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Comparative Hepatic Effects of *Hippocratea africana* Root Bark Extract on Female and Male Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author JIN designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors JIN and EGE managed the literature searches. Author JIN performed the spectroscopy and statistical analyses. Authors JIN and EGE managed the experimental process and author JIN identified the specie of plant. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To compare the effects of *Hippocretea africana* root bark extract on liver function biomarkers of female and male albino Wistar rats.
Study Design: 48 albino Wistar rats weighing 163-227 gms consisting 24 females and 24 males were randomly distributed into 4 groups of six animals each on sex basis. Group I served as normal control and were given 1 ml of distilled water. Groups II, III and IV were administered 100, 200 and 300 mg/kg body weight respectively of the extract by oral intubation.
Place and Duration of Study: Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, Uyo. The duration of the study was 14 days.
Methodology: Liver function biomarkers (AST, ALT, ALP and GGT) and Oxidative Stress markers (catalase (CAT), Superoxide dismutase (SOD) activities and MDA concentrations were assayed

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using standard procedures. Histopathological examinations of liver organs were also carried out using standard methods.

Results: Generally, there were increases in ALT and AST activities for both female and male groups II and III extract treated rats while test group IV recorded decreased activities for the female compared with the control. AST and ALP activities were significantly ($p < 0.05$ and $p < 0.01$) higher in male rats with exception of AST for group IV and GGT for groups II and III compared with the control. Treatments with extract significantly ($p < 0.05$ and $p < 0.01$) increased the activities of SOD and CAT and also significantly ($p < 0.05$ and $p < 0.01$) increased MDA concentrations in female and male rats.

Conclusion: It can be concluded that *Hippocratea africana* root bark extract has a mild toxic effect on liver tissues as seen from the concentrations of liver function enzymes, and the histopathological examination. The increased activities of SOD and CAT and MDA concentrations may probably be a way of first line defence. Thus, long-term administration of the herb may not be advised in the males even with its good antiplasmodial property.

Keywords: Hippocratea africana; antiplasmodial activity; liver enzymes; catalase; superoxide dismutase; malondialdehyde.

1. INTRODUCTION

Different parts of plants; leaves, barks, roots and even latex are used medicinally by Traditional Medicine Practitioners (TMPs) for the treatment of various body ailments such as hypertension, diabetes mellitus, ulcer, malaria, typhoid fever, gonorrhea, syphilis, cancer etc [1].

Research interest is now focused on various herbs that possess hypoglycaemic, hypolipidaemic, antiplatelet, antihypertensive, antidiabetic, antitumour, immune stimulating or antimalarial properties etc. that may be useful in complementary properties or alternative medicine [2]. This interest is fuelled in Africa (Nigeria in particular) by the rising cost of prescription drugs in the maintenance of personal health and well-being. Available evidence also shows that some herbal remedies used in the treatment of ailments are affordable and efficacious [3]. Exploration of nature as a source of new active agents is thus essential for the discovery of bioactive chemical substances from natural product for the development of novel molecular diversity of efficacious drugs. Natural products from plants, used either alone or with combinatorial synthetic methodologies, constitute a multidisciplinary approach to the current drug productivity [4,5].

Hippocratea africana is a plant that inhabits green forests and is a perennial climber with hairs (glabrous) and reproduced from seeds [6]. The plant is widely distributed in tropical Africa and the roots are used traditionally in the treatment of various ailments such as fever, malaria, body pains, diabetes and diarrhea [7]. It has been reported to possess *in vivo* antiplasmodial activity with LD50 of 2.45 mg/kg body weight in mice [7]. Okokon et al. [7] also reported that *H. africana* possesses anti-inflammatory, analgesic and anti-pyretic properties which are probably mediated via inhibition of various autocoid formation and release. Other species of *Hippocratea* which possess anti- inflammatory activity are *Hippocratea excels* [8] and *Hippocratea indica* [9]. In the phytochemical study of *Hippocratea africana* Willd. (Loes). (Hippocrateaceae), Ndem et al. [10] reported that the plant contain significant quantities of phytochemicals such as alkaloids, cardiac glycosides, tannins, anthraquinonones and flavonoids. Alkaloids are known to exhibit emetic amoebicide, expectorant, anaesthetic, antipyretic, analgesic, antihelminthic properties and are used for the treatment of stomach problems [3]. The presence of cardiac glycosides in the *H. africana*, known to be useful in the treatment of diseases associated with the heart [11], further confirms the usefulness to the Traditional Medicine Practitioners (TMPs) in the treatment of hypertension. Flavonoids elicit a wide range of therapeutic activities such as antihypertensive, antimicrobial, anti-tumour and its usefulness by herbalist confirms the presence of phytochemical properties in *Hippocratea africana* [12,13].

