

## ANTI-DIABETIC ACTIVITY OF AQUEOUS SEED EXTRACT OF (*MORINGA OLEIFERA*) IN NORMAL AND ALLOXAN -INDUCED DIABETIC RATS

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### ABSTRACT

The present study was carried out to investigate the anti-diabetic activity of aqueous extract of *Moringa oleifera* seed in both normal and alloxan induced diabetic rats. Alloxan-induced diabetic rats (150mg/kg) were administered orally with *Moringa oleifera* seed aqueous extract (200 mg/kg) for twenty eight days after which the blood glucose, lipid profile, haematological parameters, protein and albumin in the serum were measured and compared with the control. There was a significant ( $p<0.05$ ) increase in the level of blood glucose, total cholesterol, low density lipoprotein, triglyceride, eosinophil and a significant ( $p<0.05$ ) decrease in the level of PCV, Hb, WBC, PLT, NEUT, protein, albumin and body weight. Oral administration of aqueous extract of *M. oleifera* at a dose of 200 mg/kg body weight for 28 days to diabetic rats resulted in significant ( $p<0.05$ ) reduction in blood glucose, total cholesterol, low density lipoprotein and triglyceride and significant ( $p<0.05$ ) increase in body weight, high density lipoprotein, protein and albumin. These results suggested that the aqueous seed extract of *M. oleifera* possesses anti-hyperglycaemic, anti-hyperlipidaemic activity and also improved the aberrations in the blood parameters of alloxan-induced diabetic rat.

**Keywords:** *Moringa Oleifera*, Seeds, Diabetes, Haematological Parameters, Lipid Profile

### INTRODUCTION

Management of diabetes without side effect is still a challenge to the medical system. Currently available synthetic antidiabetic agents produce serious side effects such as hypoglycemic coma and hepatorenal disturbances <sup>[1]</sup>. Moreover, they are not safe for use during pregnancy <sup>[2]</sup>. Hence, the search for safer and more effective hypoglycemic agents has continued. Ethnobotanical information

indicates that plant species are used in the traditional management of diabetes [3,4,5]. *Moringa oleifera* (family *Moringaceae*) is commonly known as Drumstick tree. *Moringa* leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value [6]. It has great use medicinally both as preventative and treatment. Its bark, sap, roots, leaves, seeds, oil, and flowers are used in traditional medicine in several countries. A folk remedy for stomach complaints, catarrh, cancer [7], gastric ulcers, skin diseases, lowering blood sugar, increasing bone density, nervous conditions, diabetes, fatigue, increase lactation, hay fever, impotence, edema, cramps, hemorrhoids, headaches, sore gums; to strengthen the eyes, the brain, liver [8], hepatotoxicity [9], rheumatism, venomous bites and also for cardiac stimulation[10]. Therefore, this present study was designed to investigate anti-diabetic activity of aqueous seed extract of *Moringa oleifera* in normal and alloxan induced diabetic rats

## **MATERIALS AND METHODS**

### **PLANT MATERIAL**

The seeds of *Moringa oleifera* were obtained from Federal College of Forestry, Jos, Nigeria and were authenticated at the Herbarium of the Department of Plant Biology, University of Jos, Nigeria, where a voucher specimen was deposited at the Herbarium of the Institute.

### **CHEMICALS AND DRUGS**

Alloxan monohydrate was obtained from Sigma-Aldrich Chemical Company, St Louis, U.S.A. All the other chemicals used were of analytical grade and prepared in glass distilled water.

### **EXPERIMENTAL ANIMALS (ALBINO RATS)**

Wister male and female adult albino rats (16) weighing between 180-360g were obtained from National Veterinary Research Institute, Vom, Jos, Nigeria. The animals were housed in aluminum cages under standard conditions. They were maintained on standard animal pellets (purchased from Grand Cereal and Oil mills limited Jos, Nigeria) and water *ad libitum*. The animals were acclimatized for two weeks before the commencement of the experiment.

