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# Antidiabetic and Nephroprotective Effects of Aqueous Leaf Extracts of *Moringa oleifera* in Alloxan-Induced Diabetic Rats

Josiah JG[1]; Jiya Z[2]; Tswanya MN[3]; Adama JY[4]+; Eke SS[5]; Mathew JT[6]

[1]Department of Biotechnology, Mewar International University (MIU), Nigeria, Masaka, Nasarawa State, Nigeria
[2]Department of Science Laboratory Technology, Federal Polytechnic, Nasarawa, Nasarawa State, Nigeria.
[3]Sheda Science and Technology Complex (SHESTCO) Biotechnology Advanced Research Complex, Garki, Abuja
[4]Department of Animal Production, Federal University of Technology, Minna, Niger State, Nigeria
[+]Africa Centre of Excellence for Mycotoxin and Food Safety (ACEMFS), Federal University of Technology (FUT), Minna, Nigeria
[5]Department of Biotechnology, Federal University of Applied Sciences, Kachia, Kaduna State
[6]Department of Chemistry, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria,

*Corresponding Author: Josiah James Gana

ORCID ID: https://orcid.org/0000-0001-6983-0043

Tel: +2348036951530

**ABSTRACT**

Diabetes mellitus (DM) is a global burden, associated with life-threatening complications which include renal failure, cardiac attack and stroke. This study was carried out to investigate the antidiabetic and nephroprotective effects of aqueous leaf extracts of *Moringa oleifera* in rats with alloxan-induced diabetes. For this purpose, twenty-five adult Wistar rats with body weight of 150-250g were randomly assigned to groups of five rats each (n=5). Group A served as normal control; group B served as diabetes control; group C served as positive control and group D and E were diabetic groups administered with the aqueous leaf extracts. Blood samples were collected to determine the fasting blood glucose level, creatinine and urea. The results from this study showed that the aqueous leaf extract of *Moringa oleifera* demonstrated significant antidiabetic activity by reducing blood glucose levels by 34.7% and 36% when lower and higher doses of extracts of *M. oleifera* were administered to rats respectively and also showed an improvement in glucose tolerance in the diabetic rats. Additionally, the extract demonstrated nephroprotective effects by reducing markers of kidney damage and improving kidney functions in the diabetic rats. This was evident in the reduction of urea and Creatinine levels when compared to positive and negative control groups. The findings suggest that *M. oleifera* leaf extracts may have potential therapeutic benefits for managing diabetes and protecting against kidney damage. Further research on other parts of the plant is recommended to elucidate its mechanism of action and active compounds.

**Keywords:** Alloxan; Hyperglycemia; Hypoglycaemic; *Moringa oleifera;* Wistar rat

Corresponding Author's Email: josiah.james@miu.edu.ng

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## INTRODUCTION

Diabetes mellitus [DM] is one of the top ten causes of morbidity and mortality around the world [1]. It is a metabolic disorder of the endocrine system which is characterized by hyperglycemia, dysregulations in insulin levels, with alterations in protein, carbohydrate and lipid metabolism and in some cases, it occurs concurrently in individuals thereby leading to obesity and overweight [2,3].

The four major classifications of diabetes are Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), gestational diabetes and secondary diabetes. However, the vast majority of people with diabetes are classified into one of two broad classes: T1DM, which is caused by an absolute or near absolute deficiency of insulin, or T2DM which is characterized by the presence of insulin resistance with an inadequate compensatory increase in insulin secretion. Others are women who develop diabetes during their pregnancy and are classified as having gestational diabetes. Lastly, there are uncommon and diverse types of diabetes, which are caused by drugs, infections, pancreatic destruction, endocrinopathies and genetic defects. These unrelated forms of diabetes are included in the "Other Specific Types" that are secondary type of diabetes and are classified separately [4,5].

