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RENAL STATUS OF FEMALE AND MALE WISTAR RATS EXPOSED TO HIPPOCRATEA AFRICANA ROOT BARK EXTRACT

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ABSTRACT

The electrolyte concentrations of female and male Wistar rats exposed to Hippocratea africana root bark extract used traditionally for the treatment of malaria was studied to assess the integrity of the kidney. Forty-eight albino Wistar rats weighing between 163-227grams 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 1ml of distilled water. Groups II, III and IV were administered 100, 200 and 300mg/kg body weight respectively of the extract by oral intubation for 14 days. Sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), chloride (Cl^-), inorganic phosphorus and bicarbonate ($HC0_3^{2-}$) concentrations were assayed using standard procedures. Histopathology examinations of the kidney organs were also carried out using standard methods. The female 100 and 200mg/kg extract treatment groups recorded increase in Na^+, K^+, Ca^{2+}, Cl^- and $HC0_3^{2-}$. Only inorganic phosphorus ion recorded decrease concentration compared with the control. 300mg/kg body weight treatment group recorded however recorded significant ($P<0.05$) increase in K^+, Ca^{2+}, Cl^-, and $HC0_3^{2-}$ compared with the control with exception of Na^+ which recorded significant ($P<0.05$ and $P<0.01$) decrease. The male test groups showed significant ($P<0.05$ and $P<0.01$) decrease in Na^+ that increased with increasing concentration. There were non dose dependent significant ($P<0.05$ and $P<0.01$) increase in Ca^{2+}, Cl^-, inorganic phosphorus and $HC0_3^-$ compared with control. K^+ showed significant ($P<0.05$ and $P< 0.01$) increase for 200 and 300mg/kg treatment groups while 100 treatment groups recorded non significant ($P>0.05$) decrease. Histopathological examination of the kidney of all the test groups was normal as there were no evidence of extract induced pathology seen in the tissues. The results show that the homeostasis system was slightly distorted. The effect was more pronounced in the males. Despite its promising antiplasmodial property, its chronic usage is not advised.

1.0 INTRODUCTION

The kidneys' function is to filter the blood that passes through them several times a day. They excrete metabolic waste products and have an essential homeostatic function of controlling the body solute and water status and the acid–base balance.[1] The working of the kidney depends on its conditions and there are a number of factors that can render the kidney uniquely susceptible to toxic insults. Some of these factors include high blood flow, high metabolic activity of the renal tubular cells, countercurrent mechanisms that can raise concentration of substances (drugs) in the renal medulla to levels higher than elsewhere in the body, the largest endothelial cell surface area by weight of the kidney that can increase the potential of damage by vasoactive drugs or immune complexes which may occur from interaction with proteins etc. The kidneys also has an elaborate ion transport mechanism that facilitates drug entry into cells that can lead to generation of toxic metabolites and a large surface area of the tubular endothelium for toxin interaction and uptake.[2] Recently, there has been a general fall back on herbal remedies universally. Some are even made in tablets and capsules for convenient of administration. In Africa, Nigeria in particular these practice is a long acceptable norm especially among the peasants and low income earners. The interest in fuelled by expensive, unaffordable orthodox drugs such as the World Health Organization (WHO) recommended arthemisinin combination therapy (ACT) for the treatment of malaria. There is also report that traditional plant treatments have been used throughout the world for the treatment of ailments such as diabetes mellitus, dyslipideamia and nephrotic conditions.[3,4,5] reported that this traditional medicine evolved from environmental resources, which the people of a community adopt in desperation for survival from disease. These Plants chemicals are still the backbone of our pharmacopoeia as more than 50% of drugs used in Western pharmacopoeia are isolated from herbs or derived from a modification of chemicals first found in plants.[6,7]

