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Biochemical Effects of Ethylacetate Fraction of Root-Bark Extract of *Theobroma cacao* (Linn.) on Plasma Lipid Profiles of Rats Fed with High-Salt Diet

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ABSTRACT

The study investigated the biochemical influence of ethylacetate fraction of the root-bark extract of *Theobroma cacao* (Linn.) on plasma lipid profile of rats fed with high-salt diet, in order to ascertain the potential of the extract to manage salt-induced cardiovascular related ailments. Fresh root-bark of *T. cacao* was extracted, fractionated with solvents of increasing polarity and ethylacetate fraction (EAF) was used in this study. Rats were grouped into normal-untreated, normal-treated, salt-untreated, and salt-treated. For the first 21 days, the salt-untreated and salt-treated groups were placed on 4% salt-diet and 1% salt-water, while the normal-untreated and the normal-treated groups were fed with normal rat feed. After the first 21 days, salt-treated and normal-treated groups were orally exposed to 250 and 500 mg EAF/kg bwt for another 21 days. Phytochemical analysis showed that saponins, tannins, steroids, alkaloids, flavonoids, cardiac glycosides, xanthoproteins and triterpenes were present in EAF. The extract reduced the plasma total cholesterol, triacylglycerol, LDL-c, VLDL-c of the normal-treated and salt-treated rats, compared to the increase observed in the untreated and control groups. There was increase in plasma HDL-c in all the groups except the untreated group. In conclusion, the medicinal value of the ethylacetate extract of cocoa root could lie in its bioactive constituents that produce a definite physiological action and could be beneficial for the management of salt-induced cardiovascular diseases that are associated with free radical generation.

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Introduction

The precursor for the synthesis of bile salt and steroidal hormones, cholesterol, is an important component of the membrane of the cell. Cholesterol is present in all plasma lipoproteins and about 60% of the total cholesterol in the plasma of human subject is carried in the form of the "good cholesterol", high-density lipoproteins (HDL). Hypercholesterolaemia is a major condition that results from a snag in lipid metabolism. This complication affects the health of people globally, is implicated in the induction of oxidative stress, and it's a major contributor to heart diseases like hypertension (Bouyahya et al., 2020; D'Assante et al., 2021). Oxidative stress has been implicated in the progression of cardiovascular diseases (CVD) like Alzheimer's disease, Parkinson's disease, neurodegenerative diseases, ageing, and most importantly cancers (Saleem et al., 2020; Sen et al., 2021). A postulated and most plausible mechanism by which high level of LDL-cholesterol causes CVD is the deactivation of nitric oxide vasodilator with an increment in permeability of the endothelium which is as a result of a rise in the production of free radicals and ultimately the weakening of the cells of the endothelium (Zhazykbayeva et al., 2020; Qaddumi and Jose, 2021).

Salt has been reported has the essential cause of about 50% of the hypertensive populations (Mishra et al., 2018). There is increasing evidence, both in animal studies and human clinical trials, that high-salt consumption leads to the development of hypertension and other CVDs (Selvaraj et al., 2017). Furthermore, high-salt loading, which is associated with the western diets and low consumption of hominid diets that are rich in potassium have been reported to result in over expression of $P47^{phox}$ and $g91^{phox}$ which are components of NADPH oxidase (an enzyme which generates superoxide anion) and a reduction in the expression of an antagonist enzyme, superoxide dismutase (which is a quencher or scavenger of superoxide anion) and decreased bioavailability of antioxidants, given rise to oxidative stress (Milinković et al., 2020). Epidemiologic studies have revealed that the high phenol contents of some beverages and food has a high potential to slow down the development of atherosclerosis, cut down the predisposition to heart disease because of the antioxidant activity of phenol majorly on the "bad cholesterol", low-density lipoprotein (Stromsnes et al., 2020). The antioxidative activity of phenols is due majorly to their reduction-oxidation attributes, which confer on them the ability to reduce other molecules, donate hydrogen, chelate metals, and quench singlet oxygen (Kang et al., 2020).