## **METHODS**

### **Preparation of Plant Extract**

The plants seed was collected and air dried at room temperature under the

shade. The plant was then pounded to powdery form using local pestle and mortar. The powdery form was stored in air-tight plastic container until required for use. The preparation of the plant extract was prepared using hot water, 100g of fine powder was boiled in one (1) liter of distilled water for 15 minutes (to ensure maximum extractions of phytochemicals) using hot plate. The mixture was allowed to stand for 30 minutes before filtering using white cloth and Whatman filter paper No. 1 to remove all un-extractable matter, the filtrate was then fed 0.5ml to the induced and non-induced alloxan diabetic rats to control their diabetic level.

### **Induction of Diabetes**

Diabetes mellitus was induced to rats of group B and C (as diabetic control and diabetic treated rats respectively) by a single intro-peritoneal infection of Alloxan at 150mg/kg. Diabetes was confirmed from the fasting blood glucose after 24 hours using one touch glucometer. Adoga and Ibrahim <sup>[11]</sup>, reported that diabetes mellitus was confirmed in induced rats from when fasting blood glucose level reach 126mg/dl and accompanied with hyperglucosuric test 24 hours after streptozotocin injection. Prior to each study the animals were made to fast for 14 hours but had free access to water <sup>[12]</sup>.

### **Experimental Design**

The animals were divided into 5 groups of 4 animals each for the evaluation of anti-diabetic activity.

**GROUP A:** Normal control of four rats on normal diet for 28 days.

**GROUP B:** Diabetic control of four rats fed with normal diet for 28 days.

**GROUP C:** Diabetic treated of four rats fed with normal diet-plus extract for 28 days.

**GROUP D:** Normal treated of four rats fed with normal diet-plus extract for 28 days.

Animals in groups C and D were given 200mg/kg of the *Moringa oleifera* extract using cannula.

### **Body Weight Determination**

Each animal in the groups were at the beginning and end of the experiment with an electro weighing balance.

### **Collection of Blood Sample and Serum Preparation**

The methods described by Yakubu *et al.* [13] were used for the collection of blood sample and preparation of serum. In brief, with the animal under ether anesthesia, the neck area was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were then sharply cut with a sterile scalpel blade. Blood was collected into EDTA sample bottles for haematological assay and also collected into clean sterile sample bottles which were allowed to clot for 30 minutes. This was then centrifuged at 33.5 g for 15 minutes using a Uniscope Laboratory Centrifuge (model SM800B). The sera were aspirated with Pasteur pipettes and stored frozen overnight at -20°C before being used for the biochemical analyses.

### **Determination of some Serum Metabolites**

The protein content of serum was determined using the Biuret method. Albumin (ALB) level was determined as described by Grant and Kacchman, 1987. Serum glucose, total cholesterol (TC), low density lipoprotein (LDH), high-density lipoprotein (HDL) and triglyceride (TG) were determined by the method of Trinder, [14], Friedrickson *et al.* [15], Albers *et al.* [16], Assman *et al.* [17], Jacob and Van Demark [18] respectively. All measurements were done using Spectronic 21 spectrophotometer (Bausch and Lomb, NY).

### **Determination of Haematological Parameters**

The blood samples were obtained through cardiac puncturing of the rats for the determination of the haematological parameters: Red blood cells (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), white blood cell count (WBC) and its differential counts (neutrophil, eosinophil, basophil, lymphocyte and monocyte), using the method of Dacie and Lewis [19].

### **Statistical Analysis**

All data are expressed as mean  $\pm$  standard deviation (SD). Comparison of the data from test control groups of animals were analyzed by One Way Analysis of Variance (ANOVA) at the confidence limit of 95% and where applicable, least significant difference (LSD) was used to determine significant results; differences between groups were considered statistically significant at  $P < 0.05$ .

