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Original Research Article

Evaluation of Diuretic Potential of Aqueous Leaf Extract of *Pavetta crassipes* (K. Schum) in Rats

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ABSTRACT

Pavetta crassipes is a plant used in the treatment of hypertension. The aim of the study was to evaluate the diuretic potential of aqueous leaf extract of *P. crassipes* in rats. Four hours and twenty-four hours diuretic and natriuretic activities of 125, 250 and 500 mg/kg aqueous leaf extract of *P. crassipes* were determined with distilled water and 10 mg/kg furosemide acting as negative and positive controls respectively in rats. Urine output was measured using graduated and transparent tubes on rat's metabolic cages, while urinary sodium, potassium, and chloride assays were carried out using a spectrophotometer and standard test kits. *P. crassipes* extract at 125, 250 and 500 mg/kg lacks diuretic activity at four hours but showed diuretic activity at 24 hours when compared with furosemide. The aqueous extract (125, 250 and 500 mg/kg) significantly increased urine output when compared with the distilled water group at 24 hours (p=0.03, p=0.04, and p=0.01, respectively). All the tested doses of the extract had a lower four hours natriuretic value when compared with furosemide, but higher natriuretic values at 24 hours. The extract increased the excretion of sodium (Na^+), potassium (K^+) and chloride (Cl^-). The aqueous leaf extract of *P. crassipes* increased urine output in rats, with a late onset of diuresis and a good natriuretic activity at 24 hours, suggesting it as a possible good diuretic agent.

Keywords: Diuretic activity, Urine, Natriuretic, Sodium, Potassium, Furosemide.

Introduction

Hypertension is a global health problem.[1-3]A common phenomenon in hypertension is the derangement of multiple mechanisms involved in the maintenance of normal blood pressures.[4-5] One of such physiological dysfunctional state in hypertension is abnormal ion transport by the kidney and subsequent disruption of body fluid volumes and eventually the development of hypertension.[6-7] Treatment strategies aimed at restoring normal ion and blood volume and thus normalizing blood pressure involve the use of diuretics.

Diuretics are a class of antihypertensives that remove water and electrolytes from the body by increasing urination. The different classes of diuretics are used in the treatment of both salt/volume dependent and non-salt dependent hypertension,[8-9] edema, nephritis, liver cirrhosis and congestive heart failure.[10] Unfortunately, presently available diuretics are associated with arrays of adverse effects and diuretic resistance.[11-13] Meaning there is need to look for more effective and safer diuretic agents. Medicinal plants are readily available and being natural are term 'safer' by people that use them,[14-17] are creating renew interest currently. One of such plants used in the treatment of hypertension is *P. crassipes* (Zango Kataf, pers. com)

P. crassipes belong to the family Rubiaceae. It is found in woodlands and grasslands in sub-tropical and tropical Africa.[18] It is used to treat cough, gonorrhoeae, malaria, fever, conjunctivitis, syphilis, sores,[18] hypertension, relax sore muscle, as a food recipe (added during preparation of fermented local corn drink), as a prophylaxis for fever and to fight infection (Zango Kataf, pers.com).

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Scientific investigations show that *P.crassipes* possess in vitro antiplasmodial activity,[19] antimicrobial(inhibited the growth of *Streptococcus pyogenes*, *Corynebacterium ulcerans*, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, and *Escherichia coli*)effect,[20] inhibits spontaneous motility and elevated tone in gastrointestinal and uterine smooth muscle preparations from rabbit jejunum, guinea pig ileum and rat uterus,[21] and possesses hypotensive activity .[22]

Although, there are no folk claims on the use of *P. crassipes* as a diuretic, the fact that it is used to treat hypertension points at the possible diuretic potential of this plant. Thus, this study was conducted to evaluate the diuretic potential of the aqueous leaf extract of *P. crassipes* in rats.

Material and Methods

Animals

Adult male Swiss rats (150 – 260 g) bred in the animal house facility of the Department of Pharmacology, University of Jos, Nigeria were used for the study. The animals were maintained under standard environmental condition of humidity, temperature(24-25^{0}C), and 12 hours light/dark cycle, with access to standard food and water *ad libitum* all through breeding and during the study.

Approval for the studies was obtained from the Animal Ethics Committee of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos with approval number F17-00379. All animal experiments were in compliance with the with the National Institute of Health Guide for Care and Use of Laboratory Animals.[23]

Chemicals

Furosemide (Mancare Pharmaceuticals, Maharashtra, India), Normal saline (Fidson healthcare, Lagos, Nigeria) Sodium test kit (Spectrum diagnostic, Cairo, Egypt, Potassium and Chloride test kits (Teco diagnostic, Aneheim, U.S.A).

