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Antilipidemic Potentials of *Moringa Oleifera* Root Extract in Poloxamer 407- Induced Hyperlipidemia

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ABSTRACT

Hyperlipidemia is characterized by elevated serum total cholesterol, low density lipoprotein and concomitant decrease in high density lipoprotein all of which are known risk factors for coronary heart diseases. The paucity of scientific evidence to support ethnomedical claims of the efficacy of plants on some cardiovascular diseases necessitated the investigation of the effects of methanolic root extracts of Moringa oleifera on poloxamer 407-induced hyperlipidemia in experimental animals. Forty rats weighing 135 - 200 g were divided into 8 designated groups (n=5). Hyperlipidemia was induced in groups 2-8 animals with 1000 mg/Kg body weight of poloxamer-407. Animals in group 4 received methanol extract, while groups 5, 6, 7 and 8 received column-fractions of the extract (200 mg/kg body weight). Groups 2 and 3 received normal saline and atorvastatin (10 mg/kg), respectively, serving as hyperlipidemic and positive controls. Uninduced group 1 animals were used as negative control. Blood samples were collected after the treatment period for biochemical analysis. The methanol root extract and fractions significantly ($p < 0.05$) decreased total cholesterol, low density lipoprotein (LDL), triacylglyceride, 3-hydroxyl-3-methylglutaryl Coenzyme A (HMG-CoA) reductase activity, and increased high density lipoprotein relative to hyperlipidemic control rats. The atherogenic indices in animals treated with methanol extract (0.35±0.33; 1.26±0.05) and fraction (0.31±0.007; 1.31±0.14) were not significantly different ($p > 0.05$) compared to atorvastatin (0.33±0.004; 1.12±0.03), respectively. Moringa oleifera extract and fractions that demonstrated positive effects on lipid profile comparable to atorvastatin possess antihyperlipidemic potential. This finding validates its use in the management of dyslipidemia in ethnomedicine.

Keywords: Moringa oleifera root; Poloxamer 407; antihyperlipidemic; 3-hydroxyl-3-methylglutaryl Coenzyme A reductase; Atherogenic indices.

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INTRODUCTION

Since ancient times, medicinal plants have been used in disease management. The increasing discovery and utilization of more plants with medicinal potential have necessitated continued scientific scrutiny of their bioactivity in order to provide supportive data that would encourage responsible use.[1] Many parts of *Moringa oleifera*, commonly known as 'drumstick tree' or 'horseradish tree' have been commonly used as stimulants, antitumor, antipyretic, antiepileptic, anti-inflammatory, anti-ulcer, anti-spasmodic, diuretic, anti-hypertensive, cholesterol-lowering, antioxidant, anti-diabetic, hepatoprotective, anti-bacterial and anti-fungal agents.[2,3]

Hyperlipidemia, a condition characterised by elevated serum lipoproteins, total cholesterol (TC) and triglycerides (TG), is a major risk factor for atherosclerosis, myocardial infarction and stroke,[4] thus contributing significantly to incidences of cardiovascular disease (CVD) and associated deaths. Successes recorded in improving patient lipid profile with synthetic medications have been erratic resulting in decreased CVD mortality occasioned by atherosclerotic injuries. Although several synthetic pharmacological agents have helped to reduce plasma LDL-C levels, chronic use are associated with myraids of side effects.[5] Consequently, alternative approaches in the management of metabolic disorders[6] and related conditions are being sought after.[7] To further substantiate the scientific basis for use of many plant materials in disease management, this study was designed to investigate the potential of methanol and column-fractions of root extracts of *Moringa Oleifera* on lipid profile, atherogenic indices and HMG-CoA reductase activity in Poloxamer 407- induced hyperlipidemic experimental animal.

