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Original Research Article

Sub-Acute Toxicity Profile of Aqueous Leaf Extract of *Pavetta crassipes* (K. Schum) in Rats

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ABSTRACT

Pavetta crassipes is a plant with many ethnomedicinal uses but has limited published data on its safety. The aim of the study was to evaluate the subacute oral toxicity of aqueous leaf extract of *P. crassipes* in Wister rats. Thirty-six Wister male rats were randomly divided into 6 groups of six rats each. They were treated with; 1 mL/kg distilled water (negative control group), aqueous leaf extract of *P. crassipes* (125, 250 and 500 mg/kg test groups respectively), 10 mg/kg furosemide (positive control group 1), and 10 mg/kg lisinopril (positive control group 2) daily for 28 days. Weekly animal weight, food consumption, water intake, fecal/urine outputs, organ to body weight ratio, hematological, biochemical and histological assessment of organs were used to evaluating toxicity. There was no significant difference between all the groups in body weight, food/water intake, fecal output, plasma sodium, potassium, chloride, calcium and biochemical markers of liver and kidney function ($p>0.05$). However, *P. crassipes* leaf extract significantly increases the urine excretion, high density lipoprotein (HDL) and endogenous antioxidant ($p<0.05$) with a decrease in total cholesterol, triglyceride and low density lipoprotein ($p<0.05$) in rats. Aqueous leaves extract of *P. crassipes* is relatively safe because the biochemical parameters and organs assayed were within the values for distilled water treated rats.

Keywords: Toxicity, Biochemical, Plants, Biomarker, Organs.

Introduction

Plants represent a good source of therapeutic phytochemical.[1-2] Unfortunately, not all plants are safe,[3]and some of the so called safe medicinal plants can results in both observable and none observable toxicity. Thus, assessing the safety profile of medicinal plants is very necessary,[3] in order to help man attend a state of wellbeing and safe health while on herbal therapy. One of such plant with unique medicinal values is *Pavetta crassipes*.

P. crassipes belong to the family Rubiaceae. It is used to treat respiratory disorders and tuberculosis[4] hypertension, relax sore muscles, as a food recipe, as a prophylaxis for fever and to fight infection .[5]Scientific studies shows that *P. crassipes* has antiplasmodial,[6] antimicrobial,[7] inhibit spontaneous motility and elevated tone in gastrointestinal and uterine smooth muscle preparations,[8] possesses antihypertensive activity,[9-10] possesses anxiolytic, sedative, anticonvulsant, antipsychotic and muscle relaxant effect.[11] Previous studies shows that the leaf of *P. crassipes* is safe in rats.[12]

However, there are limited published data on the safety of this plant. Thus, this study, was conducted to evaluate the sub-acute toxicity profile of aqueous leaf extract of *P. crassipes* in other to revalidate the safety of the leaf in rats.

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Materials and Method

Animals used

Adult male Wistar rats (150 - 260g) 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 ± 1°C), and 12 hours light/dark cycle, with access to standard diet and water *ad libitum*. 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 National Institute of Health Guide for Care and Use of Laboratory Animals.[13]

Chemicals used

Furosemide (Fourrts Laboratories limited, India), lisinopril (Fredum Pharmaceutical Ltd, India), Sodium test kit (Spectrum diagnostic, Egypt), Potassium and Chloride test kit (Teco diagnostic U.S.A), Calcium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) test kit(Randox laboratories united kingdom) , total serum protein (TP), albumin, bilirubin, serum urea (UREA), serum creatinine (CREA), cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein-cholesterol (HDL), and triglycerides (TG)(Agape diagnostics Switzerland).

Plant 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.[14] 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.

Subacute toxicity studies in rats
Rats were randomly assigned into six (6) groups of six rats each. They were treated with; 1 mL/kg of distilled water (negative control group), 125, 250 and 500 mg/kg aqueous leaf extract of *P. crassipes* respectively (test groups), 10 mg/kg furosemide (positive control group 1), and 10 mg/kg lisinopril (positive control group 2). Rats were treated daily (orally) for 28 days and mean body weight, food consumption, water intake, and fecal output determined on day 7,14,21, and day 27 while urine output determine on day 7,14 and day 21.
All the rats were fasted on day 28 and sacrifice on the 29th day using chloroform. Blood samples were collected via the carotid artery in to EDTA and plan sample bottles for hematological and biochemical assessment respectively. The hearts and two kidneys were harvested, weight and preserved for histological assessment. While the liver was weight and two of its lobes preserved for histological assessment while the remaining part preserved for antioxidant assay. Information on weekly animal weight, food consumption, and water intake, fecal/urine outputs,29th day organ to body weight ratio, hematological, biochemical and gross microscopy of organs were used to evaluating toxicity.

Determination of food, water intake and fecal and urine out put
Weekly (7 days interval;) food and water intake and fecal and urine output were determined by transferring rats into metabolic cages on day(s) 6,13, 20, and day 26 after which 24 hours food consumption, water intake, fecal and urine output evaluated. Rats were eventually returned to their home cages after 24 hours observational period.

