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Toxicological studies on the aqueous leaf extract of *Pavetta crassipes* (K. Schum) in rodents

[Estudios toxicológicos sobre el extracto acuoso de *Pavetta crassipes* (K. Schum) en roedores]

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Abstract

Context: *Pavetta crassipes* (K. Schum) is used in ethnomedicine for the management of various ailments and food without scientific report of its toxicity profile.

Aims: To evaluate the toxicological effects of *Pavetta crassipes* leaves in rodents.

Methods: The leaves of *Pavetta crassipes* were collected from the suburbs of Abuja, Nigeria, dried and powdered using an electric mill. Hot aqueous extraction was done using 250 g of the powdered leaf in 1000 mL distilled water. Lethal dose 50 (LD₅₀) determination was done in mice (intraperitoneally) and rats (orally), twenty-eight-day toxicity testing in rats at doses of 400, 800 and 1600 mg/kg was also done using the aqueous extract. Biochemical, hematological and histological effects were evaluated using standard procedures.

Results: The aqueous leaf extract of *Pavetta crassipes* (AE) appears safe as the LD₅₀ for intraperitoneal route in mice and oral route in rats was greater than 5000 mg/kg. The results of the 28-day repeated exposure test produced no mortality or adverse effects on the body weights and organ/body weight ratio of rats. Histological changes occurred in the kidneys at 800 and 1600 mg/kg. There was a significant ($p < 0.01$) reduction in AST, ALT, ALP and urea. Lymphocyte (%) and platelet count increased ($p < 0.01$) but neutrophil (%) reduced ($p < 0.01$).

Conclusions: The LD₅₀ of AE was greater than 5000 mg/kg but prolonged use at high doses may produce some adverse effects.

Keywords: biochemical; hematological; histology; kidney; LD₅₀.

Resumen

Contexto: *Pavetta crassipes* (K. Schum) se utiliza en la etnomedicina para el manejo de diversas enfermedades, y en alimentos, sin informe científico de su perfil de toxicidad.

Objetivos: Evaluar los efectos toxicológicos de las hojas de *Pavetta crassipes* en roedores.

Métodos: Las hojas de *Pavetta crassipes* fueron colectadas en Abuja, Nigeria, secadas y pulverizadas usando un molino eléctrico. La extracción acuosa caliente se realizó usando 250 g de la hoja en polvo en 1000 mL de agua destilada. La determinación de la dosis letal 50 (DL₅₀) se realizó en ratones (i.p.) y ratas (p.o.), se realizó un ensayo de toxicidad de veintiocho días en ratas a dosis de 400, 800 y 1600 mg/kg utilizando el extracto acuoso. Los efectos bioquímicos, hematológicos e histológicos se evaluaron utilizando procedimientos estándares.

Resultados: El extracto acuoso de hojas de *Pavetta crassipes* (AE) parece seguro ya que la DL₅₀ para la vía intraperitoneal en ratones y la vía oral en ratas fue superior a 5000 mg/kg. Los resultados de la prueba de exposición repetida de 28 días no produjeron mortalidad ni efectos adversos sobre los pesos corporales y la proporción órgano/peso corporal de las ratas. Se produjeron cambios histológicos en los riñones a 800 y 1600 mg/kg. Hubo una reducción significativa ($p < 0.01$) en AST, ALT, ALP y urea. Los linfocitos (%) y las plaquetas aumentaron ($p < 0.01$), pero los neutrófilos (%) disminuyeron ($p < 0.01$).

Conclusiones: La DL₅₀ de AE fue superior a 5000 mg/kg, pero el uso prolongado a dosis altas puede producir algunos efectos adversos.

Palabras Clave: bioquímico; DL₅₀; hematológico; histología; riñón.

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INTRODUCTION

In recent years the interest in herbal medicine has grown steadily, major factors to this growing interest may include the rising cost of orthodox medicines, toxicity and the growing incidence of drug resistance (Onyeyilli and Egwu, 1995). It is thought that herbal medicines may be affordable and less toxic than the orthodox or synthetic medicines for the prevention and treatment of diseases. Herbal medicines contain potent phytochemicals and are employed in the treatment of various diseases. These phytochemicals are extracted by different methods and administered in most disease conditions over a long period of time without proper dosage regimen and consideration for toxic effects that might result from such a prolonged usage.

Pavetta crassipes is found in the savannah regions of west and central Africa where the leaves are eaten as food or used for the treatment of fever, schistosomiasis, mental illness, convulsions, pains, hookworms and various microbial infections (Amos et al., 1998; Abubakar et al., 2007; Ibekwe et al., 2012; Bello et al., 2014). However, the toxicological profile has not been reported hence the need for this study.

MATERIAL AND METHODS

Chemicals

Reagents and chemicals used were of analytical grade and manufactured by Sigma-Aldrich, BDH and Randox, they were all obtained from Rovet Chemicals and Reagents, Benin City, Edo state, Nigeria.

Collection, authentication, and extracts of *P. crassipes*

Pavetta crassipes leaves were harvested from the suburb of Abuja (9.0637° N, 7.3382° E), Nigeria, in the month of April, 2015. The leaves were authenticated by Mr. Ibrahim Muazzam, a taxonomist at the National Institute for Pharmaceutical Research and Development, Abuja, where a voucher specimen (NIPRD/H/6865) has been deposited. The leaves were dried and ground into coarse powder using an electric mill. A quantity (250 g) of the powdered leaf material was boiled for 15 min in 1000 mL of

distilled water, allowed to cool, filtered and concentrated to dryness in an electric oven at 50°C, weighed (51.5% w/w), packed into an airtight jar and stored in a refrigerator (2-8°C) until used. Fresh stock solution was prepared for use daily.

Animals

Adult Wistar rats weighing between 120 and 160 g and adult albino mice weighing between 24 and 28 g of both sexes were obtained from the animal house, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Nigeria. Ethical approval (NDU/PHARM/PCO/AEC/05) was obtained from the Animal Ethics Committee of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The animals were fed with standard rodent chow (Livestock Feeds Plc, Nigeria) and had free access to water. All animals were handled in accordance with EU directive (2010/63/EU) for animals.

Acute toxicity

The Lorke (1983) method was modified for the determination of the median lethal dose (LD₅₀). All animals were fasted overnight prior to the experiment but had free access to water until an hour before the experiment. Feeding resumed two hours after commencement of experiment. The experiment was divided into two phases and was carried on the oral route in rats and intraperitoneal route in mice. In phase one, Wistar rats of both sexes were weighed into four groups of six rats (three males, three females) each. Group one received ten mL/kg distilled water, group two received ten mg/kg of the aqueous extract (AE), group three received 100 mg/kg of AE and group four received 1000 mg/kg of AE. The animals were observed continuously for one hour after administration of extract and hourly intervals for eight hours then after 24 hours and signs of toxicity such as behavioural changes and mortality.

