

Original Research Article

COMPARATIVE HYPOLIPIDEMIC EVALUATION OF *AFRAMOMUM MELEGUETA* SEEDS AND *MORINGA OLEIFERA* LEAVES

ABSTRACT

Aims: The study was carried out to compare the lipid lowering effects of both crude drugs as well as deducing the extracts with the best lipid lowering property; and the fractions.

Study Design: The research was conducted with an experimental design solely based on laboratory trials which involved the use of ninety-six (96) male albino wistar rats to compare the hypolipidemic effects of both crude drugs and respective fractions.

Place and Duration of Study: Department of Pharmacognosy Laboratory, Faculty of Pharmacy, Delta State University, Abraka, Nigeria and Department of Pharmacology and Toxicology Laboratory, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu, Anambra State, Nigeria. The research was carried out from March to August, 2021.

Methodology: Ethanol extracts of both plants were prepared using soxhlet extraction. Each extract was then subjected to VLC fractionation using four solvents: n-hexane, chloroform, ethyl acetate and methanol. The fractions were bulked together after conducting thin layer chromatographic procedures and each extract was bulked into four fractions. The acute toxicity studies (LD₅₀) of both extracts were determined in the rats using Lorke's method. The crude extracts were screened for the presence and quantity of phytoconstituents using standard methods. The antilipidemic study was carried out using sixty-eight (68) rats randomized into seventeen (17) groups of four animals each. Lipid profile was determined using spectrophotometer. Liver function tests and histology was also carried out using standard procedures.

Results: Administration of various treatments (both crude extracts and fractions) evoked a significant ($p < 0.05$) reduction of TC, TG, and LDL-C as well as significant ($p < 0.05$) elevation of HDL-C when compared with the negative control. With a percentage serum lipid reduction of 45.11% TC, 48.23% TG, 63.39% LDL-C and 174.69% elevation of HDL-C, the group treated with the combination of 500 mg/kg *Aframomum melegueta* and 500 mg/kg *Moringa oleifera* produced the best hypolipidemic effect. This is closely followed by fraction MO4. Comparatively, *Moringa oleifera* extracts exerts a better antilipidemic effect than *Aframomum melegueta* seed extract. The liver function test showed that both plants has no toxic effect on the liver cells at doses of 250 mg/kg and 500 mg/kg, hence confirming the hepatoprotective effect of both crude drugs at the doses administered.

Conclusion: In conclusion, results from this study suggests that ethanol extract of *Moringa oleifera* leaves is more effective than ethanol extract of *Aframomum melegueta* seeds as a

hypolipidemic agent, however, combination of both crude drugs as a lipid lowering agent has proved to be more effective and reliable when compared to each crude drug administered independently.

Keywords: *Aframomum melegueta*, *Moringa oleifera*, hepatoprotective, hyperlipidemia, phytoconstituents, hypolipidemic, antilipidemic, extraction.

Abbreviations: TC (Total cholesterol), LDL-C (Low density Lipoprotein Cholesterol), HDL-C (High Density Lipoprotein Cholesterol), TG (Triglycerides).

INTRODUCTION

The practice of using plants to treat ailments dates back to the earliest period of known human history. The universal role of plants in the treatment of diseases is exemplified by their role in all sectors of medical practice, irrespective of the underlying philosophical premise (Evans, 2008). In recent times, healthcare providers are concerned about hyperlipidemia because of the well established association between lipid concentration and the risk of cardiovascular diseases, one of the leading cause of death in the world. In Nigeria, hyperlipidemia is highly prevalent in all geopolitical zones, with the consistent pattern being low HDL- cholesterol and high LDL- cholesterol (Oguejiofor *et al.*, 2012). Overall, the prevalence of dyslipidemia according to a study conducted by Oguejiofor *et al.*, (2012) revealed that it is common among 60% of healthy Nigerians and 89% of diabetic Nigerians.

In the traditional set up, vast majority of medicinal plants have been a good source for initiating lipid lowering properties in human and this has been made true by the increasing number of medicinal plants based supplements available for the treatment of abdominal obesity. Apparently, one can easily deduce that the increased availability of various herbal hypolipidemic supplements stems from the high cost of obtaining statins and fibrates used in tackling hyperlipidemic cases. The use of these orthodox hypolipidemic agents is very expensive and associated with side effects ranging from flatulence, constipation and dyspepsia. The use of herbs as medicine has played an important role in nearly every part of the world. Several herbs help to lower blood level of cholesterol and triglycerides.

Most cases of hyperlipidemia are asymptomatic and as such people don't take it seriously until they come up with serious cardiovascular disorders such as heart attack, stroke etc. Research on management of hyperlipidemia is limited in this part of the world, hence the need to establish a very efficacious means of curbing this menace without causing any form of adverse drug reaction or toxicity. The significance of this study is also centered on the need to establish an easily accessible natural remedy for the prophylaxis of several cardiovascular diseases. Since recent studies on *Moringa oleifera* leaves and *Aframomum melegueta* seeds have shown significant lipid lowering effect (Morakinyo *et al.*, 2019, Aborhyem *et al.*, 2016) there is an

urgent need to compare the lipid lowering properties of both crude drugs and in turn establish a possible additive, synergistic or antagonistic effect when both crude drugs are used simultaneously.

MATERIALS AND METHODS

Equipments and Apparatuses

The following equipments and apparatuses were utilized during the course of this research work; Water bath (Techmel & Techmel, Texas, USA), Micropipette (Finnipipette® Labsystems, Finland), Spectrophotometer (B. Bran Scientific & Instrument Company, England), Rotary evaporator, Analytical weighing balance (Metler H30, Switzerland), test tubes, test tube rack, round bottom flasks, beakers, measuring cylinders, TLC plates, capillary tubes, TLC spray equipment, sinta glass, soxhlet apparatus, desiccator, hot air oven(Bionics Scientifics,India) muffle furnace (Meditech,India), microscope (Olympus Optical, Japan), Whatman no 42 filter paper.

Chemicals, Reagents and Drugs

The following chemicals and drugs were used during this research work: Methanol, Ethyl-acetate, Chloroform, N-hexane, Ethanol, Sulphuric acid, Hydrochloric acid (JHD, Guangdong Schi- Tech. Ltd China), Atorvastatin (TEVA, UK). Total cholesterol, Triglyceride and High density lipoprotein cholesterol kits (Randox Laboratories Limited, Country Atrium, United Kingdom); Silica gel (60-200 mesh).

Collection and Authentication of Plant Materials

The seeds of *Aframomum melegueta* were obtained from the spice market in Effurun, Uvwie Local Government Area of Delta State. The leaves of *Moringa oleifera* were obtained from the *Moringa oleifera* tree in Abraka, Ethiope East Local Government Area of Delta State. Both were obtained in March, 2021. The leaves collection was conveniently done with the aid of a sharp knife. After collection of both plant materials, they were authenticated by Dr. Henry .A. Akinnibosun (FLS, MRSB; London), a taxonomist and academic staff in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State. Voucher numbers (UBH-M340) and (UBH-A471) were given to the *Moringa oleifera* leaves and *Aframomum melegueta* seeds respectively.

Preparation of Plant Extracts

After collecting the both plant parts (the seeds of *Aframomum melegueta* and the leaves of *Moringa oleifera*), they were both dried under room temperature for about two weeks. After drying, both plant parts were subjected to pulverization using a blender. The blender was properly cleaned to avoid influx of any adulterant or foreign particles. After blending, the powdered particles were properly stored in a refrigerator until they are needed for extraction. Both plant materials were both extracted using soxhlet extraction method. Ethanol was used as the solvent of extraction. After extraction, both extracts were carefully concentrated to dryness, with a rotary evaporator to remove the extracting solvent (ethanol). These extracts were preserved in a refrigerator at a temperature of four (4) degrees centigrade.