## RESULT

**Table 1: Effect of Aqueous extract of *Moringa Oleifera* Seed on Body Weight of both Normal and Alloxan Induced Diabetic Rats**

Group	Weight Variation (g)		
	Final	Initial	Difference
Normal control	372.00±5.88	344.50±53.08	27.50
Diabetic control	314.00±2.94 <sup>a</sup>	335.00±7.79 <sup>a</sup>	-21.00
Diabetic treated	175.00±3.56 <sup>ab</sup>	260.00±7.07 <sup>ab</sup>	-85
Normal treated	240.00±10.80 <sup>ab</sup>	180.00±6.38 <sup>ab</sup>	60

Values are expressed as mean ± SD, n= 4 for each group.

<sup>a</sup> values are significantly different from normal control (p<0.05)

<sup>b</sup> values are significantly different from the diabetic control group (p<0.05)

**Table 2: Effect of Aqueous extract of *Moringa Oleifera* Seed on Blood Glucose, Protein, and Albumin of both Normal and Alloxan induced Diabetic Rats.**

Group	Treatment	Glucose (mmol/L)	Protein (g/L)	Albumin (g/L)
A	Normal Control	3.70±0.14	69.00±1.63	30.00±0.82
B	Diabetic control	8.70±0.14 <sup>a</sup>	58.00±0.37 <sup>a</sup>	28.00±0.82 <sup>a</sup>
C	Diabetic treated	3.64±0.21 <sup>b</sup>	58.80±1.3 <sup>a</sup>	31.00±0.82 <sup>b</sup>
D	Normal treated	3.00±0.36 <sup>ab</sup>	71.00±1.41 <sup>b</sup>	30.00±0.82 <sup>b</sup>

Values are expressed as mean ± SD, n= 4 for each group

<sup>a</sup> values are significantly different from normal control (p<0.05)

<sup>b</sup> values are significantly different from the diabetic control group (p<0.05)

**Table 3: Effect of aqueous extract of *moringa oleifera* seed on serum lipid profile of both normal and alloxan induced diabetic rats.**

Group	Treatment	Lipid profile (mmol/L)			
		TC	TG	LDL	HDL
A	Normal control	3.64±0.38	1.10±0.04	2.20±0.08	2.20±0.14
B	Diabetic control	4.98±0.04 <sup>a</sup>	1.74±0.17 <sup>a</sup>	2.40±0.22	1.80±0.37
C	Diabetic treated	3.74±0.17 <sup>b</sup>	1.20±0.13 <sup>b</sup>	2.10±0.21	2.00±0.50
D	Normal treated	3.26±0.19 <sup>b</sup>	0.74±0.17 <sup>ab</sup>	2.10±0.07	2.16±0.11

Values are expressed as mean ± SD, n= 4 for each group

<sup>a</sup> values are significantly different from normal control (p<0.05)

<sup>b</sup> values are significantly different from the diabetic control group (p<0.05)

**Table 4: Effect of aqueous extract of *moringa oleifera* seed on haematological parameters of both normal and alloxan induced diabetic rats**

Parameters	GROUP			
	A Normal control	B Diabetic control	C Diabetic Treated	D Normal treated
PCV (%)	43.00±0.82	33.00±0.82 <sup>a</sup>	29.00±0.82 <sup>ab</sup>	44.00±0.82 <sup>b</sup>
HB (g/dL)	14.30±0.22	11.00±1.41 <sup>a</sup>	13.00±0.82 <sup>b</sup>	0.00±0.00 <sup>ab</sup>
WBC (μ/L)	7195.00±92.23	6000.00±355.90 <sup>a</sup>	6800.00±216.02 <sup>b</sup>	6400.00±147.20 <sup>a</sup>
PLT(10 <sup>3</sup> /L)	310.00±7.80	260.00±1.10 <sup>a</sup>	310.00±7.30 <sup>ab</sup>	396.00±6.00 <sup>b</sup>
NEUT (%)	45.00±0.82	40.00±0.82 <sup>a</sup>	43.00±0.82 <sup>ab</sup>	44.00±0.82 <sup>b</sup>
LYM (%)	53.00±1.41	56.00±2.16	55.00±2.16	56.00±2.16
MONO (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
EOS (%)	2.00±0.34	4.00±0.82 <sup>a</sup>	2.00±0.71 <sup>b</sup>	0.00±0.00 <sup>ab</sup>
BAS (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Values are expressed as mean ± SD, n= 4 for each group