Phase two consisted of four groups of two rats (one male, one female) each. Group one received ten mL/kg distilled water, group two was given 1600 mg/kg; group three received 2900 mg/kg and group

four received 5000 mg/kg of AE. The animals were observed as in phase one. The animals were thereafter removed from the study based on the observed effects and animal welfare.

Acute intraperitoneal toxicity in mice was done using the same doses and protocols described for the acute oral toxicity testing in rats but the intraperitoneal route was used to administer the extract and vehicle.

Twenty-eight-day oral toxicity testing

A total of 40 Wistar rats of both sexes were used. The rats were weighed and randomly assigned into four groups of ten rats (five males, five females) each. Group one received ten mL/kg of distilled water; group two received 400 mg/kg AE; group three was given 800 mg/kg AE while group four received 1600 mg/kg AE. Both extract and water were administered daily by means of orogastric tube for 28 consecutive days. The rats were closely monitored for signs of toxicity and mortality. The rats were sacrificed under diethyl ether anaesthesia on the 29th day.

Hematological assessment

Blood samples were collected directly from the abdominal aorta of the rats under diethyl ether anaesthesia using a 2-mL syringe. The blood was immediately transferred into EDTA-laced sample bottles and properly mixed by swirling after which it was used for analysis of hematological indices. Samples (13–18 µL) were withdrawn by means of an automated aspirator and values were read off from an automated hematology system (Sysmex Haematology-Coagulation Systems®, Model KX-21N, Sysmex Incorporation, Kobe, Japan). The parameters measured include packed cell volume (PCV, hematocrit), white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HB), platelet count (PLT), neutrophil % (NEU), lymphocyte % (LYM).

Biochemical assays

The same method applied in the collection of blood samples for haematological assessment was carried out but the blood was transferred into plain sample bottles without anticoagulant, allowed to clot and then centrifuged at 5000 rpm to separate the serum from the clotted blood samples. The se-

rum was used for the following biochemical assays: Urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), globulin (GL), total bilirubin (TB) and direct bilirubin (DB). Biochemical tests were performed in triplicates

Urea determination

This was done by mixing 10 µL of serum with 100 µL reagent 1 (EDTA, sodium nitroprusside and urease mixture) in a test tube and incubated for 10 min at 37°C. Equal volumes (2.5 mL) of reagent 2 (dil. phenol) and reagent 3 (dil. sodium hypochlorite) were added to the test tube, mixed and incubated immediately at 37°C for 15 min. The procedure was repeated with a blank and standard solution. The absorbance of the sample and standard were read against the blank at a wavelength of 546 nm in a 1 cm cuvette at 37°C. The urea concentration was calculated as shown below (Fawcett and Scott, 1960).

$$\text{Urea concentration} = \frac{\text{Absorbance sample}}{\text{Absorbance standard}} \cdot \text{Standard concentration}$$

Creatinine determination

Estimation of creatinine was done using Jaffer's reaction method (Brod and Sirota, 1948). A quantity of the sample (0.1 mL) was mixed with 1.0 mL standard reagent (picric acid and sodium hydroxide solution). Same was repeated using blank and standard solution. The absorbance was read after 30 s at 492 nm in a 1 cm light path at 37°C against air. The concentration of creatinine was calculated using the same formula for urea concentration.

Measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)

Serum ALT and AST were measured using the method of Reitman and Frankel (Reitman and Frankel, 1957). For serum ALT estimation, serum (0.1 mL) was mixed with 0.5 mL reagent 1 (phosphate buffer, L-alanine and α-oxoglutarate solution) and incubated for 30 min at 37°C. To this mixture 0.5 mL reagent 2 (2,4-dinitrophenylhydrazine) was added, mixed and allowed to stand for 20 min at 25°C. This mixture was added to 5.0 mL sodium hydroxide and

absorbance read after 5 min at 546 nm in a 1 cm cuvette at 37°C against distilled water (reagent blank) and a blank sample.

For serum AST determination, serum (0.1 mL) was mixed with 0.5 mL reagent 1 (phosphate buffer, L-aspartate and α -oxoglutarate solution) and incubated for 30 min at 37°C. To this mixture 0.5 mL reagent 2 (2,4-dinitrophenylhydrazine) was added, mixed and allowed to stand for 20 min at 25°C. This mixture was added to 5.0 mL sodium hydroxide and absorbance read after 5 min at 546 nm in a 1 cm cuvette at 37°C against distilled water (reagent blank) and a blank sample. The AST activity was read off the standard reference table.

The assay of alkaline phosphatase (ALP) activity was by the method of Walter and Schutt, (1974). Briefly, 0.05 mL serum was mixed with 3.0 mL reagent containing p-nitrophenylphosphate and diethanolamine buffer in a cuvette and the absorbance read at 405 nm maintained at 25°C against air. The reading was repeated at 1 min, 2 min and 3 min, the mean change in absorbance per minute was determined and this value was multiplied by 3300 to give the ALP activity.

Albumin (ALB)

Serum (10 μ L) was pipette into a cuvette and mixed with 3000 μ L of reagent (BCG concentrate, buffer and bromocresol green solution), incubated at 37°C for 5 min and the absorbance read at 578 nm in a 1 cm path length maintained at 25°C against the reagent blank. The procedure was repeated for blank and the standard and the absorbance calculated as shown above for urea (Dumas et al., 1971; Grant et al., 1987).

Total protein (TP) and globulin (GL)

Total protein (TP) and GL estimation was done by the Biuret method (Tietz, 1995). To measure TP levels, 0.02 mL serum was pipette into a cuvette and mixed with 1.0 mL Biuret's reagent (sodium hydroxide, sodium-potassium tartrate, potassium iodide and copper sulphate solution), incubated for 30 min at 25°C and the absorbance measured at 546 nm in a 1 cm light path maintained at 25°C against distilled water. The process was repeated for the

standard. The TP concentration was calculated with the same formula for urea. Globulin was calculated as follows: $TP - ALB = GL$

Total bilirubin (TB) and direct bilirubin (DB)

The Jendrassik-Grof method was used to estimate TB and DB (Jendrassik and Grof, 1938). Briefly, 0.2 mL serum in a cuvette was mixed with 0.2 mL reagent 1 (sulphanilic acid and HCl solution), 0.05 mL reagent 2 (sodium nitrite) and 1.0 mL reagent 3 (caffeine) and allowed to stand for 10 min at 25°C. To this mixture was added 1.0 mL reagent 4 (tartrate and sodium hydroxide solution) allowed to stand for 30 min and the absorbance read at 578 nm in a 1 cm light path maintained at 25°C against the sample blank void of reagent 2. This procedure was repeated using distilled water. The absorbance multiplied by 10.8 gave the total bilirubin concentration (mg/dL).

