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Research Article

Lipid Altering Potential of *Moringa oleifera* Lam Seed Extract and Isolated Constituents In Wistar Rats

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ABSTRACT

The use of natural products have become popular because of their minimal adverse effect, cost effectiveness and accessibility. *Moringa oleifera*, is a valued plant that has found use ethnomedicinally and economically. The leaves have been investigated in various researches for different activities. This study is aimed at evaluating the seeds for its serum lipid profile altering activities in animal models. Five groups of five animals (120-150 g) per group were made obese by feeding with a high fat diet (HFD) for 10 weeks. The basal lipid profile was determined, and treatment commenced with methanol extracts of *Moringa oleifera* seed (MOSE) at 100 and 200 mg/kg b w for 6 weeks. Control groups were the Orlistat treated (50 mg/kg b w), untreated and normal diet groups. The antihyperlipidemic activity *in-vivo* and an enzyme anti lipase assay *in-vitro* were determined respectively. The characterisation of isolated compounds and derivative was by spectroscopic techniques. A significant decrease in Very Low Density Lipoprotein cholesterol (VLDLc) at $p< 0.01$ was observed across the five groups when compared with the standard Orlistat. A significant increase in High Density Lipoprotein cholesterol (HDLc) at $p< 0.01$ was observed in the group treated with 200 mg/kg MOSE. However, a dangerous significant increase in Athereogenic index (AI) was observed in the group treated with 100mg/kg MOSE The derived 4- acetyl benzylisothiocyanate-O –α-L rhamnopyranoside (2) from isolated 4-hydroxybenzylisothiocyanate-O-α-L rhamnopyranoside (1) revealed the highest activity of 99.17% at 0.5 mg/mL. The methanol extract of *Moringa oleifera* seed could alter lipid profile and a structure activity relationship was observed with respect to the isolated compound and its derivatized analogue.

Keywords: *Serum lipid profile, Antilipase, High Density Lipoprotein, Moringaceae, High fat diet, 4- acetyl benzylisothiocyanate-O –α-L rhamnopyranoside*

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INTRODUCTION

Hyperlipidemia is a heterogeneous group of disorders characterized by high level of lipids in the bloodstream. These lipids include cholesterol, cholesterol esters, phospholipids, and triglycerides. Alternatively, the disease refers to elevated levels of lipids and cholesterol in the blood, or the manifestations of different disorders of lipoprotein metabolism (dyslipidemia). Hyperlipidemia is associated with increased levels of atherogenic lipoproteins, which is a contributing factor and primary indicators of atherosclerosis, susceptibility to coronary heart disease (CHD) and cerebrovascular accidents as well as enhanced oxidative stress in hepatocytes, renal tissue and inflammatory reactions (Suanarunsawat *et al*., 2011).

Previous studies evaluated activities of *Moringa* plant where the aqueous extract of leaves particularly was reported for wound healing (Rathi *et al*., 2006) and antiurolithiasis activity (Karadi *et al*., 2006). The methanolic crude extract of *M. oleifera* shows antibacterial activity (Nantachit, 2006) while the bark extract has been shown to possess antifungal and anti-tubercular activity (Bhatnagar *et al*., 1961). The ethanolic and aqueous extract were found to be hypotensive (Faizi *et al*., 1998). The leaves have also been evaluated for its anti-nutrient (Jain *et al*., 2010; Mehta *et al*., 2003), antioxidant (Ryter *et al*., 2007; Limon-Pacheco and Gonsebatt, 2009) and anti-inflammatory (Rana *et al*., 2007) properties. Extracts

from *M. oleifera* leaves have also been shown to modulate humoral and cellular immunity in rats and mice (Gupta *et al*., 2010; Sudha *et al*., 2010). These properties have been more extensively studied with fruit and seed extracts (Cheenpracha *et al*., 2010) with little reference to its relation to hyperlipidemia.

Currently, no pharmacological treatment provides sustained weight loss with minimal adverse effects (Hasani-Ranjbar *et al*., 2013). Literature review has revealed that various herbal plants such as *Cleistopholis patens* (Udem *et al*, 2011), *Vernonia amygdalina* (Oluwatosin *et al*., 2008), *Parkia biglobosa (*Odetola *et al*., 2006), *Nigella sativa, Camellia sinensis*, Green Tea, and Black Chinese Tea (Bhishagratna *et al*., 1991) are used in the management of hyperlipidemia. Previous researches reported that *Moringa oleifera* leaves contain phytosterols such as β-sitosterol (Jain *et al*., 2010) which works by reducing intestinal uptake of dietary cholesterol (Lin *et al*., 2010). However, *Moringa oleifera* seeds have not been evaluated for its antihyperlipidemic potential through the reduction of intestinal uptake of dietary cholesterol or lipase inhibition. This study sought to investigate the antihyperlipidemic activity of the seeds and its isolated bioactive constituents through lipase inhibition mechanism.

MATERIALS AND METHODS

Plant materials

The seeds of *Moringa oleifera* were obtained from Ibadan metropolis, Nigeria in May 2013. The authentication was done by Mr T.K Odewo of the Forest Herbarium Ibadan where voucher specimen (FHI 110098) was deposited.

