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Effect of *Hippocratea africana* Root Bark Extract on Lipid Profile of Female and Male Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JIN and MUE designed the study, wrote the protocol. Author JIN wrote the first draft of the manuscript. Authors JIN and AFU managed the literature searches, performed the spectroscopy analysis. Author JIN managed the experimental process and identified the species of plant. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

There is significant global application of plant(s) extract for curative purposes. One of such focus is herbal remedies for malaria and their safety in biological system need to be ascertained. The antiplasmodial activity of *Hippocratea africana* root bark has been studied. Its effect on the lipid parameters at graded doses of 100, 200 and 300mg/kg body weight administered to both female and male rats of groups II, III, and IV respectively for 14 days was studied. Group I served as the control and was administered distilled water. Phytochemical screening revealed the presence of flavonoids and tannins in high concentrations, alkaloids and cardiac glycosides in moderately concentrations while saponins was trace. There were significant ($p \leq 0.05$) decrease in total cholesterol and increase in TG and HDL-CH for the female test rats at 200mg/kg body weight of the extract compared with the control. There was decrease in VLDL-CH and LDL-CH concentrations that was not significant ($p \geq 0.05$) compared with the control. 200 and 300mg/kg body weight extract treatment groups for the male rats recorded significant ($p \leq 0.05$) increase in total cholesterol and TG concentrations, non significant ($p \geq 0.05$)

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increase in HDL-CH, significant ($p \leq 0.05$) increase in VLDL-CH and significant ($p \leq 0.05$) increase in LDL-CH compared with the control. The significant decrease concentration of total cholesterol with increase in TG and HDL-CH concentrations for the female rats suggest that the herb maybe safe for use its antiplasmodial property. This effect may be due to the phytochemicals present in herb. Its hypercholesterolemia for the male rats however suggests that long term administration of the herb may predispose atherosclerosis/coronary heart disease.

Keywords: Hippocratea africana; malaria; lipid profile; hypercholesterolemia; atherosclerosis.

1. INTRODUCTION

Following the World Health Organization (WHO) recommendation that multi-therapeutic approach in which artemisinin is administered along with other conventional antimalarials for the treatment of malaria, more than 60 countries have now adopted the artemisinin - based combination therapy (ACT) [1], but malaria is a tropical scourge associated mostly with low income groups and peasants who are unable to afford the very expensive ACT, thus there is a general fallback on herbal remedies. Also, there is significant global application of plant(s) extracts for curative purposes with increasing knowledge of the role of the phytochemicals present in them in the expression of the observed pharmacological activities. One of such plants is *Hippocratea africana* (Willd.) Loes. (*Hippocrateaceae*). The roots are used traditionally in the treatment of various ailments such as fever, malaria, body pains, diabetes and diarrhea [2]. It has been reported to possess *In vivo* antiplasmodial activity with LD_{50} of 2.45mg/kg body weight in mice [2].

Lipids play a critical role in almost all aspects of biological life. Most importantly, there is a strong correlation between blood lipids and coronary heart disease (CHD) [3]. When a therapeutic combination leads to an unexpected change or complication, it may be described as an interaction of potential clinical significance [4]. In the case of malaria, drugs used for its treatment example pyrimethamine, has been shown to lower the concentration of serum free fatty acids and total cholesterol [5]. The hypoglycaemic effect of quinine, which complicates malaria in hypoglycaemic patients, has also been reported [6]. Chloroquine, quinine, quinacrine and sulphydryl reagents; p-hydroxy mecuribenzoate (pHMB) and himerosal inhibit lysophospholipase activity, hence the level of free fatty acids in blood [7].