Hematological assay
Hematological parameters were evaluated using blood samples collected on EDTA bottles. Parameters evaluated included; pack cell volume (PCV), hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), and lymphocytes (LYMs). All hematological parameters were assay using standard manual method.[15]

Biochemical assay
Blood samples collected on plan sample bottles were allowed to clot, centrifuged at 3000 rpm for 15 minutes. Serum was collected using micropipette in to clean plan sample bottles and preserved under ice in a freezer for biochemical analysis. Standard diagnostic test kits were used to assay the following parameters; Serum sodium(Spectrum diagnostic, Egypt), potassium(Teco diagnostic U.S.A), chloride(Teco diagnostic U.S.A), calcium(Randox laboratories united kingdom), total serum protein (TP) (Agape diagnostics Switzerland), albumin(Agape diagnostics Switzerland), bilirubin(Agape diagnostics Switzerland), alanine aminotransferase (ALT) (Agape diagnostics Switzerland), aspartate aminotransferase (AST) (Agape diagnostics Switzerland), alkaline phosphatase (ALP) (Agape diagnostics Switzerland),endogenous antioxidant (Agape diagnostics Switzerland),serum urea (UREA) (Agape diagnostics Switzerland), serum creatinine (CREA) (Agape diagnostics Switzerland), total cholesterol (TC) (Agape diagnostics Switzerland), low-density lipoprotein (LDL) (Agape diagnostics Switzerland), high-density lipoprotein-cholesterol (HDL) (Agape diagnostics Switzerland), and triglycerides (TG) (Agape diagnostics Switzerland). All the assay were carried out following the manufacturer's instructions.

Antioxidant assay
Liver homogenate was used to determine antioxidant and lipid peroxidation in the different treatment groups. The concentration of reduced glutathione (GSH) was determine using the method of Beutler *et al*.[16] The method of Rotruck *et al.,* [17] was use to determine the concentration of glutathione peroxidase enzyme (GPX). Catalase activity was determined according to the method of Claiborne.[18] The level of superoxide dismutase (SOD) was determined by the method of Misra and Fridovich .[19] While Lipid peroxidation was determined by measuring the levels of Malondialdehyde (MDA) produced during lipid peroxidation based on the reaction between 2-thiobarbituric acid (TBA) and MDA according to the method described by Varshney and Kale.[20]
Organ to body weight ratio
Harvested organs were cleaned of any adherent tissues and kept on absorbent paper before they were weighed followed by calculation of organ to body weight ratio.

Histopathological evaluation
Liver, heart and kidney were preserved in 10% formalin and processed by conventional techniques .[21] The organs were eventually dehydrated using increasing concentration of ethanol, inserted in paraffin, and cut into sections of 4-5 µm. These sections were stained with hematoxylin-eosin and examine under microscope.

Data analysis
Statistical package for social sciences (SPSS version 25) software was used to analyze the data collected. Data was analyzed using one way 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

Effect of aqueous leaf extract of P. crassipes on body weight of rats
There was no significant difference in body weight between rats treated with *P. crassipes* and the negative and positive control groups at baseline, first, second, third, and fourth week of treatment ($p>0.05$). Although rats treated with lisinopril (10mg/kg) had a reduction in body weight on day 7th (week one), the reduction was not significant ($p>0.05$) (Table 1).
Effect of aqueous leaf extract of *P. crassipes* on food consumption
All the doses of aqueous leaf extract of *P. crassipes* did not alter food consumption when compare with rats treated with distilled water, furosemide and lisinopril at the end of first, second, third, and forth week of treatment ($p>0.05$) (Table 2).

Effect of aqueous leaf extract of P. crassipes on water intake
There was no significant difference in water intake in rats treated with 125,250 and 500 mg/kg aqueous leaf extract of *P. crassipes* and all the control rats at the end of first, second, third and four weeks ($p>0.05$) (Table 3).

Effect of aqueous leaf extract of P. crassipes on fecal output
There was no significant difference in fecal output in rats treated with 125,250 and 500 mg/kg aqueous leaf extract of *P. crassipes* and all the control rats at the end of first, second, third and four weeks ($p>0.05$) (Table 4).

Effect of aqueous leaf extract of P. crassipes on urine out put
P. crassipes aqueous extract at 125, 250 and 500 mg/kg doses increase urine output at the end of first week but the increase was significant only in rats treated with 125 mg/kg extract when compare with distilled water treated group ($p<0.05$). Furthermore, all the doses of P. crassipes produced a significant increase in urine excretion at the end of second and third week ($p<0.5$) (Table 5). This suggest that *P. crassipes* leaf possesses diuretic activity. This further supports findings from previous studies that shows that aqueous leaf extract of *P. crassipes* possesses diuretic effect following acute administration.[5]

Hematological parameters
There was no significant difference in PCV, Hb, RBCs, WBCs and lymphocytes between the rats treated with the three doses of extract when compared with distilled water, furosemide and lisinopril treated groups ($p>0.05$). These results are similar to that obtained by Bariweni et al .[12]However, furosemide and lisinopril treated rats produced a significant reduction in PCV ($p=0.03$ for furosemide and $p=0.01$ for lisinopril) when compare with distilled water treated rats. (Table.6)

Effect of P. crassipes on plasma electrolyte concentration
Plasma Na^+, K^+ Ca^{2+} and Cl^- level were similar in both 125,250 and 500 mg/Kg extract treated rats when compared with rats treated with

distilled water, and lisinopril($p>0.05$). (Figure 1). This suggest that the extract will not result in electrolyte unbalance at the doses tested following 28 days treatment. This relatively normal electrolytes values obtained and the increase urine excretion suggests a possible good diuretic agent. This result is different from that of presently available diuretics that are associated with either plasma sodium or potassium alterations.[22]

Moreover, furosemide treated rats produced a significant reduction in serum sodium ion concentration($p=0.00$) when compare with distilled water treated rats (Figure. 1). This further supports the possible superior effect of *P. crassipes* leaf on plasma sodium level.