Histological studies

After sacrificing the rats, the abdominal cavity was surgically opened and vital organs (heart, liver, lungs, kidneys and brain) excised, rinsed in normal saline, blotted with Whatmann's number one filter paper, observed for visible damage, weighed and preserved in 10% formaldehyde in normal saline for post mortem examination. Thereafter each weighed organ was standardized for 100 g body weight of the corresponding animal. Each organ was sectioned, embedded in paraffin wax and then stained with hematoxylin and eosin (Adefemi et al., 2003). Photomicrographs of the stained tissue sections were produced using a digital microscope (Olympus®) at x400 magnification.

Statistical analysis

Results are presented as mean \pm standard error of mean (SEM) and "n" represents the number of animals per group. Inferential statistical analysis was done using one-way ANOVA followed by Dunnett's multiple comparison (GraphPad Prism 6 Software, San Diego California USA.). Differences between compared data were considered significant at $p < 0.05$. LD₅₀ was estimated as a cut-off value since no mortality occurred even at 5000 mg/kg.

RESULTS

Acute toxicity test

Table 1 and Table 2 shows the results of the acute toxicity tests in mice and rats respectively. The extract did not produce any mortality even at 5000 mg/kg in both rats and mice. The LD₅₀ (oral in rats and intraperitoneal in mice) is therefore estimated to be greater than 5000 mg/kg. Signs of toxicity were absent at ten mg/kg in both rats and mice. At 100 mg/kg mice exhibited some sedation and reduced grooming. At doses greater than 100 mg/kg the animals exhibited reduced grooming and feeding, sedation was also observed. During the second phase stooling was observed in both mice and rats.

Twenty-eight day sub-acute toxicity test

Effect of oral administration of AE on body weight of rats.

The results presented in Table 3 shows the effect of oral administration of AE on body weight. There

was no statistically significant difference in mean body weight change between the treatment groups and the control.

Effect of AE on relative organ weights of rats after 28-days oral administration

The effects of the extract on relative organ to body weights ratio of rats are presented in Table 4. There were no significant differences in the relative organ to body weight ratio in the treated rats when compared to the control group

Effect of oral administration of AE on haematological parameters of rats

Table 5 shows the results of the effects of AE on the haematological parameters of treated rats. At 800 mg/kg and 1600 mg/kg LYM was significantly ($p < 0.01$) elevated while NEU was significantly ($p < 0.01$) reduced compared to the control. PLT count was also significantly ($p < 0.01$) increased at 1600 mg/kg. However, there were no significant differences in the PCV, WBC, RBC and HGB compared to the control.

Table 1. Effect of the aqueous leaf extract of *Pavetta crassipes* on acute intraperitoneal toxicity test in mice.

	Dose	Writhing	Sedation	Grooming	Diarrhea	Death
Phase 1	Control	0/6	0/6	6/6	0/6	0/6
	10 mg/kg	0/6	0/6	6/6	0/6	0/6
	100 mg/kg	3/6	5/6	1/6	0/6	0/6
	1000 mg/kg	6/6	6/6	0/6	4/6	0/6
Phase 2	Control	0/2	0/2	2/2	0/2	0/2
	1600 mg/kg	2/2	2/2	0/2	1/2	0/2
	2900 mg/kg	2/2	2/2	0/2	2/2	0/2
	5000 mg/kg	2/2	2/2	0/2	2/2	0/2

LD₅₀ > 5000 mg/kg, numerator= number affected, denominator= number tested. No mortality observed in both phases. Writhing, sedation and diarrhea occurred at doses above 1000 mg/kg.

Table 2. Effect of the aqueous leaf extract of *Pavetta crassipes* on acute oral test in rats.

	Dose	Writhing	Sedation	Grooming	Diarrhea	Death
Phase 1	Control	0/6	0/6	6/6	0/6	0/6
	10 mg/kg	0/6	0/6	6/6	0/6	0/6
	100 mg/kg	0/6	3/6	3/6	0/6	0/6
	1000 mg/kg	6/6	6/6	0/6	2/6	0/6
Phase 2	Control	0/2	0/2	2/2	0/2	0/2
	1600 mg/kg	2/2	2/2	0/2	0/2	0/2
	2900 mg/kg	2/2	2/2	0/2	0/2	0/2
	5000 mg/kg	2/2	2/2	0/2	2/2	0/2

LD₅₀ > 5000 mg/kg, numerator= number affected, denominator= number tested. Writhing and sedation occurred at 1000 mg/kg and above, diarrhea occurred at 1000 mg/kg and 5000 mg/kg. no mortality was recorded.

Table 3. Body weights of rats after 28 days oral administration of the *Pavetta crassipes* aqueous leaf extract.

Weight (g)	Control	400 mg/kg	800 mg/kg	1600 mg/kg
Initial	147.5 ± 1.32	145.8 ± 0.38	146.5 ± 1.12	145.8 ± 0.24
Final	180.6 ± 0.34	179.2 ± 1.08	179.8 ± 1.98	178.7 ± 31.8
Difference	33.1 ± 0.15	33.4 ± 0.11	33.3 ± 0.18	32.9 ± 0.12

The data represents mean ± SEM for the treatment groups n=10. No significant difference in body weight changes between the treatment groups compared to control.

Table 4. Effect of oral administration of the aqueous leaf extract of *Pavetta crassipes* on relative organ weights rats.

Organ weight (g)	Control	400 mg/kg	800 mg/kg	1600 mg/kg
Liver	2.3 ± 0.10	2.6 ± 0.30	2.5 ± 0.30	2.6 ± 0.30
Lungs	0.7 ± 0.02	0.7 ± 0.02	0.7 ± 0.02	0.6 ± 0.01
Heart	0.3 ± 0.00	0.3 ± 0.02	0.3 ± 0.01	0.3 ± 0.01
kidney	0.5 ± 0.01	0.6 ± 0.01	0.6 ± 0.02	0.6 ± 0.01

The data represents mean ± SEM for the treatment groups n=10. No significant difference in relative organ/body weight ratio of treated groups compared with control.

Table 5. Effect of daily oral treatment with doses of the aqueous leaf extract of *Pavetta crassipes* on hematological parameters.

Parameter	Control	400 mg/kg	800 mg/kg	1600 mg/kg
PCV (%)	42.2 ± 0.57	43.6 ± 0.24	41.3 ± 0.67	43.7 ± 0.30
WBC (x 10 ⁶ /μL)	13.9 ± 0.94	14.7 ± 0.20	15.7 ± 0.81	15.7 ± 0.95
RBC (x 10 ⁶ /μL)	6.9 ± 0.21	7.0 ± 0.22	6.9 ± 0.14	6.7 ± 0.23
HGB (g/dL)	12.6 ± 0.23	13.2 ± 0.43	12.8 ± 0.21	13.0 ± 0.32
PLT (x 10 ³ /μL)	779.7 ± 1.42	782.3 ± 1.16	782.8 ± 1.78	809.7 ± 1.60 ^{**b}
NEU (%)	18.7 ± 0.73	18.6 ± 0.97	14.2 ± 0.70 ^{**a}	11.7 ± 0.67 ^{**a}
LYM (%)	82.0 ± 0.26	82.1 ± 0.30	89.3 ± 0.23 ^{**b}	84.4 ± 0.25 ^{**b}

The data represents mean ± SEM for the treatment groups n=10. Hematological parameters, PLT count and LYM% increased while NEU% reduced. **P<0.01 when compared to control a= less than control, b= higher than control. PCV: packed cell volume; WBC: white blood cell count; RBC: red blood cell count; HGB: hemoglobin; PLT: platelet count; NEU: neutrophil (%); LYM: lymphocyte (%).