The drawbacks therefore, in the use of herbal remedies for their therapeutic property (ies) is/are their effect(s) on biochemical parameters. This study aimed at determining the effect of *Hippocratea africana* root bark extract traditionally used in the treatment of malaria on lipid profile of both female and male rats.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The roots of *Hippocratea africana* (Willd) Loes were harvested in February 2009 from Afaha Etok Ibesikpo forest in Ibesikpo-Asutan, L.G.A of Akwa Ibom State. They were identified and authenticated a Taxonomist in the Department of Botany, University of Uyo, Uyo. A voucher specimen of the root was deposited in the herbarium of Botany Department, University of Uyo, Uyo.

The roots were washed and the bark scrapped with a knife, air dried and crushed into powdered form using an electric blender. The powdered sample (2Kg) was stored in a dried, bottle-airtight container in a refrigerator at 4ºC. The lipid profile was carried out in the Biochemistry laboratory of the University of Uyo, Uyo.

2.2 Preparation of Plant Extract

Eighty per cent ethanol was used in the extraction. One kilogramme of the powdered *H. africana* root bark was exhaustively macerated in 2000ml of 80% ethanol. It was left overnight to allow for the solvent to interact with the extract and solubilize the active ingredients. The clear solution (filtrate) was siphoned out using syringe and needle. The filtrate was concentrated to reduce the water content by drying in a water bath at 40ºC to obtain a dry extract. The dried crude extract was stored in the refrigerator until required for use.

2.3 Experimental Animals and Animal Housing for Biochemical Studies

Forty-eight mature albino Wistar rats consisting of twenty four males and twenty four females weighing 163 - 227 grams each were obtained from the animal house of the College of Health Sciences, University of Uyo, Uyo and used in this study. The used of the animals was approved by the Ethical Committee of the University of Uyo, Uyo. The animals were divided into four groups made up of six rats to a group. They were caged in plastic cages made of stainless steel bottom. Stainless steel mesh were placed at the bottom of the cages for collection of faeces and feed droppings. The males were caged separately from the females to prevent mating during the treatment period. The weights of the animals were measured before and at the end of the experimental period. Groups II, III and IV animals were administered graded doses of 100, 200 and 300mg/kg of the crude root bark extract of *H. africana* calculated on the basis of the body weight of the animals. These doses was based on the LD_{50} of 2.5mg/kg body in mice [2]. Group I animals served as the control and were administered 1ml distilled water. The extract was administered orally once daily for fourteen (14) days by use of a canular attached to syringe. All the experimental animals were given normal rat chow (Vital Feeds, Jos) and water *ad libitum* throughout the treatment period.

2.4 Collection of Blood Samples

At the end of the fourteen days treatment, the weights of animals were taken. The animals were denied their feeds but still had water *ad libitum* for sixteen hours before they were chloroform anaesthetized and dissected. Blood sample was obtained immediately by cardiac puncture using sterile syringes and needles. The serum was obtained by centrifugation of clotted blood in a MSE table top centrifuge at 4,000 x g for 10 minutes.

2.5 Determination of Biochemical Analytes

Serum lipid profiles of the experimental animal were estimated using standard lipid profile kits obtained from Randox laboratory, United Kingdom, BT294QY. Total triacylglycerol concentration was assayed [8]. Cholesterol concentration was estimated using cholesterol enzymatic end point method [9], while HDL-cholesterol concentration was estimated using precipitant method [10]. Their absorbance was read at 546nm. The VLDL-cholesterol was obtained by dividing the serum triacylglycerols by 2.2. LDL-Cholesterol was derived differentially (LDL-cholesterol = Total-cholesterol – (TG/2.2) - HDL-cholesterol) [11]. Phytochemical tests were carried out using standard methods procedures [12,13].

2.6 Statistical Analysis

Data were expressed as mean ± SD and were analyzed using Analysis of Variance (ANOVA). Difference in mean at p≤0.05 was considered significant using Student's t- test.

3. RESULTS

The lipid profile of female and male albino Wistar rats administered graded doses of 100, 200 and 300mg/kg body weight of *H. africana* extract compared with the control is as presented on Table 2.

