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# *Moringa oleifera* leaf extract inhibits diabetogenic effect of alloxan in rats

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***Abstract:*** *This study investigated the protective and ameliorative potential of ethanol extract of Moringaoleifera leaf (50-200 mg/kg) on alloxan-induced diabetes in Wistar rats. Alloxan-induced diabetic rats were orally pretreated with extract (50, 100 or 200 mg/kg) or post-treated with extract (50, 100 or 200 mg/kg/day), glibenclamide (2.5 mg/kg/day) or metformin (200 mg/kg/day) for two weeks. Other animals received only extract, alloxan (diabetic control) or vehicle (control). Blood glucose concentrations were measured at the beginning and twice weekly. Glucose levels in extract pretreated rats were lower ($p<0.05$)than alloxan-induced levels, but when compared with control, were higher except that glucose level was normalized from the 11$^{th}$ day in 200 mg/kg extract pretreated rats. Glucose concentrations in extract, glibenclamide or metformin post-alloxan administered rats were decreased ($p<0.05$), compared to diabetic rats. But they were higher than control, except by day 14 wherein glucose level was normal or lower. When compared with glibenclamide or metformin, glucose levels of extract treated animals were higher on the 7$^{th}$ day, but lower at the end of treatment. In addition, extract treatment caused hypoglycemia after fourteen days of treatment in normal rats. The results demonstrate that Moringa leaf possesses protective and ameliorative antidiabetic potential in rats.*

***Keywords***: *Alloxan, antioxidants, diabetes, Moringa*

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## I. INTRODUCTION

Diabetes mellitus is a serious public health disease as it is a major health challenge to millions of people worldwide. Over 150 million people all over the world have diabetes, and this figure is expected to increase to 300 million by 2025 [1]. The disease, which results from abnormal control of blood glucose, is characterized by acute symptoms, as well as chronic complications which significantly affect the quality of life of affected individuals. Life expectancy of patients is even expected to be lower in the developing countries because the high prevalence of the disease is accompanied with lack of adequate treatment in these areas [1, 2]**.** Also, the disease impact is expected to be more experienced in the economically poor developing countries where majority of the population cannot afford the cost of conventional medications. There is therefore need for more affordable and cost effective means of managing the disease, probably using alternatives which are not only cheap but also have better side effect profiles than the current available drugs. In this regard, the World Health Organization, through its Expert Committee on Diabetes has considered the potential usefulness of efficacious herbs in diabetes treatment and has recommended that traditional medicinal herbs be investigated [3, 4]**.**

*Moringaoleifera*, is one of about thirteen species that belong to the Moringaceae Family. It is called the Miracle Tree as it has wide range of beneficial effects [5]**.** It has potent antioxidant activity [6, 7] and has high medicinal value. In some cases, it is referred to as 'superfood' because of its impressive nutritional profile and has been used successfully in some countries as a remedy for malnutrition [8]. Phytochemical screening of the plant has shown that it contains a wide array of phytochemical compounds. Ethanol leaf extract of Moringahas been shown to contain flavonoids, tannins, anthraquinones, cardiac glycosides, alkaloids, triterpenoids, saponins, and reducing sugars [9, 10]. The seed extract has been reported to contain all the above phytochemical constituents except anthraquinones [11]. Most of these phytochemicals (e.g., glycosides, alkaloids, terpenoids and flavonoids) have been found to possess antidiabetic properties [12, 13]. Previously, it has been reported that intraperitoneal administration with ethanol extract of *M. oleifera* leaf (250 and 500 mg/kg) reduced blood glucose levels in fasted streptozotocin-induced diabetic animals [9]. Ravi and Prasanna[14]also demonstrated

that dried leaf powder of *M. oleifera* decreased serum glucose and low density lipoproteins (LDL) levels in patients with non-insulin dependent diabetes mellitus and obesity.

