Effects of an aqueous leaf extract of *Sansevieria senegambica* Baker on plasma biochemistry and haematological indices of salt-loaded rats

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The effects of an aqueous extract of the leaves of *Sansevieria senegambica* on plasma marker enzymes, plasma chemistry and the haematological profile of salt-loaded rats were studied. The control group received only a commercial feed, whilst the four test groups received a diet consisting of the commercial feed and salt, although the reference treatment group was reverted to the normal feed at the end of 6 weeks. The extract was orally administered daily at 150 mg/kg or 200 mg/kg body weight to two test groups, respectively; whilst the test control, reference and control groups received equivalent volumes of water by the same route. The extract had no negative effects on markers of liver and kidney functions, but it did produce leukocytosis, significantly increased ($p < 0.05$) plasma calcium and potassium levels and significantly decreased ($p < 0.05$) plasma sodium and chloride levels in the test animals compared to the test control animals. This result supports the traditional use of *Sansevieria senegambica* in the management of hypertension, whilst suggesting that the extract may be a potassium-sparing diuretic whose mechanism of antihypertensive action may be achieved through alteration of plasma sodium and potassium balances, or through calcium-mediated changes in vascular muscle tone.

Introduction

Hypertension is one of the most important risk factors for cardiovascular diseases, which are the leading cause of mortality worldwide.[1] Hypertension affects about 25% of the adult population worldwide, and its prevalence is predicted to increase by 60% by 2025, when a total of 1.56 billion people may be affected.[2,3] A survey by the Expert Committee on Non-communicable Diseases in Nigeria, using 160/95 mmHg as the cut-off blood pressure, revealed the prevalence of hypertension in Nigerians aged 15 years and above to be 11.2%, with rates in the rural and urban communities of 9.8% and 14.6%, respectively.[4] Adjusting these, with the cut-off point of 140/90 mmHg,[5] would give a current prevalence rate of 17% to 20%.[6,7] Thus, hypertension has an average prevalence of 20% in Nigeria, a population of over 140 million.

Several drugs are at present available for the management of hypertension. However, according to the 2003 World Health Organization (WHO) – International Society of Hypertension (ISH) statement on hypertension (the most current), antihypertensives were not affordable in about 25% of 167 countries surveyed.[5] The other available guidelines were from the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH), which focused on European countries and did not provide any statistics on the affordability of antihypertensive drugs.[8] Thus, renewed interest in the use of herbal products[9,10] may be attributable to the downturn in the economy, as traditional medicine is perceived to be a cheaper means of treatment.[9,11] It is no wonder then that WHO has developed guidelines for the assessment of herbal medicine.[9,12] In Nigeria, a great number of plants are currently used by traditional health practitioners in the management of a wide range of illnesses. *Sansevieria senegambica*, or bowstring hemp, is one such plant.

Sansevieria senegambica Baker (Family Agavaceae or Ruscaceae) is an ornamental plant[13] used in traditional health practice in southern Nigeria for treating bronchitis, inflammation, coughs, boils and hypertension. It is also used in arresting the effects of snake bites, as well as in compounding solutions used as hair tonics. However, the biochemical basis of the use of the leaves in the management of hypertension, as well as the biochemical impact of their administration to hypertensive patients is yet to be clearly understood. Thus, in the present study, the effects of an aqueous extract of the leaves of *S. senegambica* on plasma marker enzymes and electrolyte profiles, plasma chemistry and haematological indices were investigated in salt-loaded rats.

Materials and methods

Preparation of plant extract

Samples of fresh *S. senegambica* plants were procured from a horticultural garden at the University of Port Harcourt's Abuja campus, and from behind the Ofrima complex, University of Port Harcourt, in Port Harcourt, Nigeria. The plants were cleaned of soil and the leaves were removed, oven dried at 55 °C and ground into a powder. The resultant powder was soaked in hot, boiled distilled water for 12 h, after which the resultant mixture was filtered and the filtrate was stored in a refrigerator for subsequent use. A known volume of this extract was evaporated to dryness, and the weight of the residue was used to determine the concentration of the filtrate, which was in turn used to determine the dose for administration of the extract.

