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Hypolipidemic and antioxidant effects of fruit extract of *Vitex doniana* (Black Plum) in albino rats

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Abstract

Introduction: Hyperlipidemia is a lipid metabolic disorder and the major risk factor for the development of cardiovascular diseases (CVDs). Because most of the current hypolipidemic drugs are expensive with potential side effects, the research focusing on natural alternative medicines is relevant. The fruit extract of *Vitex doniana* is used by some traditional medicine practitioners in Nigeria to treat CVDs. This study is aimed at investigating the hypolipidemic and antioxidant effects of ethanol fruit extract of *V. doniana* in high-fat diet (HFD) induced in albino rats. Oral administration of extract at 250, 500 and 1000 mg/kg body weight on HFD induced hyperlipidemia lasted for 28 days. Atorvastatin (1.2 mg/kg) served as standard drug. Hypolipidemic effect was checked by the measurement of serum lipid profile and effect on oxidative stress.

Results: The results revealed a dose dependent significant ($P<0.05$) decrease in malondialdehyde (MDA), increase in superoxide dismutase (SOD) and catalase (CAT) levels when compared to the negative control. This attests to the ability of the extract to boast the antioxidant defence mechanism in the animals. Positive effect on CVDs was further confirmed by the observed dose related significant ($p<0.05$) reduction in serum lipids (total cholesterol, total glycerides and low density lipoprotein) profile of the treated rats when compared to the negative control.

Conclusion: The fruit extract of *V. doniana* possesses serum lipid lowering effects; its folkloric use in the treatment of cardiovascular diseases could therefore be justified. Further studies need to focus on the isolation and characterization of the secondary metabolites responsible for this observed activity.

Keywords: Hyperlipidemia; Hypolipidemia; Cardiovascular diseases; Atherosclerosis

1. Introduction

The global burden of disease has dramatically shifted from communicable diseases to non-communicable diseases including cardiovascular diseases (CVDs), cancer and digestive diseases. This is expected to increase to 59% [1]. Cardiovascular diseases account for most non communicable disease deaths and is responsible for over 17.3 million deaths per annum [2].

Liver produces cholesterol and exports it to other parts of the body for the production of hormones and cell membranes. Accelerated deposition of cholesterol in the inner walls of arteries leads to atherosclerosis which is the underlying cause of cardiovascular disease. Atherosclerosis is linked to high levels of cholesterol in the blood, particularly to high levels of low-density lipoprotein (LDL)-bound cholesterol which is otherwise known as the "bad cholesterol"[3]. Furthermore, there is a negative correlation between high-density lipoprotein (HDL) levels, the "good cholesterol" and arterial

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disease [3]. Hyperlipidemia is a highly predictive risk factor for atherosclerosis, coronary artery and cerebral vascular diseases. It is characterized by elevated serum total cholesterol and very LDL cholesterol and decreased HDL levels [4]. High-fat diet (HFD) is a major cause of obesity. Excess calories in the body are converted to triglycerides which are mainly stored in the adipose tissues [5]. High triglyceride level increases the risk of atherosclerotic CVD [6].

The modern allopathic drugs used for the treatment of hyperlipidemia are effective, but are expensive and associated with side-effects leading to patient incompliance [7]. Medicinal plants are the oldest known natural source of medicine with little or no side effects. Many plant species such as *Vitex doniana* fruits extract have been used in traditional medicine to treat hyperlipidemia. In Nigeria, *Vitex doniana* fruit is commonly referred to as Black Plum. Black plum is known as *Oriri* (Yoruba), *Mbembe* (Igbo) and *Dinyan* (Hausa). The rural people of Nigeria consume the matured fruit pulp mainly as a snack and for its believed sedative effects. This study investigates the hypolipidemic and antioxidant properties of ethanol extract of *Vitex doniana* fruit in HFD induced hyperlipidemia in albino rats.

