

Research Article

***Moringa oleifera* ameliorates nephropathic changes in alloxan-induced diabetic adult wistar rats.**

H.B. Akpan, A.A. Akande, A.O. Ojewale, F.E. Oladipupo, O.F. Akinpelu and S.O. Jimoh

Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ikenne Campus, Ogun State, Nigeria.

Keywords:

Kidney, Alloxan, diabetes, nephropathy, Moringa oleifera, microarchitecture

ABSTRACT

Background: Diabetic nephropathy has been identified as a leading cause of chronic kidney disease which is a risk factor for kidney failure. *Moringa oleifera* (MO) is popularly known to possess various nutritional and health benefits. This study investigated the effects of crude aqueous extract of *Moringa oleifera* on the kidney of alloxan-induced diabetes in adult wistar rats. **Methodology:** Fifty six (56) adult wistar rats (150 – 200g) were randomly divided into 7 groups (n=8) with group A as control group, while animals in groups B, C, D, E and F were induced with multiple dosage of alloxan monohydrate (100mg/kgbw) intraperitoneally. Group B served as the diabetic group and animals in groups C, D and E were administered 100, 300 and 500 mg/kgbw of MO respectively. Further, animals in group F which received Diabinese (15mg/kgbw) served as the pharmacological control group. Group G animals were given 100mg/kgbw of aqueous extract of MO before induction of diabetes. The parameters assessed in this study include animals' weight, blood glucose levels, serum creatinine levels, and kidney histology. **Results:** The results showed that serum creatinine levels were increased as a result of diabetic nephropathy but reduced with MO administration. Histologically, kidney sections from the diabetic group presented with glomerular sclerosis, wide capsular spaces, thickening of Bowman's capsule, tubular necrosis, focal areas of massive inflammatory cells infiltration and acellular material. MO treated groups showed glomeruli and tubules in various stages of tissue repair varied on a dose dependent basis, higher concentrations being more effective. Sections from animals pretreated with MO showed that *Moringa oleifera* appeared to have slowed the action of Alloxan on the kidney. **Conclusion:** This study, therefore, concluded that treatment with *Moringa oleifera* ameliorated the acute effects of alloxan-induced diabetic complications on renal microarchitecture and probably contributed to the restoration of morphology and hence the functions of the kidney in adult Wistar rats.

© Copyright 2018 African Association of Physiological Sciences -ISSN: 2315-9987. All rights reserved

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder in the endocrine system resulting from a defect in insulin secretion, insulin action or both of them. DM contributes to several kinds of complications including diabetic retinopathy, nephropathy and neuropathy (Valmadrid *et al.*, 2000). Notably is diabetic nephropathy which is the leading cause of chronic kidney disease in patients starting renal replacement therapy and is associated with increased cardiovascular mortality (Valmadrid *et al.*, 2000).

Diabetic nephropathy has been classically defined by the presence of proteinuria (protein levels exceeding >0.3g/24 h). This stage has been referred to as overt nephropathy, clinical nephropathy, proteinuria, or macro-albuminuria (Busko, 2006). In the early 1980s, seminal studies from Europe revealed that small amounts of albumin in the urine, not usually detected by conventional methods, were predictive of the later development of proteinuria in type 1 and type 2 diabetic patients (Mogensen *et al.*, 1984). This stage of renal involvement was termed microalbuminuria or incipient nephropathy. Many traditional plant treatments for diabetes are used throughout the world but most of the evidence for their beneficial effects is anecdotal. However, after the introduction of insulin

*Address for correspondence:

Email biz.monic@yahoo.com

Tel: +2348065700406

therapy the use of traditional treatment for diabetes greatly declined, although some traditional practices are continued for prophylactic purpose and adjuncts to conventional therapy (Udoamaka *et al.*, 2013). In some societies there are strong desires to use herbs or plants for treatment, due to less side effects, easier consumption or availability and presumably low cost. However, very few of the traditional treatments for diabetes have received scientific or medical scrutiny and several have been shown to assist glycemic control in non-insulin dependent form of diabetes (Collier *et al.*, 1987).

Plants may act on blood glucose through different mechanisms; some of them may have insulin-like substances (Gray and Flatt, 1999), some may inhibit insulinase activity, others may hence increase beta cells proliferation in pancreas by activating regeneration of these cells (Abdel *et al.*, 1997). The fiber of plants may also interfere with carbohydrate absorption, thereby affecting blood glucose (Nelson *et al.*, 1991). In type-2 diabetes, there is a gradual development of insulin resistance and beta cell dysfunction, strongly associated with obesity and a sedentary lifestyle (Haffner *et al.*, 2000).

Moringa Oleifera (MO) belongs to Moringaceae family and is commonly known as Moringa or drumstick tree in English. Furthermore, in Nigeria, it is known by different names in various tribes, such as: Okwe oyibo in Igbo, Gwara or Habiwal in Hausa and Adagba maloye or Ewe Igbale in Yoruba. MO grows rapidly in most regions of Nigeria (Anwar *et al.*, 2007). It can be used for water purification and hand washing, and is sometimes, also used in herbal medicine (Folkard *et al.*, 1999). *Moringa oleifera* is an important food commodity which has had enormous attention as the 'natural nutrition of the tropics' (Anwar *et al.*, 2007). Its anti-mutagenic, anti-tumour, anti-bacterial and anti-inflammatory activities have been demonstrated (Siddhuraju and Becker, 2003). Its protective activity in simultaneous and post-treatment in relation to organ damage has also been demonstrated (Rajangam, 2001). For centuries and in many cultures around the world, the medicinal usage of *Moringa oleifera* has been employed in the treatment of asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera and many other illnesses (Khawaja *et al.*, 2010; Hamza, 2010; Singh *et al.*, 2012). It has also long been labelled for its great cosmetic value and in recent years, it has commonly been found to be used in various health care products including body and hair moisturisers and conditioners (Anwar *et al.*, 2007). It was also

discovered that *Moringa oleifera* oil was used in skin ointments ever since the Egyptian times by "Trees for Life" organization and was claimed to be 'the most nutrient-rich plant yet discovered' by Khawaja *et al.* (2010).