Experimental design

Male Wistar albino rats (weighing 180 g – 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 divided into five groups of five animals each, such that the average weight difference between groups was ±1.4 g. The animals were housed in plastic cages in the animal house of the Department of Biochemistry, University of Port Harcourt. After a 1-week acclimatisation period on guinea growers mash (Port Harcourt Flour Mills, Port Harcourt, Nigeria), the treatment commenced and lasted for 7 weeks. The control group received a diet consisting of 100% of the commercial feed, whilst the four test groups received a diet consisting of 8% salt and 92% commercial feed. The 8% dietary salt loading was adapted from Obiefuna et al.[14] We assumed that the test rats became hypertensive following 6 weeks of salt loading, on the basis of earlier reports by Obiefuna et al.[14] Nwaigwe and Sofola[15] 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, whilst the reference treatment group had its salt loading discontinued. Test group 1 received 150 mg/kg and test group 2 received 200 mg/kg body weight of the *S. senegambica* leaf extract daily by intragastric gavage. The test control, reference treatment (reference) and control groups received equivalent volumes of water by the same route. The animals were allowed food and water *ad libitum*. At the end of the 1-week treatment period, the rats were anaesthetised by exposure to chloroform. Whilst under anaesthesia, they were sacrificed and blood was collected from each rat into EDTA and heparin sample bottles. The EDTA anti-coagulated blood samples were used for the haematological studies. The heparin anti-coagulated blood samples were centrifuged at 1000 g for 10 min, after which the plasma was collected and stored for subsequent analysis.

Enzyme assays

The plasma activities of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were determined using Randox test kits (Randox Laboratories, Crumlin, England). The activities of ALT and AST were respectively measured by monitoring at 546 nm the concentrations of pyruvate and oxaloacetate hydrazones formed with 2,4-dinitrophenylhydrazine. The activity of ALP was determined by monitoring the degradation of p-nitrophenylphosphate to p-nitrophenol, at 405 nm.

Determination of plasma chemistry

Plasma total and conjugated bilirubin, urea and creatinine concentrations were determined using Randox test kits (Randox Laboratories, 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 bromcresol green dye binding method.[16] Total protein and albumin were determined at 560 nm and 630 nm, respectively.

Determination of plasma electrolytes

Plasma sodium and potassium concentrations were determined by flame photometry. Plasma calcium concentration was determinated by the cresol phthalein complexone method,[17] and the concentration of the resultant complex was measured at 575 nm. Plasma chloride and bicarbonate concentrations were determined by titrimetric methods.[18]

Determination of haematological indices

Haematological indices were determined following the procedures reported by Cheesbrough[18]. Plasma haemoglobin concentration was measured by a DTH Haemoglobinometer™ Hb523 (Gordon-Keeble Laboratory Products, Cambridge, England, UK), whilst packed cell volume was determined by the micro-haematocrit method. Red blood cell and total white blood cell counts were estimated by visual methods. A differential white blood cell count was performed using the Leishman staining technique. The mean cell haemoglobin, mean cell haemoglobin concentration and mean cell volume were also calculated, as follows:

$$\text{Mean cell haemoglobin (pg/cell)} = \frac{\text{Haemoglobin concentration (pg/cell)}}{\text{Red cell count (cell/L)}} \quad \text{[Eqn 1]}$$

$$\text{Mean cell haemoglobin concentration (g/100 mL)} = \frac{\text{[Haemoglobin] x 100}}{\text{Packed volume (\%)}} \quad \text{[Eqn 2]}$$

$$\text{Mean cell volume (fL)} = \frac{\text{Packed cell volume (\%)}}{\text{100 x Red cell count}} \quad \text{[Eqn 3]}$$

Statistical analysis

All values are reported as the mean ± standard deviation. The values of the variables were analysed for statistically significant differences using the Student's *t*-test (SPSS

Statistics 17.0 package)[19]; $p < 0.05$ was assumed to be significant.

Results and discussion

The effects of an aqueous extract of the leaves of *S. senegambica* on plasma marker enzymes of salt-loaded rats are given in Table 1. The plasma AST activity of test group 2 was significantly lower ($p < 0.05$) than that of the test control group, but not significantly lower than those of the control, reference and test 1 groups. The plasma ALT activities of both test groups 1 and 2 were significantly higher ($p < 0.05$) than that of the control group, but not different from the test control or reference groups. The plasma ALP activity of test group 2 was significantly higher ($p < 0.05$) than the reference group, but not different from the control, test control or test 1 groups.