Biochemical markers of liver health

Total plasma protein, albumin, bilirubin, ALT, AST, ALP levels were relatively normal between the extract treated groups and distilled water group. Generally, high plasma ALT, AST, ALP, are signs of liver disease or toxicity.[23]This imply that the leaf extract did not induce liver damage at the doses tested. The result obtain by Bariweni et al .[12] shows that the same plant extract significantly decrease AST and ALP in rats ,this further support the non-toxic nature of the plant on the liver. More so, normal album, total protein and bilirubin found in this study also shows that the leaf extracts had no toxic effect on the liver. While lisinopril treated rats had a significantly reduced AST value when compare with distilled water ($p=0.00$) and 250 mg/kg extract treated rats ($p=0.02$) (Figure. 2-3).

Biochemical markers of kidney function

High plasma creatinine and urea are biomarkers of acute renal injury.[24] In the present study, there was no significant change in the concentration of creatinine and urea at all doses tested when compare with distilled water, furosemide and lisinopril treated rats ($p>0.05$) (Figure. 4). Implying that the kidney is normal following treatment with the plant extract. This result is similar with that obtain by Bariweni et al. [12] that shows that aqueous leaves extract of *P. crassipes* produces a relatively normal plasma urea at 400, and 800 mg. Although in their study, the found a significant decrease in urea at 1600 mg/kg.

Lipid biomarkers of vascular health (LDL, HDL, TG and TC)

All the three doses of *P. crassipes* tested in the study increase HDL but the increase was only significant with the 250 mg/kg dose ($p=0.03$) when compared with distilled water treated group.

Table 1: Body weight of rats treated with distilled water, *P. crassipes*, furosemide and lisinopril

Treatment/Dose(mg/kg)	Weight(g)				
	Baseline	**1 week**	**2weeks**	**3 weeks**	**4 weeks**
D.W 1 ml/kg	173 ± 15.2	177 ± 15.5	191 ± 18.3	200 ± 18.1	205 ± 16.8
PC 125	177 ± 12.3	178 ± 12.9	190 ± 16.2	197 ± 14.9	202 ± 13.9
PC 250	181 ± 12.6	185 ± 12.8	193 ± 13.2	202 ± 13.1	205 ± 13.5
PC 500	180 ± 10.8	182 ± 9.4	193 ± 9.4	202 ± 10.0	204 ± 10.0
FUR 10	185 ± 12.2	188 ± 13.2	204 ± 12.8	214 ± 12.0	218 ± 10.9
LIS 10	177 ± 17.0	174 ± 14.0	189 ± 9.6	192 ± 9.5	204 ± 9.8

Result presented as mean ± SEM. D. W= distilled water, PC= *P. crassipes*, FUR= Furosemide, LIS= Lisinopril. Data analyzed using one way ANOVA followed by Bonferroni post hoc test. Values on the same column without any alphabet no significantly difference, $p>0.05$. n= 6

Table 2: Food consumption of rats treated with distilled water, *P. crassipes*, furosemide and lisinopril

Treatment/Dose(mg/kg)	**Food intake(g)**				
	Baseline	**1 week**	**2weeks**	**3 weeks**	**4 weeks**
D.W 1 ml/kg	3.25 ± 0.092	7.07 ± 0.12	7.18 ± 0.14	7.75 ± 0.40	7.43 ± 0.30
PC 125	3.72 ± 0.35	6.83 ± 0.08	7.00 ± 0.24	7.10 ± 0.25	7.20 ± 0.17
PC 250	4.40 ± 0.89	6.50 ± 0.28	6.08 ± 0.27	6.45 ± 0.33	7.15 ± 0.28
PC 500	4.65 ± 0.57	6.58 ± 0.29	6.07 ± 0.19	5.92 ± 0.43	7.02 ± 0.42
FUR 10	3.85 ± 0.37	6.65 ± 0.51	6.60 ± 0.38	7.15 ± 0.57	7.13 ± 0.32
LIS 10	4.60 ± 0.31	6.20 ± 0.38	7.00 ± 0.47	6.80 ± 0.65	7.25 ± 0.30

Result presented as mean ± SEM. D. W= distilled water, PC= *P. crassipes*, FUR= Furosemide, LIS= Lisinopril. Data analyzed using one way ANOVA followed by Bonferroni post hoc test. Values on the same column without any alphabet not significantly different, $p>0.05$. n= 6