Effect of oral administration of extract on biochemical parameters of rats

The results presented in Figs. 1 and 2 represent the effects of AE on biochemical parameters of rats following 28 days daily oral administration. Urea levels (Fig. 1) were significantly ($p<0.01$) reduced at 1600 mg/kg, but creatinine, total protein (TP), albumin and globulin (GL) levels were not different

from control. In Fig. 2, ALT concentration was significantly ($p<0.01$) decreased at 1600 mg/kg while AST was significantly ($p<0.01$) and dose dependently reduced by AE. However, only 400 mg/kg and 1600 mg/kg significantly ($p<0.05$) decreased ALP concentration. Total and direct bilirubin concentrations were not different from control at the doses of AE used in this experiment.

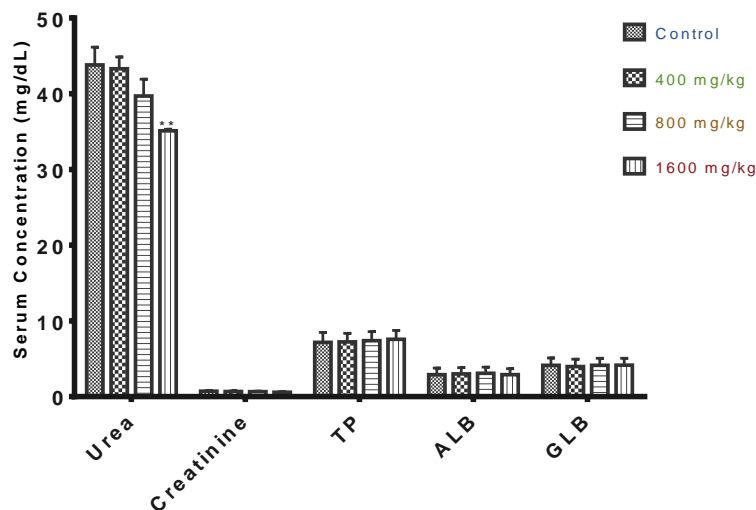


Figure 1. Effect of the aqueous leaf extract of *Pavetta crassipes* on biochemical properties of rats following 28 days daily oral administration.

The data represents mean \pm SEM for the treatment groups $n=10$. ** $p<0.01$ compared to control group (serum urea levels significantly reduced at 1600 mg/kg). TP: Total protein; ALB: Albumin; GLB: Globulin.

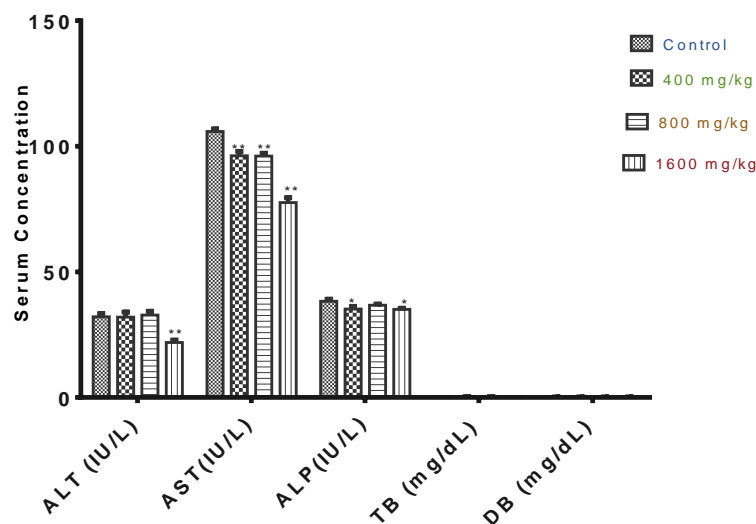


Figure 2. Effect of the aqueous leaf extract of *Pavetta crassipes* on biochemical properties of rats following 28 days daily oral administration.

The data represents mean \pm SEM for the treatment groups $n=10$. ** $p<0.01$ compared to control group (significant reductions in ALT at 1600 mg/kg, AST at all doses and ALP at 400 mg/kg and 1600 mg/kg were observed). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; TB: Total bilirubin; DB: Direct bilirubin.

Effect of oral administration of AE on histology of vital organs of rats

The effect of the extract on the histopathological features of rats is represented in Figs. 3-7, all tissues were stained with hematoxylin and eosin and viewed at x400 magnification. Fig. 3 shows the representative photomicrographs of the liver, there was no significant histological changes in the

treatment groups compared to the control. There was no treatment related histopathological changes in the brain (Fig. 4), lungs (Fig. 6) and the heart (Fig. 7) compared to control. The kidneys (Fig. 5) showed slight increase in mesangial cell proliferation and partial effacement of the glomerulus at 800 mg/kg while at 1600 mg/kg it caused minimal inter-tubular necrosis.