Extraction

Air-dried and blended *Moringa oleifera* seed (500 g) was extracted by cold maceration in 95 % methanol for 72 h. The extract was filtered and concentrated by the rotary evaporator to give a 40 g yield of the methanol extract.

In vitro anti lipase activity on extract and isolates

Inhibition of lipase was determined using a modified assay described by Smeltzer *et al*., 1992. A suspension containing 1% (v/v) of Glyceryl trioleate, and 1% (v/v) Tween 40 in 0.1 M phosphate buffer (pH 8) was prepared and emulsified. Assays were then initiated by adding 800 µL of the Glyceryl trioleate emulsion to 200 µL of porcine pancreatic lipase (0.5 gm pancreatin in 15 mL 0.1 M phosphate buffer at pH 8.0) and 200 µL of fraction (or 0.1 M Phosphate buffer, pH 8). The contents were mixed and the absorbance measured immediately at 450 nm and designated as T_0. The test tubes were incubated at 37°C for 30 mins and at the end of the incubation; the absorbance at 450 nm was recorded and designated as T_{30}.

The variation in absorbance was calculated for both control and the treatment and the % inhibition was calculated using the formula:

$$\% \text{ inhibition} = ([\Delta A450_{Control} - \Delta A450_{Extract}] / \Delta A450_{Control}) \times 100$$

Incubation at 37^0C, pH 8.9 for 1 h and Absorbance measured at 340 nm in triplicates.

Experimental animals

Twenty-five wistar rats (120-150 g) were purchased from the Vet Animal House, University of Ibadan. Nigeria and kept under standard environmental conditions.

They were grouped into 5 groups of 5 rats in a group.

Group 1: 100 mg/kg MOSE co-administered along with High fat diet (HFD)

Group 2: 200 mg/kg MOSE co-administered with High fat diet (HFD).

Group 3: Orlistat (standard) 50 mg/kg body weight was co-administered with (HFD).

Group 4: Untreated control rat group maintained on a high fat diet throughout the experiment.

Group 5: Untreated control rat group maintained on normal standard chow diet and water *ad libitum* throughout the experiment.

They were fasted for 14 h all night before the experiment. The animal experimental protocol was in conformity with the Ethics Committee Guidelines of the University of Ibadan with ethical approval number (UI-ACUREC/17/0105) as well as the US guidelines of internationally accepted principles for laboratory animal use and care. Animals were also treated in agreement with OECD guidelines for testing of chemicals (NIH publication #85-23, 1985).

In vivo lipase inhibitory assay of Extracts

In this study, a total of twenty-five rats were used and divided into five groups of 5 rats each. Groups 1-4 were fed a high fat diet continuously for 10 weeks before treatment with varying doses of MOSE. Group 5 rats were maintained on standard chow diet and water *ad libitum* for 16 weeks but were not treated.

Body weights were monitored weekly and animals were sacrificed at the end of the experiment. Capillary tubes were used to collect blood sample from the retro-orbital venous sinus of the rats into lithium heparinized bottles. The blood samples collected was centrifuged at 3000 rpm for 20 mins to obtain plasma separated for estimation of lipid profile including total cholesterol (TC), High density lipoprotein cholesterol(HDLc), Low density lipoprotein cholesterol (LDLc), Very low density lipoprotein cholesterol (VLDLc), Triglycerides (TG) and Atherogenic index (AI) by enzymatic colorimetric reactions methods using commercial kits (Randox).

Chromatographic purification of fraction

Moringa oleifera seed methanol extract (MOSE 40g) was subjected to vacuum liquid chromatography (VLC) using 1.5 L each of n-hexane, dichloromethane, ethylacetate and methanol in increasing polarities to elute. A total number of 100 fractions were collected and pooled (by TLC monitoring) into 4 pooled fractions 1-11(hexane), 12-17(dichloromethane), 18-38 (ethylacetate) and 39 (Aq/methanol) were subjected to *in-vitro* lipase inhibitory assay.

The most active VLC fraction 18-38 (ethylacetate) of MOSE **(9g)** was dissolved in methanol, the solvent was allowed to evaporate from the adsorbed material before introducing into a column (internal diameter 3.5 cm and 53 cm long) packed

with silica gel (230-400 mesh) in hexane. Elution was with acetone: hexane mixtures in increasing polarities.

Fractions (200 mLs each) were collected and separately concentrated to dryness *in vacuo* using a rotary evaporator. Fraction 11 (eluted with acetone 100%) afforded compound 1, a UV active and a golden yellow crystal, which was derivatized by acetylation to afford compound 2.

Statistical Analysis

Data were analysed using one way analysis of variance (ANOVA) followed by Turkeys multiple comparison pair and Student t test was used to determine the difference between the control and individual test groups. Results were presented as Mean ± SEM, and the differences were considered significant at $p<0.05$.

RESULTS

There was a significant increase in weight of the untreated groups when compared to the treated groups (Table 1). The *In- vitro* lipase inhibitory activity of the extrasct is shown in Table 2.

