Original Research Article

Effects of seed of different mango cultivars in Triton X-100 Induced Hyperlipdemic rats

ABSTRACT

AIM: Hyperlipidemia is the most prevalent indicator for susceptibility to atherosclerotic heart diseases. The present study investigated the hypolipidemic potential of *Mangifera haden, Mangifera piri*e and combined extracts of *Mangifera haden*and *Mangifera pirie*seeds in Triton X-100 induced hyperlipidemic rats.

Study design: Before the starting of the experiment, the animals were acclimatized to the laboratory conditions for a period of seven days. At the end of the acclimatization period, each rat was weighed and randomly divided into twelve groups of five animals each, comprising of test animals and control groups. Proximate analysis, toxicity studies, lipid profile parameters were determined in the study.

Place and Duration of Study: The research study took place in the animal house of Biochemistry Department, University of Nigeria, Nsukka from July 2020 to August 2022.

Methodology: Acute toxicity study was determined following the method described by Lorke, 1983. Proximate composition of the whole samplewere determined according to the method described by AOAC, 2005. Hyperlipidemia was induced by single intraperitoneal injection of Triton X-100 (100 mg/kg). The concentration oftotal cholesterol was determined using the method of Abell *et al*., (1952). The concentration of Low Density lipoprotein (LDL) was determined using the method of Friedwald *et al.* (1972). The concentration of High Density Lipoprotein (HDL) was determined using the method of Toth *et al*. (2013). The concentration of triacylglycerol was determined using the method of Otvos (1999). Atherogenic index of extracts was determined using the method of Chakraborty *et al.* (2012). The percentage protection for hyperlipidemia was determined using the method of Dhandapani, 2007.

Results: Proximate composition of *Mangifera haden* and *Mangifera pirie*seed extracts revealed that the (%) values of protein, lipid, crude fibre, ash, moisture, and carbohydrate are (4.82, 17.18, 4.29, 2.77, 8.39, 62.55) and (3.81, 20.20, 3.0, 1.0, 34.0, 37.99) respectively. *Mangifera haden* and *Mangifera pirie*were administered at different doses (200 mg, 300 mg, 500 mg) of the extracts per kilogram body weight of the animals while combined extract of *Mangifera haden* and *Mangifera pirie* were administered at 300 mg and 500 mg of the extract per kilogram body weight of the animals in Triton –Induced hyperlipidemic rats. Simvastatin and Atorvastatin were used as reference standards. Simvastatin was found to be an effective drug to Atorvastatin in lowering lipid profiles. The statistical analyses were carried out using one way ANOVA followed by Dunnett's Post Hoc Multiple Comparisons test. *Mangifera haden, Mangifera pirie*andcombined extracts of both species show significant decrease (($P = 0.05$) in the levels of serum Total Cholesterol, triacylglycerols, VLDL-C, LDL-C, Atherogenic index (A.I) and significant increase ($P = 0.05$) of serum HDL-C against Triton-Induced hyperlipidemic rats. The result also suggest that at 200 mg/kg *Mangifera pirie* and 300 mg/kg *Mangifera haden* body weight concentrations are an excellent lipid lowering agent.

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Conclusion: Single administration of either *Mangifera haden* or *Mangifera pirie* effectively suppressed the Triton-Induced hyperlipidemia in rats than the combined extracts. The organic extracts (*Mangifera haden* or *Mangifera pirie)* exhibited quite hypolipidemic potential when compared with one of the reference drugs, Atorvastatin which indicates that the organic extracts could be explored as an alternative therapeutic agent in the treatment of hyperlipidemia.

KEYWORDS: Hyperlipidemia, *Mangifera haden, Mangifera pirie,* Atherogenic index, Simvastatin and Atorvastatin

1.0 INTRODUCTION

Mango (*Mangifera indica*) belongs to the family Anacardiaceae and is a native of Southern Asia, especially Burma and Eastern India [Akin-Idowu et al., [1]. It is the most choicest fruits and occupies a prominent place among the fruits of the world [2]. Now, Mango is cultivated in many tropical regions and distributed widely in the world. There are as many as 1365 varieties of mango all over the world [2]. But in Nigeria, there are only three common varieties in Nigeria. They are *Mangifera haden*, *Mangifera pirie*, and *Mangifera turpentine*.

Mangifera haden is known asIndia big or purple/red/yellow mango. These are mangoes whose fruits are ready for harvesting when they are fully matured and ready for consumption when they are riped [Shah *et al*., [3]. They are sour in taste when still green but turn sweet with characteristics aroma and flavour when riped [3].

