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Moderation of hematological and plasma biochemical indices of sub-chronic salt-loaded rats, by an aqueous extract of the leaves of *Acalypha wilkesiana* 'Godseffiana' Muell Arg (Euphorbiaceae)

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ABSTRACT

Objective: To investigate the effects of an aqueous leaf extract of *Acalypha wilkesiana* (*A. wilkesiana*) on plasma chemistry and hematological indices of sub-chronic salt-loaded rats. **Method:** The control group received a diet consisting 100% of the commercial feed, while the four test groups were received a diet consisting 8% salt and 92% commercial feed all through, except for the reference treatment group that had its salt-loading discontinued after six weeks. The extract was orally administered daily at 200 and 250 mg/kg body weight; while the test control, reference and control groups received appropriate volumes of water by the same route. **Results:** The extract had no negative effects on markers of liver and kidney functions, produced hemoconcentration, significantly higher ($P<0.05$) plasma calcium and potassium levels, and significantly lower ($P<0.05$) plasma sodium and chloride levels in the test animals compared to test control. **Conclusions:** This result supports the traditional use of *A. wilkesiana* in the management of hypertension and suggests that the extract may be a potassium sparing diuretic whose mechanism of antihypertensive action may be via alteration of plasma sodium and potassium balances or calcium mediated alteration in vascular muscle tone.

1. Introduction

Herbal medicines are widely used in traditional medical practices in the management of a wide variety of illnesses. They are medicinal products that contain as active ingredients, aerial or underground parts of plants, or other materials or combinations thereof whether in the crude state or as plant preparations[1]. A great number of plants are currently used in the management of a wide range of illnesses by traditional health care practitioners. *Acalypha wilkesiana* (*A. wilkesiana*) is one of such.

A. wilkesiana Muell Arg, a member of spurge family (Euphorbiaceae), is alternatively called *A. amentaceae* and *A. tricolor*. It is commonly called copperleaf, Joseph's coat, fire dragon, beef steak plant and match-me-if-you-can[2]. It is native to Fiji and nearby islands in the South Pacific, and is a popular outdoor plant that provides color throughout the year, although it is also grown indoors as a container plant. It has antimicrobial properties[3,4,5]. Many cultivars are available with different leaf forms and colors: *A. wilkesiana* 'Godseffiana' has narrow, drooping, green leaves with creamy-white margins, 'Marginata' has coppery-green leaves with pink or crimson margins, 'Macrophylla' has larger leaves, variegated with bronze, cream, yellow and red, while 'Musaica' has green leaves that are mottled with orange and red[2,6]. The leaf-poultice is used in the treatment of headache, swellings and colds. The seeds are essential components of a complex plant mixture used empirically by traditional healers in south-west Nigeria to treat breast tumors and inflammation[7]. The expressed juice or boiled decoction is used for the treatment of gastrointestinal disorders, fungal skin infections, malaria and hypertension.

Lim *et al*[8] reported the anti-proliferative effects of ethyl acetate and hexane extracts of the leaves, against brain and lung cancer cells. Ikewuchi and colleagues had earlier reported the nutrient potential of the leaves[9-11], the hypolipidemic[12], anti-diabetic[13], diuretic[14]

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and antihypertensive[15,16] effects of aqueous extracts of the leaves, and their ability to moderate the activities of ATPases[17] and enzymes of energy metabolism[18]. However, the biochemical mechanism of the antihypertensive action of the leaves is yet to be completely understood. Thus, in the present study, the effects of an aqueous extract of the leaves of *A. wilkesiana* 'Godseffiana' on hematological indices, plasma marker enzymes and chemistry were investigated in sub-chronic salt-loaded rats.

2. Materials and methods

2.1 Chemicals

All chemicals used were of analytical grade.

2.2 Collection of plant samples and preparation of extract

Samples of the fresh *A. wilkesiana* plants (Figure 1) were collected from within the Choba and Abuja Campuses of University of Port Harcourt, Port Harcourt, Nigeria. After due identification at the University of Port Harcourt Herbarium, Port Harcourt, the identity was confirmed/authenticated by Dr. Michael C. Dike of Taxonomy Unit, Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria; and Mr. John Ibe, the Herbarium Manager of the Forestry Department, National Root Crops Research Institute (NRCRI), Umuahia, Nigeria. They were rid of dirt and the leaves were removed, oven dried at 55 ℃ and ground into powder. The resultant powder was soaked in hot, boiled distilled water for 12 h, after which the resultant mixture was filtered and the filtrate, hereinafter referred to as the aqueous extract was stored in the refrigerator for subsequent use. A known volume of this extract was evaporated to dryness, and the weight of the residue used to determine the concentration of the filtrate, which was in turn used to determine the dose of administration of the extract.

