Effects of *Moringa oleifera* Leaves Methanolic Extract on Alloxan- Induced Diabetic Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author CU designed the study performed the laboratory and statistical analysis. Author CCE analyzed the result and wrote the first draft of the manuscript. Author AN supervised the entire work interpreted the results. All authors read and approved the final manuscript.

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ABSTRACT

*Moringa oleifera*, popularly known as “miracle tree” belongs to the family, Moringaceae. It is a medicinal plant in which the leaves are the most nutritious part, being a significant source of vitamins and protein among others. This study was conceived and designed based on the gaps in the research that has been performed and what is known about the plant. In this study, the effect of *Moringa oleifera* leaves extract on alloxan induced diabetes in Wistar albino rats was investigated. A total of forty five (45) rats were acclimatized for a period of two weeks, then randomly divided into five (5) groups (1, 2, 3, 4, and 5) of nine (9) rats each and fed with standard feed and water. Group 1 which is the control was fed with just water and standard feed while Hyperglycemia was induced in groups 2, 3, 4, & 5 intra-peritoneally after an over-night fasting using alloxan at a concentration of 130 mg/kg b.w. and allowed for 48hours which resulted in a high blood glucose level between 300 mg/dl and 600 mg/dl. Group 2 was not given any treatment while Groups 3, 4, & 5 were treated with doses 100 mg/kg b.w., 200 mg/kg bw, and 400 mg/kgbw of *Moringa oleifera* leaf extract respectively for a period of four weeks. A glucometer was used to check the blood...

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1. INTRODUCTION

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period [1]. Symptoms of high blood sugar include frequent urination (polyuria), glycosuria (presence of glucose in urine) and hyperglycemia (glucose rate on an empty stomach higher than 1.2 g/l in plasma blood and confirmed in at least two occasions) increased thirst, and increased hunger [2].

Basically, there are two major clinical classes of diabetes; type 1 diabetes mellitus and type 2 diabetes mellitus. If left untreated, diabetes can cause many complications. Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes [1]. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced [3].

According to the International Diabetes Federation [4] 2014 updates, out of the world seven billion population, 387million people, aged 20–79 years worldwide are diabetic, giving a comparative prevalence of 8.3%, while 46.3% cases are undiagnosed. In every 7 seconds, a person dies of diabetes, 4.9 million deaths was recorded in 2014 [4]. Seventy seven percent (77%) of people with diabetes live in low and middle-income countries. Africa has recorded cases of 2,150,274 (5.05%) diabetic patients with over 13 million undiagnosed cases. In Nigeria, there are estimated 374,651 diabetic cases, with another 172,339 undiagnosed cases. These figures account for about 4.64% Nigerian adults between ages 20-79 living with diabetes. An estimated 105,090 Nigerians died in 2014 as a result of diabetes [4]. An average diabetic Nigerian spent about 43527.16 naira (US $178.39) in 2014 due to diabetes treatment [4]. With this alarming prevalence rate, diabetes mellitus poses a major challenge globally and accounts for a number of disabilities and deaths globally.

Medicinal plants have been identified and used throughout human history [5]. Medicinal plants are plants which can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [6]. Many important drugs used in healthcare today are directly derived from plants due to its bioactive constituents such as; alkaloids, tannins, steroids, etc [5]. Medicinal Plant materials have been shown to have various chemicals also known as phytochemical at various concentrations. These phytochemicals play vital roles in the medicinal and otherwise properties of the plant materials. Plants may act on blood glucose through different mechanisms. Some plants may contain insulin-like substances [7], inhibit insulinase activity or increase beta β-cells in the pancreas by activating the regeneration of these cells [8,9], or some may serve as antioxidants by reducing the oxidative stress due to free radicals in the pancreas [10,11].

Moringa oleifera is one of the most widely distributed species of the Moringaceae family throughout the World, especially in Asian countries, having a remarkable range of pharmacological properties in addition to significant nutritional value. Moringa derives from the Tamil word, 'murungai', referring to a twisted pod found in young fruit [12]. M. oleifera is a fast-growing, deciduous tree that can reach a height of 10–12 m (32–40 ft) and trunk diameter of 45 cm (1.5 ft). The bark has a whitish-grey color and is surrounded by thick cork while the shoots have purplish or greenish-white, hairy bark. The tree has an open crown of drooping and fragile branches. The flowers are fragrant and bisexual, surrounded by five unequal, thinly veined, yellowish-white petals. The fruit is a hanging, three-sided brown capsule of 20-45 cm size which holds dark brown, globular seeds with a diameter around 1 cm. The seeds have three whitish papery wings and are dispersed by wind and water [13].