Recently, approximately 150 million people have diabetes worldwide, a number expected to reach 300 million by 2025 [6]. Currently, the available remedy or management of diabetes involves treatment with insulin and other oral anti-diabetic agents such as metformin, sulfonylurea and biguanid. Many of these oral anti-diabetic agents have a number of serious with adverse effect, thus management of diabetes without side effect is still a challenge [7]. The rapid increase in cases of diabetes mellitus, most especially T2DM, raises concerns, and also highlights an urgent need to investigate effective therapies to curb this disease [8].

Herbs have become reliable substitutes and have played a significant role in the management of various disorders. One of these herbs is *Moringa oleifera. Moringa oleifera,* popularly called "the miracle tree", is a monogeneric plant of the family *Moringaceae* [9]. It is a medicinal plant that has gained a lot of interest for its diverse phytochemical properties. A review of available literature indicates the biological capabilities of this plant expand to protecting against complications linked with cancer, heart disease, diabetes mellitus and fatty liver [10,11].

Different parts of moringa plant contain important minerals such as K, Ca, P, Fe, and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolics such as zeatin, quercetin, β-sitosterol, caffeoylquinic acid and kaempferol and high concentrations of natural dietary antioxidants: Vitamins A, C and E [12]. The leaves are known to be used mostly for medicinal purpose, and they are a great source of antioxidant flavonoids and prominent anti-inflammatory [13].

Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes mellitus due to their effectiveness, less side effects and relatively low cost. However, most of the plants prescribed for diabetes mellitus are not edible and therefore, the studies on edible plants which have a hypoglycemic effect would be of great value in the dietary management of the disease. Therefore, this study aimed at investigating the antidiabetic and nephroprotective effect of aqueous leaf extracts of *Moringa oleifera* in alloxan-induced diabetic rats.

## METHODOLOGY

### Sample Collection and Preparation

The plant materials *(*leaves of *Moringa oleifera)* were sourced from Nasarawa town in Nasarawa Local Government Area of Nasarawa State, Nigeria. The identification and authentication were done by a taxonomist in the department of Agricultural Technology, Federal Polytechnic, Nassarawa and the voucher number was assigned and deposited in the same Department. The preparation of the leaves sample was carried out using the method of Josiah *et al.* [14]. The sample was washed under running tap water and then air dried on top of the laboratory bench and thereafter crushed with a mortar and pestle into fine powder. The powdered leaves (500 g) were extracted by cold maceration in distilled water for 48 hours at room temperature with manual intermittent agitations after every 3 hours. Thereafter, the extract was filtered using Whatmann No. 1 filter paper and concentrated at 40˚C in a hot air oven. The resulted extract was stored in a capped bottle in refrigerator at 4˚C until required.

### The Experimental Animals and Design

The method of Josiah *et al*. [15] was used to calculate the sample size. A total of twenty-five (25) wistar strain of albino rat (WSAR) of both sexes, weighing between 150-250 g were obtained from the animal house of the Department of Pharmaceutical Science, Ahmadu Bello University (ABU) Zaria, Nigeria. The rats were kept and maintained in well-ventilated cages under standard laboratory conditions and were maintained at ambient temperature and relative humidity which was between 22°C, 12 hours light and 12-hour dark and 30-35% humidity respectively [16]. They were maintained on grower's mash (Vital feeds Nigeria Ltd) and

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provided with water *ad libitum*. They were allowed to acclimatize to the laboratory conditions for two weeks before the experiment [14]. The animal ethical committee in the Department of Laboratory Technology, Federal Polytechnic, Nasarawa approved the animal studies.

After the acclimatization, the 25 WSAR rats were randomly divided into five groups of five rats each:

Group A: Normal rats administered with only distilled water (positive control).

Group B: Diabetic-induced rats administered without treatment (negative control).

Group C: Diabetic-induced rats administered with a standard drug (metformin).

Group D: Diabetic-induced rats administered with 200 mg/kg aqueous extract of *Moringa oleifera* (Low Dose)

Group E: Diabetic-induced rats administered with 400 mg/kg of aqueous extracts of *Moringa oleifera* (High Dose)

After 28 days of administration, the animals were humanely sacrificed and blood was collected.