Furthermore, a number of studies have shown that *M. oleifera* has a very good safety profile [15-17]. Bakre et al. [15] determined oral lethal dose of ethanol extract of *M. oleifera* leaf in mice as > 6.4 g/kg, Une et al. [16] reported zero mortality in Wistar rats after oral administration of 2-5 g/kg of ethanolic extract of *M. oleifera* pod. As a result of its high safety margin, most studies employ fairly high doses, 300-1000 mg/kg [9, 14, 18]. In addition, most of the antidiabetic studies on Moringadid not evaluate if the plant possesses any protective capacity for the disease.

This study was therefore intended to evaluate the protective and ameliorative potential of low dose levels of *M.oleifera* ethanol leaf extract (50-200 mg/kg) on alloxan induced diabetes in Wistar rats.

## II. Materials and Methods

### 2.1 Plant extraction

Fresh leaves of *M. oleifera*were harvested from a botanical garden at Aluu, Rivers State, Nigeria. They were washed, air dried and ground to fine powder after identification by Mr. C. C. Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The dried powder was weighed and then extracted by refluxing in 75% ethanol at room temperature. The extract was dried in a rotary evaporator, weighed and preserved in a refrigerated at 4$^{o}$C for the experiments.

### 2.2 Animals and Experimental Design

Sixty-five male Wistar rats weighing 180-220 g were used for the study. They were obtained from the Animal House of the Department of Pharmacology, University of Port Harcourt, Nigeria. The rats were maintained under natural room temperature (27±5$^{o}$C) and lighting condition, housed in cages in a ventilated room, fed with standard rodent diet and given tap water *ad libitum*. They were cared and handled humanly and all experimental procedures were in line with approved guideline of the Research Ethics Committee of the University of Port Harcourt, Nigeria.

The animals were randomized into thirteen groups (1-13) containing 5 rats each.
Group 1: Animals were given 0.5 ml DMSO plus distilled water (2:3) daily for 2 weeks. Blood was sampled at the beginning (day 1) and twice weekly (on days 3, 7, 11 and 14) to measure glucose levels in animals.
Group 2: Animals were injected intraperitoneally with a single dose of alloxan (120 mg/kg) to induce diabetes [19]**.** The rats were fasted overnight for 12-14 h but allowed access to water before alloxan administration. Blood glucose level was measured at the beginning and twice weekly for 2 weeks.
Groups 3, 4 and 5: Animals were pretreated with Moringa extract (50, 100 or 200 mg/kg, po) daily for 14 days, followed by induction of diabetes with alloxan and observed for 2 weeks. Blood glucose level was measured at the beginning and twice weekly after alloxan administration.
Groups 6, 7, 8, 9 and 10: Animals were injected alloxan, followed by treatment with Moringa extract (50, 100 or 200 mg/kg, po), glibenclamide (2.5 mg/kg, po) or metformin (200 mg/kg, po) daily for 2 weeks. Blood glucose level was measured at the beginning and twice weekly after extract and drugs administration.
Groups 11, 12 and 13: Animals were administered Moringa extract (50, 100 or 200 mg/kg, po) daily for 14 days. Blood glucose level was measured at the beginning and twice weekly.

The doses of metformin and glibenclamide used are equivalent to their therapeutic dose levels [20, 21]. The drugs and alloxan were dissolved in distilled water, while extract was dissolved in 40% DMSO. Extract and drug solutions were administered with a 1 ml syringe and oropharyngeal cannula. Blood glucose level was measured with an Accu-Check® active glucometer. Animals were fasted overnight for 12-14 h on days of sampling, and blood samples were obtained using the tail nipping method [22]**.**

### 2.4 Statistical Analysis

All the data are expressed as Mean±SEM (standard error of mean) and analyzed by one way ANOVA using GenStat Software, 2009 (VSN International Ltd., UK).Values were considered significant at $p<0.05$.