Hippocratea africana (Willd.) Loes from the Hippocrateaceae family is a green forest perennial climber without hairs and reproduces from seeds. The plant is widely distributed in tropical Africa and commonly known as African paddle – pods.[8] The phytochemical and anatomical studies of H. africana show that it contains significant quantities of phytochemicals such as alkaloids, cardiac glycosides and flavonoids.[9,10] [11] reported that the plant (root) possesses in vivo anti-plasmodial property, analgesic and anti-inflammatory[12], antidiarrheal and antiulcer activities.[13] Other biological activities include; anti-diabetic and hypolipidemic property.[10,14] In this study, we report the electrolyte concentrations as an index of renal integrity of female and male albino Wistar rats exposed to the root bark extract of Hippocratea africana used traditionally by the people in the South- Eastern part of Nigeria to treat malaria

2.0 MATERIALS AND METHODS

2.1 Collection of plant materials

The roots of Hippocratea africana (Willd) Loes were harvested from Afaha Etok Ibesikpo forest in Ibesikpo-Asutan, L.G.A of Akwa Ibom State. The plant was identified and authenticated in the Department of Botany University. The voucher specimen was deposited at the herbarium.

2.2 Preparation of plant extracts

The fresh root bark (2Kg) of the plant was washed off sand using tap water. The bark was scraped with a knife, dried and pulverized using an electric blender. One kilogramme of the pulverized H. africana root bark was exhaustively macerated in 80% ethanol (2000ml) and allowed to stand for 72 hours for the solvent to solubilize the active ingredients. The orange filtrate was carefully siphoned using a tube and concentrated in vacuum at 40^0C to obtain a dry orange crude extract. The dried crude extract was stored in the refrigerator at 4^0C and used for the analyses.

2.3 Experimental Animals and Design

Forty-eight healthy adult female and male albino Wistar rats consisting of twenty four female and twenty four males weighing between 163 - 227 grams were obtained from the animal house of the College of Health Sciences, University of Uyo, Uyo and were used for the experiment. They were housed in plastic cages made of stainless steel at the bottom that separated the animals from the faeces and feed droppings. The animals were randomly divided into four groups of six rats each on a sex basis. The males were caged separately from the female to prevent mating during the treatment period. The animals were fed daily with commercial rat mash and drinking water was allowed ad libitum. The weights of the animals were measured before and at the end of the experimental period. The treatment groups were as follows:

Group 1: Normal control rats received 1ml distilled water
Group 2: Normal treated rats received 100mg/kg bw H. africana root bark
Group 3: Received 200mg/kg bw H. africana root bark
Group 4: Received 300mg/kg bw H. africana root bark

2.4 Administration of Plant Extract

The extract was administered orally once daily for fourteen (14) days by use of a canular attached to a syringe.

2.5 Collection of Blood Samples

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^oC.

2.6 Histopathological examinations

The kidney 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. Histopathological analysis was carried out according to the procedure recommended by.[15] The slides were examined using a light microscope and the histopathological changes especially morphological changes were recorded.

2.7 Chemicals

All chemicals used were of analytical grade. Serum concentrations of sodium, potassium, chloride and carbon-dioxide (CO_2) were carried out using Teco diagnostic kit reagent. Sodium and potassium concentrations determination were based on method by[16], chloride concentration was based on method by[17] while carbon-dioxide (CO_2) concentration was by method of.[18] Serum calcium and inorganic phosphorus concentrations were carried out using Randox laboratory kit reagent based on methods by[19-16] respectively.

2.8 Statistical Analysis

Results of Biochemical estimations 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.

2.9 RESULTS

The kidney weights for the female and male test groups II, III and IV compared with the control group 1 rats are presented in Table 1. For the female, only test group IV kidney weight was significantly ($p < 0.05$) higher compared with the control group1 while for the male,

test group III recorded significant ($p < 0.05$ and $p < 0.01$) increase kidney weight compared with control group I. Test group IV only recorded significant increased kidney weight compared with control group I at ($p < 0.05$).