MATERIALS AND METHODS

Collection of Plant Materials

Moringa oleifera roots were collected from its natural habitat at the Federal University of Agriculture Makurdi (now Joseph Sarwuan Tarka University), Benue State, Nigeria, authenticated at the Department of Botany. A voucher specimen (FUAM/BOT/OG/0113) was deposited at the University herbarium. The roots were washed properly and air-dried at ambient temperature for two weeks. The dried roots were pulverised into fine powder using a mortar and pestle, and the powdered samples were preserved at 4°C in sealed plastic packet for further use.

Preparation of *Moringa oleifera* root extract and fractions

The root extract of *Moringa oleifera* was prepared by adopting the Das *et al*. method.[8] Extraction was carried out based on sample to solvent ratio of 1:10 (w/v). A 100 g of the powdered sample was suspended in 1000 ml of 70% methanol on a shaker for 48 hours at room temperature. The extract was filtered through a sterilized Buchner funnel and Whatman filter paper no.1. The filtrate was concentrated by drying in a hot water bath maintained at a temperature of 45°C until a brownish black residue was obtained. Percentage yield was determined. The procedure was concurrently run for all samples, and the extracts were kept in sealed containers and refrigerated at -20°C from which aliquots were reconstituted for the experiment.

Fractionation was carried out by column chromatography the method described by Seelinga *et al*.[9] The crude extract derived from methanolic root extract was disolved in n-Hexane and 6 g of the weighed extract placed on top of 2 × 30 cm silica gel column. Thin layer chromatography (TLC) was used to check the fractions and those with similar solvent fronts were combined to yield four fractions designated F1 to F4. The collected eluates (fractions) were evaporated under vacuum, and further dried on a lyophilizer. Weights of the eluates were determined as similarly described for methanol extract.

Experimental Animals

Forty rats weighing between 135 - 200 g were obtained from the Animal House of the College of Health Sciences, Benue State University, Makurdi and used for the study. Ethical approval for use of animals for this study was obtained from the Institutional Research and Ethics Committee of the College of Health Sciences,

Benue State University, Makurdi. The animals were maintained at standard conditions (temperature of 25 ± 2 °C; 40-50 ± 5% humidity with 12-hour light/12-hour dark cycle) for acclimitization for 1 week while allowing them access to standard feeds (Chikum feed PLC, Lagos, Nigeria), and water ad libitum.

Acute toxicity test (LD_{50})

Acute toxicity test of the extract was carried out using up and down method.[10] This method entails sequential administration of a specific dose of a test substance to a single animal over a 48-hour period. After the first dose, the next dose is determined based on the result of the previous dose administered. If an animal survives, the subsequent dose is adjusted upward and vice versa by a constant factor. Testing is usually halted when a typical upper limit (2000 – 5000 mg/kg) has been reached without mortality or when the LD_{50} has been established through computer analysis. In this test, a total of 10 rats were used with a single rat dosed sequentially with the crude extract as well as fraction and observed for signs of toxicity such as depilation and diarrhoea. The LD_{50} was calculated using AOT 425 Statistical program.

Induction of hyperlipidemia

Hyperlipidemia was induced by a single intraperitoneal injection of freshly prepared solution of 1000 mg/kg poloxamer 407 (prepared by dissolving 2 g in 20 ml normal saline) after overnight fasting.[11] Forty-eight-hour post induction, hyperlipidemia was confirmed at 245 mg/dl total cholesterol and 198 mg/dl triglyceride levels with no reversal at the expiration of the test period.

Experimental design

The animals were randomly divided into 8 groups of 5 rats each with group 4 – 8 treated orally with methanol and column-fractions of methanol extract along with standard drug for 21 days as indicated below:

Group 1: not induced and not treated; Normal control (NC).