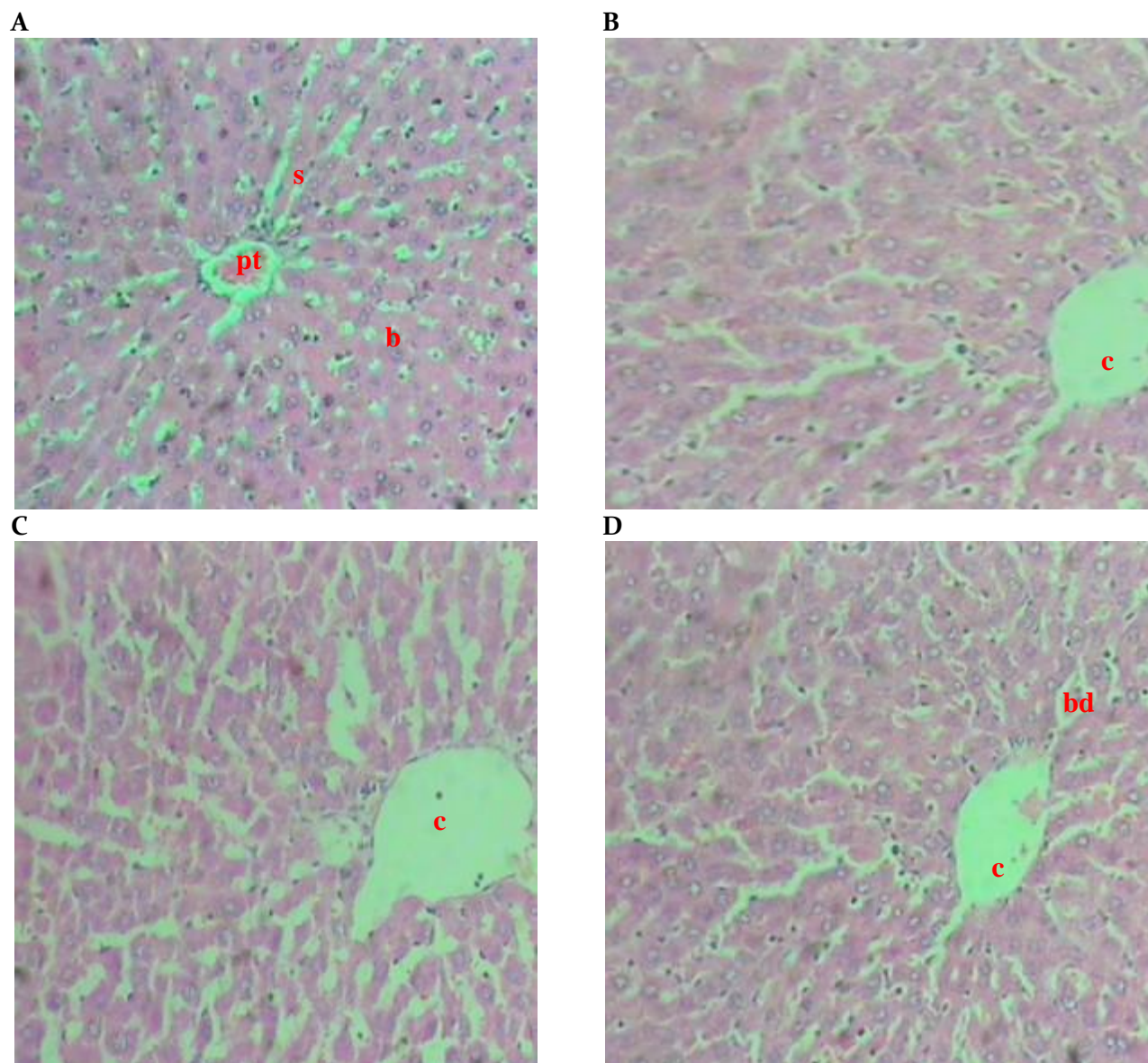


Figure 3. Effect of 28 days oral administration of the aqueous leaf extract of *Pavetta crassipes* on the liver of rats.

A: Control: photomicrograph showing normal portal triad (pt), sinusoid (s) and hepatocyte (b); **B:** 400 mg/kg: showing normal central hepatic vein (c); **C:** 800 mg/kg: a normal central hepatic vein (c) is shown; **D:** 1600 mg/kg: liver photomicrograph showing normal bile duct (bd) and central hepatic vein (c). (hematoxylin and eosin stain, magnification x400).

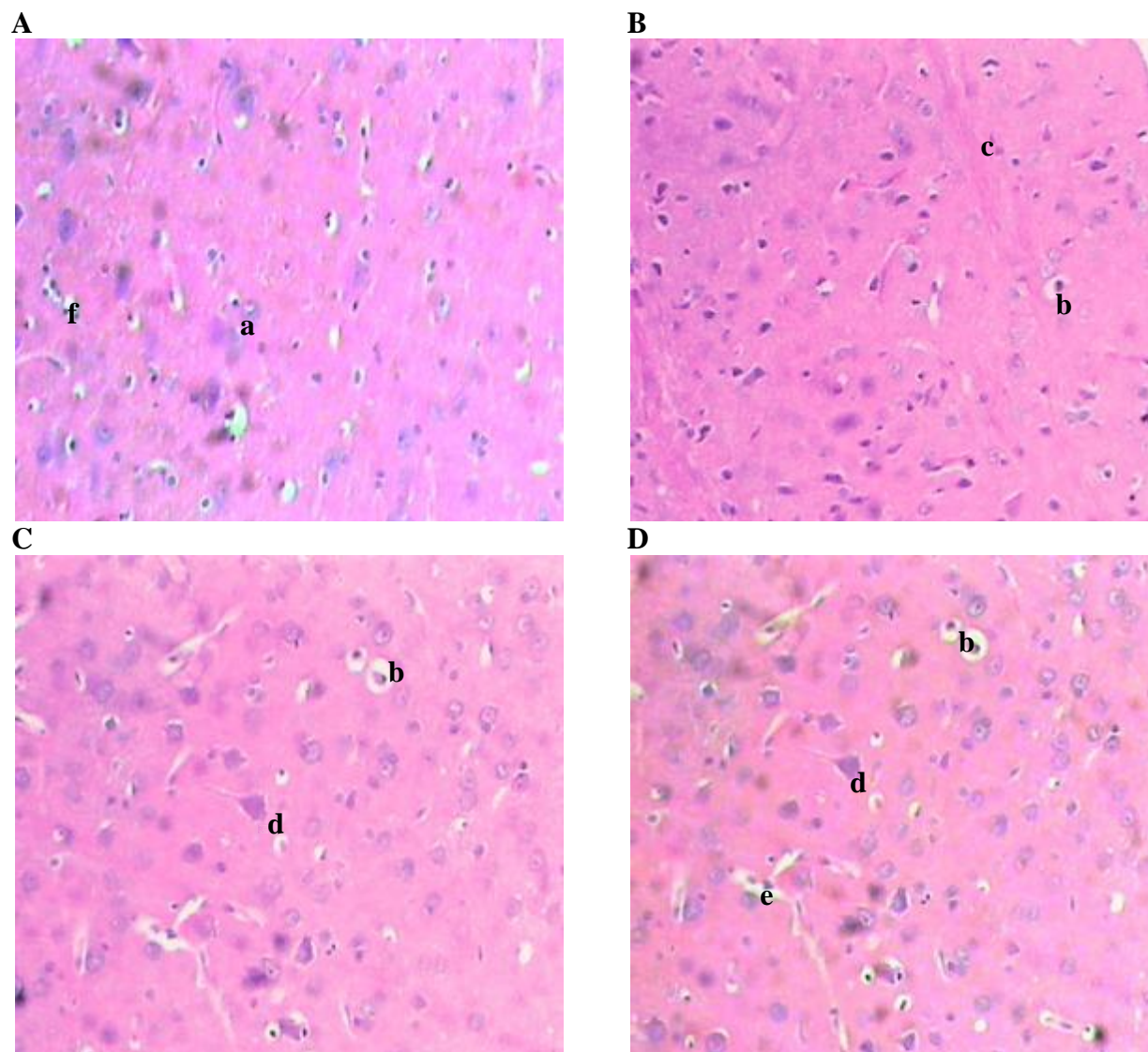


Figure 4. Effect of 28 days oral administration of the aqueous leaf extract of *Pavetta crassipes* on the brain of rats.