Nutritional composition of *Moringa oleifera* include many essential nutrients like, vitamins, minerals, amino acid, beta-carotene, antioxidants, anti-inflammatory nutrients and omega 3 and 6 fatty acid (Fahey, 2005; Hsu *et al.*, 2006; Kasolo *et al.*, 2010). These components have been reported to play essential roles in its medicinal and therapeutic properties (Al-Kharusi *et al.*, 2009). In vitro and in vivo studies with the plant have recommended its effectiveness in treating inflammation, hyperlipidemia, and hyperglycemia (Fahey, 2005; Mbikay, 2012).

Despite the widespread use of aqueous leaves extract of MO as a nutraceutical to manage DM and its complications, its protective effects on the renal system has not been elaborated. Therefore, the present study was designed to investigate some effects of aqueous crude extract of MO leaves on the kidney of alloxan - induced diabetes in adult wistar rats.

MATERIALS AND METHODS

Plant Material

Fresh *Moringa oleifera* leaves were collected from Twotees farm at Boluwaji, Ibadan, Nigeria. The plant was authenticated at the Forest Research Institute of Nigeria (FRIN), Ibadan and a voucher specimen was deposited in their herbarium with the herbarium number FHI. 110283.

Plant extraction

The plant leaves were washed, air dried under shade and grinded into fine powder using an electric blender. The aqueous crude extract was prepared by extracting the powdered leaves (100g) with 1litre of distilled water in an electric shaker for 48 hours. After 48 hours, the extract was filtered through Whatman filter paper No. 4 and evaporated under reduced pressure using a rotary evaporator. The residue was kept in a bottle with a tight-fitting cover.

Animals

All animal experiments were carried out under protocols approved by the Institutional Animal House of the Olabisi Onabanjo University, Ago-Iwoye, Nigeria. Fifty six rats ranging in weight from 150-200g of both sexes were used for this study. These rats were randomly assigned to seven groups of eight (n=8) animals each as groups A, B, C, D, E F and G.

Acclimatization of experimental animals

The animals were housed in plastic cages and acclimatized for a period of 2 weeks (14 days) in the research laboratory of the Faculty of Basic Medical Sciences, Olabisi Onabanjo University in strict compliance with the animal handling ethics of the University. This acclimatization was done to enable the rats adapt to their new environment before the commencement of the experiment. The rats were fed with rat feed and water *ad libitum* throughout the research period.

Induction of Diabetes

Alloxan was dissolved in 0.9% normal saline and diabetes was induced at 100mg/kg body weight by intraperitoneal injection of a freshly prepared solution.

intraperitoneal injection of a freshly prepared solution. Animals in Groups B, C, D, E, F and G were induced. Induction was monitored by taking blood glucose level measurements at zero hour (prior to induction) and forty-eight (48) hours after induction. Animals with fasting glucose level greater than 200mg/dl after 48 hours were considered diabetic. Thereafter, the animals were followed up for three weeks with a weekly assessment of the blood glucose levels. The blood sample was obtained by sequential snipping of the tail (Fluttert *et al.*, 2000) and a glucometer (Accu-Check, by Roche, manufactured in USA) was used to measure the blood glucose levels using Accu-Check customized glucose strips.

Table 1: Experimental design

Groups	Alloxan administration (100mg/kgbw)	Duration (Days)	Moringa <i>oleifera</i> intervention (mg/kg/bw)	Diabinese	Duration (Days)
A-Control	NO	42	NO	NO	NO
B-Alloxan	YES	42	NO	NO	NO
C-Alloxan+100mgMO	YES	21	100	NO	21
D-Alloxan+300mgMO	YES	21	300	NO	21
E-Alloxan+500mgMO	YES	21	500	NO	21
F-Alloxan+Diabinese	YES	21	NO	YES	21
G-100mgMO+Alloxan	YES	21	YES(Pre-treated)	NO	21

Post- treated)

Intervention and administration of extract and drug

After three (3) weeks of uninterrupted diabetic condition, animals in groups C, D and E were treated orally with graded doses of aqueous extract of MO at low dose (100mg/kgbw), medium dose (300mg/kgbw), and high dose of 500mg/kgbw respectively and to group F, Diabinese (15mg/kgbw) a conventional drug using orogastric gavage for another three (3) weeks. Diabinese served as the pharmacological control agent.

Sacrifice and serum collection

At the end of six acid weeks, all animals were sacrificed under exsanguination. Blood was collected by cardiac puncture with 5ml syringe and needle from the apex of the heart. The blood withdrawn was expelled into plain serum bottles and left for about 30 minutes for the blood to properly clot before centrifugation. The blood was centrifuged for 10 minutes at 4000 rpm to obtain a clear supernatant for serum creatinine analysis. The kidneys were thereafter harvested and immediately fixed in 10% Bouin's fluid and processed for light microscopic studies. The

processed sections of kidney were stained using Haematoxyline and Eosin (H&E) (Elias, 1974), Masson's trichrome (MT) (Masson, 1929) and Periodic-Schiff (PAS) (McManus, 1948) staining technics.

