

Hypocholesterolemic and Hypoglycaemic Effects of Ethanolic Extract Of Leaf of *Moringa Oleifera* Lam in High Fat Diet Fed Wistar Rats

Sule, O.J.* and Arhoghro, E.M.

Accepted 1 June, 2016

Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

ABSTRACT

Hypocholesterolemic and hypoglycaemic activity of ethanolic extract of *Moringa oleifera* leaves was assessed. Thirty male Wistar albino rats were used, comprising of five groups of six animals each. Rats in groups (1 to 4) were fed with formulated fatty diet mixed with grower's match, while rats in group 5 were fed with grower's match only for fifteen day. Rats in groups 2, 3 and 4 were treated with extracts of *M. oleifera* (100, 200 and 300 mg/kg), respectively for ten days, while group 5 received normal saline for ten days. Blood samples were collected from the tail of animals on days 15 and 25 of the study. Samples were used to determine the cholesterol and glucose concentrations using standard kits. Weight changes were also determined. The results showed significant increases in the weight, cholesterol and glucose levels of rat fed with high fatty diets without extract compared to rats in normal group. Also, there was significant decrease in the levels of these parameters in rats fed with fatty diets with extracts, compared to untreated group. Conclusively, the leaves of *M. oleifera* may possess hypocholesterolemic and hypoglycemic activity and may be the basis for exploring its medicinal values by the traditionalist especially in Nigeria.

Key words: Hypocholesterolemic, Hypoglycemic and *Moringa oleifera*.

*Corresponding author. E-mail: j_sule@yahoo.com.

INTRODUCTION

Moringa oleifera Lam is an angiosperm that has been utilized throughout history as food and medicine in Indian subcontinent (Kannel et al., 1995). *M. oleifera* is a rapidly growing tree also known as the horseradish tree, drum stick tree, Benz olive tree, Kelor, Morango, Mlonge, Moionga, Mulangay, Nebeday and Sajan. Ben oil tree and drumstick from the appearance of the long, slender, triangular seed pods (Markkar et al., 2007). It is widely cultivated and has become neutralised in many locations in the tropics. *Moringa* is a perennial soft tree with timber of low quality and was utilised by the ancient Romans, Greeks and Egyptians. All parts of *moringa* tree are edible and have long been consumed by humans. The tree is slender, with dropping branches that grow to approximately 10 m in height. The leaves of *moringa* is

particularly rich in potassium, calcium, phosphorous, iron, vitamins A and B, essential amino acids, and antioxidant such as β -carotene, vitamin C and flavonoids (Suanarunsawat et al., 2010). *M. oleifera* is variably labelled as miracle tree, tree of life, and mother's best friends. In many region of Africa, it is widely consumed for self-medication by patient affected by diabetes, hypertension or HIV/AIDS (Dangi et al., 2002).

In Nigeria, *M. oleifera* leaves are eaten as vegetables without any side effects being reported (Stevens et al., 2013). These leaves are also eaten commonly as a food by infants and children in south India, because the high content of b-carotenes helps to prevent the development of vitamin A deficiency blindness (Answar et al., 2007; Jed and Fahey, 2005). Cholesterol is a sterol, a sort of

fat. It is one of three major classes of lipids which all animal cells utilize to construct their membranes and thus manufacture by all animal cells (Durrington, 2003). Cholesterol is a fat (lipid) which is produced by the liver is crucial for normal body functions and is the precursor of the steroid hormones, bile acids and Vitamin D, transported in the blood plasma within protein particles (Hooper et al., 2012). Cholesterol plays an important role in the body such as building and maintaining cell membranes (outer layer). It prevents crystallization of hydrocarbon in the membrane as well as performing cell membrane permeability functions (Bhatnager et al., 2008). Cholesterol is involved in the production of sex hormones and also essential for the production of hormones released by the adrenal glands (Grundy et al., 1998). Cholesterol aids in the production of bile, converts sunshine to vitamin D, and is important in the metabolism of fat soluble vitamins, including vitamin A, D, E and K and insulates nerves fibres (shields and shields, 2008). Cholesterol is often regarded as the cause of many deadly diseases such as coronary heart disease and stroke (Ravi, 2015). This can happen when there is a high concentration of cholesterol in the blood called hypercholesterolemia. LDL (low density lipoprotein) is called bad cholesterol because elevated levels of LDL cholesterol are associated with an increased risk of coronary heart disease (Bligh, 1959). Coronary atherosclerosis is the hardening and narrowing of the arteries that supplies blood to the heart muscle. Coronary atherosclerosis is the major cause of sudden and unexpected death in humans and heart attacks are also a significant cause of heart failure due to weakened heart muscle (Woo et al., 2004). The heart attack can be lowered or prevented by lowering high blood pressure, controlling diabetes and high cholesterol. Research has shown that lowering LDL cholesterol reduces the risk of heart attacks, stroke and peripheral arteries diseases (Suarez, 1999). Eggs are laid by females of many different species including birds, reptiles, amphibians and fish and are probably eaten by mankind for millennia. More than half the calories found in eggs come from the fat in the yolk; a large (50 grams) chicken egg contains approximately 5 grams of fat. However, only 27% of the fat in egg is saturated fats that contain LDL cholesterol (Weggemans et al., 2001). This form the basis for using egg yolk in formulating fatty diet fed to test the animals as well as investigating the effect of *M. oleifera* on the exposed animals in the study.