Mangifera pirie is reffered to as German mango or green mango. These are mangoes whose fruits are ready to be harvested and consumed when they are still green and have unique nutty taste, with little or no sour taste[Torres-Leon *et al*., [4]. They may be mature but not yet softened. Some cultivars may be eaten when they are half-ripe. A green mango have a high water content, the amount of carbohydrate by weight makes the glycemic load score low [4].

Mango seed is a single flat oblong seed that can be fibrous or hairy on the surface depending on the cultivar [leanpolchareanchai*et al*., [5]. Mango seed consists of a tenacious coat enclosing the kernel. During the processing of mango, by-products such as peel and kernel are generated. Kernels take up about 17-22% of the fruit. The major components of mango seed are starch, fat, and protein [5]. The oil of mango seed kernel consist of about 44-48% saturated fatty acids (majority staearic) and 52-56% unsaturated [4]. Mango seed kernels have a low content of protein but they contain the most of the essential amino acids, with highest values of leucine, valine and lysine [Kumar *et al*., [6]. Mango seed kernels were shown to be a good source of polyphenols, phytosterols as campesterol, sitosterol and tocopherols. In addition, mango seed kernel could be used as a potential source for functional food ingredients, antimicrobial compounds and cosmetic due to its high quality of fat and protein as well as high levels of natural antioxidants [Kumar *et al*., [6].

Hyperlipidemia is a heterogenous group of disorders characterized by abnormally elevated levels of lipids and lipoproteins in the bloodstream (Tung-Ting Sham *et al.,* [7]. In fact, it has become one of the major killers around the world. It was projected that by 2030, there will be about 23.3 million CVD deaths world-wide (Tung-Ting Sham *et al.,*[7]. Also, CVD has imposed great medical burden to different societies around the world. The global burden of CVD is beginning to be viewed as high as infectious diseases. Apart from CVD, hyperlipidemia is also closely associated with diabetes, insulin resisitance and obesity.

Statins are known collectively as 3-hydroxyl-3 –methylglutaryl-CoA(HMG-CoA) reductase inhibitors used to lower lipid levels and also reduce the risk of cardiovascular diseases [Sanjai, S. [8]. They are drugs used to lower serum cholesterol as a means of reducing the risk of

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cardiovascular disease including myocardial infarction and stroke. These drugs include simvastatins, atorvastatins, provastatin, rosuvastatin, fluvastatin and lovastatin etc

Simvastatin is a lipid – lowering drug derived synthetically from the fermentation product of *Aspergillus terreus*[9]. It competitively inhibit the enzyme hydroxymethyl-glutaryl-coenzyme A (HMG-CoA) Reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid [Liu et al., [10]. This is the third step in the sequence of metabolic reactions involved in the production of several compounds in lipid metabolism and transportation of cholesterol, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) [10].It belongs to the statin class of medications, which are used to lower the risk of cardiovascular disease and manage abnormal lipid levels by inhibiting the endogenous production of cholesterol in the liver [Qui *et al*., [11].

Atorvastatins are lipid lowering agents, which act by inhibiting HMG-CoA reductase, which catalyses the conversion of HMG-CoA to mevalonate, an important rate –limiting step in cholesterol biosynthesis [Qui *et al*., [11]. They are used in the treatment of hyperlipidemia, atherosclerosis or cardiovascular complications like coronary heart disease [Ye *et al*., 12]. It inhibit endogenous production of cholesterol in the liver which are the first-line treatment options for dyslipidaemia [Yahya *et al*., [13].

There is a dramatic increase in the number of people living with atherosclerosis and cardiovascular diseases in our society due to major changes in the type ofdiets consumed, reduction in physical activity and increase in overweight and obesity. Hyperlipidemia can be treated or rather managed by the use of synthetic drugs such as statin, fibrates, niacin, bile acid sequestrants etc. These drugs are costly and exhibit high undesirable side effects. Thus, natural products from medicinal plants need to be investigated for hypolipidemic activities.

2.0 Material and Methods

2.1 Plant Material

The plant materials are the seeds of *Mangiferahaden* and *Mangifera pirie*.

2.2 Methods

2.2.1 Collection, Preparation and Extraction of Mango Seeds

The plant materials, *Mangifera pirie*and *Mangifera haden* seeds were collected from Mile One market, Port-Harcourt, Rivers State, Nigeria. The seed samples were identified and authenticated by Mr. Alfred Ozioko of the Bioresources Development and conversation Programme (BDCP), Nsukka. The specimen (*Mangifera haden*) voucher number is InterCEDD/908. (International Centre for Ethomedicine and Drug Development, InterCEDD). They were washed and air-dried. The kernel and kernel sheathes were removed manually from the seeds. Fresh kernel seeds and kernel sheathes were chopped and blended with distilled water at a ratio of sample/water of 1:3 (W/V). After filtration, the filtrate was lyophilized with a freeze –dryer. The extract was then stored in an airtight container in a refrigerator until use.

