

Evaluation of Anti-hyperlipidemic Effect of Aqueous **Leaves** Extract of *Moringa oleifera* **Leaves** in Alloxan Induced Diabetic Rats

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ABSTRACT

Aims: The purpose of this study was to evaluate the effect of administration of aqueous leaf extract of *Moringa oleifera* (Moringaceae) on plasma glucose level, total cholesterol level, triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in male albino rats.

Study design: Experimental method was adopted

Place and Duration of Study: Adeleke University, Ede, Nigeria and University of Ibadan, Nigeria between September 2012 and January, 2013.

Methodology: We made use of twenty-four male albino rats of the Wistar strain weighing between 160 and 200 g. These were randomly assigned into 3 groups of 8 animals per group as normal control, diabetic control and diabetic treated with extract of *Moringa oleifera*. Diabetes was induced by 100 mg/kg of alloxan monohydrate. The control and the diabetic groups received distilled water while the diabetic treated group was administered with 400 mg/kg body weight of aqueous leaf extract of *Moringa oleifera* for 28 days. At the end of the experiment, plasma glucose level, cholesterol, Triglycerides (TG), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) were determined in all the experimental animals after 12 hours fast.

Results: The result showed significant increases ($P=0.05$) in plasma cholesterol, TG and LDL level of the diabetic control group when compared with the normal control group while there were no significant differences in the *Moringa oleifera* -treated diabetic group and the normal control group. The HDL however was not different in all the three groups.

Conclusion: Oral administration of aqueous leaf extract of *Moringa oleifera* may reduce the plasma lipid imbalances associated with diabetes mellitus.

Key words: *Moringa oleifera*, alloxan-induced diabetes, blood glucose, lipid imbalances.

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1. INTRODUCTION

The consumption of a variety of local herbs and vegetables by man is believed to contribute significantly to the improvement of human health, in terms of prevention, and or cure of diseases because plants have long served as a useful and natural source of the therapeutic agents[1].

Moringa oleifera (family Moringaceae) is commonly known as Drumstick tree. Most of the parts of the plant possess antimicrobial activity [2]. They are well known for their pharmacological actions too and are used for the traditional treatment of diabetes mellitus [3] hepatotoxicity [4], rheumatism, venomous bites and also for cardiac stimulation [5].

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycaemia resulting from a variable interaction of hereditary and environmental factors due to defects in insulin secretion, insulin action, or both [6]. Hyperglycemia resulting either due to defective production or action of insulin leads to a number of complications; cardiovascular, renal, neurological, ocular etc. [7]. The ailment may result in the development of further metabolic and anatomic disturbances among which is lipemia, hypercholesterolaemia, loss of weight, ketosis, arteriosclerosis, gangrene, pathologic changes in the eye, neuropathy, renal disease, and coma [8, 9].

Dyslipidaemia is common in diabetes, as both insulin deficiency and insulin resistance affects enzymes and pathways of lipid metabolism [10]. Dyslipidaemia, as defined by the World Health Organization [11], is considered in circumstances whereby the fasting plasma triglyceride is between 150 and 400 mg/dL (1.7 to 4.5 mmol/L), total cholesterol (TC) > 200 mg/dL (>5.2 mmol/L), low-density lipoprotein cholesterol (LDL-C) > 135 mg/dL (>3.5 mmol/L), high-density lipoprotein cholesterol (HDL-C) < 35 mg/dL (<0.9 mmol/L) in men or <40 mg/dL (<1.0 mmol/L) in women, and a ratio of total cholesterol to HDL-cholesterol > 5 [12]. Characteristic abnormalities in lipids in type 2 diabetes mellitus include elevated triglycerides (TG) levels, decreased atheroprotective high density lipoprotein cholesterol (HDL-C) levels and increased levels of small dense LDL-C [13,14].

World Health Organisation (WHO) [15] has emphasized strongly on the rational use of traditional and natural indigenous medicines, for treating diabetes mellitus. Patients with diabetes mellitus have been treated orally by folklore with a variety of plant extracts [16]. More than 1200 plants species are used worldwide in diabetes phytotherapy, and experimental studies support the hypoglycaemic activity of a large number of these plants [17]. In addition to correction of blood glucose levels, several plants with hypoglycaemic properties have potential in ameliorating lipid metabolism abnormalities of diabetes mellitus [18].