MATERIALS AND METHODS

Thirty (30) male albino rats were purchased from Pharmacology Department, College of Health Sciences, Niger Delta University, kept in Medical Biochemistry Department (research room under normal room temperature) for 2 weeks to acclimatize and allowed

access to water and feed *ad libitum*. After which, the rats were grouped into five of six members each, numbered from groups 1 to 5. Groups 1 to 4 were fed with formulated fatty diet (egg yolk mixed with grower's mash in ratio of 40: 60%) for 15 days; group 5 was fed on growers mash.

Chemicals

The chemicals used for this study were sourced from reputable chemical companies of high analytical standards. Absolute ethanol was purchased from BDH chemical company Ltd. (Poole, England). Cholesterol kits and glucose kits were purchase from Randox Laboratory Ltd. Other materials include; Rat chow (Pfizer Nigeria Plc), Raw (chicken) eggs, also purchased from Agricultural Farm, Niger Delta University.

Extract Preparation

M. oleifera leaves were obtained from the Pharmacognosy herbarium, Niger Delta University, after identification by Professor K. Ajibeshin in the Department of Parmacognosy, Faculty of Pharmarcy, Niger Delta University. The extraction was done in the Medical Biochemistry Laboratory, College of Health Sciences, Niger Delta University. 100 gram of *M. oleifera* leaves was thoroughly washed and homogenized with mortar and pestle. It was soaked inside 1000 ml beaker with 500 ml ethanol and kept open for 24 h. The mixture was thoroughly mixed and then filtered. The filtrate was evaporated in a water bath at 40°C, until the extract became concentrated. The concentrate was mixed with 5% (v/v) ethanol and stored in a refrigerator at 4°C until used.

Feeding Procedure /Extract Administration

The rats in groups 1 to 4 were fed with formulated fatty diet (egg yolks mixed with grower's match in a ratio 40:60%) for 15 days, rats in group 5 were fed with normal grower's match also for 15 days. On the 16th day the following treatments were given to the various animal groups for ten days: Group 1 received normal saline water, Group 2 received (100 mg/kg/day) of the extract orally. Group 3 received (200 mg/kg/day) of the extract orally, Group 4 received (300 mg/kg/day) of the extract orally and Group 5 received normal saline water.

Sample Collection

Blood samples were collected from the rat tails by cleaning it with cotton wool wet in methylated spirit. The tail was rubbed gently to increase blood flow and the tip cut with sterilized scissors. The blood was then collected into universal bottles, on the 15th and 25th days of the study; spin for 5 min at 2500 rpm in a centrifuge, the

Table 1. Effect of *M. Oleifera* on weight changes.

Groups	Treatment	Change in weight after high fatty diet (g)	Change in weight after <i>M. oleifera</i> extract administration (g)
1	Fed with high fatty diet without treatment	30.2 ± 18.404 ^a	75.6 ± 13.334 ^b
2	Fed with high fatty diet + 100 mg/kg extract	40.2 ± 60.909 ^a	32.8 ± 36.953 ^b
3	Fed with high fatty diet + 200 mg/kg extract	60.2 ± 70.311 ^a	52.8 ± 11.563 ^b
4	Fed with high fatty diet + 300 mg/kg extract	32 ± 33.061 ^a	30.2 ± 25.283 ^b
5	Fed with grower's match without Treatment.	38.4 ± 22.305 ^a	40.2 ± 2.283 ^b

Values are mean ± standard deviation, where n=6. Values with different superscripts are significantly different, p≤0.05.