The female rats recorded significant (p≤0.05) decreases in serum total cholesterol concentration of 1.45±0.06, 1.39±0.04 and 1.48±0.05 mmol/l for test groups II, III and IV rats respectively compared with control group I concentration of 1.95±0.19mmol/l. There were also decreases in triacylglycerols concentrations of 0.84±1.15, 0.74±0.10 and 0.89±0.10mmol/l respectively for test groups II, III, IV compared with the control group I concentration of 1.07±0.13mmol/l. The decreases were significant (p≤0.05) only for group III compared with the control. The serum HDL-cholesterol concentration showed dose-dependent increases of 0.58±0.03, 0.63±0.07 and 0.65±0.05mmol/l for test groups II, III and IV rats respectively and the increase was only significant (p≤0.05) for test group IV rats compared with the control. There was no significant changes in VLDL-cholesterol at all the doses administered compared with the control. The serum LDL-cholesterol showed a non-significant (p≥0.05) decreases but not in a dose dependent manner compared with the control group I. The decreases in triacylglycerol, total cholesterol, VLDL- cholesterol and LDL-cholesterol lipid fraction with increase in HDL-cholesterol denote good lipid profile female.

The male rats recorded different concentrations of total cholesterol. Compared with the control. Test group II rats recorded a decrease in total cholesterol concentration of 1.45 ± 0.26mmol/l compared with the control concentration of 1.49±0.10mmol/l while test groups III and IV rats recorded increases of 1.83±0.15 and 2.47±0.21mmol/l respectively that showed significant (p≤0.05), only when test group IV concentration was compared with the control. The serum triacylglycerol result showed significant (p ≤ 0.05) increases of 0.44 ± 0.02 and 0.82±0.19mmol/l for test groups III and IV respectively compared with the control group I concentration of 0.37±0.03mmol/l. Test group IV concentration recorded further significant (p≤0.01) increase compared with the control. There was no significant (p≥0.05) decrease in triacylglycerol concentration for test group II rats compare with the control. The HDL-cholesterol concentrations were 0.54±0.03, 0.62±0.07, 0.60±0.03 and 0.59±0.05mmol/l for control group I and test II, III and IV respectively. The increases in the test groups were not significant (p≥0.05) compared with the control. The serum VLDL-cholesterol concentration result showed significant (p≤0.05) dose-dependent increases of 0.20±0.01 and 0.37±0.09mmol/l for test groups III and IV compared with 0.17±0.01mmol/l concentration for the control group I rats. Test group II rats recorded a decrease concentration of 0.16±0.01mmol/l compared with the control that was not significant (p≥0.05). Test group II recorded a decreased LDL-cholesterol concentration for test group II was 0.67±0.19mmol/l. The value was not significant (p≥0.05) compared with the control group I concentration of 0.78±0.10mmol/l. Test groups III and IV, however recorded increasing concentrations of 1.03±0.13 and 1.51±0.21mmol/l LDL-cholesterol concentrations respectively. Only test group IV concentration was significant (p≤0.05) compared with the control. The serum lipid profile of the male rats showed an increase in HDL-cholesterol and LDL-cholesterol exception of

group II for LDL- cholesterol. There was a decrease in total cholesterol, TG, and VLDL-cholesterol for group II and an increase in total cholesterol, TG, and VLDL-cholesterol for group lV. Long term administration of the herb may have adverse impact on the male lipid profile.

4. DISCUSSION

In this study, variations in lipid parameters following administration of graded doses of *H. africana* root bark extract to both female and male albino Wistar rats was observed. The serum total cholesterol for the female test rats showed significant decreases while the male test rats showed increases in total cholesterol concentration at doses of 200 and 300mg/kg body weight of extract treatment. The 100mg/kg body weight of the extract treatment however recorded slight non significant decrease concentration. The relationship between cholesterol and atherosclerotic coronary disease is curvilinear [14]. Many studies have confirmed that free and esterified cholesterol accumulates in the aorta, coronary arteries and cerebral veins.