Phytochemical Analysis

The phytochemical analytical techniques carried out on both plant extracts were both qualitative and quantitative. Each plant extract was carefully assessed for the presence of various secondary metabolites. The presence or absence of alkaloids, tannins, saponins, glycosides, steroids, quinines, flavonoids, quinones, anthraquinones, and terpenoids were evaluated using the method of Dhivya and Manimegalai (2013).

Some secondary metabolites present in both *A. melegueta* seeds and *M. oleifera* leaves were quantified using diverse standard procedures viz: Alkaloids (Harborne, 1973), Phenols (Hagermann *et al.*, 2000), Flavonoids (Kumaran and karunakaran, 2006), Tannin (Van-Burden and Robinson, 1981), Saponin (Obdoni and Ochuko, 2001), Cardiac glycosides (El-olemy *et al.*, 1994).

Fractionation of Plant Extracts via Vacuum Liquid Chromatography

Each of the plant extract (Ethanol extract of both *Aframomum melegueta* seeds and *Moringa oleifera* leaves) was fractionated using vacuum liquid chromatographic technique. 30 g of each plant extract was carefully weighed with a top loading balance, then 60 g of silica gel 60-200 mesh was measured into a compounding mortar, then a pestle was used to mix the extract and the silica gel to get a homogenous powdered mixture, after this process, the mixture was covered to prevent influx of foreign particles and then allowed to dry for about 24 hours. After 24 hours, about 30 g of silica gel G was measured and emptied into the sinta glass, then Whatman no 42 filter paper was placed on it, then the mixture of the extract and the silica gel 60-200 mesh was added to the sinta glass, this was then lined with another filter paper. The sinta glass is connected to the round bottom flask serving as the receiving flask. The above set up is connected to a vacuum pump with the aid of a long flexible hollow tube. Solvent mixture was prepared using hexane, chloroform, ethyl acetate and methanol in order of increasing polarity. Thirteen (13) solvent systems were prepared accordingly; these solvents can be outlined as follows:

1). 300 ml hexane 2). 225 ml hexane + 75 ml chloroform 3). 150 ml hexane + 150 ml chloroform 4). 75 ml hexane + 225 ml chloroform 5). 300 ml chloroform 6). 225 ml chloroform + 75 ml ethyl acetate 7). 150 ml chloroform + 150 ml ethyl acetate 8). 75 ml chloroform + 225 ml ethyl acetate 9). 300 ml ethyl acetate 10). 225 ml ethyl acetate + 75 ml methanol 11). 150 ml ethyl acetate + 150 ml methanol 12). 75 ml ethyl acetate + 225 ml methanol 13). 300 ml methanol.

After preparing the above solvent mixtures, they were properly covered to avoid evaporation. The extract in the sinta glass was subjected to defatting by gradually adding the 300 ml hexane and the vacuum pump switched on to apply negative pressure to the set up, once the vacuum pump starts supplying negative pressure to the set up, the first fraction (mainly fat components) are released into the receiving flask. This will continue until the solvent applied has completely drained. The vacuum pump is then switched off and the set up disengaged to collect the fraction in the receiving flask. This process is repeated for each of the solvent system until the thirteenth fraction has been utilized and all the thirteen fractions collected for each plant extract. The twenty six (26) fractions from both plant extracts were covered with aluminum foil until they are needed for chromatographic profiling.

Thin layer chromatography procedure was carried out on thirteen (13) fractions of *Moringa oleifera* and thirteen fractions of *A. melegueta*. At the end of the procedure, the similarity and difference in colour observed for each fractions formed the basis for bulking up the fractionated extracts as the fractions having similar colour was bulked together because they are likely to contain similar components. The *Aframomum melegueta* fractions were bulked as follows; fraction AM1, fraction AM2, fraction AM3 and fraction AM4. The *Moringa oleifera* fractions were bulked as follows; fraction MO1, fraction MO2, fraction MO3 and fraction MO4. To determine the number of component present in the bulked up fractions, the aforementioned thin layer chromatography procedures was also carried out. Finally, the bulked up fractions was allowed to concentrate to dryness.

Experimental Animal Handling

96 Wistar albino rats weighing between 120-150g were supplied from the Animal house of the Faculty of Pharmaceutical sciences, University of Nigeria, Nsukka. They were placed under standard conditions ($23^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and 12h light-dark cycle) in the Animal house of the Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Agulu. These animals were allowed to acclimatize for two weeks. During this period, all the rats were fed with normal feeds and water. All animals were cared for in compliance with the internationally accepted guide for the care and use of laboratory animals, published by the US National Institutes of Health (NIH Publication, Revised in 2010).

Induction of Hyperlipidemia

Ninety-one (91) Wistar albino rats were subjected to hyperlipidemia. Hyperlipidemia was induced by feeding the animals on high fat diet composed of 35% Vital grower feed, 10% egg yolk, 5% crayfish, 35% Palm kernel cake (P.K.C) and 15% Margarine for four weeks (Pankaj *et al.*, 2016). The remaining five (5) rats serving as the control was given normal standard diet devoid of fat feeds. After four weeks of successfully inducing hyperlipidemia, the rats were allowed to fast overnight for 10-12hrs before collecting their blood samples. Blood samples were collected intraperitoneally from all the rats, the serum obtained using a centrifuge for 10 minutes at a speed of 3000 rpm and stored in the refrigerator at a temperature of -20⁰c until they are needed for lipid profiling. The level of hyperlipidemia attained was measured using Randox commercial assay kits (Ezeigho, 2016). Blood lipid levels including TC, TG, HDL and LDL was measured accordingly.

Acute Toxicity Testing

The method used to determine the toxicity was the method described by Lorke (1983). A total of twenty-six (26) rats were used for this particular study involving two different extracts (crude ethanol extract of *Aframomum melegueta* seeds *Moringa oleifera* leaves. This procedure involves two different phases. In phase one, the Wistar albino rats were weighed and sorted into three groups of three (3) rats each. In phase two, the rats are sorted into four groups of one rat each, such that their average weights were approximately equal. The rats were kept in cages in the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu, Anambra state. They were allowed to acclimatize for a period of one (1) week before commencement of the study. In phase one, each group of animals were administered with different doses (10, 100 and 1000 mg/kg) of each the test substance (*Aframomum melegueta* seeds and *Moringa oleifera* leaves). The animals were placed under observation for 24 hours to monitor their behavior and also confirm if any mortality will occur. The absence of any form of toxicity or mortality after 24hrs led to progression to the next phase, in this phase, 1600, 2900 and 5000 mg/kg doses of each the test substance were administered to the animal in the three groups and then observed for 24 hours for behavior as well as mortality.

Then the LD₅₀ was calculated using the formula below:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D₀ = Highest dose that gave no mortality,

D₁₀₀ = Lowest dose that produced mortality.

Dose Determination Based On LD₅₀

The dose of each extract to be administered to the rats was ascertained by calculating 1/10th and 1/20th of the calculated LD₅₀, as described by Neharkar and Galkwad (2011).