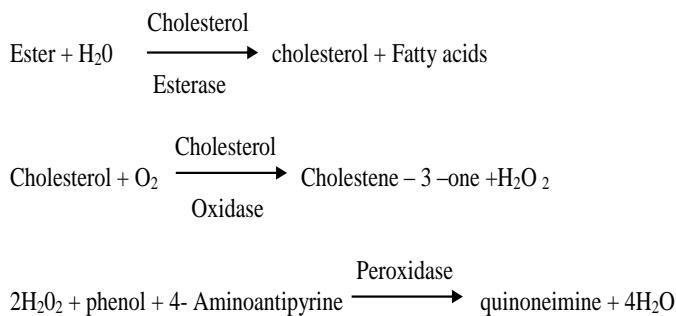
serum was collected for biochemical analysis.

Estimation of Serum Cholesterol

Cholesterol determination as reported by Abell and Harrison (1952) and Zlatkis et al. (1953), which involves the extraction of cholesterol by organic solvents and subsequent alkaline hydrolysis of the cholesterol esters. The use of cholesterol oxidase following specimen saponification as described by Richmond (1973) provided the first step toward totally enzymatic procedure for cholesterol determination replacing chemical saponification with enzymatic saponification.

Assay Principle

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase which is measured spectrophotometrically at 560 nm.

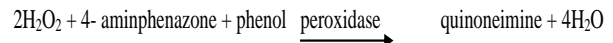


Principle of Glucose Oxidase Test

Glucose oxidase reagent contains glucose oxidase, peroxidase and o-toluidine. The latter reagent is an oxygen reagent acceptor and changes from a colourless reduced form to a blue oxidized form. Glucose is oxidized by glucose oxidase to glucuronic acid. Hydrogen peroxide is also formed in the reaction and is decomposed by peroxidase to water and oxygen. The liberated oxygen is accepted by orth-toluidine and

therefore a red-violet coloration (quinoneimine) which was measured spectrophotometrically at absorbance of 560 nm.

Reaction Principle



Statistical Analysis

The results were expressed as mean ± SD. Data was analysed by one-way analysis of variance. Sequential differences among means were calculated at the level of P < 0.05, using Turkey contrast analysis as needed.

RESULTS

Effects of *M. Orlifera* on Weight Changes

In this study, values obtained from the group 1 (fed on high fatty diet without extract) are compared with those of the normal group 5, while values for those that were fed high fatty diet with extract (groups 2, 3 and 4) are compared with those of the high fat exclusive group 1. Rats fed on high fatty diet without extract showed significant increase in weights (30.2 ± 18.2- 75.6 ± 13.3) when compared to rat on a normal diet (38.4 ± 22.3 – 40.2 ± 2.3) , p≤ 0.05. However, there were significant decreases in the weights of rats treated with extracts (groups 2, 3 and 4); (40.2 ± 18.4- 32.8 ± 37.0; 60.2 ± 70.3 -52.8 ± 11.6; and 32.0 ± 33.1 – 30.2 ± 25.3), respectively, when compared with rats in normal and untreated groups, p≤0.05 (Table 1).

Effects of Moringa on Cholesterol Levels

Cholesterol level increased significantly, in rats in group 1, (7.4 ± 1.7- 9.2 ± 4.0) when compared to rats in normal

Table 2. Effect of *M. oleifera* on cholesterol level.

Groups	Treatment	Cholesterol level after feeding with high fatty diet mmol/l	Cholesterol level after <i>M. oleifera</i> administration mmol/l
1	Fed with high fatty diet without treatment	7.388 ^a ± 1.724	9.209 ^b ± 3.972
2	Fed with high fatty diet + 100 mg/kg extract	7.380 ^a ± 1.724	4.178 ^c ± 2.342
3	Fed with high fatty diet + 200 mg/kg extract	6.662 ^d ± 3.188	6.281 ^d ± 2.198
4	Fed with high fatty diet + 300 mg/kg extract	5.889 ^d ± 2.559	5.760 ^d ± 2.887
5	Fed with grower's match without Treatment.	4.55 ^e ± 1.479	4.579 ^e ± 1.487

Values are mean ± standard deviation, where n=6. Values with different superscripts significantly different, p≤0.05.

Table 3. Effect of *M. oleifera* on glucose level.

