British Journal of Pharmacology and Toxicology 5(3): 103-108, 2014
ISSN: 2044-2459; e-ISSN: 2044-2467

Submitted: September 28, 2013 Accepted: October 14, 2013 Published: June 20, 2014

Lipid Lowering and Appetite Suppressive Effect of Leaves of *Moringa oleifera* Lam. in Rats

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Abstract: This six-week study evaluates the effect of *Moringa oleifera*on the lipid profile, body weight and appetite of adult male albino Wistar rats. The experimental rats were fed on a High Fat Diet (HFD) for four weeks and those found to be significantly hyperlipidemic were subdivided into five groups (A-E). Group A (control) received only distilled water; B, C, D received aqueous extract of *M. oleifera* leaves at varied doses of 100, 200 and 300 mg/kg, respectively; E were given the standard drug- atorvastatin (4 mg/kg, *p.o*.) along with the HFD. *M. oleifera* and atorvastatin were found to lower the serum cholesterol, triacylglyceride, VLDL, LDL, body weight and atherogenic index, but increased the HDL as compared to the HFD fed-untreated group (control). Interestingly too, it was found that the leaf extract did not precipitate high glucose and liver enzyme levels unlike those treated with the anti-hyperlipidemic drug. Extract exhibited a weight loss and appetite reducing property in the treated rats whist nourishing them as well. Thus, the study demonstrates that *M. oleifera* possesses a lipid lowering effect and also suppresses appetite, hence can be useful in the management of hyperlipidemia and associated health conditions.

Keywords: Appetite, cholesterol, high fat diet, hyperlipidemia, *Moringa oleifera*, weight loss

INTRODUCTION

Hyperlipidemia is an elevation of one or more of the plasma lipids, including cholesterol, cholesterol esters, triglycerides and phospholipids (Iyera *et al*., 2012). Hyperlipidemia is a risk factor for cardiovascular diseases due to its influence on atherosclerosis progression (Levin and Keany, 1995; Nelson, 2013). The prevalence of hyperlipidemia has dramatically increased worldwide due to modern lifestyles which bring about increase in the consumption of high-fat diets (Jacobson *et al*., 2007). Certainly, diets high in saturated fats have been shown to induce weight gain and hyperlipidemia in humans and animals (Hill *et al*., 1992).

Statins are agroup of lipid lowering agents. They act by inhibiting HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver. (Raasch, 1988). They are used in the treatment of hyperlipidemia, atherosclerosis or cardiovascular complications like coronary heart disease. Among the available HMG-COA reductase inhibitors, atovastatin is one of the majorlipid lowering drug used in hyperlipidemic conditions (Lea and Mctavish, 1997). Since its approval in 1996, the drug has been one of the top best selling branded pharmaceuticals globally (Crain's New York Business).

In order to compare the lipid lowering activity of *M. oleifera* leaf extract with that of atovastatin, we induced hyperlipidemia in rats successfully using a standard cholesterol diet. Inducing hyperlipidemia in rats is often through a high fat, high cholesterol diet, with the fat source varying from lard to canola, coconut, soybean or palm oil. Commercial rations supplemented with cholesterol have also been used for these investigations (Doucet *et al*., 1987).

M. oleifera (lam) is the most widely cultivated species of a monogeneric family, the Moringaceae. This rapidly-growing tree is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan; but is now widely cultivated and has become naturalized in many locations in the tropics. *M. oleifera* is gaining increasing popularity amongst lovers of natural products. All parts of the Moringa tree are edible and have long been consumed by humans (Orwa *et al*., 2009).

The present study was aimed at investigating the effect of the aqueous extract of *Moringa oleifera* on the lipid profile, body weight and appetite using male wistar albino rats placed on high fat diet as hyperlipidemic models.

MATERIALS AND METHODS

Plant materials: Healthy leaves of *Moringa oleifera* were bought from a herbal market in Port Harcourt, Rivers State, Nigeria, in March, 2013. They were

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identified and authenticated at the herbarium of Plant Science and Biotechnology department, University of Port Harcourt, Rivers State, Nigeria. The leaves were washed and air-dried till constant weight is attained. They were kept away from direct sun light to avoid destroying active compounds. The dried leaves were pulverized to a powdery fine texture using a mechanical grinder.