Acute Antilipidemic Study

The hypolipidemic study was conducted with sixty-eight (68) male albino Wistar rats. This study commenced 24hrs after the acute toxicity test. The rats were randomly divided into seventeen (17) groups of four (4) animals per group. Each group received different concentrations or fractions of each plant extract as well as normal rat feeds and water. Group 1 served as the normal control group, the animals in this group was not induced with high fat feed; they were given normal rat feeds and water. No treatment was also initiated. Group 2 served as the negative control, hence the animals in this group did not receive any treatment after induction of hyperlipidemia; they were given normal rat feeds. Group 3 served as the positive control, hence received a dose of 1.2 mg/kg of Simvastatin. Group 4 received 250 mg/kg of *A. melegueta* crude extract. Group 5 received 500 mg/kg of *A. melegueta* crude extract. Group 6 received 250 mg/kg of *M. oleifera* crude extract. Group 7 received 500 mg/kg *M. oleifera* crude extract. Group 8 received equal ratio of 250 mg/kg *M. oleifera* and 250 mg/kg *A. melegueta*. Group 9 received equal ratio of 500 mg/kg *M. oleifera* and 500 mg/kg *A. melegueta*. Group 10 received 500 mg/kg fraction AM1. Group 11 received 500 mg/kg fraction AM2. Group 12 received 500 mg/kg fraction AM3. Group 13 received 500 mg/kg fraction AM4. Group 14 received 500 mg/kg fraction MO1. Group 15 received 500 mg/kg fraction MO2. Group 16 received 500 mg/kg fraction MO3. The last group, Group 17 received 500 mg/kg fraction MO4. The extracts and control drug were administered orally once daily for two weeks.

Determination of Serum Lipid Profile

At the end of the 14th day treatment, the animals were allowed to fast overnight after which the animals were sacrificed using chloroform anaesthesia and blood samples were collected from the tail vein for the determination of the serum lipid profile such as total cholesterol, triglyceride, high density lipoprotein cholesterol and low density lipoprotein cholesterol (Akah and Okolo, 2011; Salim and Dikko 2016; Mohammed *et al.*, 2016) using Randox commercial assay kit.

Determination of liver function enzymes

On the 14th day, blood samples were also randomly collected from the retro-orbital plexus of the rats in each group for the determination of serum liver function enzymes. Blood samples were collected from rats across the seventeen groups. All the blood samples in the different sample containers were allowed to clot for 30 minutes and then subjected to the process of centrifugation at 3000 rpm for 10 minutes. The serum (supernatant) obtained from this process was used for the assays. Standard procedures were employed in determining the following liver function test:

Alanine aminotransferase (ALT) activity, Aspartate aminotransferase (AST) (Reitman and Frankel, 1957), Alkaline Phosphatase (King and Armstrong, 1934).

Statistical analysis

Data obtained from the study were analyzed using Statistical Package for Social Sciences (SPSS-21). Results were presented as mean \pm standard error of mean (SEM) of sample replicates. Raw data were subjected to one way analysis of variance (ANOVA) followed by post hoc turkey's test. $P=.05$ were considered to be statistically significant.

RESULTS

The blood lipid lowering effects of the crude and fractionated ethanolic extracts of *A. melegueta* seeds and *M. oleifera* leaves are presented in Figure 1 below. The crude and fractionated extracts significantly ($P=.05$) reduced the blood lipid levels in hyperlipidemic albino rats. Both the crude extracts of *Aframomum melegueta* seeds and *Moringa oleifera* leaves drastically reduced the TC, TG, and LDL-C blood level of the albino rats. Similarly, the HDL-C level was also increased, though not all doses of extracts showed a significant increment. Nearly all crude extracts and fractions exhibited a dose related and significant ($P=.05$) reduction in blood lipid level.

However, the combination of 500 mg/kg *Aframomum melegueta* seeds and 500 mg/kg *Moringa oleifera* leaves crude extract showed a better blood lipid lowering effect compared to others. Combination of 500 mg/kg of *Aframomum melegueta* seeds and 500 mg/kg *Moringa oleifera* leaves was able to produce a blood lipid lowering effect almost at same level with that of the positive control (Atorvastatin 1.2 mg/kg). The reduction in TC, TG and LDL-C is almost at par with that of the positive control (Atorvastatin).

Also, Atorvastatin and 500 mg/kg/500 mg/kg *Aframomum melegueta* and *Moringa oleifera* combination therapy produced same level HDL-C increment. Amongst the crude extract and fractionated extracts of both crude drugs, 250 mg/kg *Aframomum melegueta* and *Moringa oleifera* fraction MO3 produced the least level reduction in TC, TG, LDL-C as well as the most insignificant increase in HDL-C.

The comparative effect of the single therapy crude extract of *Aframomum melegueta* seeds and *Moringa oleifera* leaves on blood lipid level are presented in Figure 2 below. The highest reduction ($P=.05$) of blood lipid level among the single drug crude extracts was clearly seen in 500 mg/kg *Moringa oleifera* leave extract with percentage reduction of 43.31% (TC), 46.845(TG), 47.84% (LDL-C). This also led to this same dose having the highest level of HDL-C elevation (85.23%) among the single drug crude extracts. 250 mg/kg *Aframomum melegueta* seed extract produced the least blood lipid lowering effect with a percentage reduction of 33.31% (TC), 36.21% (TG), 23.68% (LDL-C) and a percentage HDL-C elevation of (11.74%). It is pertinent to note that the positive control (1.2 mg/kg Atorvarstatin) resulted in slightly higher blood lipid lowering effect in comparison with the single drug crude extracts having the greatest blood lipid lowering effect. Atorvarstatin produced a better percentage reduction in blood lipid levels as follows: 47.04% (TC), 48.55% (TG), 63.51% (LDL-C), and it has a higher percentage increase of HDL-C (170.58%).