Plants collection and authentication
P. crassipes leaves were collected in November 2020 from Zonkwa, Kaduna State, Nigeria. The plant was authenticated by a Taxonomist in the Department of Forestry and Wildlife, University of Agriculture, Markudi, Nigeria and a voucher number UAM/FH/0336 was assigned.

Plant preparation and extraction
Fresh Leaves of *P. crassipes* were air-dried under shade for two weeks. They were then size-reduced mechanically using a clean mortar and pestle into powder. The powdered leaves were extracted by decoction method.[24] 200 g of powdered plant was boiled in 2000 ml of distilled water for 10 minutes and allowed to stay for 24 hours. Afterwards, the extract was filtered with a clean white cloth. The filtrate was evaporated to dryness in an oven set at 45°C. This gave a percentage yield of 24.3% w/w. The dried extract was kept in a refrigerator in an air tight container until use.

Acute toxicity study
The aqueous leaf extract of *P. crassipes* was screen for acute toxicity using the method of Lorke[25] via the oral route. The method consists of two phases. In the first phase, nine (9) mice were divided into three groups of three mice each. They were treated with *P. crassipes* extract at doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg respectively and observed for signs of toxicity and death for 24 hours. In the second phase, mice were also divided into three groups of one mouse each. 1600 mg/kg, 2900 mg/kg and 5000 mg/kg doses of extract were administered to the respective groups and observed for 24 hours. The oral median lethal dose (LD_{50}) was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animals survive.

Screening for diuretic activity in rats
Diuretic study was conducted using a modified version of Kau method and Fekadu method.[26,27] Rats were randomly assigned into five (5) groups of six rats each. They were placed individually in metabolic cages 24 hours prior to initiation of the experiment for acclimatization and then fasted overnight with free access to water *ad libitum*. The metabolic cages contained graduated and transparent tubes to collect urine and determine urine volume every hour.
Overnight fasted rats were treated with 1 mL/kg of distilled water (negative control group), 10 mg/kg furosemide (positive control group), and 125 mg/kg, 250 mg/kg and 500 mg/kg aqueous leaf extract of *P. crassipes*, respectively. Immediately after dosing, rats were treated with 10 mL/kg normal saline orally and were returned to their respective metabolic cages with food and water restriction throughout the test period. The urine was collected; individual cumulative volumes(ml) were measured at one, two, three, four, and twenty-four hours. Four hours and 24 hours (last 20 hours) urine samples from rats in the same group were eventually collected in one container and 4 and 24 hours urinary excretion, diuretic index, and diuretic activity were calculated. Diuretic activity was rated good if the values were >1.5, moderate if the values are between 1.00 and 1.5, least/small if the values range between 0.72 and 0.99, and nil if the values < 0.72.[27-29] The formulas used for calculations are shown below:

$$\text{Urinary excretion} = \frac{\text{Total urinary output for a group}}{\text{Total normal saline administered to the group}} \times 100(\%)$$

$$\text{Diuretic index} = \frac{\text{Urinary excretion of treatment groups}}{\text{Urinary excretion of control group}}$$

$$\text{Diuretic activity} = \frac{\text{Urinary excretion of treatment groups}}{\text{Urinary excretion of control group}}$$

Urine samples were stored at -20°C for electrolyte (potassium, sodium and chloride) analysis.

Determination of urinary Na^+, K^+, and Cl^- concentration
Four hours and 24 hours urinary sodium, potassium and chloride concentrations of negative control (distilled water), positive control (furosemide) and aqueous *P. crassipes* extracts were analyzed spectrophotometrically (Jenway 6310 spectrophotometer Essex, England) using sodium, potassium, and chloride diagnosis test kits following manufacturer's instructions. The following parameters: saliuretic index, natriuretic and kaliuretic activities were calculated using the results of urinary ionic concentrations obtained.[29-31]
Saliuretic index = C_t/C_c. Where C_t is the concentration of electrolyte in the urine of test group and C_c is the concentration of electrolyte in the urine of control group.
Natriuretic activity Na^+/K^+ ratio = C_{Na}/C_k. Where C_{Na} is the concentration of Na^+ in the urine of a group and C_k is the concentration of K^+ in the urine of the same group.
Kaliuretic activity K^+/Na^+ ratio = C_k/C_{Na}. Where C_k is the concentration of K^+ in the urine of a group and C_{Na} is the concentration of Na^+ in the urine of the same group.