Figure 1 Fresh fruits and leaves of *Vitex doniana*

2. Material and methods

2.1. Plant materials

2.1.1. Sample collection and Identification

The fresh fruits of *Vitex doniana* were purchased from Ekwulobia in Anambra state of Nigeria. The fruit was authenticated by a Taxonomist at the department of Botany, Nnamdi Azikiwe University, Awka, Nigeria, and a voucher specimen number (NAUBT 3218) was assigned to it.

2.1.2. Chemicals, reagents and drugs:

Atorvastatin,((Lipitor by Pfizer, United states of America), 96 % v/v ethanol analytical grade (Zigma, India), chloroform (Zigma, India), Elisa reagents (Harman Finochem Limited India).

2.1.3. High fat feed materials

Grower feed (Top Feed Premier Feed Mills Sapele, Delta state, Nigeria), egg yolk, crayfish, Palm kernel cake (P.K.C) and Margarine

2.1.4. Animals

Thirty albino rats (130-150 g) of both sexes were purchased from the laboratory animal house of the Department of Pharmacology and Toxicology, Chukwuemeka Odumegwu Ojukwu University Igbariam campus Anambra state Nigeria, were used for the study. The rats were housed in clean plastic cages, supplied with clean drinking water and fed with Grower feed (Top Feed Premier Feed Mills Sapele, Delta state, Nigeria). Ethical approval number PHACOOU/AREC/2023/033 was assigned to attest the animals were cared for according to the Faculty of Pharmacy

(COOU) Animal Research Ethics Committee guidelines (PHACOOUAREC), which are in line with the National Institute of Health (NIH), USA, guidelines for the care and use of laboratory animals.

2.1.5. Dosage selection

Dose of extract administered to animals were 1/20th, 1/10th and 1/5th of the estimated LD_{50} [8] and the fruit extract was administered orally.

2.1.6. Formulation of the high fat feed

The mixture of following ingredients in their various proportion were used to fed the animals; 35% Top Feed, 10% egg yolk, 5% crayfish, 35% Palm kernel cake (P.K.C) and 15% margarine. the feeding lasted for four weeks [9].

2.2. Extraction of plant material

Extraction was done using a method described by [10]. The fruits were cleaned to remove sand and other debris. After removing the thin mericarp, the fleshy juicy mesocarp was scraped off from the seeds and dried for a period of two weeks under room temperature. This dried mesocarp was grinded into powder with the help of an electrical grinder. About, 800 g of the powdered material was cold macerated in 80 % ethanol. The mixture was agitated intermittently for three days (72 hours). The filtrate was recovered and concentrated to dryness using water bath at 40 ^{0}C. The percentage yield of the extract was calculated and the extract stored in a refrigerator until when needed.

2.3. Qualitative phytochemical analysis

Screening for the presence of secondary metabolites was carried out following the phytochemical tests as demonstrated by [11]. The following secondary metabolites were tested for: tannins, alkaloids, reducing sugars, flavonoids, glycosides, anthraquinones, saponins, acidic compounds and proteins.

2.4. Acute toxicity study

The toxicity test was conducted using the up and down procedure (UDP) adopted by [12] and revised by [13]. Using this method, the animals were dosed one at a time and the doses were dependent on the response of the first animal to the initial dose. The second animal receives a lower dose if the first animal dies (the initial dose is decreased by a factor of 3.2) or the second animal receives a higher dose if the first animal survives (the initial dose is increased by a factor of 3.2). Three albino rats (130-150 g) were used as starting point. Two rats served as negative control having received 10 ml/kg of distilled water orally while the test animal received a default oral dose of 5000 mg/kg of the extract. The animals were then observed continuously for 4 hours for changes in behaviour and for any other obvious signs of toxicity and subsequently daily for a total of 14 days for delayed toxicity

2.5. Induction of hyperlipidemia

Hyperlipidemia was induced by feeding the animals on high fat diet composed of 35 % Top grower feed, 10 % egg yolk, 5 % crayfish, 35 % Palm kernel cake (P.K.C) and 15 % Margarine for two weeks [9]. The treatment pattern was as seen stated below:

