

Effects of Aqueous Extract of *Moringa oleifera* Seeds on Alloxan Induced Hyperglycemia

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Abstract This study examines the effect of aqueous extract of *Moringa Oleifera* seeds on alloxan induced mild and severe hyperglycemia relative to its route of administration. The animals were divided into six groups (n = 6). Group I animals received only rat chow and water (served as control group). Group II animals received only alloxan and were not treated (served as Hyperglycemic group), Group III received alloxan and after three hours were treated with 400mg/kg body weight of aqueous extract of *moringa oleifera* seed orally (served as mild hyperglycemic group A), Group IV received alloxan and after three hours were treated with 400mg/kg body weight of aqueous extract of *moringa oleifera* seed intraperitoneally (served as mild hyperglycemic group B). Group V received alloxan and after two days, began receiving treatment with 400mg/kg body weight aqueous extract of *moringa oleifera* seed orally for two weeks (served as severe hyperglycemic group A). Group VI received alloxan and after two days, began receiving treatment with 400mg/kg body weight aqueous extract of *moringa oleifera* seed intraperitoneally for two weeks (served as severe hyperglycemic group B). The result of the study showed a significant decrease in the blood glucose level after six hours and also after fourteen days of both oral and intraperitoneal treatment of the mild hyperglycemia with *moringa oleifera* seed extract. Also there was a 48.6% and 42.8% decrease in the blood glucose level of the mildly hyperglycemic rats on treatment with both oral and intraperitoneal *moringa oleifera* seed extracts and a 69.7% and 89.6% decrease in the blood glucose level of the severely hyperglycemic rats on treatment with both oral and intraperitoneal *moringa oleifera* seed extracts respectively. The study shows that *Moringa oleifera* seed extract exhibited a hypoglycemic effect on both the mild and severe alloxan induced hyperglycemic rats.

Keywords Alloxan, Hyperglycemia, *Moringa oleifera* seed, Rats

1. Introduction

Moringa Oleifera (drumstick or horseradish in English) is a member of Moringaceae family. It is an edible plant and it is grown extensively in many Southeast Asian countries particularly in Thailand, India, Philippines and Pakistan [1]. It has long been known as a food plant and as an ingredient of Indian traditional medicine [2, 3]. Phytochemical analyses have shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as such known antioxidants such as β -carotene, vitamin C, and flavonoids [4, 5]. Aside the nutritional benefits of *Moringa oleifera* it has been reported to have medicinal values. *Moringa oleifera* has anti-inflammatory [6] and thyroid status regulator [7] efficacies and researchers reported its hypoglycemic potential [8].

Hyperglycemia can be defined as excess sugar (glucose)

in the blood. It is a common complication of critical illness, regardless of a history of diabetes mellitus. It has an estimated prevalence of approximately 40% in hospitalized patients [9]. Hyperglycemia resulting either due to defective production or action of insulin leads to a number of complications; cardiovascular, renal, neurological, ocular etc [10]. In general, the normal range of glucose for most people (fasting adults) is about 80 to 110 mg/dl or 4 to 6 mmol/l. (where 80 mg/dl is "optimal".) An individual with a consistent range above 126 mg/dl or 7 mmol/l is said to have hyperglycemia, whereas a consistent range below 70 mg/dl or 4 mmol/l is considered hypoglycemic. In fasting adults, blood plasma glucose should not exceed 126 mg/dL. An individual is diagnosed as diabetic when his blood glucose level is chronically ≥ 126 mg/dL after an overnight fast and ≥ 200 mg/dL 2h after an oral glucose load of 75g Oral (Glucose Tolerance Test, OGTT [11]. Sustained higher levels of blood sugar cause damage to the blood vessels and to the organs they supply, leading to the complications of diabetes [12]. Blood glucose levels can also get too high if cells are unable to respond to insulin properly (insulin resistance). Diabetes mellitus is a disease that occurs when the body can't use glucose properly. Hyperglycemia is a

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symptom of diabetes; however, one can have hyperglycemia without having diabetes.

Alloxan is a drug used to induce experimental diabetes due to the selective destruction of the insulin-producing pancreatic beta – islets [13]. Alloxan induces a multiphasic blood glucose response when injected into an experimental animal, which is accompanied by corresponding inverse changes in the plasma insulin concentration followed by sequential ultrastructural beta cell changes ultimately leading to necrotic cell death [14]. In one of the multiphasic phases of alloxan, it has shown that one hour after the administration of alloxan, there is a rise in blood glucose level with a decrease in insulin concentration at the same time. This is known as the first hyperglycaemic phase after first contact of the alloxan with the pancreatic beta cells. This hyperglycaemic phase lasts for about 2-4 hours. [15]. This study therefore aimed to determine the anti-hyperglycemic activity of *Moringa oleifera* seed extract on alloxan induced mild and severe hyperglycemia in rats.