Table 3: Water intake of rats treated with distilled water, *P. crassipes*, furosemide and lisinopril

Treatment/Dose(mg/kg)	**Water intake(ml)**				
	Baseline	**1 week**	**2weeks**	**3 weeks**	**4 weeks**
D.W 1 ml/kg	17.5 ± 1.71	16.8 ± 3.07	17.5 ± 4.17	17.5 ± 2.81	17.5 ± 1.71
PC 125	19.2 ± 0.83	21.7 ± 1.67	21.7 ± 1.05	20.3 ± 0.42	23.3 ± 2.7
PC 250	20.0 ± 3.41	20.0 ± 2.24	21.7 ± 1.17	20.8 ± 1.54	21.7 ± 2.11
PC 500	17.5 ± 1.71	18.3 ± 3.07	23.3 ± 2.11	19.2 ± 2.01	24.2 ± 1.53
FUR 10	19.2 ± 2.01	21.7 ± 2.47	21.67 ± 1.05	20.0 ± 1.29	25.0 ± 3.16
LIS 10	18.3 ± 2.79	20.0 ± 2.89	23.3 ± 1.67	20.0 ± 2.24	24.2 ± 1.54

Result presented as mean ± SEM. D. W= distilled water, PC= *P. crassipes*, FUR= Furosemide, LIS= Lisinopril. Data analyzed using one way ANOVA followed by Bonferroni post hoc test. Values on the same column without any alphabet no significant difference, $p>0.05$. n= 6

Table 4: Fecal output of rats treated with distilled water *P. crassipes*, furosemide and lisinopril

Treatment/Dose(mg/kg)	Fecal output (g)				
	Baseline	1 week	2weeks	3 weeks	4 weeks
D.W 1 ml/kg	1.86 ± 0.51	2.93 ± 0.39	5.45 ± 0.34	4.57 ± 0.43	5.50 ± 0.72
PC 125	2.47 ± 0.64	3.65 ± 0.30	6.38 ± 0.22	5.85 ± 0.26	5.45 ± 0.67
PC 250	2.12 ± 0.50	3.20 ± 0.27	4.48 ± 0.71	5.32 ± 0.34	5.60 ± 0.80
PC 500	2.03 ± 0.47	3.52 ± 0.32	5.21 ± 0.82	5.28 ± 0.32	5.48 ± 0.45
FUR 10	2.35 ± 0.67	3.12 ± 0.33	5.23 ± 0.39	4.93 ± 0.31	6.10 ± 0.49
LIS 10	2.02 ± 0.62	2.65 ± 0.48	4.05 ± 0.66	5.15 ± 0.45	5.51 ± 0.24

Result presented as mean ± SEM. D. W= distilled water, PC= *P. crassipes*, FUR= Furosemide, LIS= Lisinopril. Data analyzed using one way ANOVA followed by Bonferroni post hoc test. Values on the same column without any alphabet no significant difference, p>0.05. n= 6

Table 5: Urine output of rats treated with distilled water *P. crassipes*, furosemide and lisinopril

Treatment/Dose (mg/kg)	Urine volume (ml)		
	1 week	2 weeks	3 weeks
D.W 1 ml/kg	1.07 ± .25a	1.35 ± 0.37a	1.73 ± 0.33a
PC 125	4.67 ± 0.62b	3.50 ± 0.56b	4.33 ± 0.50b
PC 250	2.67 ± 0.33ac	4.00 ± 0.45b	4.00 ± 0.73b
PC 500	2.83 ± 0.48ab	3.50 ± 0.43b	3.67 ± 0.33ab
FUR 10	3.42 ± 0.46ab	3.83 ± 0.54b	4.00 ± 0.45b

Result presented as mean ± SEM. D. W= distilled water, PC= *P. crassipes*, FUR= Furosemide. Data analyzed using one way ANOVA followed by Bonferroni post hoc test. Values on the same column with different alphabet significant difference p<0.05, while those with same alphabet represent no significant difference. n= 6

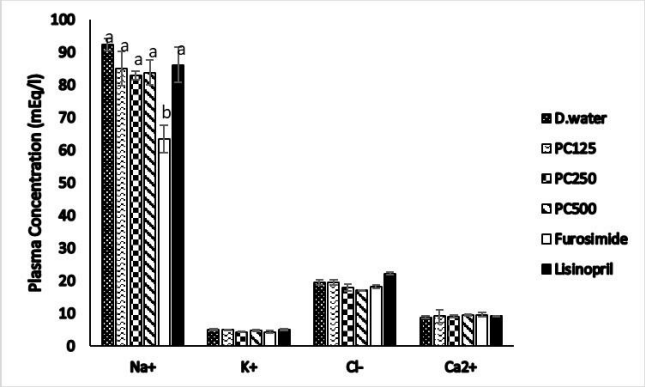

Figure 1: Effect of P. crassipes on serum Na^+, K^+ and Cl^- concentration.