The blood promising schizontocidal activity of *H. africana* both in early and established malaria infection at oral doses of 200 to 600 mg/kg/day in mice have been reported [7] and owing to the wide spread use of the root bark of this plant as an antiplasmodial herb by the Niger Delta people of Nigeria (Akwa Ibom State), its effect on the

liver as the major organ of xenobiotic metabolism was studied to ascertain the possible toxic or adverse effects that may be associated with its use.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Extract

The roots of *Hippocratea africana* (Willd) Loes were harvested from Afaha Etok Ibesikpo forest in Ibesikpo-Asutan, L.G.A of Akwa Ibom State in Nigeria. They were identified and authenticated by 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 using tap water and the bark scrapped with a knife, cut into very tiny pieces, air dried and pulverised using an electric blender. The powdered sample (2 Kg) was stored in a dried, airtight container at 37℃.

One kilogramme of the powdered *H. africana* was exhaustively macerated in 2000 ml of 80% ethanol. It was left overnight to allow for the solvent to interact with the extract and solubilise the active ingredients. The clear orange filtrate was carefully siphoned using a tube and concentrated in vacuum at 40℃ to obtain a dry orange crude extract. The dried crude extract was stored in the refrigerator at 4℃ until required for use.

2.2 Experimental Animals

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. The animals were divided into four groups of six rats each on sex basis. 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.

2.3 Administration of Plant Extract

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 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 feeds but still had water *ad libitum* for sixteen hours before they were chloroform anaesthetized and dissected. Blood sample was obtained by cardiac puncture using sterile syringes and needles and collected into plain sample bottles for serum separation. The serum was obtained by centrifugation of clotted blood in a MSE table top centrifuge at 4,000 rpm for 10 minutes. The separated sera were stored in a refrigerator at 4℃.

2.5 Assessment of Serum Activities of Oxidative Stress Biomarkers and Assay of Serum Activities of Liver Function Enzymes

Serum activities of superoxide dismutase (SOD), catalase (CAT), as well as lipid peroxidation (LPO) measured in terms of malondialdehyde (MDA) concentration was assessed.

Superoxide Dismutase and catalase activities were assayed based on the method by Wheeler et al. [14]. Assessment of lipid hydroperoxide (LPO) measured in terms of malonyldialdehyde (MDA) concentration was carried out based on method by Mihaljevic et al. [15]. They were all done using Cayman laboratory research kits.

Serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatise (ALP), and gamma glutamyl transferase (GGT), were assessed. ALT, AST and ALP activities were determined using Randox laboratory reagent kit, UK, BT 29 4QY based on method by Reitman and Frankel [16]. Serum activity of gamma-glutamyl-transferase (GGT) activity was estimated using Randox laboratory kit reagent, UK, BT294QY based on method by Szassz [17].

2.6 Histological Examinations

The liver tissues were extracted by the use of forceps and scissors. They were placed on clean filter paper to mop up excess blood, weighed and stored in screw tight containers containing 10% buffered formaldehyde solution. Histological analysis was carried out according to the procedure recommended by Drury and Wallington [18]. The slides were examined using a light microscope and the histopathological changes especially morphological changes were

recorded. Photomicrographs of prominent pathological lesions were taken.

2.7 Statistical Analysis

The results of all determinations were expressed as Mean ± standard deviation. Data between treatment groups were analysed using one way analysis of variance (ANOVA). Pairwise comparison was done using the student's t-test. Values of $p < 0.05$ and $p < 0.01$ were regarded as being significant.

3. RESULTS

3.1 Effect of Extract on Serum Activities of Superoxide Dismutase (SOD), Catalase (CAT) and Concentration of Malonyldialdehyde (MDA) of Female and Male Albino Wistar Rats

Tables 1 and 2 shows the serum activities of superoxide dismutase (SOD), catalase and concentration of MDA of female and male rats administered 100, 200 and 300 mg/kg bw of *H. africana* root bark extract to test groups II, III and IV respectively against control group I rats administered distilled water.

For the female rats, the result in Table 1 depicts a dose- dependent significant ($p < 0.05$ and $p < 0.01$) increase in superoxide dismutase activity of 1.26±0.04, 1.88±0.07 and 2.23±0.08 U/ml for test groups II, III and IV respectively administered 100, 200 and 300 mg/kg bw respectively of *H. africana* root bark extract compared with the control group I activity of 1.17±0.04 U/ml. Catalase activity showed significant ($p < 0.05$ and $p < 0.01$) increases of 14.52±0.45, 18.35±0.54 and 17.58±0.04 nmol/min/ml for test groups II, III and IV respectively compared with the control group I activity of 12.49±0.5 nmol/min/ml, although test group III recorded a non-significant higher value than test group IV. MDA concentration also depicts significant ($p < 0.05$ and $p < 0.01$) increasing activities of 1.92± 0.01, 2.46±0.06 and 2.35±0.04 μM for test groups II, III and IV respectively compared with the control group I concentrations of 1.52±0.02 μM.