Effect of extract on lipid profile parameters in treated rats and control groups: A significant reduction in Total Cholesterol (TC) at $p<0.05$ was observed in the groups of rats treated with *Moringa* 100 mg/kg (87.92 mg/dL) and Orlistat 50 mg/kg (81.49 mg/dL) as compared to the HFD group (111.24 mg/dL). However, the reduction in TC in the other treatment group (*Moringa* 200 mg/kg) was not statistically significant (Fig 1).

The treatment groups (*Moringa* 100 mg/kg and 200 mg/kg) revealed a significant increase in High density lipoprotein cholesterol HDLc when compared to the Orlistat treated group (Fig 2). The increase was significant in the *Moringa* 200 mg/kg treated group at $p<0.01$ when compared to the three control groups suggesting a dose dependent activity.

Table 1

Determination of percentage mean weight increase of *Moringa oleifera* treated groups

Parameter	HFD+ MOSE 100 mg/kg	HFD+ MOSE 200 mg/kg	HFD+ Orlistat	HFD+ Tween 80	Normal diet
% Difference in weight	3.07	3.08	1.05	44.23	36.70

Key
HFD- High fat diet
MOSE- Moringa oleifera seed extract

Table 2.

In- vitro lipase inhibitory activity of plant extract compared to reference standard

Plant extract (0.005 g/mL)	Absorbance (Mean ±SEM)	% Inhibition
Moringa oleifera seed	0.445 ± 0.146	36.0
Orlistat	1.35 ± 0.004	57.0

$$\%\ Inhibition = A_{control} - A_{extract} / A_{control} \times 100$$

A non-significant decrease in low density lipoprotein cholesterol (LDLc) was observed in the *moringa* treated groups although to different degrees which suggests a dose dependent activity (fig 3). The Normal diet group, however revealed an expected reduction in LDLc level that is significantly different from the other two control groups (HFD and Orlistat 50 mg/kg).

The treatment groups *Moringa* 100 mg/kg and 200 mg/kg, significantly reduced the VLDLc levels at $p< 0.01$ (fig 4) when compared to Orlistat 50 mg/kg treatment group supporting the claim of *Moringa* seed antihyperlipidemic activity .