Each bar represents mean ± SEM. Data analyzed using one ANOVA followed by Bonferroni post hoc test. Bars with different alphabet for Na^+ significantly different p‹0.05, while those with same alphabet for Na^+ and those without alphabet for K^+, Cl^- and $Ca2^+$ no significant difference p›0.05. n=6

Also, all the doses of extract, furosemide and lisinopril treated rats produced a significant reduction in total cholesterol and LDL (p=0.00 in all the groups for both cholesterol and LDL with only furosemide group having a p=0.01 for LDL). While as, 250 and 500 mg/kg extract treated rats significantly reduce TG (p=0.002, p=0.008 respectively) when compare with distilled water treated rats (figure.5). Suggesting that the plant extract has antiatherosclerosis like effect and is good for vascular health.

Markers of oxidative stress and lipid peroxidation

Aqueous leave extract of *P. crassipe* at 125, 250 and 500 mg/kg doses significantly increase liver GPX (p=0.01, p=0.00, and p=0.004 for 125, 250 and 500 mg/kg extract respectively) and GSH (p=0.00 for all three doses) concentrations. While as, 500 mg/kg extract and lisinopril treated rats significantly increased catalase concentration (p=0.00, and p=0.002 respectively) in the liver when compared with distilled water treated rats. More so, liver SOD concentration increased significantly in lisinopril (p=0.01), 250 (p=0.00), and 500 mg/kg (p=0.00) extract treated rats (Figure. 6,7).

The increase liver SOD, catalase, GPX and GSH concentrations when compare with distilled water treated rats found in this study shows that *P. crassipes* leaf reduce free radical in the body. SOD protect cells against free radical injury mediated by superoxide ,[25,26] while catalase catalyzes the degradation of hydrogen peroxide (H2O 2) to water and molecular oxygen.[26-28] GPX remove both hydrogen peroxide and lipid peroxide reactive species .[26,29] Reduced glutathione directly mops off reactive oxygen species .[26,30] Thus, this study shows that *P. crassipes* has good safety profile . The result is in line with the works of other scholars that shows the antioxidant activity of plants like; *Centella asiatica* (L.),[31] *Choerospondias axillaris* fruit,[32] and Taraxacum officinale.[33]

Malonaldehyde level in the liver was significantly reduced in the 250 and 500 mg/kg extract treated rats (p=0.02 for both doses) when compared with distilled water treated group (figure 8). High malonaldehyde is a sign of cell membrane disruption, that promote the leaking of cellular contain and enzymes into the bloodstream.[34] The reduction of malonaldehyde concentration in this study suggest membrane protective potentials of *P. crassipes* leaf extract.

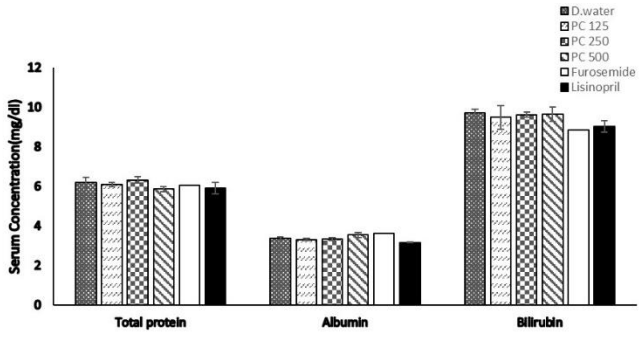

Figure 2: Effect of *P. crassipes* on total protein, albumin and bilirubin.

Data analyzed using one way ANOVA followed by Bonferroni post hoc test. Bars with different alphabet significantly different p<0.05, while those without alphabet no significant difference p>0.05. n=6

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Table 6: Hematological parameters of rats treated with distilled water *P. crassipes*, furosemide and lisinopril

Treatment/Dose(mg/kg)	Hematological Parameters				
	PCV (%)	HB (g/L)	RBCs (x10^{12}/L)	WBCs (x10^{9}/L)	LYM (%)
D.W 1 ml/kg	55.0 ± 2.56a	18.4 ± 0.89a	5.33 ± 0.22a	4.13 ± 0.70a	59.33 ± 2.38a
PC 125	49.3 ± 1.48ac	17.33 ± 0.87a	5.23 ± 0.08a	7.40 ± 0.86a	52.67 ± 6.00a
PC 250	49.3 ± 0.76ac	16.68 ± 0.92a	5.60 ± 0.07a	8.33 ± 1.52a	61.67 ± 2.79a
PC 500	50.7 ± 0.76ac	18.8 ± 1.03a	5.27 ± 0.04a	8.90 ± 1.89a	53.33 ± 2.77a
FUR 10	48.7 ± 0.76bc	17.6 ± 0.69a	5.87 ± 0.31a	8.07 ± 1.12a	55.00 ± 3.19a
LIS 10	46.3 ± 0.42bc	15.2 ± 0.15a	5.15 ± 0.10a	7.73 ± 1.36a	55.00 ± 3.19a

Result presented as mean ± SEM. D. W= distilled water, PC= *P. crassipes*, FUR= Furosemide, LIS= Lisinopril. Data analyzed using one way ANOVA followed by Bonferroni post hoc test. Values on the same column with different alphabet significantly different p<0.05, while those with at least one same alphabet represent no significant difference. n=6.