A: Control: photomicrograph showing normal glia cells (a) and central canal (f); **B:** 400 mg/kg: brain histology showing normal astrocyte (b) and blood vessel (c); **C:** 800 mg/kg: brain histology showing normal astrocyte (b) and cell body (d); **D:** 1600 mg/kg: normal brain tissue showing astrocyte (b), cell body (d) and pia matter (e) (hematoxylin and eosin stain, magnification x400).

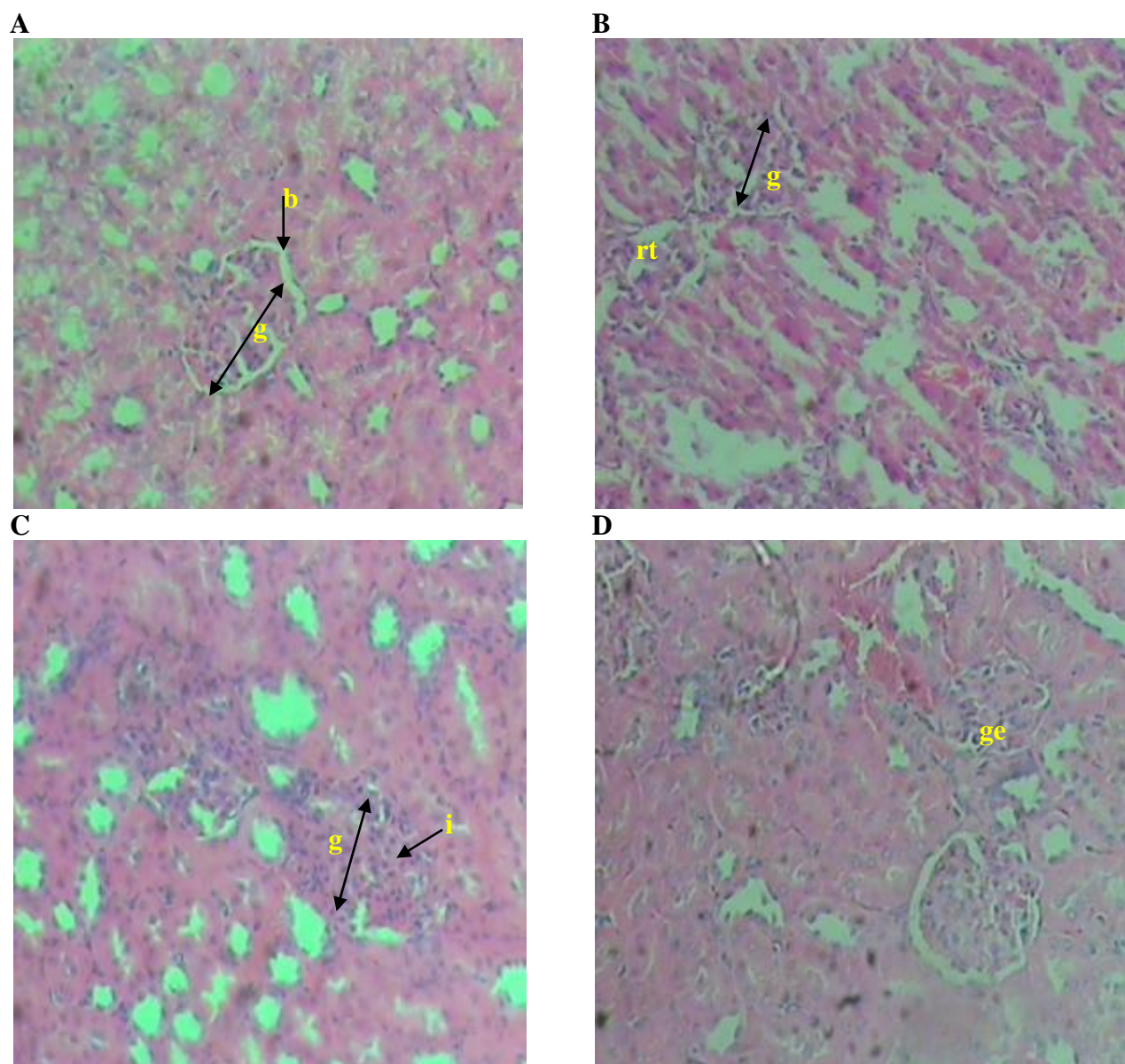


Figure 5. Effect of 28 days oral administration of the aqueous leaf extract of *Pavetta crassipes* on the kidney of rats.

A: Control: section of the kidney showing normal glomerulus (g) and Bowman's capsule (b); **B:** 400 mg/kg: photomicrograph showing normal glomerulus (g) and renal tubule (rt); **C:** 800 mg/kg: photomicrograph showing glomerulus (g) filled with inflammatory cells (i); **D:** 1600 mg/kg: photomicrograph showing glomerular effacement (ge). (hematoxylin and eosin stain, magnification x400).