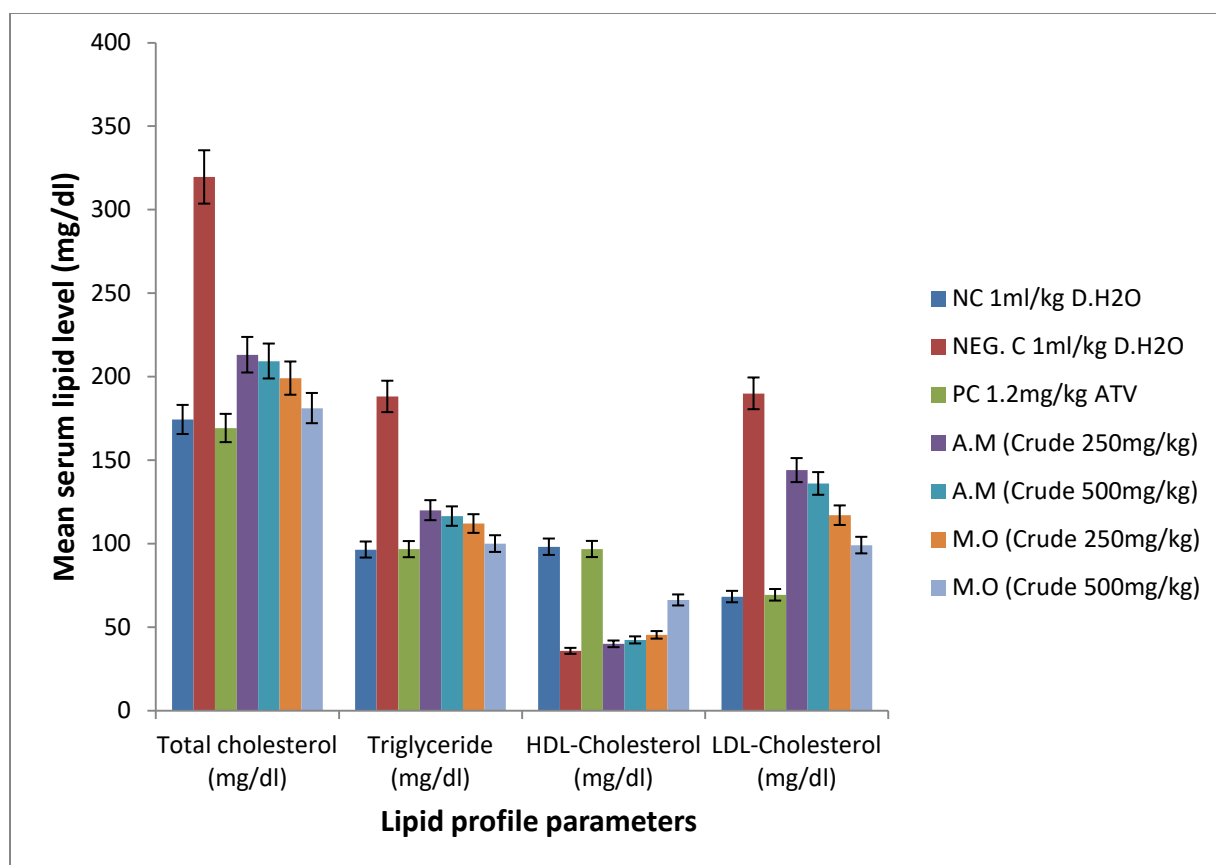


Figure 2: Comparative effect of different doses of single therapy crude extracts of *A. melegueta* seeds and *M. oleifera* leaves on blood lipid level of albino rats

NC: Normal control, NEG.C: Negative control, PC: Positive control, A.M: *Aframomum melegueta* seed extract, M.O: *Moringa oleifera* leaf extract, D.H₂O: Distilled water, ATV: Atorvastatin

The comparative effect of combination of *Aframomum melegueta* seeds and *Moringa oleifera* leaves on blood lipid level are presented in fig. 3 below. The positive control (Atorvastatin 1.2 mg/kg) produced a better blood lipid lowering effect than the combination therapy of 250mg/kg of *Aframomum melegueta* seed extract and 250 mg/kg *Moringa oleifera* leaf extracts. The combination of 500 mg/kg *Aframomum melegueta* seed extract and 500 mg/kg *Moringa oleifera* leaf extract produced slightly similar blood lipid lowering effect. Although the percentage reduction of TC, TG and LDL-C and the percentage increase of HDL-C exhibited by Atorvastatin is slightly higher, the combination of 500 mg/kg *Aframomum melegueta* seed extract and 500 mg/kg *Moringa oleifera* leaf extract produced a slightly higher percentage increase of HDL-C than that of Atorvastatin.

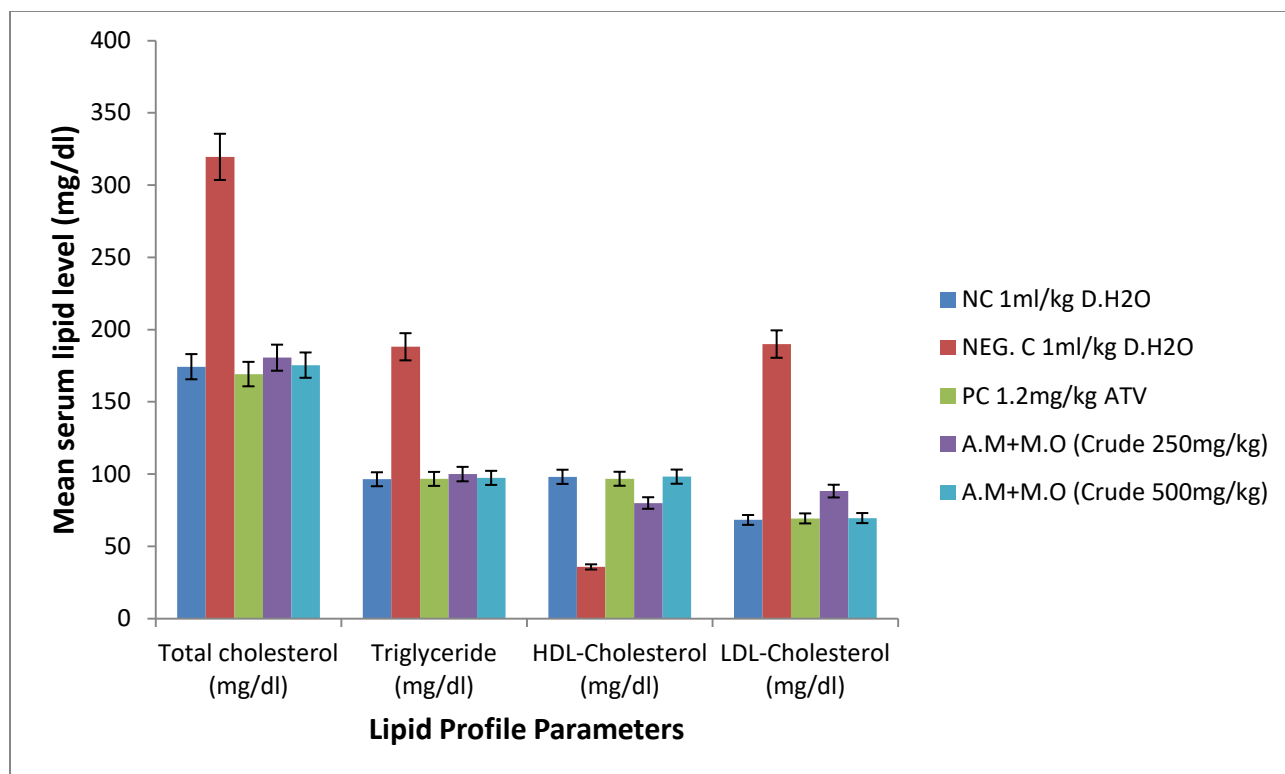


Figure 3: Comparative effect of the combination of *Aframomum melegueta* seeds and *Moringa oleifera* leaves on blood lipid level

NC: Normal control, NEG.C: Negative control, PC: Positive control, A.M: *Aframomum melegueta* seed extract, M.O: *Moringa oleifera* leave extract, D.H₂O: Distilled water, ATV: Atorvastatin

The effect of the VLC-fractionated extracts of *Aframomum melegueta* seeds and *Moringa oleifera* leaves on blood lipid level are presented in Figure 4 below. Generally, the hypolipidemic effect of fraction MO4 of *Moringa oleifera* leaves extract is almost at par with that of the positive control (Atorvastatin), although the percentage increase of HDL-C exhibited by Atorvastatin (170.58%) is slightly higher than that of fraction MO4 (146.08%). The four fractions of each plant extract showed a significant reduction in blood lipid level of the albino rat. Amongst the four fractions of *Aframomum melegueta* seed extract, fraction AM3 exhibited a better percentage reduction when compared with other *Aframomum melegueta* fractions. The fraction of the *Moringa oleifera* leaf extract with the best lipid lowering effect is fraction MO4.

