Research Article

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

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**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.

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
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., 2007).

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 (Kharusi 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. 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 (Fluttet *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.

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<td>G-100mgMO+Alloxan</td>
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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.

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 anti-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.
highly significant decrease with reference to alloxan group; * = 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).

**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

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**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
**M. oleifera** and neuropathic changes in diabetic rats

the groups during the period of uninterrupted hyperglycaemic condition post-induction with AL; however, the trend appeared to have been slowed by 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, Sangkittikomol 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 glycemic control and anti-hyperglycemic 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 hyperglycemia 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 hyperglycemic 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 border 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 hypercellularity 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 glucorominin, 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, hypoglycemic, hypotensive, antidiabetic, 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.

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