Groups	Treatment	Glucose level after feeding with high fatty diet mmol/l	Glucose level after <i>M. oleifera</i> administration mmol/l
1	Fed with high fatty diet without treatment	5.984 ^a ± 1.391	7.554 ^b ± 1.463
2	Fed with high fatty diet + 100 mg/kg extract	4.573 ^c ± 1.586	3.987 ^d ± 1.223
3	Fed with high fatty diet + 200 mg/kg extract	8.275 ^e ± 1.906	7.449 ^b ± 1.230
4	Fed with high fatty diet + 300 mg/kg extract	6.033 ^a ± 2.184	5.966 ^a ± 2.187
5	Fed with grower's match without Treatment.	3.899 ^f ± 0.807	3.922 ^f ± 0.813

Values are mean ± standard deviation, where n=6. Values with different superscripts are significantly different, p≤0.05.

group 5, (4.6 ± 1.5 – 4.6 ± 1.5) p ≤ 0.05. Also, these values decreased significantly in rats treated with extracts (groups 2, 3 and 4); (7.4 ± 1.7- 4.2 ± 2.3; 6.7 ± 3.2 -6.3 ± 2.2; and 5.9 ± 2.6 – 5.8 ± 2.3), respectively, when compared with rats in normal and untreated groups, p ≤ 0.05 (Table 2).

Effect of Moringa on Glucose Levels

The results showed a significant increase in rats in group 1, (6.0 ± 1.4 – 7.6 ± 1.5) as compared to rats in normal group 5, (3.9 ± 0.8 -3.9 ± 0.8). Meanwhile, the glucose levels decreased significantly in rats treated with extract (groups 2, 3 and 4); (4.6 ± 1.6-4.0 ± 1.2; 8.3 ± 1.9 – 7.4 ± 1.2 and 6.0 ± 2.2 -6.0 ± 2.1), respectively, when compared with rats in normal and untreated groups, p ≤ 0.05 (Table 3). However, these decreases were not concentration dependent.

DISCUSSION

Ethanollic extract of leaf of *M. oleifera* Lam has been shown to possess both hypoglycemic and hypocholesterolemic activities as well as weight lowering effect. Doses of extract at 100, 200 and 300 mg/kg had glucose and cholesterol-reducing effect in serum of rats fed with high fatty diet compared to untreated high-fat fed group. The decreases were significant in serum glucose and cholesterol levels. Ghasi et al. (2000) earlier reported and concluded that leaves of *M. oleifera* have definite pharmacological basis for employing them for this purpose in India. The decrease in the cholesterol concentration in the extract treated groups is in

agreement with the earlier report by Raida et al. (2008), that *M. oleifera* leaves were found to lower serum cholesterol, triglycerides, VLDL and LDL. Komal (2003) also reported increased excretion of faecal cholesterol showing that it possesses a hypolipidemic effect. The leaves extract of *M. oleifera* with atenolol has been revealed to possess profound hypolipidemic activity (Naznin, 2003). In the report by Saluja et al. (1978), b-sitosterol was isolated from the stem of a hybrid variety of *M. oleifera* Lam and b-sitosterol is a plant sterol with a structure similar to that of cholesterol, except for the substitution of an ethyl group at C24 of its side chain. This led to the believe that *M. oleifera* lowers cholesterol by lowering plasma concentrations of LDL (Kane and Malloy, 1982). Therefore b- sitosterol may be a bioactive phyto constituent in the leaves of *M. oleifera* Lam. Conclusively, the observed cholesterol-reducing action of the crude leaf extract of *M. oleifera* Lam indicates that this leafy vegetable possesses some potential medicinal value and could validate and explain its ethno medical use on the obese and heart patients in Nigeria.

REFERENCES

- Abell K, Hrrison M (1952) Extraction of cholesterol by organic solvent. Indian J. Exp. Biol. 44: 898-90
- Answar F, Latif S, Ashraf M, Gilani AH(2007). *Moringa oleifera* with multiple medicinal uses. Phytoether. Res. 21(1): 17-25.
- Bhatnagar D, Soran H, Durrington PN (2008). Hypercholesterolaemia and its management. BMJ, 337: a993. doi: 10.1136/bmj.a993.
- Bligh R, RT van der Knaap, HC, Duchateau GS, Meijer L, Zock PL, Geleijnse, JM, Trautwein EA (1959). Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake. J. Nutr. 139(2): 271-84.
- Dangi SY, Jolly Cl, Narayannan S (2002). Antihypertensive activity of