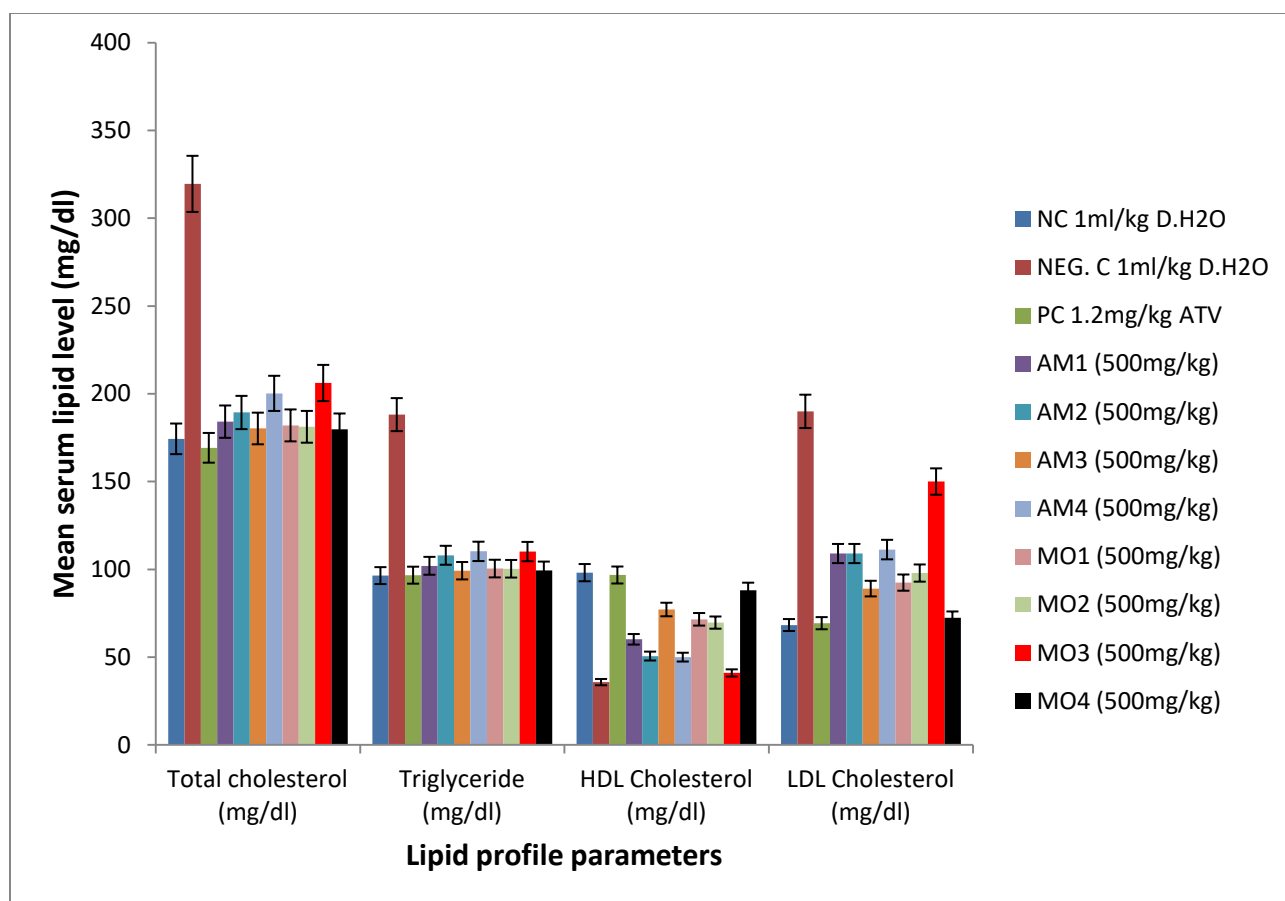


Figure 4: Effect of the VLC fractionated extracts of *Aframomum melegueta* seeds and *Moringa oleifera* leaves on blood lipid level

NC: Normal control, NEG.C: Negative control, PC: Positive control, A.M: *Aframomum melegueta* seed extract, M.O: *Moringa oleifera* leave extract, D.H₂O: Distilled water, ATV: Atorvastatin

The effect of the extracts of *A. melegueta* seeds and *M. oleifera* leaves on serum AST, ALT and ALP concentration of hyperlipidemic albino Wistar rats are presented in Figure 5 below. In comparison with the negative control (rats that did not receive any treatment with plant extracts), A.M + M.O (500 mg/kg) produced a better reduction in serum AST (62.68%), closely followed by *M. oleifera* fraction MO4 (62.01%) and A.M + M.O 250 mg/kg (59.30%). A.M + M.O (500 mg/kg) produced a percentage reduction in AST concentration almost at the same level as the positive control (Atorvastatin) which showed a percentage reduction of 63.18%. *A. melegueta* seeds crude extract 250 mg/kg produced the least percentage reduction of AST serum concentration (17.93%) when compared with the negative control. Similarly, A.M + M.O (500 mg/kg) produced a better reduction of serum ALT concentration in comparison with the negative control. The percentage reduction of A.M + M.O 500 mg/kg (28.84%) is slightly higher than that of the positive control (Atorvastatin) (28.30%). This is closely followed by *M. oleifera* fraction

MO4 with a percentage ALT reduction of (28.23%). The least reductive effect was exhibited by *A. melegueta* seeds crude extract 250 mg/kg (8.16%).

Also, the percentage reductive effect of serum ALP concentration showed that the positive control (Atorvarstatin) and A.M +M.O (500 mg/kg) produced a better effect. Although, that of the positive control (60.70%) is slightly higher than that of A.M + M.O 500 mg/kg (60.02%). The least serum ALP concentration reductive effect was exhibited by *M. oleifera* crude extract 250 mg/kg (11.73%)