Table 2 depicts the serum activities of superoxide dismutase (SOD) and catalase and concentration of MDA for male albino rats. SOD activity against concentration doses of 100, 200 and 300 mg/kg bw *H. africana* root bark extract showed a significant ($p < 0.05$) dose dependent increases of 1.34±0.01, 1.40±0.01 and 1.73±0.10 U/ml for test groups II, III and IV respectively compared with the control group I activity of 1.11±012 U/ml. Test groups II, III, and IV rats showed significant ($p < 0.05$ and $p < 0.01$) increasing catalase activity of 23.18±0.03, 31.29±0.57 and 26.12±0.38 nmol/min/ml compared with the control group I activity of 20.80±0.39 nmol/min/ml. Although, test group III rats recorded higher value than test group II rats. Graded concentrations of 100, 200, 300 mg/kg

Table 1. Serum activities of superoxide dismutase (SOD) and catalase (CAT) and concentration of Malonyldiadehyde (MDA) of female albino Wistar rats administered graded doses of ethanol extract of *Hippocratea africana* root bark

Group/Dosage	Superoxide dismutase (SOD) (U/ml)	Catalase (nmol/min/ml)	Malonyldialdehyde (MDA) (μM)
Group I			
Control	1.17±0.04	12.49±0.53	1.52±0.02
(distilled water)			
Group II	*,**	*,**	*,**
H.A	1.26±0.04	14.52±0.45	1.92±0.01
(100 mg/kg body weight)			
Group III	*,**	*,**	*,**
H.A	1.88±0.07	18.36±0.54	2.46±0.06
(200 mg/kg body weight)			
Group IV	*,**	*,**	*,**
H.A	2.23±0.08	17.58±0.04	2.35±0.04
(300 mg/kg body weight)			

*Results are presented as Mean ± SD, n = 6; H.A = Hippocratea africana root bark extract; *= Significantly different from control value at p < 0.05, ** =Significantly different from control value at p < 0.01*

Table 2. Serum activities of superoxide dismutase (SOD) and catalase (CAT) and concentration of malonyldiadehyde (MDA) of male albino Wistar rats administered graded doses of ethanol extract of *Hippocratea africana* root bark

Group/Dosage	Superoxide dismutase (SOD) (U/ml)	Catalase (nmol/min/ml)	Malonyldialdehyde (MDA) (μM)
Group I Control (distilled water)	1.11±0.12	20.80±0.39	1.19±0.05
Group II H.A (100 mg/kg body weight)	* 1.34±0.01	*,** 23.18±0.37	*,** 1.47±0.02
Group III H.A (200 mg/kg body weight)	* 1.40±0.01	*,** 31.29±0.57	*,** 1.55±0.02
Group IV H.A (300 mg/kg body weight)	* 1.73±0.10	*,** 26.12±0.38	*,** 1.52±0.01

Results are presented as Mean ± SD, n = 6, H.A= Hippocratea africana root bark extract
=Significantly different from control value at p < 0.05,=Significantly different from control value at p < 0.01*

bw administered to test groups II, III and IV rats respectively recorded significant ($p < 0.05$ and $p < 0.01$) increasing MDA concentrations of 1.47±0.02, 1.55±0.02, and 1.52±0.01 μM compared with the control group I concentration of 1.19±0.05 μM.

3.2 Effect of Extract on Activities of Serum Liver Enzymes and AST/ALT Ratio of Albino Wistar Rats

The effects of graded doses of ethanol extract of *H. africana* root bark on the activities of serum liver enzymes and AST/ALT ratio of female and male albino Wistar rats are depicted in Tables 3 and 4 respectively.

The female rats (Table 3) recorded Alanine aminotransferase (ALT) activities of 17.06±1.84, 22.84±3.56, 18.43±3.75, and 16.55±1.62 u/l for the control group I and test groups II, III and IV animals respectively. Only test group II increase activity was statistically significant ($p < 0.5$ and $p < 0.01$) compared with the control group I activity.

The aspartate aminotransferase (AST) activity for test groups II, III and IV were 191.43±1.96, 190.78±2.22 and 186.68 u/l respectively while the control group I rats recorded activity of 189.17±3.01 u/l. The result showed a non-significant ($p > 0.5$) dose-dependent reduction in AST activity compared with the control. Test groups II, III, and IV rats recorded dose-dependent decreases in Alkaline phosphatase (ALP) activity of 114.73±4.30, 111.29±3.74 and 109.18±4.67u/l that were all significant ($p < 0.5$ and $p < 0.01$) compared with the control group I activity of 125.71±1.16 u/l. Gamma glutamyl transferase (GGT) activity showed a non-significant ($p > 0.5$) decreasing values of 1.51 ±1.14, 2.28±0.88 and 1.93±0.55 u/l for test groups II, III and IV respectively compared with the control group I activity of 2.32 0.54u/l. The AST/ALT ratio were 8.38, 10.35 and 11.27 for test groups II, III and IV rats compared with control group rats ratio of 11.09.