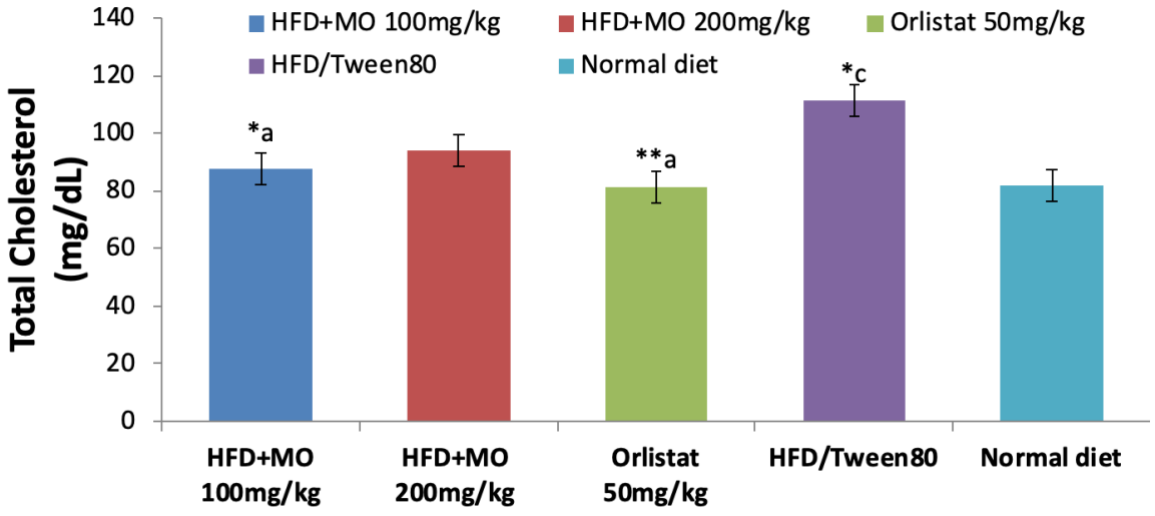

Figure 1.
Effect of Moringa seed extract on Total Cholesterol

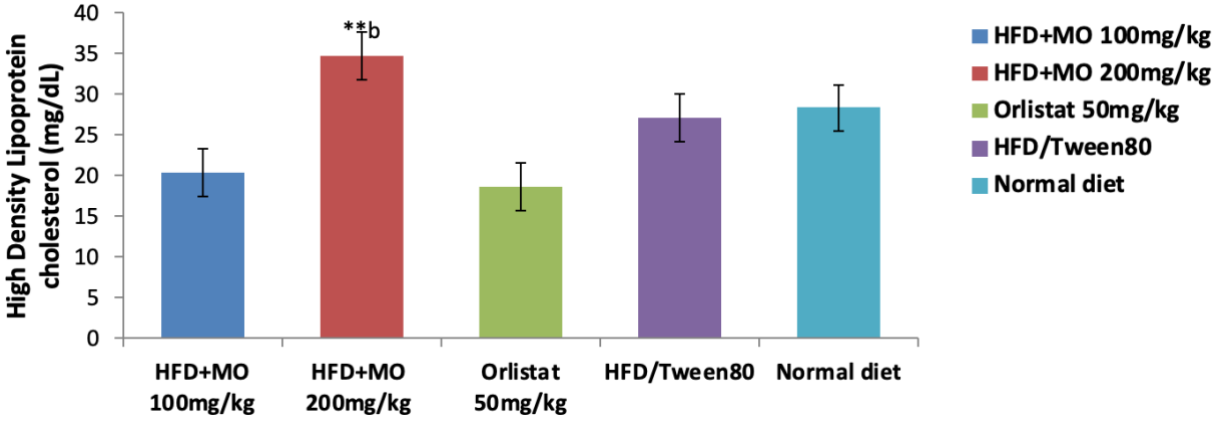

Figure 2.
Effect of *Moringa* seed extract on High density lipoprotein cholesterol (HDLc)

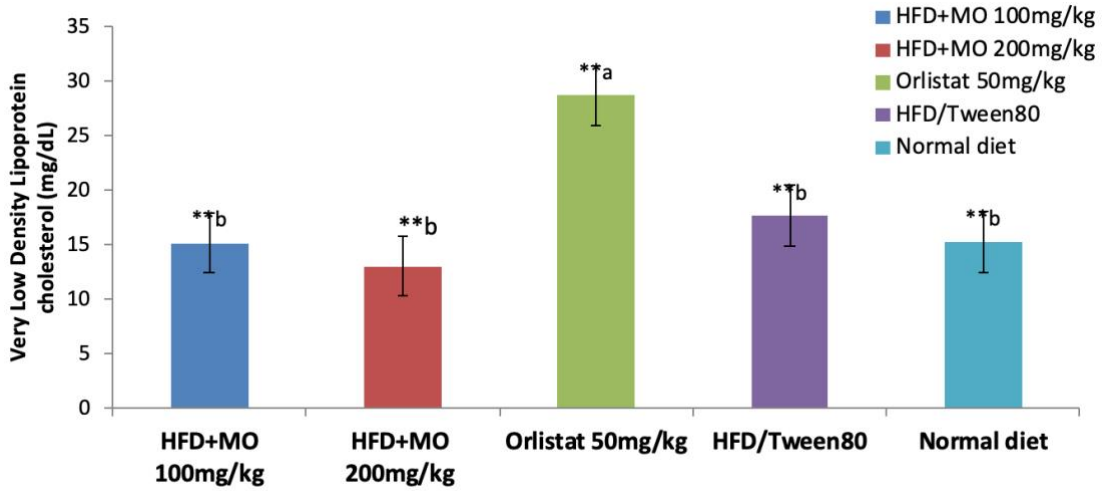

Figure 3.
Effect of moringa seed extract on low density lipoprotein cholesterol (LDLc)

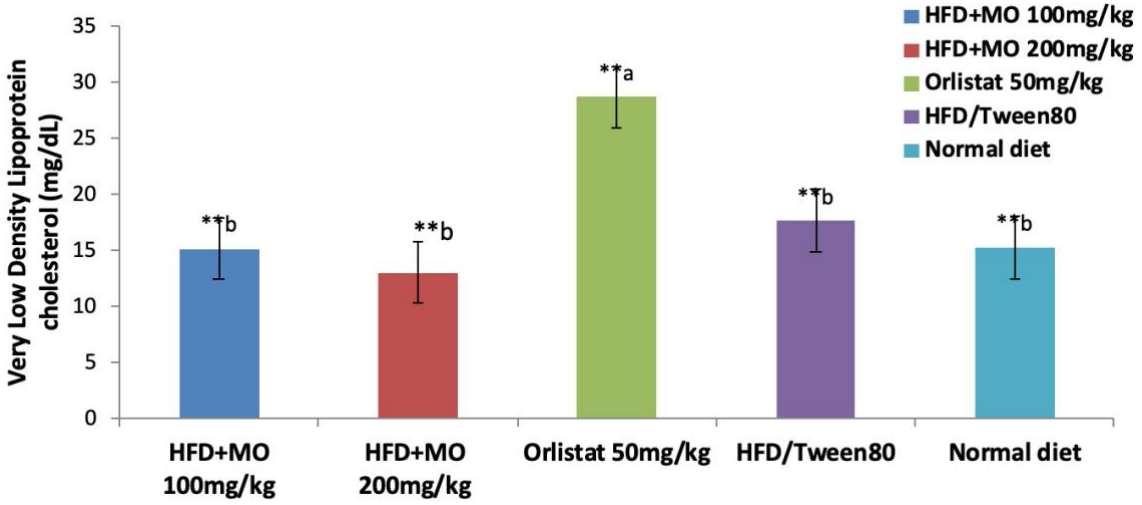

Figure 4.
Effect of *Moringa* seed extract on Very low density lipoprotein cholesterols (VLDLc)

According to lipid hypothesis, 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 CHD (Idemudia *et al.*, 2013). The increase observed in atherogenic index in the *moringa* 100 mg/kg treatment group was statistically significant at $p<0.05$ when compared to the normal diet group (fig 5). This is a pointer to a risk that should

be double checked despite all some other parameters revealing significant reduction.