Group 2: induced but not treated; hyperlipidemic control

Group 3: induced and treated with 10 mg/kg b.wt atorvastatin standard drug

Group 4: induced and treated with 200 mg/kg b.wt crude extract

Group 5: induced and treated with 200 mg/kg b.wt of fraction 1

Group 6: induced and treated with 200 mg/kg b.wt of fraction 2

Group 7: induced and treated with 200 mg/kg b.wt of fraction 3

Group 8: induced and treated with 200 mg/kg b.wt of fraction 4

Collection of blood samples and preparation for analysis

At the end of 21-day treatment, the rats were sacrificed by decapitation following chloroform anesthesia. Blood samples were drawn from the heart puncture into plain bottles which were allowed to clot before the serum was separated by centrifugation (using Labofuge 300 centrifuge Heraeus) at 3000 rpm for 10 minutes. The supernatant (serum) was collected for biochemical analysis. The liver was quickly excised, trimmed of connective tissues, and rinsed with saline used to assay 3 Hydroxyl-3-methylglutaryl Coenzyme A (HMG-CoA) reductase activity.

Biochemical assays

Lipid profile (Total cholesterol (TC), Triglycerides (TG), Low density lipoprotein cholesterol (LDL-C) and High-density lipoprotein cholesterol (HDL-C)) was assayed using Randox assay kits (Randox Laboratories, UK) as described previously.[12] Risk indices were calculated using Friedewald formula:

Atherogenic indices (Castelli's Risk Index-I (CRI-I) = TC/HDL-C; Castelli's Risk Index-II (CRI-II) = LDL-C/HDL-C while Atherogenic Index of Plasma (AIP) = log10(TG/HDL-C).

Similarly, HMG-CoA reductase activity estimation was carried out according to method described by,[13] where liver tissue was removed as quickly as possible and a 10% w/v homogenate prepared in saline arsenate solution. The homogenate was deproteinized using an

equal volume of dilute perchloric acid and allow to stand for 5 min, followed by centrifugation. To 1 mL of the filtrate, 0.5 mL of freshly prepared (alkaline hydroxylamine reagent in the case of HMG-CoA was added. It was mixed and 1.5 mL of ferric chloride reagent was added after 5 min. The absorbance was read after 10 min at 540 nm with treated saline arsenate as the blank. The ratio of HMG-CoA/mevalonate was thereafter calculated.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) and analyzed by analysis of variance (ANOVA) using SPSS program (version 20.0 SPSS Inc., Chicago, IL, USA). Significance was defined as $P < 0.05$. The difference between the various extracts and animal groups was compared using the Duncan Multiple Range Test.

RESULTS

Effect of extract of *Moringa oleifera* root and fractions on serum lipid profile of Poloxamer 407-induced hyperlipidemic rats

The effect of root extract of *Moringa oleifera* and fractions on serum lipid profile of experimental rats is shown in Table 1 below.

Effect of *Moringa oleifera* Root Extract and Purified Fractions on Serum Atherogenic Indices of Poloxamer 407-induced Hyperlipidemic Rats

The effects of daily oral administration *Moringa oleifera* root extract and fractions on serum atherogenic indices of poloxamer 407-induced hyperlipidemic rats is as presented in Table 2. The results showed that hyperlipidemic control had significant ($p < 0.05$) reduction in HDL-C/TC ratio and a significant ($p < 0.05$) increase in LDL-C/HDL-C and Log (TG/HDL-C) ratio when compared with all other groups.

Effect of *Moringa oleifera* Root Extract and Purified Fractions on Liver Function Parameters and HMG-CoA Reductase Activity of Polaxomer 407-induced Hyperlipidemic Rats

The effect of *Moringa oleifera* root extract and fractions on HMG-CoA reductase activities of polaxomer 407-induced hyperlipidemic rats is presented in Table 3. The result shows that serum levels of HMG-CoA reductase activity in hyperlipidemic untreated rats was significantly ($p < 0.05$) increased when compared to negative control

Table 1: Effect of *Moringa oleifera Root Extract and Fractions on Serum Lipid Profile of poloxamer 407-induced Hyperlipidemic Rats*