Experimental rats: Twenty-five male albino wistar rats of an average weight of 184g were procured from the animal house of the department of Pharmacology, University of Port Harcourt. They were divided into five groups (5 animals per group), labeled and kept in cages and maintained in a well ventilated room. During the period of acclimatization, they were fed with grower's mash by Top feed, Nigeria and water *ad libitum.*

Equipments, reagents and assay kits: Activity cages (Ugo Basile, Italy), Stuart Scientific Orbital Shaker (SO1, Stone, UK.), Uniscope Laboratory Centrifuge (model SM800B, Surgifriend Medicals, Essex, England), Syringes (1 mL, 5 mL), Oral cannula, Cotton wool, centrifuge tubes, Whatman No. 1 filter paper (Maidstone, Kent, UK), Dissecting kit and board, Weighing balance (Mettler AL 204), Hand gloves, Distilled water, Picric acid, 10% tannic solution, Potassium mercuric iodide, Ferric chloride solution, Hydrochloric acid, Chloroform, Sodium hydroxide, Tetraoxosulphate (V1) acid, Dragendorff's reagent, Diethyl ether, Assay kits for cholesterol, HDL-C, LDL-C, triglycerides, PCV, WBC. Hb, GGT, ALT and AST (Randox Laboratories Ltd., Ardmore, Co. Antrim, UK).

Phytochemical testing: The extract solution were tested for the presence of phytochemicals-alkaloids, saponins, glycosides, tannins, steroids, flavonoids and anthraquinones, were carried out using the protocol described by Trease and Evans (1989).

Preparation of extract and drug: 400 g of the plant material (*M. oleifera* leaves) were extracted in 2 liter of distilled water using the cold maceration method for 48 h. Thereafter, it was filtered with Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure and the extract obtained was weighed to determine yield. The resultant yield (11.6%) was reconstituted in distilled water to give the required doses of 100, 200 and 300 mg/kg body weight used in this study.

High fat diet (HFD) preparation: The high fat diet was prepared by mixing cholesterol (100 g) and cholic acid (50 g) in 1 liter of soybean oil supplemented with egg yolk.

Animal grouping: The experimental rats were fed on a high fat diet, every morning for four weeks, thereafter blood was withdrawn from the tail vein to analyze them for lipid profiles [Total Cholesterol (TC), triglycerides (TG), Low Density Lipoproteins (LDL-C) and High Density Lipoproteins (HDL-C)] to confirm the induction of hyperlipidemiaand those found were to be significantly hyperlipidemic were subdivided into five groups (A-E) of five animals each. Group A (control) received only distilled water; B, C, D received aqueous extract of *M. oleifera* leaves at varied doses of 100, 200 and 300 mg/kg, respectively; E were given the standard drug-atorvastatin (4 mg/kg, *p.o.*, once daily) along with the continued high fat diet (HFD), for 2 weeks. At the end of every week throughout the duration of the study, all experimental animals were weighed and the average weights for each group recorded accordingly. The dose of extract was according to the results of a pilot LD50 studies in rats of both sexes.

Body weight and appetite determination test: All test animals were weighed on day 1 and weekly and their weights were recorded. At the end of the experiment, the average weights of the test group were calculated and then compared with that of the control. The rats were housed in activity cages, so that they can be observed properly and the exact amount of feed consumed were thus determined daily by giving 100 g of feed to each animal in a group and measuring the remaining quantity the following day to determine the quantity eaten by each animal and the average was determined for each group.

Collection of blood samples and serum preparation: On the 15^{th} day were sacrificed after overnight starving. First, they were anaesthetized in a jar containing cotton wool soaked in diethyl ether and when rats became unconscious, incisions were made into their thoracic cavity. Blood samples were collected by cardiac puncture using a centrifuge tubes. The blood samples were allowed to clot for 10 min at room temperature and subsequently centrifuged at 3000 rpm for 5 min with Uniscope Laboratory Centrifuge. The sera were aspirated with pasteur pipette and used for the determination of the assay within 12 h of preparation.

Biochemical assays for lipids: TC, HDL-C and TG were determined in the serum of the rats by adopting the protocol outlined in the manufacturer's assay kit from Randox Laboratories Ltd, Ardmore, Co. Antrim, UK. LDL-C was calculated using the Friedewald formula – LDL-C = TC- (HDL-C+TG)/5.

