



Effects of Ethanol Extracts of Healthy and Infected *Panicum maximum* (Jacq.) Floret on liver and kidney function profile and histopathology in Sprague-dawley rats

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Abstract

There is concern that consumption of infected *Panicum maximum* florets may result in poisoning in livestock. This study investigated the effects of ethanol extracts of healthy and infected *P. maximum* florets (Poaceae) on selected indices of liver and kidney functions, haematological and histopathological parameters in female Sprague-Dawley rats. The rats were fed with different doses of lyophilized extracts for 21 days and effect of the plant on tissues of liver and kidney were macroscopically examined. Also the effects on the biochemical and haematological parameters were evaluated. The healthy floret extract significantly reduced ($P < 0.05$) aspartate aminotransferases (AST), alanine aminotransferases (ALT), and alkaline phosphatase (ALP), creatinine, urea, albumin and total protein at moderate to high doses. There were no significant changes in red blood cell (RBC), haemoglobin levels (HB) and packed cell volume (PCV) when compared with control. The infected floret extract significantly reduced ALT, AST and ALP at low to moderate (100 – 500mg /kg body weight) but induced significant increase in ALT level at the highest dose of 750mg/kg body weight when compared with control. Total protein and creatinine levels were not significantly ($P < 0.05$) affected while urea level was reduced at all doses. Red blood cell, HB and PCV increased as doses increased. Histopathological examination revealed marked pathological lesions on liver and kidney at high dose administration of the infected extracts. However healthy floret extracts did not induce any pathological lesions on liver and kidney. Phytochemical screening revealed presence of alkaloids, tannins, saponins and flavonoids.

Keywords: *Panicum maximum*, histopathology, liver, phytochemical, Sprague-dawley.

Introduction

Panicum maximum (Jacq.) (poaceae) is indigenous to Africa and widely distributed throughout the tropics and subtropics. This plant belongs to family poaceae, subfamily panicioideae and tribe paniceae which is highly diverse in morphological and physiological characters^{1,2,3}.

Ethanol leaf extract of *P. maximum* showed antidiabetic and antibacterial activities against clinically important microbial pathogens has also been reported suggesting that this plant can be used in treating diseases caused by these pathogens⁴. *Panicum maximum* has also been reported to be used as folk remedy for tympanitis in cattle⁵. *Panicum maximum* is the best forage and pasture grass in the tropics. The grass is drought-resistant and has been reported to be highly nutritive, palatable and acceptable to livestock^{6,7,8,9}. Fungal attack of parts of *P. maximum* (stem, leaves and florets) has been reported by several workers. *Cercospora fusimaculans* and *Fusarium* spp attack the stem causing stem blight, *Phyllachora* spp causes black spots (tar spots) while *Curvularia* spp and *Colletotrichum*

graminicola cause leaf spot disease. Smut disease of the floret is caused by *Tilletia ayresii*. During infection by this fungus, it colonizes the ovary and replaces the contents with fungal mycelia and spores^{10,11,12}. *P. maximum* is often grazed upon by camels, horses and sheep and since these animals do not discriminate between infected and health plants during grazing, naturally infected grass forms part of their diet. Studies have shown that ingestion of infected grass often result in changes in physiology of the animals probably due to presence of toxic substances in the infected grasses^{13,14}. The present study, was designed to investigate the effect of ethanolic floret extracts of healthy and infected *P. maximum* on indices of liver and kidney functions in experimental animals.

Material and methods

Collection of plant material: Fresh, healthy and infected *P. maximum* florets were collected within the premises of main campus of University of Lagos, Akoka, Nigeria during the months of June-November (2006-2010) and were authenticated by Prof. Olowokudejo of the Department of Botany, University

of Lagos. The voucher specimen (LUTH 3687) was deposited in University of Lagos herbarium. Diseased florets were confirmed by a Mycologist in University of Lagos, Nigeria.

Preparation of extract: Two kilogrammes samples of dried powdered healthy and infected floret was extracted separately with ethanol (80%)¹⁵. Each was soaked in the solvent for 7 days with constant stirring. The extracts were filtered with Whatman no 1 filter paper and the resulting filtrate were concentrated in the rotatory evaporator under reduced pressure and controlled temperature (40°C). The dried solid product was weighed to give yields of 4.5% and 0.9% for healthy extracts and infected extracts respectively.