2.2.2 Animal Preparation

Sixty (60) Adult albino wistar rats were used for the study. The animals were acclimatized to the laboratory conditions for a period of seven (7) days. The animals were grouped and housed in aluminium cages and maintained at an ambient temperature (25 ±2^{0}C) and relative humidity (40-60%), with 12/12 hours of light and dark cycle in a well –ventilated animal house. Bedding material (saw dust) was removed and replaced as often as necessary to keep the animals clean

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and dry. The animals were fed with commercial grower's mash feed and water provided *ad libitum.* At the end of the acclimatization period, they were weighed and randomly divided into twelve (12) groups of five animals each, comprising of test animals and control groups.

2.2.3 Toxicity Studies

2.2.3.1 Acute Toxicity Test

The mean lethal dose (LD_{50}) for acqueous extract of the seeds of *Mangifera haden* and *Mangifera pirie*were determined following the method described by Lorke (1983)[14]. Eighteen(18) experimental animals (albino mice with weight range 30 – 35g) were used for the study. This was carried out in two phases:

Phase 1: Three groups of animals containing three albino mice each were given 10, 100 and 1,000 mg extract/kg body weight respectively, orally via a cannula. The animals were closely observed for 24 hour for lethality or any behavioural changes.

Phase 2: However, after 24 hour, three groups of three wistar rats each were administered higher doses of 1500, 2900 and 5000 mg extract/kg body weight orally. They were also observed for 24 hour for any behavioural changes or death.

The geographic mean of the least dose that killed the mice and the highest dose that did not kill the mice was taken as the median lethal dose.

2.2.4 Proximate Analysis of the Seeds of *Mangifera haden* and *Mangifera pirie*

The whole sample of *Mangifera haden* seeds were analyzed for proximate composition using standard analytical methods.

Proximate Analysis

The proximate analysis of whole sample of *Mangifera haden* seeds was carried out using the method of Association Official of Analytical Chemists.

2.4.1. Determination of Moisture Content

This method is based on moisture evaporation. Here the aluminum dishes were washed dried in oven and in desiccators for cooling. The weight of each dish was taken. 5.0 g of ground samples were weighed into a sterile aluminium dish, weight of the dish and weight of un-dried sample (in triplicate) were taken. This was transferred into an oven set at 80°C for 2 hrs and at 105°C for 3

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hrs respectively. This was removed and cooled in desiccators. Then the weight was measured using a measuring scale balance. It was transferred back into the oven for another one hour and then reweighed. The process continued until a constant weight was obtained. The difference in weight between the initial weight and the constant weight gained represents the moisture content.

Calculation: The loss in weight multiplied by 100 over the original weight is percentage moisture content.

Moisture content (g/100 g) = loss in weight ((W2-W3)/(W2-W1)) x 100

W1= initial weight of empty crucible, W2= weight of crucible + food before drying,

W3 = final weight of crucible + food afterdrying.,

% Total solid (Dry matter) (%) = 100- moisture [15]

2.4.2. Ash Content

The ash represents the inorganic component (minerals) of the sample after all moisture has been removed as well as the organic material. The method is a destructive approach based on the decomposition of all organic matter such that the mineral elements may be lost in the process. Twenty grams (20 g) of each of the samples were weighed into a clean dried and cooled platinum crucible. It was put into a furnace set at 550 °C and allowed to blast for 3 hrs. It was then brought out and allowed to cool in desiccators and weighedagain.

Calculation: Percentage weight is calculated as weight of ash multiplied by 100 over original weight of the samples used.

Ash content = (weight of ash/ weight of original sample used) x100.

Loss in weight ((W3-W1)/(W2-W1)) x 100

Where W1 = weight of empty crucible, W2 = weight of crucible + food before drying and or ashing, W3 = weight of crucible + ash. [15]

2.4.3. Determination of Lipid Content

The method employed was the soxhlet extraction technique described by [15]. 15 g of the samples were weighed and carefully placed inside a fat free thimble. This was covered with

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cotton wool to avoid the loss of sample. Loaded thimble was put in the Soxhlet extractor, about 200 ml of petroleum ether were poured into a weighed fat free soxhlet flask and the flask was attached to the extractor. The flask was placed on a heating mantle so the petroleum ether in the flask refluxed. Cooling was achieved by a running tap connected to the extractor for at least 6hrs after which the solvent was completely siphoned into the flask. Rotary vacuum evaporator was used to evaporate the solvent leaving behind the extracted lipids in the soxhlet. The flask was removed from the evaporator and dried to a constant weight in the oven at 60°C. The flask was then cooled in a desiccator and weighed. Each determination was done in triplicate. The amount of fat extracted was calculated by difference.