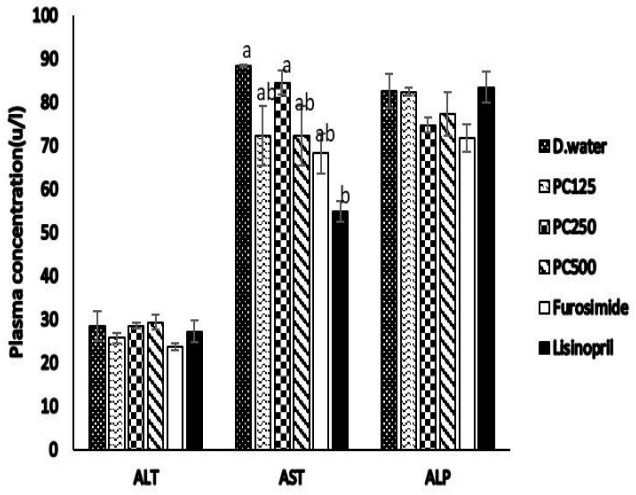

Figure 3: Effect of *P. crassipes* on enzyme markers of liver function.
Data analyzed using one way ANOVA followed by Bonferroni post hoc test. Bars with different alphabet significantly different p‹0.05, while those without alphabet for ALT and ALP and those with at least one same alphabet no significant difference for AST, p>0.05. n=6

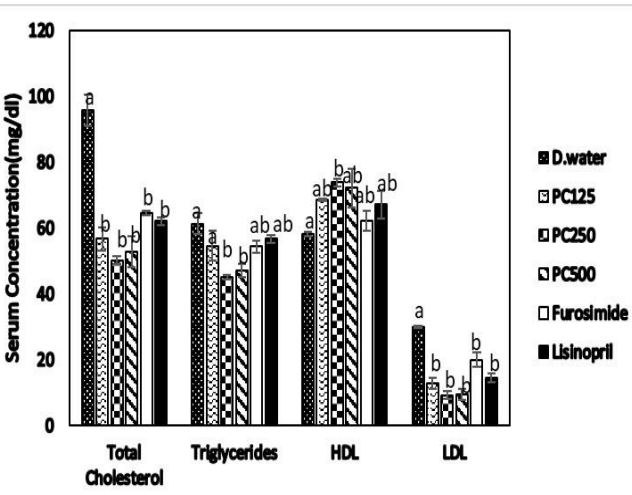

Figure 5: Effect of *P. crassipes* on lipid profile of rats.
Data analyzed using one way ANOVA followed by Bonferroni post hoc test. Bars with different alphabet significantly different p‹0.05, while those without alphabet or with at least one same alphabet no significant difference p›0.05. n=6

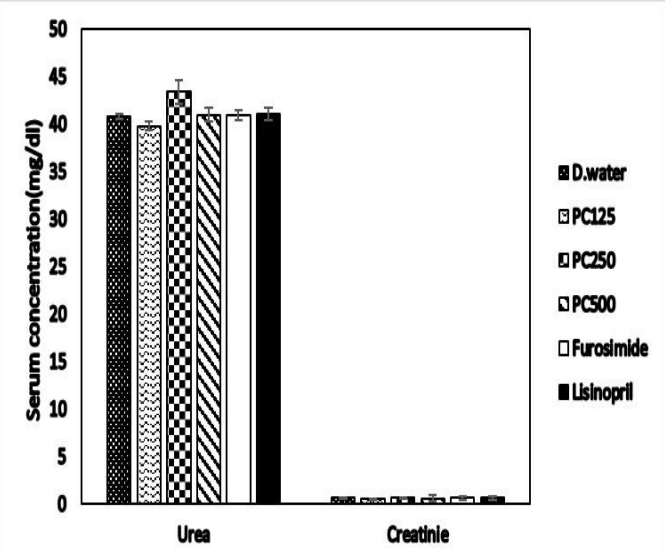

Figure. 4: Effect of *P. crassipes* on serum urea and creatine concentration.
Data analyzed using one-way ANOVA followed by Bonferroni post hoc test. Bars with different alphabet significantly different p<0.05, while those without alphabet no significant difference p›0.05. n=6

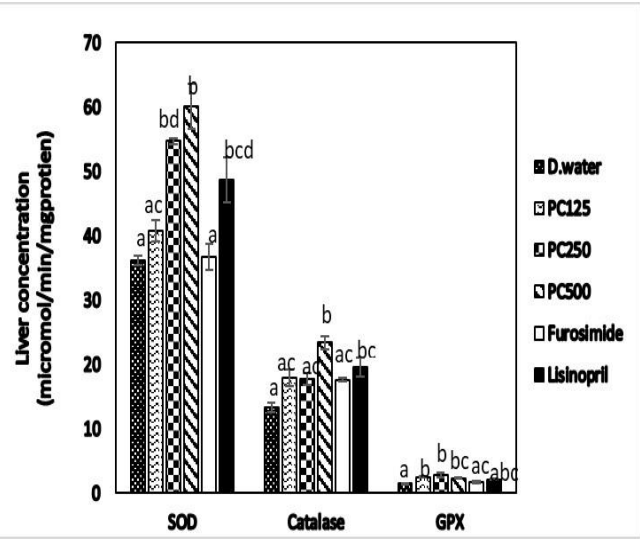

Figure 6: Effect of *P. crassipes* on liver SOD, GPX and catalase level in rats.
Data analyzed using one way ANOVA followed by Bonferroni post hoc test. Bars with different alphabet significantly different p‹0.05, while those with at least one same alphabet no significant difference p›0.05. n=6