- Group 1: Normal control (NC)
- Group 2: Negative control (NEG.C)
- Group 3: Positive control (1.2 mg/kg Atorvastatin)
- Group 4: 250 mg/kg extract + High fat feed
- Group 5: 500 mg/kg extract + High fat feed
- Group 6: 1000 mg/kg extract + High fat feed

All the groups were treated with the extract except the negative control group. In the same manner all the groups were treated with high fat diet (induction of hyperlipidemia) except the normal control group.

Both the standard Atorvastatin and fruit extract were administered orally once daily for 28 days. On the 28th day, the animals were fasted overnight and were humanely sacrificed with excess chloroform. The blood sample were collected for biochemical tests while the liver was harvested for histopathological examination.

2.5.1. Biochemical assays

Determination of serum total cholesterol:

Serum total cholesterol (TC) was evaluated using Randox commercial assay kits, applying the methods described by [14]. One milliliter (1ml) of the working Cholesterol reagent (1ml) was added into tubes labelled blank, standard and test groups. Ten microlitres of standard cholesterol reagent, and samples were added into their respective tubes. They were mixed and allowed to stand for 10 minutes at room temperature. Absorbance of samples and standard were read with the aid of a spectrophotometer at 500 nm. Total cholesterol level in sample was calculated using the formula

$$\text{Total cholesterol in sample (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ x Concentration of standard.}$$

Determination of serum triglyceride

Serum triglyceride was evaluated according to the methods described by [15]. One millilitre (1 ml) of the working triglyceride reagent was added into tubes labeled blank, standard and test groups. Ten microlitres of standard triglyceride reagent, and samples were added into their respective tubes. They were mixed and allowed to stand for 10 minutes at room temperature. Absorbance of samples and standard were read with the aid of a spectrophotometer at 500 nm. Total cholesterol level in sample was calculated using the formula;

$$\text{Total triglyceride in sample (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ x Concentration of standard}$$

Determination of serum high density lipoprotein cholesterol (HDL-cholesterol):

Serum HDL-cholesterol was evaluated according to the methods developed by National Institute of Health Consensus Development Conference Statement. One hundred microlitres (100 ul) of samples and standard cholesterol reagent were dispensed into test tubes containing 250 ul of HDL cholesterol precipitate (R1). The mixture was centrifuged at 4000 rpm for 10 minutes. Thereafter, 100 uL of samples and standard supernatants were added to another set of test tubes labeled samples and standard containing cholesterol reagent. The mixture was incubated for 10 minutes at room temperature and absorbance of standard and samples were measured against reagent blank at 500 nm within 60 minutes using Spectrophotometer. HDL-cholesterol level in sample was calculated using the formula below;

$$\text{HDL cholesterol in sample (mg/dL)} = \frac{\textit{Absorbance of sample}}{\text{Absorbance of standard}} \text{ x Concentration of standard.}$$

Determination of serum low density lipoprotein cholesterol (LDL-cholesterol):

Low density lipoproteins (LDL) cholesterol in serum was calculated using the equation described by [16]. The Friedewald's equation estimates the value of HDL-C using the values of total cholesterol, triglyceride and HDL-cholesterol. [16].

$$\text{LDL cholesterol (mg/dl)} = \text{Total cholesterol} - \frac{\textit{Triglycerides}}{5} - \text{HDL cholesterol.}$$

2.5.2. Effect of the extract on oxidative stress

Determination of plasma Thiobarbituric acid:

Thiobarbituric acid reactive substances (TBARS) assay is an established method for quantifying lipid peroxides. Lipid peroxidation generates peroxide intermediates which upon cleavage releases malondialdehyde, a product which reacts with thiobarbituric acid [17]. The activity of TBARS was determined by the methods of [18].