Phytochemical Screening: The plant extracts were subjected to phytochemical screening for alkaloids, saponins, flavonoids and tannins^{16,17}.

Animal Management and Extract Administration: Forty – five female Sprague-Dawley rats weighing 160-200g obtained from animal house of College of Medicine, University of Lagos were housed in clean metabolic cages of dimensions 90cm x 40cm x 35cm contained in a well ventilated laboratory at temperature 28 ± 37°C; photoperiod; 12h natural light and 12hr dark. The rats were completely randomized into nine groups of five each and acclimatized for two weeks. The animals were fed with standard rat chow (Nimeth livestock feeds, Ikeja) and water *ad libitum* and then fasted before drug administration.

The control group (group1) received 0.5ml of vehicle (0.5% Tween 80 solution) orally once a day for 21 days. The 1st four treated groups (groups 2-5) received 100, 250, 500 and 750 mg/kg body weight doses of healthy floret extract while groups 6-9 received similar doses of infected floret extract respectively for 21 days^{18,19}.

Drugs were withdrawn on the 21st day and animals were fasted overnight (18hr). All animals were sacrificed by cervical dislocation. Blood was collected via cardiac puncture into heparinized capillary tubes for analysis of haematological parameters and EDTA and plain bottles for collection of plasma and serum respectively for biochemical estimations^{20,21}.

Histological preparation: Histological tissue studies of liver and kidney from each animal group were fixed in 10% formaldehyde and processed for haematoxylin eosin staining. Photomicrographs of the prepared slides haematoxylin – eosin stained tissue sections were taken with a camera attached to the compound light microscope in the Department of Medical anatomy, College of medicine, University of Lagos.

Statistical analysis: The SPSS (version 11.0) software was employed for data entry and validation. Statistical analysis of data was carried out using the Students t-test. A $p < 0.05$ was considered statistically significant.

Results and discussion

The percentage yield of extract was 4.5% and 0.9% for healthy extracts and infected extracts respectively. Phytochemical screening of the plant extracts revealed the presence of alkaloids, saponins, tannins and flavonoids in both the healthy and infected florets. However, there was a significant increase in the concentrations of the secondary metabolites, particularly the alkaloid and tannin content in the infected floret compared to the healthy²² (table 1 and 2). In the groups that were treated with healthy floret extracts there was significant reduction in AST at all doses when compared with control (Table 2). Activity of ALT increased significantly ($p < 0.05$) in animals only at the highest dose of the extract. The extracts induced significant reduction in creatinine, urea and albumin levels when compared with control. Alkaline phosphatase level reduced at all doses of the extract. On the other hand, animals treated with infected floret extract produced marked reduction in AST and ALT at low to moderate doses but increased significantly only at the highest dose. The level of ALP decreased significantly ($p < 0.05$) at all the doses. Creatinine and total protein were not affected while marked reduction of urea occurred at all doses administered. The healthy floret extract did not reduce levels of RBC, HB and PCV while infected floret extract increased the level of red blood cell, haemoglobin and packed cell volume when compared with control. Histopathological studies of the tissue sections of the liver and kidney of rats administered with healthy extract showed no gross tissue damage at all doses (100-750mg/kg body weight) when compared with the control rats while the infected extracts altered the anatomical structure of liver and kidney only at highest dose (750mg/kg body weight) of the extract (Figures 1-4).

At low to moderate doses (100-500mg/kg body weight) of the infected extract the liver sections showed normal plates of hepatocytes without congestion of the sinusoids nor necrosis of the cells while at highest dose (750mg/kg body weight) of the extract induced severe sinusoidal congestion. The kidney also showed normocellular glomerular tufts displayed on background containing tubules at low to moderate doses of the extract while at the highest dose there was congestion with distension of vascular channels with blood. High density lipoprotein cholesterol (HDL – Chol) and low density lipoprotein cholesterol (LDL – Chol) were not significantly affected. However the total cholesterol level reduced significantly.