Therefore, the purpose of this study is to evaluate the effect of administering the aqueous *Moringa oleifera* (*M. oleifera*) leaf extract on total cholesterol level, triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in male albino rats based on the reported activities of most hypoglycaemic plant agents at ameliorating lipid metabolism abnormalities [19].

2. MATERIAL AND METHODS

2.1 Preparation of plant material

Fresh leaves of *M. oleifera* (5 kg) were collected from Oyo town in Nigeria and were taken to the Botany Department, University of Ibadan, Nigeria, for authentication. A voucher specimen (FHI, 142807) has been submitted earlier in the Forestry Research Institute of Nigeria (FRIN) herbarium. The leaves were air-dried and reduced to powdered form; the powdered leaves were percolated in distilled water for 12 h and filtered; the filtrate was subsequently evaporated to dryness and yielded a 22.5% dark green concentrate.

2.2 Animals

Twenty-four male albino Wistar rats weighing between 160 and 200 g were used for this study. They were maintained at standard laboratory condition and fed with commercial pellet diet (Bendel Feeds, Nigeria) and water *ad libitum*. The animals were subjected to natural photoperiod of 12 h light: dark cycle. All experimental protocols and handling were in compliance with the National Institutes of Health (NIH) publication No. 85-23 guidelines [20]. The animals were acclimatized to laboratory condition for one week before commencement of experiment.

2.2.1 Acute oral toxicity study of *M. oleifera* extract and Fruit Juice

Acute toxicity study was carried out according to Organization for Economic Co-operation and Development (OECD) guideline 423 [21]. None of the rats showed observable signs of toxicity upon single administration of *M. oleifera* extract and fruit juice (2 g /kg, *p.o.*) on day one. Observations twice daily for 14 days also did not reveal any drug related

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observable changes. The study was repeated with another set of animals for 14 days and no signs of toxicity were observed.

2.2.2 Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of 100 mg/kg of alloxan monohydrate obtained from Sigma Chemical Co. (St. Louis, MO, USA). Diabetes was confirmed by glucose oxidase method using the glucometer (One Basic, Inc.) after 72 h of alloxan injection, rats with plasma glucose level ≥ 200 mg/dl were separated and used as diabetic in this study.

2.3 Experimental design

The rats were randomly distributed into three groups of eight rats each:

- Group A: normal and received distilled water – (Normal control)
- Group B: diabetic and received distilled water– (Diabetic Control)
- Group C: diabetic treated with 400 mg/kg aqueous extract of *M. oleifera* (Diabetic + Extract)

The rats were treated for 28days. At the end of the experimental period, they were fasted for 12 h and blood was collected by cardiac puncture under light anesthesia. The blood was transferred into sample bottles containing EDTA for plasma collection. Plasma was collected from the blood samples after centrifuging at 3000 x g for 10 min using a bench centrifuge.

2.3.1 Blood Glucose estimation

Blood glucose was estimated on 0, 7, 14, 21 and 28th day by Glucometer (One Basic, Inc.).

2.3.2 Determination of plasma lipid

Plasma triglycerides and total cholesterol levels were measured on the final day of experiment using enzymatic colorimetric diagnostic kits obtained from Randox Laboratories, UK, in which the GPO-PAP method of Trinider [22] was employed. Absorbance was measured at 500 nm. The phosphotungstate precipitation method of Richmond [23] as applied in Randox kit was used for the determination of HDL-cholesterol. The LDL-cholesterol was estimated using Friedewald [24] formula:

$$\text{LDLc} = \text{Total cholesterol} - \text{HDLc} - \text{TG}/5$$

Where; LDLc = LDL-cholesterol, HDLc = HDL-cholesterol and TG = triglycerides

2.4 Statistical Analyses

All results were expressed as mean $\pm$ SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) and Duncan new multiple range test (DMRT). Differences in means were considered significant at $P=0.05$ and $P<0.01$. All analyses were performed using SPSS 15.

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3. RESULTS AND DISCUSSION

3.1 RESULTS

In acute oral toxicity studies *M. oleifera* extract did not show any mortality and toxic effects up to the dose of 2000 mg/ kg body weight. So one-fifth of the safe dose is used for the experiments.

Alloxan-induced diabetic rats exhibiting persistent hyperglycemia (Blood Glucose >200 mg dL $^{-1}$) were selected for assessing the effect of *M. oleifera* aqueous extract. Oral daily administered dose of 400 mg/ kg body weight of the extract led to significant decrease in hyperglycemia as shown in Figure 1 and Table 1.