The male rats (Table 4) recorded a significant ($p < 0.5$ and $p < 0.01$) increased ALT activities of 20.53±2.18 and 19.46±2.06 u/l for test groups II and III rats compared with the control group I activity of 14.14±1.68 u/l. Test group IV rats however recorded a non-significant ($p > 0.05$) decreased activity of 13.14±2.61 u/l compared with the control. The result shows increasing AST activities of 200.54±1.73, 186.33±4.06 and 205.55±2.91 u/l for test groups II, III and IV respectively compared with the control group I activity of 163.45 u/l. The increases though statistically significant ($p < 0.5$ and $p < 0.01$) were not dose-dependent. ALP activity depicts a dose-dependent significant ($p < 0.5$ and $p < 0.01$) increasing activities of 165.17±5.52, 185.53±8.04 and 207.00±8.59 u/l for test groups II, III and IV rats respectively compared with the control group I activity of 151.19±7.15 u/l. The GGT activity showed decreasing values of 2.32±0.02 and 2.70±1.08 u/l for test groups II and IV rats respectively compared with the control group 1

activity of 2.83±0.23 u/l. Test group III activity however showed a non-significant ($p > 0.05$) increase activity of 2.90±2.45 u/l compared with control group I activity. The decreases were only significant ($p < 0.5$ and $p < 0.01$) when test group II activity was compared with control group I activity. The AST/ALT ratio was 9.54, 9.58, 15.65 for test groups II, III and IV rats respectively and 11.56 for control group rats.

There were increases in ALT activity of female group II and III extract treated rats while test group IV recorded decrease activities. AST and ALP activity were significantly higher in male rats with exception of AST for group IV (300 mg/kg bw) and GGT for group II and III, enzyme activities in the male followed the same pattern as the female rats.

3.3 Histological Evaluations of the Liver Tissues of albIno Wistar Rats Administered Graded Doses of *H. africana* Root Bark Extract

Sections from the liver were taken from all the animals used in this study after 14 days extract administration period and analysed for histopathological effects.

Table 3. Activities of serum liver enzymes and AST/ALT ratio of female albino Wistar rats administered graded doses of ethanol extract of *Hippocratea africana* root bark

Group/ Dosage	ALT (U/l)	AST (U/l)	ALP (U/l)	GGT (U/l)	AST/ALT
Group I					
Control (distilled water)	17.06±1.84	189.17±3.01	125.71±1.16	2.32±0.54	11.09
Group II	*,**		*,**		
H.A (100 mg/kg body weight)	22.84±3.56	191.43±1.96	114.73±4.30	1.51±1.14	8.38
Group III	*,**		*,**		
H.A (200 mg/kg body weight)	18.43±3.75	190.78±2.22	111.29±3.74	2.28±0.88	10.35
Group IV	*		*,**		
H.A (300 mg/kg body weight)	16.55±1.62	186.68±2.27	109.18±4.67	1.93±0.55	11.27

Results are presented as Mean ± SD, n = 6, H.A=Hippocratea africana root bark extract
*ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = Alkaline phosphatise; GGT = Gamma glutamyl transferase; * = Significantly different from control value at p < 0.05; ** = Significantly different from control value at p < 0.01*

Table 4. Activities of serum liver enzymes and AST/ALT ratio of male albino Wistar rats administered graded doses of ethanol extract of *Hippocratea africana* root bark

Group/Dosage	ALT (U/l	AST (U/l)	ALP (U/l)	GGT (U/l)	AST/ALT
Group I					
Control (distilled water)	14.14±1.68	163.45±5.09	151.19±7.15	2.83±0.23	11.56
Group II	*,**	*	*,**	*,**	
H.A (100 mg/kg body weight)	20.53±2.18	200.54±1.73	165.17±5.52	2.32±0.02	9.54
Group III	*,**	*,**	*,**		
H.A (200 mg/kg body weight)	19.46±2.06	186.33±4.06	185.53±8.04	2.90±2.45	9.58
Group IV		*,**	*,**		
H.A (300 mg/kg body weight)	13.14±2.61	205.55±2.91	207.00±8.59	2.70±1.08	15.65

Results are presented as Mean ± SD, n = 6; H.A.= Hippocratea africana root bark extract
*ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = Alkaline phosphatise; GGT = Gamma glutamyl transferase; * = Significantly different from control value at p < 0.05; ** = Significantly different from control value at p < 0.01*