Parameter / Group	Serum TC (mg/dl)	Serum TAG (mg/dl)	Serum LD (mg/dl)	L-C Serum HDL-C (mg/dl)
1	86.25 ± 9.66^{a}	83.91 ± 6.51^{b}	71.60 ± 5.25^{b}	69.40 ± 31.81^{c}
2	293.26 ± 21.43^{e}	191.57 ± 11.91^{e}	135.38 ± 10.94^{e}	31.80 ± 7.82^{a}
3	99.56 ± 9.72 (65)^{a}	70.38 ± 3.34 (61)^{a}	57.41 ± 8.27^{a}	67.40 ± 6.70^{c}
4	188.93 ± 50.91 (32)^{d}	141.67 ± 52.85 (27)^{d}	72.16 ± 4.73^{b}	50.80 ± 36.95^{b}
5	130.41 ± 35.14 (56)^{b}	105.02 ± 22.29 (47)^{c}	90.09 ± 9.51^{d}	69.40 ± 23.70^{c}
6	160.81 ± 40.95 (46)^{c}	109.85 ± 24.11 (40)^{c}	68.30 ± 7.07^{b}	69.00 ± 27.80^{c}
7	152.22 ± 33.98 (45)^{c}	84.37 ± 10.99 (57)^{b}	76.12 ± 10.61^{b}	74.60 ± 23.31^{d}
8	149.96 ± 46.46 (49)^{c}	91.43 ± 8.16 (51)^{b}	84.12 ± 8.12^{c}	$71.00 \pm$

Key: Values are mean ± SD of 3 determinations. Values with different superscripts in the column are significantly different at $P < 0.05$. Values in parenthesis indicates percentage reduction. 1= Normal Control Rat, 2= Hyperlipidemic Control Rat, 3= Hyperlipidemic + Standard Drug (atorvastatin 10 mg/kg), 4= Hyperlipidemic Rat + Crude Extract (200 mg/kg), 5= Hyperlipidemic Rat + Fraction 1 (200 mg/kg), 6= Hyperlipidemic Rat + Fraction 2 (200 mg/kg), 7= Hyperlipidemic Rat + Fraction 3 (200 mg/kg), 8Hyperlipidemic Rat + Fraction 4 (200 mg/kg). TC= Total Cholesterol, TAG= Triacyglycerol, LDL-C= Low Density Lipoprotein Cholesterol, HDL-C= High Density Lipoprotein Cholesterol.

Table 2: Effect of Moringa *oleifere* Root Extract and Fractions on Serum Atherogenic Indices of Poloxamer 407-induced Hyperlipidemic Rats

Parameter / Group	(HDL-C/TC)	(LDL-C/HDL-C)	Log (TA G/HDL-C)
1	0.52 ± 0.07^{d}	0.57 ± 0.06^{a}	0.25 ± 0.04^{a}
2	0.19 ± 0.02^{a}	0.72 ± 0.33^{b}	0.96 ± 0.04^{d}
3	0.33 ± 0.04^{c}	1.12 ± 0.03^{c}	0.55 ± 0.04^{c}
4	0.35 ± 0.03^{c}	1.26 ± 0.05^{c}	0.44 ± 0.04^{b}
5	0.31 ± 0.07^{c}	1.31 ± 0.14^{c}	0.51 ± 0.04^{c}
6	0.36 ± 0.05^{b}	1.30 ± 0.17^{c}	0.51 ± 0.07^{c}
7	0.46 ± 0.08^{d}	1.25 ± 0.01^{c}	0.42 ± 0.06^{b}
8	0.39 ± 0.07^{b}	1.25 ± 0.02^{c}	0.57 ± 0.02^{c}

Key: Values are mean ± SD of 3 determinations. Values with different superscripts in the column are significantly different at $P<0.05$. 1= Normal Control Rat, 2= Hyperlipidemic Control Rat, 3= Hyperlipidemic + Standard Drug (atorvastatin 10 mg/kg), 4= Hyperlipidemic Rat + Crude Extract (200 mg/kg), 5= Hyperlipidemic Rat + Fraction 1 (200 mg/kg), 6=Hyperlipidemic Rat + Fraction 2 (200 mg/kg), 7= Hyperlipidemic Rat + Fraction 3 (200 mg/kg), 8= Hyperlipidemic Rat + Fraction 4 (200 mg/kg). TC= Total Cholesterol, TAG= Triacyglycerol, LDL-c= Low Density Lipoprotein Cholesterol, HDL-c= High Density Lipoprotein Cholesterol.