Figure 1. *A. wilkesiana* Muell Arg.

2.3 Experimental design

Wistar albino rats (weighing 185-200 g at the start of the study) were obtained from the animal house of the Department of Physiology, University of Nigeria, Enugu Campus. Studies were conducted in compliance with the applicable laws and regulations for handling experimental animals. The rats were sorted into five groups of five animals each, so that the average weight difference was < 1.5 g. The animals were housed in plastic cages in the animal house of the Department of Biochemistry, University of Port Harcourt. After a one-week acclimatization period on guinea growers mash (Port Harcourt Flour Mills, Port Harcourt, Nigeria), the treatment commenced and lasted for seven weeks. The control group received a diet consisting 100% of the commercial feed, while the four test groups received a diet consisting 8% salt and 92% commercial feed. The 8% dietary salt-loading was adapted from Obiefuna et al[19]. We assumed that the test rats became hypertensive following 6 weeks of salt loading, on the basis of earlier reports by Obiefuna et al[19] and Nwaigwe and Sofola[20]. The latter reported that blood pressure increased significantly, from 84.3±5.3 mmHg to 167.5±6.5 mmHg, in rats on this regime. At the end of the sixth week, the rats were weighed, before commencing the administration of the extract, while the reference treatment group had its salt-loading discontinued. Test group 1 (AW1) received 200 mg/kg and test group 2 received 250 mg/kg body weight of the *A. wilkesiana* leaf extract daily by intra-gastric gavage. The test control, reference treatment (reference) and control groups received equivalent volumes of water by the same route. The dosage of administration of the extract was adopted from Ikewuchi and Ikewuchi[12]. The salt removal therapy as a treatment for hypertension, which was used here as the reference treatment, was adapted from O'Shaughnessy and Karet[21]. The animals were allowed food and water ad libitum. At the end of the one week treatment period, the rats were anaesthetized by exposure to chloroform. While under anesthesia, they were painlessly sacrificed and blood was collected from each rat into EDTA and heparin sample bottles. The EDTA anti-coagulated blood samples were used for the hematological analysis. The heparin anti-coagulated blood samples were centrifuged at 1 000 *g* for 10 min, after which their plasma was collected and stored for subsequent analysis.

2.4 Enzyme assays

The plasma activities of alanine transaminase, aspartate transaminase, and alkaline phosphatase, were determined using Randox test kits (Randox Laboratories Ltd., Crumlin,

England, UK). The activities of alanine and aspartate transaminases were respectively measured by monitoring at 546 nm, the concentrations of pyruvate and oxaloacetate hydrazones formed with 2,4-dinitrophenylhydrazine. The activity of alkaline phosphatase was determined by monitoring the degradation of p-nitrophenylphosphate to p-nitrophenol, at 405 nm.

2.5 Determination of plasma chemistry

Plasma total and conjugated bilirubin, urea, creatinine, total protein and albumin concentrations were determined using Randox test kits (Randox Laboratories Ltd., Crumlin, England, UK). The wavelength for the determination of conjugated bilirubin and urea was 546 nm, that of total bilirubin was 578 nm and creatinine was 482 nm. Plasma total protein was determined by the Biuret method, whilst plasma albumin was determined using the bromocresol green dye binding method[22]. Total protein and albumin were determined at 560 nm and 630 nm, respectively.

2.6 Determination of plasma electrolytes

Plasma sodium and potassium concentration were determined by flame photometry, according to AOAC Official Method 956.01[23]. Plasma bicarbonate and chloride concentrations were determined by the titrimetric methods[24]. Plasma calcium concentration was determined by the cresol phthalein complexone method[25], and the concentration of the resultant complex was measured at 575 nm. The plasma albumin 'corrected' calcium levels were calculated according to the method of Crook[26] as follows:

2.7 Determination of hematological indices

These were carried out according to methods adopted from Cheesbrough[27]. The hemoglobin concentration was measured with DTH HemoglobinometerTM Hb523 (Gordon-Keeble Laboratory Products, Cambridge, England, UK). Packed cell volume or hematocrit was measured with micro-hematocrit, with 75 mm × 16 mm capillary tubes filled with blood and centrifuged at 3 000 *g* for 5 min. The red blood cell and total white blood cell (WBC) counts were estimated by visual methods. Differential WBC count was carried out using Leishman staining technique. The mean cell hemoglobin, mean cell hemoglobin concentration and mean cell volume were also calculated.

