

# Hypolipidaemic effect of ethanol leaf extract of *Moringa oleifera* Lam. in experimentally induced hypercholesterolemic wistar rats

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**Abstract:** The hypolipidaemic effect of ethanol leaf extract of *Moringa oleifera* in experimentally induced hypercholesterolemic rats was investigated. Thirty six (36) wistar rats of both sexes weighing  $130.53 \pm 4.86$  were used for the study. The animals were completely randomized into six groups (A-F) comprising 6 animals each. Groups A, B and C comprise female rats administered 1 ml of distilled water, high dose of 600 mg/kg and low dose of 300 mg/kg body weight of the extract respectively. Groups D, E and F comprise male rats administered 1 ml of distilled water, high dose of 600 mg/kg and low dose of 300 mg/kg body weight of the extract respectively. Hypercholesterolemia was induced by feeding the animals with high fat diet for 21 days before administration of the extract. After the 21 days of feeding, administration of extract lasted for 14 days. Preliminary phytochemical screening revealed that the ethanol leaf extract of *M. oleifera* contains alkaloids, tannins, carbohydrates and cardiac glycosides. Only the high dose female group (600 mg/kg body weight) lost or maintained their body weight significantly ( $p < 0.05$ ), the rest did not. Body weight was not significantly ( $p > 0.05$ ) altered in the male group administered low dose and high dose, showing that the dose of the extract slightly affected their weight. For serum lipids, serum total cholesterol concentration in both male and female reduced significantly ( $p < 0.05$ ), both in those given low and high doses of the extract. Serum high density lipoprotein cholesterol (HDL) level significantly ( $p < 0.05$ ) increased both in male and female rats that were administered high dose of 600 mg/kg body weight of the extract, but was not significantly ( $p > 0.05$ ) affected in other groups. Serum low density lipoprotein cholesterol (LDL) level also reduced significantly ( $p < 0.05$ ) in both male and female rats that were administered high dose of the extract, but was not significantly ( $p > 0.05$ ) altered for those that received low doses (300 mg/kg body weight) of the extract. There was no significant ( $p > 0.05$ ) reduction in the LDL of the male rats. Serum triacylglycerol (TAG) concentration in male and female rats reduced significantly ( $p < 0.05$ ), in those that received low and high doses of the extract. Overall, findings from the present study suggest that the ethanol leaf extract of *M. oleifera* has hypolipidaemic effect. Therefore, the leafy vegetable may be recommended to patients that have problems with high serum lipid profiles and also for people that want to lose or maintain body weight.

**Keywords:** *Moringa Oleifera*, Body Weight, High Fat Diet, Hypercholesterolemia, Serum Lipid, Hypolipidaemic Effect

## 1. Introduction

*Moringa oleifera* Lam. is the most widely cultivated species of the monogeneric family Moringaceae, which includes 13 species of trees and shrubs distributed in sub-

Himalayan ranges of India, Sri Lanka, North-eastern and South-western Africa, Madagascar and Arabia [11]. *Moringa* is also native to parts of West Africa particularly Nigeria [29]. It is commonly known in Hausa (Northern Nigeria) as Zogale. English common names include moringa and drumstick tree [36]. *M. oleifera* is one of the

