

Moderation of Haematological Indices, Plasma Electrolytes and Markers of Hepato-Renal Function in Sub-Chronic Salt-Loaded Rats by an Aqueous Leaf Extract of *Tridax procumbens* Linn (Asteraceae)

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ABSTRACT

The effect of aqueous leaf extract of *Tridax procumbens* on plasma chemistry and haematological indices of sub-chronic salt-loaded rats was investigated. The control group received a diet consisting 100% of the commercial feed, while the four test groups received an 8% salt-loaded diet all through, except for the reference treatment group that had its salt-loading discontinued after six weeks. The extract was orally administered daily at 150 and 200 mg/kg body weight; while the test control, reference and control groups received appropriate volumes of water by the same route. The extract had no negative effects on the liver and kidney functions, produced haemo-concentration and leukocytosis, significantly ($P < 0.05$) higher plasma calcium and potassium levels, and significantly ($P < 0.05$) lower plasma sodium and chloride levels in the test animals compared to the test control group. This result supports the use of *T. procumbens* in the management of hypertension and suggests that the extract may be a potassium sparing diuretic whose antihypertensive action may be mediated via alteration of plasma sodium and potassium balances or calcium induced alteration in muscle tone.

(Keywords: haematological indices, hepatospecific markers, plasma chemistry, plasma electrolytes, *Tridax procumbens*)

INTRODUCTION

Thirty percent of modern conventional drugs are derived from plant sources and about eighty percent of the world's population depends on plants to treat many common ailments. Herbs have been used safely and effectively for many centuries, and are free of most of the side effects

associated with synthetic drugs (Murray, 2004). *Tridax procumbens* Linn (Compositae) is a native of Central America and tropical South America, though now widespread in the tropical and subtropical parts of the world (Jahangir, 2001). Traditionally, it is used for the treatment of bronchial catarrh, dysentery, stomachache, malaria, hemorrhage, high blood pressure, and as an hair tonic. It has antiseptic, insecticidal, anti-protozoal, parasiticidal, anti-inflammatory, antioxidant and hepatoprotective properties, and marked depressant action on respiration (Salahdeen *et al.*, 2004; Ravikumar *et al.*, 2005; Saxena and Albert, 2005; Bhagwat *et al.*, 2008; Hemalatha, 2008). The nutritional potential (Ikwuchi *et al.*, 2009; Ikwuchi and Ikwuchi, 2009a,b), weight reducing, hypocholesterolemic (Ikwuchi and Ikwuchi, 2009c; Ikwuchi *et al.*, 2011a), hepatoprotective (Ikwuchi, 2012b), anti-diabetic (Ikwuchi, 2012a) and hypotensive (Ikwuchi *et al.*, 2011b) properties of the leaves have been investigated. In the present study, the effect of aqueous extract of the leaves of *Tridax procumbens* on plasma hepato-renal function markers, electrolytes and haematological indices was investigated in sub-chronic salt-loaded rats.

MATERIALS AND METHODS

Preparation of Plant Extract

Samples of the fresh *Tridax procumbens* plants were collected from within the Choba and Abuja Campuses of University of Port Harcourt, Nigeria. After due identification at the University of Port Harcourt Herbarium, Port Harcourt, Nigeria, the identity was confirmed/authenticated by Dr. Michael C. Dike of the 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. The samples were rid of dirt and the leaves removed, oven dried at 55 °C 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 was stored in a 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.

Experimental Design

Wistar albino rats (weighing 180-200 g at the start of the study) were collected from the animal house of the Department of Physiology, University of Nigeria, Enugu Campus, Enugu, Nigeria. Studies were conducted in compliance with applicable laws and regulations. The rats were sorted into five groups of five animals each, so that the average weight difference was ≤ 1.6 g. The animals were housed in plastic cages. 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 other four groups received a diet consisting 8% salt and 92% commercial feed. The 8% dietary salt-loading was adapted from Obiefuna *et al.* (1991). 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.