2.8 Statistical analysis of data

All values are quoted as the mean±s.e.m. (standard error in the mean). The values of the variables were analyzed for statistical significant differences using the Student's *t*-test, with the help of SPSS Statistics 17.0 package (SPSS Inc., Chicago Ill). $P<0.05$ was assumed to be significant.

3. Results

The effect of aqueous extract of *A. wilkesiana* on plasma

Table 1
Effects of an aqueous extract of the leaves of *A. wilkesiana* on plasma marker enzymes of sub-chronic salt-loaded rats.

Enzymes	Activity (U/L)				
	Control	Test control	Reference	AW1	AW2
Aspartate transaminase	56.42±16.00^{a,b}	90.16±11.25^{a}	48.63±12.66^{a,b}	52.58±8.99^{b}	35.75±6.66^{b}
Alanine transaminase	14.61±2.98^{a}	21.23±4.95^{a}	23.51±3.90^{a}	21.79±3.93^{a}	19.05±3.99^{a}
Alkaline phosphatase	10.76±3.27^{a}	8.05±1.58^{a}	6.44±1.20^{a}	4.14±1.07^{a}	6.90±0.62^{a}

Values are mean±s.e.m., $n=5$, per group. ^{a,b}Values in the same row with the different superscripts are significantly different at $P<0.05$.

Table 2
Effects of an aqueous extract of the leaves of *A. wilkesiana* on plasma chemistry of sub-chronic salt-loaded rats.

Parameter	Magnitude				
	Control	Test control	Reference	AW1	AW2
Creatinine (μ mol/L)	56.348±2.376^{a}	50.829±2.640^{a,b}	34.126±5.104^{b,c}	50.090±2.992^{a,c}	38.474±4.048^{b}
Urea (mmol/L)	6.063±0.677^{a}	7.463±0.668^{a,b}	6.111±0.784^{a,b}	7.637±0.388^{a,b}	10.605±1.313^{b}
Blood urea nitrogen (mg/dL)	17.055±1.904^{a}	20.993±1.879^{a,b}	17.190±2.205^{a,b}	21.483±1.091^{a,b}	29.831±3.693^{b}
Total bilirubin (μ mol/L)	14.245±6.424^{a}	20.538±4.728^{a}	8.973±2.587^{a}	17.671±6.492^{a}	14.338±2.501^{a}
Conjugated bilirubin (μ mol/L)	6.907±2.381^{a,c}	11.902±1.644^{b}	4.971±2.244^{c}	8.513±2.484^{a}	8.390±1.627^{a,c}
Unconjugated bilirubin (μ mol/L)	7.338±4.419^{a}	8.637±4.197^{a}	4.043±1.199^{a}	9.158±4.043^{a}	5.947±1.644^{a}
Unconjugated/ conjugated bilirubin ratio	0.884±0.320^{a}	0.718±0.336^{a}	0.814±0.209^{a}	0.925±0.151^{a}	0.771±0.230^{a}
Total protein (g/L)	0.057±0.002^{a}	0.059±0.001^{a}	0.058±0.002^{a,b}	0.064±0.001^{b}	0.060±0.003^{a,b}
Albumin (g/L)	0.035±0.001^{a}	0.036±0.001^{a}	0.037±0.001^{a}	0.037±0.001^{a}	0.037±0.001^{a}

Values are mean±s.e.m., $n=5$, per group. ^{a,b}Values in the same row with the different superscripts are significantly different at $P<0.05$.

Table 3

Effects of an aqueous extract of the leaves of *A. wilkesiana* on plasma electrolyte levels of sub-chronic salt-loaded rats.