Medicinal uses of cocoa are reported to originate from the Mexican Ayurveda, with more than hundred uses documented in the medical (Montagna et al., 2019). Cocoa bean, as chocolate (remedy for cold, cough, enhance digestion, fecundity, strengthen the performance of brain and an anti-sadative), cocoa root-bark (blood supplement, nerve tonic and membrane stabilizer) (Oyedapo et al., 2004), butter from cocoa (antimicrobial), pulp and flowers of cocoa (refreshing juice), and leaves of cocoa, all of which are produced by cocoa tree (*Sterculiaceae* or *Malvaceae*). *Theobroma* in Greek word means "the food of the gods" (Dillinger et al., 2000). This study was designed to assess the biochemical effects of the ethylacetate fraction from the root-bark extract of *T. cacao* on plasma lipid profiles of rats fed with high-salt diet.

Materials and Methods

Plant materials

Fresh and new roots from *Theobroma cacao* (L.) were harvested from Cocoa Plantation at Babajakan Village in Ayedaade Local Government of Osun State, Nigeria. The plant was identified and authenticated at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, with voucher number IFE-172418. The roots were washed, drained, the root-bark peeled, and then cut into bits.

Reagents and chemicals

Analytical grade reagents were used for this study. Distilled water was used to prepare all reagents, solutions, and buffers used, and all were kept in the fridge.

Methods

Preparation and fractionation of extract

The aqueous extract of the root of *T. cacao* was prepared using the procedure earlier described by Dillinger et al. (2000) with little modification. Typically, fresh root cut into bits was boiled for 30 min with 6.0 L of distilled water. The infusion was allowed to settle overnight at 25 ^{o}C, after which it was filtered. The filtrate was evaporated to dryness at 40 ^{o}C on vacuum pump (designed by Edward High, Crawley, England) to yield brown residue termed aqueous extract. The aqueous extract was solubilised in hot distilled water and

partitioned with n-hexane in a separating funnel, to get rid of the fatty components in it. The aqueous layer was collected, further partitioned with ethylacetate and the ethylacetate layer was collected and evaporated to dryness to yield ethylacetate fraction (EAF). The dried solid residues were weighed and kept in air-tight container for biological assays.

Phytochemical screening

The ethylacetate extract of *T. cacao* root was comprehensively screened for phytochemicals such as xanthoproteins, saponins, alkaloids, phlobatanins, triterpenes, flavonoids, tannins, cardiac glycosides, and steroids using standard procedures (Oyedapo et al., 2004; Sofowora, 2006).

Experimental animals

Thirty (30) healthy albino rats were bought from Faculty of Health Sciences, Obafemi Awolowo University, Ile-Ife, housed in the Animal House, Department of Biochemistry and Molecular Biology, OAU Ile-Ife under standard conditions. The thirty animals were acclimatised for two weeks and granted access to standard rat feed purchased from Ladokun Feeds Limited, Ibadan, and clean water *ad libitium*, at the experimental site.

Preparation and processing of diet

The rat diet was prepared according to the method described by Onwumelu et al. (2013) by mixing 96 g of ground standard rat chow with 4 g of finely ground sodium chloride salt (Sigma Aldrich sodium chloride salt) to make (4% w/w) salt-diet, after which it was pelletized and moderately dried. The rat drinking water also contained 1% (w/v) of salt (freshly prepared). The rats were fed constantly for a period of 21 days. The body weights of the rats were monitored before, during and after the treatments.

Preparation and administration of extract

Two concentrations of 250 and 500 mg EAF/kg bwt were used for this study by dissolving appropriate weight of EAF in corresponding volume of distilled water (Onwumelu et al., 2013). The rats were grouped into six with five rats in each group (n = 5):

I: Rats + salt-free diet/water (control);
II: Rats + 4 (w/w) salt-diet/ 1 % salt water;
III: Rats + 4 % (w/w) salt-diet/1% salt water + 500 mg EAF/kg bwt;
IV: Rats + 4% (w/w) salt-diet/1% salt water + 250 mg EAF/kg bwt;
V: Rats + salt-free diet/water +250 mg EAF/kg bwt; and
VI: Rats + salt-free diet/water + 500 mg EAF/kg bwt

The experimental animals were orally administered with the extract for a period of 21 days, during which the rats were also fed with the salt-diet and salt-water regularly.