## III. Results

3.1 Effect of Moringa extract pretreatment on blood glucose levels in diabetic rats (study on protective effect)
Blood glucose levels in all extract pretreated groups were lower ($p<0.05$) when compared with the levels in rats that received alloxan alone (Table 1). However, the glucose levels in pretreated groups were higher ($p<0.01$) compared to the control except 200 mg/kg pretreated group where blood glucose levels became comparable with control on days 11 and 14 (Table 1). Intra-group comparison showed that glucose levels in 50 or 100 mg/kg extract administered groups were higher than the 200 mg/kg extract treated group (Table 1).

3.2 Effect of Moringa extract post-treatment on blood glucose levels in diabetic rats (study on ameliorative effect)

Blood glucose levels in rats that were administered extract were decreased ($p<0.05$) dose-dependently over the 14 days treatment period, compared to alloxan alone treated rats (Table 2). However, glucose levels in extract treated rats on sampling days 3, 7 and 11 were higher than control. On day 14, glucose level in the control (75.00±1.66 mg/dl) was comparable ($p>0.05$) with that in group that received 100 mg/kg extract (85.00±2.89 mg/dl), but lower ($p<0.05$) in the group that received 200 mg/kg extract, 43.00±1.73 mg/dl (Table 2). In addition, glucose levels in glibenclamide and metformin administered rats were reduced ($p<0.05$) over the treatment period compared to alloxan alone treated rats. Also, whereas glucose level in glibenclamide treated rats was not significantly different from control on day 14, all other glibenclamide and metformin induced glucose levels were higher than control (Table 2). When compared with glibenclamide or metformin, glucose levels of extract treated animals were not different on days 3 or 11, but higher on day 7 (Table 2). On the $14^{th}$ day, glucose level in rats that received the smallest dose of extract (50 mg/kg) was higher, while the level in rats that received the highest dose (200 mg/kg) was lower when compared to glibenclamide or metformin (Table 2).

3.3 Effect of Moringa extract on blood glucose levels in normal Wistar rats

Rats that were treated with Moringa extract had normal blood glucose levels that ranged from 75.00±2.89 to 78.00±4.62 mg/dl at the beginning (first day) of experiment. Blood glucose levels in these animals declined during the period of extract administration, but the values obtained were not significant compared to control except those that were obtained on the $14^{th}$ day, $p < 0.05$ (Table 3).

## IV. Discussion

Blood glucose level of fasting animals gives indication of both the circulating levels of insulin and sensitivity of peripheral tissues to the action of insulin. The normal blood glucose level in *Rattusnovergicus*rats (Wistar rats) is known to be 50-135mg/dl, and levels > 200mg/dl are generally considered to have severe hyperglycemia and induce diabetes [23].

Dimethyl sulfoxide (DMSO) plus water was used as vehicle for the extract and used as control in this study. From the results, blood glucose levels in control rats were within the above stated normal range, while alloxan caused over 400 % elevation in glucose level relative to the control. The alloxan treated rats showed persistent hyperglycemia (> 400 mg/dl) throughout the duration of the experiment. This is consistent with previous studies that have reported that alloxan induces irreversible diabetes mellitus after 24 h following its administration [23, 24]. It was further observed in this study that the elevated blood glucose levels of diabetic rats were reduced by extract in a dose- and time-dependent manner. Glibenclamide and metformin are standard oral antidiabetic drugs that are among the most commonly prescribed drugs to treat type 2 diabetes. Metformin is a biguanide that lowers blood glucose levels primarily by improving insulin sensitivity in the liver, i.e., inhibits gluconeogenesis, while glibenclamide is a sulfonylurea which lowers blood glucose levels by stimulating insulin secretion. Expectedly, they equally caused reduction in alloxan induced hyperglycemia as the extract. Moringa extract at 50 mg/kg was not as effective as glibenclamide in reducing blood glucose in diabetic rats, 100 mg/kg was as effective, whereas 200 mg/kg appeared to be more effective than glibenclamide. On the other hand, 100 and 200 mg/kg concentrations of the extract produced greater levels of reduction in diabetic rat's blood glucose level than metformin. The low concentration (50 mg/kg) of extract was less effective than metformin. It thus implies that doses above 100 mg/kg of the extract may produce comparable or better antidiabetic activity than metformin or glibenclamide. Antidiabetic activity of Moringa has been reported in earlier studies, but most of such studies used higher doses. Jaiswal et al. [25] observed dose-dependent blood glucose lowering activity of aqueous extract of Moringa leaf (100-300 mg/kg). Al-Malki and El Rabey [26] reported that low doses (50 and 100 mg/kg) of *M. oleifera seed* powder restored pancreatic histology of streptozotocin induced hyperglycemic rats to normal.