Parameter	Concentration				
	Control	Test control	Reference	AW1	AW2
Bicarbonate (meq/L)	25.100±0.640[a]	24.400±0.510[a,b]	23.600±0.510[a,b]	22.760±0.371[b]	24.260±0.487[a,b]
Calcium (mg/dL)	9.664±0.124[a]	8.656±0.088[c]	8.264±0.092[b]	9.528±0.104[a]	10.144±0.148[a]
Albumin 'corrected' Calcium (mg/dL)	3.584±0.005[a]	3.543±0.004[c]	3.528±0.004[b]	3.578±0.004[a]	3.603±0.006[a]
Chloride (meq/L)	99.700±1.655[a,d]	107.400±0.510[c]	111.400±0.510[b]	98.750±0.371[a]	102.750±0.487[d]
Potassium (mg/dL)	17.511±1.342[a,c]	13.572±0.433[b]	12.558±0.144[b]	19.110±0.589[a]	15.795±0.086[c]
Sodium (mg/dL)	320.620±1.173[a]	342.700±2.183[c]	356.960±4.26[d]	311.075±1.686[b]	330.050±4.853[a,c]

Values are mean±s.e.m., n=5, per group. [a,b]Values in the same row with the different superscripts are significantly different at P<0.05.

Table 4

Effects of an aqueous extract of the leaves of *A. wilkesiana* on the hematological profile of sub-chronic salt-loaded rats.

Parameter		Magnitude				
		Control	Test control	Reference	AW1	AW2
Hematocrit (%)		39.50±1.67[a,b]	35.40±1.50[a]	35.00±2.93[a,b]	40.00±1.76[a,b]	41.25±0.86[b]
Hemoglobin (g/dL)		13.28±0.61[a,b]	12.00±0.47[a]	11.78±0.95[a,b]	13.40±0.64[a,b]	13.93±0.28[b]
Mean cell hemoglobin concentration (g/dL)		33.51±0.27[a]	33.88±0.31[a]	33.92±0.29[a]	33.92±0.22[a]	33.76±0.13[a]
Red blood cell count ($\times10^{12}$ cells/L)		4.78±0.23[a]	3.96±0.22[b]	4.06±0.40[a,b]	4.68±0.40[a,b]	5.10±0.11[a]
Mean cell hemoglobin (pg/cell)		28.62±1.32[a,b]	30.43±0.67[a]	29.21±0.66[a]	29.11±1.05[a,b]	27.32±0.24[b]
Mean cell volume (fL)		85.41±3.94[a]	89.70±1.96[a]	86.14±1.96[a]	85.84±3.26[a]	85.37±3.32[a]
White blood cell count	Total ($\times10^9$ cells/L)	5.54±0.38[a]	4.96±0.32[a,b]	4.24±0.10[b]	5.88±0.33[a]	6.25±0.56[a]
	Basophils (%)	0.10±0.10[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	Eosinophils (%)	1.10±0.40[a]	0.80±0.58[a]	0.80±0.37[a]	1.00±0.55[a]	0.75±0.37[a]
	Lymphocytes (%)	44.40±2.32[a]	47.20±2.40[a]	47.80±3.60[a]	48.75±3.47[a]	51.00±1.82[a]
	Monocytes (%)	3.50±1.50[a,b]	3.00±1.05[a,b]	2.20±0.66[a]	4.75±0.86[b]	0.75±0.37[a]
	Neutrophils (%)	50.90±2.19[a]	49.00±2.53[a]	48.80±3.98[a]	45.50±2.94[a]	47.50±1.47[a]

Values are mean±s.e.m., n=5, per group. [a,b]Values in the same row with the different superscripts are significantly different at P<0.05.

marker enzymes of salt-loaded rats is shown in Table 1. The plasma aspartate transaminase activity of the test groups were each significantly lower (P<0.05) than the test control, but not significantly different from the control and reference. Table 2 shows the effects of an aqueous extract of the leaves of *A. wilkesiana* on plasma chemistry of sub-chronic salt-loaded rats. The plasma creatinine levels of the test groups were each not significantly (P<0.05) different from the control, test control and reference treatment. The plasma urea levels of AW2 was significantly higher (P<0.05) than the control, but not significantly different from AW1, test control and reference. The plasma conjugated bilirubin of the test groups were each significantly lower (P<0.05) than the test control, and significantly higher (P<0.05) than the reference, but not different from the control. There were no significant differences in the plasma total and unconjugated bilirubin, conjugated/unconjugated bilirubin ratio and albumin levels of all the animals. The plasma total protein levels of AW1 was significantly higher (P<0.05) than the control and test control, but not different from the reference and AW2.