Ether extracts (100g) dry matter = (weight of extracted lipids/ weight of dry sample)x100

2.4.4. Protein Determination

Total protein was determined by the kjeldahl method by [15]. The analysis of a compound of its protein content by kjeldahl method is based upon the determination of the amount of reduced nitrogen present. About 20 g of the samples were weighed into a filter paper and put into a kjedahl flask, 10 tablets of Na_2SO_4 were added with 1 g of $CuSO_4$ respectively. Twenty millilitre (20 ml) of conc. H_2SO_4 were added and then digested in a fume cupboard until the solution becomes colourless. It was cooled overnight and transferred into a 500 ml flat bottom flask with 200 ml of water. This was then cooled with the aid of packs of ice block. About 60 to 70 ml of 40% of NaOH were poured into the conical flask which was used as the receiver with 50 ml of 4% boric acid using 3 days of screened methyl red indicator. The ammonia gas was then distilled into the receiver until the whole gas evaporates. Titration was done in the receiver with 0.01M HCl until the solution becomes colourless.

Calculation: The percentage protein is calculated as follows:

$$\% \text{ Protein} = \frac{1.4 \times \text{D.F} \times \text{Normality of acid} \times 100 \times 6.25}{\text{Original weight of Sample (mg)}}$$

Where:

1.4 = Nitrogen equivalent of 1.0ml of 0.1N of 0.1N H_2SO_4

2.4.5. Crude Fiber

The bulk of roughages in food is referred to as fiber and is estimated as crude fiber. Twenty grams (20 g) of the different samples were defatted with diethyl ether for 8 hrs and boiled under reflux for exactly 30 minutes with 200 ml of 1.25% H_2SO_4. It was then filtered through cheese

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cloth on a flutter funnel. This was later washed with boiling water to completely remove the acid. The residue was then boiled in a round bottomed flask with 200 ml of 1.25% sodium hydroxide (NaOH) for another 30 minutes and filtered through previously weighed couch crucible. The crucible was then dried with samples in an oven at 100°C, left to cool in a desiccator and later weighed (C_1). This was later incinerated in a muffle furnace at 600°C for 2 to 3 hrs and later allowed to cool in a desiccator and weighed (C_2). [15]

The loss in weight of sample on incineration = $C_1 - C_2$

$$\% \text{ Crude Fiber} = \frac{C_1 - C_2 \times 100}{\text{Weight of the sample}}$$

2.4.6. Carbohydrate Determination: Carbohydrate composition was estimated by the difference method described by [16]. The sum of the percentage moisture, ash, crude protein, and crude fibre was subtracted from hundred (100).

Total Carbohydrate (%) = 100- (protein (%) + Moisture (%) + Ash (%) + Fibre (%) + Fat (%)).

2.4.7. Calorific Value Determination

Energy or Caloric Value (KJ/100g) = (Protein X 16.7) + (Lipids X 37.7) + (Carbohydrate X 16.7) This was determined using the method of [17].

2.4.8 Induction of Hyperlipidemia

Hyperlipidemia was induced in wistar albino rats by single intraperitoneal injection of freshly prepaid solution of Triton X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 hours.

2.4.9 Experimental Design

Group 1: Feed + water (Normal Control)

Group 11: Feed + Water + Triton X-100

Group IIIA: Feed + Water + Triton X-100 + Atorvastatin (10 mg/kg)

Group IIIB: Feed + Water + Triton X-100 + Simvastatin (10 mg/kg)

Group IV: Feed + Water + Triton X-100 + 200 mg/kg of *Mangifera pirie* extract

Group V: Feed + Water + Triton X-100 + 300 mg/kg of *Mangifera pirie* extract

Group VI: Feed + Water + Triton X-100 + 500 mg/kg of *Mangifera pirie* extract

Group VII: Feed + Water + Triton X-100 + 200 mg/kg of *Mangifera haden* extract

Group VIII: Feed + Water + Triton X-100 + 300 mg/kg of *Mangifera haden* extract

Group IX: Feed + Water + Triton X-100 + 500 mg/kg of *Mangifera haden* extract

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Group X: Feed + Water + Triton X-100 + 300 mg/kg of (*Mangifera pirie* + *Mangifera haden)* extract

Group XI: Feed + Water + Triton X-100 + 500 mg/kg of (*Mangifera pirie* + *Mangifera haden)* extract

2.4.10 Administration of Extracts

The different doses of each sample were given orally by gavage using intubation cannular. The administration was carried out once daily for 3 days. At the end of the treatment period, the animals were allowed to fastfor 18 hours before sacrifice. The serum was used for further studies.