Table 2 shows the effect of an aqueous extract of the leaves of *S. senegambica* on the plasma chemistry of salt-loaded rats. The plasma creatinine concentration of test group 1 was significantly lower ($p < 0.05$) than that of the control and test control groups, but not different from the reference and test 2 groups. There were no significant differences in plasma urea, total bilirubin, conjugated bilirubin, unconjugated bilirubin, albumin and unconjugated to conjugated bilirubin ratios between the groups. The plasma total protein concentrations of the test groups were not significantly different from that of the control, test control or reference groups, although plasma total protein was significantly lower ($p < 0.05$) in test group 1 than in test group 2. No negative effects on liver and kidney functions of the test animals (Tables 1 and 2) were observed as a result of administration of the extract.

The effects of an aqueous extract of the leaves of *S. senegambica* on the plasma electrolyte levels of salt-loaded rats are shown in Table 3. There were no significant differences in the plasma bicarbonate concentrations between the groups. The plasma calcium levels of the test groups were significantly higher ($p < 0.05$) than those of the test control and reference groups. Plasma calcium levels of test group 1, but not those of test group 2, were also not significantly different from that of the control group. The plasma chloride and sodium levels of the test groups were significantly lower ($p < 0.05$) than those of the test control and reference groups, but not different from those of the control group. The plasma potassium levels of

TABLE 1: Effects of an aqueous extract of *Sansevieria senegambica* on plasma marker enzymes (U/L) of salt-loaded rats.

Enzyme	Control		Test control		Reference		Test 1		Test 2	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Aspartate transaminase	$56.42^{a,b}$	35.77	90.16^{a}	25.16	$48.63^{a,b}$	28.31	$82.37^{a,b}$	45.04	33.00^{b}	3.67
Alanine transaminase	14.61^{a}	6.66	$21.23^{a,b}$	11.06	$23.51^{a,b}$	8.72	30.51^{b}	5.59	23.52^{b}	9.32
Alkaline phosphatase	$10.76^{a,b}$	7.31	$8.05^{a,b}$	3.54	6.44^{a}	2.68	$7.02^{a,b}$	4.62	12.27^{b}	2.46

Values in the same row with different superscripts are significantly different at $p < 0.05$.
s.d., standard deviation.
$n = 5$ per group.

TABLE 2: Effects of an aqueous extract of the leaves of *Sansevieria senegambica* on the plasma chemistry (mg/dL) of salt-loaded rats.

Aspect of plasma chemistry	Control		Test control		Reference		Test 1		Test 2	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Creatinine	0.64^{a}	0.06	$0.58^{a,c}$	0.07	$0.39^{b,c}$	0.13	0.48^{b}	0.06	$0.51^{a,b}$	0.19
Urea	36.47^{a}	9.10	44.89^{a}	8.98	36.76^{a}	10.54	40.45^{a}	11.91	76.81^{a}	45.33
Total bilirubin	0.83^{a}	0.84	1.20^{a}	0.62	0.52^{a}	0.34	0.62^{a}	0.15	0.76^{a}	0.22
Conjugated bilirubin	0.40^{a}	0.31	0.70^{b}	0.22	0.29^{a}	0.29	$0.37^{a,b}$	0.18	0.32^{a}	0.21
Unconjugated bilirubin	0.43^{a}	0.58	0.50^{a}	0.55	0.24^{a}	0.16	0.25^{a}	0.17	0.45^{a}	0.31
Unconjugated to conjugated bilirubin ratio	0.88^{a}	0.71	0.72^{a}	0.75	0.81^{a}	0.47	0.93^{a}	1.00	1.41^{a}	1.44
Total protein	$5.73^{a,b}$	0.38	$5.90^{a,b}$	0.16	$5.84^{a,b}$	0.49	5.78^{a}	0.28	6.03^{b}	0.18
Albumin	3.54^{a}	0.23	3.58^{a}	0.38	3.72^{a}	0.13	3.58^{a}	0.43	3.80^{a}	0.07

Values in the same row with different superscripts are significantly different at $p < 0.05$.
s.d., standard deviation.
$n = 5$ per group.