Assay of antioxidant activity of superoxide dismutase (SOD):

The activity of superoxide dismutase (SOD) was determined using serum from the experimental rats. The activity of superoxide dismutase was assessed using the NWLSS™, superoxide activity assay. The method was based on monitoring the auto-oxidation rate of haematoxylin as described by [19]. The sample's SOD activity was determined by measuring the ratio of auto-oxidation rate in the presence and absence of the sample and expressed as traditional 'McCord-Fridovich.

Assay of antioxidant activity of catalase (CAT)

The catalase activity of the hemolysate (a solution of lysed red blood cells) was determined by adopting the method of [20]. The assay is based on the disappearance of H_20_2 in the presence of the enzyme source at 26 ^{0}C. In brief, the hemolysate was prepared from lysed RBC suspension and further diluted using phosphate buffer (pH 7.0). The reaction mixture containing 0.05 M phosphate buffer (pH 7.0), 1.2 mM H_20_2 and 0.2 ml of diluted hemolysate was allowed to stand for 25 minutes. At the end of 25 minutes the reaction was stopped by the addition of 2.5 ml of peroxidase reagent containing peroxidase and the chromogen system. Peroxidase reduced the H_20_2 to give a red colored compound and absorbance was measured at 505 nm. With each assay a suitable blank which contained the phosphate buffer to maintain the pH of the mixture, hydrogen peroxide which is same concentration as the test sample and distilled water instead of the hemolysate and a control which contained l ml sodium azide, a catalase inhibitor was provided.

2.5.3. Determination of body mass

The animals were weighed at the beginning of the experiment and then weekly for four weeks. Weights gained by the rats were calculated as the difference between initial and final weights and the results were presented as a percentage of the initial weights.

2.6. Statistical analysis

Data obtained from the study was analyzed using Statistical Package for Social Sciences (SPSS-25). Results were presented as mean ± Standard error of mean (SEM) of sample replicates. Raw data was subjected to one-way analyses of variance (ANOVA) followed by post hoc turkey's test. $p<0.05$ was considered to be statistically significant.

3. Results

3.1. Yield of extract

The weight of the *V. doniana* fruits after the removal of the seeds was 765 g and the crude extract obtained from it was 333 g. The percentage yield was 43.8%

3.2. Acute Toxicity test (LD_{50}) of the extract

Oral administration of the fruit extract up to 5000 mg/kgbw dose produced no change in behaviour, neither was there any mortality in any of the groups. Therefore, the LD_{50} of ethanol fruit extract of *Vetex doniana* was above 5000 mg/kgbw.

3.3. Phytochemical screening of the extract

Phytochemical analysis of the extract revealed the abundance presence of tanins, flavonoids, glycosides, saponins, steroids, anthraquinones, and carbohydrates while alkaloids, and reducing sugars were in moderate presence (Table 1).