It was also observed that the triglyceride level (TG) was significantly higher in the orlistat treated group (143.20 mg/dL) (fig 6) when compared to other control groups HFD (88.82 mg/dL) and normal diet (76.20 mg/dL) suggesting that TG was not hydrolysed as lipase which normally should hydrolyze Triglycerides (TG) to free fatty acids and stored as fat was effectively inhibited. However, the TG levels in *Moringa* treated groups at 100 mg/kg (75.65 mg/dL) and 200 mg/kg (65.08 mg/dL) were not significantly different from the control groups which could be due to the differences in the mechanism of action of moringa extracts and that of the standard drug.

Isolation of compounds from Moringa oleifera

Compound 1: $_1$H-NMR spectral data (CD_3OD, 500 MHz) δ_{ppm}; 1.20 (d, 3H, H-6´), 3.30 (s, 2H, H-7), 3.45(t, 1H, H-5´), 3.65(m, 1-OH, H-4´), 3.80(t, 1-OH, H- 3´), 4.00 (s, 1-OH, H- 2´), 5.40 (s, 1H, H- 1´), 7.10 (dd, 2H, H-3, H-5), 7.30 (dd, 2H, H-2, H-6).
ESI-MS m/z (% relative intensity) 311 (M_+, 100). Accurate mass measurement; Found:311.0511, $C_{14}H_{17}NO_5S$ requires 311.3534.

Compound 2: $_1$H-NMR spectral data (CD_3OD, 500 MHz) δ_{ppm}; 0.9 (m, 3H, H-6´), 1.20 (m, 2H, H-7), 2.1(m, 1H, H-5´), 3.7(s, 2-$OCOCH_3$, H-4´), 3.95(s, 1-OH, H- 3´), 5.15 (d, 1-OH, H- 2´), 5.50 (m, 2H, H- 1´), 7.10 (dd, 2H, H-3, H-5), 7.30 (dd, 2H, H-2, H-6).
ESI-MS m/z (% relative intensity) 438 [M_++H], accurate mass measurement; Found:437.1439, $C_{20}H_{23}NO_8S$ requires 437.1469.