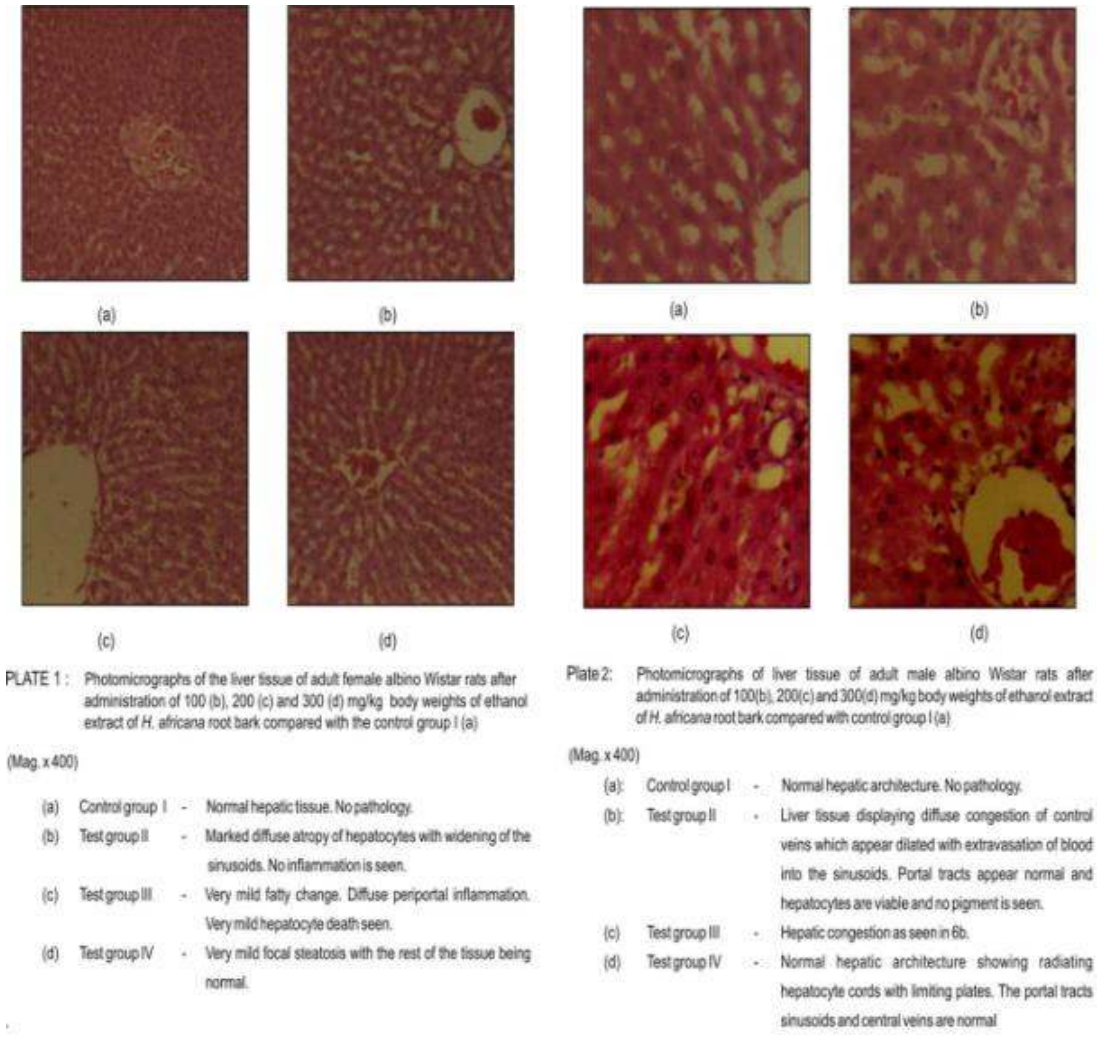

PLATE 1 : Photomicrographs of the liver tissue of adult female albino Wistar rats after administration of 100 (b), 200 (c) and 300 (d) mg/kg body weights of ethanol extract of *H. africana* root bark compared with the control group I (a)

(Mag. x 400)

(a) Control group I - Normal hepatic tissue. No pathology.
(b) Test group II - Marked diffuse atropy of hepatocytes with widening of the sinusoids. No inflammation is seen.
(c) Test group III - Very mild fatty change. Diffuse periportal inflammation. Very mild hepatocyte death seen.
(d) Test group IV - Very mild focal steatosis with the rest of the tissue being normal.

Plate 2: Photomicrographs of liver tissue of adult male albino Wistar rats after administration of 100(b), 200(c) and 300(d) mg/kg body weights of ethanol extract of *H. africana* root bark compared with control group I (a)

(Mag. x 400)

(a): Control group I - Normal hepatic architecture. No pathology.
(b): Test group II - Liver tissue displaying diffuse congestion of control veins which appear dilated with extravasation of blood into the sinusoids. Portal tracts appear normal and hepatocytes are viable and no pigment is seen.
(c) Test group III - Hepatic congestion as seen in 6b.
(d) Test group IV - Normal hepatic architecture showing radiating hepatocyte cords with limiting plates. The portal tracts sinusoids and central veins are normal

Fig. 1. Photographs of liver tissues of female and male experimental rats

4. DISCUSSION

Interest in medicinal plants as re-emerging health therapy have been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well-being, and the bioprospecting of new plant-derived drugs [19]. One of such refocus interest in Africa, especially Nigerian (in both rural and urban areas) is herbal treatment for diseases. Despite the many orthodox drugs in the market, the upsurge in the number of people that rely on herbs for treating ailments has necessitated increased efforts to authenticating the efficacy and safety of some herbal claims. One of such herbs is *Hippocratea africana* root bark.