Serum creatinine assay.

The Randox creatinine kit was used to determine creatinine according to the manufacturer's instructions. Absorbance readings were taken using the spectrophotometer at a wavelength of 492 (490-510nm). The working reagents were picric acid and sodium hydroxide and the principle is that creatinine in an alkaline solution reacts with picric acid to form a colored complex. The amount of formed is directly proportional to the creatinine concentration.

Statistical Analysis

The mean and standard deviation were calculated for all values and comparisons between the control and the treated groups were analyzed using One-way analysis

of variance (ANOVA) with the 16.0 version of SPSS package. Significance level was set at $P < 0.05$

RESULTS

Average Animal Body Weight

The average weight of animals in the alloxan treated groups C, D, E and F decreased during induction by 24.32%, 19.13%, 19.78%, and 9.68% respectively. However, upon treatment, body weight increased by 10.26%, 8.6%, 10.98% and 13.40% for the aforementioned groups respectively. In group A, there was 11.11% weight gain, while in group B, the negative control, 44.7% weight loss was recorded during the experiment (Fig.1). The treatment with *MO* ameliorated the impact of induced diabetes on the body weight and restored them to near normal levels with reference to control rat weights. This observation was however found to be dose- dependent. With graded doses of 100, 300 and 500 mg/kgbw of *MO* leaf aqueous extract, rats in the group that received the highest dose of 500mg/kgbw of *MO* leaf aqueous extract showed most significant weight gain compared to other treatment groups and the control. Group F that received 15mg/kgbw of diabinese showed high weight gain compared with group E. Furthermore, group G that received 100mg/kgbw of *MO* leaf aqueous extract before induction of diabetes showed a slow weight loss compared with groups that were treated with alloxan before intervention with *MO* leaf aqueous extract. *MO* contains calcium and iron that contribute to improvements in bone density, a major factor in weight determination.

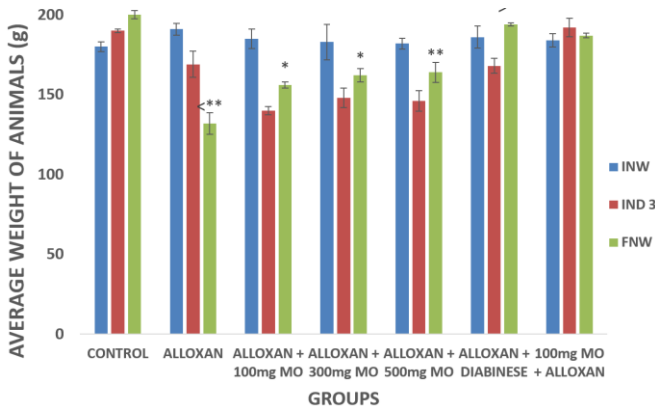


Fig. 1: Average weight (g) of the rats. INW = Initial weight; IND 3 = Induction week 4; FNW = Final weight: <math>< ** </math> = significant decrease; $** >$ = significant increase; $**$ = highly significant; * = slight significant difference

Average Blood glucose level

The graph below (figure 2) represents the blood glucose levels at three strategic points during the experiment viz: initial blood glucose level (INBGL),

third week after alloxan administration (IND3) and the final blood glucose level (FNBGL) taken after three weeks of administration of *MO* extract. The results show that in all groups except the control administration of alloxan resulted in statistically significant increases ($p < 0.05$) in blood glucose levels (*) of the rats during first three weeks when the animals remained in an uninterrupted hyperglycemic state. Administration of *MO* extract resulted in statistically significant decreases ($p < 0.05$) in blood glucose levels (**) during the intervention period. However, in the diabetic control group in which animals did not receive any intervention agent, there was a highly significant elevation ($p < 0.05$) in average blood glucose level (***) as a result of alloxan administration. In alloxan induced diabetic rats, the extract produced significant ant-hyperglycemic effect, giving a percentage ($p < 0.05$) reduction in blood sugar levels of 34.35%, 42.54% and 58.27% for 100, 300 and 500 mg/kg doses respectively of the extract while diabinese exhibited 43.49% ($P < 0.05$) reduction of the blood sugar levels.

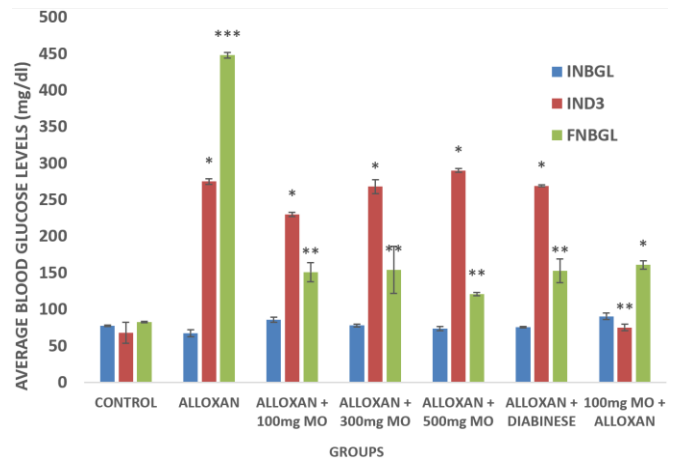


Fig. 2: Average blood glucose level of the rats. INBGL = initial blood glucose level; IND3 = induction week 3; FNBGL = final blood glucose level.

