International Journal of Modern Science and Technology, 2020;5(7):172-181.
ISSN: 2456-0235.
www.ijmst.co

Research Article

Phytochemical Analysis and effect of methanolic root extract of *Moringa oleifera* on Haematological parameters and Body weight in poloxamer 407 Induced hyperlipidemic albino rats

Itodo Matthew Ocheleka[1]*, Inalegwu, Bawa[2], Agnes Ogbene Abutu[3]

[1]Department of Biochemistry, Federal University of Agriculture Makurdi, Benue State, Nigeria.
[2]Department of Biochemistry, Federal University of Agriculture Makurdi, Benue State, Nigeria.
[3]Plataue State College of Health Technology, Zawan, Plataue State, Nigeria.

*Corresponding author's e-mail: ocheleka1@gmail.com

Abstract

The present research was aimed to study the phytochemical analysis and effect of methanolic root extract of *Moringa oleifera* on Haematological parameters and Body weight in Poloxamer 407 induced Hyperlipidemic Albino rats. Albino rats were grouped into 8; Group 1: Normal control given only food and water, Group 2: Hyperlipidemic control not treated, Group 2 to 8 was induced with Poloxamer 407 1000 mg/kg body weight, and however group 3 was treated with standard drugs atorvastatin (10 mg/kg). Group 4 treated with crude root extract (200 mg/kg) of *Moringa oleifera* and group 5 to 8 were treated with purified fraction from column chromatography. The study showed the multiple biological activities including antilipidemic, hepatoprotective, antiatherosclerotic, oxidative DNA damage protective, cardioprotective, as well as folk medicinal uses of *Moringa oleifera* root are attributed to the presence of functional bioactive compounds, such as flavonoids, alkaloids, saponins, tannins, cardiac glycoside. The methanolic root extract at 200 mg/kg body weight significantly ($p \leq 0.05$) increase packed cell volume (PCV), haemoglobin (Hb), and significantly ($p \geq 0.05$) decrease white blood cell (WBC) of the treated groups. This study supports the popular sayings about the tradomedicinal use of *Moringa oleifera* in the treatment of cardiovascular and other related diseases. However it can be used in optimal concentrations to sustain animals infected with abnormal lipid in the absence of synthetic chemical drugs to prevent death to cardiovascular disease.

Keywords: *Moringa oleifera*; Poloxamer 407; Phytochemical analysis; Haematology; Body weight.

Introduction

Moringa oleifera (MO) has many common names such as ben oil, drumstick, horseradish, and miracle tree. *Moringa oleifera* is a widely distributed species of the family Moringaceae. It is a small graceful, deciduous plant with thin foliage and can grow up to 8 m height. Moringa is native to Western and sub Himalayan regions, India, Pakistan, Asia and Africa and it is distributed throughout the world in arid and semi-arid climate [1].

Moringa trees are having a remarkable range of medicinal properties as high as nutritional values. Most parts of the plant, leaves, seeds, fruit or pods, roots, stem, and bark are used as medicines or foods in various countries with especial references to the traditional communities [2].

Arteries are normally smooth and unobstructed on the inside, but in case of increased lipid level, a sticky substance called plaque is formed inside the walls of arteries. This leads to reduced blood flow, leading to stiffening and narrowing of the arteries. It has been proved that elevated higher lipids level, especially cholesterol (Tc) and triglycerides (TAG) leads to hyperlipidemia (HL) and plasma levels of cholesterol and of low density lipoprotein cholesterol (LDL-c) are responsible for atherosclerosis in man, and epidemiological data suggests that elevated plasma levels of High density lipoprotein cholesterol (HDL-c) have a protective effect. So they have been developed many methods previously using some plants extract to increase the protective effect [3].

Various animal models have been used to study the pathophysiological effects of

Received: 28.05.2020; Received after Revision: 23.06.2020; Accepted: 24.06.2020; Published: 28.07.2020

hyperlipidemia. Genetic variants of hyperlipidemia, such as the obese Zucker rat are available. Alternatively hyperlipidemia can be induced by dietary means (e.g. chronic feeding of a high fat diet or by treatment with compounds such as Triton or poloxamer 407 (P407). Poloxamer 407, a non-ionic synthetic copolymer surfactant, provides an attractive means of inducing hyperlipidemia because of its rapid onset and seeming lack of overt toxicity; within 24 h of its intraperitoneal (i.p.) injection a profound hyperlipidemic state is achieved [4].

