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Effect of *Hippocratea africana* root bark extract on some biochemical indices of male and female albino Wistar rats

J. I. Ndem[1]*, M. U. Eteng[2], M. I. Akpanabiatu[1], A. F. Uwah[1], O. Otitoju[3] and E. O. Akpanyung[1]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria.
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria.
[3]Department of Biological Sciences, Faculty of Pure and Applied Sciences, Federal University of Wukari, Taraba State, Nigeria.

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The benefits of medicinal plants may not be without some biochemical impediments. Anti-plasmodial activity of *Hippocratea africana* has been studied; its effects on some biochemical parameters were examined in rats in this study. It was observed that in the female test groups, there was no significant ($p > 0.05$) changes in urea concentration but there was significant ($p < 0.05$) increase in glucose concentration. There was also a slight increase in total protein and albumin concentrations which was not significant ($p > 0.05$). The male treatment groups however recorded significant ($p < 0.05$) increases in glucose, total protein, urea and albumin except for serum bilirubin which showed slight but non-significant ($p > 0.05$) decrease. The result generally does not suggest any biochemical imbalance and therefore the herb may be safe for use for its anti-plasmodial property.

Key words: *Hippocratea africana*, biochemical imbalance, glucose, total protein, urea, albumin, bilirubin, anti-plasmodial property, health benefit.

INTRODUCTION

There has been wide acceptability of phyto-remedies because of their availability, efficacy and affordability for the treatment of ailments, especially among the peasants and low income earners. Available evidence shows that herbal remedies have been used in the treatment of ailments for time immemorial and have continued to play significant roles in the general provision of good heath to people all over world (Farombi, 2003). All 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, syphiis, cancer etc. (Farombi, 2003). Many botanicals are now sold in United States as dietary supplements (Borchers et al., 2000). Research interest is now focused hypolipidaemic, anti-platelet, antihypertensive, anti-diabetic, anti-tumour, immune stimulating or anti-ma-larial proterties etc. That may be useful in complementary properties or alternative medicine (Borchers et al., 1997). One of such plants is *Hippocratea africana* (Willd.) Loes. (Hippocrateaceae). The plant inhabits green forests and is a perennial climber with hairs (glabrous), and reproduced from seeds (Dalziel, 1956). The plant is widely distributed in tropical Africa. It is called “godyi” in Hausa, “ponju owiwi” in Yoruba, and “ipungwa” in Tiv tribes of Nigeria. The Ibibio tribe of the Niger Delta region of Nigeria calls it “Mba enang enang”. In Ghana, the Akan-Asante call it “nnoto” and Fula-Pulaa in Senegal calls it “Rdelbi” while the Loko in Sierra Leone call it “njabo” (Burkill, 1985). The roots are used traditionally in

* Corresponding author. E-mail: jessndem@yahoo.com.

Table 1. Distribution of experimental groups.

Group	Number of animals		Treatment
	Female	Male	(mg/kg body weight)
I	6	6	Control
II	6	6	*H. africana* (100)
III	6	6	*H. africana* (200)
IV	6	6	*H. africana* (300)

Control = distilled water

the treatment of various ailments such as fever, malaria, body pains, diabetes and diarrhea (Okokon et al., 2006). It has been reported to possess in *vivo anti*-plasmodial activity with lethal dosage (LD_{50}) of 2.45 mg/kg body weight in mice. Okokon et al. (2006) also report 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 *H. excelsa* (Perez et al., 1995) and *H. indica* (Ogbole et al., 2007). In the phytochemical and anatomical studies of *H. africana* Willd. (*Celastraceae*), Essiet et al. (2006) reported that the plant contains significant quantities of phytochemicals such as alkaloids, cardiac glycosides and flavonoids.

Okokon et al. (2006) have reported the anti-malarial activity of *H. africana* in mice. Studies carried out showed that ethanolic root extract of *H. africana* possess promising blood schizontocidal activity, both in early and established infection at oral doses of 200 to 600 mg/kg/day in mice. The chemo-suppressive effect of *H. africana* at 400 and 600 mg/kg were 81.8 and 90.9%, respectively while that of chloroquine at 5 mg/kg was 92.5%. In this study, the effect of graded doses of root bark extract of *H. africana* on biochemical indices of both female and male albino Wistar rats were assayed.

MATERIALS AND METHODS

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 to 227 g each were obtained from the animal house of the College of Health Sciences, University of Uyo, Uyo and used in this study. The animals were randomly divided into four groups of six rats to a group 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.