2.4.11 Collection and Preparation of the Blood Sample

On the 4th day after fasting for 18 hours,the animals were anesthetized with chloroform and blood was withdrawn by cardiac puncture. Serum was separated by centrifugation of blood at 3000rpm/10 minutes for estimation of Biochemical parameters. The clear supernatant (serum) was aspirated using a Pasteur pipette and kept for further studies. Serum total cholesterol, HDL-C, and Triglycerides were estimated spectrophotometrically using ready-use-test kits from Randox. LDL-C was estimated using Friedwald formula.

2.5 Lipid Profile Tests

2.5.1Determination of Total Cholesterol Concentration

The concentration of total cholesterol was determined using the method of Abell *et al.* [18].

Principle

Cholesterol concentration was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxidase and 4-aminoantipyrine in the presence of phenol and peroxidise.

Test procedure

Three (3) test tubes were set up in a test tube rack and labeled blank, standard and sample respectively. To the blank, was added 10 µl distilled H_2O, 10ul standard specimen to the standard test tube and 10µl sample (serum) to the sample test tube. To each of these test tubes was added 1000 µl of the cholesterol reagent. It was thoroughly mixed and incubated for 10 minute at room temperature (20-25^{0}C). The absorbance of the sample (A sample) against the blank was taken within 60 minute at 500nm.

Conc. of cholesterol in sample (mg/dl) =

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$$\frac{\Delta A_{sample} x}{\Delta A_{standard}} \text{ conc. of standard}$$

2.5.2 Determination of Low-Density Lipoprotein Concentration

The concentration of low density lipoprotein (LDL) was determined using the method of Friedwald *et al.* [19].

Calculation:

LDL-C (mg/dl) = Total Cholesterol –High Density Lipoprotein – Triglyceride/5

2.5.3 Determination of High-Density Lipoprotein Concentration

The concentration of high-density lipoprotein (HDL) was determined using the method of Toth *et al.* [20].

Principle:

LDL and VLDL (low and very low density lipoproteins) are precipitated from serum by the action of a polysaccharide in the presence of divalent cations. Then, high density lipoproteins (HDL) present in the supernatant is determined.

Procedure:

The precipitant solution (0.1ml) was added to 0.3 ml of the serum sample and mixed thoroughly and allowed to stand for 15 minute. This was centrifuged at 2,000 x g for 15 minute. The cholesterol concentration in the supernatant was determined.

Calculation

Concentration of HDL cholesterol in sample

$$= \frac{\Delta \text{Asample x concentration of Standard}}{\Delta \text{ Astandard}}$$

Where concentration of the standard = 52.5 mg/dl

2.5.4 Determination of Triacylglycerol Concentration

The concentration of triacylglycerol (TAG) was determined using the method of Otvos [21].

Clinical significance:

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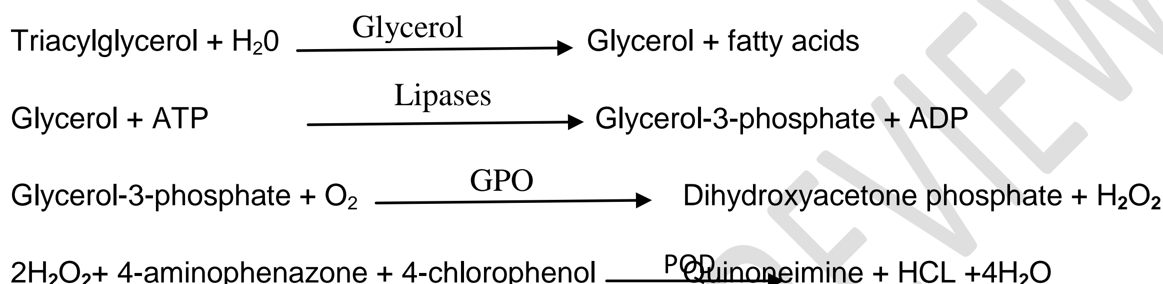
Triacylglycerols measurements are used in the diagnosis and treatment of diseases involving lipid metabolism and various endocrine disorders e.g diabetes mellitus, nephrosis and liver obstruction.

Principle:

The triacylglycerols are determined after en natic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.