Biochemical and toxicological evaluations of crude drugs (herbs) and sometimes histopathological evaluations of tissue sections to compliment the changes that have occurred at the molecular level are necessary for the safety use of the drugs. The changes observed in these clinical biochemical indices may reveal the presence or absence of any tissue pathology. In the present study, biochemical and toxicological evaluations following administration of graded doses of *Hippocratea africana* root bark extract in both adult female and male albino Wistar rats, was undertaken.

Results obtained revealed increased activity of serum ALT for the female test rats at doses of 100 and 200 mg/kg bw of *H. africana* root bark extract. The dose of 300 mg/kg bw of the extract however had decreased ALT activity. The AST activity showed increases for 100 and 200 mg/kg bw of the extract treated groups. The 300 mg/kg bw of the extract treated group showed decrease

activity. The slight increases in the ALT and AST activities at doses of 100 and 200 mg/kg bw of the extract observed indicate mild cellular damage in the female. ALT and AST are found in large amount in the liver and also small amount are found in the heart, kidney and muscles. When the liver is injured or inflamed as the case may be via its exposure to various forms of toxic substances, the level of ALT and AST in the blood is usually elevated. The level of these enzymes in the blood is directly related to the extent of the tissue damage [20-22]. The observed decrease in the activities of ALT and AST for 300 mg/kg bw treated group may be attributed to the dose-dependent protective effect of the antioxidant flavonoid phytochemical present in the herb Catechin, a flavonoid from *Uncaria gambri*, Roxbury and other plants used in the treatment of acute hepatitis have been reported to have a lowering effect on plasma aminotransferase activity compared with the control [23]. Vandenbergh et al. [24] have also reported a decrease in levels of transaminase in the liver of rats treated with bidisomide, an antiarrhythmic agent.

The male rats showed the same effects observed in the female rats for ALT and AST activities except that there was a significant increase and not decrease in AST activity following 300 mg/kg bw extract administration suggesting mild toxicity of the herb to males. Comparatively, sex appear to have impact on the enzyme activity with the increases in ALT and AST activities imposed by the herb affecting the male more than the female at 100 and 200 mg/kg bw dose of the extract and for all the test groups (100, 200 and 300 mg/kg bw of the extract) for AST activity.

The female rats recorded significant dose-dependent decreases in serum ALP activity. The male test groups' rats however, recorded dose-dependent increases in ALP activity. The ALPs are a group of enzymes that hydrolyse organic phosphate at alkaline pH. They are present in most tissues but are in particularly high concentration in the osteoblasts of bone and the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta [25]. In adults, plasma ALP is derived mainly from bone and liver in approximately equal proportions; the proportion due to the bone fraction is increased when there is increased osteoblastic activity that may be physiological [25]. Increased concentrations of vitamin C also elevate its concentration [25]. Serum ALP activity may also be raised due to primary neoplasm at site other than liver and non-neoplastic hepatobiliary diseases, as in the case of drugs and other chemically induced hepatoxicity [26]. Transient elevations may be found during healing of bone fractures and physiological bone growth elevates ALP in serum [27]. Low plasma ALP activity have been observed in arrested bone growth conditions (such as achondroplasia, hypothyroidism, and severe vitamin C deficiency), hypophosphatasia and treatment of hyperlipidaemia with a fibrate drug e.g. bezafibrate [25]. The significant dose-dependent decreases in ALP activity for the female rats observed in this study is likely due to the fact the phytochemicals present in the herb may have promoted bone growth and the herb may contain high concentration of vitamin C. while the significant increases in male may be attributed to disturbances in the tissue integrity of the experimental animals.

Non-significant ($p > 0.05$) decrease in GGT activity was observed in all the female treatment groups' rats in this study (Table 3). The male test rats also showed decrease in GGT activity for 100 and 300 mg/kg bw extract treatment. The 200 mg/kg bw extract treated group however, recorded a slight non significant ($p > 0.05$) increased activity (Table 4). GGT is the enzyme actively involved in the extracellular catabolism of glutathione, in the main mammalian [28]. GGT is present on the outer surface of plasma membrane of most cell types and in blood, where it forms complexes with several plasma components especially with albumin and lipoprotein [29]. The determination of serum GGT activity is a well-established diagnostic test for hepatobiliary diseases, and is used as a sensitive marker of liver damage. Increased serum GGT activity is associated with diseases of the liver, biliary system and pancreas [30,29]. The decrease in GGT activity in female suggest non toxicity, however, the slight non significant increase activity at 200 mg/kg bw of the extract for the male rats together with the significant increase in ALT and AST activities suggest toxicity. Long term administration is cautioned in males.