Cholesterol levels below 200mg/dl are classified as desirable blood cholesterol. Those 200 to 239 mg/dl as borderline high blood cholesterol and those 240mg/dl and above as high blood cholesterol. Cholesterol levels are fairly constant but triglycerides level fluctuate considerable from day to day and are highly 1 to 4 hr after meal [5]. The aim of the present study is to investigate the phytochemical analysis and effects of methanolic root extract of *Moringa oleifera* on haematological parameters and body weight in poloxamer 407 induced hyperlipidemic albino wistar rats.

Material and methods

Plant material

The *Moringa oleifera* root was collected from its natural habitat at the University of Agricultural Makurdi, Benue State, Nigeria and washed properly with tap water and air dried. Finally roots powdered was obtained by grinding the roots and kept at 4°C with sealed plastic packet until experiment to avoid the microbial contamination.

Extraction

Extraction was carried out on the powdered sample using the method [6]. The collected plant sample was rinsed in clean water and dried at room temperature for two weeks. The dried plant sample was ground into powder using a mortar and pestle, the powder obtained was used to prepare the extracts. Based on the sample to solvent ratio of 1:10 (w/v), 100g of each of the ground samples root was suspended in 1000ml of 70% methanol on a shaker for 48 hr at room temperature. Each extract was filtered using a sterilized Buchner funnel and Whatman No. 1 filter papers. The filtrates was concentrated by drying in a water bath maintained at a temperature of 45°C until a brownish black residue was obtained and the weights of the extracts were determined as the percentage weight of the extract to the original weight of the sample used, using the formula below. The extracts were kept in the sealed containers and refrigerated at 24°C from where aliquot was reconstituted for the experiment.

$$Percentage\ Yield = \frac{Weight\ of\ Extract}{Weight\ of\ Sample} \times 100$$

Phytochemical qualitative screening of the root of Moringa oleifera plant

Chemical tests were carried out on the powdered sample using standard procedures to identify the constituents that are active as described in the literature [7-9].

Test of flavonoids

5ml of dilute ammonia solution was added to 5g extract, followed by the addition of concentrated sulphuric acid. The yellow color indicates the presence of flavonoids.

Test for saponins

10g of the extract was mixed with 5 ml of distilled water and shaken vigorously for stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion.

Test for cardiac glycosides

To 5 g of extract of 2 ml of glacial acetic acid and one drop of ferric chloride solution was added followed by 1ml of concentrated sulphuric acid. The formation of brown ring indicates the presence of deoxy sugars.

Alkaloids

Powdered sample 2 g was stirred with 5 ml of 1% aqueous HCL on a steam bath and filtered. Then 1 ml of the filtrate was treated with a few drops of Mayers reagent and another 1ml portion similarly treated with Wagner reagent. The cream or pale yellow precipitate was observed as evidence of alkaloids.

Tannins

Lead Sub-Acetate Test: Lead sub-acetate solution (3drops) was added to a solution of 1 g of the extract. A red precipitate indicates tannins. Ferric Chloride Test: About 0.5 ml of extract was dissolved in 10 ml of distilled water, and then filtered. Few drops of ferric chloride solution were added to the filtrate. Formation of

a blue-black precipitate indicates hydrolysable tannins and green precipitates indicate the presence of condensed tannin.

Quantitative phytochemical analysis of methanolic root extract of Moringa oleifera

Standard methods were used to determine the amount of the required bioactive constitutes in the *Moringa oleifera* root based on gravimetric tests. Three replicates were used for each test to ensure the accuracy.

Flavonoids

Flavonoid determination was done using the standard method [1], 10 g of the extract was extracted with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper no 42 (125 mm), the filtrate was later transferred into a crucible and Was evaporated to dryness in a water bath and weighed as flavonoids.

$$\%\ Flavonoids = \frac{Weight\ of\ flavonoids}{Weight\ of\ sample} \times \frac{100}{1}$$

Saponins

A gravimetric method was used as illustrated in literature [10], employing the use of a Soxhlet extractor and two different organic solvents was used. The first solvent (Acetone) extracts lipids and interfering pigments while the second solvent (methanol) extracts saponin. Two grams each of the extract was weighed into a thimble and put in a soxhlet extractor with a condenser fitted on top. Extraction was done with acetone in a 250 ml round bottom flask for 3hrs, after which the other weighed 250 ml round bottom flask containing methanol was fitted to the same extractor continued for another 3hrs. At the end of second extraction, the methanol was recovered by distillation and the flask oven-dried to remove the remaining solvent in the flask. The flask was allowed to cool in a desiccator and weighed.

$$\%\ Saponin = \frac{A-B}{W} \times \frac{100}{1}$$

Where A = weight of flask and extract (Saponin)
B = weight of empty flask
W = weight of sample.