The liver and kidney are known to play significant roles in various metabolic processes. The liver play important role in xenobiotic function and the kidneys are the main organs involved in drug elimination, therefore it is particularly exposed to the toxic effect of exogenous compounds. The transaminases (AST and ALT) are two enzymes that are associated with hepatocellular damage and so are used as biomarkers for predicting possible toxicity²³. Although both are common liver enzymes and their concentration in hepatocytes is high, only

ALT is remarkably specific for liver function since AST is mostly present in myocardium, skeletal muscles, brain and kidneys²⁴.

Damage to parenchyma liver cells or hepatic cell due to the presence of drugs or toxic substances often result in elevation in both of these transaminases in the serum. Therefore enzyme measurement provides a valuable tool for clinical diagnosis of liver damage as well as toxicity studies²⁵. Mild elevation of alkaline phosphatase has been reported to be associated with liver injury or myocardial infarction²⁶.

From this study, reduction of AST and ALP at all doses of infected floret extract and ALT only at low dose suggests that there was no liver damage. However, an increase of ALT at the highest dose of these extract indicates nephrotoxicity²⁷. Significant reduction in creatinine, urea and albumin level indicates that the kidney function was not impaired.

Loss of liver lobular radiation, obliteration of sinusoids and agglutination of cells with clumps of blood which occurred after feeding the guinea pig in other works with large quantity of infected grass could be as a result of haemorrhage in the liver. Non- reduction of red blood cell, haemoglobin and packed cell volume by healthy and infected floret extracts indicate that the extracts did not regenerate anaemia²⁸. Increase in the percentage of RBC, HB and PCV suggests that RBC was not lysed which is often indicated by reduction of RBC. The presence of tannins in the extract which binds to protein and carbohydrate which are components of erythrocyte membrane and prevented breakdown of erythrocyte membrane may have contributed to non-lysing of red blood cell. Haemoglobin metabolism was however not adversely affected.

Since the levels of AST and ALT showed no appreciable increase except at the highest dose, it implies that the extract can cause damage only at very high consumption of the infected grasses. The non –significant adverse effect of the extracts on creatinine, urea and albumin showed that the renal function was not affected. However, reduction of protein at the highest dose of the extract is sign of renal impairment. This result is confirmed by the histology study of liver and kidney which showed pathological changes only in animals that were treated with high doses of extracts. The pathological effects of the extracts suggest that the extracts contain high concentration of toxic substances at high dose while a significant decrease in plasma total cholesterol level might be due to the presence of hypolipidemic agents in the extract²⁹.

Conclusion

The ethanolic extract of healthy and infected *Panicum maximum* florets exhibits selective toxicity in Sprague-dawley rats. It is suggested that intake of the extracts should be in low to moderate doses.

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Table – 1

Phytochemical profile of ethanolic extracts of healthy and infected *Panicum maximum* florets

Phytochemical component	Healthy <i>P.maximum</i> Extract	Infected <i>P.maximum</i> extract
Alkaloids	+	++
Tannins	+	++
Saponins	++	+
Flavonoids	++	+

++=highly present, +=present

Table – 2

Phytochemical composition of aqueous, ethanol and chloroform extracts of healthy and infected *Panicum maximum* florets

Plant Extracts	Alkaloids%	Tannins%	Saponins%	Flavonoids%
Aqueous extract (healthy)	0.50	0.90	0.33	0.75
(infected)	0.98	1.07	0.74	1.01
Ethanol extract (healthy)	0.31	0.90	1.83	0.78
(Infected)	1.15	1.03	1.14	0.69
Chloroform extract (healthy)	0.41	0.19	1.93	nil
(infected)	1.07	0.92	0.29	nil

Table – 3

Effect of Ethanol extract of healthy *P.maximum* florets on parameters of Liver and kidney functions of Sprague-dawley rats