Experimental design and administration of extract for biochemical studies

Group I animals served as the control and were administered distilled water while Groups II, III and IV animals were administered graded doses of 100, 200 and 300 mg/kg of the crude root bark extract of *H. africana* calculated on the basis of the body weight of the animals. The experimental design is as shown in Table 1. The extract was administered orally once daily for fourteen (14) days by the use of a canular attached to syringe. All the experimental animals were given normal rat chow and water *ad libitum* throughout the treatment period.

Collection of blood samples

At the end of the fourteen days treatment, 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 by cardiac puncture using sterile syringes and needles 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 min and used for analyses.

Biochemical assays

Serum concentration of glucose

The serum glucose concentration was determined using enzymatic colorimetric test. Randox laboratory reagent kit method was used in this test based on method by Barham and Trinder (1972). Glucose is determined after enzymatic oxidation in the presence of glucose oxidase (GOD). The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye which was measured spectrophotometrically at 546 nm.

Serum total protein

The serum total protein concentration was estimated by biuret method using Randox Laboratory reagent kit method (Tietz, 1995). Cupric ions in an alkaline medium interacted with protein peptide bonds resulting in the formation of a coloured complex which was measured spectrophotometrically at 540 nm. This was used to quantify the protein in the sample.

Serum urea concentration

Randox laboratory reagent kit method (Fawcett et al., 1960; Weatherburn, 1967; Chaney and Marbach, 1962; Mackay and Mackay, 1927) was used to determine the serum urea concentration. Urea in serum was hydrolysed to ammonia in the presence of urease. The ammonia was then measured photometrically at 546 nm by Berthelot's reaction.

Serum albumin concentration

Serum albumin concentration was determined by the use of Randox laboratory reagent kit method (Grant, 1987). The measurement of serum albumin is based on its quantitative binding to the indicator 3, 3^1, 5, 5^1 - tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The albumin - BCG - complex was measured spectrophotometrically at 630 nm, the absorbance being directly proportional to the concentration of albumin in the sample.

Serum total bilirubin concentration

Serum total bilirubin was determined using Randox Laboratory

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

Grouping/dosage	Glucose (mmol/l)		Total protein (g/l)		Urea (mmol/l)		Albumin (g/l)		Total bilirubin (µmol/l)	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
I	4.14±0.20	3.27 ±0.32	68.53±3.47	63.37±1.24	10.22±0.72	7.34±0.61	43.19±1.87	38.31±2.41	29.14±2.88	26.64±3.08
II	4.63±0.70*	3.32±0.35*	73.56±2.14	73.36±1.83*#	9.44±0.26	8.85±0.97*#	46.03±3.53	44.57±2.83*#	22.36±2.06*#	24.18±1.49
III	5.75±0.71*#	3.68±0.77*	71.97±2.91	66.66±2.01*	10.68±1.59*	7.74±0.76*	44.76±2.69	41.73±1.75*	26.89±1.15*	26.46±3.08
IV	6.01±0.53*#	5.66±0.93*#	69.75±2.53	71.24±1.05*#	10.04±0.88	8.84±1.37*	45.28±3.08	41.66±1.74*	24.45±1.67	23.19±2.38

Results are presented as mean ± SD; n = 6, * = significantly different from control at $p < 0.05$, # = significantly different from control at $p < 0.01$, HA = *Hippocratea africana* root bark extract; Group I = control, Group II = *H. africana* root bark extract (100 mg/kg body weight), Group III = *H. africana* root bark extract (200 mg/kg body weight), Group IV = *H. africana* root bark extract (300 mg/kg body weight).

reagent kit method (Jendrassik and Grof, 1938; Sherlock, 1951). Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazatised sulphanilic acid. This was measured spectrophometrically at 578 nm.

Statistical analysis

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

RESULTS

The impact of graded doses of extract on glucose (mmol/l), total protein (g/l), urea (mmol/l), albumin (g/l) and total bilirubin (µmol/l) of female and male biochemical imbalance following the administration of *H. africana* root bark extract. The results are as presented in Table 2. The female rats recorded significant ($p < 0.05$) increase in glucose concentrations of 4.63 ± 0.70, 5.75 ± 0.71 and 601 ± 0.53 mmol/l for test groups II, II and IV administered 100, 200 and 300 mg/kg body weight of *H. africana* root bark extract compared with the control group I rats concentration of 4.14 ± 0.20 mmol/l, administered distilled water. Test groups III and IV also showed significant increases ($p < 0.01$) compared with the control group I rats. The result showed no significant ($p > 0.05$) increase in total protein concentration of 73.56 ± 2.14, 71.97 ± 2.91 and 69.75 ± 2.53 g/l for test groups II, III and IV compared with 68.53 ± 3.17 g concentration for the control group I rats.