TABLE 1: **Weights and relative weights of Kidney of rats administered graded doses of ethanol extract of Hippocratea africana root bark**

Group/Treatment	Female		Male	
	Kidney Weight (g)	Kidney Weight as % bw	Kidney Weight (g)	Kidney Weight as % bw
Group I Control (Distilled Water)	0.87 ± 0.14	0.52 ± 0.08	1.09 ± 0.02	0.53 ± 0.03
Group II H.A (100mg/kg body weight)	0.95 ± 0.06	0.56 ± 0.04	1.16 ± 0.13	0.57 ± 0.06
Group III H.A (200mg/kg body weight)	0.94 ± 0.06	0.56 ± 0.04	*,** 1.28 ± 0.04	0.58 ± 0.02
Group IV H.A (300mg/kg body weight)	* 1.05 ± 0.09	0.57 ± 0.07	* 1.29 ± 0.17	0.58 ± 0.08

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 = body weight, % = percentage

The results of electrolyte concentrations of both adult female and male albino Wistar rats administered graded doses of 100, 200 and 300mg/kg bw of H. africana root bark extract compared with the control are presented in Tables 2 and 3 respectively.

The female rats test group II rats recorded increase in sodium concentration that was significant ($p < 0.05$) compared with the control group 1. Test group IV concentration showed a significant ($p < 0.05$ and $p < 0.01$) decrease compared with the control. The result for potassium shows a dose dependent increasing concentrations of 3.76 ± 0.33, 4.20 ± 0.13 and 4.23 ± 0.25 mEq/L for test groups II, III and IV respectively compared with the control group 1 concentration of 3.58 ± 0.10 mEq/L. Only test groups III and IV increases showed significant ($p < 0.05$ and $p < 0.01$) difference compared with the control. Calcium recorded a dose dependent significant ($p < 0.05$) increasing concentrations of 2.37 ± 0.12, 2.40 ± 0.09 and 2.51 ± 0.05 mmol/l for test groups II, III and IV respectively compared with control group I concentration of 2.15 ± 0.12 mmol/l. Chloride concentration also showed increasing concentrations of 95.38 ± 1.21, 103.50 ± 1.25 and 102.70 ± 0.79 mEq/L for test groups II, III and IV respectively compared with the control group I concentration of 88.83 ± 1.55 mEq/L that was not dose-dependent. Only test groups III and IV concentrations was significant ($p < 0.05$) compared with the control. Inorganic phosphorus concentration showed significant ($p < 0.05$) decreases of 1.87 ± 0.10 and 1.85 ± 0.11 mmol/l for test group II and III compared with the control group 1 concentration of 2.28 ± 0.24 mmol/l. The results for bicarbonate concentration showed a non dose-dependent significant ($p < 0.05$) increase concentrations of 44.25 ± 1.27, 42.75 ± 1.35 and 47.21 ± 1.94 mmol/l for test groups II, III and IV respectively compared with the control group I concentration of 39.88 ± 1.31 mmol/l. Test group IV showed further significant increase at ($p < 0.01$). The result for electrolytes concentrations for the male albino Wistar rats (Table 3) shows significant ($p < 0.05$ and $p < 0.01$) decrease in sodium concentration that increased with increase dose of extract compared with the control group 1. There were non dose-dependent significant ($p < 0.05$ and $p < 0.01$) increases calcium, chloride, inorganic phosphorus and bicarbonate compared with the control. Potassium concentration showed significant ($p < 0.05$ and $p < 0.01$) increases of 4.25 ± 0.22 and 4.65 ± 0.22 mEQ/L for test group III and IV compared with control group I concentration of 3.56 ± 0.57 mEQ/L.