Glucose and liver enzyme activities [Gamma Glutamyl Transferase (GGT), alanine amino transferase (ALT) and aspartate amino transferases (AST)] were also determined using assay kits and protocol from the

same manufacturer. Hematology values [Packed Cell Volume (PCV), hemoglobin (Hb) and White Blood Cell count (WBC) were also assayed. The atherogenic index (AI) was calculated by using the formula, atherogenic index = TC/HDL-C

Statistical analysis: Data were analyzed using the SPSS version 17 software package for ANOVA and $p<0.05$ was considered as the level of significance.

RESULTS AND DISCUSSION

The phytochemical screening of the leaves of *M. oleifera* revealed mainly the presence of flavonoids and saponins (Table 1), both of which has been reported to increase HDL-C concentration and decrease in LDL and VLDL levels in hypercholesteremic rats (Yanping *et al*., 2005). Chávez-Santoscoy *et al*. (2013) in their work reported that flavonoids and saponins lowers cholesterol absorption by the inhibition of cholesterol micellar solubility. Thus, flavonoids and saponins found in our aqueous extract could be instrumental to its hypolipidemic effect.

A high fat diet significantly elevated levels of serum cholesterol (135.7%, $p<0.05$), triacylglyceride (124.7%, $p<0.05$) and LDL-C (106.3%, $p<0.05$) and decreased the level of HDL-C in the high fat diet fed animals when compared to normal fed rats (-48.1%, $p<0.05$) (Table 2).

Supplementation of the HFD with 100, 200 and 300 mg/kg of aqueous extracts of leaves of *M. oleifera*, significantly decreased the levels of serumTC by 19.7, 40.7 and 46.9%; TG, 19.6, 34.9 and 40.8%; LDL- C, 11.9, 24.3 and 32.6%, respectively, compared to rats fed HFD alone (Table 3). Treatment with the aqueous extract caused increase in HDL-C by13.9, 22.6 and 116.1% at 100, 200 and 300 mg/kg of the extract, respectively. Atherogenic index was significantly reduced in the *M. oleifera* as well as the atorvastatin treated groups (Table 3). Elevated level of blood cholesterol especially LDL-C is a known major risk factor for CHD whereas HDL- C is cardio-protective

Table 1: Preliminary qualitative phytochemical screening of *M. oleifera* aqueous leaf extract

Phytochemical constituent	Status
Flavonoids	+++
Alkaloids	+
Saponins	++
Glycosides	+
Steroids	+
Tannins	-
Anthraquinones	-

Table 2: Effect of High Fat Diet (HFD) on serum lipid profile of albino wistar rats

Parameters (mmol/l)	Normal control rats	HFD fed rats
TG	1.27±0.11	3.61±0.09 (↑124.7 %)
HDL-C	0.52±0.08	0.27±0.13 (↓- 48.1%)
LDL-C	1.59±0.14	3.28±0.11 (↑106.3%)
TC	1.50±0.20	3.37±0.18 (↑135.7%)

and is referred to as good cholesterol carrier (Eren *et al*., 2012). Atherogenic index is an indication of the degree of deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronaries, aorta, liver and kidneys. The higher the atherogenic index, the higher the risk of Coronary Heart Disease (CHD) (Idemudia *et al*., 2013).

Atorvastatin which was used as standard control in this study is a HMG-CoA reductase inhibitor. HMG-CoA reduces serum triglyceride levels through the modulation of apolipoprotein C-III and lipoprotein lipase. Rats treated with atorvastatin showed marked reduction in all serum lipoproteins and increase in HDL level as compared with HFD untreated group.

Figure 1 shows the body weight gain of the rats during the test period. There were only slight differences in the weight gain pattern of the extract treated animals on HFD diet and those on standard drug treatment. The data shows that the HFD fed-extract treated animals did not gain weight as much as HFD fed-untreated group while the standard drug treated group gained weight initially but lost it abruptly.

