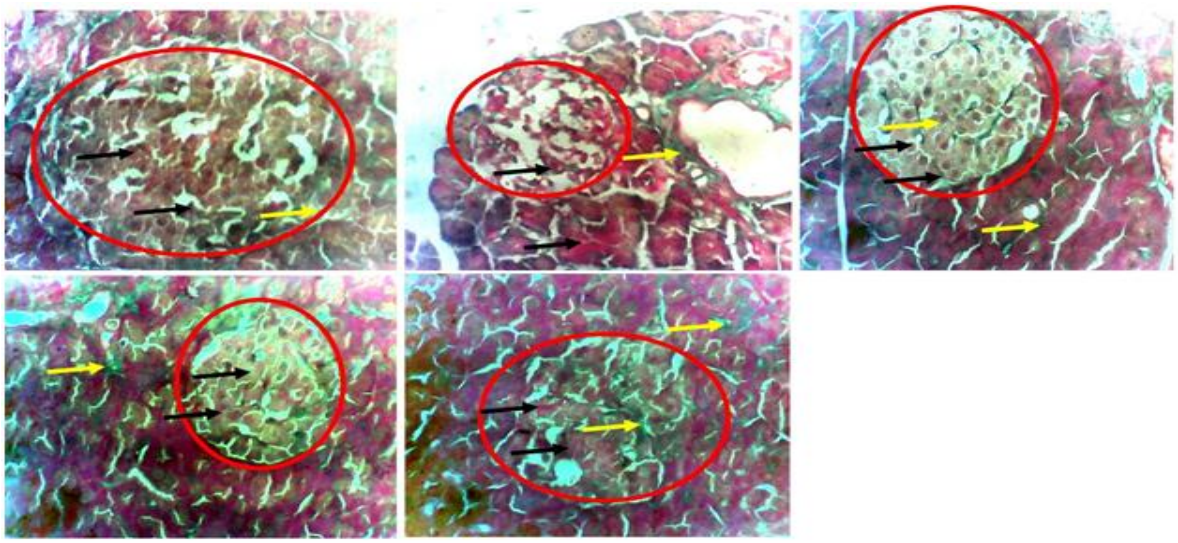

Figure 6: Photomicrograph of a cross section of the pancrease of Adult Male Wistar rats. It was obserevd that groups A, C, D and E clearly demonstrate Collagen fibres as comapre to B. Legend:- Group A: Control group, Group B: Diabetic Animal Model (120mg/kg single Alloxan intraperitoneal injection), Group C: Aqueous seed extract of *M. oleifera* (100 mg/kg bw) Group D: *M. oleifera* treatment Diabetic Model (120mg/kg Alloxan + 100 mg/kg bw Aqueous seed extract of *M. oleifera,* Group E: Glibenclamide treatment Diabetic Model- Animals were pretreated with 120mg/kg single Alloxan intraperitoneal injection 24 hours before given 5 mg/kg body weight of Glibenclamide for 14 days, feed and water *ad libitum. Black Circle: Islet Cell of Langerhans, Yellow arrows: Nuleus of the Beta Cells and Red arrows: Vacuolation within the Islet cells and* Collagen fibres stained green. Masson and Trichrome stain at Mag x 400 Light Microsocpy. Scalebar x50 µm.

DISCUSSSION

Moringa has long been recognized in traditional medicine worldwide as having value both as a preventative and treatment agent of several health conditions, including the treatment of inflammation, infectious diseases, cardiovascular, gastrointestinal and haematological disorders.[53] The protective role of MO against alloxan induced toxicity was assessed by stress markers MDA, antioxidant enzymes SOD, and histology demonstration of Islet cells and histochemistry of collagen fibre using Masson Trichrome stain. Diabetes is a major health problem which according to International Diabetes federation a total of 382 million people are currently suffering from this disorder and this a chance it will increase to 592 million by 2035[54] hence the need to continuous search for alternative therapy. Notably, alloxan is far less expensive and more readily available than streptozotocin. On this ground, one will logically expect a preference for use of alloxan in experimental diabetes studies, diabetogenicity of alloxan is underlined by its selective cellular uptake by beta cells of the pancreas and consequent accumulation in these cells.[55] This animal model provide an important tool for study of the pathogenesis, prevention and treatment of diabetic complications.[56] In adult rats, diabetes is accompanied with a reduction in body weight.[57] Alloxan induced diabetes significantly decreased body weight of the diabetic untreated rat (Fig 2) during treatment as compared to the control and MO treated groups. It supports reports that DM is associated with increased glycogenolysis, lipolysis, gluconeogenesis and this events results in loss of tissue protein and muscle wasting.[58] Significant weight loss is a clinical feature of DM which is associated with the degeneration of the adipocytes and muscle tissues to make up for the energy lost from the body due to frequent urination and over conversion of glycogen to glucose, this condition is a very serious issues in the management of diabetes mellitus.[59,60] Alloxan induced loss of weight in our study support other reports.[57,61] Although reports have that antioxidant and antidiabetes agents suppresses loss of body weights,[61] that was not notable in this study. The reduction in body weight observed in D and E is probably associated with reduced initial weight at the beginning of the study or changes in appetite of experimental animals during the study. We can also deduce that MO treatment had no significant effect on differences between initial and final weight of Groups D. However, report made by Al-Maliki and Rabey,[42] who stated that 100 mg/kg *Moringa* seeds powder significantly increased the body. Obembe and Raji,[40] also reported that 100mg/kg of MO seed extract increases body and organ weight. MDA is one of the most prevalent byproducts of lipid peroxidation during oxidative stress, therefore the content of MDA could be used as an index of the lipid peroxidation degree.[62-64] In diabetes, chronic hyperglycemia can induce carbonyl stress, which would lead to the increase of lipid peroxidation.[65] Alloxan induced DM caused an elevation in MDA as compared to the control groups.[64] But MO treated attenuates this elevation which is linked to its antioxidant property as a results of its richness in polyphenolics and/or flavonoids phytochemicals which have been reported to be effective against ROS-related damage by their antioxidant activities. This supports reporrs by Al-Maliki and Rabey,[42] where 100 mg/kg of *Moringa* seeds reduces lipid peroxides in serum and Tissue homogenate. Phenolics are good for scavenging various free radicals leading to reduced lipid peroxidation.[42] The antioxidant activity of *Moringa* seed powder is due to its content of phenolics and flavonoids that have scavenging effect on the free radicals .These three phytochemicals of *Moringa* possess antioxidant, hypoglycemic, hypotensive, antidyslipidemic, anticancer, and anti-inflammatory properties. The antidiabetic activity of the higher dose of *Moringa* seeds powder (100 mg/kg b.w.) was more efficient.[42] Over the years, studies have evidently documented that oxidative stress involvement in the pathogenesis of DM.[66] An elevation in the serum or tissue homogenate concentration of free radicals and increased lipid peroxidation would inevitably result in decrease of antioxidant defense ability in biological systems milieu.[64] SOD is a strong antioxidant vital to mob off free radicals to overcome oxidative stress.[67] Our study shows that alloxan Diabetic model had a significant reduction in SOD activity linked with the recorded elevation in MDA, which shows that the alloxan has activated ROS generation leading to disruption of lipid bi-layer of Islet cells increasing perioxdiation of lipids.