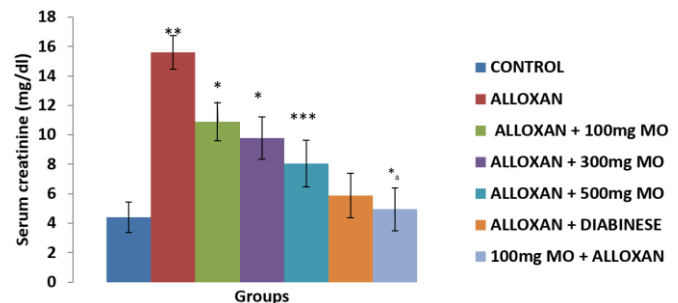


Fig. 3: The bar chart of average serum creatinine level (mg/dl). Data expressed as Mean \pm SEM $p < 0.05$. ** = highly significant increase compared with the control; * = significant decrease compared with alloxan group; *** =

highly significant decrease with reference to alloxan group; *_a = no significant difference compared with control and diabinese groups (higher and lower respectively)

Serum creatinine

The serum creatinine level of all the treated groups significantly decreased (P<0.05) compared with group B (Fig. 3).

Renal histoarchitecture

In this study, sections from control group showed normal glomerulus (G), normal PCT (P) and normal DCT (D) (Plate 1A). The microscopic appearance of kidneys of alloxan-treated diabetic rats (Plate 1B) showed glomeruli with severe sclerosis (blue arrows) and wide capsular space (red arrow), The renal tubules showed some obvious cases of tubular necrosis affecting the proximal convoluted tubules (PCT) and distal convoluted tubules (DCT), with widened lumen (green arrows) as well as focal areas of massive inflammatory cells infiltration (INF – plate 1H). Sections of kidneys from animals treated with MO (Plates 1C, D, E and G) showed glomeruli (black arrows), PCTs (P) and DCTs (D) at various stages of restoration. *Moringa* pre-treated group showed a mild preservation of normal cellularity and reduced inflammatory cell infiltration (B – plate 1I).

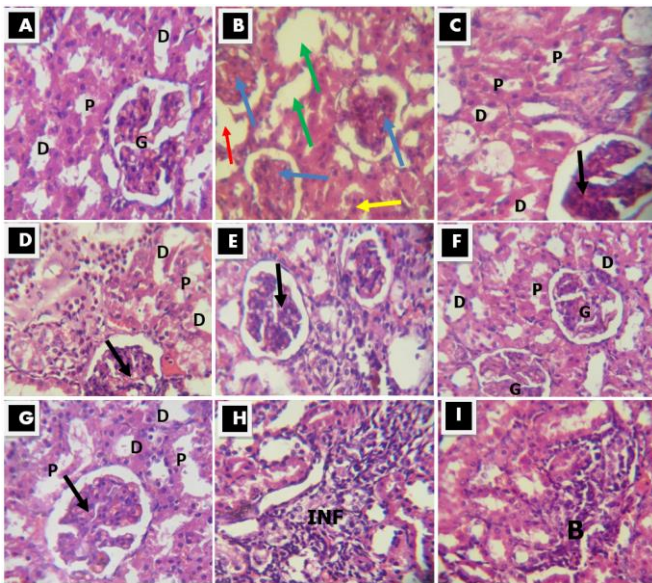


PLATE 1: A: Control showing normal glomerulus (G), normal PCT (P), normal DCT (D) H&E x400; B: Alloxan group – showing necrotic tubules (green arrows), glomerular sclerosis (blue arrows) H&E x400; C: Alloxan+100mgMO group H&E x400; D: Alloxan+300mgMO group H&E x400; E: Alloxan+500mgMO group H&E x400; F: Alloxan+Diabinese Diabinese (features comparable with control group) H&E x400; G: 100mgMO+alloxan H&E x400; H: Alloxan group showing inflammatory cell

infiltration (INF). H&E x400; I: *Moringa* pre-treated group showing a mild preservation of normal cellularity and reduced inflammatory cell infiltration (B) H&E x400