2. Methodology

2.1. Collection of Plant Material and Extraction

25g of the seeds of the plant *Moringa oleifera* bought from Masakka market in Nasarawa state of Nigeria was shade dried, milled and ground into coarse powder using a laboratory mortar. The coarse powder seed was soaked with 100ml of distilled water for 20min and filtered with a sieve. The filtrate was then centrifuged and the supernatant decanted, collected in a storage bottle, and stored at temperature of -4°C.

2.2. Acute Oral Toxicity Study of *M. oleifera* Extract

Acute toxicity study was carried out according to Organisation for Economic Co-operation and Development (OECD) guideline 423 [29]. None of the rats showed observable signs of toxicity upon single administration of *M. oleifera* seed extract (2000mg/kg body weight) on day one. Observations twice daily for 14 days also did not reveal any drug related observable changes. A dose of 400mg/kg body weight was used for the study which is one fifth of the dose used for the toxicity study. It has been reported to have anti-hyperglycemic properties by increasing blood glucose tolerance in the normal rat [30].

2.3. Animals

36 Healthy adult female albino Wistar rats weighing between 200g - 225g were procured from National Institute of Pharmacological and Research Development (NIPRED). The animals were housed under laboratory conditions (12h light and 12h dark cycle). They were fed with rat chow and water *ad libitum* and acclimatized for two weeks.

2.4. Induction and Treatment of Mild and Severe Hyperglycemia

The baseline blood glucose levels were determined

before the induction of hyperglycemia. The rats were fasted overnight prior to injection of alloxan dissolved in normal saline at a dose of 150mg/kg body weight given intraperitoneally [23]. After 3 hours, a period that targets the first hyperglycaemic phase after the first contact of the pancreatic cells with the toxin [24, 25], rats with blood glucose levels greater than 135mg/dl were considered as mildly hyperglycaemic. While after 3 days, a period that targets the last and 4th phase of the blood glucose response [24, 25, and 26] rats with blood glucose levels greater than 135mg/dl were considered as severely hyperglycemic and were used for the investigation [16]. The treatment with 400mg/kg body weight aqueous extract of *moringa oleifera* seed extract [27, 28] was done for fourteen days in which blood glucose levels and body weight of the rats were taken on day 0, 7 and 14 of administration.

2.5. Experimental Design

The rats were randomly divided into 6 groups consisting of 6 animals each:

Group I Control animals received only rat chow and water

Group II Hyperglycemic animals that received only alloxan and no treatment

Group III (mild hyperglycemic group): Animals received alloxan and after three hours were treated with 400mg/kg body weight of aqueous extract of *moringa oleifera* seed orally.

Group IV (mild hyperglycemic group): Animals received alloxan and after three hours were treated with 400mg/kg body weight of aqueous extract of *moringa oleifera* seed intraperitoneally.

Group V (severe hyperglycemic group): Animal received alloxan and after two days, began receiving treatment with 400mg/kg body weight aqueous extract of *moringa oleifera* seed orally for two weeks.

Group VI (severe hyperglycemic group): Animal received alloxan and after two days, began receiving treatment with 400mg/kg body weight aqueous extract of *moringa oleifera* seed intraperitoneally for two weeks.

2.6. Measurement of Blood Glucose Level

Blood glucose levels were measured by the Medisense Precision PCx (Abbott Medisense Division, Bedford, MA) blood glucose testing system (glucose strip method). The tip of the tail was snipped with sharp scissors and gently squeezed for a drop of blood. The strip was inserted into the machine, and the drop of blood was placed on the strip. Within 20s, the instrument measured and displayed the blood glucose level. The blood glucose level of the rats were taken just before the administration of alloxan (for the group that received alloxan) to induce mild and severe hyperglycemia.

For Group III and IV, their blood glucose level was checked three hours after inducing hyperglycemia, before the total damaging of the pancreatic beta cells by the alloxan and was immediately treated with aqueous extract

of *moringa oleifera* seed orally and intraperitoneally respectively in order to see the immediate effect of the extract on hyperglycemia before the complete damaging of the beta cells of the pancreas and also to compare the effectiveness of both routes of administration in treatment. The blood glucose level of the treated groups was observed three hours after treatment.

For Group V and IV, their blood glucose level were checked two days after the administering alloxan after which they began receiving treatment with aqueous extract of *moringa oleifera* seed orally and intraperitoneally respectively for two weeks. A weekly record of the blood glucose was observed after oral and intraperitoneal administration where the rats had already developed hyperglycemia.