most useful tropical trees [12]. The Moringa tree is a multi-function plant. It has been cultivated in tropical regions all over the world for the following characteristics: high protein, vitamins, mineral and carbohydrate content of entire plants; high value of nutrition for both humans and livestock; high oil content (42%) of the seed which is edible, and with medicinal uses; the coagulant of seeds could be used for wastewater treatment [8]. Previous studies by Amaglo *et al.* [3], Limon-Pacheco and Gonsebatt [19], and Mahajan and Mehta [20] have reported the pharmacological, antioxidant, and anti-inflammatory property of *M. oleifera* respectively. Furthermore, Awodele *et al.*, [4] worked on the toxicological evaluation of the aqueous extract of *Moringa oleifera* Lam (Moringaceae). Oyedepo *et al.*, [26] evaluated the anti-hyperlipidemic effect of aqueous leaves extract of *Moringa oleifera* while Gupta *et al.*, [15] worked on the evaluation of antidiabetic and antioxidant activity of *Moringa oleifera* in experimental diabetes. Again, Jaiswal *et al.* [17] worked on the role of *Moringa oleifera* in regulation of diabetes-induced oxidative stress while Choudhary *et al.*, [7] assessed the antiulcer potential of *Moringa oleifera* root bark extract in rats. Although there have been several reports on the cholesterol reducing effect of the aqueous and ethanol leaf extracts of *M. oleifera* in rats, there is still paucity of information on the hypolipidaemic activity of the extract at the doses investigated in the present study. Again, considering the use of *M. oleifera* for the treatment of various ailments, there is the need to investigate the possible effect of the plant on some biochemical parameters as it relates to weight and lipid in animals. In this study, we therefore present information on the hypolipidaemic effect of ethanol leaf extract of *Moringa oleifera* on the body weight and serum lipid profile using experimentally induced hypercholesterolemic wistar rats as model.

## 2. Materials and Methodology

### 2.1. Materials

#### 2.1.1. Plant Materials and Authentication

Fresh leaves of *Moringa oleifera* was procured at Mrs. Bali's home farm at No. 10 Kuchikau Road, Auta Balefi, Karu LGA, Nasarawa State, Nigeria, and was authenticated by the Botany Department of Federal University of Technology, Yola with voucher specimen number: BC/FUTY/DD02/2004.

#### 2.1.2. Experimental Animals

Wistar rats (*Rattus norvegicus*) of both sexes weighing  $130.53 \pm 4.86$  g were obtained from the Animal House of Federal College of Animal Health and Production Technology (FCAH & PT), VOM, Jos, Plateau State, Nigeria.

#### 2.1.3. Assay Kits

The assay kits for the determination of serum total cholesterol, high-density lipoprotein cholesterol (HDL),

low-density lipoprotein cholesterol (LDL) and triacylglycerol (TAG) were products of Randox Laboratory Ltd, Co-Atrium, United Kingdom.

#### 2.1.4. Other Reagents

All other chemicals and reagents used which were of analytical grade were products of sigma Aldrich Ltd., Buchs, Canada and are prepared in volumetric flask using glass wares with distilled water except otherwise stated.

### 2.2. Methodology

#### 2.2.1. Formulation of High Fat Diet

The high fat diet was prepared by adopting the procedure described by [25] with little modification. Briefly, composition of the experimental diet include: 50.0% (Cholesterol rich butter + hydrogenated sunflower oil), 25.0% Garri, 20.0% (Bonga fish + egg yolk), 1.5% Fiber, 2.5% Mineral mixture and 1.0% Vitamin mixture making up 100%, which gives an energy requirement of 6.35 KCal/g. This was done by mixing these components in an experimental bowl. After preparation, it was dried at room temperature, and then administered to the animals to induce hypercholesterolemia.

#### 2.2.2. Preparation of Ethanolic Leaf Extract of *Moringa Oleifera*

Fresh leaves of *Moringa oleifera* were air dried under a shade until a constant weight was obtained. This was thereafter pulverized in a blender (PHILIPS, Model HR-1724, Brazil). A known weight (250 g) of the powder was extracted in 1000 ml of 70 % ethanol for 72 hours at room temperature. The extract was filtered with Whatman No. 1 filter paper (Maidstone, UK) and the resulting filtrate concentrated in a Rotary Evaporator, where some ethanol was recovered. The mixture was further transferred into steam bath where it was evaporated to give the required brownish-black residue. This was then reconstituted in distilled water to give the required low and high doses (300 and 600 mg/kg body weight) used in the present study.