Effect of P. crassipes on organ to body weight ratio

The results of organ to body weight ratio of rats treated with *P. crassipes* leaf extract shows that each of the organ (liver, heart, left and right kidney) weight to body ratio where comparable between the control and treatment groups ($p>0.05$) (Figure. 9). This means that the extract has no detrimental effect on liver, heart and kidney. This data is similar with results obtain from previous study.[12]

Histology

Gross microscopic examination of the heart, liver, and kidney (right and left) shows no pathological changes in the heart, liver and kidney following 28 days treatment with the three different doses of *P. crassipes* extract, distilled water and lisinopril in rats (Figure 10). The normal liver, heart and kidney further supports the normal organ to body weight ratio obtained in this study. Previous studies showed a normal liver and heart but small histological changes in the kidney at 800 and 1600 mg/kg dose .[12] Indicating that the extract could cause toxicity at higher dose in the kidney.

Conclusion

P. crassipes leaf extract at the doses tested is relatively safe, with no harmful effect on rats kidney, liver and heart. The leaf is good for health because it increases the concentrations of endogenous antioxidants; catalase, glutathione peroxidase, glutathione, superoxide dismutase and decreases the level of Malonaldehyde. The extract also reduces total cholesterol, triglyceride, and low-density lipoprotein with an increase in high density lipoprotein. These findings suggest that aqueous leaf extract of *Pavetta crassipes* is non-toxic.

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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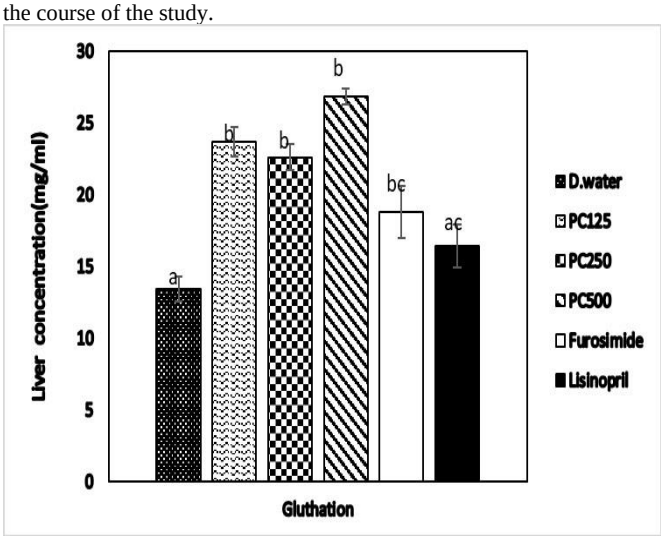

Figure 7: Effect of *P. crassipes* on liver glutathione level in rats.
Data analyzed using one way ANOVA followed by Bonferroni post hoc test. Bars with different alphabet significantly different $p<0.05$, with at least one same alphabet no significant difference $p>0.05$. n=6

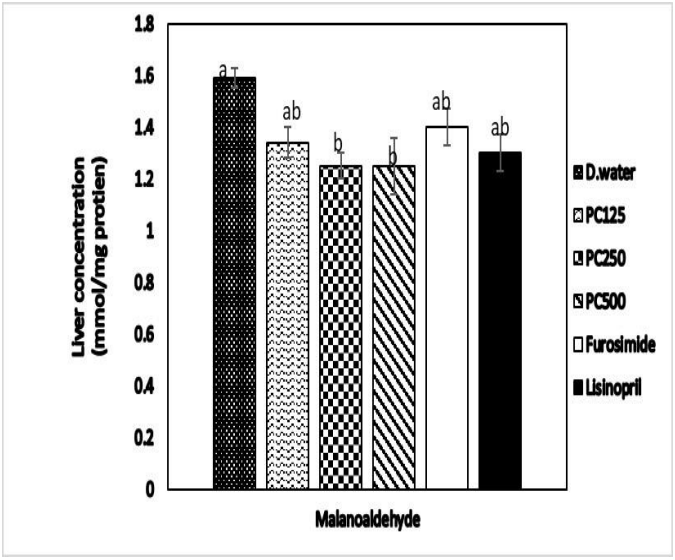

Figure 8: Effect of *P. crassipes* on liver malonaldehyde level in rats.
Data analyzed using one way ANOVA followed by Bonferroni post hoc test. Bars with different alphabet significantly different $p<0.05$, while those with at least one same alphabet no significant difference $p>0.05$. n=6