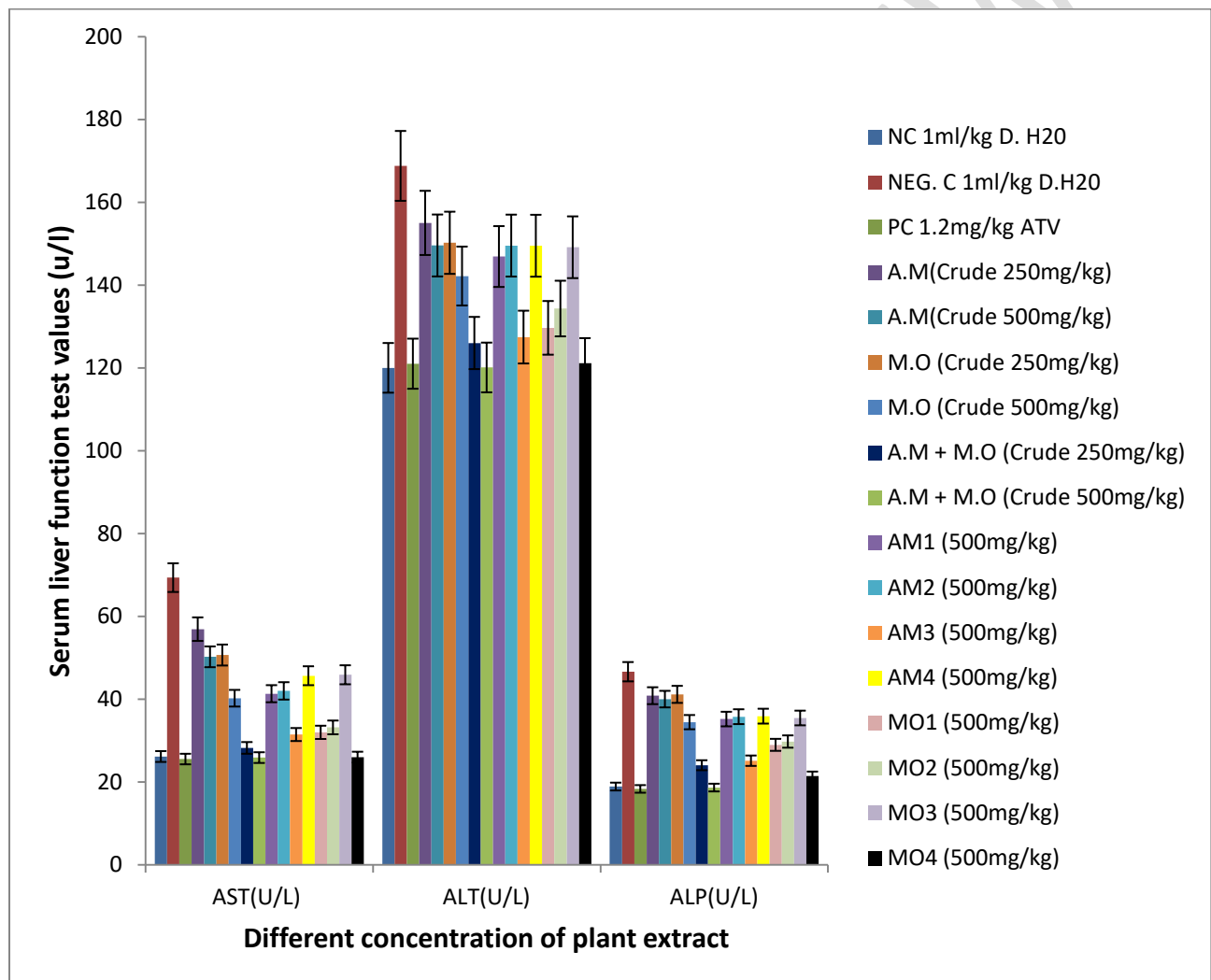


Figure 5: Effect of the extracts of *A. melegueta* seeds and *M. oleifera* leaves on serum AST, ALT and ALP concentration of hyperlipidemic albino Wistar rats.

NC: Normal control, NEG.C: Negative control, PC: Positive control, A.M: *Aframomum melegueta* seed extract, M.O: *Moringa oleifera* leaf extract, D.H₂O: Distilled water, ATV: Atorvastatin

The effects of various treatments on body weight are presented in Figure 6 below. The reduction in body weight of test animals ranges from high to very minimal. The normal control that was neither induced with hyperlipidemia nor subjected to any form of treatment exhibited a very minimal increase in body weight (0.84%), the negative control group that was induced with hyperlipidemia without being subjected to any form of treatment showed a marked increase in body weight (77.52%). Weight reduction was more in the group treated with *Moringa oleifera* Fraction MO4 (26.91%), closely followed by the positive control group with a percentage reduction of 26.55%. Other percentage reduction in body weight across the treatment groups are in the following order: A.M +M.O 500 mg/kg (26.54%), *Moringa oleifera* fraction MO1 (18.03%), *Moringa oleifera* fraction MO2 (17.96%), A.M +M.O 250 mg/kg (17.34%), *Aframomum melegueta* fraction AM3 (16.57%), *Moringa oleifera* 500 mg/kg (9.97%), *Aframomum melegueta* fraction AM4 (8.83%), *Moringa oleifera* fraction MO3 (7.91%), *Aframomum melegueta* fraction AM1 (7.20%), *Aframomum melegueta* fraction AM3 (2.53%), *Aframomum melegueta* 250 mg/kg (2.30%), *Moringa oleifera* 250 mg/kg (2.02%), *Aframomum melegueta* 500 mg/kg (1.98%).

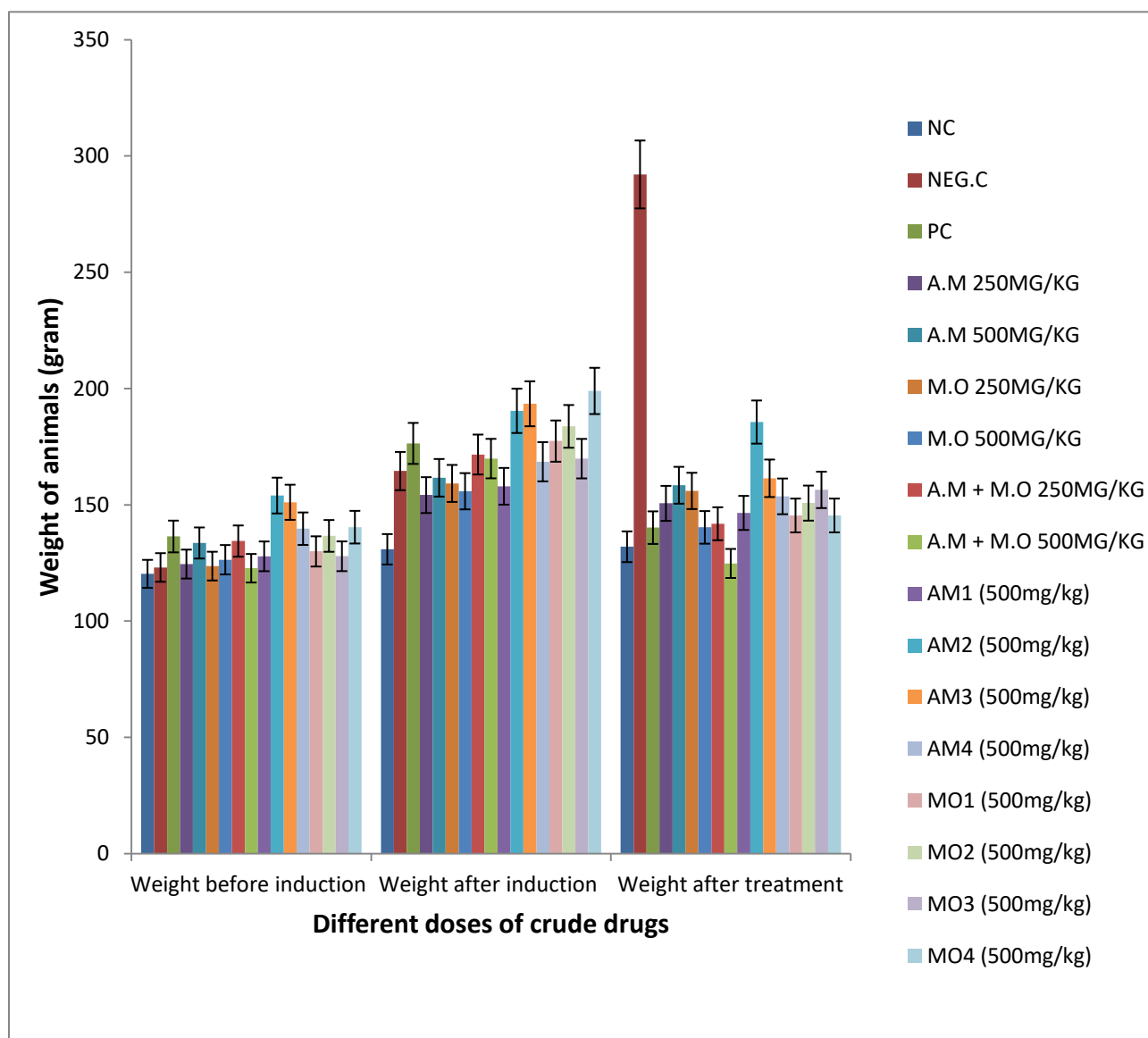


Fig. 6: Final change in body weight of Wistar rats in different treatment groups

NC: Normal control, **NEG.C:** Negative control, **PC:** Positive control, **A.M:** *Aframomum melegueta* seed extract, **M.O:** *Moringa oleifera* leave extract, **D.H₂O:** Distilled water, **ATV:** Atorvastatin

The results of the qualitative and quantitative phytochemical screening ethanolic extract of *Aframomum melegueta* seeds and *Moringa oleifera* leaves are presented in table 1 and 2 below respectively. The qualitative phytochemical screening results reveals that the ethanolic extract of *M. oleifera* leaves and *A. melegueta* seeds contains some bioactive constituents such as tannins, saponins, flavonoids, alkaloids, phenols, terpenoids, steroids, quinines and cardiac glycosides. The quantitative phytochemical screening reveals that *Moringa oleifera* leaves contains more of

cardiac glycosides, saponins and flavonoids while *Aframomum melegueta* seeds contains more of cardiac glycosides and phenols.