Cardiac glycosides

A Previousely published methodology was employed [10], these was used in the determination of cardiac glycosides. Five (5) grams of extract was placed in a 200ml conical flask; 50ml of distilled water was added. The content of the flask was stirred and allowed to stand for 4hrs. The filtrate was steam distilled into 20ml of 2.5% NaOH. About 75ml of the distillate was collected. This was titrated with 0.02N $AgNO_3$ (Silver nitrate) after the addition of 8ml of 6N Ammonium hydroxide and 2ml of 2% potassium iodide. Permanent turbidity indicated end-point. The cardiac Glycosides was calculated by multiplying the volume of 0.02N $AgNO_3$ used (titre value) by 1.08 mg, using the equation below.
1 ml 0.02N $AgN0_3$ = 1.08 mg HCN Therefore cardiac Glycosides content in the sample = titre value x 1.08 mg

Tannins

A methodology reported previously was used [10] to determine concentration of tannin. Two (2) grams of the extract was boiled with 300 ml of distilled water. This was diluted in a standard volumetric flask and filtered through a non-absorbent cotton wool. Twenty five millilitre (25 ml) of the infusion was measured into a 2 litre porcelain dish and titrated with 0.1N potassium permanganate (0.1N potassium permanganate was standardized against 0.1N Oxalic acid) until the blue solution changed to green, Then 0.5 ml of 0.1N potassium permanganate was added at a time until the solution turned golden yellow.
The tannin content in the sample was calculated by multiplying the volume of 0.1N potassium permanganate used (titre value) by 0.0066235 g. Using the equation below;
1ml of 0.1N potassium permanganate (titre value) = 0.0066235 g Tannins Therefore Tannins content in the sample = titre value x 0.0066235 g.

Alkaloids

Alkaloid was determined based on a previously established protocol [1]. Five (5 g) of the aqueous extract was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hr. This was filtered and the filtrate was concentrated on a water bath to one-fourth of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the

precipitates was collected and washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed. The alkaloid content was determined using the formula: % alkaloid = final weight of the sample / initial weight of the extract x 100.

Column chromatography

Fractionation was carried out as reported in a literature [11], the crude extract derived from methanolic root extract was dissolved in n-Hexane and 6g of the weighed extract placed on top of 2x30cm silica gel column, The mobile phase were used as illustrated in the literature [12]. Compounds were eluted with the mobile phase. Collected fractions were checked by thin layer chromatography (TLC) and those with similar solvent front were recombined to yield four fractions. The collected 4 ml eluates was evaporated to near evaporator under vacuum, and dried further on a lyophilizer and the weights of the eluates was determined as the percentage weight of the eluates to the original weight of the sample used, using the formula below.

$$Percentage\ Yield = \frac{Weight\ of\ Extract}{Weight\ of\ Sample} \times 100$$

Acute toxicity of the plant (LD50)

The median lethal dose (LD50) of the plant extract was conducted in order to determine a suitable dose for the evaluation of the effects of the extract. This was done using up and down method [13,14]. This method involves the sequential dosing of a single animal with a test substance within a time interval of 48 hr. After the administration of the first dose, the next dose is determined by the outcome of the subsequent dose administered. If the animal survivals the sequent dose is adjusted upward, but when mortality is recorded, subsequent dose is adjusted downward. The adjustment is by a constant factor. Testing is terminated when the upper limit (2000 – 5000 mg/kg) have been reached without mortality or when the LD50 established from the test.

Induction of hyperlipidemia

The poloxamer 407 solution was prepared by dissolving 3 g in 30 ml of cold normal saline. Then kept overnight in a refrigerator to facilitate its homogeneity. The syringes and needles are kept in the fridge likewise. Hyperlipidemia was induced in wister albino rats by single intraperitoneal injection of freshly prepared solution of poloxamer 407 (1000 mg/kg) in physiological saline after overnight fasting for 24 hr by a method [12]. After 48 hr of induction hyperlipidemia and liver damage was confirmed to be 245 mg/dl of total cholesterole and 198 mg/dl of triglyceride, there was no reversal of hyperlipidemia using 1000 mg/kg body weight of poloxamer 407 for induction [12].

Groupings and treatment of rats

A total of 40 rats were randomly divided into 8 groups of 5 rats each. The rats weighing between 135 – 200g, group 4 – 8 were treated orally with crude and purified extract.