Also, when the total cholesterol concentration is high, the incidence and prevalence of coronary heart disease are also high [15]. The significant decrease concentrations of total cholesterol observed for the female rats at doses of 100, 200 and 300mg/kg body weight of the extract treatment suggest that the use of the herb for its antiplasmodial property is maybe safe for the females as it does not induce hypercholesterolemia or predispose to coronary heart disease. Its hypercholesterolemia effect at doses of 200 and 300mg/kg body weight for the male rats however suggest that long term administration of the herb may predispose atherosclerosis and should not be administered to diabetic males. Plant products can lower cholesterol level by many mechanisms such as by increase removal of very low density lipoprotein by peripheral tissues and by an increased excretion of bile in faeces [1]. There is also reported significant reduction in the cholesterol level of rats receiving a low dose of garlic (11-14%) that was suggestive of modulation in its absorption [16]. Decreases in total cholesterol may also be due to decrease of cholesterol synthesis and cholesterol ester hydrolyses enzymes which metabolizes cholesterol and vice versa. Decrease in total cholesterol concentration following pyrimethamine administration at a dose of 5mg/kg have been reported [17]. The hypocholesterolemic effect observed for the female rats may be due to the phytochemicals present in herbs and possibly, by unknown mechanism for the synthesis of female productive hormone. Phytochemical screening of the root bark of *H. africana* revealed high concentration of tannins and flavonoids. Alkaloids and cardiac glycosides were of moderate concentration while saponin concentration was trace (Table 1). Flavonoids prevent the oxidation of low density lipoprotein, lowers the blood cholesterol and triacylglycerols levels and thus reduce the risk of developing atherosclerosis [18]. Flavonoids have vaso-dilatory and inhibitory effects on platelets aggregation which in turn help in preventing coronary heart diseases [19]. Tannins are polymeric phenolic substances that are capable of tanning leathers or precipitating gelatin from solution. There is a report that consumption of beverages containing tannins such as green teas and red wines may have positive effect on illnesses including heart related diseases [20]. Alkaloids have been reported to possess antimalarial, analgesic, antibacterial and antihypertensive properties [21] while cardiac glycosides are cardioactive compounds with their inherent property on the aglycone portion of the sugar moiety that exert a number of effects on neural tissue thereby indirectly influencing the mechanical and electrical activities of the heart, modifying vascular resistance and capacitance [22]. Saponins on the other hand have been reported to have health benefits such as lowering cholesterol level and having anti cancer properties [23]. There was observed decrease in TG concentrations, increase in HDL-cholesterol

concentrations that was only significant ($p \leq 0.05$) at 200mg/kg body weight of the extract, non significant ($p \geq 0.05$) decrease in VLDL-cholesterol and LDL-cholesterol concentrations for all the female treatment groups compared with the control. Study on the effect of antimalarial agents on the fasting lipid profile in systemic lupus erythematosus showed significantly lower concentrations of total cholesterol, VLDL-cholesterol and LDL-cholesterol in patients taking antimalarials, including patients taking concomitant prednisolone [24].