Method: A quantity of the sample (0.1 ml) was pipetted into a clean labelled tube and 1.0 ml of trichloroacetic acid (TCA) was added to it, mixed and then centrifuged at 250 rpm for 10 minute. The supernatant was decanted and reserved for use. The assay procedure was carried out as shown below. Three test tubes were set up and labelled as blank, standard and sample. To the blank test tube was added distilled water (0.5 ml), TCA (0.5 ml) and reagent mixture (1 ml). To the standard tube was added, standard solution (0.5 ml), TCA (0.5 ml) and reagent mixture (1 ml) while the sample tube was filled with 1 ml of the supernatant and 1ml of the reagent mixture. The mixtures were allowed to stand for 20 minute at 25 ^{0}C and the absorbance of the sample and standards read against the blank was taken at 540 nm.

Calculation: The concentration of triacylglycerol in serum was calculated as follows:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{standard conc.}}{1} \text{ (mg/dl)}$$

Where concentration of the standard = 196 mg/dl

2.5.6 Determination of Atherogenic Index

Atherogenic index of aqueous extractwas determined using the method of Chakraborty *et al.* [22].

Atherogenic Index = <u>TC - Total serum HDL-C</u>

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Total serum HDL-C

2.5.6Determination of Percentage Protection of Aqueous Extract

The % protection of aqueous extract from hyperlipidemia was determined using the method of Dhandapani, [23].

$$\% \text{ Protection} = \frac{\text{AI of control} - \text{AI of treated group}}{\text{AI of Control}} \times 100$$

2.5.7 Determination of VLDL- Cholesterol(Tietz, [24]

$$\text{VLDL-C} = \frac{\text{Triglyceride}}{5}$$

Statistical Analysis

The results obtained from various analyses were analyzed using a statistical program SPSS/PC+, version 16.0. The results were expressed as Mean ± SD. A one-way ANOVA was employed for comparison among the twelve groups followed by Dunnet's Post-Hoc Multiple Comparisons tests. $P<0.05$ was considered as statistically significant.

3.0. Results

3.1. Mean Lethal Dose (LD_{50}) of Aqueous Extract of *Mangifera haden* Seeds

A 24-hour acute toxicity test of orally administered aqueous extract of *Mangifera haden* seeds in albino mice showed that the group fed with 5000 mg/kg body weight of the extract appeared very weak and one (1) death was recorded as shown below in table 1.

Table 1: LD_{50} of Aqueous Extract of *Mangifera haden* Seeds

DAY 1:

Group	Dose (mg/kg)	No. of animals	No of death
1.	10 mg/kg	3	0
2.	100 mg/kg	3	0
3.	1000 mg/kg	3	0

DAY 2:

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Group	Dose (mg/kg)	No. of animals	No of death
1.	1600 mg/kg	3	0
2.	2900 mg/kg	3	0
3.	5000 mg/kg	3	1

3.2. Mean Lethal Dose (LD_{50}) of Aqueous Extract of *Mangifera pirie* Seeds

A 24-hour acute toxicity test of orally administered aqueous extract of the seeds of *Mangifera pirie*in albino mice did not produce any toxic symptoms or mortality up to the dose level of 5000 mg/kg body weight in mice.

DAY 1:

Group	Dose (mg/kg)	No. of animals	No of death
1.	10 mg/kg	3	0
2.	100 mg/kg	3	0
3.	1000 mg/kg	3	0

DAY 2:

Group	Dose (mg/kg)	No. of animals	No of death
1.	1600 mg/kg	3	0
2.	2900 mg/kg	3	0
3.	5000 mg/kg	3	0

3.3 Proximate Analysis of the Seeds of *Mangifera haden* and *Mangifera pirie*

Table 2. The proximate composition of *Mangifera haden and Mangifera pirie.*

Proximate Analysis	*Mangifera haden* seeds	*Mangifera pirie* Seeds
Protein	4.82±0.02	3.81±0.03
Lipid	17.18±0.03	20.20±0.05
Crude Fibre	4.29±0.01	3.00±0.27
Ash Content	2.77±0.03	1.00±0.36
Moisture Content	8.39±0.02	34.00±0.30
Carbohydrate	62.55±0.07	37.96±4.65

Mean±SEM; n=5; P<0.05

3.4 Lipid Profile Analysis on Triton X – 100 Induced Hyperlipidemic Rats

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Table 3. present the lipid profile analysis of the study.