Group 1: Normal Rats control was given feed and water only. This served as the normal control group (NC)

Group 2: Hyperlipidemic Rats induced with P407 (1000 mg/kg b.wt) hyperlipidemic control (HYPER C)

Group 3: Hyperlipidemic Rats were treated with 10 mg/kg b.wt atorvastatin (HYPER + SD) standard control

Group 4: Hyperlipidemic Rats were treated with 200 mg/kg b.wt methanolic crude extract (HYPER + Crude)

Group 5: Hyperlipidemic rats, administered 200 mg/kg b.wt of fraction 1 (F1)

Group 6: Hyperlipidemic rats, administered 200 mg/kg b.wt of fraction 2 (f2)

Group 7: Hyperlipidemic rats, administered 200 mg/kg b.wt of fraction 3 (F3)

Group 8: Hyperlipidemic rats, administered 200 mg/kg b.wt of fraction 4 (F4)

Preparation of standard drugs

Atorvastatin was purchased in a tablet form at a strength of 10mg.Tablet was dissolve in 10ml of normal saline to desired concentrations and administered orally. This preparation was done daily at time of administration. Treatments were done with a standard drug (Atorvastation) 10 mg/kg for 21 days served as standard [15].

Determination of live weight

Albino rats in all groups were weighed in grams (g) on the day before induction of poloxamer 407, and weighed again on day 21 before sacrifice using weighing balance (sensitive electronic weighing balance with model number: Labtech BL20001). Mean weights from each replication was also recorded to establish the

growth rates, effect of poloxamer 407, and plant extract on body weight.

Collection and preparation of samples

At the end of 21 days of treatment, the rats were sacrificed by decapitation after using chloroform anesthesia and blood samples were collected from the heart puncture in a Ethylene diamine tetraacetic acid (EDTA) coated bottles for haematological parameters.

Haematological parameters

Packed cell volume (PCV)

The PCV is the volume of red blood cells (RBC) expressed as a fraction of the total volume of the blood. The microhaematocrit method was used [16-18]. Blood from EDTA bottle was allowed by capillary action to flow through the capillary tube and one end of the tube was sealed by flaming. It was then centrifuged at a speed of 3000 rpm for 10 min. The PCV was estimated using a microhaematocrit reader and expressed as percentage erythrocytes the blood contain.

Estimation of haemoglobin

The purpose of estimating haemoglobin is to determine the oxygen carrying capacity of blood. The results help in detecting diseases which causes deficiency or excess of haemoglobin. Acid haematin method (sahli) was used as described [17]. The graduated tube was filled up to the mark 20 with 0.1 N/HCL. Fill haemoglobin pipette exactly up to the 20 cu.mm mark with well mixed blood by gentle controlled sucking, holding the pipette horizontal. Empty the pipette into the acid in the tube gently without causing bubbles. Rinsed the pipette 2-3 times by drawing in and discharging the fluid. Mix the acid haematin solution in the tube and allow to stand for 5-10 min. Place the tube in the sahli comparator and start adding 0.1 N/HCL or distilled water drop by drop. Mix between each addition, continues till the colour matches that of the standard. Read the volume of solution in the graduated tube, and express as grams/100ml (g/dl) or as percentage.

Use of counting chamber (Haemocytometer) for cell counting

Haemocytometer consist of a counting chamber, a coverglass for the counting chamber and the diluting pipettes. Improved neubauer counting chamber was used as described [17]. Prepared the 1:20 dilution of the blood in the diluting fluid in a Thomas pipette or using unopette. Alternatively, add 0.02 ml of blood to 0.38 ml of diluting fluid. Charge the improved Neubauer counting chamber with the mixed diluting blood. Thomas pipette was used; first 3-5 drops were discarded before charging the chamber. Allow the cells to settle in a moist chamber for 3-5 min. Using 10 × objective of the microscope, locate the large corner square. The area of these squares is 4mm. Check that the cells are evenly distributed. Count the total number of white cells in the four large corner squares in the same pattern.

$$Total\ cell\ count = \frac{number\ of\ cell\ counted \times diluting\ factor \times converting\ factor}{area\ counted \times depth\ of\ chamber}$$

Diluting factor = 20
Converting factor = 10^6
Area counted = 4
Depth of chamber = 0.1
Note: Number of white cells counted in the four larger corner squares of the microscope during viewing is the number of cell counted.

Statistical Analysis

The results were expressed as mean ± standard deviation (SD). The data was analyzed by the analysis of variance (ANOVA) using SPSS program (version 16.0 SPSS Inc., Chicago, IL, USA). The p value less than 0.05 was considered as significant ($p<0.05$). The difference between the various extracts and animal groups was compared using the Duncan Multiple Range Test.

Result and discussion

Qualitative analysis on phytochemical constituents of powdered sample of root extract of Moringa oleifera

Qualitative analysis on phytochemical constituents of powdered sample of root extract of *Moringa oleifera* is shown in Table 1. The results revealed the presence of alkaloids, saponins, flavonoids, tannins and cardiac glycosides in powdered sample of the root of *Moringa oleifera*. The presence of the secondary metabolites, alkaloids, saponins and flavonoids which have been reported to be responsible for protecting the plants [19] and some associated with numerous physiological activities in mammalian cells in various studies, may explain

the various uses of *Moringa oleifera* for traditional medicine [20].