Keywords: Moringa oleifera; diabetic rats; hyperglycemia alloxan.

The results of Groups 3, 4, & 5 (172.0±4.75 mg/dl, 142.9±47.25 mg/dl, 70.6±24.46 mg/dl respectively) showed a significant decrease (p< 0.05) in blood glucose level of the induced rats when compared with Group 2 (316±47.17 mg/dl) which was induced only alloxan. It can therefore be concluded that this study has shown that the extract of Moringa oleifera leaves offers an anti-diabetic effect in Wistar albino rats.

M. oleifera leaves offers an anti-DM activity or increase beta β-cells in the pancreas by activating the regeneration of these cells [8,9], or some may serve as antioxidants by reducing the oxidative stress due to free radicals in the pancreas [10,11].
The leaves are the most nutritious part of the plant, being a significant source of B vitamins, vitamin C, pro-vitamin A as beta-carotene, vitamin K, manganese, and protein, among other essential nutrients [14]. The therapeutic use of *M. oleifera*, leaves has been evaluated in diabetes because of their possible capacity to decrease blood glucose concentrations after ingestion because they contain polyphenols such as quercetin-3-glycoside, rutin, kaempferol and glycosides [15,16,17].

Several biological activities of the *Moringa oleifera* leaves have been reported such as anti-septic, antioxidant, antihypertensive, larvicidal, fungicidal, hypolipidemic amongst others [18]. Several studies have shown that *Moringa oleifera* leaves presented anti-diabetic properties [17,19, 20,21]. Other parts of the plant such as the pods and seed have also shown to exhibit anti-diabetic property [22].

At the moment, there are a growing researches on herbal remedies considered to be less toxic and have negligible side effect for the management of diabetes mellitus, especially in countries where access to conventional treatment of the disease is inadequate [23]. The present study evaluates the anti-diabetic effect of the leaf extracts of *M. oleifera* on alloxan-induced wistar albino rats with a view to providing more information on the clinical treatment of diabetes.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Fresh large quantity of leaves of *Moringa oleifera* plant was gotten from a farm in Elele, River State, Nigeria. The botanic identification andauthentication (MU/PHGSY/05/001) was done in faculty of pharmacy Madonna University, Elele campus. *Moringa oleifera* leaves were dried under room temperature and ground with manual grinder. Cold extraction was carried out by soaking 400 g of *M. oleifera* leaf powder in 2L of methanol for 72 hrs. The mixture was subsequently filtered using Whatman filter paper. The residue was re-extracted in 2L of methanol for 48 hrs and then concentrated using the rotator evaporator and dried. The extract was stored at -20°C until use.

2.2 Animal Experiment

A total of 45 albino rats weighing between 120-180g were obtained from the animal house of Madonna University, Elele, Nigeria and used for the study. They were housed in a photoperiod cycle of 12 h: 12 h (Light and dark), at room temperature (28°C) and fed with standard laboratory diet and distilled water for a period of two weeks for acclimatization.

Groups 2, 3, 4, and 5: were induced with diabetes by the intraperitonal (IP) injection 130 mg/kg body weight of alloxan monohydrate solution. They rats were assigned into five (5) groups of nine (9) rats per group as shown below:

- **Group 1:** Normal Untreated rats (negative control)
- **Group 2:** received Untreated Diabetic rat (positive control)
- **Group 3:** Diabetic rats treated with 100 mg/kg of leaf extract.
- **Group 4:** Diabetic rats treated with 200 mg/kg of leaf extract.
- **Group 5:** Diabetic rats treated with 400 mg/kg of leaf extract.

2.3 Induction of Diabetes

The baseline blood glucose levels of the rats were determined before they were induced with diabetes by intraperitonal (IP) injection of 130 mg/kg body weight of alloxan monohydrate solution [24]. After a period of 48 hours, the rats were tested to ascertain the onset of diabetes. The blood glucose levels of the animals were determined using an Acc-Check glucometer. The blood glucose levels of the rats were determined on a weekly basis for four (4) weeks of administration of the extracts. The body weights of the rats before induction, after induction and at intervals during the extract administration were noted. The treatment was withdrawn after a 28 days regime.