#### 2.2.3. Phytochemical Screening

Preliminary phytochemical screening to detect the presence of alkaloids, saponins, tannins, carbohydrates were carried out by adopting the methods described by Harborne [16], Walls *et al.*, [37], Odebiyi and Sofowora [24], Trease and Evans [34] respectively. Steroids, anthraquinones and cardiac glycosides were carried out by adopting the procedures described by El-Olemy *et al.*, [8], Mainasara *et al.*, [21] and Sofowora [32] respectively.

#### 2.2.4. Determination of Body Weight

The weight of individual rat of each group was measured 'only' before and after administration of extract.

#### 2.2.5. Animal Grouping, Feeding and Extract Administration

A total of thirty six (36) wistar rats, housed in clean aluminum cages contained in well ventilated standard

housing conditions (temperature: 28-31°C; photoperiod: 12 hours; humidity: 50-55%) was used for the study. The animals were allowed free access to rat pellets (Premier Feed Mill Co. Ltd., Ibadan, Nigeria) and tap water *ad libitum*. The cages were also cleaned on daily basis. The animals were acclimatized for two weeks before the commencement of the experiment. The animals were completely randomized into six groups (A-F) comprising 6 animals each. Groups A, B and C comprise female rats administered 1 ml of distilled water, high dose of 600 mg/kg and low dose of 300 mg/kg body weight of the extract respectively. Groups D, E and F comprise male rats administered 1 ml of distilled water, high dose of 600 and low dose of 300 mg/kg body weight of the extract respectively. Hypercholesterolemia was induced by feeding the animals with high fat diet for 21 days before administration of any extract. After the 21 days of feeding, administration of extract lasted for 14 days using metal oropharyngeal cannula. The animals were handled humanely according to the guidelines of European convention for the protection of vertebrate animals and other scientific purposes- ETS-123 [10].

#### 2.2.6. Collection of Serum

The rats were anaesthetized in a glass jar containing cotton wool soaked in diethyl ether. The unconscious rats were quickly removed and the neck area cleared of fur. The jugular vein which was slightly displaced (to avoid contamination of the blood with interstitial fluid) was cut with a sterile scalpel blade and an aliquot of the blood was collected into a sample bottle. The blood was then left undisturbed for 10 minutes at room temperature to clot. The blood was thereafter centrifuged at 224x g for 10 minutes using Uniscope Laboratory Centrifuge (Model 800D, New Life Medical Instrument, England). The sera were later aspirated with Pasteur pipette into dry, sample bottles and used within 12 hours of preparation for the determination of serum lipid profile.

#### 2.2.7. Statistical Analysis

Results were expressed as the mean  $\pm$  SEM of six determinations. The data were analyzed using Duncan Multiple Range Test and complemented with Student's t-test. The differences were considered statistically significant at  $p < 0.05$ . All these analyses were done using SPSS 20.0 Software (Statistical Package for Social Sciences, Inc., Chicago, IL, USA).

### 3. Results

Preliminary phytochemical screening of the ethanol leaf extract of *Moringa oleifera* revealed the presence of alkaloids, tannins, carbohydrates and cardiac glycosides while saponins, steroids and anthraquinones were not detected (Table 1).

Only the high dose (600 mg/kg body weight) female group lost or maintained their body weight significantly ( $p < 0.05$ ), the rest did not. However, body weight was not

significantly ( $p > 0.05$ ) different in the male group administered low and high doses, showing that these doses of the extract did not produce any change on their body weight (Figure 1).

The study revealed that serum total cholesterol levels in both male and female reduced significantly ( $p < 0.05$ ), both in those that received low (300 mg/kg body weight) and high (600 mg/kg body weight) doses of the extract (Figure 2).

Serum high density lipoprotein cholesterol (HDL) level significantly ( $p < 0.05$ ) increased both in male and female rats that were administered high dose (600 mg/kg body weight) of the extract, but was not significantly ( $p > 0.05$ ) affected in other groups (Figure 3).