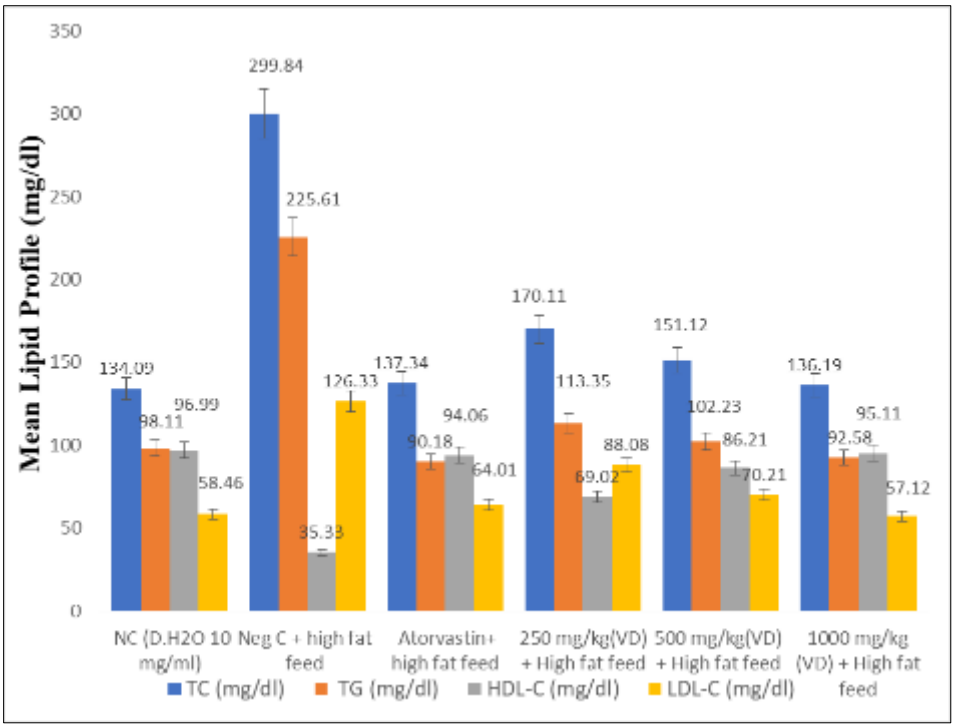
Table 1 Phytochemical screening of the extract

Constituent	Alk	Tan	Flav	Gly	Sap	Anthr	Ste	Carb	Acid cpds	Red sugar
Presence	++	+++	+++	+++	+++	+++	+++	++	+++	++

Key: Alk = alkaloids, Tan= tannins, Flav= flavonoids, Gly= glycosides, Anthr= anthraquinones, Sap=saponin, Ste= steroids, Carb = carbohydrates, Red sugar=reducing sugar, (++) = moderately present, (+++) = abundance

3.4. Effect of extract on serum lipid profile

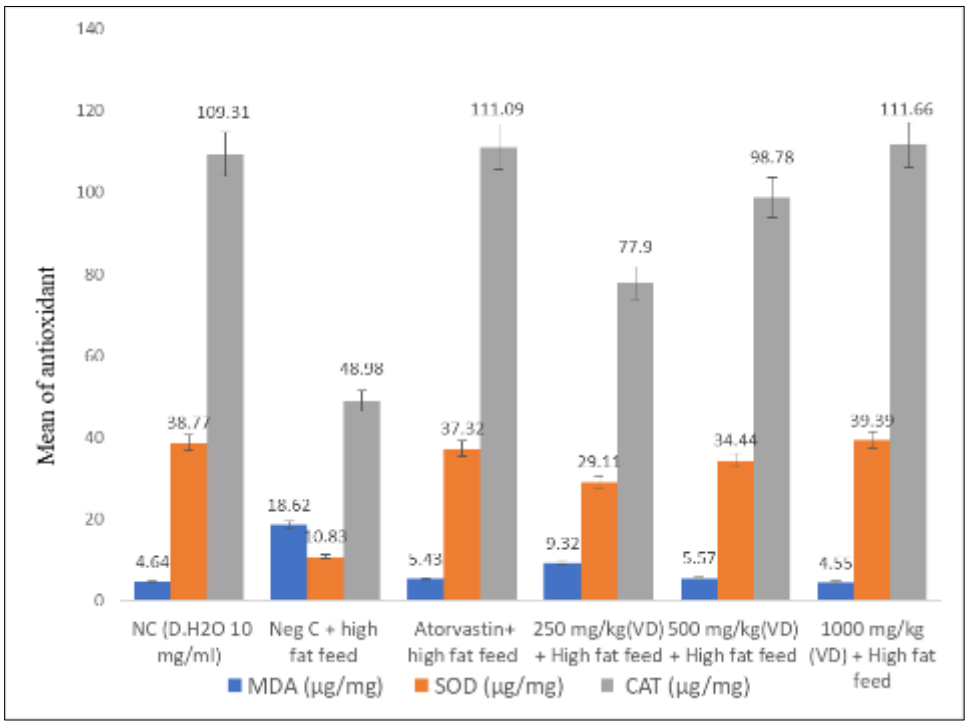
The fruit extract caused a dose related significant ($P<0.05$) decrease in TC (total cholesterol), TG (total glycerides), LDL (low density lipoprotein) and increase in HDL (high density lipoprotein) when compared to the negative control (Figure 2)