2.4 Administration of Extracts

With the aid of a gavage tube, the prescribed doses of plant extracts were orally administered to the rats daily, for 28 days of experiment.

3. RESULTS

The results obtained from the study were expressed as mean ± standard deviation and comparison of differences in their various groups were done using one-way analysis of variance.
4. DISCUSSION

Over the past years, numerous experimental and epidemiological studies have shown that a wide variety of plant materials have anti-diabetic properties. Natural products mainly from the plant kingdom among which we have *Moringa oleifera*, offer a wide range of biologically active compounds that act as natural antioxidants with potential in anti-diabetic drug discovery and development.

Results from Table 1 shows that the weight of the animals after the induction with alloxan and during the treatment with *Moringa oleifera* leaf extract revealed that there was no significant difference between the initial body weights (P< 0.05).

Whereas the normal rats (group 1) gained weights, the untreated diabetic rats (group 3). This weight loss could be attributed to the action of diabetes. The ability of alloxan induced diabetes to induce weight loss in diabetic untreated rats mimics what is commonly observed in clinical diabetes [1].

Within the *Moringa oleifera* treated groups (groups 3, 4 and 5), it would be noticed that there was an initial loss of weight amongst these groups of rats probably as a result of onset of diabetes (week two). However, as treatment was introduced, it could be noticed that they rats showed little weight gain. This suggests a possible short-term positive effect of the extracts on body weight of diabetic rats. At this point, it could be noticed that group 5 with the highest extract dose exerted the highest percentage weight gain (2.94%), while group 3 with the lowest extract dose presented the lowest percentage gain (0.15). This suggests a possible dose dependent weight gain. It seems that as the dose of *Moringa* was increased, the weight gained increased.

At week four, the treated groups continued to gain weight, however the trend in earlier week didn’t continue. Group 4 with the middle extract dose scored the highest percentage weight gain (2.04%), while group 3 still maintained it lowest percentage weight gain (0.91%). This suggests that in the long run, a more moderate extract dose would exert a better effect on weight gain. Thereby, suggesting a possible deleterious effect of increased dosage on weight in the long run. The restoration of body weight by *Moringa* seems to be due to its lowering blood sugar property by increased glucose metabolism, and this may be due to the protective effect of the extract in controlling muscle wasting, by reversal of gluconeogenesis [25].

Diabetic *M. oleifera* treated rats increased in body weight. This result is consistent with previous studies. Olayaki et al. [25] observed that oral administration of extract of *M. oleifera* inhibits weight loss in alloxan induced diabetic rats.

Table 2 shows that there is no significant difference (p > 0.05) in the glucose levels in all the groups before diabetes was induced. This implies that the blood Glucose level in all groups before induction of diabetes is comparable.

The blood glucose level at week 1 (Table 2) shows that there is no significant difference in the mean glucose levels (p > 0.05) among the Groups. Group 2 and Group 4 had near same blood glucose levels (70.8±5.12 mg/kg and 70.9±5.99 mg/kg respectively), while Group 3 and 5 had similar glucose levels (66.3±7.57 mg/kg and 67.7±6.78 mg/kg respectively). Group 1 had the highest blood glucose level (73.2±8.26 mg/kg).

At week two, there is a significant different between Group 1 and the other groups signifying diabetes. Within the diabetic Group, there is no significant difference between them.

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Table 1. The mean value of the weight (g) of all the rats treated throughout the experimental period

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>129.77±20.52</td>
<td>131.77±12.09</td>
<td>139.0±12.86</td>
<td>148.0±16.59</td>
</tr>
<tr>
<td>2</td>
<td>140.77±18.97</td>
<td>127.33±5.26</td>
<td>119.5±10.94</td>
<td>74.77±14.62</td>
</tr>
<tr>
<td>3</td>
<td>119.67±14.20</td>
<td>110.23±17.50</td>
<td>110.4±17.55 (↑0.15)</td>
<td>111.4±11.30 (↑0.91)</td>
</tr>
<tr>
<td>4</td>
<td>124.56±14.37</td>
<td>116.2±20.40</td>
<td>117.6±11.70 (↑1.20)</td>
<td>120.0±25.36 (↑2.04)</td>
</tr>
<tr>
<td>5</td>
<td>119.57±10.40</td>
<td>108.8±14.20</td>
<td>112.0±8.47 (↑2.94)</td>
<td>113.8±11.90 (↑1.61)</td>
</tr>
</tbody>
</table>

Mean ± SD. Figures in parenthesis indicate percentage decrease↓ or increase↑ in body weights.