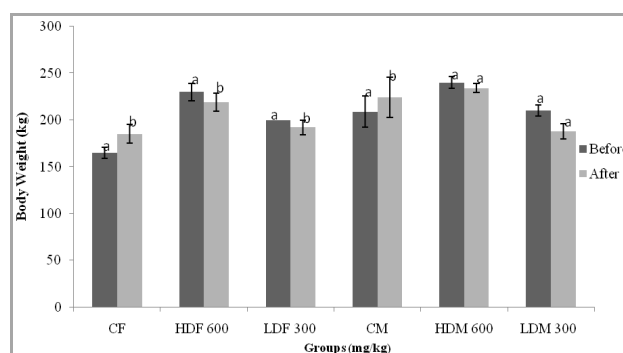
Serum low density lipoprotein cholesterol (LDL) also reduced significantly ( $p < 0.05$ ) in both male and female rats that were administered high dose (600 mg/kg body weight) of the extract, but was not significantly ( $p > 0.05$ ) altered in those that received low doses (300 mg/kg body weight) of the extract. There was no significant ( $p > 0.05$ ) reduction in the LDL of the male rats (Figure 4).

Serum triacylglycerol (TAG) levels in both male and female rats decreased significantly ( $p < 0.05$ ), in those that received low and high doses (300 and 600 mg/kg body weight respectively) of the extract (Figure 5).

**Table 1.** Phytochemical constituents of ethanol leaf extract of *Moringa oleifera*

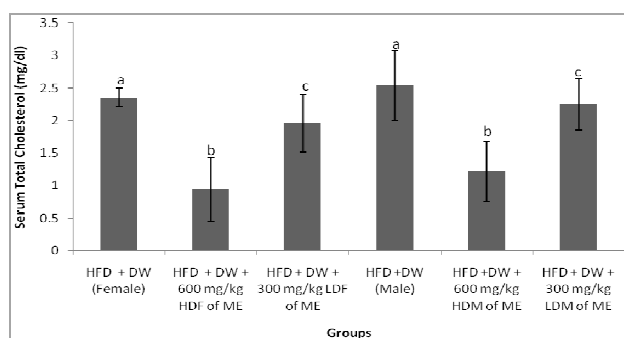
Constituents	Result
Alkaloids	+++
Saponins	-
Tannins	+++
Carbohydrates	++
Steroids	-
Anthraquinones	-
Cardiac glycosides	++

Key: (+) = Present (-) = Absent



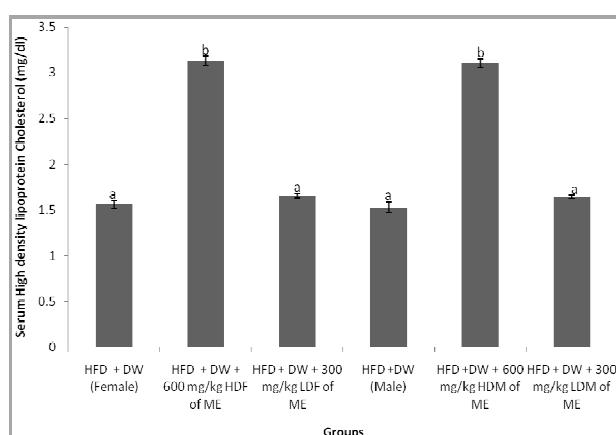
**Figure 1.** Effect of administration of ethanol leaf extract of *Moringa oleifera* on body weight of rats

Key: CF = Control Female, HDF= High Diet Female, LDF= Low Diet Female, CM= Control Male, HDM= High Diet Male, LFM= Low Diet Male



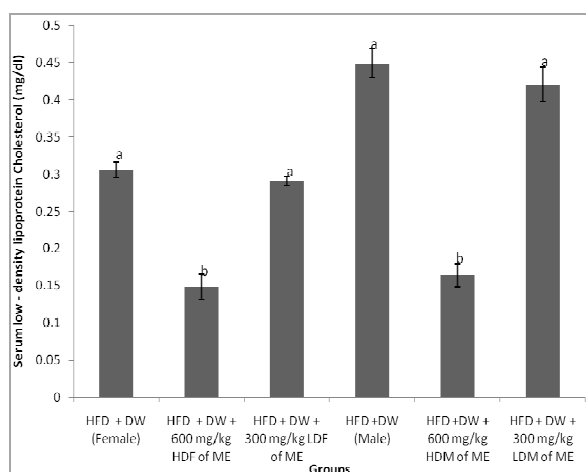
**Figure 2.** Effect of administration of ethanol leaf extract of *Moringa oleifera* on serum total cholesterol of rats