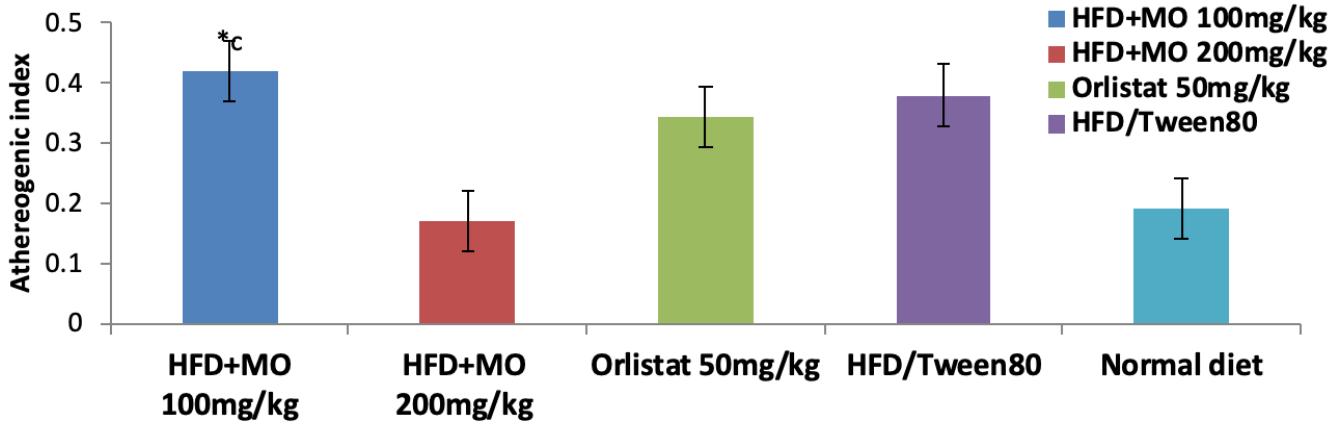

Figure 5.
Effect of *Moringa* seed extract on Atherogenic index

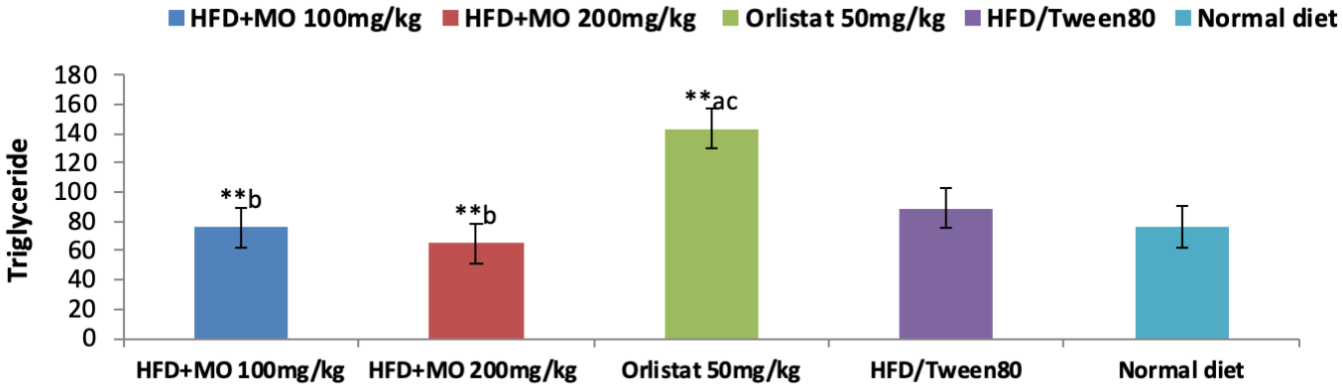

Figure 6
Effect of *Moringa* seed extract on Triglycerides (TG)
*p<0.05, **p<0.01; MO = Moringa seed extract; a= compared with HFD; b = compared with Orlistat; c = compared with Normal diet*

DISCUSSION

Besides the use of synthetic orthodox hypolipidemic drugs (Eliot and Jamali, 1999; Ochani and D'mello, 2009), numerous plant extracts have been previously demonstrated to possess anti-hyperlipidemic effects in animal models in vivo (Al-Dosari, 2011; Raghuveer *et al*., 2011; Suanarunsawat *et al*., 2011; Giri *et al*., 2012; Durendic-Brenesel *et al*., 2013; Kaur and Meena, 2013). The findings in this study are supported by several previous studies in which the therapeutic potentials of the plant extracts have been attributed to their phytochemical and nutritive contents. Accordingly, phytochemical studies by previous researches have revealed the presence of bioactive principles in *Moringa oleifera*

involved in readjustment of lipid metabolism to the advantage of experimental animals (Kumar *et al*., 2012).

Table 3:
$_{13}$CNMR of compound 1 in comparison with published data

Carbon number	Cpd 1	Reported data(Faizi *et al*., 1994)
1	156.41	156.03
2,6	117.05	116.65
3,5	129.31	129.23
4	124.79	132.64
7	21.7	22.9
8	118.8	123.72
1´	98.82	97.96
2´	72.78	70.9
3´	71.20	71.7
4´	70.98	73.5
5´	69.69	68.8
6´	17.01	17.5

Table 4:
$_{13}$CNMR of compound 2 in comparison with published data

Carbon number	Cpd 2	Reported data (Consolacion *et al*., 2012)
1	155.52	156.65
2, 6	129.24	129.25
3, 5	116.83	116.87
4	124.00	131.70
7	22.88	22.91
8	116.95	123.70
1´	95.60	97.19
2´	69.56	70.65
3´	70.83	70.13
4´	68.80	75.49
5´	67.25	66.36
6´	17.40	17.46
OAc	170.06	172.01
OAc	169.96	172.01
OAc	20.88	21.05

Table 5:
In vitro antilipase activity of isolated compounds

S/No	Bioactive compounds	% lipase inhibition at 0.25 mg/mL	%lipase inhibition at 0.5 mg/mL
1	4-hydroxybenylisothiocyanate-O-α-L- rhamnopyranoside (1)	**	**
2	4-acetylbenzylisothiocyanate-O-α-L- rhamnopyranoside (2)	94.21	99.17
3	Orlistat® (Control)	67.98	54.00

**** No activity**

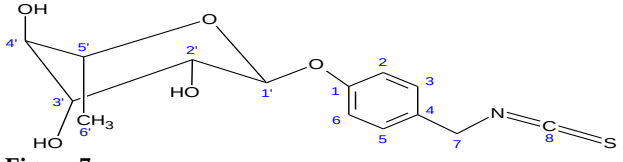

Figure 7:
4- hydroxybenzylisothiocyanate-O-α-L-rhamnopyranoside (1)

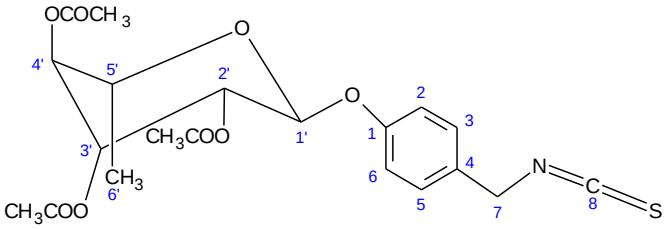

Figure 8:
4- acetyl benzylisothiocyanate-O –α-L rhamnopyranoside (2)

The phytochemical screening of the crude methanolic extract of *Moringa oleifera* seed has been reported by Ogunjinmi *et al*. 2012, to contain alkaloids, flavonoids, saponins, and steroids, which confer several biological properties. Saponins have been demonstrated to possess hypocholesterolemic property and effectiveness in the control of high blood pressure associated with hyperlipidemia (Oakenfull and Sidhu, 1990)

A general increase in the mean weight of the experimental animals was observed throughout the experiment, especially the High Fat Diet (HFD) groups indicating the expected effect of the High Fat Diet on the animals. However, the percentage weight increase in the *Moringa* treated groups is comparable to the Orlistat treated group suggesting some management in the weight increase when compared to the untreated group after the experiment. The untreated group on normal diet however, maintained a steady minimum increase in weight throughout the experiment as expected as shown in Table 1. A comparable percentage lipase inhibition was observed in the *Moringa oleifera* seed when compared to Orlistat, the reference standard as shown in Table 2.