TABLE 3: Effects of an aqueous extract of the leaves of *Sansevieria senegambica* on the plasma electrolyte levels (mmol/L) of salt-loaded rats.

Electrolyte	Control		Test control		Reference		Test 1		Test 2	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Bicarbonate	25.10^{a}	1.43	24.40^{a}	1.14	23.60^{a}	1.14	24.75^{a}	1.48	25.33^{a}	1.08
Calcium	2.42^{a}	0.07	2.16^{c}	0.05	2.07^{d}	0.05	$2.49^{a,b}$	0.08	2.57^{b}	0.10
Chloride	99.70^{a}	3.70	107.40^{b}	1.14	111.40^{c}	1.14	101.50^{a}	0.50	102.25^{a}	1.08
Potassium	4.49^{a}	0.77	3.48^{b}	0.25	3.22^{c}	0.08	4.08^{a}	0.16	3.93^{a}	0.18
Sodium	139.40^{a}	1.14	149.00^{c}	2.12	155.20^{b}	4.15	142.75^{a}	2.28	140.33^{a}	1.08

Values in the same row with different superscripts are significantly different at $p < 0.05$.
s.d., standard deviation.
$n = 5$ per group.

the test groups were significantly higher ($p < 0.05$) than those of the test control and reference groups, but not different from those of the control group.

This study confirms earlier reports by McCarron[20] and Young et al.[21], that the concentration of calcium in body fluids and its handling by cellular proteins are disturbed in patients and experimental animals with arterial hypertension. The extract countered the lowering of plasma calcium levels induced by salt-loading. It may have achieved this by altering parathyroid hormone secretion. The raised plasma calcium may, in turn, have great impact on arterial muscle tone, because cardiac muscle relies on extracellular calcium for contraction.[22] In healthy animals, there is a direct relationship between myogenic tone in isolated arteries and blood pressure.[23] Therefore, the basis of the antihypertensive activity of the extract may be through moderation of muscle tone, brought about by increases in plasma calcium concentration. This is in turn modulated by reducing the entry of calcium into the cells or increasing its removal from the cells into the extracellular space.

One of the mechanisms of action of antihypertensive drugs, especially the diuretics, is the reduction of plasma sodium and chloride concentrations.[24,25] Antihypertensive therapy usually achieves this by diminishing the reabsorption of these electrolytes at different sites in the nephrons. Of note amongst these therapies are the potassium-sparing diuretics, which inhibit either aldosterone directly, or the Na^+/K^+ exchange mechanisms in the distal tubules and collecting ducts.[24,25] The overall effect is the loss of sodium in the urine and the retention of potassium in the blood, culminating in lowered plasma sodium and raised plasma potassium levels. In this study, the leaf extract produced reduced plasma sodium and increased plasma potassium levels. This finding suggests that the extract may have potassium-sparing diuretic effects and may also be a β-antagonist.

Table 4 shows the effects of an aqueous extract of the leaves of *S. senegambica* on the haematological profile of salt-loaded rats. There were no significant differences in the packed cell volume, haemoglobin concentration, mean cell volume, and basophil and eosinophil counts. The mean cell haemoglobin concentration of test group 2 was significantly lower ($p < 0.05$) than that of the test control group, but not different from that of the control, reference or test 1 groups. The red cell count of the test control group was significantly lower ($p < 0.05$) than that of the control group, but not significantly lower than that of the reference and test groups. The mean cell haemoglobin of test group 2 was significantly lower ($p < 0.05$) than that of the reference group, but not significantly lower than that of the control, test control and test 1 groups. The total white cell count of test group 2 was significantly higher ($p < 0.05$) than that of the control, test control and reference groups, but not different from that of test group 1. The lymphocyte count of test group 1 was significantly higher ($p < 0.05$) than that of the control group, but not significantly different from that of the test control, reference or test 2 groups. The monocyte count of test group 2 was significantly lower ($p < 0.05$) than that of the test control group, but not different from that of the control, reference or test 1 groups. The neutrophil count of test group 1 was significantly lower ($p < 0.05$) than that of the control group, but not different from that of the test control, reference or test 2 groups.