TABLE 2: Electrolyte concentrations of female albino Wistar rats administered graded doses of ethanol extract of Hippocratea africana root bark

Group/Dosage	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mmol/l)	Chloride (mEq/L)	Inorganic Phosphorus (mmol/l)	Bicarbonate (mmol/l)
Group I Control (Distilled water)	60.28±0.30	3.58 ± 0.10	2.15±0.12	88.83±1.55	2.28 ± 0.24	39.88 ± 1.31
Group II H.A(100mg/kg body weight)	* 64.76±0.14	3.76 ± 0.33	* 2.37±0.12	95.38±1.21	* 1.87 ± 0.10	* 44.25 ± 1.27
Group III H.A(200mg/kg body weight)	60.53±0.69	*,** 4.20± 0.13	* 2.40±0.09	* 103.50±1.25	* 1.85 ± 0.11	* 42.75 ± 1.35
Group IV H.A(300mg/kg body weight)	*,** 47.76±0.27	*,** 4.23 ± 0.25	* 2.51±0.05	* 102.70±0.79	2.03 ± 0.17	*,** 47.21 ± 1.94

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 3: Electrolyte concentrations of male albino Wistar rats administered graded doses of ethanol extract of Hippocratea africana root bark

Group/Dosage	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mmol/l)	Chloride (mEq/L)	Inorganic Phosphorus (mmol/l)	Bicarbonate (mmol/l)
Group I Control (Distilled water)	138.0±0.90	3.56±0.57	1.87±0.14	91.50±1.14	1.82 ± 0.13	14.21±0.07
Group II H.A (100mg/kg body weight)	*,** 66.17±0.66	3.35±0.05	*,** 2.39±0.09	* 106.80 ±1.18	*,** 2.33 ± 0.07	*,** 32.07±0.55
Group III H.A (200mg/kg body weight)	*,** 83.41 ± 1.50	*,** 4.25±0.22	*,** 2.28±0.06	*,** 113.57 ±1.28	*,** 2.28 ± 0.12	*,** 22.50±1.64
Group IV H.A (300mg/kg body weight)	*,** 113.60±1.97	*,** 4.65±0.22	*,** 2.55±0.04	* 110.37 ±1.59	*,** 2.52 ± 0.18	*,** 23.25±1.19

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 $p < 0.01$

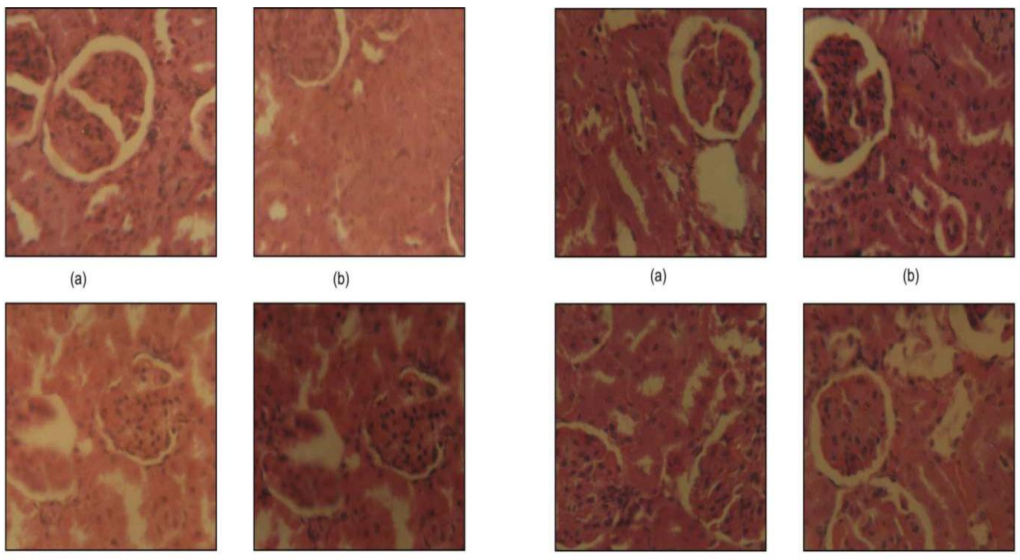

Plate 1: Photomicrographs of the kidneys of adult female albino Wistar rats after administration of 100 (b), 200 (c) and 300 (d) mg/kg body weights of ethanol extract of *H. africana* root bark compared with control group I (a) mag. x 400