Administration of *H. africana* root bark extract at the different doses to both adult female and male albino Wistar rats significantly raised the serum activities of SOD and CAT and concentration of MDA in both sexes although the raised activities were not dose-dependent. The significantly ($p < 0.05$ and $p < 0.01$) raised activities of the serum oxidative stress enzymes (as shown in Tables 1 and 2) may be a way of the antioxidant defence systems of the rats attempting to combat the mild

toxic effect of the extract by increasing the activities of the antioxidant enzymes. Xenobiotics have been reported as a source of reactive oxygen species because some of them can enhance the production of oxyradicals within the cells by inhibition of mitochondrial electron transport and subsequent accumulation of reduced intermediates [31], inactivation of antioxidant enzymes such as catalase and depletion of radical scavengers [32,33]. Massive oxidative stress, particularly in mitochondria induces necrosis [34]. No necrosis was observed in the histopathological analysis of the liver in both female and male Wistar rats which further support the mild toxic effect of the extract also indicating that long-term administration of the extract may be very toxic.

Administration of 100 mg/kg bw of the extract to female rats (Plate 1b) showed pronounced diffuse degeneration of the liver cells indicating possible toxicity, although no inflammation was seen. The liver is involved in about 8% of reports of adverse drug reactions, reflecting its central role in the metabolism and excretion of many drugs [35]. The pronounced diffuse degeneration of the liver cells observed at 100 mg/kg dose may be explained as first line effect of administration of foreign compound. The liver is usually the target of the immune system because of the production of drug metabolites that bind to proteins which are degraded to peptides presented on the surface with major histocompatibility complex class I [36]. Cell death is the crucial event leading to the clinical manifestation of drug-induced hepatoxicity. The pathogenesis of clinical drug hepatitis reflects either an immune-mediated attack on the liver or a biochemical effect of toxic metabolites leading to a loss of cell viability [37]. Plate 1 c of rats administered 200 mg/kg bw of the extract shows very mild fatty change. Diffuse periportal inflammation and very mild hepatocyte death is seen, indicating further mild toxic insult of the extract to the liver (Plate 1d) for test group IV rats administered 300 mg/kg bw shows mild focal steatosis with the rest of the tissue being normal.

The male, test groups II and III rats (Plates 2b and 2 c) administered 100 and 200 mg/kg bw of the extract liver tissues, depict diffuse congestion of central veins which appear dilated with extravasations of blood into the sinusoids. The dilation may obstruct the normal functioning at the liver of draining the blood i.e. carrying deoxygenated blood away from the liver. This may lead to accumulation of toxic materials and subsequently causing cell death [37]. However, the portal tracts appeared normal and the hepatocytes were viable with no pigment seen. Test group IV rats administered 300 mg/kg bw of the extract (Plate 2d) shows normal hepatic architecture showing radiating hepatocyte cords with limiting plates and the portal tract sinusoids and central veins appearing normal. Collectively, the overall results obtained from the histopathological examination of the liver tissues corroborate the results obtained for the liver enzyme activities.

5. CONCLUSION

The findings from this study show that *Hippocratea africana* root bark extract has a mild toxic effect on liver tissues as seen from the liver function enzyme results and from the liver histopathological examinations. Thus, long-term administration of this extract may be very toxic despite its blood promising schizontocidal activity and should be used with caution. Further study on its effect on the renal status is suggested.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that the “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) were followed as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Farombi EO. African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. African Journal of Biotechnology. 2003;2(12):662-671.
2. Borchers AT, Halkman RM, Keen CL, Steru JS, Gershwin ME. Complementary medicine a review of immunodilatory effects of Chinese herbal medicine. American Journal of Clinical Nutrition. 1997;66:1302-1321.