Sacrifice and collection of blood from experimental animals

On day 43 (after three weeks feeding with the high-salt diet and salty water only and three weeks administration of extract and high-salt diet/salty water), the animals were put to sleep by light chloroform anaesthesia. Blood was collected into tubes that have anticoagulant (3.8% (w/v) tri-sodium citrate) from the hearts of unconscious rats (while the hearts were still beating) by cardiac puncture.

Preparation of plasma

The blood was spun at 3000 rpm for 10 min at 25 ^{o}C on a tabletop centrifuge, and the plasma (supernatant) was carefully collected. The plasma was used for lipid profile estimations.

Biochemical assays

The estimation of plasma lipid profiles were carried out using Randox diagnostic kits as earlier described: total cholesterol (Richmond, 1973), triacylglycerol (Tietz, 1990). The plasma HDL-c was precipitated using phosphotungstic acid (0.55 mM)/ Manganese Chloride (25 mM) solution complex and estimated using Randox diagnostic kit.

The concentrations HDL-c in the plasma was obtained, using the expression:

$$\text{HDL} - \text{c (mg/dL)} = \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{standard}}} \text{x standard conc(mg/dL)}$$

The concentration of LDL-c in the plasma was obtained using the expression:

$$\text{LDL} - \text{c (mg/dL)} = \text{Total cholesterol (mg/dL)} - (1.5 \text{ x supernatant cholesterol (mg/dL)})$$

The concentrations of plasma VLDL-c was calculated as Triacylglycerol/5 as described by Chattopadhyay and Bandyopadhyay (2005).

Statistical Analysis: The data were expressed as Mean ± SEM, n = 5 readings. Differences between the mean values of control and treated groups were evaluated by One-way ANOVA followed by Tukey multiple comparison test using GraphPad Prism 5. The values of $P < 0.05$ were considered statistically significant.

Results

The phytochemical screening of EAF of *T. cacao* root-bark discovered the presence of secondary metabolites like flavonoids, steroids/phytosterols, alkaloids, cardiac glycosides, saponins, triterpenes, tannins, and xanthoproteins.

Table 1 showed the weights of the rats as monitored over the six weeks period of the study. The consumption of salty diet/salty water led to an increase in the weight of the rats in Group II which is higher than the increase brought about by the rat feed only (in Group I) for the six weeks experimental period. The rats fed with normal rat feed throughout the six weeks and administered 250 and 500 mg EAF/kg bwt for the last three weeks of the experiment (Groups V and VI respectively) also had an increase in body weight but with a percentage lower than as it is in the control group (Group I). Groups III and IV fed with salty diet/salty water for the first three weeks and administered extract (500 and 250 mg EAF/kg bwt respectively) with salty diet/salty water for the last three weeks experienced an increase in weight for the first three weeks and a reduction from the last three weeks.

As shown in Figures 1 to 4, the EAF reduced the concentration of plasma total cholesterol (TC), triacylglycerol (TRIG), and low-density lipoprotein-cholesterol (LDL-c), but increased the concentration of high-density lipoprotein-cholesterol (HDL-c) in the plasma of the treated rats. The extract had a relatively lowering effect on the concentration of very low-density lipoprotein-cholesterol (VLDL-c) concentrations of the rats (Figure 5). The consumption of the extract had a significantly ameliorative effect on the toxic effect of the high-salt diet while it had a significantly positive effect on the normal rats treated with it.