<sup>a</sup> values are significantly different from normal control (p<0.05)

<sup>b</sup> values are significantly different from the diabetic control group (p<0.05)

## DISCUSSION

Prolonged exposure to hyperglycemia is now recognized as the primary casual factor in the pathogenesis of diabetic complications as well as induces a large number of alterations in vascular tissue that potentially promote or accelerated atherosclerosis. In this study, there was a significant decrease in the level of blood glucose of rats in the diabetic group. Oral administration of aqueous seed extract of *M. oleifera* to the diabetic rats significantly reduced the blood glucose level compared with the control. Thus, the results of this study of the aqueous extract of *M. oleifera* on the blood glucose levels of normal and alloxan induced diabetic rats are in consonant with the findings of earlier researchers that plant extracts have hypoglycemic and insulin release stimulatory effects which in turn reversed alloxan induced hyperglycemia in <sup>[20]</sup>, <sup>[21]</sup>. The possible mechanism by which aqueous extract brings about its hypoglycemic action may be by induction of pancreatic insulin secretion from  $\beta$  cells of islets of Langerhans or due to enhanced transport of blood glucose to peripheral tissue <sup>[22]</sup>, insulin resistance reducing <sup>[23]</sup>, inhibition of intestinal glucose absorption <sup>[24]</sup>, it may contain biomolecules that can modify or stimulate insulin receptors, may modify the structure of glucose transport protein (GLUT 4) and it may inhibit insulin antagonist within the body <sup>[25]</sup>.

In the present study, diabetes induced in the experimental animal by alloxan produced significantly decreased in body weight <sup>[26]</sup>. The loss in weight in the diabetic groups is attributed to the alloxan that was injected into the animals. Alloxan is known to destroy the beta-cells of the islets of the Langerhans of the pancreas that function in insulin regulation, producing type 1 diabetes <sup>[27]</sup>. The destruction of the pancreas results in the utilization of non-carbohydrate moieties such as protein for the synthesis of glucose. The loss of structural proteins in increased gluconeogenesis together with increased lipolysis and increased synthesis of ketone bodies results in severe weight loss <sup>[28]</sup>. Furthermore, the weight loss observed in alloxan-diabetic rats can be due to a reduction of food intake <sup>[29]</sup>. However, administration of aqueous extract of *M. oleifera* seed was found to be effective in ameliorating the weight loss observed in the diabetic rats compared with the control. Diabetes affects both glucose and lipid metabolism <sup>[30]</sup>. In the post prandial state elevated serum insulin increases lipoprotein lipase activity in adipose tissue and promotes fuel storage as triglycerides in normal metabolism <sup>[31]</sup>. The deficiency of insulin depletes the activity level of lipoprotein lipase, thus leading to deranged lipoprotein metabolism during diabetes <sup>[32]</sup>. In the present study, alloxan induced diabetic

untreated rats showed significantly increased serum lipid profiles except HDL compared with the control rats. The elevated TG, TC, LDL level and decreased HDL level in alloxan-induced diabetic rats observed in this study is in agreement with the previous reports regarding alteration of these parameters under diabetic condition <sup>[21]</sup>. This may be due to the increase in the mobilization of free fatty acids (FFA) from the peripheral depots, since insulin inhibits the hormone sensitive lipase <sup>[33]</sup>. Serum FFA concentration are a result of the balance between the release from lipolysis, neosynthesis and disposal and represent the major determinant of insulin effect on FFA oxidation and non-oxidative metabolism <sup>[34]</sup>. Oral administration of aqueous seed extract of *M. oleifera* to the diabetic rats significant reduced the level of TG, TC, and LDL and significantly increases the level of HDL. The result of our study is in accord with the findings of other researchers who reported that many plants extracts have potential therapeutic value in combating arteriosclerosis which is one of the major complications of diabetes by lowering serum lipids particularly total cholesterol, triglyceride and low density lipoprotein level <sup>[21]</sup>.