The urea concentrations were 10.22 ± 0.72, 9.44 ± 0.26, 10.68 ± 1.59 and 10.54 ± 0.88 mmol/l for control group I rats, test groups II, III and IV, respectively. The test groups II, III and IV concentrations showed no significant ($p > 0.05$) difference compared with control. There was no significant ($p > 0.05$) increase in albumin concentrations of 46.03 ± 3.53, 44.76 ± 2.69 and 45.28 ± 3.08 g/l for test groups II, III and IV rats, respectively compared with the control group I concentration of 43.19 ± 1.87 g/l. The result showed significant ($p > 0.05$) reductions of 22.36 ± 2.06, 26.89 ± 1.15 and 24.45 ± 1.69 µmol/l in total bilirubin concentrations for test groups II, III and IV rats, respectively compared with the control group I concentration of 29.14 ± 2.88 µmol/l. Test group II rats concentration also showed significant reduction at $p < 0.01$. Ge-nerally, there were no significant changes in urea concentration but significant increase in glucose concentration was observed. However, there was slight increase in total protein and albumin and decrease in serum bilirubin concentrations in the female. The male Wistar rats recorded significant ($p < 0.05$) increase in glucose concentrations of 3.32 ± 0.35, 3.68 ± 0.77 and 5.66 ± 0.93 mmol/l for test groups II, III and IV rats administered 100, 200 and 300 mg/kg body weight of *H. africana* ethanol root bark extract compared with the control group I rats concentration of 3.27 ± 0.32 mmol/l. Test groups IV concentration further showed significant increase at $p<0.01$ compared with the control.

Significant ($p < 0.05$) increase in total protein concentrations of 73.36 ± 1.83, 66.66 ± 2.01 and 71.24 ± 1.05 g/l were recorded for test groups II, III and IV, respectively compared with the control group I albino Wistar rats were used to assess for the concentration of 63.37 ± 1.24 g/l. The increase in test groups II and IV further showed significant difference at ($p < 0.01$) compared with the control group. The urea concentration depict significantly ($p < 0.05$) increased values of 8.85 ± 0.97, 7.74 ± 0.76 and 8.84 ± 1.37 mmol/l for test groups II, III and IV, respectively compared with the control group I concentration of 7.34 ± 0.61 mmol/l. Test group II further showed significant increase at $p < 0.01$ compared with the control. The albumin concentrations were 38.31 ± 2.41, 44.57 ± 2.83, 41.73 ± 1.75 and 41.66 ± 1.74 g/l for the control group I, test groups II, III and IV, respectively. The test groups II, III and IV increasing concentrations

were all significant ($p < 0.05$) compared with the control. Test group II value further showed significant different at ($p < 0.01$). The test groups II, III and IV rats recorded non dose-dependent and non significant ($p > 0.05$) reductions in total bilirubin concentrations of 24.18 ± 1.49, 26.46 ± 3.08 and 23.19 ± 2.38 µmol/l compared with the control concentration of 29.14 ± 2.88 µmol/l. For the males, there was significant increase in all biochemical parameters except for total serum bilirubin which showed slight decrease.

DISCUSSION

Administration of graded doses of *H. africana* root bark extract to both female and male rats of the test groups showed hyperglycemic effect compared with the control. The glucose level is regulated in a special way in the liver and other extra hepatic tissues and the role of hormonal systems cannot be ignored (Crook, 2006). Plant extracts such as *Eleophobia drupifera* (Eno and Itam, 1996), *Gongronema latifolium* (Ugochukwa, 2003), and *Vernonia amygdalena* (Gidado et al., 2005) have been reported in related studies to have hypoglycemic effects in experimental animals. Anti-malarial such as quinine and xenobiotics such as bidisomide have also been reported to have hypoglycemic effect (Vandenberghe et al., 1995). The hyperglycemic effects observed in this study suggest that there may be phytochemical constituent(s) present in the herb that has glucagon-like activity. Various liver diseases result in decrease blood glucose (Bolarin, 1997). The observed increase in glucose concentration is favorable to the organisms owing to the metabolic roles of glucose in several tissues.