Table 1: Weights of Animals Before, During and After Treatment

	Weight (g)							
Groups / Period	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Percentage change**
Group I	98.56 ± 2.51	105.78 ± 5.11	110.95 ± 1.69	114.45 ± 1.23	119.11 ± 2.78	124.23 ± 4.76	128.17 ± 3.33	23.10↑
Group II	197.35 ± 3.94	203.08 ± 4.22	218.19 ± 0.77	235.43 ± 1.51	249.26 ± 0.91	266.66 ± 2.93	278.09 ± 1.79	29.03↑
Group III	151.61 ± 5.23	155.26 ± 4.95	152.77 ± 5.33	171.77 ± 1.83	173.89 ± 1.76	154.51 ± 1.42	142.47 ± 0.44	11.74[a]↑/20.56[b]↓
Group IV	170.15 ± 2.77	168.88 ± 2.94	167.47 ± 4. 72	179.04 ± 5.37	168.57 ± 3.92	166.44 ± 5. 31	165.78 ± 4.72	4.97 [a]↑/8.04 [b]↓
Group V	115.76 ± 4.55	121.38 ± 1.22	126.72 ± 3.78	129.29 ± 0.56	124.56 ± 2.31	129.78 ± 5.21	133.77 ± 3.11	3.34 ↑
Group VI	150.67 ± 1.11	158.12 ± 0.78	162.57 ± 2.42	165.69 ± 4.75	173.98 ± 5.22	180.71 ± 4.43	185.17 ± 2.11	18.63↑

Week 0: Before treatment of rats; **Weeks 1-3**: When Groups II, III and IV rats were treated with salt-diet/salt water and Groups I, V and VI rats were fed with normal rat feed; **Weeks 4-6**: When Groups V and VI were treated with the extract, Groups III and IV were treated with the extract in addition to the salt-diet/salt-water, Group II rats were constantly fed with salt-diet/salt-water only, and Group I rats fed with normal rat feed.

Group I: Rats + salt-free diet/water (control); Group II: Rats + 4 % (w/w) salt diet/ 1 % salt water; Group III: Rats + 4 % (w/w) salt diet/1% salt water + 500 mg EAF/kg bwt; Group IV: Rats + 4% (w/w) salt diet/1% salt water + 250 mg EAF/kg bwt; Group V: Rats + salt-free diet/water +250 mg EAF/kg bwt; Group VI: Rats + salt-free diet/water + 500 mg EAF/kg bwt

[a] is percentage change from week 0 to Week 3, [b] is percentage change from Week 3 to Week 6, is increase, decrease ↑ ↓

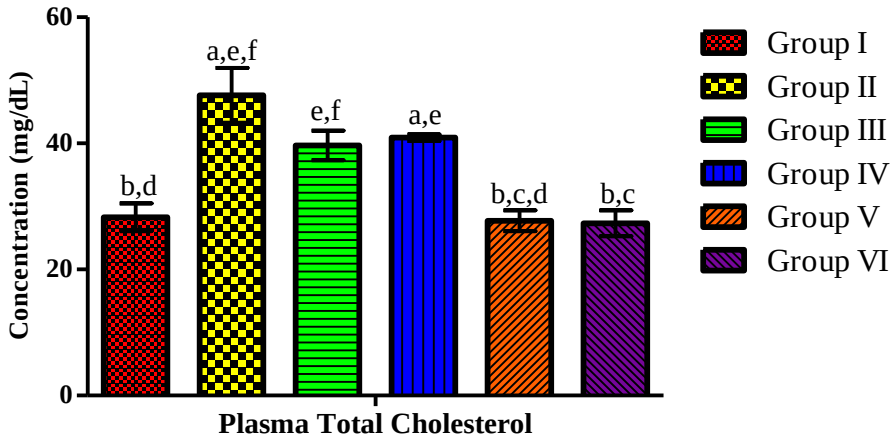

Figure 1: Concentration of Total Cholesterol in Plasma

Each value is expressed as Mean ± SEM of n = 5. The values with asterisk alphabets are statistically significant at $P < 0.05$. a compares Group I with others, b compares Group II with others, c compares Group III with others, d compares Group IV with others, e compares Group V with others, f compares Group VI with others.