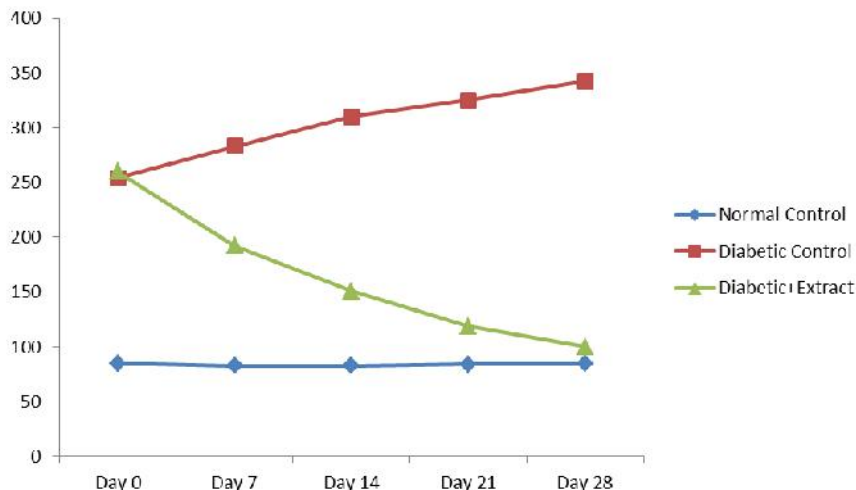


Fig. 1: Effect of aqueous extract of *M. oleifera* leaves on blood glucose in diabetic rats

Table 1. Effect of aqueous extract of *M. oleifera* leaves on blood glucose in diabetic rats

Groups	Blood glucose (mg/dl)				
	0 Day	7 Day	14 Day	21 Day	28 Day
Normal control	85±1.22	84±1.12	84±0.09	83±2.44	85±1.02
Diabetic control	255±2.80	285±2.87	312±2.66	324±4.22	344±2.55
Diabetic + extract	262±2.11	190±2.11**	153±2.80**	121±3.23**	102±1.23**

All values are expressed as mean ± SEM (n=5). *: $P < 0.05$, **: $P < 0.01$ as compared to diabetic control.

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Table 2 shows the mean values of plasma glucose level, total plasma cholesterol, TG, HDL and LDL in the control, diabetic untreated and diabetic treated with *M. oleifera*.

The total cholesterol level was significantly increased ($P=0.05$) in the diabetic group when compared with the control group, while there was no significant difference in the cholesterol level of the *M. oleifera*-treated diabetic group and the control group. The TG level was significantly increased ($P=0.05$) in the diabetic group with no significant difference in the *M. oleifera* -treated diabetic group when both were compared with the control group. Though there was no significant difference in the HDL level of the three groups, the diabetic group had a significantly higher ($P=0.05$) LDL when compared with the control while there was no difference in the LDL level of the *M. oleifera* -treated group and the control group.

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Table 2. Effect of aqueous leaf extract of *M. oleifera* on lipid profile of alloxan induced diabetic rats.

Variable	Control (n=7)	Diabetic Control (n=7)	Diabetic + Extract (n=7)
Total cholesterol (mmol/L)	4.62±0.254	6.01±0.188*	4.77±0.356
Triglycerides (mmol/L)	0.97±0.102	1.70±0.065*	1.25±0.412
HDL (mmol/L)	1.54±0.212	1.25±0.198	1.60±0.320
LDL (mmol/L)	2.77±0.153	4.12±0.102*	3.43±0.226

Values are expressed as mean ±SEM (n=7) * P<0.05 when compared to control, **significantly reduced when compared to diabetic untreated group.

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3.2 DISCUSSION

Alloxan-induced hyperglycaemia has been described as a useful experimental model to study the activities of hypoglycemic agents [25]. The purpose of the present study was to assess the effect of the aqueous extract of *M. oleifera* leaves on glycemic control, total cholesterol, triglycerides (TG) and low density lipoprotein (LDL) levels. Aqueous extract of *M. oleifera* leaves normalizes the high blood glucose level in diabetic rats by the 28th day of the experiment. Since good activity has been seen in severely diabetic rats with damaged islets, it is likely to be expected that the aqueous extract of leaves has some direct effect. We are suggesting the mechanisms of actions could be either by increasing the tissue utilization of glucose [7], by inhibiting hepatic gluconeogenesis or absorption of glucose into the muscles and adipose tissues [26].