Table 3 shows the effects of an aqueous extract of the leaves of *A. wilkesiana* on the plasma electrolyte profiles of sub-chronic salt-loaded rats. The plasma bicarbonate level of AW1 was significantly lower than control, but not different from test control, reference treatment and AW2. The plasma measured and albumin 'corrected' calcium levels of the test groups were significantly higher than the test control and reference treatment group, but not different from the control. The plasma chloride levels of the test groups were significantly lower (P<0.05) than the test control and reference treatment groups, but not different from the control. The plasma potassium levels of the test groups were significantly higher (P<0.05) than the test control and reference treatment groups, but not different from the control. The plasma sodium levels of the test groups were significantly lower (P<0.05) than the test control and reference treatment, but not different from the control.

The effects of an aqueous leaf extract of *A. wilkesiana* on the hematological indices of sub-chronic salt-loaded rats is given in Table 4. The packed cell volume, hemoglobin concentration and red cell count of AW2 were significantly higher (P<0.05) than the test control, but not different from the control, reference treatment and AW1. There were no significant differences in the mean cell hemoglobin concentration, mean cell volume, basophils, eosinophils, lymphocytes and neutrophils profiles of all the rats. The mean cell hemoglobin of AW2 was significantly lower (P<0.05) than that of test control and reference treatment, but not different from the control and AW1. There was no significant difference in the monocytes profile of the test

groups and the control, test control and reference treatment groups. The total white blood cell counts of the test groups were significantly higher ($P<0.05$) than the reference treatment, but not different from the control and test control.

4. Discussion

The extract had no negative effects on the liver and kidney functions of the test animals. This study confirmed earlier reports that the concentration of Ca^{2+} in body fluids and its handling by cellular proteins are disturbed in patients and experimental animals with arterial hypertension[28,29]. The extract countered the salt-loading induced lowering of plasma calcium levels. Recall that calcium fluxes modulate neuromuscular activities and mediate hormonal effects on target organs through several intracellular signaling pathways[26,30]. The extract may have evoked the present effect by altering parathyroid hormone secretion. The improved plasma calcium may impart greatly on arterial muscles tones, since cardiac muscle relies on extracellular Ca^{2+} for contraction[31]. In intact animals, there is a direct relationship between myogenic tone in isolated arteries and blood pressure[32]. Thus, the mechanism of the antihypertensive action of the extract may be via moderation of muscle tone, brought about by increases in plasma calcium concentration, which in turn is produced by reducing its entry into the cells or increasing its removal from the cells into the extracellular space.

Reduction in plasma sodium and chloride concentrations is one of the mechanisms of action of antihypertensive drugs, especially the diuretics[26,33]. These diuretics decrease plasma levels of these electrolytes by diminishing their reabsorption at different sites in the nephrons. Amongst them are the potassium-sparing diuretics, which inhibit either aldosterone directly, or the Na^{+}/K^{+} exchange mechanisms in the distal tubules and collecting ducts[26,33]. The net effect is the loss of sodium in the urine and the retention of potassium in the blood, resulting in lowered plasma sodium and increased plasma potassium levels. In this study, the leaf extract produced a low plasma sodium and increased plasma potassium levels. This suggests that it may be a potassium-sparing diuretic and may contain a β-antagonist; thus confirming earlier report of a similar observation in normal rabbits[34].

The extract had a positive effect on the hemopoietic system of the test rats. Raised hematocrit indicates hemoconcentration, often due to increased red cell mass. This is confirmed by the observed level of the red cell count and hemoglobin concentrations. The ability of the extract to inhibit the hypertension-induced anemia in the test animals may be attributable to the presence of iron[10] and quercetin[13] in the leaves and their extract. Quercetin has an established anti-anemic activity[35,36].

Poisoning from drugs and stress are among the main causes of raised white blood cell count. As posited by some experimental and pathological studies, white blood cells play important roles in the destabilization of coronary artery plaques at the onset of acute coronary syndrome[37-40]. However, an elevated white blood cell count in peripheral blood is a known risk factor of coronary artery disease, and an independent predictor of cardiovascular morbidity in hypertensive patients[39,41,42]. In this study, the extract did not significantly alter the white blood cell count in the animals.

In conclusion, this result suggests that the extract may be a diuretic that causes hemoconcentration, without altering liver and kidney functions, at least at the doses at which it was administered in this study. It also supports the use of *A. wilkesiana* in the management of hypertension, and in addition, suggests that its antihypertensive action may be mediated via alteration of plasma sodium and potassium levels or increases in muscle tone brought about by changes in plasma calcium levels.

Conflict of interest statement

I declare that I have no conflict of interest.

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