In pretreated rats, blood glucose levels were decreased over time relative to diabetic rats that did not receive any treatment. Although, only the pretreatment group that received 200 mg/kg of extract became normoglycemic on the last treatment day. Animals were pretreated with the extract daily for 14 days before diabetes induction, to study its protective antidiabetic activity. The result therefore suggests that *M. oleifera* may possess some form of protective role on pancreatic β cells, which is dose- and time-dependent. This finding is novel as no similar report on the plant exists. Mechanisms of alloxan induced diabetes has been shown to involve generation of reactive oxygen species (ROS) in pancreatic tissues that result in cell damage [27]. The observed inhibitory or ameliorative capacity of Moringa could be due to the presence of potent antioxidant phytochemicals like flavonoids and glycosides [6, 7, 28] which reduce oxidative stress by antagonizing or neutralizing the deleterious effects of ROS. The mechanism may also involve improvement or enhancement of the sensitivity of cells to insulin or exhibition of insulin like activity, i.e., the extract may have pancreatic and extra pancreatic effects.

In addition, the extract caused a gradual decrease in blood glucose levels of normoglycemic rats over time. The reduction was not significant initially, but by the end of the second week of extract administration, blood glucose levels became significantly lower than control glucose level (hypoglycemia). Some studies have reported that *M. oleifera* has no effect on blood glucose levels of normoglycemic rats; but this may be because most of those studies were carried out for shorter durations. Tende et al. [9] reported that there was no observed hypoglycemic effect of Moringa after 7 h treatment. Similarly, Ples et al. [29] reported no significant change in the glucose levels of normal subjects 2 h after they were given *M. oleifera* tea while significant reduction in blood glucose levels was observed in treated hyperglycemic patients. The above observations were consistent with our results that were obtained in treatment periods that were less than 14 days, but hypoglycemic effect was demonstrable after 14 days of administration of the extract in the present study. The present study thus reveals that short term administration of Moringa may not affect basal blood glucose level, but repeated administration over long duration may induce hypoglycemia. This agrees with the recent findings of Ravi and Prasanna [14] who demonstrated that dried leaf powder of Moringa produced hypoglycemia in patients with non-insulin dependent diabetes mellitus and obesity. Although, this may be an undesirable side-effect in diabetes treatment, human and animal toxicity studies have shown that Moringa is generally well tolerated with minimal side effects [17, 30]. This is obviously an advantage over many orthodox antidiabetic drugs that have several adverse effects, aside from hypoglycemia.

## V. Conclusion

The results demonstrate that *M. oleifera* leaf possesses protective and ameliorative antidiabetic potentials in rats which may be due to its antioxidant activity.

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**Conflicts of Interest**

None

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**Table 1:** Blood glucose levels after injection of alloxan (Allox) in ethanol *Moringaoleifera*leaf extract (EML) pretreated Wistar rats

| Group | Blood glucose concentration (mg/dl) | | | | |
|---|---|---|---|---|---|
| | Day 1 | Day 3 | Day 7 | Day 11 | Day 14 |
| Control | 74.00±2.46 | 73.00±1.00 | 71.00±2.66 | 70.00±0.33 | 75.00±1.66 |
| Alloxan | 74.00±1.23 | 440.00±7.12*** | 420.00±3.33*** | 410.00±0.33*** | 380.00±2.33** |
| EML (50 mg/kg)+Allox | 72.00±4.62 | 290.00±5.77**$^{\#}$ | 242.00±1.15**$^{a}$ | 202.00±1.15**$^{a}$ | 182.00±1.15*$^{a}$ |
| EML (100 mg/kg)+Allox | 75.00±2.89 | 287.00±9.81**$^{\#}$ | 239.00±5.20**$^{a}$ | 190.00±11.55*$^{a}$ | 143.00±1.73*$^{a}$ |
| EML (200 mg/kg)+Allox | 75.00±8.66 | 289.00±5.20**$^{\#}$ | 192.00±1.15* | 100.00±11.55$^{\#}$ | 70.00±2.89$^{\#}$ |