Albumin is a major protein of human plasma and represents about 25% of total hepatic protein synthesis and half its secreted proteins. Its synthesis is depressed in variety of diseases, particularly those of the liver 35. Table 2 shows that there was a significant decrease in the concentration of albumin and total serum protein of the untreated alloxan induced diabetic rats when compared with the control and the treated groups. This observation may be attributed to numerous effects of hyperglycemia in alloxan-induced diabetes. Hyperglycemia increases gluconeogenesis and as such leads to excess protein breakdown as well as excess loss of nitrogen resulting in negative nitrogen balance <sup>[35]</sup>. A decline in total protein level in diabetic rats have been attributed to inhibition of oxidative phosphorylation which leads to decrease in protein synthesis, increase in catabolic processes and reduction in protein absorption <sup>[36]</sup>. Also, decrease in the total protein of alloxan induced rat, might may due to decrease due to microproteinuria which are important clinical markers of diabetic nephropathy <sup>[37]</sup>, and/or may be due to increased protein catabolism <sup>[38]</sup> as a result of insulin deficiency from free radical generation due to alloxan induction, since it has been established that insulin stimulates the incorporation of amino acids into protein <sup>[38]</sup>. The results showed that administration of the extracts caused a remarkable increase in the serum total protein and albumin levels in the diabetic rats. These observations may be due to the presence of some compounds which help in provision <sup>[39]</sup> of a reserved store of protein <sup>[39]</sup>.



Blood examination is a good way of assessing the health status of animals as it plays a vital role in physiological, nutritional and pathological status of organisms [40]: [41]. Assessment of haematological parameters can be used to determine the extent of deleterious effect on blood constituents of an animal [42]: [43]. It can also be used to explain blood relating functions of chemical compounds/plant extract [44]. During diabetes the excess glucose present in blood reacts with haemoglobin to form glycosylated haemoglobin. The present study thus revealed that alloxan induced diabetic untreated rats showed abnormalities in the haematological parameters (PCV, Hb, RBC, WBC, PLT, NEUT and EOS) i.e. In diabetic untreated rats, there was significant ( $p < 0.05$ ) reductions in PCV, Hb RBC, WBC, PLT, NEUT and a significant increase in EOS. Some of these abnormalities might be due to destruction of matured red blood cells leading to the low Hb counts accompanied by the fall in the RBC and PCV (Moss [45]: [46]. White blood cell differentials are indicators of the ability of an organism to eliminate infection. An increase in the number of circulating leukocytes is rarely due to an increase in all the types of leukocytes [47]. The significant increase in eosinophils levels of diabetic rats may be attributed to diabetic complications. It has been demonstrated that severity of diabetes and the development of retinopathy are associated with increased numbers of polymorphonuclear leukocytes [48]. Administration of the extract elicits a positive change in the haematological parameters (PCV, Hb, RBC, WBC, PLT, NEUT and EOS). The reversal of this derangement in diabetic rats administered with aqueous extract of *Moringa oleifera* seed may signify the protective effect of the intervention [46].

## CONCLUSION

This study has demonstrated that aqueous seed extract of *M. oleifera* induce significant reduction in the blood glucose, lipoproteins and increase in haematological parameters in experimentally induced diabetic rats. Therefore, the plant has a hypoglycaemic, hypolipidaemic effects and improve hematological abnormalities associated with pathophysiology of diabetes mellitus on alloxan-induced diabetic rats.

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**Reference** to this paper should be made as follows: Rafiu, A. A. and Luka, C.D. (2015), Anti-Diabetic Activity of Aqueous Seed extract of (*Moringa Oleifera*) in Normal and Alloxan -Induced Diabetic Rats. *J. of Biological Science and Bioconservation*, Vol. 7, No. 1, Pp. 24 - 37.

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