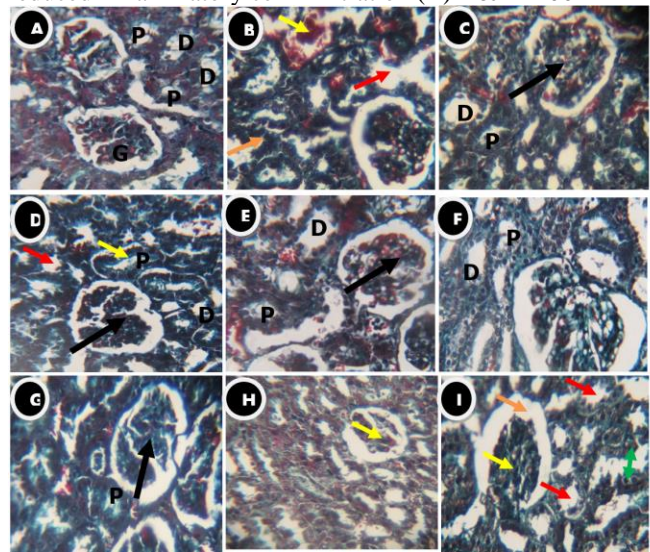


PLATE 2: Sections showing the microarchitecture of the kidney: A=Control showing normal glomerulus (G), normal PCT (P), normal DCT (D) MT x400; B: Alloxan group – showing necrotic tubule (red arrow), the deposit of a homogeneous, acellular material (yellow arrow) and increased interstitial material between the tubes (brown arrow). MT x400; C: Alloxan+100mgMO group MT x400; D: Alloxan+300mgMO group MT x400; E: Alloxan+500mgMO group MT x400; Groups C, D, E and G present glomeruli (black arrows, PCT- P and DCT-D at various stages of repair); F: Alloxan+Diabinese (features comparable with control group MT x400; G: 100mgMO+alloxan, *Moringa* pre-treated group showing a mild preservation of normal kidney microarchitecture MT x400; H: Alloxan group showing sclerotic glomerulus (G), wide capsular space (yellow arrow). MT x400; I: Alloxan group showing sclerotic glomerulus (yellow arrow), widened DCT (red arrow), widened PCT (green double pointer arrow), and wide capsular space (brown arrow). MT x400.

DISCUSSION:

This present study demonstrated that alloxan successfully induced diabetes mellitus in the experimental animals and this was confirmed with the consistent rise in blood glucose level and after the intraperitoneal administration of alloxan into the experimental animals. The weight loss observed in this study appeared to be consistent with a previous report of the World Health Organization, that diabetes mellitus is often characterized by rapid and significant weight loss leading to fatigue which is not easily reversed (WHO, 1985). This experiment showed that there was significant decrease in the body weight of the rats in all

the groups during the period of uninterrupted hyperglycaemic condition post-induction with AL; however, the trend appeared to have been slowed by

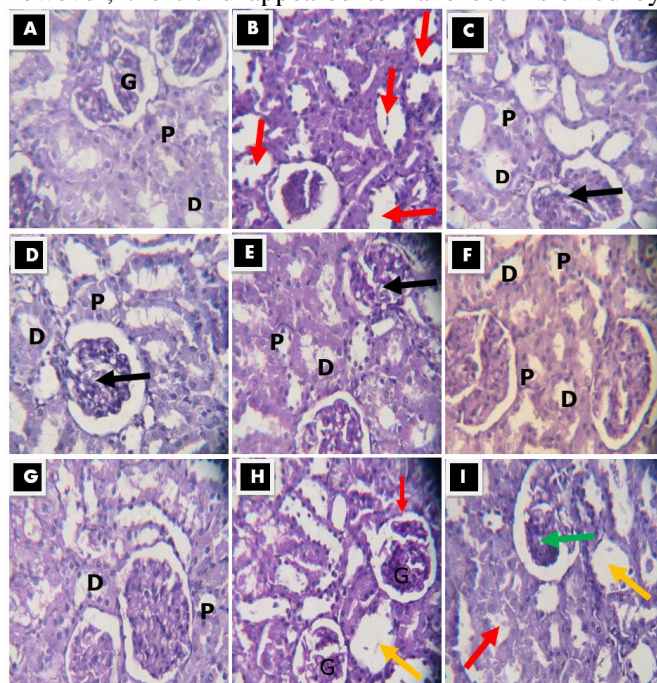


PLATE 3: sections showing the microarchitecture of the kidney: A: Control showing normal glomerulus (G), normal PCT (P), normal DCT (D) PAS x400; B: Alloxan group – showing necrotic tubule (red arrow), the deposit of a homogeneous, acellular material (yellow arrow) and increased interstitial material between the tubes (brown arrow). PAS x400; C: Alloxan+100mgMO group PAS x400; D: Alloxan+300mgMO group PAS x400; E: Alloxan+500mgMO group PAS x400; Groups C, D, E and G present glomeruli (black arrows, PCT- P and DCT-D at various stages of repair); F: Alloxan+Diabinese (features comparable with control group PAS x400; G: 100mgMO+alloxan, *Moringa* pre-treated group showing a mild preservation of normal cellularity PAS x400; H: Alloxan group showing sclerotic glomeruli (G), widened DCT (yellow arrow) and thickened basement membrane of Bowman’s capsule (red arrow). PAS x400; I: Alloxan group showing sclerotic glomerulus (green arrow), widened DCT (yellow arrow) and widened PCT (red arrow). PAS x400

the intervention with *MO*. A pointer to the mechanism underlying the weight loss is the result of the investigations by Locatto *et al.*, 1993 who reported that AL-treated rats showed an important reduction in body and bone mass, with a greater impact on soft tissues. The result obtained in this study is also in agreement with Viswanathaswamy *et al.*, (2011), who reported that alloxan-induced diabetes significantly decreased the body weight of diabetic untreated rats as the study duration increased compared with the treated groups

and normal control rats. Furthermore, with the compromised carbohydrate metabolism (Akpan *et al.*, 2007, 2017), there is evidence of having to draw energy from stored sources in the body thereby impacting significantly on the animal body weight.