Group I: Rats + salt-free diet/water (control); Group II: Rats + 4 % (w/w) salt diet/ 1 % salt water; Group III: Rats + 4 % (w/w) salt diet/1% salt water + 500 mg EAF/kg bwt; Group IV: Rats + 4% (w/w) salt diet/1% salt water + 250 mg EAF/kg bwt; Group V: Rats + salt-free diet/water +250 mg EAF/kg bwt; Group VI: Rats + salt-free diet/water + 500 mg EAF/kg bwt

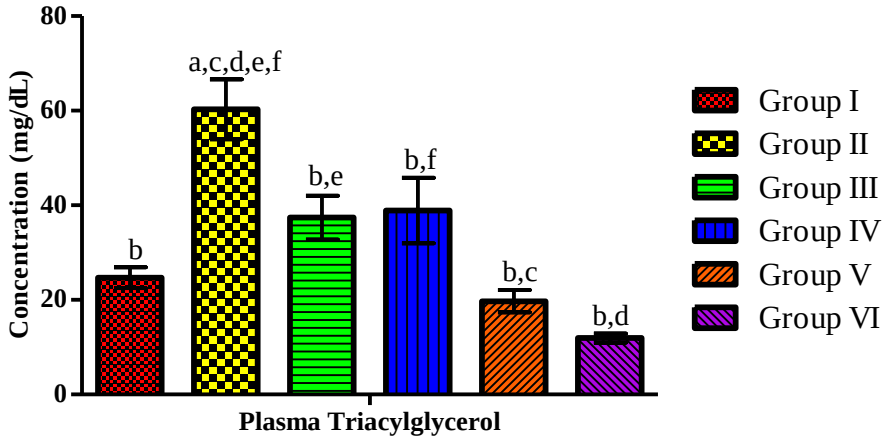

Figure 2: Concentration of Triacylglycerol in the Plasma

Each value is expressed as Mean ± SEM of n = 5. The values with asterisk alphabets are statistically significant at $P < 0.05$. a compares Group I with others, b compares Group II with others, c compares Group III with others, d compares Group IV with others, e compares Group V with others, f compares Group VI with others.

Group I: Rats + salt-free diet/water (control); Group II: Rats + 4 % (w/w) salt diet/ 1 % salt water; Group III: Rats + 4 % (w/w) salt diet/1% salt water + 500 mg EAF/kg bwt; Group IV: Rats + 4% (w/w) salt diet/1% salt water + 250 mg EAF/kg bwt; Group V: Rats + salt-free diet/water +250 mg EAF/kg bwt; Group VI: Rats + salt-free diet/water + 500 mg EAF/kg bwt

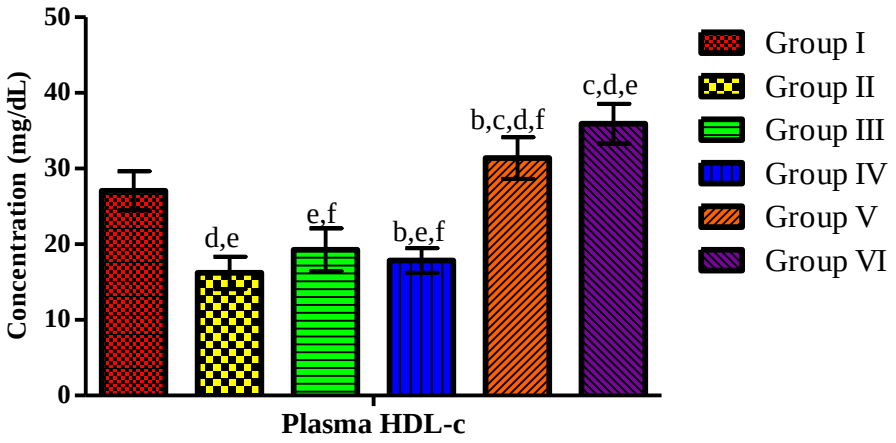

Figure 3: Concentration of HDL-c in the Plasma

Each value is expressed as Mean ± SEM of n = 5. The values with asterisk alphabets are statistically significant at P < 0.05. a compares Group I with others, b compares Group II with others, c compares Group III with others, d compares Group IV with others, e compares Group V with others, f compares Group VI with others.