Statistical analysis
Statistical Package for Social Sciences (SPSS version 25) software was used to analyze the data collected. Data were analyzed using two-way repeated measure ANOVA followed by Bonferroni post hoc for multiple comparison. $P < 0.05$ was considered statistically significant. Results were reported as mean ± standard error of mean.

Results and Discussion

Acute toxicity
The oral median lethal dose (LD_{50}) of the aqueous leaf extract of *P. crassipes* was greater than 5000 mg/kg.

Effect of aqueous leaf extract of P. crassipes on urine output and urine electrolyte concentration in rats
Urine output of rats treated with distilled water, furosemide, *P. crassipes* leaf extract (125, 250, and 500 mg/kg) were not significantly different ($p > 0.05$) between the five groups at 1 hour. Furosemide (10 mg/kg) produced a significant higher urine output at 2, 3, and 4 hours ($p < 0.05$) when compared with both the distilled water group and all the 3 doses of the aqueous extract. At 24 hours, both furosemide (10 mg/kg) and the 3 different doses of *P. crassipes* extract produced significant increase in urine output ($p = 0.01$ for furosemide, $p = 0.03$, $p = 0.04$, and $p = 0.01$ for 125, 250, and 500 mg/kg *P. crassipes* respectively) when compared with distilled water group (Table 1).
The aqueous leaf extract of *P. crassipes* produced an inverse graded dose increase in diuretic index at four hours with 125 mg/kg extract producing the greatest diuretic index, followed by 250 mg/kg and lastly 500 mg/kg. At 24 hours, the diuretic index and diuretic activity of *P. crassipes* were not dose-dependent with 250 mg/kg dose producing the least effect, followed by 125 mg/kg and the greatest diuretic index and diuretic activity produced by 500 mg/kg leaf extract (Table 2).The aqueous leaf extract of *P. crassipes* increased 24 hours urine output when compared with the distilled water group. However, all the tested doses of *P. crassipes* extract lacked diuretic activity and had low urine output at 4 hours, but high urine output and small diuretic activity at 24 hours when compared with furosemide. This shows that *P. crassipes* possesses delayed onset diuresis effect and it is not as potent as furosemide in terms of urine excretion.
All the three doses of *P. crassipes* used in the study increased both 4 hours and 24 hours urine concentration of sodium, potassium and chlorine in rats (Tables 3 and 4). This shows that *P. crassipes* promotes the excretion of water, Na^+, K^+, and Cl^-, suggesting that the extract may have acted via similar mechanism as furosemide. Furosemide inhibits the $Na^+/K^+/2Cl^-$ co-transporter pump in the thick ascending loop of Henle resulting in increasing water, Na^+, K^+, and Cl^- excretion.[32]
All the rats treated with the three doses of *P. crassipes* extract had high urinary potassium concentration (kaliuresis) at four hours when compared with furosemide and distilled water treated groups. This implies that the plant extract promotes the excretion of potassium.

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Table 1: Effect of aqueous leaf extract of *P. crassipes* on urine output in rats

Treatment/Dose (mg/kg)	Urine output (ml/h) 1 h	2 h	3 h	4 h	5 h
DW (mL/kg)	0.067 ± 0.03[a]	0.067 ± 0.03[a]	0.100 ± 0.05[a]	0.100 ± 0.05[a]	0.317 ± 0.07[a]
Furo 10	0.183 ± 0.10[a]	0.733 ± 0.22[b]	1.00 ± 0.24[b]	1.267 ± 0.33[b]	3.517 ± 0.85[b]
PC 125	0.100 ± 0.05[a]	0.100 ± 0.05[a]	0.217 ± 0.05[a]	0.318 ± 0.07[a]	2.985 ± 0.56[b]
PC 250	0.133 ± 0.07[a]	0.138 ± 0.07[a]	0.172 ± 0.05[a]	0.272 ± 0.08[a]	2.985 ± 0.56[b]
PC 500	0.002 ± 0.02[a]	0.050 ± 0.02[a]	0.192 ± 0.04[a]	0.242 ± 2.0[a]	3.317 ± 0.66[b]

Values on the same column with different alphabet are significantly different, $p < 0.05$, while those with the same alphabet are not statistically different, $p > 0.05$. Values represent mean ± SEM (n = 6). DW = distilled water, Furo = Furosemide, PC = *P. crassipes*.