Table 3: Effect of *Moringa oleifera* Root Extract and Fractions on HMG-CoA reductase activities of Poloxamer 407-Induced Hyperlipidemic Albino Rats

Group / Treatment	HMG -CoA/mevalonate ration (u/l)
1	2.07 ± 0.27^{b}
2	3.28 ± 0.23^{c}
3	1.05 ± 0.51^{a}
4	1.45 ± 0.28^{a}
5	1.77 ± 0.61^{a}
6	2.05 ± 0.11^{b}
7	1.90 ± 0.12^{a}
8	1.99 ± 0.09^{a}

Values are mean ± SD of 3determinations. Values with different superscripts in the column are significantly different (P<0.05). 1= Normal Control Rat, 2= Hyperlipidemic Control Rat, 3= Hyperlipidemic + Standard Drugs (atorvastatin 10 mg/kg), 4=Hyperlipidemic Rat + Crude Extract (200 mg/kg), 5= Hyperlipidemic Rat + Fraction 1 (200 mg/kg), 6= Hyperlipidemic Rat + Fraction 2 (200 mg/kg), 7= Hyperlipidemic Rat + Fraction 3 (200 mg/kg), 8= Hyperlipidemic Rat + Fraction 4 (200 mg/kg). HMG-CoA= 3 hydroxyl 3 Methylglutaryl Coenzyme A.

DISCUSSION

Poloxamer 407 caused significant ($P < 0.05$) alteration of lipid profile manifested by increase in total cholesterol (TC), triglyceride (TAG) and LDL-cholesterol with concomitant decrease in HDL-cholesterol of treated groups compared to normal control. However, with the administration of crude extract, there was significant ($p > 0.05$) reduction in total cholesterol, triglyceride and LDL-cholesterol as well as increase in HDL-c in the treated groups (3 – 8) compared to the hyperlipidemic control (group 2). The results also showed that fractions 1 and 3 (F1and F3) significantly ($P > 0.05$) lowered TC and TAG respectively, and more effective compared to fractions F2 and F4. Observed increase in serum lipoproteins caused by Poloxamer 407 is suggestive of inhibition of lipoprotein lipase enzyme, which facilitates the hydrolysis of triglycerides.[14] Poloxamer 407 is also reported to cause indirect stimulation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase which is involved in cholesterol biosynthesis.[15] Since LDL (commonly known as the bad cholesterol) is responsible for transporting cholesterol to the body cells, the observed high levels of LDL in the hyperlipidemic control group may indicate increased availability of cholesterol in the blood, which has been shown to be correlative with increased risk of heart disease.[16]

Methanol and fractions of *Moringa oleifera* roots extract exhibited significant reduction in LDL-C levels which aligns with previous report [17]. HDL, otherwise referred to as good cholesterol is produced by the liver to carry cholesterol and other lipids from tissues back to the liver for degradation. High levels of HDL cholesterol at concentrations of 60 mg/dL or higher have been considered as optimal and considered as a good indicator of a healthy heart, whereas, HDL concentrations below 40 mg/dL are considered as major risk factor for cardiovascular diseases (CVDs). However, HDL is often interpreted in the context of TC and LDL concentrations, and hence may be regarded as less significant when LDL is low. HDL-C acts as cholesterol scavengers by mopping up excess cholesterol and cholesterol esters from the blood and peripheral tissues thereby limiting formation of atherosclerotic plaque in the aorta.[18,19] The observed effect of *Moringa oleifera* root extract in increasing the level of HDL-C is consistent with previous studies.[20,21] The higher antihyperlipidemic effects of fraction 1 on TC, and fraction 3 on TAG is suggestive of availability of more beneficial active principles of *Moringa oleifera* root in these fractions that would warrant further investigation. These may include saponins, which forms insoluble complexes with cholesterol or their bile salt precursor, thus making them unavailable for absorption.[22] The present studies show significant increase in HDL-C by the root extract of fraction 1 and fraction 3. This could possibly be due to increasing activity of lecithin-cholesterol acyl transferase (LCAT), an enzyme responsible for incorporating free cholesterol into HDL-C as previously suggested.[23] Thus, these findings are consistent with documented mechanistic insights in support of many phytochemicals in modulating lipid homeostasis.[24]