- total alkaloids from the leaves of *Moringa oleifera*. J.Pharm. Biol. 40(2): 144-148.
- Durrington P (2003). Dyslipidaemia. The Lancet, 362(9385): 717-31.
- Easterday J (2005). High contain of cholesterol in egg yolk .The Coyle Egg-Safety Carton. Hiway16 Magazine. [http://www.bcnorth.ca/magazine/ page s/Jim/egg/egg1.htm](http://www.bcnorth.ca/magazine/page%20s/Jim/egg/egg1.htm)
- Ghasi S, Nwobodo E, Ofili JO (2000). Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high fat diet fed wistar rats. J. Ethnopharmacol.69(1): 21-25.
- Grundt SM, Greenland S, Pastemak RC, Lenfant C (1998). Problems on the pathway from risk assessment to risk reduction. Circulation, 97(18): 1761-2.
- Hooper L, Summerbell CD, Thompson R (2012). Reduced or modified dietary fat for preventing cardiovascular disease. Cochrane Database Syst. Rev. 5: CD002137.
- Jed W, Fahey SCD (2005). *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic and prophylactic properties part 1. Trees for life Journal, 1:5
- Kane JP, Malloy MJ (1982). Treatment of hypercholesterolemia. Med. Clin. North Am. 66, 537-550.
- Kannel WB, Castelli WP, Gordon T (1995). Cholesterol in the prediction atherosclerotic disease. New perspectives based on the Framingham study. Ann.Intern. Med. 90(1): 85-91.
- Komal A (2003). Vulnerables' perceptions of disaster and a disaster management project: a case study from Nepal. In. Third DPRI-IIASA international symposium on integrated disaster risk management- Coping with regional vulnerability, 3-5, July, 2003. Kyoto, Japan.
- Markkar HPS, Linder P, Lignin M (2007). A cholesterol lowering agent. Lancet, 2:1259.
- Naznin A (2003). Antihypertensive activity of total alkaloids from the leaves of *Moringa oleifera*. J. Pharm. Biol. 40(2): 144-148.
- Raida K, Nizar A, Barakat S (2008). The Effect of *moringa oleifera* aqueous extract in rabbits fed with high cholesterol diet. Eur. J. Sci. Res. 22: 352-360.
- Ravi KU (2015). Emerging risk Biomarkers in cardiovascular diseases and disorders. J. of lipid, 971453.p.50.
- Saluja MP, Kapil RS, Popli SP (1978). Studies in medicinal plants: part VI. Chemical constituents of *Moringa oleifera* Lam. (hybrid variety) and isolation of 4-hydroxymellein. Indian J.Chem. B 16B (11): 1044-104.
- Shields C, Shields J (2008). Eyelid, conjunctival, and orbital tumors: atlas and textbook. Hagerstown, Maryland: Lippincott Williams & Wilkins.
- Stevens GC, Baiyeri KP, Akinnnagbe O (2013).Ethno-medicinal and culinary uses of *Moringa oleifera* Lam in Nigeria. J. Med. Plant Res. 7(13): 79-804.
- Suanarunsawat T, Boonnak T, Na Ayutthaya WD, Thirawarapan S. (2010). Anti-hyperlipidemic and cardioprotective effects of *Ocimum sanctum* L. fixed oil in rats fed a high fat diet. J. Basic Clin. Physiol. Pharmacol. 21 (4): 387-400.
- Suarez EC (1999).Relations of trait depression and anxiety to low lipid and lipoprotein concentrations in healthy young adult women. Psychosom. Med. 61(3): 273-9.
- Weggemans RM, Zock PL, Katan MB (2001). Dietary cholesterol from eggs increases the ratio of total cholesterol to high-density lipoprotein cholesterol in humans: a meta-analysis. Am J. Clin. Nutr.73(5): 885-91.
- Woo D, Kissela BM, Khoury JC, Sauerbeck LR, Haverbusch MA, Szafarski JP, Gebel JM, Pancioli AM, Jauch EC, Schneider A, Kleindorfer D, Broderick JP. (2004). Hypercholesterolemia, HMG-CoA reductase inhibitors, and risk of intracerebral hemorrhage: a case-control study. Stroke, 35(6): 1360-4
- Zlatkis A, Zak B, Boyle GJ (1953). A new method for direct determination of serum cholesterol. J. Lab. Clin. Med. 41:486.