Parameters	Control (ml)	Infected Ethanol extract (mg/kg)			
	0.5(0.5% Tween80)	100	250	500	750
AST(i.u/L)	148.6±1.10	117.80±0.00*	139.00±1.04*	116.00±0.11*	89.60±1.53*
ALP(i.u/L)	290.01±0.59	262.70±0.67	174.04±0.46*	134.10±0.55*	121.10±1.00*
ALT (i.u/L)	150.00±0.58	100.40±1.00*	99.00±1.42*	73.00±2.03*	78.73±1.05*
Creatinine (mg/L)	37.60±0.28	32.80±0.40	31.39±0.50	31.20±0.46	30.33±0.52
Urea(mg/dL)	9.07±0.00	7.53±0.47*	8.07±0.18*	7.77±1.00*	6.78±1.22*
Albulmin(g/dL)	40.26±0.31	34.77±1.36*	32.52±0.84*	31.50±0.40*	31.56±0.26*
T.Protein (g/dL)	78.57±0.38	79.43±0.18	78.00±0.84	77.3±0.61	78.47±0.61
HDL-chol. (mg/dL)	0.90±0.58	0.90±0.58	0.97±0.09	1.00±0.06	1.00±1.00
LDL- chol.(mg/dL)	0.25±0.03	0.23±0.01	0.20±0.03	0.23±0.01	0.22±0.04
CHOL (mg/dL)	1.52±0.02	1.15±0.00*	1.28±0.01*	1.31±0.01*	1.30±0.01*

Values are expressed as Mean±SEM.*P<0.05 significantly different compared to control. Means without *are not significantly different compared to control, AST: Aspartate aminotransferase, ALP: Alkaline phosphate, ALT: alanine amino transferase, HDL-chol: high -density lipoprotein cholesterol, LDL-chol: low-density lipoprotein cholesterol; T.protein-Total protein

Table – 4

Effect of Ethanol extract of infected *P.maximum* florets on parameters of Liver and kidney functions of Sprague-dawley rats

Parameters	Control (ml)	Infected Ethanol extract (mg/kg)			
	0.5(0.5% Tween80)	100	250	500	750
AST(i.u/L)	224.50±0.22	156.40±0.90*	138.20±9.01*	176.00±3.35*	224.40±18.62
ALP(i.u/L)	314.60±0.19	79.72±0.31*	84.02±0.74*	99.30±1.24*	145.4±2.51*
ALT (i.u/L)	101.90±0.58	76.40±1.19*	58.00±4.42*	43.47±2.03*	223.73±3.4
Creatinine (mg/L)	45.15±0.10	44.00±1.06	44.01±0.96	24.51±6.79*	46.16±0.86*
Urea(mg/dL)	10.97±0.15	7.53±0.47*	8.07±0.18*	9.43±0.33*	10.00±0.12
Albulmin(g/dL)	33.83±0.20	40.63±0.09*	38.60±0.07*	38.80±1.29*	31.53±0.77*
T.Protein (g/dL)	83.03±0.58	83.43±0.18	79.00±2.84	73.30±2.84	66.55±3.84*
HDL-chol. (mg/dL)	0.90±0.58	0.90±0.58	0.97±0.09	1.00±0.06	0.43±0.04*
LDL- chol.(mg/dL)	0.25±0.03	0.23±0.01	0.20±0.03	0.23±0.01	0.22±0.04
CHOL (mg/dL)	1.52±0.02	1.15±0.00*	1.28±0.01*	1.31±0.01*	1.30±0.01*

Values are expressed as Mean±SEM.*P<0.05 significantly different compared to control. Means without *are not significantly different compared to control, AST: Aspartate aminotransferase, ALP: Alkaline phosphate, ALT: alanine amino transferase, HDL-chol: high -density lipoprotein cholesterol, LDL-chol: low-density lipoprotein cholesterol; T.protein-Total protein