Table 1. Qualitative phytochemicals present in the root of methanol extract of *Moringa oleifera*

Phytochemical	**Root**
Alkaloids	+
Saponins	+
Flavonoids	+
Tannins	+
Cardiac glycosides	+
Terpenoides	-
Anthraquinones	-

Key: + = Present; - = Absent

Quantitative phytochemical constituents of methanolic root extract of Moringa oleifera

The quantitative phytochemical constituents of Methanolic root extract of *Moringa oleifera* is presented in table 2. The amount of saponins was 20.15%, flavonoids 3.94%, alkaloids 1.94%, tannins 1.10% and cardia glycoside 0.06%. The quantitative analysis of phytochemical constituents of methanolic root extract of *Moringa oleifera* revealed that the saponins is significantly ($p \leq 0.05$) higher than the alkaloids and flavonoids. This may be responsible for the observed antihyperlipidemia as it has been reported to possesse hyperlipidemic activity [1].

It was reported that some isoflavones (a type of flavonoid) increase resistance to LDL-c oxidation, like soybean isoflavones and genistein derivatives. Flavonoid acts as a cofactor of the enzyme cholesterol esterase, enhancing its activity. Flavonoids act on body lipid constituents like steroids and bile acids, and influence lipid metabolism. They increase bile acid excretion because cytochrome P- 450 enzymes bind some compounds to the bile acids and therefore decrease cholesterol level in the body [21]. A number of studies have shown that saponins when supplemented in diets reduce cholesterol content in blood and tissues in monogastric mammals, such as rats, gerbils and humans [22] Alkaloids possess anti-hyperglycemic and antilipidemic effects on blood glucose and serum lipid profiles in streptozotocin-induced diabetic rats [22].

Table 2. Quantitative phytochemical constituents of methanolic root extract of *Moringa oleifera*

Parameters	Root
Saponins %	20.15 ± 1.59
Flavonoids %	3.94 ± 0.06
Alkaloids %	1.94 ± 0.10
Tanins %	1.10 ± 0.66
Cardia glycoside %	0.06 ± 0.03

Values are mean ± SD for triplicate determination

Percentage yield of fractions of methanolic root extracts of Moringa oleifera from column chromatography

Percentage yield of fractions of methanolic root extracts of *Moringa oleifera* from column chromatography is presented in Table 3. Percentage yields of the four fractions (F1-F4) showed that fraction 4 had yields of (5.67%) with fraction 3 recording the highest yield (6%). Fraction 1 and 2 recorded the least of 5.33% and 5.17%. The total percentage yield was 22.11%.

Table 3. Percentage yield of fractions of methanolic root extract *Moringa oleifera* from column chromatography

Fractions	Weight (g)	Percentage Yield (%)
F1	0.32 ± 0.19	5.33
F2	0.31 ± 0.15	5.17
F3	0.36 ± 1.25	6.00
F4	0.34 ± 0.66	5.67
TOTAL	1.33 ± 0.03	22.11

Values are mean + SD for five determination

Effect of Moringa oleifera root extract and purified fractions on haematological parameters of Poloxamer 407 induced hyperlipidemic rats

The effect of *Moringa oleifera* root extract and purified fractions on haematological parameters of Poloxamer 407 induced Hyperlipidemic rats represented in table 4. The result shows a significant ($p \geq 0.05$) decrease in the levels of packed cell volume and haemoglobin (Hb) of hyperlipidemic control group when compared with all other groups and significant ($p \leq 0.05$) increased in the white blood cell (WBC) of

hyperlipidemic group when compared with all other group. The crude extract and the four fraction (F1-F4) significantly ($p \leq 0.05$) increased the packed cell volume (PCV) and haemoglobin (Hb) when compared to hyperlipidemic control group and significant ($p \leq 0.05$) decreased was recorded when compared to the normal control.
On day 1, when hyperlipidemia have been conformed before treatment after 48 hr of induction the PCV (32.21, 30.12, 31.45, 31.95, 33.23, 30.34, 32.64) were recorded in the hyperlipidemic group (HYPER C) to fraction 4 (HYPER + F4). Hb (10.70, 10.12, 10.50, 10.35, 10.80, 10.13, 10.30) were recorded in the hyperlipidemic group (HYPER C) to fraction 4 (HYPER + F4). WBC (13.90, 12.34, 11.13, 13.67, 12.00, 11.24, 10.92) were recorded in the hyperlipidemic group (HYPER C) to fraction 4 (HYPER + F4). These result shown significant ($p \leq 0.05$) decrease in PCV, Hb, and significant ($p \leq 0.05$) increase in WBC of hyperlipidemic group (induced and non-treated group) when compared to normal control group from day 1 to day 21 recorded in table 4 while there is significant ($p \geq 0.05$) increase in the PCV, Hb, and decrease in WBC recorded across the induced and treated group when compared to hyperlipidemic group.