Table 2: Diuretic effect of aqueous leaf extract of *P. crassipes* in rats

Treatment/Dose (mg/kg)	4 hours effect				24 hours effect			
	CUV(ml)	UE(%)	DI	DA	CUV(ml)	UE(%)	DI	DA
D. water 1 mL/kg	0.6	5.2	1	-	1.9	17	1	-
Furosemide 10	7.6	65	12.5	1	21.1	180	10.6	1
P. crassipes 125	1.91	16.3	3.1	0.25	17.9	153	9	0.85
P. crassipes 250	1.63	13.3	2.6	0.21	17.6	143	8.4	0.79
P. crassipes 500	1.45	11.5	2.2	0.18	19.9	158	9.3	0.88

D. water = Distilled water,CUV = cumulative urine volume (mL), UE = urinary excretion, DI = diuretic index, DA = diuretic activity

Table 3: Effect of *P. crassipes* on 4 hours urine electrolyte concentration in rats

Treatment (mg/kg)	Na^+ mEq/l	K^+ mEq/l	Cl^- mEq/l	NU	KU	Saliuretic index Na^+	K^+	Cl^-
DW mL/kg	44.9	18.7	31.1	2.4	0.42	-	-	-
Furo 10	110.4	53.9	108.4	2.1	0.49	2.5	2.9	3.5
PC 125	98.4	64.0	70.4	1.5	0.65	2.2	3.4	2.3
PC 250	97	65.8	63.3	1.5	0.67	2.2	3.6	2.0
PC 500	81	69.0	48.1	1.2	0.85	1.8	3.7	1.6

NU = natriuretic, KU = kaliuretic, DW = distilled water, Furo = Furosemide, PC = *P. crassipes*

Table 4: Effect of *P. crassipes* on 24 hours urine electrolyte concentration in rats

Treatment (mg/kg)	Na^+ mEq/l	K^+ mEq/l	Cl^- mEq/l	NU	Ku	Saliuretic index Na^+	K^+	Cl^-
DW mL/kg	48.6	19.5	47.7	2.5	0.40	-	-	-
Furo 10	116.8	61.1	88.5	1.9	0.52	2.4	3.1	1.9
PC 125	118.9	35.9	86.7	3.3	0.31	2.5	1.8	1.8
PC 250	113.9	37.5	87.3	3.0	0.33	2.3	1.9	1.8
PC 500	114.7	39.3	87.0	2.9	0.34	2.4	2.0	1.8

NU = natriuretic, KU = kaliuretic, DW = distilled water, Furo = Furosemide, PC = *P. crassipes*

Several studies reported that high potassium content in diet results in increased potassium excretion.[33-34] The suggested high potassium content of the extract also suggest that another possible mechanism of diuresis with *P. crassipes* may be via K^+ induced natriuresis. High K^+ diet induced natriuresis is associated with increased urinary K^+ excretion,[35-37] which was the case in our study with four hours 125, 250 and 500 mg/kg extract treated rats excreting 64, 65, 69 mEq/l potassium respectively as against 53.9 mEq/l in furosemide and 18.9 mEq/l in distilled water treated rats. However, the urinary excretion of potassium in all the extract treated rats decreased at 24 hours, suggesting a lesser tendency of causing potassium wasting with time.

Urinary Na^+/K^+ ratio rating scale of >1, 2, and 10 for satisfactory natriuresis, a favorable natriuresis, and favorable K^+ sparing activity, respectively[30,31] was used to determine natriuretic activity of the plant extract. Accordingly, all the three doses of *P. crassipes* showed a satisfactory and favorable natriuretic index at four and twenty four hours respectively. Also, the saliuretic index of all the electrolytes tested in the extract treated rats was above one showing that the excretion of these ions is higher in the extract treated groups when compared with the distilled water control group.

P. crassipes was found to contain flavonoids, sugars, tannins, saponins, glycosides, alkaloids and polyphenols in a previous study.[21] The presence of flavonoids in the plant may be responsible for its diuretic like activity because the findings of Mareck *et al.*[38] shows that some flavonoids inhibit $Na^+/K^+/2Cl^-$ co-transporter, as well as increase natriuresis and kaliuresis.

Conclusion

The aqueous leaf extract of *Pavetta crassipes* increased urine output in rats, with a late onset of diuresis and a good natriuretic activity. This could partly explain the traditional use of *P. crassipes* in the treatment of hypertension.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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