Glucose is a major fuel for animal cells. It is supplied to the organism through dietary carbohydrates and, endogenously, through hepatic gluconeogenesis and glycogenolysis (Gylfe *et al.*, 2014). Glucose absorption from the gastrointestinal tract (GIT) into blood is regulated by a variety of neuronal signals and enterohormones (incretins), as well as by meal composition and the intestinal flora (Owens *et al.*, 2013). Glucose homeostasis reflects a balance between glucose supply and its utilization. Physiologically, this balance is determined by the level of circulating insulin and tissue responsiveness to it. It stimulates glucose uptake and utilization by tissues, especially by liver, skeletal muscle, and adipose tissue (Owens *et al.*, 2013; Gylfe *et al.*, 2014). Insulin is secreted by pancreatic islet β cells (Owens *et al.*, 2013). Medicinal plants attract growing interest in the therapeutic management of diabetes mellitus. The present study assessed the probable anti-diabetic effects of an aqueous extract of *MO* leaves in treating alloxan-induced diabetic Wistar rats. The reduction of blood glucose level in this experiment confirmed reports that the active compounds of *MO* have strong glucose lowering potential (Jain *et al.*, 2010; Abd El Latif *et al.*, 2014, Sangkitikomol *et al.*, 2014 and Yassa & Tohamy, 2014). The extract showed a dose dependent effect since more pronounced anti-hyperglycemic effect was observed with increase in dosage. Tight control of blood sugar level has been found to be the major factor in preventing micro albuminuria which if left unattended would progress to macro albuminuria and subsequently, end stage renal disease.

From the results obtained, it could be inferred that *Moringa oleifera* had effects comparable with the reference drug diabinese. The comparable effects of *MO* with diabinese on hyperglycemic animals suggests similar modes of action. Alloxan destroys the pancreatic β -cells which implies that fewer of these cells will be available to elaborate insulin needed to aid the removal of glucose from the blood milieu. The result of this is the accumulation of glucose in the blood which leads to the hyperglycaemic state observed in alloxan-treated animals. However, in this study, the extract successfully lowered blood sugar levels in rats previously administered alloxan, an indication that the extract has pro-pancreatic effects. Earlier studies by Malviya *et al.* (2010), reported that the therapeutic actions of alkaloids, flavonoids, saponins, and tannins,

as found in MO, were additive in the context of their collective capacities to exert glycaemic control and anti-hyperglycaemic capabilities. The polyphenolic proanthocyanidin, commonly referred to as condensed tannin, has also been reported to exhibit considerable antidiabetic properties (Xie, 2005; Yokozawa *et al.*, 2012). According to Yokozawa *et al.*, 2012, proanthocyanidin protected STZ-diabetic rats against hyperglycaemia and related disorders as well as hyperlipidemia through modulation of general metabolism. These previous findings corroborate the outcomes of the current study in which previously hyperglycaemic rat had lowered blood glucose levels following administration of MO extract.

The very high blood creatinine level as observed in the alloxan group was probably the result of diabetic nephropathy which is considered a major complication of diabetes (Sayed *et al.*, 2012). The implication is that the ability of the kidney to filter creatinine from the blood is somewhat impeded which allow the product to remain in the blood. Diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria, progressive decline in the glomerular filtration rate (GFR), and elevated arterial blood pressure (Kanwar *et al.*, 2008). However, treatment of experimentally diabetic rats with MO extract revealed a dose-dependent decrease in the serum creatinine level, an indication of an enhanced functionality of the kidneys of these animals. Creatinine is not reabsorbed into the body from the kidneys. Hence, any failure on the part of the kidneys to excrete creatinine results in rise of creatinine levels in the blood. Variations from normal creatinine levels could indicate kidney dysfunction and muscle problems

Compromise in PCT microstructure translates to impaired function of about seventy-five percent water reabsorption from the glomerular filtrate. This, obviously, is the mechanism underlying the frequent urination experienced in the diabetic condition. Sodium reabsorption coupled with hydrogen and potassium secretion is compromised in DCT necrosis and these have adverse effects in the general well-being of diabetics. Sections stained with PAS showed thickened glomerular basement membrane and lack of brush boarders in the tubules, in agreement with Yassin *et al.*, (2004) who noticed that in diabetic rats the glomerular tufts were obviously contracted, lobulated, degenerated and infiltrated by chronic inflammatory cells and RBCs. The glomeruli were more or less shrunken and the urinary space became wide. According to Yanardag *et al.*, 2002, the treatment of rats with alloxan showed some histopathological changes in the kidney in the

form of degeneration, inflammation, necrosis, mesangial hyper cellularity and deformed renal tissue architecture. Also, Selvant *et al.* (2008) reported that in diabetic rats the kidney showed degenerative changes in cortex, medulla and necrosis of tubules. In addition Zeeuw *et al.*, (2006) observed that in diabetics, the kidney sections showed damaged glomeruli, proximal tubules and interstitial inflammation. The foregoing, which are in agreement with the findings of this study, underlie major anatomical disruptions in the kidney structure and are pointers to gradual deterioration in the physiological capability of this very important organ in the body of the animals.

Significant restructuring which include increase in animal body weight, reduction in blood glucose level, regeneration in the glomeruli, DCT and PCT among others were observed following the treatment of rats with MO extract. These observations also followed a dose-dependent pattern and agree with the findings of Ndong *et al.*, 2007, Kumbhare *et al.*, 2012, Yassa & Tohamy, 2014 and Parikh *et al.*, 2014, who separately, noticed that the radical scavenging effect of MO was found to be increased with increasing concentrations. Also, Abdulraham *et al.*, 2015 reported the antidiabetic activity of the higher dose of Moringa seeds powder to be more efficient than that of a lower dose.

These ameliorative effects as observed in this study could be attributed to the availability of the active beneficial constituents present in MO leaf (Vongsak *et al.*, 2013).