The first test group (Test 1) received daily by intra-gastric gavages 150 mg/kg body weight of the *Tridax procumbens* extract; the second group (Test 2) received 200 mg/kg body weight of the extract; while the other three groups, test control, reference and control groups received appropriate volumes of water by the same route. The dosage of administration of the extract was adapted from Bhagwat *et al.* (2008) and Ikewuchi *et al.* (2011a, b). The salt removal as a therapy for hypertension was adopted from O'Shaughnessy and Karet (2004). 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 haematological analysis. The heparin anti-coagulated blood samples were centrifuged at 1000 g for 10 min, after which their plasma was collected and stored for subsequent analysis.

Determination of Haematological Indices

These were carried out as reported by Cheesbrough (2006a). Plasma haemoglobin concentration was measured with DTH Haemoglobinometer™. Packed cell volume was measured with micro-haematocrit, with 75×16 mm capillary tubes filled with blood and centrifuged at 3000 g for 5 min. The red blood cell and total white blood cell counts were estimated by visual methods. Differential white blood cell count was carried out using Leishman staining technique. The mean cell haemoglobin, mean cell haemoglobin concentration and mean cell volume were also calculated, as follows:

$$\begin{aligned} &\text{Mean cell haemoglobin (pg/cell)} \\ &= \frac{\text{Haemoglobin concentration (pg/L)}}{\text{Red blood cell count (cell/L)}} \end{aligned}$$

$$\begin{aligned} &\text{Mean cell haemoglobin concentration (g/dL)} \\ &= \frac{\text{Haemoglobin concentration (g/dL)}}{\text{Packed cell volume (\%)}} \end{aligned}$$

$$\begin{aligned} &\text{Mean cell volume (fL)} \\ &= \frac{\text{Packed cell volume (\%)}}{\text{Red blood cell count (cell/L)}} * 10 \end{aligned}$$

Enzyme Assays

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

Determination of Plasma Chemistry

Plasma total and conjugated bilirubin, urea, creatinine, total protein and albumin 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 that of creatinine was 492 nm. Plasma total protein was determined by the Biuret method, and the concentration of the resultant colored complex was measured at 560 nm; while plasma albumin was determined by the bromocresol green dye binding method, and the resultant protein-dye complex was measured at 630 nm.

Determination of Plasma Electrolytes

Plasma sodium and potassium concentration was determined by flame photometry, according to AOAC Official Method 956.01 (AOAC International, 2006). Plasma chloride and bicarbonate concentrations were determined by titrimetric methods (Cheesbrough, 2006b). Plasma calcium concentration was determined by the cresol phthalein complexone method (Baginsky *et al.*, 1973). The plasma albumin 'corrected' calcium levels were calculated (Crook, 2006) as follows:

$$\begin{aligned} \text{Corrected calcium (mg/dL)} \\ = 4\{\text{measured calcium (g/L)} \\ + 0.02[40 - \text{albumin (g/L)}]\} \end{aligned}$$

Statistical Analysis of Data

All values are quoted as the mean \pm S.E.M. (standard error in the mean). The values of the variables were analyzed for statistically 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.

RESULTS

The effect of aqueous extract of the leaves of *Tridax procumbens* on plasma hepatospecific marker enzymes of sub-chronic salt-loaded rats is shown in Table 1. The plasma aspartate transaminase activity of Test 1 was significantly lower ($P < 0.05$) than test control, but not different

from control, reference and Test 2. There were no significant differences in the plasma alanine transaminase and alkaline phosphatase activities of the test groups and the control, test control and reference.

Table 2 shows the effect of aqueous extract of the leaves of *T. procumbens* on plasma chemistry of sub-chronic salt-loaded rats. The plasma creatinine level of Test 1 was significantly lower ($P < 0.05$) than control and test control, but not different from reference and Test 2. The plasma urea level of Test 1 was significantly higher ($P < 0.05$) than test control, but not different from control, Test 2 and reference. There were no significant differences in the plasma total protein, albumin, total, conjugated and unconjugated bilirubin, and unconjugated/conjugated bilirubin ratio.