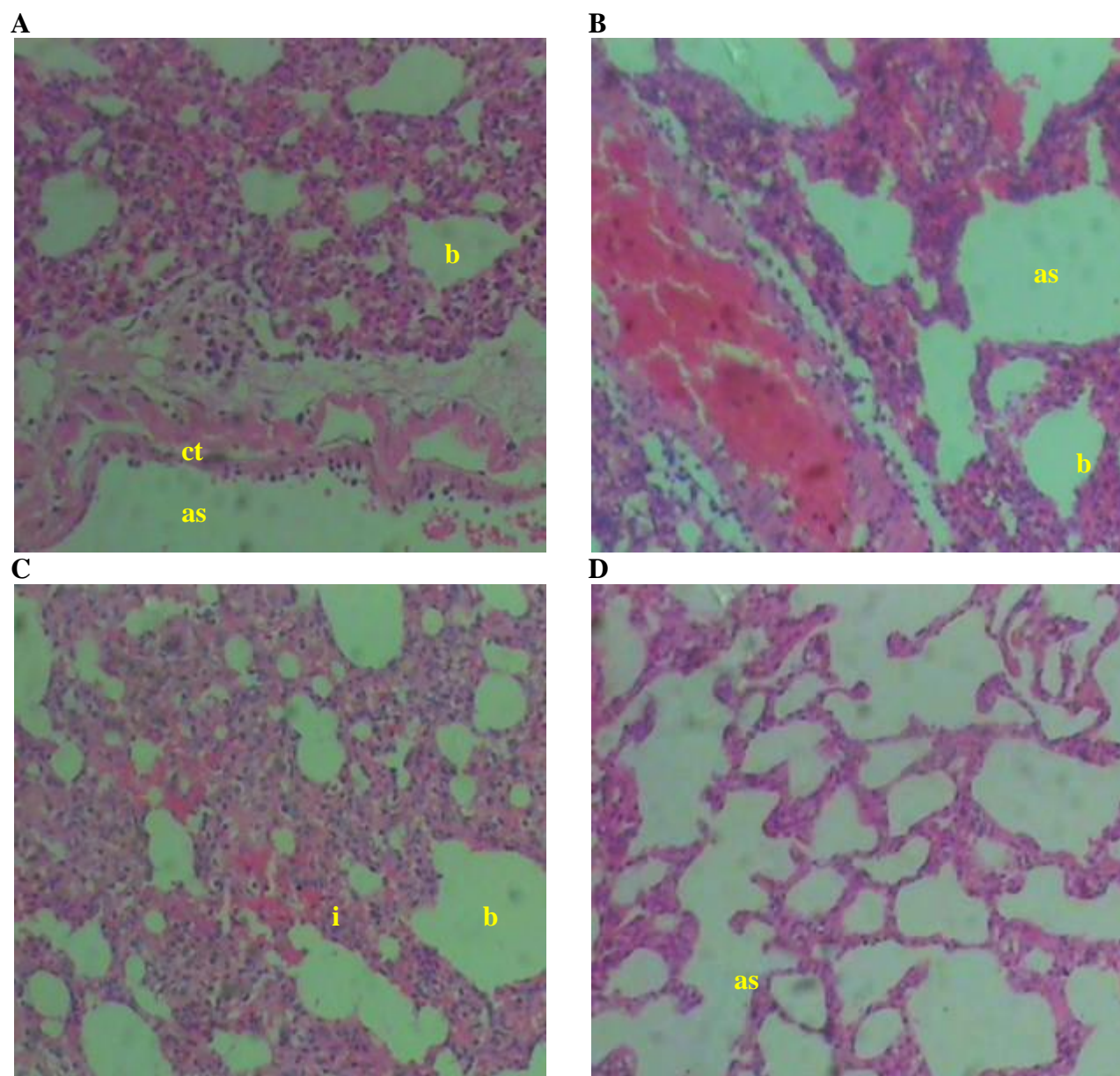


Figure 6. Effect of 28 days oral administration of the aqueous leaf extract of *Pavetta crassipes* on the lungs of rats.

A: Control: normal alveolar space (as), bronchiole (b) and connective tissue (ct); **B:** 400 mg/kg: normal bronchiole (b) and alveolar space (as) with connecting duct; **C:** 800 mg/kg: normal bronchiole (b) and interstitia; **D:** 1600 mg/kg: normal alveolar space (as) with connecting ducts. (hematoxylin and eosin stain, x400 magnification).

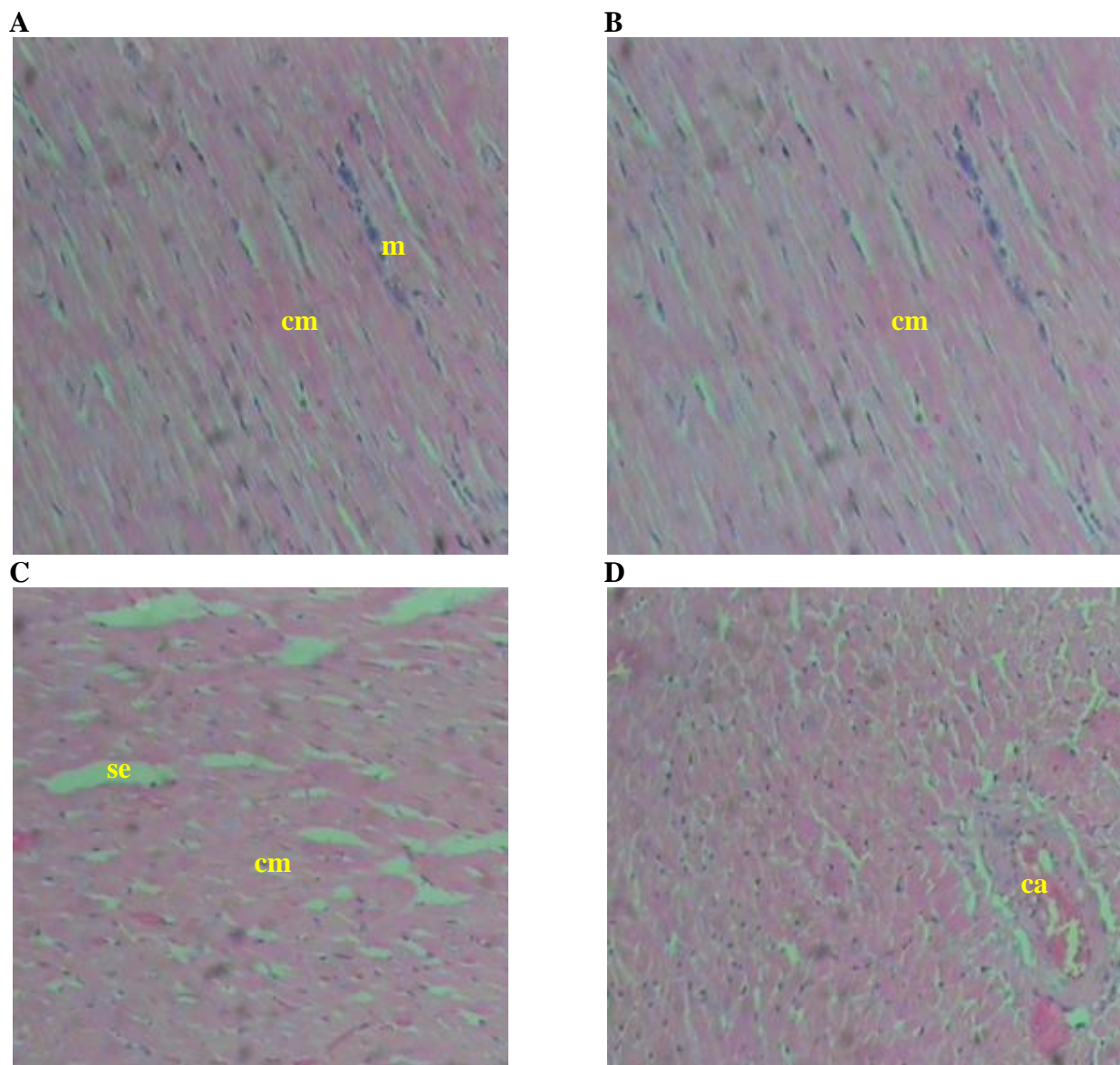


Figure 7. Effect of 28 days oral administration of the aqueous leaf extract of *Pavetta crassipes* on the heart of rats.

A: Control: photomicrograph showing normal myocyte (m) and cardiac muscle (cm); **B:** 400 mg/kg: photomicrograph of heart with normal cardiac muscle (cm); **C:** 800 mg/kg: section of heart showing normal septae (se); **D:** 1600 mg/kg: section of the heart showing normal coronary artery (ca). (hematoxylin and eosin stain, magnification x400).