**Induction of Diabetes**
After the rats were fasted for 18 hours, they were induced by single intraperitoneal injection of freshly prepared Alloxan (120 mg/kg) in 0.1 M citrate buffer (pH 4.5) for type 2 diabetes [17]. Diabetes were confirmed in these rats after a period of 7 days. The control animals were administered citrate buffer (pH 4.5). To overcome the initial hyperglycaemic shock, which may occur as a result of the Alloxan administration, diabetic rats were given 5% glucose solution ad libitum for 24 hours. The method of Okoli *et al.* [17] with modification was used to collect the blood from the tail and the glucose level of each rat was determined. Rats with a fasting blood glucose range of 15–20 mmol/L were considered diabetic and included in the study [17] The fasting blood glucose level was determined using glucometer (Acc-check Advantage. Boche Diagnostic GmbH, Germany) after fasting the rats for 12 hours [18].

**Collection for Biochemical Analysis of Hepatic and Renal Functions**
Blood samples were collected during the experiment via retro-orbital bleeding under light ether anaesthesia. Blood samples collected were left to clot at room temperature, and then centrifuged at 3000 rpm for 15 min. Sera were then separated and stored at -20 °C as aliquots for further biochemical analysis [19].

Sera were used to evaluate serum levels of hepatic and renal function biomarkers. Serum creatinine and urea were determined according to Larsen *et al.* respectively [20, 21]. Rats were administered with the extracts at various concentrations daily for four weeks experimental period, while blood glucose level was determined weekly for the period of four weeks. The rats were fasted overnight and anesthetized with chloroform and were sacrificed 24 hours after the last treatment. The serum collected was used to determine blood urea nitrogen (BUN), and creatinine. Creatinine present in the serum was determined using the Randoxlaboratories$^{TM}$ assay kit as described by Myes *et al.* [22]. Serum urea concentration was measured using spectrophotometer (UV-V is spectrophotometer - Gold Spectrum lab 54 produced from Angstron Advanced Inc, Buston USA) according to Berthelot's reaction, using (Randox assay kit) according to Fawcett and Scout [23].

**Data Analysis**
The results were presented as mean±SEM of 5 rats in each group. Analysis of variance (ANOVA) was used to test for significance differences between the treated and untreated groups. Values of $P< 0.05$ was considered significance and Multiple comparism test (Tukey's Honestly Significant Difference (HSD) test) was used [24].

## RESULTS
The results indicate that aqueous leaf extracts of *Moringa oleifera* exhibit significant hypoglycemic activity in alloxan-induced diabetic Wistar rats. This is evidenced by the reduction in fasting blood glucose levels, as shown in Table 1. A significant difference was observed between pre- and post-treatment glucose levels in all groups except Group A. The percentage reduction of blood glucose level of the treatment of alloxan-induced diabetic rats with metformin and different concentration of aqueous extracts of *M. oleifera* are shown in Table 2. The groups (D and E) treated with extract of *M. oleifera* had reduction of blood glucose level at 34. 7% and 36% respectively while the highest reduction of blood glucose level at 44.1% was found in group C treated with metformin which is the standard drug. On the other hand, group A which was positive control, showed increase in blood glucose level while group B which was negative control showed reduction in blood glucose level at 2.9% and 18.6% respectively.

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**Table 1: Effect of aqueous leaf extracts of *Moringa oleifera* on fasting blood sugar level in albino wistar rats**

| Treatment | Initial (mg/dl) | After induction (mg/dl) | After treatment (mg/dl) |
|---|---|---|---|
| Group A | 83.7±15.65$^{a}$ | 86.3±16.34$^{ab}$ | 88.8±16.67$^{ab}$ |
| Group B | 86.9±16.37$^{a}$ | 157.3±26.53$^{c}$ | 128±23.60$^{b}$ |
| Group C | 90.5±18.65$^{a}$ | 259.3±37.98$^{c}$ | 145±25.85$^{b}$ |
| Group D | 85.4±16.26$^{a}$ | 194.9±30.53$^{c}$ | 127.2±23.46$^{b}$ |
| Group E | 81.2±14.92$^{a}$ | 184.3±28.50$^{c}$ | 118±21.70$^{b}$ |