The increase in the total cholesterol, TG and the LDL levels of diabetic rats observed in this study are in accordance with earlier report in diabetic subject [27]. Diabetes-induced hyperlipidemia has been attributed to excess mobilization of fat from the adipose due to underutilization of glucose [28].

The significant reduction in TG, LDL and cholesterol levels of the diabetic rats when treated with aqueous leaves extract of *M. oleifera* in this study supports the findings of Coon and Ernst [18] who stated that most hypoglycaemic plants have potentials of ameliorating diabetic lipid metabolism anomalies. This cholesterol lowering effect is supported by the earlier work of Oyedepo and Babarinde [29] who used *Citrus maxima* and Iweala and Obidoa [30] using leaves of *Ocimum gratissimum* as supplementary diet for six months to achieve the same effect. However this study is contrary to the report by Owoyele et al [31] observing a discrepancy due to administration length of *Ocimum gratissimum* extract.

This hypolipidemic effect of *M. oleifera* could be related to its chemical composition, which shows the presence of alkaloids, flavonoids, saponin and cardiac glycosides. All these components are known to reduce serum lipid level in animals [32, 33]. Saponins may lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption, and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase in its fecal excretion [34]. The increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol [13].

Although the precise mechanism by which the leave extract exerts it hypolipidemic effect is not clearly known nor studied, it could not be excluded that the control of glycaemia is a contributing mediator since control of glycaemia is a major determinant of total cholesterol and triglyceride levels [35]. Accordingly, the evolution of glycaemia was parallel to lipid parameters in both the normal/control and the diabetic rats of this study.

4. CONCLUSION

The present study reveals that aqueous extract of *M. oleifera* leaves has significant hypoglycemic and anti-diabetic potential. From this study, it could be concluded that prolonged oral administration of aqueous leaf extract of *M. oleifera* may reduce the plasma lipid imbalances associated with diabetes mellitus which support its traditional use in the treatment of diabetes and cardiovascular diseases. A daily intake of this plant may help to prevent hyperglycemia and hyperlipidemia. However, the precise mechanisms and sites of action require further studies. *Moringa oleifera* may play

important role for inhibition of α -glucosidase, pancreatic cholesterol esterase activity, as well as bile acid binding and inhibiting the formation of cholesterol micellization. However, further studies are needed to clarify this important hypothesis.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Roberts JE, Tyler VE. Tyler's herbs of choice. The therapeutic use of phytomedicinals. New York :The Haworth Herbal Press; 1999.
2. Caceres, A., Cabrera, O., Morales, O., Mollinedo, P., Mendia, P. Pharmacological properties of *Moringa oleifera* 1: preliminary screening for antimicrobial activity. Journal of Ethnopharmacology 1991; 33: 213–216.
3. Babu, R., Chaudhuri, M.. Homewater treatment by direct filtration with natural Coagulant. Journal of Water and Health 2005; 3:27–30.
4. Ruckmani K, Kavimani S, Anandan R, Jaykar B. Effect of *Moringa oleifera* Lam. on paracetamol induced hepatotoxicity. Indian Journal of Pharmaceutical Science 1998; 60: 33–35.
5. Chaudhary RD, Chopra RD. Herbal Drug Industry: A Practical Approach to Industrial Pharmacognosy. New Delhi: Eastern Publishers;1996.
6. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025; Prevalence, numerical estimates and projections. Diabetes Care 1998; 21: 1414-20
7. Gray AM, Abdel-Wahab YH, Flatt PR. The traditional plant treatment, *Sabucus nigra* (Elder) exhibits insulin like and insulin releasing actions in vitro. Journal of Nutrition 2000; 130: 15–20.
8. Swanson-Flatt SK, Day C, Bailey CJ, Flatt PR. Traditional plant treatments for diabetes. Studies in normal and Streptozotocin diabetic mice. Diabetologia 1990; 33: 462-4.
9. Andrew IR, Scott BE, Clarke HH, Michael DE, Scott C. Microvascular Complications in Cystic fibrosis-Related Diabetes mellitus: a case report. J. Pancreas 2000; 14: 208-210.
10. Gibbons GF. Hyperlipidaemia of diabetes. Clin Sci 1988; 71:477- 486.
11. World Health Organization. Report of WHO Scientific Group –Cardiovascular disease risk factors; new areas for research. WHO Technical Report Series No. 841, Geneva: 1994; 60:55-63.
12. Jisieike-Onuigbo NN, Unuigbo EI, Kalu OA, Oguejiofor CO, Onuigbo PC. Prevalence of dyslipidemia among adult diabetic patients with overt diabetic nephropathy in Anambra state South-East Nigeria. Niger. J. Clin. Pract. 2011; 14: 171-5
13. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology and management. JAMA 2002; 287:2570-81.
14. Navab M, Berliner JA, Watson AD, Hama SY, Territo MC, Lusis AJ, Shih DM, Van Lenten BJ, Frank JS, Demer LL, Edwards PA, Fogelman AM. The yin and yang of oxidation in the development of the fatty streaks: a review based on the 1994 George Lyman Duff Memorial Lecture. Arterioscler. Thromb. Vasc. Biol. 1996; 16: 831-842.
15. World Health Organization. Report of WHO Scientific Group –Diabetes Mellitus. WHO Technical Report Series No. 844, Geneva: 1994, 60:78-79.
16. Ajgaonkar SS. Herbal drugs in treatment of diabetes mellitus: a review. Int. Diabetes Fed. Bull. 1979; 24: 10-9.
17. Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomedicine 1995; 2: 137-189.
18. Coon T, Ernst E. Herbs for serum cholesterol reduction: a systematic view. J Fam Pract. 2003; 52(6): 468-78
19. Thompson Coon JS, Ernst E. Herbs for serum cholesterol reduction: A systematic view. J. Fam. Pract. 2003; 52: 468-478.
20. National Institutes of Health. Guide for the Care and Use of Laboratory Animals. NIH Publication; 1985: No. 85-23.
21. Organisation for Economic Co-operation and Development (OECD), Test No. 423: Acute Oral toxicity - Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals, OECD Publishing, 2002, Section 4, doi: 10.1787/9789264071001-en
22. Trinider P. Triglycerides estimation by GPO-PAP METHOD. Clin. Chem. 1969; 6: 24-27
23. Richmond W. Cholesterol enzymatic colorimetric test chop-PAP method of estimation of total cholesterol in serum. Clin. Chem. 1973; 191: 1350-1356.