Data expressed as mean±SEM, n=5 rats per group
* Significant compared to control at p<0.05
** Significant compared to control at p<0.01
*** Significant compared to control at p<0.0001
$^{\#}$ Significant compared to alloxan at p<0.05
$^{a}$Significant compared to extract (200 mg/kg) at p<0.05

**Table 2:** Effects of ethanol *Moringaoleifera*leaf extract (EML), metformin (Met) and glibenclamide (Glib) on alloxan (Allox)-induced hyperglycemia in Wistar rats (study on ameliorative effect)

| Group | Blood glucose concentration (mg/dl) | | | | |
|---|---|---|---|---|---|
| | Day 1 | Day 3 | Day 7 | Day 11 | Day 14 |
| Control | 74.00±1.33 | 73.00±1.00 | 71.00±2.66 | 70.00±0.33 | 75.00±1.66 |
| Allox | 70.00±1.66 | 440.00±7.12*** | 420.00±3.33*** | 410.00±0.33*** | 380.00±2.33*** |
| Allox+ EML (50 mg/kg) | 70.00±5.20 | 456.00±3.46*** | 310.00±5.77** $^{a\ b}$ | 200.00±11.55** | 134.00±2.31* $^{a\ b}$ |
| Allox+ EML (100 mg/kg) | 73.00±1.73 | 452.00±1.15*** | 290.00±2.89** $^{a\ b}$ | 165.00±8.66*$^{\#}$ | 85.00±2.89$^{\#\ b}$ |
| Allox+ EML (200 mg/kg) | 73.00±3.46 | 430.00±17.32*** | 284.00±2.31** $^{a}$ | 120.00±11.55*$^{\#\ b}$ | 43.00±1.73*$^{\#\ a\ b}$ |
| Allox+Glib | 74.00±2.31 | 476.00±17.32*** | 254.00±2.31** | 162.00±1.15* | 88.00±4.62 |
| Allox+Met | 71.00±0.58 | 443.00±24.83*** | 266.00±3.46** | 192.00±1.15* | 110.00±5.77* |

Data expressed as mean±SEM, n=5 rats per group
* Significant compared to control at p<0.05
** Significant compared to control at p<0.01
*** Significant compared to control at p<0.0001
$^{\#}$ Significant compared to alloxan at p<0.05
$^{a}$Significant compared to glibenclamide at p<0.05

[b]Significant compared to metformin at $p<0.05$.

**Table 3:** Blood glucose levels in normal Wistar rats following 14 days daily treatment with ethanol extract of *Moringaoleifera*leaf

| Dose (mg/kg) | Blood glucose concentration (mg/dl) | | | | |
|---|---|---|---|---|---|
| | Day 1 | Day 3 | Day 7 | Day 11 | Day 14 |
| Control | 76.00±2.46 | 71.00±0.33 | 71.00±2.33 | 71.00±2.66 | 74.00±1.33 |
| 50 | 78.00±4.62 | 70.00±0.33 | 65.00±2.89 | 52.00±5.15 | 43.00±1.73* |
| 100 | 75.00±2.89 | 71.00±0.58 | 70.00±5.77 | 57.00±4.04 | 43.00±1.73* |
| 200 | 73.00±8.66 | 70.00±5.77 | 63.00±1.73 | 50.00±5.77 | 41.00±0.58* |

Data expressed as mean±SEM, n=5 rats per group
*Significant compared to control at $p<0.05$.

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