Table 1: Qualitative Phytochemical Screening of ethanolic extract of *Moringa oleifera* leaves and *Aframomum melegueta* seeds

S/N	Phytoconstituents	<i>Moringa oleifera</i> leaves	<i>Aframomum melegueta</i> seeds
1	Tannins	++	+
2	Saponins	++	++
3	Flavonoids	+	++
4	Alkaloids	++	++
5	Phenols	++	++
6	Terpenoids	+	++
7	Steroids	+	++
8	Quinones	+	+
9	Cardiac glycosides	+++	+++

(+) = Present in small concentration

(++) = Present in moderately high concentration

(+++)= Present in high concentration

Table 2: Quantitative Phytochemical Screening of *Moringa oleifera* leaves and *Aframomum melegueta* seeds

Phytoconstituents (%)	<i>Moringa oleifera</i> leaves	<i>Aframomum melegueta</i> seeds
Tannins	2.63%	3.60%
Phenols	4.82%	14.43%
Flavonoids	11.6%	7.53%
Alkaloids	7.50%	5.78%
Saponins	17.6%	6.26%
Cardiac glycosides	11.86%	14.58%

DISCUSSION

Herbal medicines have been reported to reduce blood lipid level in humans (Chattopadhyay and Bandyopdhyay, 2005). Recently, herbal supplements have been widely utilized in managing

hyperlipidemia and this has led to a rapid decline in the prevalence of atherosclerosis and cardiovascular related diseases. This antilipidemic study was able to ascertain that amongst the extracts, the A.M 500 mg/kg + M.O 500 mg/kg produced the most significant ($P=.05$) reduction of serum lipid profile. The positive control drug (Atorvastatin 1.2mg/kg) produced a decrease of TC, TG, and LDL-C and corresponding increase of HDL-C slightly higher than that of A.M 500 mg/kg +M.O 500 mg/kg, although the difference in the lipid lowering effect between both is not significant, it can be simply interpreted that A.M 500 mg/kg +M.O 500 mg/kg has similar antilipidemic effect with the positive control drug (Atorvastatin 1.2 mg/kg). Other treatments that produced extremely significant reduction in TC, TG, LDL-C as well as increase in HDL-C are fraction MO4, A.M 250 mg/kg + M.O 250 mg/kg, A.M fraction AM3, fraction MO2, fraction MO1, M.O crude extract 500 mg/kg. Information on the lipid profile of animals and humans provide clinicians with concrete guide on lipid metabolism and predisposition of animals and humans to cardiovascular disorders. Increased cholesterol level has been associated with cardiovascular disorders and statins have been clinically indicated for the prevention and treatment of this disease, hence the need to use Atorvastatin in the antilipidemic study as a positive control drug. HMG-CoA reductase enzyme has been implicated in the biosynthesis of cholesterol and Atorvastatin acts by competitively inhibiting this enzyme. However, since all members of the statin group are similar to HMG-CoA on a molecular level, Atorvastatin substitutes HMG-CoA in the enzyme and reduce the rate of mevalonate synthesis, which is responsible for biosynthesis of cholesterol (Ray *et al.*, 2001).

The phytochemical screening carried out on the ethanol extract and fractions of *Aframomum melegueta* seeds and *Moringa oleifera* leaves revealed the presence of tannins, saponins, flavonoids, alkaloids, phenols, terpenoids, steroids, quinones, cardiac glycosides. Previous studies on phytochemical screening of roots of the aqueous extract of *Moringa oleifera* revealed the aforementioned secondary metabolites including anthraquinone glycosides (Kasolo *et al.*, 2011), while that of *Aframomum melegueta* seed extract does not show the presence of anthraquinone glycosides (Okwu and Njoku, 2010; Groblacher *et al.*, 2012). The presence of these bioactive phytoconstituents are known to be clearly responsible for the several general and specific functions in plants and may exhibit different biochemical and pharmacological actions in different species of animals when administered. The action ranges from cell toxicity to cell protective effect (Trease and Evans, 1996)

On the other hand, the quantitative phytochemical screening carried out revealed that amongst all the secondary metabolites present in *Moringa oleifera* leaf extract, saponins, cardiac glycosides and flavonoids were found to be present in a much larger proportions compared to alkaloids, phenols and tannins which were found to be present in lesser quantities. Similarly, *Aframomum melegueta* seed extract possesses a greater proportion of cardiac glycosides, it also contains a considerable high quantity of phenolic compounds. Other phytoconstituents such as saponins, alkaloids, flavonoids as well as tannins were found to be present in lesser quantities when compared with cardiac glycosides and phenols. Saponin is a membrane active secondary

metabolite, which can complex cholesterol in animal membranes (Frenkel *et al.*, 2014). Saponins have been known to lower blood triglyceride level due to their lytic role and it is well established that VLDL cholesterol are the main transporter of triglycerides in the serum (Hertog *et al.*, 1993). Saponins are also responsible for facilitating enterohepatic circulation of bile acids, making it unavailable for re-absorption back to the intestine (Fuhrma *et al.*, 2002). The ability of the *Moringa oleifera* leaf extract to drastically lower the total cholesterol, triglycerides, low density lipoproteins and markedly increase the blood HDL-C level can be attributed to its high saponin content.

Several studies have elucidated the impact of phenolics and flavonoids in reducing the risk of arterosclerosis development (Costa and Martinez, 1997). Apart from promoting arterosclerosis in blood vessels, hyperlipidemia may directly affect the heart, leading to increased ischemia and reperfusion injury and weakened response to cardiac protective interventions such as ischemic preconditioning and post conditioning (Balakumar *et al.*, 2012). Furthermore, since oxidative stress has been responsible for the improvement of cardiovascular and neurodegenerative diseases, phenolics being a potent antioxidant can be very vital in averting cardiovascular disorders that might arise from hyperlipidemia (Heo and Lee 2006; Tarozzi *et al.*, 2013; wiczowski *et al.*, 2013). The presence of phenolic compounds in both crude drugs indicates that the test drugs are potent antilipidemic agents because they are standards with which other antioxidants are compared. Specifically, flavonoids have been directly linked with inhibition of LDL oxidation, platelet aggregation, promoting vasodilation as well as modification of eicosanoid synthesis (Sesso *et al.*, 2003). These biological actions are closely linked with lipid lowering effects of the test drugs.

M.O crude extract exhibited a better TC reduction than A.M crude extract; this can be outrightly linked to the quantity of phytoconstituents present in both crude extracts. The result of the quantitative phytochemical screening revealed that *Moringa oleifera* leaves contains more saponins and flavonoids than *Aframomum melegueta* seeds while *Aframomum melegueta* seeds contains more phenols and cardiac glycosides than *Moringa oleifera* leaves (Table 2). Phytochemicals are generally defined as physiologically active compounds with great potentials for therapeutic uses. It is important to note that medicinal plants contain mixtures of different chemical compounds acting individually, or synergistically to improve health (Gurib-fakim, 2006). Saponins exhibit hypocholesteremic activity in animals and humans, thereby contributing to the low incidence of atherosclerosis (Johns and Chapman, 1995; Johns 1996). The ability of saponins to lower blood cholesterol level is centered on its ability to bind either bile acids or cholesterol in the intestinal lumen. Binding with cholesterol will make it more easily reabsorbed causing a reduction in enterohepatic circulation of bile acid in the liver (Kritcheroky, 1997). There is also an extreme significant reduction of triglyceride exhibited by fractionated extracts of *Aframomum melegueta* seeds and *Moringa oleifera* leaves. Comparatively, the A.M fraction AM3 and M.O fraction MO4 exhibited the best serum TG reduction amongst all the fractionated extracts of both plants. The significant reduction of triglyceride by various doses of the crude and

fractionated extracts of A.M and M.O can be attributed to the presence of bioactive principles. Typical examples of these antitriglyceride bioactive compounds include Tannins(ellagic acid), Alkaloids(sanguinarine), Saponins(sinigrin and sinalbin) Flavonoids(narigin) (Nijveldt *et al.*, 2001; Rathbone and Bruce, 2002).