The effect of aqueous extract of the leaves of *T. procumbens* on plasma electrolyte levels of sub-chronic salt-loaded rats is shown in Table 3. There was no significant difference in the plasma bicarbonate levels of the animals in all five groups. The plasma measured and albumin 'corrected' calcium levels of the test groups (Test 1 and Test 2) were significantly higher ($P < 0.05$) than those of the test control and reference groups, but not different from the control. The plasma chloride levels of the test groups (Test 1 and Test 2) were significantly lower ($P < 0.05$) than those of the test control and reference, but higher than that of control. The plasma potassium levels of the test groups (Test 1 and Test 2) were significantly higher ($P < 0.05$) than those of the test control and reference groups, but not different from that of control. The plasma sodium levels of the test groups (Test 1 and Test 2) were significantly lower ($P < 0.05$) than those of the test control and reference groups, but significantly higher ($P < 0.05$) than that of control.

Table 4 shows the effect of aqueous leaf extract of *Tridax procumbens* on the haematological indices of sub-chronic salt-loaded rats. The packed cell volume of Test 1 was significantly higher ($P < 0.05$) than those of control, test control and reference, but not different from that of Test 2. The haemoglobin concentration of Test 1 was significantly higher ($P < 0.05$) than those of test control and reference, but not different from those of control and Test 2. The mean cell haemoglobin concentration of Test 1 was significantly lower ($P < 0.05$) than that of reference, but not different from those of control, test control and Test 2.

Table 1: Effect of Aqueous Extract of the Leaves of *Tridax procumbens* on Plasma Hepatospecific Marker Enzymes of Sub-Chronic Salt-Loaded Rats.

Enzymes	Activity (U/L)				
	Control	Test control	Reference	Test 1	Test 2
Aspartate transaminase	56.416±15.997 ^a	90.157±11.251 ^a	48.634±12.660 ^a	55.850±10.667 ^a	61.888±22.366 ^a
Alanine transaminase	14.608±2.980 ^a	21.230±4.946 ^{a,b}	23.510±3.901 ^{a,b}	17.590±3.217 ^b	25.650±2.794 ^a
Alkaline phosphatase	10.764±3.271 ^{a,b}	8.050±1.583 ^{a,b}	6.440±1.200 ^a	8.050±3.179 ^{a,b}	10.810±0.534 ^b

Values are Mean ± S.E.M., n=5, per group.

^{a,b}Values in the same row with different superscripts are significantly different at $P<0.05$.

Table 2: Effect of Aqueous Extract of the Leaves of *Tridax procumbens* on Plasma Hepatospecific Marker Molecules of Sub-Chronic Salt-Loaded Rats.

Parameters	Magnitude				
	Control	Test control	Reference	Test 1	Test 2
Total bilirubin (µmol/L)	14.245±6.424 ^{a,b}	20.538±4.728 ^a	8.973±2.587 ^{a,b}	8.647±2.724 ^{a,b}	5.159±0.925 ^b
Conjugated bilirubin (µmol/L)	6.907±2.381 ^{c,d}	11.902±1.644 ^b	4.971±2.244 ^{a,d}	5.314±2.398 ^{b,c,d}	2.655±0.600 ^{a,c}
Unconjugated bilirubin (µmol/L)	7.338±4.419 ^a	8.637±4.197 ^a	4.043±1.199 ^a	2.333±0.497 ^a	2.504±0.394 ^a
Unconjugated/conjugated bilirubin ratio	0.884±0.320 ^a	0.718±0.336 ^a	0.814±0.209 ^a	0.722±0.300 ^a	0.942±0.097 ^a
Total protein (g/L)	0.057±0.002 ^a	0.059±0.001 ^a	0.058±0.002 ^a	0.057±0.002 ^a	0.062±0.001 ^a
Albumin (g/L)	0.035±0.001 ^a	0.036±0.001 ^a	0.037±0.001 ^a	0.037±0.001 ^a	0.038±0.001 ^a
Creatinine (µmol/L)	56.338±2.376 ^a	50.829±2.640 ^{a,c}	34.126±5.104 ^{b,c}	31.944±3.080 ^b	45.021±3.872 ^{b,c}
Urea (mmol/L)	6.063±0.677 ^{a,c}	7.463±0.668 ^a	6.111±0.784 ^{a,c}	8.333±0.608 ^c	7.496±0.746 ^{a,c}

Values are Mean ± S.E.M., n=5, per group.