There was a non significant ($p > 0.05$) increase in protein concentration in all the female test groups' rats and significant increase for the rats in the male test groups compared with the control. In a study, Iwu et al. (1986) showed that *A. indica* has a hyper-proteinaemic effect on chicks. Acetaminophin and an anti-malarial, sodium artesunate, are known to bind plasma proteins (Miller and Panosium, 1997). The reported hypo-proteinaemic effects of *A. indica* and acetaminophen were attributed to glutathione (GSH) decrease resulting in increase glutathione disulfide (GSSG) concentration known to inhibit the incorporation of essential amino acids into protein. The reverse was observed for *H. africana* root bark extract. Why there was increase in the serum protein in this study is not known but may imply that the extract did not affect protein absorption and that there was no possible upset in liver function.

The same pattern was observed in the serum urea concentration except that the female 100 mg/kg body weight extract treatment group recorded non significant reduced urea concentration compared with the control. Excess amino acids cannot be stored, thus the liver break them into nitrogen containing amino parts which it then converts to urea for excretion by the kidney; and carbon compounds (Keto acids) which it converts into glucose, glycogen or fats in the process known as deamination (Crook, 2006). Serum urea levels and blood urea nitrogen (BUN), alongside other parameters like serum creatinine level, are used to assess renal functions (Crook, 2006). The non significant decrease/increase observed in female test rats implied that glomerular functions of the renal tubules of the female experimental animals were not distorted by the extract. The significant increase observed in the male test rats compared with control may suggest extract induced increased protein absorption and catabolism, resulting in the increase urea concentration and not toxicity. Histopathology of the kidney showed normal kidney.

The study revealed a non significant ($p > 0.05$) increase in albumin concentrations that was not dose-dependent for the female test groups rats compared with the control, and significant ($p < 0.05$) increase for all the male test groups compared with the control. Albumin is the major protein present in the blood and is synthesized primarily by the hepatic parenchymal cells, except in early foetal life when it is synthesized largely by the yolk sac (Gitlin and Perricelli, 1970). It is a major synthetic protein and is a major market for the ability of the liver to synthesize protein (Quinn and Johnston, 1997). The synthetic rate is controlled primarily by colloidal osmotic pressure (COP) and secondarily by protein intake (Peter (Jr), 1996; Rothschild, 1972). The observed increase in albumin concentration may be attributed to extract induced protein intake, a secondary reason for its synthetic rate. Markedly increased levels of albumin are seen only in acute dehydration. As a result, the finding of elevated values has no clinical utility. This is because the synthetic rate and intravascular-extravascular shifts usually occur fairly rapidly to stabilize relative osmotic pressures (Burtis and Ashwood, 1999).

Physical examination and observed weight increase in all the test groups animals (both female and male) does not indicate dehydration and therefore the increase may not imply toxicity.

Bilirubin, the end product of haem degradation by the mononuclear phagocytes system, when released into the blood, is transported to the liver bound to serum albumin (conjugated bilirubin) or free (unconjugated bilirubin). The unconjugated bilirubin is a small fraction which may increase in severe haemolytic disease or when protein binding chemicals displace bilirubin from albumin. Both conjugated and unconjugated bilirubin in the serum (total serum bilirubin) accumulate systematically and deposit in tissues, resulting in the yellow discolouration of jaundice (Crook, 2006; Burtis and Ashwood, 1999) that is, bilirubin production exceeds the hepatic capacity to excrete it or conjugate it (or if there is obstruction in the biliary flow) (Crook, 2006).

Generally, both conjugated and unconjugated, bilirubins are increased in the serum in hepatitis (Crook,

2006). In this study, the level of total serum bilirubin was observed to be significantly ($p < 0.05$) decreased in all the female test groups rats compared with the control. There was observed non significant ($p > 0.05$), non dose-dependent decrease for all the male test groups rats compared with the control. The observed decrease indicate that the extract did not cause haemoglobin lyses and that the hepatic capacity to excrete or conjugate bilirubin was not compensated, it may also indicate that there was no impaired intrahepatic and extrahepatic bile flow induced by the herb in both female and male test groups rats.

Conclusion

Although there was hyperglycemia and hyperproteinaemia, the other biochemical indices taken together do not suggest kidney and liver malfunctioning or derangement in critical tissues or organ function. Hence the herb may be safe for use for its anti-plasmodial property.

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