Key: HFD = High Fat Diet, DW = Distilled Water, ME = Moringa Extract, HDF = High Dose Female, LDF = Low Dose Female, HDM = High Dose Male, LDM = Low Dose Male



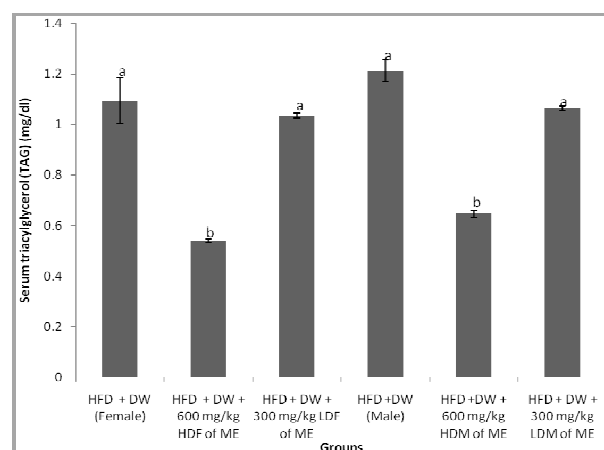
**Figure 3.** Effect of administration of ethanol leaf extract of *Moringa oleifera* on serum high density lipoprotein cholesterol (HDL) of rats

Key: HFD = High Fat Diet, DW = Distilled Water, ME = Moringa Extract, HDF = High Dose Female, LDF = Low Dose Female, HDM = High Dose Male, LDM = Low Dose Male



**Figure 4.** Effect of administration of ethanol leaf extract of *Moringa oleifera* on serum low density lipoprotein cholesterol (LDL) of rats

Key: HFD = High Fat Diet, DW = Distilled Water, ME = Moringa Extract, HDF = High Dose Female, LDF = Low Dose Female, HDM = High Dose Male, LDM = Low Dose Male



**Figure 5.** Effect of administration of ethanol leaf extract of *Moringa oleifera* on serum triacylglycerol (TAG) of rats

Key: DW = HFD = High Fat Diet, Distilled Water, ME = Moringa Extract, HDF = High Dose Female, LDF = Low Dose Female, HDM = High Dose Male, LDM = Low Dose Male

## 4. Discussion

The incidence of obesity, which results from intake of high fat diet and other metabolic disorders, is rising at an alarming rate and is becoming a major public health concern with incalculable social cost. Indeed, obesity facilitates the development of metabolic disorders such as diabetes, hypertension, and cardiovascular diseases such as stroke, osteoarthritis, apnea, some cancers and inflammation-based pathology [14]. Alterations in the concentration of major lipids like serum total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and triacylglycerol (TAG) could give useful information on the lipid metabolism as well as predisposition of the heart to atherosclerosis and its associated coronary heart diseases [38].

The hypolipidaemic activity of several medicinal plants has been associated to bioactive agents like alkaloids, tannins, and cardiac glycosides. Alkaloids may mediate hypolipidaemic action either by up regulation of activities of lipolytic enzymes or by stimulating faecal bile acid excretion in agreement with previous findings by Mathur *et al.*, [22]. Also, other phytoconstituents and  $\beta$ -sitosterol present in the extract could be the major cholesterol-reducing components of the ethanol leaf extract of *M. oleifera* as it may work singly or in synergy with other bioactive agents [33].