*Result= mean value ± SD of duplicate determinations. Different superscript in a row means that the difference is significant at probability value p = 0.05 (95% confidence interval), otherwise, there is no significant difference.*

Group A= Normal control (Positive)
Group B = Negative Control
Group C = Metformin
Group D = DE + 200mg/kg
Group E = DE +400mg/kg

**Table 2: Percentage reduction of blood glucose level after treatment of experimental animals with aqueous leaf extracts of *M. oleifera* and metformin**

| Treatment | After induction (%) | After treatment (%) |
|---|---|---|
| Group A | 100 | -2.9 |
| Group B | 100 | 18.6 |
| Group C | 100 | 44.1 |
| Group D | 100 | 34.7 |
| Group E | 100 | 36 |

Note: Group A which is positive control shown 2.9% increase in blood glucose level

Table 3 shows the effect of aqueous leaf extracts of *M. oleifere* on urea and creatinine level in WSAR. The result indicates reduction in the value of urea and creatinine of WSAR treated with *M. oleifera* when compared to normal and metformin control and with significant difference. The values of creatinine also showed reduction in the WSAR rats treated with the leaf extract of M. *oleifera* when compared to the normal control with significant difference. However, the value of metformin control was lower than the values of creatinine treated with aqueous leaf extracts with significant difference.

**Table 3: Effect of aqueous leaf extracts of *Moringa oleifera* on urea and creatinine levels in Wistar rats**

| | NC | DC | MC | LD | HD |
|---|---|---|---|---|---|
| UREA | 24.0 ± 3.51$^{a}$ | 14.0 ± 1.41$^{b}$ | 29.2 ± 3.44$^{a}$ | 11.1 ± 1.72$^{b}$ | 12.7 ± 2.05$^{b}$ |
| CREATININE | 0.70 ± 0.070$^{ad}$ | 0.83 ± 0.124$^{a}$ | 0.10 ± 0.078$^{c}$ | 0.45 ± 0.111$^{bcd}$ | 0.58 ± 0.181$^{ab}$ |

*Key: NC = Normal control; DC = Diabetic control; MC = Metformin control; LD = Low dose; HD = High dose; Different superscript in a row means that the difference is significant at p<0.05*

## DISCUSSION

Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both [25]. The results obtained suggest that aqueous leaf extracts of *Moringa oleifera* exhibited significant hypoglycemic activities in alloxan induced diabetic Wistar rats. This was evidenced by reduction of fasting blood glucose level observed in Table 1. The significant increase in serum glucose levels in the diabetic control group (86.9 ± 16.37a to 157.3 ± 26.53c) compared to the normal control group (83.7 ± 15.65a to 86.3 ± 16.34ab) may be attributed to alloxan administration, which damages pancreatic β-cells. The administration of alloxan monohydrate which are known to induces diabetes by damaging insulin secreting cells of the pancreas leads to hyperglycemia [26, 27]. There is selective necrosis of the β-cell of the islet of langerhans on the pancreas so that the production of insulin is totally or partially inhibited, which is dependent on the concentration of the alloxan [28].

There was significant ($p<0.05$) decrease in the level of fasting blood glucose in diabetic rats with 34.7% and 36% reduction of rats treated with 200 mg/kg and 400 mg/kg of aqueous leaf extract of *M. oleifera* respectively when compared to group C treated with conventional drug (metformin) with 44.1% reduction in blood glucose level. The Treatment with metformin (which is the drug of reference) recorded a non-significant but slightly higher hypoglycemic effect (44.1%) when compared with the aqueous leaf extracts within the same period of time.