24. Friedewald WT, Levy RT, Fredrickson DS. Estimation of LDL-cholesterol without the use of plasma ultracentrifuge. Clin. Chem. 1972; 18: 499-520.
25. Junod A, Lambert AE, Stauffacher W, Renold AE. Diabetogenic action of streptozotocin: Relationship of dose to metabolic response. J. Clin. Invest. 1969; 48: 2129-2139.
26. Kamanyi A, Djamen D, Nkeh B. Hypoglycemic properties of the aqueous root extracts of *Morinda lucida* (Rubiaceae) study in the mouse. Phytotherapy Research 1994; 8: 369–371.
27. Oberley LW. Free radicals and diabetes. Free Radical. Biol. Med. 1988; 5: 113-124
28. Nimenibo-Uadia R. Effect of aqueous extract of *Canavalia ensiformis* seeds on hyperlipidaemic and hyperketonaemia in alloxan-induced diabetic rats. Biokemistri 2003; 15:7-15.
29. Oyedepo TA, Babarinde SO. Effects of Shaddock (*Citrus maxima*) Fruit Juice on Glucose Tolerance and Lipid Profile in Type-II Diabetic Rats. Chem Sci Trans., 2013; 2(1):19-24.
30. Iweala EEJ, Obidoa O. Studies on some biochemical and histological changes associated with long term consumption of leaves of *Ocimum gratissimum* L. in male rats. Am. J. Food Technol.2010; 5(6): 376-384.
31. Owoyele BV, Funsho MA, Soladoye AO. Effect of aqueous leave extract of *Ocimum gratissimum* on alloxan induced diabetic rat. PHCOG MAG 2005; 1(2): 62-64
32. Tomita T, Lacy P, Matschinsky FM, Mcdaniel ML. Diabetes 1974; 23: 517.
33. Ezekwe CI, Obidoa O. Biochemical effect of *Vernonia amygdalina* on rats liver microsome. Niger. J. Biochem. Mol. Biol. 2001; 16: 1745-1798.
34. Rotimi SO, Omotosho OE, Rotimi OA. Persistence of acidosis in alloxan-induced diabetic rats treated with the juice of *Asystasia gangetica* leaves. Phcog Mag 2011; 7:25-30
35. Markku L. Epidemiology of diabetes dyslipidemia. Diabetes Rev.1995, 3: 408-422.