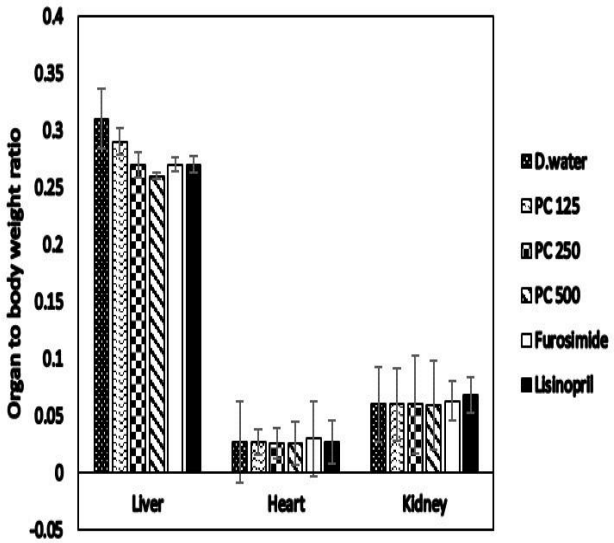

Figure 9: Effect of *P. crassipes* on organ to body weight ratio.
Data analyzed using one way ANOVA followed by Bonferroni post hoc test. Bars with different alphabet significantly different $p<0.05$, while those without alphabet $p>0.05$. n=6

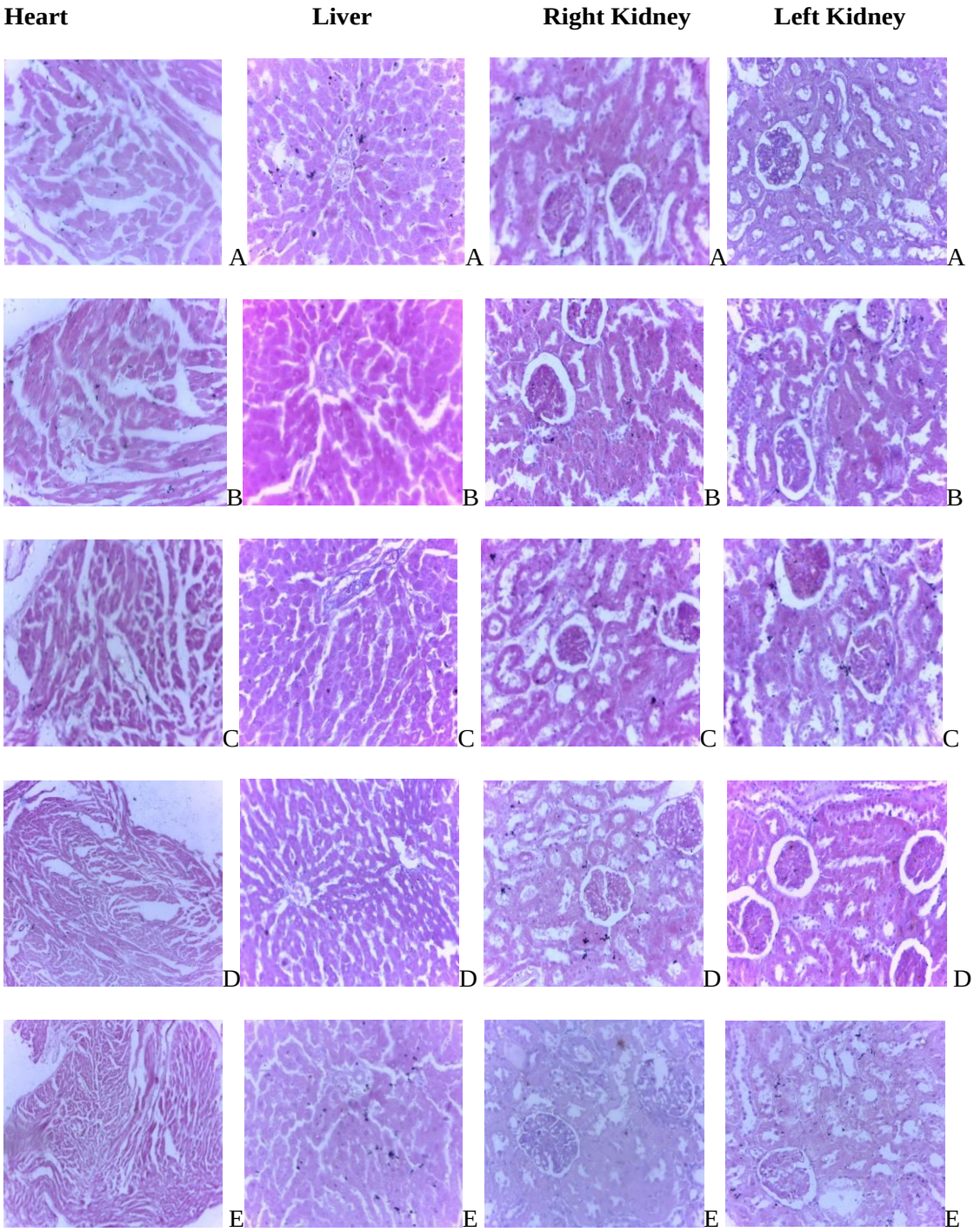

Figure 10: Photomicrograph of heart, liver, and kidney (right and left) sections of male rats showing normal organs following 28 days treatment with distilled water(A), 125, 250, 500 mg/kg aqueous leaf extract of *P. crassipes* (B, C, D respectively) and 10 mg/kg lisinopril(E)

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