Treatment Groups	TC(mg/dl)	TG(mg/dl)	HDL-C(mg/dl)	VLDL-C(mg/dl)	LDL-C(mg/dl)
Normal Control	96.57±2.67	117.16±2.10	41.95±0.35	23.43±0.42	31.19±3.06
Triton X –100	124.55±1.87	240.26±7.94	25.48±0.62	45.88±1.58	51.82±3.81
Triton X-100 + Simvastatin	101.32±1.07	173.49±0.70	47.59±4.35	34.70±0.14	19.30±4.78
Triton X-100 + Atorvastatin	109.21±1.22	140.92±1.42	30.54±3.47	28.18±0.35	50.49±3.88
Triton X-100 + 200 mg/kg *M. pirie*	90.48±8.40	104.70±15.97	52.38±3.71	20.94±3.19	17.16±8.57
Triton X-100 + 300mg/kg *M. pirie*	82.93±4.58	99.25±5.16	49.38±4.60	19.85±1.03	13.71±7.09
Triton X-100 + 500mg/kg *M. pirie*	112.22±1.23	132.10±3.32	54.80±1.85	26.42±0.66	31.01±2.80
Triton X-100 + 300mg/kg(*M. pirie* +*M. haden)*	104.76±2.68	111.84±4.87	46.07±4.47	22.37±0.97	36.32±6.96
Triton X-100 + 300mg/kg(*M. pirie* +*M. haden)*	95.54±1.10	99.64±0.38	24.30±0.35	19.93±0.07	50.17±1.05
Triton X-100 + 200mg/kg *M. haden*	77.84±4.65	157.50±13.00	34.47±0.10	31.50±2.60	11.87±4.33
Triton X-100 + 300mg/kg *M. haden*	81.27±0.56	135.78±1.54	46.50±0.15	27.16±0.30	7.62±0.36
Triton X-100 + 500mg/kg *M. haden*	74.41±1.22	122.90±2.79	42.35±1.19	24.58±0.55	7.48±2.05

Values are expressed as mg/dl; Mean±SEM; n=5; P<0.05

3.5 Atherogenic Index (A.I) and % Protection

Table 4. The atherogenic index and % protection of the studies.

Treatment Groups	**Atherogenic Index (A.I)**	**(%) Protection**
Normal Control	1.30±0.74	-----
Hyperlipidemic Control (Triton	3.91±0.18	-----

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X-100)		
Triton X-100 + Simvastatin	1.20±0.20	69.31
Triton X-100 + Atorvastatin	2.72±0.32	30.43
Triton X-100 + 200mg/kg *M. pirie*	0.63±0.14	83.89
Triton X-100 + 300mg/kg *M. pirie*	0.78±0.28	80.05
Triton X-100 + 500mg/kg *M. pirie*	1.06±0.07	72.89
Triton X-100 + 300mg/kg (*M. pirie +M. haden)*	1.41±0.34	63.94
Triton X-100 + 500mg/kg (*M. pirie +M. haden)*	2.87±0.06	26.60
Triton X-100 + 200mg/kg *M. haden*	1.26±0.13	67.77
Triton X-100 + 300mg/kg *M. haden*	0.75±0.013	80.82
Triton X-100 + 500mg/kg *M. haden*	0.76±0.057	80.56

Values are expressed as %; Mean±SEM; n=5; P<0.05

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4.0. Discussion

Hyperlipidemia is associated with heart disease which is a leading cause of death in the world. This study was planned, as there have been reports that currently available hypolipidemic drugs have been associated with a number of side effects. The consumption of synthetic drugs lead to nausea, gastric irritation, myositis, diarrhoea, hyperuricemia, flushing, dry skin and abnormal liver function. To overcome the adverse effects of these drugs, there is an urgent need for the development of new hypolipidemic drugs from natural resources. An indigenous approach for hyperlipidemia having no side effects, locally available, relatively cheap would be a choice for people in developing countries. Bioavailability of *Mangifera haden* and *Mangifera pirie* seeds are major concerns which limits its therapeutic utility.

According to the American Society for testing and materials, any chemical substance or test compound with LD_{50}estimate greater than 2000-5000mg/kg body weight/oral route could be considered of low toxicity and safe. Hence the extracts were considered to be safe and non-toxic for further pharmacological screening.

This study revealed that all Triton X-100 induced rats displayed hyperlipidemia as shown by their elevated levels of serum Total Cholesterol (TC), Triacylglycerol (TG), Very Low Density Lipoproteins (VLDL-C), Low Density Lipoproteins (LDL-C), Atherogenic Index (A.I) and the reduction in High Density Lipoprotein (HDL-C) level. The large increase in serum cholesterol and triglycerides is mainly due to an increase of VLDL-C secretion by the liver accompanied by strong reduction of VLDL-C and LDL-C catabolism.

Triton X-100 acts as surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extra hepatic tissues. This result tend to increased blood lipid concentration, increased hepatic synthesis of cholesterol and induction of hyperlipidemia.

The administration of *Mangifera haden* and*Mangifera pirie* seeds extracts significantly lowered the levels of serum TC, TG, VLDL-C, LDL-C, A.I, and increased HDL-C level. The decrease in cholesterol mayindicate increased oxidation of mobilized fatty acids or lipolysis. The hypolipidemic potentials of these extracts may be due to their antioxidant and antihyperlipidemic effects carried out by mangiferin.