Key: TC= Total Cholesterol, TG= Triglycerides, HDL-C= High density lipoproteins, LDL-C= Low density lipoproteins, NC= Normal control, and H_2o= Negative control

Figure 2 Effect of ethanol fruit extract of *Vitex doniana* on serum lipid profile

3.5. Effects of extract on oxidative stress parameters



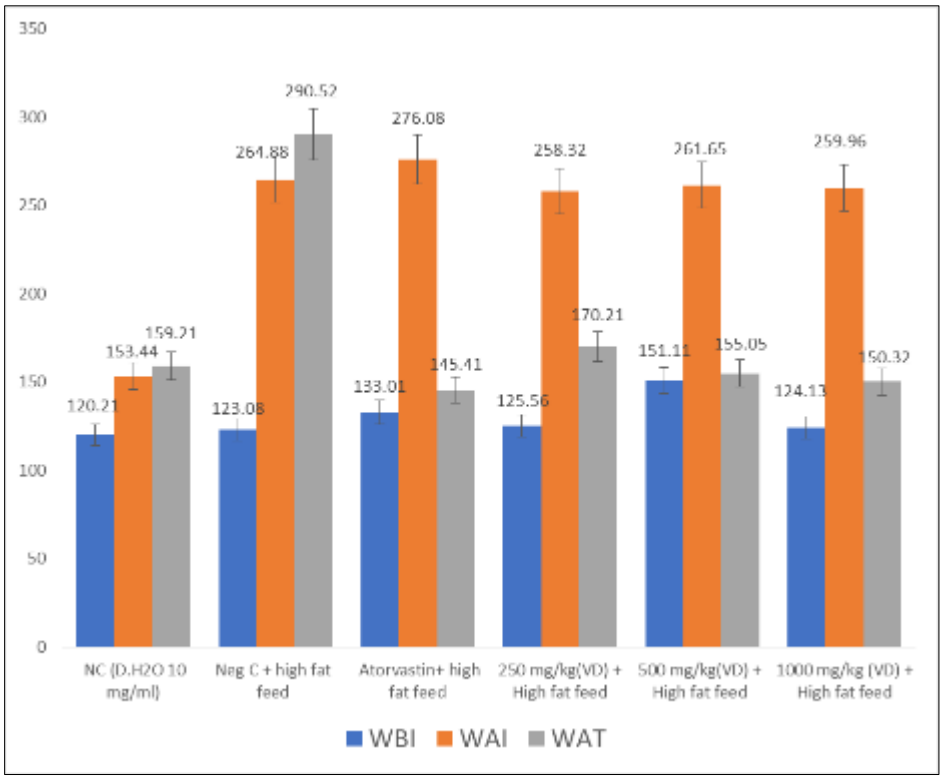
Key: MDA= Malondiadehyde, SOD=super oxide dismutase, CAT =catalase activity, NC= Normal control, and H2o= water

Figure 3 Effect of ethanol fruit extract of *Vitex doniana* on oxidative stress parameters

The extract caused a dose dependent significant ($P<0.05$) decrease in malondialdehyde (MDA), (a product of lipid oxidation), increase in superoxide dismutase (SOD) which neutralizes free radical, and increase in catalase (CAT) which protects cells from oxidative damage when compared to the negative control group (Figure 3)

3.6. Effect of extract on body weight

Considering the weight after induction(WAI) of hyperlipidaemia and weight after 28 days treatment(WAT), the extract caused a dose related significant (P<0.05) decrease in weights of the animals in the treated groups when compared the initial weight after induction in all the groups.