Medically, high levels of HDL-C can reduce any tendency of coming down with heart disease and stroke. Experts believe HDL-C scavenges, removes and recycles the LDL-C by transporting it to the liver where it can be reprocessed. They also help to prevent any form of damage to the inner walls of the blood vessels because damage to the endothelium (inner wall) is usually the first step in the process of atherosclerosis. This is the reason why constant efforts should also be made to boost blood HDL-C level. The antilipidemic study clearly shows that A.M 500 mg/kg + M.O 500 mg/kg exhibited a better HDL-C increment when compared to the positive control drug (Atorvastatin) and A.M 250 mg/kg exhibited the least percentage increment of HDL-C (11.74%). As in other lipid profile parameters, phytochemicals are also largely implicated in the HDL-C surge. Craig et al (2010) reported that phytochemicals such as phenolic compounds are bioactive compounds found abundantly in plant foods and protect against cardiovascular events by reducing prothrombic and inflammatory status and improving endothelial function.

Reduction of LDL-C also followed similar pattern with that of TC and TG. LDL-C is the primary target of most hypolipidemic therapy because it is termed “Bad cholesterol” and a major atherogenic lipoprotein. Nevertheless, this study has been able to deduce the lipid lowering effect of *Moringa oleifera* leaves and *Aframomum melegueta* seeds when used individually and when used as combination therapy. Apart from the A.M 500 mg/kg + M.O 500 mg/kg having a percentage reduction almost similar with that of Atorvastatin, it is also vital to note that the M.O fraction MO4 produced a better serum LDL-C reduction than A.M 250 mg/kg + M.O 250 mg/kg (Figure 1). Summarily, the M.O crude extracts produced a better hypolipidemic effect than the A.M crude extracts, the highest dose (500 mg/kg) of the combination of extracts of *Aframomum melegueta* seeds and *Moringa oleifera* leaves produced the best hypolipidemic effect, almost similar with that of Atorvastatin. Besides saponin, flavonoid, and phenolics, cardiac glycosides were also present in considerably high quantities in both plant extracts (Table 2). In April, 2017, scientists at the Medical University of South Carolina (MUSC) discovered that cardiac glycosides plays a big role in reducing LDL-C by degrading apolipoprotein B (ApoB), a molecule used by liver cells to produce LDL-C and was suggested as a potential remedy for familial hypercholesterolaemia irrespective of statin therapy. Since this study revealed high cardiac glycoside content and some groups of crude and fractionated extracts of both crude drugs, especially the combination therapies resulted in an extremely significant blood LDL-C reduction, these groups could be suggested as a potential solution for familial hypercholesterolaemia. Despite the aforementioned discovery, these group of natural compounds still exhibits a low therapeutic index, thereby having a very small margin of safety, hence toxicity can emanate at a dose slightly higher than the experimented safe dose. This justifies the need for acute toxicity testing (LD50). On the other hand, alkaloids usually have marked

physiological action on humans or animals. Tannins could be responsible for the slightly hot, bitter and pungently taste of *Aframomum melegueta* seeds because tannins are known to have stringent properties (Okwu, 2004).

According to the American Heart Association, high cholesterol can affect anyone, regardless of their weight; however, excess body weight can also trigger increased cholesterol levels. The group treated with M.O fraction MO4 produced the best reduction in body weight (26.91%); this was closely followed by the groups treated with Atorvastatin and A.M 500 mg/kg + M.O 500 mg/kg. The negative control group induced with hyperlipidemia without treatment produced a very drastic increase in body weight while the normal control group that was neither induced nor treated produced a very slight increase in body weight. The standard diet caused a slight increase in body weight in the normal control group, while the drastic increase in body weight amongst the negative control group was due to the intake of high fat feeds. The reduction of body weight can be linked to the treatment with both crude drugs and their fractions. From the body weight result, these two plants combines together to produce a synergistic body weight reduction effect when compared with each crude drug administered individually. Generally, *Moringa oleifera* leaves have been known to promote stable blood sugar through its high fiber content, as well as secondary metabolites such as flavonoids and phenols-which inhibits the activity of amylase, an enzyme that helps breakdown of starch into glucose (Leone *et al.*, 2018). *Moringa oleifera* leaves is also involved in weight loss by preventing the gut from absorbing starch and glucose, sending them out as digestive waste products (Leone *et al.*, 2018). Similarly, this study is in tandem with the reports of Mojekwu *et al.* (2011), they reported the ability of *Aframomum melegueta* seeds to inhibit α -amylase and α -glucosidase enzymes. Since research by American Heart Association (AHA) has indicated that a weight loss of 5-10% of body weight may significantly reduce LDL-C levels in people at higher risk of cardiovascular disorders, it is noteworthy to suggest that both single and combination therapies of both crude and fractionated extracts should be considered effective as lipid lowering agents.

In a bid to monitor or screen for any possible hepatotoxicity and side effects associated with the crude and fractionated portion of both crude drugs, liver function tests were carried out. This test measures the level of certain enzymes and proteins in the blood. These enzymes, also known as biomarkers of liver damage include alanine transferase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). These three enzymes are naturally found in the liver and they help to metabolize proteins and amino acids. However, an increased level of these enzymes in the blood stream signifies possible liver damage or disease. In this present study, none of the crude and fractionated extracts of both *Aframomum melegueta* seeds and *Moringa oleifera* leaves caused an increase in the blood levels of these biomarkers, hence they are all considered safe and devoid of any form of hepatotoxicity. Specifically, the dose of extracts that produced the best antilipidemic effect together with the positive control drug (Atorvastatin) exerted the best percentage reduction of these three enzymes in comparison with the negative control group. These extracts include: A.M 500 mg/kg + M.O 500 mg/kg and M.O fraction MO4 (Figure 5).

This study correlates with the results of previous study which clearly stated that *Moringa oleifera* leaves improved the carbon tetrachloride (CCl₄) induced liver injury and therefore can be used as a futuristic hepatoprotective drug (El-bakry *et al.*, 2016). Also, aqueous extracts of *Aframomum melegueta* seeds have been reported to be free from toxic effects on the liver cells in both high or low doses, thus has protective properties (Obike *et al.*, 2014).

CONCLUSION

Ethanol extract of *Moringa oleifera* leaves and *Aframomum melegueta* seeds exhibited various degrees of dose dependent hypolipidemic activities. The combination of *Aframomum melegueta* seed extract and *Moringa oleifera* leave extract produced a better dose dependent hypolipidemic effect when compared with each crude extract administered individually. The M.O fraction MO4 produced a very good antilipidemic effect almost similar with the combination therapies.

The combination of both crude drugs produced positive interaction in reducing blood lipid level, body weight as well as protection against liver damage. Synergism was evident in the ability of both plants to combine together to reduce body weight.

In a nutshell, the findings of this study indicates that combination of *Aframomum melegueta* seed extract and *Moringa oleifera* leave extract produces an additive blood lipid lowering effect when compared to each crude drug given independently. This also confirms the synergistic effect of both crude drugs in the reduction of body weight and protection from hepatotoxins. This study further confirms and establishes a firm scientific basis for the use of both plant parts by the *Urhobos* in Delta state as a natural supplement for weight loss as well as prophylactic agent against cardiovascular disorders.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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