2.7. Statistical Analysis

The data were expressed as mean \pm S.E.M. Data were analysed using Student t-test and ANOVA was used for more than two groups. Data were considered significant when $p < 0.05$.

3. Results

Table 1 show a significant decrease in the blood glucose level after six hours of both oral and intraperitoneal treatment of the mild hyperglycemia (that was induced by alloxan) with *moringa oleifera* seed extract.

Table 1. Percentage change in the fasting blood glucose levels of the rats after six hours of treatment with *Moringa Oleifera* seed extract as compared with the untreated group for mild hyperglycemia

Treatment	Mild Hyperglycemia	% Change in Blood Glucose Levels
Alloxan only	233.4	
Alloxan + moringa (oral)	146.8	37.1%
Alloxan + moringa (IP)	133.4	42.8%

Table 2 shows that there was a 69.7% and 89.6% decrease in the blood glucose level of the severely hyperglycemic rats on treatment with both oral and intraperitoneal *moringa oleifera* seed extracts.

Table 2. Percentage change in the fasting blood glucose levels of the rats after treatment with *Moringa Oleifera* seed extract as compared with the untreated group for severe hyperglycemia

Treatment	Severe Hyperglycemia	% Change in Blood Glucose Levels
Alloxan Only	601	
Alloxan + Moringa (oral)	182.0	69.7%
Alloxan + Moringa (IP)	62.4	89.6%

4. Discussion

Induction of diabetes using alloxan has been described as a useful experimental model for studying the effects of hypoglycemic agents [13]. Alloxan and the products of its

reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with simultaneous massive increase in cytosolic calcium concentration, resulting in the destruction of pancreatic beta cells and severe hyperglycemia [13]. The untreated hyperglycemic rats had a significantly higher fasting blood glucose level than control rats that received only rat chow and water *ad libitum*. This confirms induction of hyperglycemia by alloxan. Table 1 showed that in the mildly hyperglycemic rats, only a little increase in the fasting blood glucose was noted.

Alloxan induces a multiphasic blood glucose response when injected into to an experimental animal, which is accompanied by corresponding inverse changes in the plasma insulin concentration followed by sequential ultrastructural beta cell changes ultimately leading to necrotic cell death. The first phase that comes into view within the first minutes after alloxan injection is transient hypoglycemic phase that lasts maximally for 30 minutes. The 2nd phase appearing one hour after administration of alloxan leads to rise in blood glucose concentration. Moreover, the plasma insulin concentration has been noted to decrease at the same time. This is the first hyperglycemic phase after the first contact of the pancreatic beta cells with the toxin. This hyperglycemic phase lasts for 2-4 hours which is accompanied by decreased plasma insulin concentrations. These changes are a result of inhibition of insulin secretion from the pancreatic beta cells that is attributed to the induction due to their beta cell toxicity [23, 24, and 27]. From this study it is suggested that *moringa oleifera* seed extract was able to reverse the inhibition of insulin secretion from the pancreatic beta cells since only a few of the pancreatic beta cells may have been destroyed before treatment with the extract began three hours after induction of hyperglycemia with alloxan compared to the control group.

The 3rd phase is again a hypoglycemic phase that is noted 4-8 hours after the alloxan injection, which lasts for several hours. The flooding of circulation with insulin occurs as a result of the alloxan-induced secretory granule and cell membrane rupture resulting in severe transitional hypoglycemia. In addition, other subcellular organelles are also ruptured that include cisternae of rough endoplasmic reticulum and the golgi complex. Moreover, the outer and inner membranes of the mitochondria loose structural integrity in this particular phase. These changes are irreversible and highly characteristic for a necrotic cell death of pancreatic islets [25].

The last and the 4th phase of the blood glucose response is the final permanent diabetic hyperglycemic phase during which complete degranulation and loss of the integrity of the beta cells within 24-48 h after administration of the alloxan takes place [26]. The extract demonstrated antihyperglycemic effect by causing a significant decrease in the fasting blood glucose level of the alloxan induced severely hyperglycemic rats which were targeted for the 4th

phase of blood glucose response of this present study, in line with the study of [17] on the regulation of insulin production by pancreatic beta cells.