DISCUSSION

Safety is a key issue that precedes the use of medicines be it orthodox or herbal. In screening natural products for pharmacological activity, assessment of the toxic characteristics of the extract is an initial step. Although various biological effects of *P. crassipes* have been published, there are no data on the toxicological profile of the plant. According to Kennedy et al. (1986), any substance with LD₅₀

greater than 5000 mg/kg can be considered non-toxic. The Organisation for Economic Cooperation and Development (OECD) stipulates that a limit dose of 2000 mg/kg be used and 5000 mg/kg under special regulatory needs. The OECD under its Globally Harmonised Classification System (GHS) for chemical substances and mixtures categorises substances with LD₅₀ >2000 – 5000 mg/kg as unclassified or category 5 (OECD, 2001). The aqueous leaf extract of *P. crassipes* appears safe as the LD₅₀ for in-

traperitoneal route in mice and oral route in rats is greater than 5000 mg/kg.

Substances administered in chronic disease conditions may need repeated dosing toxicological evaluation since daily use may result in accumulation in the body with gradual effects on tissues and organs. Repeated dosing toxicity testing is useful in assessing target organ effects of extracts since these effects are mostly not observable in acute toxicity testing. Furthermore, it is necessary to establish human safety particularly in the development of pharmaceuticals. Statistically significant body weight changes are an indication of the general health status of experimental animals (El-Hilaly et al., 2004). The results for the 28-day repeated exposure test showed no mortality or adverse effects on the body weights of rats. The body weight changes were statistically insignificant; this suggests that the extract, which has been reported to have nutritive values did not adversely affect the nutritional state of the animals.

Organ weight changes in toxicity studies are sensitive indicators of toxicity, effects on enzymes, physiologic disturbances and target organ injury (Michael et al., 2007). An increase in organ weight suggests the occurrence of hypertrophy while a decrease suggests necrosis in the target organ (Teo et al., 2002). The results showed no significant difference in the relative organ-to-body weight ratios of the analysed organs in the test animals compared to the vehicle treated group. This further corroborates its oral safety in rodents.

Liver and kidney functions are essential for survival of animals. Their functionality can be assessed by serum biochemical analysis, which are vital tests in the toxicological evaluation of xenobiotics. Serum liver function tests provide insight into the state of the liver. The aminotransferases (ALT and AST) describe its cellular integrity, alkaline phosphatase (ALP) describes its link with the biliary tract while albumin and protein levels describe its functionality (Boyde and Latner, 1961; Adeoye and Oyedapo, 2004). A significantly high level of liver enzymes (AST, ALT and ALP) are signs of hepatocellular disease or toxicity (Brautbar and Williams, 2002), while a decrease in the serum levels may indicate enzyme inhibition (Akanji et al., 2013). These enzymes are mainly synthesised by the liver cells and structural

or secretory alterations occurring in the liver may lead to an increase in the serum level of these enzymes (Adedapo et al., 2004). However, ALT is the most sensitive marker of liver damage or toxicity since AST is also found in abundance in kidneys, testes, cardiac and skeletal muscles, and ALP is also abundant in growing bone (Friedman et al., 1996). In this study, there was a significant ($p < 0.01$) reduction in AST, ALT and ALP. The reduction in the enzyme activity may serve a protective function as the alterations did not result in histological changes in the liver. This may also be attributed to the presence of alkaloids and flavonoids in the extract as they have been reported to possess liver and kidney protective properties by preventing lipid peroxidation (Fraga et al., 1987; Laughton et al., 1989; Sanz et al., 1994).

Apart from liver enzymes, the functionality of the liver can also be assessed by the serum protein, globulin and albumin levels since they are synthesized and metabolised by the liver (Ganong, 2001). A reduction in serum levels of proteins, globulin and albumin is a sign of reduced synthetic function, which occurs in liver disease or damage but an increase occurs in cancerous conditions, or following high protein diet (Tietz et al., 1994). In this study, there was no significant difference in the serum protein, globulin or albumin levels in the AE treated groups compared to the control group. This further corroborates the possible hepato-protective nature of the extract.

Kidney function can be assessed by measurement of biochemical parameters such as urea, creatinine and bilirubin levels, which are indicators of glomerular filtration rate (Davis and Bredt, 1994). Although there was a reduction in bilirubin and creatinine levels in this study, these reductions were not statistically significant. However, there was a significant reduction in urea levels. Low urea levels occur in acute liver failure or overhydration (Lum and Leal-Khoury, 1989), but with normal liver histology the decrease in serum urea could have been due to its accumulation in the kidneys or increased renal excretion.

Histomorphological changes in target organs are first hand indication of toxicity of a chemical or biological substance (Kwan et al., 2013). Histomorphological examination of the liver, heart, lungs and brain showed no hypertrophy or structural changes in the

cells of the organs in both the treated groups and the control group. However, the kidneys showed slight structural changes at 800 mg/kg and 1600 mg/kg, these changes may be due to accumulation of urea in the kidneys.

The hemopoietic system is an important target for xenobiotics and is a sensitive index of toxic or pathological conditions. Haematological analysis reveals the physiological and pathological status of humans and animals in toxicological studies (Adeneye et al., 2006; Oduola et al., 2007). Haematological indices can be altered by phytochemical constituents of plants when ingested (Ajagbona et al., 1999; Adedapo et al., 2004). In this study, administration of AE did not significantly affect RBC, HBG and PCV even though slight increases were noticed. Therefore, the extract seems to have little or no effect on circulating red blood cells. Although WBC count was not significantly affected, there was a significant increase LYM while NEU was significantly reduced. Platelet count was also significantly elevated. White blood cells mediate the first line of defence against foreign substances, tissue injury and inflammation (Kwan et al., 2013). Platelets play an important role in blood haemostasis. An abnormally high platelet count may point to haemostatic disorders (Osei-Bimpong et al., 2012) and result in cardiovascular risks (Adeneye et al., 2010) while a decrease may infer liver disease (Adams, 2010). Lymphocytes are dynamic cells and mediate immune response to foreign substances (Pearce et al., 2013). Lymphocytes also produce antibodies enabling the destruction of intracellular microbes and cancer cells (Ganong, 2001). Neutrophils play a defensive role in microbial attacks and are also mobilised in acute inflammatory conditions (Guyton and Hall, 2006). The lymphocytosis seen in this study indicates a possible immuno-stimulatory effect. This may be due to the immune response of the rats to the presence of the extract. A reduction in neutrophil suggests inhibition of neutrophil migration and anti-inflammatory effect of the extract, which could be due to flavonoids contained in the plant extract.

CONCLUSIONS

The aqueous leaf extract of *Pavetta crassipes* appears safe and its LD₅₀ is greater than 5000 mg/kg in rodents. The extract possesses protective effects

on the liver, slightly toxic to the kidney but appears to have no adverse effects on the heart, lung and brain cells. It also exhibited immune boosting activity and has no deleterious effects on circulating red blood cells.

In conclusion, the aqueous leaf extract of *P. crassipes* appears safe but chronic use especially in high doses should be avoided as it may result in some renal toxicity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Concepts or ideas	X		X
Design		X	
Definition of intellectual content	X	X	X
Literature search	X		
Experimental studies	X	X	
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Data analysis	X	X	X
Statistical analysis	X		
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Manuscript editing			X
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