^{a,b,c}Values in the same row with different superscripts are significantly different at $P<0.05$.

Table 3: Effect of Aqueous Extract of the Leaves of *Tridax procumbens* on Plasma Electrolyte Profiles of Sub-Chronic Salt-Loaded Rats.

Parameters	Concentration				
	Control	Test control	Reference	Test 1	Test 2
Bicarbonate (meq/L)	25.100±0.640 ^a	24.400±0.510 ^a	23.600±0.510 ^a	24.000±0.949 ^a	24.260±0.662 ^a
Calcium (mg/dL)	9.664±0.124 ^a	8.656±0.088 ^b	8.264±0.092 ^c	9.752±0.148 ^a	9.904±0.160 ^a
Albumin 'corrected' calcium (mg/dL)	3.584±0.005 ^a	3.543±0.004 ^c	3.528±0.004 ^b	3.587±0.006 ^a	3.593±0.007 ^a
Chloride (meq/L)	99.700±1.655 ^{a,e}	107.400±0.510 ^d	111.400±0.510 ^c	104.800±0.583 ^d	104.250±0.371 ^d
Potassium (mg/dL)	17.511±1.342 ^{a,c}	13.572±0.433 ^b	12.558±0.144 ^b	15.522±0.144 ^a	15.210±0.277 ^a
Sodium (mg/dL)	320.620±1.173 ^a	342.700±2.183 ^c	356.960±4.267 ^d	333.500±1.260 ^b	334.075±0.853 ^b

Values are Mean ± S.E.M., n=5, per group.

^{a,b,c}Values in the same row with different superscripts are significantly different at $P<0.05$.

Table 4: Effect of Aqueous Extract of the Leaves of *Tridax procumbens* on the Haematological Profile of Sub-Chronic Salt-Loaded Rats.

Parameters	Magnitude				
	Control	Test control	Reference	Test 1	Test 2
Packed cell volume (%)	39.500±1.673 ^a	35.400±1.503 ^a	35.000±2.933 ^a	44.400±2.015 ^b	39.200±1.463 ^{a,b}
Haemoglobin (g/dL)	13.280±0.610 ^{a,b}	12.000±0.465 ^a	11.780±0.952 ^a	14.760±0.651 ^b	13.180±0.462 ^{a,b}
Haemoglobin (%)	91.000±4.195 ^{a,b}	82.000±3.209 ^a	80.600±6.431 ^a	100.800±4.532 ^b	90.200±3.121 ^{a,b}
Mean cell haemoglobin concentration (g/dL)	33.512±0.265 ^{a,b}	33.881±0.311 ^{a,b}	33.915±0.289 ^a	33.252±0.217 ^b	33.637±0.229 ^{a,b}
Red cell count (x10 ¹² cells/L)	4.780±0.229 ^{a,c}	3.960±0.223 ^{b,d}	4.060±0.404 ^{a,b}	5.340±0.388 ^{c,d}	4.620±0.306 ^{a,b,c}
Mean cell haemoglobin (pg/cell)	28.618±1.318 ^a	30.428±0.670 ^a	29.211±0.655 ^a	27.906±0.955 ^a	28.796±1.038 ^a
Mean cell volume (fL)	85.410±3.937 ^a	89.704±1.955 ^a	86.142±1.963 ^a	83.920±2.805 ^a	85.531±3.140 ^a
White blood cell count					
a) Total (x10 ⁹ cells/L)	5.540±0.378 ^a	4.960±0.317 ^{a,c}	4.240±0.103 ^c	6.720±1.243 ^{a,b,c}	8.240±0.527 ^b
b) Differential (%)					
i. Basophils	0.100±0.100 ^a	0.000±0.000 ^a	0.000±0.000 ^a	0.000±0.000 ^a	0.000±0.000 ^a
ii. Eosinophils	1.100±0.400 ^a	0.800±0.583 ^a	0.800±0.374 ^a	1.000±0.316 ^a	0.200±0.200 ^a
iii. Lymphocytes	44.400±2.315 ^a	47.200±2.396 ^a	47.800±3.597 ^a	46.200±1.241 ^a	48.000±1.517 ^a
iv. Monocytes	3.500±1.500 ^a	3.000±1.049 ^a	2.200±0.663 ^a	3.200±1.281 ^a	1.800±0.735 ^a
v. Neutrophils	50.900±2.193 ^a	49.000±2.530 ^a	48.800±3.980 ^a	49.800±0.583 ^a	50.000±1.304 ^a

Values are Mean ± S.E.M., n=5, per group.