(a) Control group I - Normal kidney showing normal cortex and medulla. The glomerulus, vascular and interstitial component are normal.
(b) Test group II - Normal renal tissue. No pathology
(c) Test group III - Normal renal tissue. No pathology
(d) Test group IV - Normal renal tissue. No pathology

Plate 2: Photomicrographs of the kidneys 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 kidney showing normal cortex and medulla. The glomerulus, vascular and interstitial component are normal.
(b) Test group II - Normal renal tissue. No pathology
(c) Test group III - Normal renal tissue. No pathology
(d) Test group IV - Normal renal tissue. No pathology

Fig 1: Photomicrographs of Kidney tissues of female and male experimental rats

4.0 DISCUSSION

The concentrations of the various electrolytes in the body fluids are maintained within a narrow range. However, the optimal concentrations differ in the extracellular and intracellular fluid. Certain disease conditions and xenobiotics may lead to a deficiency of one or more electrolytes and to an imbalance among them[20,21] There were variations following administration of graded doses of H. africana root bark extract to experimental animals in the concentration of serum electrolytes. Potassium concentration for the female rats of treatment groups reflected a dose-dependent significant ($p < 0.05$) increase at 200 and 300mg/kg extract dose for both female and male treatment groups compared with the control. 100mg/kg extract dose in the female recoded non significant ($p > 0.05$) increase while the male recorded non significant ($p > 0.05$) decrease compared with the control. Concentration gradients of sodium and potassium across the cell membrane produce the membrane potential and provide the means by which electrochemical impulses