Hyperlipidemia a well known risk factor for cardiovascular disease, especially atherosclerotic coronary artery disease (CAD) and throughout sub-Saharan Africa, the incidence of coronary heart

Table 3: Effect of *Moringa oleifera* aqueous extract on lipid profile of HFD induced hyperlipidemic rats

	Mean±sem (% change in lipid profile)				
Parameters (mmol/L)	HFD fed control	HFD fed-Extract treated (100 mg/kg)	HFD fed-Extract treated (200 mg/kg)	HFD fed-Extract treated (300 mg/kg)	HFD fed -Atorvastatin treated (4 mg/kg)
TG	3.73±0.13	3.00±0.11 (↓- 19.6%)	2.43±0.14 (↓- 34.9%)	2.21±0.10 (↓- 40.8%)	1.53±0.10 (↓- 59.0%)
HDL-C	0.31±0.12	0.36±0.13 (↑13.9%)	0.38±0.11 (↑22.6%)	0.67±0.12 (↑116.1%)	0.79±0.21 (↑154.8%)
LDL-C	3.71±0.09	3.27±0.20 (↓- 11.9%)	2.81±0.13 (↓- 24.3%)	2.50±0.17 (↓- 32.6%)	2.01±0.11 (↓- 45.8%)
TC	3.86±0.10	3.10±0.13 (↓- 19.7%)	2.29±0.10 (↓- 40.7%)	2.05±0.11 (↓- 46.9%)	1.93±0.11 (↓- 50.0%)
AI	12.45±0.17	8.61±0.11	6.03±0.10	3.06±0.09	2.44±0.17

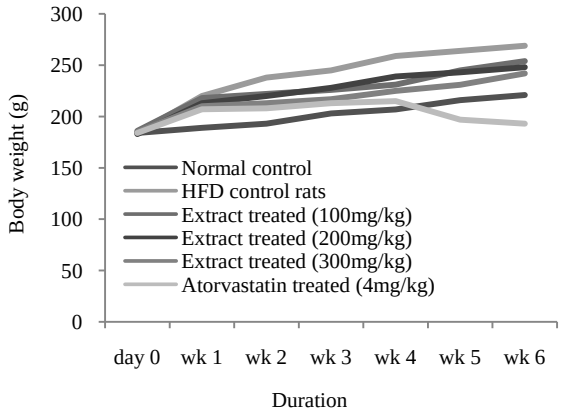

Fig. 1: Mean body weight of normal and HFD induced-extract treated hyperlipidemic rats

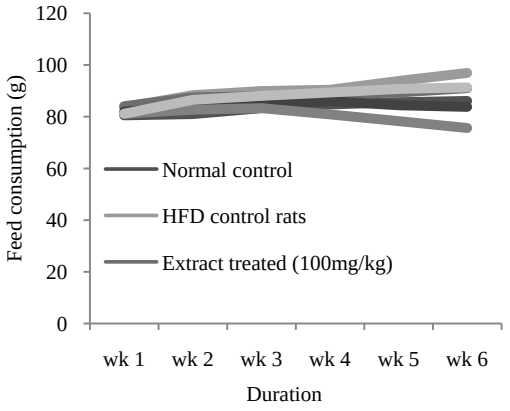

Fig. 2: Mean feed consumption of normal and HFD induced-extract treated hyperlipidemic rats

disease is rising in keeping with westernization (Opie, 2006). Also, it has been well established that nutrition playsan important role in the etiology of hyperlipidemias and atherosclerosis. Bhandari *et al*. (2011) have demonstrated that HFD feeding of Wistar rats increased the serumlipids. The addition of cholic acid to the HFD diet is due to its emulsifying property which improves cholesterol absorption (Dhulasavant *et al*., 2010).

The feed consumption result and direct observation of test animals using activity cages shows a reduction in daily feed consumption rate and appetite of the extract treated group when compared the HFD fed untreated groups (Fig. 2). It thus seems that, in addition to the hypolipidemic property, *M. oleifera* has an appetite reducing effect, whist nourishing the test animals as well. A comparison of the hematology values of the control and treated animals reveals that administration of *M. oleifera* builds blood and improves the WBC index more than the standard treated group (Table 4). The nourishing effect observed in the extract treated groups may be due to the highly nutritious nature of Moringa leaves, they are considered as source of vitamins, protein and numerous minerals (Makkar and Becker, 2007). The result suggests that the extract is a natural appetite suppressant without any adverse effects.