Group I: Rats + salt-free diet/water (control); Group II: Rats + 4 % (w/w) salt diet/ 1 % salt water; Group III: Rats + 4 % (w/w) salt diet/1% salt water + 500 mg EAF/kg bwt; Group IV: Rats + 4% (w/w) salt diet/1% salt water + 250 mg EAF/kg bwt; Group V: Rats + salt-free diet/water +250 mg EAF/kg bwt; Group VI: Rats + salt-free diet/water + 500 mg EAF/kg bwt

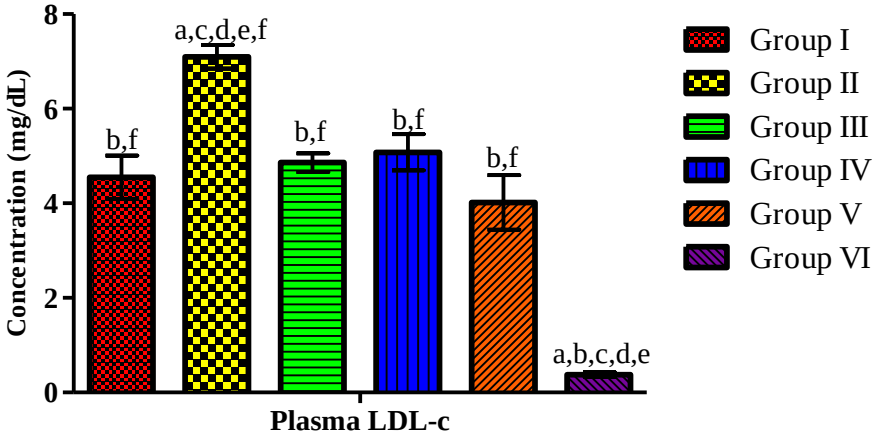

Figure 4: Concentration of LDL-c in the Plasma

Each value is expressed as Mean ± SEM of n = 5. The values with asterisk alphabets are statistically significant at P < 0.05. a compares Group I with others, b compares Group II with others, c compares Group III with others, d compares Group IV with others, e compares Group V with others, f compares Group VI with others.

Group I: Rats + salt-free diet/water (control); Group II: Rats + 4 % (w/w) salt diet/ 1 % salt water; Group III: Rats + 4 % (w/w) salt diet/1% salt water + 500 mg EAF/kg bwt; Group IV: Rats + 4% (w/w) salt diet/1% salt water + 250 mg EAF/kg bwt; Group V: Rats + salt-free diet/water +250 mg EAF/kg bwt; Group VI: Rats + salt-free diet/water + 500 mg EAF/kg bwt

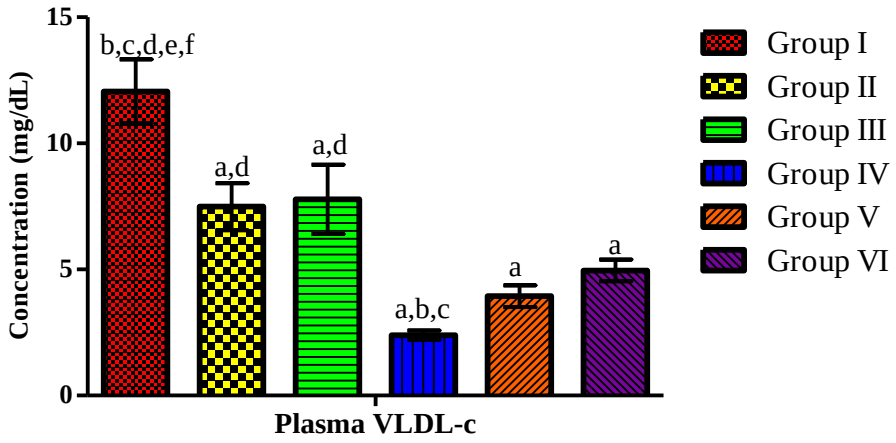

Figure 5: Concentration of VLDL-c in the Plasma

Each value is expressed as Mean ± SEM of n = 5. The values with asterisk alphabets are statistically significant at $P < 0.05$. a compares Group I with others, b compares Group II with others, c compares Group III with others, d compares Group IV with others, e compares Group V with others, f compares Group VI with others.