The disparity in the significant reduction observed in Total Cholesterol in the two treated groups as compared to the HFD group suggests that the TC lowering activity could be dose dependent. This finding is supported by previous researches that established phytosterols, specifically β-sitosterol to possess blood cholesterol-lowering activity by impeding intestinal cholesterol absorption routes and has inhibitory action on hepatic cholesterol biosynthetic pathways (Duester, 2001; Mayes and Botham, 2003). Expectedly, the intake of plant materials rich in β-sitosterol results in reduction in total serum cholesterol levels in the experimental animals (Moghadasian and Frohlich, 1999; Al-Dosari, 2011).

The observed elevation of HDLc in rats administered *Moringa oleifera* however, indicates its beneficial effect against cardiovascular disease as HDLc is secreted by the liver and intestinal cells where it removes excess cholesterol from circulation and carries it back to the liver where degradation or conversion into bile acids takes place (Rahilly, 2011).

A non-significant decrease in low density lipoprotein cholesterol (LDLc) observed in the *moringa* treated groups suggests a dose dependent activity. The Normal diet group, however revealed an expected reduction in LDLc level that is significantly different from the other two control groups (HFD and Orlistat 50 mg/kg). This corroborates the fact that consumption of a HFD will result in a high LDLc level and accumulating evidence over the last decades had linked elevated total cholesterol and low-density lipoprotein-

cholesterol (LDLc) levels and reduced HDL levels to the development of Coronary heart disease (CHD) (Segal *et al*., 1972).

Very low density lipoprotein cholesterols (VLDLc) are triglyceride-rich lipoproteins, but contains 10–15 % of the total serum cholesterol (Howell *et al*., 1997). The major apolipoproteins of VLDLc are apo B-100, apo Cs (C-I, C-II, and C-III), and apo E. VLDLc are produced by the liver and are precursors of LDLc; some forms of VLDLc, particularly VLDLc remnants, appear to promote atherosclerosis, similar to LDL (Howell *et al*., 1997). The treatment groups *Moringa* 100 mg/kg and 200 mg/kg, significantly reduced the VLDLc levels at $p< 0.01$ when compared to Orlistat 50 mg/kg treatment group supporting the claim of *Moringa* seed antihyperlipidemic activity.

The overall result points to the fact that *Moringa oleifera* seed extract possess a dose independent relationship all through the study. This could be attributable to some factors which could include experimental errors as well as biological variation in response of animals tested. Of note is the observation in the groups treated with both doses (100 and 200 mg/kg) revealing a significant decrease at $p<0.01$ in TG and VLDLc levels when compared to the control group on orlistat. The group treated with 100 mg/kg MOSE showed a significant decrease of AI at $p<0.05$ when compared to the control group on normal diet, while the group treated with 200 mg/kg of MOSE showed a significant decrease of HDL at $p< 0.01$ when compared to all the control groups. However, the two treated groups showed no significant change in LDLc when compared to controls. There was an observation of a high atherogenic index which is used as a diagnostic indicator when the other risk parameters appear normal (Nwagha and Igweh, 2005). These observations may be due to pharmacokinetics of gastrointestinal absorption of extract which may also change with increased dose of extract administered leading to a fall in the absorption rate which in turn affects the desired effect. At other times doubling the dose of extract administered may lead to a decrease in effect other than doubling the tissue concentration of a particular sample under testing (Veng-Pedersen et al., 1991).

Compound 1 have also been isolated from *Moringa stenopetala* (Faizi *et al*., 1994) and *Noccaea caerulenscens* (Rob *et al*., 2014). It is the rhamnose derivative of sinalbin (4-hydroxybenzyl glucosinate) (Amaglo *et al*., 2010; Bennette *et al*., 2003). The ^{13}CNMR spectra (Table 3) indicated that there are 14 carbon atoms present. The signal identified as due to a methyl carbon and the most shielded carbon atom (δ 17.0) in the ^{13}CNMR is assignable to C-5′ located on the rhamnose sugar. This is closely followed by a methylene (δ 21.7).The ^{13}CNMR spectra also revealed the presence of five methine carbons assignable to C-1′, C-2′, C-3′, C-4′ and C-5′ all located on the rhamnose moeity. The methine carbon assigned as C-1′ (δ 98.82) is an anomeric carbon linking the rhamnose to the benzene ring. There are four other methine carbons appearing on the benzene ring and are assigned as C-2 and C-6(δ 129.31), C-3 and C-5 (δ 117.05). The most de- shielded is assigned to C-2 and C-6 δ 129.31 due to the proximity to the C-1 which directly links the oxygen atom connecting the rhamnose. The spectra also revealed the presence of three quaternary carbons appearing at δ 156.41, 124.79 and 118.85 assignable to C-1, C-4 and C-8.