Observation of both the alloxan induced mild and severe hyperglycemic rats after six hours and fourteen days of treatment with *moringa oleifera* seed extract of same dose of 400mg/kg body weight in all groups, showed that there was a significant decrease ($P < 0.05$) in the fasting blood glucose level as shown in figures 1 and 2. For the mildly hyperglycemic group, there was a 37.1% decrease in fasting blood glucose level, when the extract was given orally. While a 42.8% decrease in fasting blood glucose level was noted when the extract was given intraperitoneally. Also a 69.7% and 89.6% decreases in fasting blood glucose level were noted when the extract was administered orally and intraperitoneally respectively to the severely hyperglycemic group of rats. The ability of the seed extract of *moringa oleifera* to significantly reduce hyperglycemia induced by alloxan may be as a result of its phytochemical and micronutrient constituents. A major phytochemical constituent of the extract that have been reported is flavonoids, which has been further characterized by structure and functional relationships as; flavans, flavanones, flavones, flavanols, flavanonols, cetechnins, anthocyanidins and isoflavones. Bioflavonoids are well known for their multi-directional biological activities including hypoglycemic effects [13]. Also the *moringa oleifera* contain many powerful antioxidant phytochemicals, especially quercetin and kaempferol. Kaempferol has been shown to have hypoglycemic activities [18, 19]. Also, the mechanisms of actions could be either by increasing the tissue utilization of glucose [31], by inhibiting hepatic gluconeogenesis or absorption of glucose into the muscles and adipose tissues [32].

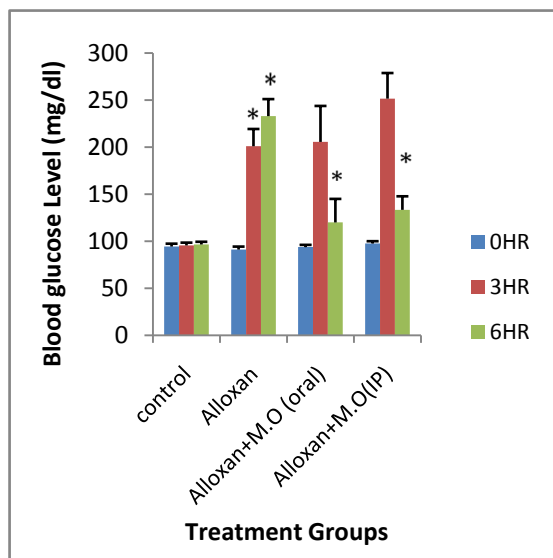


Figure 1. Effect of *Moringa Oleifera* Seed Extract on Glucose Level of Alloxan Induced Mild Hyperglycemia

The experiment also showed a decrease in the fasting blood glucose which was dependent on the route of

administration. It was noted that in both the mildly and severely hyperglycemic rats the intraperitoneal route of treatment was more effective since the percentage decrease in fasting blood glucose level were higher than when the treatment was done orally. This can be related to the bioavailability of the drug, which is a key pharmacokinetic parameter that expresses the proportion of a drug administered by any nonvascular route that gains access to the systemic circulation [20]. When a drug is administered orally, it is potentially subjected to first-pass metabolism initially in the small intestine and then the liver before it reaches systemic circulation [21]. Intraperitoneally administered drugs enter the blood stream directly providing relatively higher concentration, large absorbing surface, and longer half-life of a drug in the peritoneal cavity [22].

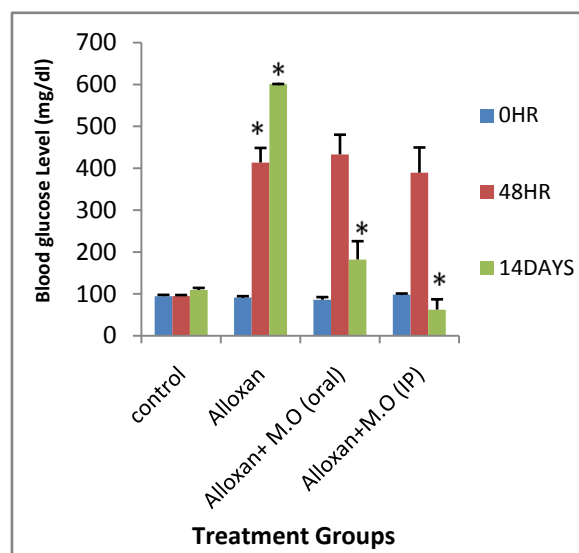


Figure 2. A sample line graph using colors which contrast well both on screen and on a black-and-white hard copy

5. Conclusions

The result of this study has shown that *Moringa oleifera* seed extract exhibited a hypoglycemic effect on both the mild and severe alloxan induced hyperglycemic rats, indicating that it can be used as a curative plant for the treatment of hyperglycemia and diabetes as well. We therefore recommend that *Moringa Oleifera* seed be taken as functional foods or nutraceuticals as it is readily available and widely grown.

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