Group I: Rats + salt-free diet/water (control); Group II: Rats + 4 % (w/w) salt diet/ 1 % salt water; Group III: Rats + 4 % (w/w) salt diet/1% salt water + 500 mg EAF/kg bwt; Group IV: Rats + 4% (w/w) salt diet/1% salt water + 250 mg EAF/kg bwt; Group V: Rats + salt-free diet/water +250 mg EAF/kg bwt; Group VI: Rats + salt-free diet/water + 500 mg EAF/kg bwt

Discussion

Foods that contain phytochemicals with antioxidant activities have been proven through numerous scientific investigations to have potent protective strength against the risk of major diseases like CVDs and cancer (Guldiken et al., 2018; Jakubczyk et al., 2020). These phytochemicals are important components of diet and have found applications in medicinal therapies to ameliorate the deleterious effects of free radicals on biological tissues. Due to their safety in consumption, herbs and spices are major focus in the search for antimicrobials and antioxidants from natural sources (Ostad et al., 2001; Pakniat et al., 2019). Plant-derived compounds especially phenols and flavonoids have been reported to exhibit a number of biological activities such as anti-cancer, anti-ageing, anti-diabetes and reversal of hypertension (Velu et al., 2012; Adegbaju et al., 2019). Very high quantities of phenols, flavonoids, vitamin C, and vitamin E have been reported in fruits and veggies (Cartea et al., 2011; Sarker and Oba, 2020). Phenolics are known to have anti-cancer, antiviral, anti-mutagenic, and antibacterial activities and play protective roles against the progression of chronic diseases, retard lipid oxidation, and decrease the risk of heart diseases (Njintang et al., 2012; Sun et al., 2018; Magrone et al., 2020).

In this study, the phytochemical screening revealed that the phytoconstituents of EAF from *T. cacao* root-bark include alkaloids, flavonoids, cardiac glycosides, saponins, triterpenes, tannins, steroids/phytosterols and xanthoproteins. Previous studies have documented the potent and appreciable antioxidant and anti-inflammatory activities of flavonoids and saponins (Aquila et al., 2009; Oyedapo et al., 2010; Akinpelu et al., 2012).

In the present study, the plasma total cholesterol (TC) concentrations of the rats on high salt diets and water were investigated (Figure 1). The plasma cholesterol level of the normal-treated with 250 and 500 mg extract/ kgbwt (Groups V and VI) rats, as well as the salt-treated groups (Groups III and IV) decreased significantly ($p < 0.05$), compared to the increase observed in the untreated group (Group II).

Previous studies (Song and Jiang, 2017; Oyetayo et al., 2020) indicated that the mechanism for the lipid-lowering action of natural products may be through the enhancement of the activity of the enzyme plasma lecithin cholesterol acyltransferase (LCAT), increase in the excretion of faucal bile acid, inhibition of

biosynthesis of cholesterol in the liver, and a drop in the intestinal and peripheral tissues absorption of lipid. The reduction of plasma cholesterol concentrations observed in the normal-treated with 250 and 500 mg EAF/kg bwt (Groups V and VI) and salt-treated rats (Groups III and IV) could be the result of reduced lipid absorption in the intestine and peripheral tissues, caused by the phytosterols present in the EAF of *T. cacao* root-bark extract or inhibition of hydroxymethylglutaryl CoA (HMG-ScoA) in the liver.

Studies on hyperlipidaemia have shown positive association between increased plasma cholesterol concentration and coronary heart diseases (CHD) (Owunari et al., 2018). The increased plasma cholesterol concentrations observed in the untreated rats group could be an indication of cholesterol metabolism disorder.