The antioxidant activity of MO leaf powder is due to its content of phenolics and flavonoids that have scavenging effect on free radicals (Ghiridhari *et al.*, 2011). MO contains three classes of phytochemicals, viz: glucosinolates such as glucomoringin, flavonoids such as quercetin and kaempferol, and phenolic acids such as chlorogenic acid; all of these classes have medicinal benefits (Mbikay *et al.*, 2012). These three phytochemical groups of MO possess antioxidant, hypoglycaemic, hypotensive, antidyslipidemic, anticancer, and anti-inflammatory properties (Lako *et al.*, 2007) and would likely have contributed to the restoration of weight and blood glucose levels observed in this study.

CONCLUSION

This study, therefore, concluded that treatment with *Moringa oleifera* ameliorated the acute effects of alloxan-induced diabetic complications on renal microarchitecture and probably contributed to the

restoration of morphology and hence the functions of the kidney in adult Wistar rats.

REFERENCES

- Abayomi A.L, Adewoye E.O, Olaleye S.B, and Salami A.T (2011).Effect of Magnesium pre-treatment on Alloxan induced hyperglycemia in rats. *African Health Science*. **11**(1): 79–84.
- Abd El Latif A., El Bialy Bel S., Mahboub H. D., Abd Eldaim M. A (2014). Moringa oleifera leaf extract ameliorates alloxan-induced diabetes in rats by regeneration of β cells and reduction of pyruvate carboxylase expression. *Biochemistry and Cell Biology*; **92**(5):413–419.
- Abdel Moneim A, El-Feki M, Salah E (1997). Effect of Nigella Sativa, Fish oil and Gliclazide on alloxan diabetic rats, 1-Biochemical and Histopathological studies. *Journal Egyptian German Society Zoology*; **23**:237–265.
- Abdulrahman L, Malki A.L, Hadad A. Rabel E.L (2015). The antidiabetic effect of low doses of Moringa oleifera lam. Seed on streptozotocin induced diabetes and diabetes nephropathy in male rats. *Biological Medical Research International*; **2015**:1-13.
- Akpan, H.B., Adefule, A.K., Fakoya, F., Caxton-Martins, E. (2007) Evaluation of LDH and G6PDH activities in Auditory Relay Centres of Streptozotocin induced Diabetic Wistar Rats. *Journal of Anatomical Sciences*, **1**(1): 21-25.
- Akpan, H.B., Shallie, P.D., Adefule, A.K., Adenowo, T.K. (2017) Lipid Peroxidation is Key to Neuronal Cell Death in the Inferior Colliculus of STZ-Induced Diabetic Rats. *Journal of Applied Life Sciences International*; **10**(1): 1-15
- Al-Kharusi LM, Elmardi MO, Ali A, et al. (2009). Effect of mineral and organic fertilizers on the chemical characteristics and quality of date fruits. *International Journal of Agriculture and Biology*. **11**: 290-296.
- Anwar, F., Latif, S., Ashraf, M. & Gilani, A.H (2007). Moringa oleifera: a food plant with multiple medicinal uses. *Phytochemical Research*; **21**(1):17-25.
- Barminas JT, Charles M, Emmanuel D (1998). Mineral composition of non-conventional leafy vegetables. *Plant Foods for Human Nutrition*. **53**: 29–36.
- Busko M. (2006) Macroalbuminuria, Not Low GFR, Predicted Kidney Function Decline in Population Screening. *Journal of American Society of Nephrology*; **17**:2582-2590
- Collier E, Watkinson A, Cleland CF, Roth J (1987). Partial purification and characterization of an insulin-like material from spinach and lemna gibba G3. *Journal of Biological chemistry* **262**(13): 6238–6241.
- Fahey, J. W (2005). Moringa oleifera: Moringa oleifera: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees for Life Journal.
- Ghiridhari W.A, Malhati D, and Geetha K, “Anti-diabetic properties of drumstick (*Moringa oleifera*) leaf tablets,” *International Journal of Health and Nutrition*, **2**:1–5.
- Gylfe E., Gilon P (2014). Glucose regulation of glucagon secretion. *Diabetes Research and Clinical Practice*. **103**(1):1–10.
- Haffner SM, Mykkanen L, Festa A, Burke JP, Stern MP (2000): Insulin resistant prediabetic subjects have more atherogenic risk factors than insulin sensitive prediabetic subjects: implications for preventing coronary heart disease during the prediabetic state. *Circulation* **101**: 975–980.
- Hamza A.A (2010) “Ameliorative effects of Moringa oleifera Lam seed extract on liver fibrosis in rats,” *Food and Chemical Toxicology*, vol. **48**:345–355.
- Hsu R, Midcap S, Arbainsyah DWL (2006). Moringa oleifera: Medicinal and Socio Economical Uses. *Internationa Course on Economic Botany, National Herbarium Leiden, the Netherlands*.
- Jain P. G., Patil S. D., Haswani N. G., Girase M. V., Surana S. J (2010). Hypolipidemic activity of *Moringa oleifera* Lam., *Moringa ceae*, on high fat diet induced hyperlipidemia in albino rats. *Brazilian Journal of Pharmacognosy*; **20**(6):969–973.
- Johnson DR (1982): Role of renal cortical sulfhydryl groups in development of mercury- induced renal toxicity. *Journal of Toxicology and Environmental Health*, **9**: 119 -126.
- Junqueira, L. C. & Carneiro, J. Basic Histology: Text & Atlas. 11th ed. New York, McGraw-Hill, 2007. Chapter **19**.
- Kanwar Y.S, Wada J, Sun.L et al., (2008) “Diabetic nephropathy: mechanisms of renal disease progression,” *Experimental Biology and Medicine*, vol. **233**: 4–11.
- Kasolo J.N, Bimenya G.S, Ojok L, Ochieng J, and Ogwal-Okeng J.W (2010), “Phytochemicals and uses of Moringa oleifera leaves in Ugandan rural communities,” *Journal of Medicinal Plants Research*, **4**:753–757.
- Khawaja TM, Tahira M, Ikram UK (2010). Moringa oleifera: a natural gift - A review of *Journal Pharmaceutical Science Research*. **2**: 775-781.
- Kumbhare M.R, Guleha V, and Sivakumar T (2012), “Estimation of total phenolic content, cytotoxicity and in-vitro antioxidant activity of stem bark of