Although Poloxamer 407 treatment destabilized atherogenic indices, treatment with extract and F1 - F4 resulted in significant ($p < 0.05$) increase in the HDL-C/TC and decreased LDL-C/HDL-C cum Log

(TG/HDL-C) ratios in comparison with the hyperlipidemic untreated group. When compared to the normal control, group that received 200 mg/Kg of root extract had a significant ($p < 0.05$) reduction in HDL-C/TC, significant increase ($p < 0.05$) in LDL-C/HDLC and Log (TG/HDL-C) while group receiving 200 mg/kg of crude extract recorded significant ($p < 0.05$) reduction in HDL-C/TC and significant ($p < 0.05$) reduction in LDL-C/HDL-C as well as significant ($p < 0.05$) increase in Log (TG/HDL-C) ratio. Atherogenic risk predictor indices (HDL-c/TC, LDL-C/HDL-C and log (TG/HDL-C) are mathematical relationships between TC, TG, LDL-C and HDL-C that have been successfully used as markers for assessing atherosclerosis development.[25] They indicate deposition of plaques or fatty infiltration in the cardio routes, thus, the higher the value, the higher the risk of oxidative damage to the heart and associated organs.[26] Instructively, raising the level of HDL-C and decreasing the atherogenic indices as observed in this study are important measures in reducing the risk of atherosclerosis. Our study showed that the crude extract and purified fractions significantly increased HDL-C/TC ratio, lowered LDL-C/HDL-C and log (TG/HDL-C) ratio when compared with the hyperlipidemic group. These results suggest anti-atherogenic potential of *Moringa oleifera* in reducing the development of coronary atherosclerosis, which is in agreement with earlier report.[27]

Interestingly too, treatment of experimental rats with methanol extract of *Moringa oleifera* significantly ($p < 0.05$) lowered serum levels of HMG-CoA reductase in comparison with hyperlipidemic control rats. Similarly, the four fractions (F1-F4) and standard drug show significant ($p < 0.05$) lowering effect in hyperlipidemic rats compared to normal control consistent with previous reports.[28,29] Mechanisms for maintenance of cholesterol homeostasis is exercised at the levels of HMG-Co-A reductase activity in its synthesis and/or cholesterol absorption from either dietary or transported source from the liver through biliary secretion. The HMG-Co-A/mevalonate ratio has a direct relationship to the activity HMG-Co-A reductase,[30] which was observed in our study consequent on the treatment with *Moringa oleifera* root extract and fractions evidenced by the increase in the ratio of measured parameters in the hyperlipidemic group.

CONCLUSION

The study demonstrated that methanolic root extract and column-fractions of extract of *Moringa oleifera* plant possess antihyperlipidemic activity evidenced by reduction in total cholesterol and triacylglyceride. Additionally, the observed lowering effect on atherogenic risk predictors are pointers to the efficacy of the plant in modulating lipid profile for healthy cardiovascular status. This work thus provides the scientific basis for the phytotherapeutic potential of methanolic root extract of *Moringa oleifera* and column-fractions that may be needed by scientists and researchers to fully maximise the potential of *Moringa* as a nutraceutical agent.

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