Key: WBI= weight before induction, WAI= weight after induction, WAT=weight after treatment, NC= normal control, and H2o= water

Figure 4 Effect the extract on body weight

4. Discussion

Cholesterol is a vital part of all cell membrane and it is essential molecule for all living beings [21]. Large amount of cholesterol is found in breast milk, where it plays part in infant nourishment and brain development [22]. It helps in the healing and repair of blood vessels [23]. However, the ccelerated deposition of cholesterol in the inner walls of arteries leads to atherosclerosis, the underlying cause of cardiovascular disease. Atherosclerosis is linked to high levels of cholesterol in the blood, particularly, the low-density lipoprotein bound cholesterol (LDL-C) the ‘‘bad cholesterol’’ [3]. Atorvastatin decreases the amount of LDL-C in the blood thereby preventing the events associated with cardiovascular disease [24]. It does this by acting as a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the enzyme involved in hepatic cholesterol biosynthesis.

High serum levels of TC and LDL are associated with an increased risk for atherosclerosis [25]. The treatment with the fruit extract of V. doniana for 28 days, caused a dose dependent significant (P<0.05) reduction in TC, TG, LDL as well as a dose dependent significant (P<0.05) increase in HDL. Hypercholesterolemia and hypertriglyceridemia are independent risk factors that alone or together can accelerate the development of coronary artery disease and the progression of atherosclerotic lesions [26]. Amirkhizi [27] demonstrated increase in the bioavailability of free fatty acids can increase lipid peroxidation. In this study, the fact that this fruit extract was able to cause a decrease in TC, TG LDL and an increase in HDL concentrations suggests the fruit of *Vitex doniana* may play a protective role against hyperlipidemia.

Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS), free radicals and their scavenging by the natural antioxidant system [28]. Oxidative stress is associated with hyperlipidemia, which accelerates atherosclerosis [29]. High fat diet (HFD) can led to an increase in free radical production in vivo, followed by oxidative stress [30]. The administration of the extract caused increased activity of the antioxidant defense system in the treated groups in a dose dependent manner. This was revealed by the observed dose-dependent significant (P<0.05) increase

in SOD and CAT levels and reduction in the MDA level. Catalase(CAT) is an enzyme found in red blood cells that breaks down the free radical hydrogen peroxide (H2O2) into water and oxygen. This observed antioxidant property of *Vitex doniana* fruit extract confirms the report by [31][32]. Antioxidants have the potentials to positively affect hyperlipidemia by reducing oxidative stress, which is a key contributor to the development of the hyperlipidemia by neutralizing free radicals. (https://www.nature.com). These result attest the ability of this fruit extract to reduce oxidative stress which may have contributed to its hypolipidemic effect since obesity has been found to be an independent risk factor for increasing lipid peroxidation and decreased activity of cytoprotective enzymes [33].

The qualitative phytochemical screening of the fruit extract revealed the abundance of secondary metabolites like flavonoids, anthraquinone, and tannins. The antioxidant and hypolipidemic activities of anthraquinones and flavonoids have previously been reported [34] [35]. Hence, the hypolipidemic and antioxidant activities exhibited by *V. doniana* fruit extract could be attributed to the synergistic action of the bioactive compounds present in it.

5. Conclusion

The fruit extract of *V. doniana* possesses serum lipid lowering effects, its folkloric use in the treatment of cardiovascular disease could therefore be justified. We suggest that further studies should include the isolation of the secondary metabolites responsible for these observed activities.

Compliance with ethical standards

Disclosure of conflict of interest

The authors wish to confirm that there is no known conflict of interests associated with this paper and there has been no significant financial support for this work that could have influenced its outcome.

Statement of ethical approval

Ethical approval number PHACOOU/AREC/2023/033 was assigned to attest the animals were cared for according to the Faculty of Pharmacy (COOU) Animal Research Ethics Committee guidelines (PHACOOUAREC), which are in line with the National Institute of Health (NIH), USA, guidelines for the care and use of laboratory animals.

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