The administration of 1000 mg/kg body weight of P407 resulted in splenomegaly from red pulp expansion due to the infiltration of macrophages, which contained phagocytized lipid [23]. PCV provides information on the general pathophysiology of the blood and reticuloendothelial system. It increases circulating inflammatory monocytes counts and renders the cells more prone for emigration into atherosclerotic lesions [23]. In these studies, the reduction in the levels of PCV observed in induced not treated groups may be due to damage to the entire reticuloendothelial system by accumulation of triglycerides and other fats. However, there was a restoration of the levels of PCV and Hb and WBC in all the induced treated groups on administration of the extracts and atorvastatin compared to the hyperlipidemic control.

Table 4. Effect of *Moringa oleifera* root extract and purified fractions on haematolagical parameters of Poloxamer 407 induced hyperlipidemic rats

Group N = 8	PCV (%)	Hb (g/dl)	WBC (L)
NC	43.17 ± 1.68^{d}	14.06 ± 0.37^{c}	5.93 ± 0.56^{a}
HYPER C	29.51 ± 1.06^{a}	9.66 ± 0.25^{a}	17.90 ± 1.07^{d}
HYPER + SD	38.41 ± 2.47^{b}	12.23 ± 0.60^{b}	13.35 ± 0.43^{c}
HYPER + Crude (200mg/kg BW)	37.81 ± 1.64^{b}	12.10 ± 0.89^{b}	14.09 ± 1.31^{c}
HYPER + F1 (200mg/kg BW)	36.98 ± 0.69^{b}	12.08 ± 0.78^{b}	12.30 ± 0.77^{c}
HYPER + F2 (200mg/kg BW)	40.71 ± 2.41^{c}	13.50 ± 0.60^{b}	11.01 ± 0.72^{b}
HYPER + F3 (200mg/kg BW)	39.08 ± 2.50^{c}	13.56 ± 0.57^{b}	12.77 ± 0.87^{c}
HYPER + F4 (200mg/kg BW)	36.98 ± 0.69^{b}	13.50 ± 0.60^{b}	11.01 ± 0.72^{b}

Values are means ± SD of five determinations. Values with different superscripts in the column are significantly different ($p<0.05$). NC: Normal Control Rat, HYPER C: Hyperlipidemic Control Rat, HYPER + SD: Hyperlipidemic + Standard Drugs (atorvastatin 10 mg/kg), HYPER + Crude: Hyperlipidemic Rat + Crude Extract (200 mg/kg), Hyper + F1: Hyperlipidemic Rat + Fraction 1 (200 mg/kg), Hyper + F2: Hyperlipidemic Rat + Fraction 2 (200 mg/kg), Hyper + F3: Hyperlipidemic Rat + Fraction 3 (200 mg/kg), Hyper + F4: Hyperlipidemic Rat + Fraction 4 (200 mg/kg). PCV: Packed Cell Volume, Hb: Haemoglobin, WBC: White Blood Cell.

Effect of root extract of moringa oleifera and purified fractions on body weight of Poloxamer 407 in Induced Hyperlipidemic Rats

The effect of administration of root extract of *Moringa oleifera* and purified Fractions on body weight of poloxamer (407) induced hyperlipidemic rats is shown in Table 5. The result reveals that hyperlipidemic control rats shows a significant ($p \geq 0.05$) decrease in body weight when compared to all other groups. The treated hyperlipidemic groups shows that all the

extracts including standard drug significantly ($p \leq 0.05$) increased body weight when compared to hyperlipidemic control.

The initial body weight of Albino rats before induction of poloxamer 407 (125.8, 175.2, 167.2, 170.8, 153.6, 190.6, 141.6, 150.8) were recorded in the normal (NC) and hyperlipidemic group (HYPER C) to fraction 4 (HYPER + F4). Final body weight of albino rat on day 21. After treatment completed (135.8, 150.4, 148.8, 157.0, 138.2, 174.6, 129.6, 136.0) were recorded in the normal (NC) and hyperlipidemic group (HYPER C) to fraction 4 (HYPER + F4).

These result shown significant ($p \leq 0.05$) increase in the normal control group (non-induced and none treated) and significant ($p \leq 0.05$) decrease in the hyperlipidemic group (induced and non-treated group) from day 1 to day 21 recorded in table 5. While there was a significant ($p \geq 0.05$) increase in the induced and treated group of plant extract (crude and purified fractions) and atorvastatin (Standard group).