P<0.05=Significant, p>0.05=Not significant
This finding collaborates with the report of Sai et al. [26] which clearly revealed that aqueous extract of *M. oleifera* leaf possesses potent antihyperglycemic and antihyperlipidemic effect in both insulin resistant and insulin deficient rat models. Similarly, after treating diabetic rats with both aqueous and ethanolic extracts of *Moringa* leaf for 14 days, Ezeigbo et al. [19], reported a percentage reduction of 45.2% and 33.7% respectively in blood glucose of the diabetic rats. In another study by Olayaki et al. [27], they observed that oral administration of extract of *M. oleifera* significantly reduces blood glucose concentration. Studying the effect of the consumption *Moringa* by diabetic rats, Villarruel-López et al. [21] reported that consumption of the leaves showed a hypoglycemic effect in diabetic rats. A further research by Lopez et al. showed that the tested doses revealed no lethal dose and no significant differences in genotoxicity parameter. Another study by Basyony et al. [20] suggested that *Moringa oleifera* seeds extract was able to reverse the inhibit ion of insulin secretion from the pancreatic beta cells and reduced the blood glucose level. Furthermore, Ghiridhari et al. [28] reported that medication with *M. oleifera* gives diabetic patients better glucose tolerance by increasing treatment time.

The anti-diabetic effects of *Moringa* leaf extracts indicate the presence of hypoglycaemic agents in the plant. *M. oleifera* contains three classes of phytochemicals, that is, glucosinolates such as glucororingin, flavonoids such as quercetin and kaempferol, and phenolic acids such as chlorogenic acid; all of these classes have medicinal benefits [29,30]. These three phytochemicals of *Moringa* possess antioxidant, hypoglycemic, hypotensive, antidyshlipidemic, anticancer, and anti-inflammatory properties [31, 32,33]. Hypoglycemic effect of Moringa leaf may probably be due to contents of elements such as calcium, magnesium, potassium, sodium, zinc, and chromium [34]. These elements play role in blood glucose homeostasis by regulating the key enzymes involved in gluconeogenesis in the liver.

### Table 2. The mean value of the glucose concentration (mg/kg) of all the rats treated throughout the experimental period

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73.2±8.26</td>
<td>68.2±7.41</td>
<td>71.4±3.67</td>
<td>74.8±14.62</td>
</tr>
<tr>
<td>2</td>
<td>70.8±5.12</td>
<td>465.2±79.7</td>
<td>394.2±56.6</td>
<td>316.1±47.17</td>
</tr>
<tr>
<td>3</td>
<td>66.3±7.57</td>
<td>460.4±74.46</td>
<td>275.3±92.63 (↓40.20)</td>
<td>172.0±45.75 (↓37.52)</td>
</tr>
<tr>
<td>4</td>
<td>70.9±5.99</td>
<td>477.8±80.75</td>
<td>357.2±140.16 (↓25.24)</td>
<td>142.9±47.25 (↓59.99)</td>
</tr>
<tr>
<td>5</td>
<td>67.7±6.78</td>
<td>495.2±56.19</td>
<td>190.2±63.77 (↓61.59)</td>
<td>70.6±24.46 (↓62.88)</td>
</tr>
</tbody>
</table>

Mean ± S.D. P<0.05=Significant, p>0.05=Not significant. Figures in parenthesis indicate percentage decrease↓ in blood glucose level within the weeks.

At the end of week three, within the diabetic Groups, Group 2 indicated the highest mean blood glucose level (394.2±56.6 mg/kg). All rats treated with *Moringa oleifera* presented an appreciable level of percentage decrease in their blood glucose showing a possible anti-diabetic effect. Within the *Moringa oleifera* treated Groups, from Table 2, Group 5 presented the highest percentage decrease in blood glucose level (↓61.59%). This probably indicates that higher doses of *Moringa oleifera* can reduce blood glucose more than lower doses, indicating a dose dependant relationship.