There were mild elevations in the ALT and ALP levels of test animals and more significant increase in the GGT value of HFD fed-untreated group. Ruttmann *et al*. (2005) also reported a correlation between GGT and risk of death from cardiovascular disease. Furthermore, liver enzyme levels were elevated in the

Table 4: Effect of *Moringa oleifera* aqueous extract on hematology values of HFD induced hyperlipidemic rats

Parameters	Normal control	HFD fed control	HFD fed-Extract treated (100 mg/kg)	HFD fed-Extract treated (200 mg/kg)	HFD fed-Extract treated (300 mg/kg)	HFD fed - Atorvastatin treated (4 mg/kg)
Hb (g/dl)	13.60±1.30^{a}	10.12±1.90^{b}	11.10±1.14^{b}	13.36±1.30^{a}	16.00±1.32^{c}	8.74±2.01^{d}
RBC (x 10^{12}/L)	7.05±1.24^{a}	6.34±1.07^{b}	7.36±1.13^{a}	7.89±1.21^{a}	9.67±1.13^{c}	6.29±0.21^{b}
PCV (%)	42.50±1.29^{a}	38.71±2.02^{b}	35.26±1.29^{b}	39.01±1.33^{b}	43.01±0.78^{a}	37.03±0.27^{b}
WBC (x 10^{9}/L)	11.8±1.51^{a}	9.04±2.11^{b}	10.91±2.00^{a}	10.98±1.72^{a}	11.15±1.53^{a}	10.11±1.32^{a}

Values are expressed as mean ± SEM; Values not sharing a common superscript letter across a column differ significantly at p< 0.05

Table 5: Effect of *Moringa oleifera* aqueous extract on liver enzymes (U/L) of HFD induced hyperlipidemic rats

Parameters (U/L)	Normal control	HFD fed control	HFD fed-Extract treated (100 mg/kg)	HFD fed-Extract treated (200 mg/kg)	HFD fed-Extract treated (300 mg/kg)	HFD fed - Atorvastatin treated (4 mg/kg)
AST	42.21±2.18^{a}	43.73±2.43^{a}	44.00±2.33^{a}	43.43±2.74^{a}	42.19±2.10^{a}	55.09±2.28^{b}
GGT	17.40±2.80^{a}	39.75±2.18^{b}	28.11±2.15^{c}	23.38±2.41^{c}	19.35±2.32^{a}	40.32±2.18^{b}
ALT	31.3±2.10^{a}	33.71±2.09^{a}	36.25±2.30^{a}	37.11±2.18^{a}	37.50±2.17^{a}	44.08±2.31^{b}

Values are expressed as mean ± SEM; Values not sharing a common superscript letter across a column differ significantly at p< 0.05

Table 6: Effect of *Moringa oleifera* aqueous extract on serum glucose concentration (mmol/L) of HFD induced hyperlipidemic rats

Normal control	HFD fed control	HFD fed-Extract treated (100 mg/kg)	HFD fed-Extract treated (200 mg/kg)	HFD fed-Extract treated (300 mg/kg)	HFD fed - Atorvastatin treated (4 mg/kg)
5.98±0.39^{a}	3.85±0.21^{b}	5.02±0.17^{a}	5.13±0.16^{a}	5.27±0.10^{a}	9.53±1.07^{c}

Values are expressed as mean ± SEM; Values not sharing a common superscript letter across a column differ significantly at p< 0.05

HFD fed-atorvastatin treated (Table 5), thus confirming the scientific reports on the effect of atorvastatin on liver enzymes (Armitage, 2007; Gillett and Norrell, 2011).

The major reason for the significant decline ($p<0.05$) in serum glucose in the HFD fed untreated rats is not clear but it may be that the high serum cholesterol increases the level of glucagon-likepeptide-1 which enhances insulin secretion from the pancreatic beta-cells leading to hypoglycemia (Prasad, 2008). The study also confirms the hyperglycemic effect of atorvastatin treatment (Table 6); studies have shown that use of atorvastatin is associated with a slight increase in the risk of developing diabetes (Sattar *et al*., 2010; Miao *et al*., 2012).

CONCLUSION

Result of present study revealed that the aqueous extract of leaves of *Moringa oleifera* Lam. improved the serum lipidprofile in hyperlipidemic rats by decreasing serum TC, TG, LDL-C and increasing serum HDL-C, thus improving the atherogenic index. It was also noted that the treatment with the extract, reduced appetite and body weight of test animals even as it was providing needed body nutrients. This finding provides some biochemical basis for the use of leaf extract of *M. oleifera* as antihyperlipidemic agent and in weight loss regimen. Further, studies are required to elucidate its possible mechanism of action.

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