The plasma triacylglycerol concentrations of the rats were investigated (Figure 2). The extract decreased the plasma triacylglycerol levels of the rats that were solely administered with it (Groups V and VI) when compared to the control group. Treatment with 250 and 500 mg extract/ kgbwt alongside intake of high-salt diet and salty water showed an ameliorative effect with a significant decrease in the groups (III and IV) when compared with the group that was administered only high-salt diet and salty water without treatment.

Triacylglycerol accounts for approximately 95% of dietary fat found in nature and their primary function is to provide energy for the cell (Choi et al., 2020). It can also be derived from endogenous synthesis and are stored in the liver, from where they are secreted in the blood as VLDL during gluconeogenesis and ketogenesis. Triglyceridaemia is a disorder of lipid metabolism in which the triacylglycerol level in the plasma are greatly elevated accompanied with slight or moderate elevation of cholesterol concentrations (Patrick et al., 2015).

The study also investigated the plasma HDL-c concentrations of the rats (Figure 3). Increased plasma HDL-c levels were noticed in the normal-treated (Groups V and VI) when compared with the significant reduction noticed in the untreated (Group II) relative to the control (Group I). The administration of the extract with treatment with high-salt diet and salty water also increased the concentration of HDL-c in those treated groups (Group III and IV) when compared with the untreated group administered high-salt diet and salty water (Group II). This indicated that the extract increased the plasma HDL-c levels of the rats.

HDL offers the removal of excess cholesterol from the peripheral tissues (and other lipoproteins) and transports them to the liver and steroidogenic tissues for metabolism and excretion (Rysz et al., 2020). Studies have shown that individuals with lower plasma HDL levels are apparently more susceptible to premature coronary heart disease (Bermúdez-López et al., 2016; Silva et al., 2018; Ossoli et al., 2019). The decreased plasma HDL-c levels observed in this study could be an indication of inhibition of excess cholesterol clearance from the peripheral tissues and blood macrophages, hence resulting to high accumulation of cholesterol in the plasma, which could result to atherosclerotic lesion (Nessler et al., 2018).

The plasma LDL-c concentrations of the rats were investigated (Figure 4). The extract decreased the plasma LDL-c levels of the rats relative to the rats not treated with the extract. LDL constitutes about two-third of the total plasma cholesterol. The synthesis of cholesterol is possible in virtually all the tissues of the body but cholesterol that are transported are majorly synthesised by the liver and the intestine. Deficiency in LDL-receptors leads to defects in the breakdown of LDL and extreme LDL concentration in the plasma as a result of the failure of the tissues to internalise, bind, break down and control the synthesis of cholesterol (Allen et al., 2014; Zhong et al., 2021).

The study investigated the liver and plasma VLDL-c concentrations of the rats (Figure 5). The extract decreased the plasma VLDL-c levels of the normal-treated, salt-treated and untreated rats, compared to the control group. Chylomicrons are the major form in which dietary or exogenous fat (chiefly triglycerides and cholesterol) are transported in the system. VLDL is a large triglyceride-rich complex that contains cholesterol and apoproteins (B, C and E). They are synthesized in the liver from fatty acids of adipose tissue or glucose. The inability of the liver to metabolise chylomicrons and VLDL always result in the increased concentration of both cholesterol and triglyceride in the plasma of patients. Hypertriglyceraemia may be due to overproduction of VLDL-triglyceride arising from increased liver synthesis, which could predispose to CVDs.

The reduction in the body weights of rats (Table 1) especially treated with EAF after and with the administration of salty diet/salty water further corroborated the therapeutic value of the extract.
In conclusion, the medicinal value of the ethylacetate extract of cocoa root lies in its bioactive constituents that restore lipid profiles derangement caused by excessive salt both in the diet and water. As such, it could be applied in the management and remedy of salt-induced related cardiovascular diseases caused by free radical generation due to excessive salt intake.

Conflict of Interest: None

Ethical Approval: Ethical clearance was obtained from the Health Research Ethics Committee (HREC) of the Institute of Public Health (IPH), Obafemi Awolowo University, Ile-Ife.

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