The significant reduction in body weight by the extract in female rats fed with high fat diet may be partly attributed to inhibition of cholesterol deposition in body tissues or inhibition of HMG CoA reductase activity which is the key regulatory enzyme in cholesterol biosynthetic pathway [1, 2, 27]. This implies that the extract may be recommended for persons who want to lose weight. For the male rats, since the extract at both doses did not significantly affect the body weight when compared with the control animals, it "may" also be recommended in maintaining body weight.

The phytochemical constituents and  $\beta$ -sitosterol present are the major cholesterol-reducing components of the *Moringa oleifera* leaf.  $\beta$ -sitosterol helps in reducing cholesterol levels by limiting the amount of cholesterol that is able to enter the body, by inhibiting cholesterol absorption in the intestines [5, 13]. The structure of  $\beta$ -sitosterol is similar to that of cholesterol.  $\beta$ -sitosterol takes the place of dietary and biliary cholesterol in micelles produced in the intestinal lumen and this reduces cholesterol absorption in the body. Therefore, instead of cholesterol being absorbed, the  $\beta$ -sitosterol in *moringa oleifera* will be absorbed. Another way cholesterol can be reduced is by reduction in LDLC. LDLC carries cholesterol from the liver to the blood stream. Therefore, when LDLC is reduced, the amount of cholesterol being carried will also reduce [30]. Chlorogenic acid and moriginine are also constituents of *Moringa oleifera* and they reduce serum total cholesterol [6]. Similarly, the decrease in serum total cholesterol observed with the extract at 600 mg/kg body weight (female) may be due to decrease in the concentration of acetyl CoA arising probably from reduced  $\beta$ -oxidation of fatty acid, since acetyl-CoA is a key substrate in the biosynthesis of cholesterol [31]. Low blood cholesterol concentration is one of the important beneficial factors that do not predispose an individual to cardiovascular diseases [35]. Therefore, such decrease in the serum lipid contents may be beneficial to the animals as it may not enhance obesity, atherosclerosis and hypertension [9].

High density lipoprotein cholesterol (HDL) is considered to have anti-atherogenic properties. It has also been shown that increase in HDL correlates inversely with coronary heart disease [23]. In the present study, administration of 600 mg/kg body weight of the extract to both female and male animals, which showed significant increase in serum HDL when compared to the control animals is beneficial, and 'may' not predispose the animals to cardiovascular risk.

For low density lipoprotein cholesterol (LDL), it is known that for being effective antihyperlipidemic agent, the compound should reduce the plasma levels of LDL, as it transports 70% of plasma cholesterol in humans [28]. Epidemiological and clinical studies have demonstrated positive correlation in LDL concentration in serum and risk of coronary heart diseases [5]. This result showed significant decrease in serum LDL level as a function of treatment with the ethanol leaf extract of *M. oleifera*. Part of the insignificance can be attributed to lower concentration of bioactive phytochemicals involved in inhibition of the uptake of dietary and biliary cholesterol, thereby reducing LDL levels.

Triacylglycerols are the main storage form of fatty acids. The decrease in serum triacylglycerol by the ethanol leaf extract of *M. oleifera* may be due to reduced lipolysis. This may deplete the store of fatty acids. Also, the reduced serum triacylglycerol (TAG) level in the treated animals could be co-related to elevated lipoprotein lipase activity which is in agreement with previous studies by Phil-Sun *et al* [28] and Kannel *et al* [18] on mushroom extracted exo-

biopolymer. Overall, the study showed that there was reduced level of all lipids except the HDL-C, and this may be beneficial to the animal as this pattern of alterations are associated with reduced risk of atherosclerosis and its coronary heart diseases.

## 5. Conclusion

The result of the present study has shown that ethanol leaf extract of *Moringa oleifera* has hypolipidaemic effect mainly in terms of reduction in serum lipids and body weight loss. Cholesterol-reducing action of the extract indicates that this leafy vegetable possess medicinal value which could validate and explain its ethno-medical use on obese persons as well as other related cardiovascular diseases. Therefore, ethanol leaf extract of *M. oleifera* may be recommended to patients having problems with high serum lipid profiles, and also for people that want to lose or maintain body weight.

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