^{a,b,c}Values in the same row with different superscripts are significantly different at $P<0.05$.

The red cell count of Test 1 was significantly higher ($P<0.05$) than those of test control and reference, but not different from those of control and Test 2. There were no significant differences in the mean cell haemoglobin, mean cell volume, as well as the basophils, eosinophils, lymphocytes, monocytes and neutrophils counts of all the animals. The total white blood cell count of Test 2 was significantly higher ($P<0.05$) than those of control, test control and reference, but not different from that of Test 1.

DISCUSSION

The extract had no negative effects on the liver and kidney functions of the test animals (Tables 1 and 2). The present result lends credence to earlier claims by Ravikumar *et al.* (2005), Hemalatha (2008) and Ikewuchi (2012b), of a hepatoprotective activity of the plant. In patients and experimental animals with arterial hypertension, the concentration of Ca^{2+} in body fluids and its handling by cellular proteins are disturbed (McCarron, 1982; Young *et al.*, 1988). The extract reversed the salt-loading induced lowering of plasma calcium levels. Calcium fluxes are involved in neuromuscular activities and mediation of hormonal effects on target organs through several intracellular signaling pathways (FAO, 2004; Crook, 2006). The extract may have achieved the present effect by

affecting parathyroid hormone secretion (?). A hormone that increases the renal tubular reabsorption of calcium, promotes intestinal calcium absorption by stimulating the renal production of 1,25-dihydroxyvitamin D, and, if necessary, resorbs bone (Brown and Hebert, 1997; Crook, 2006). The increased plasma calcium may impart greatly on the tones of the arterial muscles, since according to Murray (2003), cardiac muscle relies on extracellular Ca^{2+} for contraction. There is a direct relationship between myogenic tone in isolated arteries and blood pressure in intact animals (Blaustein *et al.*, 2006). Thus, the mechanism of the anti-hypertensive 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 anti-hypertensive drugs, especially the diuretics (Rang *et al.*, 2005; Crook, 2006). These diuretics decrease plasma levels of 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 (Rang *et al.*, 2005; Crook, 2006). 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 level and increased plasma potassium levels. This suggests that it may be a potassium-sparing diuretic and may contain a β -antagonist.

The extract had a positive effect on the haemopoietic system of the test rats. Raised haematocrit indicates haemoconcentration, often due to increased red cell mass. This is reflected by the observed level of the red cell count and haemoglobin concentrations; and negates Bhagwat *et al.* (2008) earlier report of a slightly, but not significantly lowered haemoglobin value in animals treated with the plant. The ability of both extracts to inhibit the salt-loading induced anemia in the test animals may be attributable to the presence of iron (Ikewuchi and Ikewuchi, 2009b) and quercetin (Ikewuchi, 2012b) in the leaves and their extracts. Quercetin has an established anti-anemic activity (Sen *et al.*, 2005).

Poisoning (from chemicals, drugs, etc.), stress, etc., are among the main causes of leukocytosis (raised white blood cell count). Some experimental and pathological studies have posited that white blood cells play important roles in the destabilization of coronary artery plaques at the onset of acute coronary syndrome (Moreno *et al.*, 1994; van der Wal *et al.*, 1994; Libby, 2001; Takeda *et al.*, 2003). On the other hand, an elevated white blood cell count in peripheral blood is a known risk factor of coronary artery disease (Takeda *et al.*, 2003). Thus, the observed higher white blood cell count in the test rats, in this study, has two implications. First, protection against the onset of acute coronary syndrome, and secondly, increased risk of coronary artery disease.

CONCLUSION

This result suggest that the extract may be a diuretic that causes haemoconcentration and leukocytosis, 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 *T. procumbens* in the management of hypertension, and in addition, suggests that its anti-hypertensive 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.

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