The extract had no significant effect on the haemopoietic system of the rats, but raised the total white cell count whilst lowering the monocyte count. Drug poisoning and stress are among the main causes of raised white blood cell counts. According to reports from some experimental and pathological studies, white blood cells play an important role in the destabilising of coronary artery plaques at the onset of acute coronary syndrome.[25,26,27,28] Nevertheless, an elevated white blood cell count in peripheral blood is a known risk factor for coronary artery disease.[28,29,30] Therefore, the observed higher white blood cell count in the animals that

TABLE 4: Effects of an aqueous extract of the leaves of *Sansevieria senegambica* on the haematological profile of salt-loaded rats.

Variable	Control		Test control		Reference		Test 1		Test 2	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Packed cell volume (%)	39.50^{a}	3.74	35.40^{a}	3.36	35.00^{a}	6.56	38.40^{a}	2.30	37.33^{a}	2.27
Haemoglobin (g/dL)	13.28^{a}	1.37	12.00^{a}	1.04	11.78^{a}	2.13	12.70^{a}	0.88	12.33^{a}	0.48
Haemoglobin (%)	91.00^{a}	9.38	82.00^{a}	7.18	80.60^{a}	14.38	86.80^{a}	6.02	84.67^{a}	3.34
Mean cell haemoglobin concentration (g/dL)	33.51^{a,b}	0.59	33.88^{a}	0.70	33.92^{a,b}	0.65	33.06^{a,b}	0.48	33.09^{b}	0.70
Red blood cell count (x 10^{12} cells/L)	4.78^{a}	0.51	3.96^{b}	0.50	4.06^{a,b}	0.90	4.34^{a,b}	0.45	4.47^{a,b}	0.29
Mean cell haemoglobin (pg/cell)	28.62^{a,b}	2.95	30.43^{a,b}	1.50	29.21^{a}	1.47	29.43^{a,b}	2.80	27.73^{b}	1.59
Mean cell volume (fL)	85.41^{a}	8.80	89.70^{a}	4.37	86.14^{a}	4.39	89.02^{a}	8.55	83.88^{a}	5.47
White blood cell count										
a) Total (x10^{9} cells/L)	5.54^{a}	0.84	4.96^{a,c}	0.71	4.24^{c}	0.23	6.42^{a,b}	1.28	6.57^{b}	0.78
b) Differential (%)										
i. Basophils	0.10^{a}	0.22	0.00^{a}	0.00	0.00^{a}	0.00	0.20^{a}	0.45	0.00^{a}	0.00
ii. Eosinophils	1.10^{a}	0.89	0.80^{a}	1.30	0.80^{a}	0.84	0.60^{a}	0.55	0.67^{a}	0.82
iii. Lymphocytes	44.40^{a}	5.18	47.20^{a,b}	5.36	47.80^{a,b}	8.04	50.20^{b}	4.76	50.00^{a,b}	4.30
iv. Monocytes	3.50^{a,b}	3.35	3.00^{a}	2.35	2.20^{a,b}	1.48	3.60^{a,b}	2.30	1.00^{b}	1.23
v. Neutrophils	50.90^{a}	4.90	49.00^{a,b}	5.66	48.80^{a,b}	8.90	45.40^{b}	6.19	48.33^{a,b}	5.49

Values in the same row with different superscripts are significantly different at $p < 0.05$.
s.d., standard deviation.
$n = 5$ per group.

received the extract has two implications: it implies either protection against the onset of acute coronary syndrome or increased risk of coronary artery disease.

Conclusion

Results from this study suggest that the extract may be a diuretic that causes leukocytosis without altering liver and kidney functions or activities of the haemopoietic system, at least at the doses at which it was administered in this study. This study also supports the use of *S. senegambica* in the management of hypertension, whilst suggesting that its antihypertensive activity may be mediated through alteration of plasma sodium and potassium levels, or increases in muscle tone brought about by changes in plasma calcium levels.

Competing interests

We declare that we have no financial or personal relationships which may have inappropriately influenced us in writing this article.

Authors' contributions

All the authors participated in the planning and design of the research. C.C. Ikewuchi and J.C. Ikewuchi performed the experiments and wrote the manuscript, which was edited by E.N. Onyeike and E.O. Ayologu.

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