At the end of the fourth weeks of treatment (Table 2), there is no significant difference (p > 0.05) between Groups 1 and 5 (74.8±14.62 mg/kg and 70.6±24.46 mg/kg). Within the diabetic Groups, Group 2 indicated the highest mean blood glucose level (316.1±47.17 mg/kg). Groups 3, 4, and 5 treated with *Moringa oleifera* presented percentage decrease in their blood glucose (↓37.52, ↓59.99 and ↓62.88 respectively) showing an anti-diabetic property of the plant sample. Within the *Moringa oleifera* treated Groups, from Table 2, Group 5 presented the highest percentage decrease in blood glucose level (↓62.88), while Group 3 with lowest dose showed the smallest percentage decrease (↓37.52). This pattern tends to show that as the dose is reduced, the anti-diabetic property of the leaf is reduced.

As shown in the Table above, *Moringa oleifera* exerted an blood glucose lowering activity on diabetic rats. Groups with higher doses presented a better percentage decrease showing the possibility that higher doses can reduce blood glucose more than the smaller doses.

The hypoglycemic effect of *Moringa olifera* leaf observed in the present study agrees with results of earlier studies. This finding collaborates with the report of Sai et al. [26] which clearly revealed that aqueous
e.g. glucose-6- phosphatase, fructose-1, 6-bisphosphatase and phosphoenolpyruvate carboxykinase, thereby blocking gluconeogenesis and enhancing glucose utilization in the body [34]. The leaf may also contain certain hypoglycemic agents such as phytochemicals like tannins. It might also contain insulin stimulatory substances such as insulin receptors substrate (IRS), glycogen synthase, the β3 adrenergic receptor, glucose dependent insulinotropic polypeptide (GIP) receptor [34]. However, the mechanism by which the extract lowered the blood glucose level in alloxan induced diabetic rats is still unclear. It could be by stimulating peripheral utilization of glucose by inhibiting absorption in the gastrointestinal tract (GIT), increasing glucose metabolism, or regenerating the pancreatic tissue or potentiating the insulin secretion by the surviving B-cells [35]. It has been established that alloxan monohydrate destroys the pancreatic β-cells [36,37] hence, M. oleifera leaf extract might exhibit anti-diabetic property by the regeneration of β-cells to release insulin [38,39]. This ameliorates the effect of the alloxan and thereby normalizes the elevated serum level of glucose [38].

Reports from several studies have also shown that diabetes can be managed by herbal approaches with results comparable with the result obtained in the present study. A 2018 study on hypoglycaemic and biochemical effects of the aqueous and methanolic extract of Persea americana seeds on alloxan-induced albino rats by Ejiofor et al. [40] concluded that The effects of different doses (200 mg/bw, and 300/bw) of both water and methanol extracts of P. americana seed were comparable to those of a reference drug, insulin. About 94% seed diet of Acacia arabica showed hypoglycemic effect in rats through release of insulin. Acacia arabica seed powder at 2, 3 and 4g/kg, b.wt., exerted a significant hypoglycemic effect in normal rabbits by stimulating the release of insulin from pancreatic beta cells [41]. Plants such as Gallega officinalis [42,43,44] Syzygium cumini [45,46], are reported to have anti diabetic properties. Interestingly, these studies were carried out with different parts of the plants such as seed, leaves and even flower. Anti hyperglycemic activity of Allium sativum (garlic) was reported to be most potent when administered at 0.25 mg/kg b.wt dose level which was due to increased insulin-like activity [47]. Oral administration of the juice, ethanol extract, and oil of A. sativum has remarkably blood sugar lowering effect in normal and alloxan-induced diabetic rats or rabbit suggesting stimulation of insulin secretion from parital cells of pancreas [48].

5. CONCLUSION

Results from this study indicate indicates that extracts of M. oleifera leaf exerts significant anti-diabetic property in rats. These observations provide a pharmacological basis for the traditional use of M. oleifera leaf in the management of diabetes mellitus. However, further studies are required to identify the active ingredient responsible for the anti-diabetic properties of the leaf extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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