are transmitted in nerve and muscle fibres.[22] The stability of the electrolyte balance depends on adequate intake of water and the electrolyte, and on homeostatic mechanisms within the body that regulate the absorption, distribution and excretion of water and its dissolved particles.[23] The increases in potassium concentration observed for the female test groups rats and at 200 and 300 mg/kg bw dose of the extract for the male is an indication that the herb may have possibly affected the membrane channels. The increases however did not reflect hyperkalemia. The Histological of the kidney for both female and male rats of the test groups show normal kidney architecture; the cortex and medulla, the glomerulus, vascular interstitial components were all normal. Sodium is associated with blood pressure and in many hypertensive patients a reduction in sodium intake lowers blood. The reduction in sodium concentrations imposed by herb in both female and male test groups compared with the control suggest that the herb is not toxic to both sexes especially hypertensive patients and may help reduce blood pressure possibly by mediating a vasodilatory effect. A normal calcium level is essential for normal cardiac functions, transmission of nerve impulses and muscle contraction. It is involved in blood coagulation.[23] Administration of graded doses of H. africana to both female and male albino Wistar showed significant ($p < 0.05$) increase in calcium concentrations in the female test group rats and significant increases both at $p < 0.05$ and $p < 0.01$ in the male rats compared with the control that may not imply hypercalcimia. The concentration of calcium, phosphate and magnesium in plasma are dependent on the net effect of bone mineral deposition and reabsorption regulated by parathyroid hormone (PTH) and 25-dihydroxy-vitamin D.[24] The serum chloride concentration observed in this study, showed significant ($p < 0.05$) increases for the female test groups rats at doses of 200 and 300 mg/kg bw of the extract and at 100, 200 and 300 mg/kg bw for the males test groups rats. The 200mg/kg bw dose of the extract for the male test group rats recorded additional significant increase at $p < 0.01$ compared with the control. Chloride is the major anion found in the ECF and in the blood. The serum chloride concentration can be elevated above the normal range - hyperchloremia either by the addition of excess chloride to the ECF or by the loss of water from this compartment and vice-versa resulting in hypochloremia.[25] Drugs such as acetazolamide, androgens, cholestyramine, diazoxide etc. have been reported to raise chloride concentration.[26,27] Increases are also seen in dehydration, renal tubular acidosis, and acute renal failure.[26,27] Histopathological screening of the kidney of both female and male test rats administered graded doses of the extract showed normal kidney (fig. 1). The increases observed can be said to be drug induced and may not imply toxicity. Also total body chloride stores cannot be evaluated from the serum chloride concentration alone; other clinical parameters must be used in conjunction with the serum chloride values to assess the significance of hypochloremia or hyperchloremia.[25] Administration of 100 and 200mg/kg bw of H. africana root bark showed significant ($p < 0.05$) decreases in inorganic phosphate concentration and a non-significant ($p > 0.05$) increase at 300 mg/kg bw of the extract in the female rats compared with the control (Table 2). The male test group's rats all recorded significant increases at both $p < 0.05$ and $p < 0.01$ compared with the control. Disorders of phosphate homeostasis occur in a wide range of clinical conditions. Both hyper and hypophosphatemia can be caused by cellular shifts of phosphate.[28] The three primary conditions that lead to phosphate dysfunction are dietary intake, gastrointestinal and renal status.[29] Alterations in the concentrations of inorganic phosphate following administration of graded doses of the extract to both female and male albino Wistar rats may be attributed to cellular shift. Histopathology of the kidney for both female and male test group rats showed no renal failure (fig. 1). The increases observed in the male rats may also be attributed to phosphate load impose by the extract. This therefore implies that long term administration of the herb may cause hyperphosphatemia in male and may result in volume contraction and impaired parathyroid hormone secretion. Administration of 100, 200 and 300 mg/kg bw of H. africana root bark extract to female albino Wistar rats showed significant ($p < 0.05$) increase in bicarbonate concentration compared with the control that was not dose-dependent (Table 2). The male test groups' rats all showed significant increase both at $p < 0.05$ and $p < 0.01$. Increase in serum CO_2 content for the most part reflects increase in serum bicarbonate (HCO_2^-) concentration rather than dissolved CO_2 gas or PCO_2. Increased serum bicarbonate is seen in compensated respiratory acidosis and in metabolic acidosis.[27] Diuretic (thiazides, ethacrynic acid, furosemide, mercurials), corticosteroids (in long term use), and laxatives (when abused) may cause increased bicarbonate.[27] The observed increases in bicarbonate concentration in all the test groups' rats both female and male indicated compensated acidosis. The effect was more in the male than in the female test rats. There is a report of non significant ($p > 0.05$) decreases and increases in the concentration of urea for female rats and significant increase for male rats ($p < 0.05$) exposed to same graded doses of Hippocratea africana root bark extract.[30] The non-significant decrease/increase observed in the female test rats was reported as not implying distortion of glomerular functions of the renal tubules by the extracts. The photomicrograph of the female kidneys further confirms the report (fig.1). The significant increase observed in the male compared with the control was suggestive of extract induced increased protein adsorption and catabolism which resulted in increased urea concentration and did not imply toxicity.[30] This is further confirmed by the photomicrograph the male kidneys (fig.1). The general increase in kidney weights in both the male and female test groups which was only significant for test group IV rats in the female and test groups III and IV compared with the control may suggest dose dependent toxicity.[31] reported that difference in weight and relative weight of

the kidney and other organs or tissues could be as a result of exposure to drugs and other xenobiotics and does not necessarily imply toxicity.

4.0 CONCLUSION

The study shows that Hippocratea africana root bark extract distorted the homeostatic system of the experimental animals which was more pronounced in the males. However, this did not suggest toxicity as the histopathological examinations of the kidney organs were normal for both sexes. Despite its promising schizontocidal property and popularity as an antiplasmodial remedy, chronic usage of the extract is not advised especially in the male. Study on the creatinine concentration is suggested to assess the glomerular filtration rate and further confirm the renal function integrity.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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