These assignments were further supported by comparison with reported data (Faizi *et al*., 1994). The presence of oxygenated groups at δ 3.3, 3.45, 3.65 and 3.8 in the ^{1}HNMR is further confirmed in the ^{13}CNMR with signals appearing at δ 117.05 assignable to C-3 and C-5, δ 118.85 ppm assignable to C-8, δ 124.79 ppm assignable to C-4 and δ 129.31 assignable to C-2 and C-6. The 500 MHz ^{1}HNMR spectra signals in CD_3OD revealed the presence of a para substituted aromatic benzene ring in the molecule at 7.1 (d, J= 8.7Hz) and 7.3 (d, J =8.6Hz). Other protons include 3 protons doublet at signal δ 1.2 (J=6.2 Hz) which is due to the methyl group on the rhamnose.

Compound (2) was obtained as a yellow substance by the acetylation of compound 1. It was identified as 4- acetyl benzylisothiocyanate-O –α-L rhamnopyranoside (2) (Fig viii) with a melting point of 236 ^{o}C (Lit m.pt 234^{o}C) (Consolacion *et al*., 2012) and a molecular formula of $C_{20}H_{23}NO_8S$. The accurate mass found was 437.1439 while the calculated mass is 437.1469. The ^{13}C NMR (Table 4) confirms the presence of equivalent carbon atoms (δ 20.76 and 29.67) shifted more downfield than normal as in the parent compound assignable to the carbon atoms of the acetate attached to (C-2′, C-3′ and C-4′). The presence of 1,4 disubstituted benzene ring was also confirmed by resonances at δ 116.8 (C-3 and C-5) and 129.2(C-2 and C-6). The presence of a carbonyl group of the acetate was observed at δ 170.064ppm assignable carbons attached to C- 2′, C- 3′ and C-4′.

The ^{13}CNMR reveals the presence of three quatenary carbons resonating at δ 155.52, 124.00 and 116.95 assignable to C-1, C-4 and C-8 with the most de-shielded resonating at δ 155.52. This suggests that the position of the anomeric carbon (δ 95.6 assigned C-1′) of the glycoside was attached to the benzene ring through this carbon. The least de-shielded quatenary carbon is that attached to the sulphur and Nitrogen because of the pulling effect on the carbon which leaves it electron deficient.

The oxygenated methine protons were assigned to resonances at δ 70.8, 69.6, 68.8 and 67.2 assigned as C-5′, C-4′, C-3′and C-2′. The methylene carbon resonance appeared at δ 17.4 assignable to C-6′, the acetate signals appear at δ 20.88 assignable to the carbonyl attached to C-3′ and δ 170.06 and 169.96 assignable to the carbonyl attached to C-2′ and C-4′ and are more de-shielded due to their proximity to the oxygen atom linking the rhamnose to the benzene ring. The ^{13}C NMR spectra indicated that there are 20 carbon atoms present as methyls, (OMe) and (CMe). There are three quaternary carbons at δ 155.5, 124.0 and 116.95 assignable to C-1, C-4 and C-8. This was further confirmed by comparing data with already reported data (Consolacion *et al*., 2012).

A signal at 1.3 (d) ppm of the ^{1}HNMR data was assigned to the CH_3 group of rhamnose (C-6′). The 500MHz ^{1}HNMR spectra signals in CD_3OD revealed the presence of a para substituted benzene ring in the molecule at 7.1 (d, J =8.7 Hz) and 7.3 (d, J= 8.6 Hz). The proton is shifted more downfield than normal and appears as a doublet as it is coupling with the C-2′ proton (δ 170.06 ppm).

The *in-vitro* lipase inhibitory evaluation of isolates as shown in Table 5 revealed that Compound 2, resulting from

the derivatization of the naturally occurring compound 1 gave the highest lipase inhibitory activity of 99.17% at 0.5 mg/mL and 94.21% at 0.25 mg/mL when compared to the Orlistat® control at both concentrations (54% at 0.5 mg/mL and 67.98% 0.25 mg/mL). This suggests a structure activity relationship between the active derivatized compound 2 and the isolated compound 1 which showed no activity. The derivatized compound (2) is however, being reported for antihyperlipidemic activity in this study thereby validating the hypolipidemic claim in ethnomedicine.

In conclusion, results here are comparable to previous research in which rats with induced hyperlipidemia were used where aqueous leaf extract of *Moringa oleifera* demonstrated an ameliorative effect on lipid profile (Pratik *et al*., 2013) and is attributed to its potential to control the mechanisms involved in lipids elimination from the body (Naznin *et al*., 2008). This study revealed that *Moringa oleifera* at lower doses of 100 mg/kg and 200 mg/kg, were able to alter the serum lipid profile although may be ameliorative and at the same time aggravative and to different degrees. Summarily, the use of the seed of *Moringa oleifera* should be with some caution as supported by claims from previous researches (Ajayi et al., 2016). It may however, be a potent template or lead for future lipid altering agents.

Conflict of interest

The authors declare no conflict of interest

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