This reports support similar study from Ohnishi et al[68] and Yin et al.[64] Degradation of antioxidant enzymes such as SOD, catalase and glutathione peroxidase, and their associated co-factors are known to be essential for decrease of pancreatic insulin secretion.[68] Elevated SOD activity in MO treated contributes to regeneration or protection of the pancreatic Islet cells making them viable in the presence of alloxan toxicity to continue secretion of insulin required to avert hypoglycaemia. This proposed mechanism of action of MO via antioxidant activity support report of Ohnishi et al[68] and Al-Maliki and Rabey,[42] 100 mg/kg of *Moringa* seeds powder can increase SOD activity and other antioxidant enzymes in the serum and tissue homogenate. The antidiabetic activity of Moringa seed powder has been observed in rat models with the decreased glucose and the amelioration of levels of lipid peroxide, the diminish levels of IL6, and immunoglobulins A in comparison with diabetic positive control in both insulin resistant and insulin deficient bioassays.[24,69] Pancreas of the control rats shows the normal lobular histological structure of pancreatic acini and Langerhans islets cells (Fig 4A). Langerhans islets are interspersed among the pancreatic acini, as compact spherical masses with intact interlobular connective tissue and interlobular ducts. But alloxan caused a disruption of this normal architecture Figure 4(b) shows pancreatic tissues of diabetic rat with necrosis and vacuolations of pancreatic acini and

Langerhans islets cells. Treatment with 100 mg/kg b.w of *Moringa* seeds powder restored the pancreatic tissues to their normal histology with no histopathological changes as shown in Figure 4(c). This report is similar to that obtained from Al-Maliki and Rabey.[42] Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycaemias.[70] Alloxan, a β-cytotoxin destroys the β-cells of the islets of Langerhans of pancreas.[46] Treatment with Moringa has showed after histological examination of pancreas from diabetic rats, a significantly damage reversed in the histoarchitectural of the islet cells.[71] In the present study, the anti-diabetic activity of the aqueous extract of M. oleifera leaf was evaluated in alloxan-induced diabetic male Wistar rats. Moringa leaf possesses protective and ameliorative antidiabetic potential in rats.[50] Significant difference was observed in the islet of Langerhans of the diabetic untreated group and diabetic treated groups, showing the regenerative action of MO on the islet of Langerhans. Glibenclamide helped to upgrade activity of *β*-cells of the pancreas for higher release of insulin. Aqueous seed extract of MO shows its antidiabetic effect by not just mobbing off free radicals but upgrading the sensitivity of *β*-cells to glucose, secreting the elevated insulin. Sections of pancreas were stained with Masson tri-chrome stain[72] to demonstrate Collagen fibres. Masson's trichrome, in control animals the green staining collagen fibers are seen around the interlobular and interlobar pancreatic ducts, in diabetic rat, collagen fibers stained green increased in the periductal collagen fibers within the interlobular and interlobar connective tissue and aslo within the exocrine acinar cells this is associated with changes in Islet cell function linked to fibrosis and thickening of islets capillaries as reported by Abdelrahim.[73] However, treatment with MO and GB showed few collagen fibers around the islets and pancreatic acini compared with the diabetic group.

CONCLUSION

Collectively, the mechanism behind is intestinal glucose inhibition, insulin release as well as decrease in insulin resistance probably regeneration of β-cells of pancreas, increase in glutathione and reduction in malondialdehyde.

Conflict of interest statement

We declare that we have no conflict of interest.

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