- Moringa oleifera,” *Asian Pacific Journal of Tropical Disease*, **2**:144–150.
- Lako J, Trenerry V.C, Wahlqvist M, Wattanapenpaiboon N, Sotheeswaran S, and Premier R (2007), “Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods,” *Food Chemistry*, **101**:1727–1741.
- Locatto M E, Abranzon H, Caferra D, Fernandez MC, Alloatti R, Puche RC (1993). Growth and development of bone mass in untreated alloxan diabetic rats. Effects of collagen glycosylation and parathyroid activity on bone turnover. *Laboratorio de Biologia Osea, Facultad de Ciencias Medicas, Rosario, Argentina*. **23**(2):129-144
- Malviya N, Jain S, Malviya S (2010). Antidiabetic potential of medicinal plants *.Acta Poloniae Pharmaceutica*, **67**:113–118
- Mbikay M, (2012). Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review. *Frontiers in Pharmacology*. **3**(24): 1-12.
- Mogensen CE, Christensen CK (1984): Predicting diabetic nephropathy in insulin-dependent patients. *New England Journal of Medicine*. **311**:89–93.
- Nelson RW, Ihle SL, Lewis LD, Salisbury SK, Bottoms GD (1991). Effects of dietary fiber supplementation on glycemic control in dogs with alloxan-induced diabetes mellitus. *American Journal Veterinary Research*; **52**:2060–2066.
- Owens D. R., Monnier L., Bolli G. B (2013). Differential effects of GLP-1 receptor agonists on components of dysglycaemia in individuals with type 2 diabetes mellitus. *Diabetes and Metabolism*: **39**(6):485–496.
- Parikh H, Parikh P.K, and Kothari C (2014), “Indigenous plant medicines for health care: treatment of Diabetes mellitus and hyperlipidemia,” *Chinese Journal of Natural Medicines*, **12**:335–344.
- Sangkitikomol W., Rocejanasaroj A., Tencomnao T (2014). Effect of *Moringa oleifera* on advanced glycation end-product formation and lipid metabolism gene expression in HepG2 cells. *Genetics and Molecular Research*; **13**(1):723–735
- Sayed A.A.R (2012), “Ferulsinaic acid modulates SOD, GSH, and antioxidant enzymes in diabetic kidney,” *Evidence-Based Complementary and Alternative Medicine*, **2012**:1-9.
- Selvant VT, Mamikandam L, Kumar SG, Kakoti SR, Kumar GP, Shaha P, Gupto M and Muzumder VK (2008): *International Journal of Applied Research in Natural Products*, **(1)** 25 -33.
- Thakran S, Siddiq MR and BaquerWZ (2004): Trigonella foenum graecum seed powder protect against histopathological abnormalities in tissues of diabetic rats. *Molecular and Cellular Biochemical*. **(1)**151 -159.
- Thurber, M. D. & Fahey, J. W (2009). Adoption of Moringa oleifera to combat under-nutrition viewed through the lens of the "Diffusion of innovations" theory. *Ecol. Food and Nutrition Research*, **48**(3):212-25.
- Udoamaka F, Ezuruike, Jose M, Prieto (2013): The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological consideration. *Journal of Ethnopharmacology*, **155**:857-924
- Valmadrid CT, Klein R, Moss SE, Klein BE (2000): The risk of cardiovascular disease mortality associated with microalbuminuria and gross proteinuria in persons with older-onset diabetes mellitus. *Archives of International Medicine* **160**: 1093–1100.
- Xie D.Y, Dixon R.A (2005). Proanthocyanidin biosynthesis—still more questions than answers? *Phytochemistry Journal*, **66**:2127–2144
- Yanarday R, Colac H. (1998). Effect of chard (*Beta vulgaris L. var cicla*) on blood glucose level in normal and alloxan-induced diabetic rabbit. *Journal Ethnopharmacology*. **4**:309-311.
- Yassa H.D and Tohamy A.F (2014), “Extract of Moringa oleifera leaves ameliorates streptozotocin-induced Diabetes mellitus in adult rats,” *Acta Histochemica*, vol. **116**:844–854.
- Yassin MM, Ashour AR and Elyazji NR (2004): Alternations in body weight, protein profile, non-protein nitrogen constituents and kidney structure in diabetic rats under glibenclamid treatment. *Journal of the Islamic University of Gaza (Natural Science Series)*.
- Yokozawa T, Cho E.J, Park C.H, Kim J.H (2012). Protective effect of proanthocyanidin against diabetic oxidative stress. *Evidence-Based Complementary and Alternative Medicine*, **2012**: 623-879.