Administration of 1000 mg/kg body weight of P407 resulted in significant reduction in body weight (b.wt) observed in hyperlipidemic rats could be as a result of damages done to the liver and kidney by high level of triacylglyceride and other fats, thus impairing the functions of the organs [23] [24]. This probably may lead to reduction in the food intake and degradation of structural proteins. However, hyperlipidemic treated groups shows a significant ($P \leq 0.05$) increase in body weight compared to hyperlipidemic control. The increase in body weight may be due to increasing in glucose uptake in peripheral tissues or inhibition of catabolism of fat and structural protein.

Table 5. Effect of root extract of *moringa oleifera* and purified fractions on body weight of P407 in induced hyperlipidemic rats

Group N = 8	Initial BW (g)	Final BW (g)	Difference BW (g)	% Difference BW (%)
NC	125.8	135.8	10.0	8.09 ± 2.659^{d}
HYPER C	175.2	150.4	24.8	-14.15 ± 4.209^{a}
HYPER + SD	167.2	148.8	18.4	-11.00 ± 2.370^{b}
HYPER + CRUDE (200mg/kg BW)	170.8	157.0	13.6	-796 ± 2.886^{c}
HYPER + F1 (200mg/kg BW)	153.6	138.2	15.4	-10.02 ± 1.714^{c}
HYPER + F2 (200mg/kg BW)	190.6	174.6	16.0	-8.39 ± 1.240^{c}
HYPER + F3 (200mg/kg BW)	141.6	129.6	12.0	-8.47 ± 1.442^{c}
HYPER + F4 (200mg/kg BW)	150.8	136.0	14.8	-9.81 ± 4.964^{c}

Values are means ± SD of five determinations. Values with different superscripts in the column are significantly different ($P<0.05$). NC: Normal Control Rat, HYPER C: Hyperlipidemic Control Rat, HYPER + SD: Hyperlipidemic + Standard Drugs (atorvastatin 10mg/kg), HYPER + Crude: Hyperlipidemic Rat + Crude Extract (200mg/kg), Hyper + F1: Hyperlipidemic Rat + Fraction 1 (200mg/kg), Hyper + F2: Hyperlipidemic Rat + Fraction 2 (200mg/kg), Hyper + F3: Hyperlipidemic Rat + Fraction 3 (200mg/kg), Hyper + F4: Hyperlipidemic Rat + Fraction 4 (200mg/kg). BW: Body Weight.

Conclusions

This study shown the presence of the secondary metabolites, alkaloids, saponins and flavonoids, tannins and cardiac glycoside which have been reported to be responsible for protecting the plants also is associated with numerous physiological activities in mammalian cells. These may explain the various uses of *Moringa oleifera* for traditional medicine. The quantitative analysis of phytochemical constituents of methanolic roots extract of *Moringa oleifera* revealed that the saponins is significantly increase ($p \leq 0.05$) higher than the alkaloids and flavonoids, tannins and cardiac glycoside This may be responsible for the observed antihyperlipidemia as it has been reported to possess antihyperlipidemic activity.

In these studies, the reduction in the levels of PCV observed in induced not treated groups may be due to damage to the entire reticuloendothelial system by accumulation of triglycerides and other fats. However, there was a restoration of the levels of packed cell volumn (PCV) and Haemoglobin (Hb) and White blood cell (WBC) in all the induced treated groups on administration of the extracts and atorvastatin compared to the hyperlipidemic control. Administration of 1000mg/kg body weight of P407 resulted in significant reduction in body weight (b.wt) observed in hyperlipidemic rats could be as a result of damages done to the liver and kidney by high level of triacylglyceride and other fats, thus impairing the functions of the organs. This study supports the popular sayings about the tradomedicinal use of *Moringa oleifera* in the treatment of cardiovascular and other related diseases. However it can be used in optimal concentrations to sustain animals infected with abnormal lipid in the absence of synthetic chemical drugs to prevent death to cardiovascular diseases.

Conflict of interest

Authors declared no conflict of interests.

References

[1] Fahal EM, Rani AMB, Aklaku MD, Chanu TI, Saharan N. Qualitative and Quantitative Phytochemical Analysis of *Moringa oleifera* pods. International Journal of Current Microbiology and Applied Sciences 2018;7(5):657-65.

[2] Oboma Y, Asuqwo EE. Histology, Hyperglycemia and Dyslipidemia Evaluation of Aqueous Extract of *Moringa oleifera* leaves on Adult Wistar Rat. International Journal of Pharma Sciences and Research 2015;6(9):1234-40.

[3] Radha K, Syed M, Rao DS. Evaluation of Antihyperlipidemic Activity of Methanolic Extract of *Carica papaya* seeds on wistar rats. Indo American Journal of Pharmaceutical Sciences 2014;1(5):328-32.