The findings in this study agreed with the findings of Ezeigbo *et al.* [16] and Sai *et al.* [29] who revealed clearly that the aqueous extract of *M. oleifera* leaf possesses antihyperglycemic and antihyperlipedemic effect in both insulin deficient and insulin resistant in rat models. The hypoglycaemic influence of the *M. oleifera* observed in the present study is also in conformity with Hegde *et al.* who observed 36% reduction in blood glucose levels in alloxan induced diabetic rats maintained on the *M. oleifera* [30]. Varga-Sanchez *et al.* also had a similar result in *reduction* of blood glucose level after 6 weeks experimental duration by feeding rats with the *M. oleifera* leaves extract [31]. The hypoglycemic activity of the extract could be due to pancreatic cell regeneration that were partially destroyed by alloxan, which may be by stimulation of the residual pancreatic mechanism, probably by increasing peripheral utilization of glucose [32, 33].

Diabetic hyperglycemia is known to induce elevated blood urea and serum creatinine levels, which serve as significant markers of renal dysfunction [34]. The balance impairment of nitrogen coupled with reduction in protein synthesis can leads to increase in concentration of urea in the blood and the increased in plasma creatinine level and blood urea are indication of the development of diabetic nephropathy in rats [35].

In the present study (Table 3), there was a significant reduction in the blood urea and serum creatinine levels from the group treated with low dose (11.1 ± 1.72 and 0.45 ± 0.111) and high dose (12.7 ± 2.05 and 0.58 ± 0.181*)* of aqueous leaf extracts *of M. oleifera* respectively, when compared with normal control group (24.0 ± 3.51 and 0.70 ± 0.070). This study revealed that the administration of alloxan to rats prevented the development of diabetic nephropathy by lowering blood urea and serum creatinine. This could be explained that there was increased clearance of blood urea and creatinine in the kidney due to antioxidant or anti-inflammatory properties of *M. oleifera* which might contribute to renal protection or that there was decreased protein degradation. This result agrees with the findings of Gross *et al.* [36].

It was observed in this study that there was increase in urea concentration (29.2 ± 3.44) and decrease in creatinine concentration (0.10 ± 0.078) of the metformin control group when compared with the diabetic control group for urea and creatinine (24.0 ± 3.51 and 0.70 ± 0.070). This was also observed by Hasan *et al*. [37]. This report suggested that *Crateva adansonii* prevented the progression of diabetic nephropathy and protected the kidney from further damage. The end-stage of diabetic renal disease is usually characterized by changes in both proteinuria and subsequent gradual decline in glomerular filtration rate (often progressing over 10–20 years). Therefore, the development of lesions in the glomerular capillaries of the kidneys allows protein to escape because of changes in the basement.

## CONCLUSION

The aqueous leaf extracts of *Moringa oleifera* exhibited promising hypoglycemic and nephroprotective effect in alloxan induced diabetic wistar rats. Hence the leaf can be helpful in the management of diabetes mellitus and its other associated complication. It is recommended that further investigation using other part of the plant may be more useful to explicate the mechanism of action and other active principles to project this plant as a therapeutic target in diabetic research.

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## ETHICAL APPROVAL
The animal ethical committee in the Department of Laboratory Technology, Nasarawa Federal Polytechnic approved the animal studies with Protocol number: H20371. The principles of the laboratory animal care were followed.

## CONFLICT OF INTEREST
The authors declare no conflicts of interest in this research.

## AUTHOR'S CONTRIBUTION
JJG and JZ designed the experiment, oversaw data collection and execution, and contributed to manuscript preparation; TMN participated in experimentation, collation of data and manuscript preparation; AJY participated in design of experiment and manuscript preparation; ESS and MJT participated in sample collection, experimentation and data collation

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## LIST OF ABBREVIATIONS
ANOVA: Analysis of Variance; BUN: Blood Urea Nitrogen; DC: Diabetic Control; DM: Diabetes Mellitus; HD: High Dose; HSD: Honestly Significant Difference; IDF: International Diabetes Federation; LD: Low Dose; MC: Metformin Control; NC: Normal Control; SEM: Standard Error of Mean; STZ: Streptozotocin; T1DM: Type 1 Diabetes Mellitus; T2DM: Type 2 Diabetes Mellitus; WHO: World Health Organization; WSAR: Wistar Strain of Albino Rats.

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