The 200 and 300mg/kg body weight extract treatment groups for the male rats recorded significant increase in TG, non significant increase in HDL-cholesterol, significant increase in VLDL-cholesterol and significant increase in LDL-cholesterol compared with the control. Only 100mg/kg body weight extract treated rats recorded non significant ($p \geq 0.05$) decrease in TG, non significant increase in HDL-cholesterol, non significant ($p \geq 0.05$) increase in VLDL-cholesterol and non significant ($p \geq 0.05$) decrease in LDL-cholesterol concentrations compared with the control. Triacylglycerol concentration is used in the diagnosis and treatment of patient with hyperlipidaemia. Significant decrease in serum TG, increases in HDL-cholesterol, decreases in VLDL and decreases in VLDL is an indication of a favorable lipid hence, less formation of atherosclerotic plaques and a resultant low pressure as seen in all the female treatment rats and 100mg/kg body weight extract treated male rats. HDL is involved in the reverse transport of cholesterol from the atherosclerotic plaque and high plasma concentrations of HDL cholesterol are associated with decreased risk of coronary heart disease (CHD) [25]. The ratio of LDL/HDL is used as an index of risk of CHD [26]. Coronary artery disease and other metabolic disorders are strongly associated with low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol as well as triacylglycerol concentration [27]. There is confirmation that treatment causing an increase of HDL-C levels are associated with a reduction in the coronary risk and with a significant regression of arterial lesion [28,29]. The reverse was observed for male test groups III and IV rats administered 200 and 300mg/kg body weight of the extract. High TG, high VLDL-C together with LDL-C has been reported to directly promote atherosclerosis. Very low density lipoproteins are rich in TG and so an elevated VLDL levels results in hypertriacylglycerolemia which may result in atherosclerosis. However, hyperglycemic effect following administration of graded doses of *H. africana* root bark extract to both female and male rats compared with the control have been reported [30]. The hyperglycemic effects observed was suggested to be due to the phytochemical constituent(s) present in the herb that may have glucagon-like activity. The observed increase in glucose concentration was suggested to be favorable to the organisms owing to the metabolic roles of glucose in several tissues. There is a lot of literature on the inverse relationship between the intake of polyphenols that is highly present in *H. africana* extract (Table 1) and cardiovascular diseases [31,32].

Table 1. Phytochemicals present in *H. africana* root bark extract

Chemical constituent	Inference
Alkaloids	++
Saponins	+
Tannins	+++
Phlobatannins	-
Anthraquinones	-
Flavonoids	+++
Cardiac glycosides	++

Key: +++ = Highly concentrated or highly present, ++ = Moderately present, + = Trace, - = Absent

Table 2. Serum lipid profile of female and male albino Wistar rats administered graded doses of ethanol extract of *Hippocratea africana* root bark

Group/Dosage	Serum Total Cholesterol (mmol/l)		Serum Triacylglyce-rols (mmol/l)		Serum HDL-Cholesterol (mmol/l)		Serum VLDL-Cholesterol (mmol/l)		Serum LDL-Cholesterol (mmol/l)	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Group I Control (Distilled water)	1.95±0.19	1.49 ± 0.10	1.07±0.13	0.37±0.03	0.53±0.02	0.54±0.03	0.49±0.06	0.17±0.01	0.93±0.18	0.78±0.10
Group II H.A (100mg/kg body weight)	∗1.45±0.06	1.45 ± 0.26	0.84±1.15	0.33±0.01	0.58±0.03	0.62±0.07	0.38±0.07	0.16±0.01	0.28±0.05	0.67±0.19
Group III H.A (200mg/kg body weight)	∗1.39±0.04	1.83±0.15	∗0.74±0.10	∗0.44±0.02	0.63±0.07	0.60±0.03	0.34±0.05	∗0.20±0.01	0.41±0.03	∗1.03±0.13
Group IV H.A (300mg/kg body weight)	∗1.48±0.05	∗2.47±0.21	0.89±0.10	∗0.82±0.19	∗0.65±0.05	0.59±0.05	0.40±0.04	∗0.37±0.09	0.42±0.04	∗1.51±0.21

*Results are presented as Mean ± SD, n = 6, H.A. = Hippocratea africana root bark extract, * = Significantly different from control value at p≤0.05*

5. CONCLUSION

Interest in medicinal plants as a re-emerging health aid has been filled by the rising costs of prescription drugs in the maintenance of personal health and well-being and the bioprospecting of new plant-derived drugs. In this study, there was reduction in the markers of atherosclerotic coronary disease for the female test rats by the extract as seen in favorable lipid profile parameters (with increase concentration of HDL-cholesterol) but there is need to avoid high dosage and long term administration of the herb to prevent hypertriacylglycerolemia observed in the male rats even with its good lipid lowering potential observed in the female rats.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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