[4] Chaudhary HR, Brocks DR. The single Dose Poloxamer 407 Model of Hyperlpidemia. Systemic Effects on Lipids Assessed using Pharmacokinetic Methods and its Effects on Adipokines. J Pharm Pharmaceut Sci 2013;16(1):65-73

[5] Hill RD, Smith RB. Examination of the Extremities: 3rd edition; The history, physical, and laboratory examination; Boston, Butter Worths 1990.

[6] Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research 2010;4(2):104-11.

[7] Sofowara EA. Medicinal Plants and Traditional medicine in Africa. 1st edition. John Wiley and Sons 1993;114-191:202-9.

[8] Trease GE, Evans WC. Pharmacognosy. 16th Edition, Saunders Elsevier, Edinburgh London 2009;135-137.

[9] Adegoke AA, Iberi PA, Akinpelu DA, Aiyegno OA, Mboto CI. Study on phytochemical Screening and antimicrobial potential of *Phyllanthus amarus* against multiple antibiotics resistance bacteria. International Journal of Applied Research in Natural Products 2010;3 (3):6-12.

[10] AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists. 14th edition, Arlington, Virginia 1984;114-9.

[11] Seelinga M, Popesco R, Seephohonkai P, Singhube J, Giessrigl B, Unger C. Fractionation of an extract of *Pluchea oderata* seperate a property indicative for the induction of cell plasticity from one that inhibit neoplastic phenotype: Evidence-Based Complementary and Integrative Medicine, 2012; ID 70192.

[12] Bawa I, Ocheleka MI, Itolo O. Effect of methanolic crude and purified root extracts of *Moringa oleifera* on liver enzymes activities, Kidney function and Histopathology in poloxamer 407-induced hyperlipidemic albino rats. International Journal of Modern Science and Technology 2020;5(5):128-36.

[13] OECD. Organisation for Economic Cooperation and development. Guild lines for the testing of chemical, 423.

[14] Chinedu E, Arome D, Ameh SF. A new Method Determining Acute Toxicity in animal Models. Toxicology International 2013;20(3):224-6.

[15] Inalegwu B, Ornuga G, Etim, EE. Effect of the Aqueous Extracts of *Grewiavenusta*

leaves on poloxamer 407 Induced Hyperlipidemc rats. International Journal of science and Research. 2018;7(5):398-404.

[16] Cheesbrough M. District Laboratory practice in Tropical countries: Tropical Health Technology. Cambridge University Press. 2006; pp. 333-363.

[17] Ochei JO, Kolhatkar AA. Medical Laboratory Science: Theory and Practical. Tata McGraw- Hill Publishing Company Limited. 2008; pp. 169-287.

[18] Udo EJ, Manji MM, Ikpa TF, Imandeh NG. In-Vivo Evaluation of Trypanocidal Efficacy of *Phyllanthus amarus* Root Extracts in mice experimentally infected with Trypanosoma congolense. International Journal of Modern Science and Technology 2020;5(2):65-71.

[19] Vergara-Jimenez, M, Almatrafi MM, Fernandez ML. Bioactive Component in *Moringa oleifera* Leaves Protect against Chronic Diseases. Antioxidants 2017;6:91. doi: 10.3390/antiox6040091.

[20] Kumar ARI, Lavarasa T, Jayachandra M, Decaraman P, Aravindhan N, Padmanabhan G, Krishnan MRV. Phytochemical investigation on a tropical plant, *Syzygium cumini* from Kattuppalyam, Erode District, Tamil Nadu, Chupandia South India. Pakistan Journal of Nutrition 2009;8:83-5.

[21] Boham BA, Kocipal-Abyazan R. Flavonoids and condensed tannins from leaves of *Haiwaiian Vaccinium vaticulatum* and *Vaccinium calycinium*. Asian Journal of Pacific Science 1974;48:458-63.

[22] Dineshkumar B, Analava M, Manjunatha, M. Antidiabetic and hypolipidemic effects of mahanimbine (carbazole alkaloid) from murraya koenigii (rutaceae) leaves. International Journal of Phytomedicine 2010;2(11):22-30.

[23] Asheneni VP. Anti-Hyperlipidemic potential of *Vitex doniana* Ethanol Extracts on Poloxamer 407 Induced Hyperlipidemic and Normal Rats. M.Sc. Thesis Report. Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria. 2014.

[24] Subhashi D, Singh J. Safety Assessment of Poloxamer 101 – 407, 405 Benzoate, and used Poloxamer 182 Dibenzoate as used in Cosmetic. International Journal of Toxicology 2018;27(2):93-128.

****