The combined extract at 300mg/kg has more hypolipidemic potential when compared to the 500mg/kg combined extract. This may be due to its ability to inhibit lipase enzyme at 500 mg/kg combined extract. hence, at 500mg/kg, it exhibited a poor hypolipidemic ability. Single administration of *Mangifera pirie* or *Mangifera haden* is more effective therapeutic agent than its combination. Since, *Mangifera pirie* or *Mangifera haden* reduce lipid profiles but raise HDL-C, they are considered anti-atherogenic and gives protection against cardiac problems, obesity and stroke.

Simvastatin and Atorvastatin are antihyperlipidemic drugs. They are competitiveinhibitor of HMG-COAR which blocks cholesterol biosynthesis and stimulates the synthesis of LDL receptors present on hepatic cell. This however, lowers LDL and cholesterol concentration.The decrease in serum lipid levels was more in Simvastatin treated group than Atorvastatin treated group. This may be attributed to the ability of Simvastatin to increase lipoprotein lipase activity in animals.

Atherogenic Index (A.I) was considerably decreased in the plant extract treated groups when compared to hyperlipidemic control. At 200mg/kg of *M. pirie* kilogram body weight of the animal, confers the most protective effect of the plant extract (83.89%) against hyperlipidemia while at

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500mg of the combined extract per kilogram body weight of the animal exhibited the least percentage protection (26.60%) against hyperlipidemia.

This study revealed that the administration of *Mangifera pirie*and *Mangifera haden*extracts significantly lowered (P<0.05) the serum Total Cholesterol (TC), Triacylglycerols (TG), and Low Density Lipoprotein (LDL-C), Atherogenic Index (A.I) and significantly increased (P<0.05) High Density lipoprotein. However, Atherogenic Index was remarkably and significantly decreased in the plant extracts treated group when compared to hyperlipidemic control. These results were in agreement with the results reported by Muruganandan*et al.* [25] which established that mangiferin (10 and 20 mg/kg, intraperitoneal) showed significant antihyperlipidemic and antiatherogenic activities as evidenced by significant decrease in plasma total cholesterol, triacylglycerols, low-density lipoproteins (LDL-C) level and diminution of atherogenic index in diabetic rats.

The results showed that Simvastatin significantly reduced (*P = 0.05*) total cholesterol and Low Density Lipoprotein more than Atorvastatin. However, Atorvastatin significantly suppressed (P<0.05) serum triglyceride than Simvastatin. The serum Total Cholesterol (TC), Triglycerides (TG) and Low Density Lipoprotein (LDL-C) levels in Simvastatin were 101.32, 173.49, and 19.30. Similarly, the serum TC, TG, and LDL-C levels in Atorvastatin were 109.21, 140.92 and 50.49. This result was in contrast with the results of Rajyalakshimi*et al.*[26] which reported that the decrease in plasma TC, TG and LDL-C levels was more in Simvastatin treated group than Atorvastatin treated group.

According to Rajyalakshimi*et al.*[26], the HDL-C levels were significantly higher (*P = 0.05*) in Atorvastatin treated group (69.5) than Simvastatin treated group (67.89). This was in contrary to my studies, since HDL-C levels were significantly increased in Simvastatin treated group (47.59) than Atorvastatin treated group (30.54).

5.0 Conclusion

The values for the negative control (group 2) i.e hyperlipidemic rats not treated with extract were high; these are atherogenic and undesirable. These values reduced in groups of rats treated with varying doses of aqueous extract of *Mangifera pirie* and *Mangifera haden*. This shows that the extracts are capable of reducing lipids in acute triton – induced hyperlipidemic rats probably by reducing absorption of lipids. In addition, the extracts have the potential to reduce the risk of development of coronary heart disease and atherosclerosis. The hypolipidemic activity of these extracts could be attributed to the presence of mangiferin in the extract which act as a valuable source of polyphenolic compounds. This finding is in agreement with previous reports that

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mangiferin a xanthone exhibits a variety of pharmacological activities including the anti-atherogenic effect.

Treatment with *Mangifera*pirie or *Mangifera haden* exhibited quite competitive hypolipidemic potential when compared with one of the reference drugs, Atorvastatin. Although, Simvastatin was found to be more effective drug in lowering lipid profiles than Atorvastatin. But, it has a lot of undesirable side effects. Hence, these extracts could be explored as an alternative therapeutic agent agent in the treatment of hyperlipidemia.

Ethical approval: The Department of Biochemistry, University of Nigeria, Nsukka approved the use of animals for this research study. All the experiments has been examined and approved by the approprioate ethics committee.

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