3. Fabricant DS, Farnsworth NR. The value of plants in traditional medicine for drug discovery. Environmental Health Perspectives. 2001;109(1):69-75.
4. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. Journal of Natural Products. 2003;66(7):1022-1037.
5. Cos P, Vlietinck AJ, Berghe DV. and Maes L. Anti-infective potential of natural products: How to develop a stronger *in* vitro 'Proof-of-Concept'. Journal of Ethno-pharmacology. 2006;106(3):290-302.
6. Dalziel JM. Useful plants of west tropical African. London: Crown Agents for Overseas Government; 1956.
7. Okokon JE, Ita BN, Udokpoh AE. The *in-vivo* antimalarial activities of *Uvariachamae* and *Hippocratea africana.* Annals of Tropical Medicine and Parasitology. 2006;100(7):585-590.
8. Perez RM, Zawala MA, Salazar M. Anti-inflammatory activity of the bark of *Hippocratea excelsa.* Journal of Ethno-pharmacology. 1995;47:85-90.
9. Ogbole OO, Ekor MN, Olumeri BB, Ajaiyeobu AA, Gbolade AA, Ayoola MA. and Adeyemi AA. Anti-inflammatory and anti-microial activities of *Hippocratea indica* root ark and *Poga oleosa* fruits. African Journal of Traditional and Complementry Alternative Medicine. 2007;4(3):372-380.
10. Ndem JI, Eteng MU, Uwah AF. Effect of *Hippocratea africana* root bark extract on the lipid profile of female and male albino Wistar rats. Journal of Scientific Research & Reports. 2014;3(19):2574-2583.
11. Trease A, Evans WC. Pharmacognosy 13th ed. London: Bailliere Tindall; 1989.
12. Latha PG, Panikkar KR. Inhibition of chemical carcinogenesis in mice by *Ixora coccineae* flowers. Pharmaceutical Biology. 2000;38(12):152-156.
13. Pieta PG. Flavanoids as antioxidants. Journals of Natural Product. 2000;63(7): 1035-1042.
14. Wheeler CR, Salzman JA, Elsayed N. Automated assays for superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase activity. Analytical Biochemistry. 1990;184: 193-199.
15. Mihaljevic B, Katusin-Razen B, Razem D. The re-evaluation of the ferric thiocyanate assay for lipid hydroperoxides with special considerations of the mechanism aspects of the response. Free Radical Biology and Medicine. 1996;21:53- 63.
16. Reitman S, Frankel S. Serum-transaminase levels. American Journal of Clinical Pathology. 1957;28:56-63.
17. Szasz G, Bergmeyer HU. Method of enzymatic analysis. Weinheim: Verlag Chemie; 1974.
18. Drury RAB, Willington EA. Carleton's histological technique. 4th ed. London: Oxford University Press; 1967.
19. Hoareau L, Dasila EJ. Medicinal plants: A re-emerging health aid. Electronic Journal of Biotechnology. 1999;2:2-5.
20. Lum G, Gambino SR. Serum gamma-glutamyl transpeptidase activity as an indicator of disease of liver, pancreas, or bone. Clin Chem. 1972;18(4):358-362.
21. Halim A, El-Almady O, Hassab-Allah S, Abdel-Gelil F, Hafez Y, Derivis A. Biochemical effect of antioxidants on lipids and liver function in experimentally-induced liver damage. Annals of Clinical Biochemistry. 1997;34:656-663.
22. Udosen EO, Ojong AS. The biochemical, histological and hepatotoxic activity of the extract of *Sacoglothis gabonensis* in rats. Pharmaceutical Biology. 1998;36:467-469.
23. Thabrew MI, Hughes R. Phytogenic agents in the therapy of liver disease. Phytotherapy Research. 1996;10:461-467.
24. Vandenberghe Y, Mason, Palate B. and Roba J. Effects of folate supplementation on clinical chemistry and haematologic changes related to bidisomide administration in the rat. Drug Chemisty and Toxicology. 1995;18:L235-L270.
25. Crook MA. Clinical chemistry and metabolic medicine. 7th ed. New Delhi: Edward Arnold; 2006.
26. Bodansky O. Enzymes in cancer: The phosphohydrolases. In: Bodansky O. (Ed.), Biochemistry of human cancer. New York: Academic Press. 1995;61-92.
27. Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry. 3rd ed. Tokyo: W. B. Saunders; 1999.
28. Huseby NE. Multiple forms of gamma-glutamyltransferase in normal human liver, bile and serum. Biochim Biophys Acta. 1978;522(2):354-362.
29. Huseby NE. Multiple forms of serum gamma-glutamyltransferase. Association of the enzyme with lipoproteins. Clin Chim Acta. 1982;124(1):103-112.
30. Betro MG, Oon RC, Edwards JB. Gamma-glutamyl transpeptidase and other liver

function tests in myocardial infarction and heart failure. Am J Clin Pathol. 1973; 60(5):679-683.

31. Nohl H. and Hegner D. Identification of free hydroxyl radicals in respiring heart mitochondria by spin trapping with nitrone. DMPO Federation of European Biochemical Societies Letter. 1981;123: 241-244.
32. Hasspieler BM, Behar JV, DiGiulio RT. Glutathione-dependent defense in channel catfish (*Ictalurus punctatus)* and brown bullhead *Ameriurus neblosus*). Ecotoxicology and Environmental Safety. 1994;28:82-90.
33. Kano Y, Fridovich I. Inhibition and reactivation of Mn-catalase. Implications for valence changes at the active site manganese. Journal of Biology and Chemistry. 1983;258:13646-13648.
34. Kaplowitz N. Mechanisms of liver cell injury. Journal of Hepatolog. 2001;32: 39-47.
35. Graffin JP. Manual of adverse drug reactions. 3rd ed. Bristol: J. Wright; 1988.
36. Robin M, LeRoy M, Descatoire V, Pessayre D. Plasma membrane cytochromes P450 as neoantigens and autoimmune target in drug-induced hepatitis. Journal of Hepatology. 1997;26: 23-30.
37. Kaplowitz N. Cell death at the millennium: Implications for liver diseases. Clinical Liver Disease. 2002;4:1-23.

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