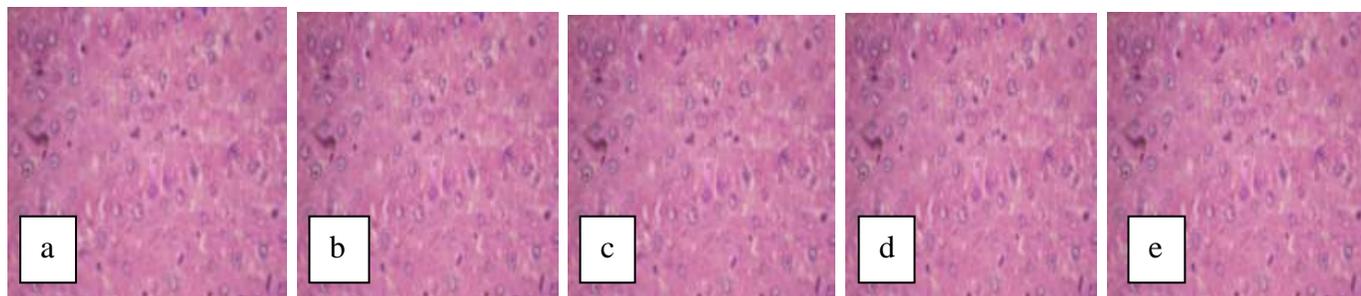


Figure - 1
Histological section of liver of rats administered with varied doses of healthy extract (a)100mgkg⁻¹ b)250mgkg⁻¹ c)500mgkg⁻¹ d)750mgkg⁻¹ e)control(no extract) (H and E stain)mag.x400

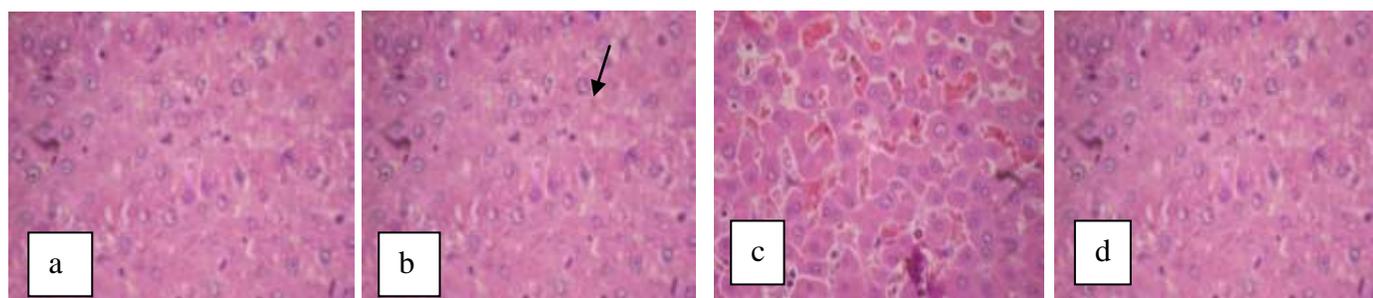


Figure - 2
Histological section of liver of rats administered with varied doses of infected extract (a)250mgkg⁻¹ b)500mgkg⁻¹ c)750mgkg⁻¹ d)control(no extract) (H and E stain)mag.x400

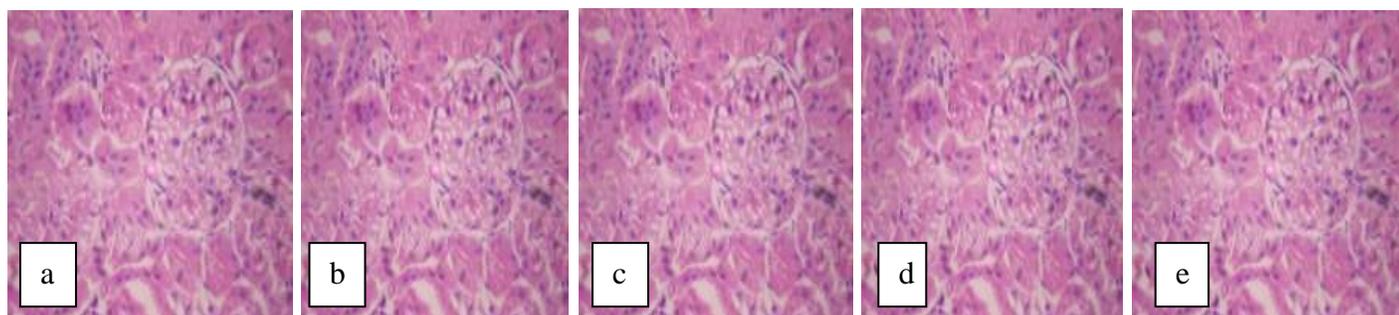


Figure - 3
Histological section of kidney of rats administered with varied doses of healthy extract (a)100mgkg⁻¹, b)250mgkg⁻¹ c)500mgkg⁻¹ d)750mgkg⁻¹ e)control(no extract) (H and E stain)mag.x400

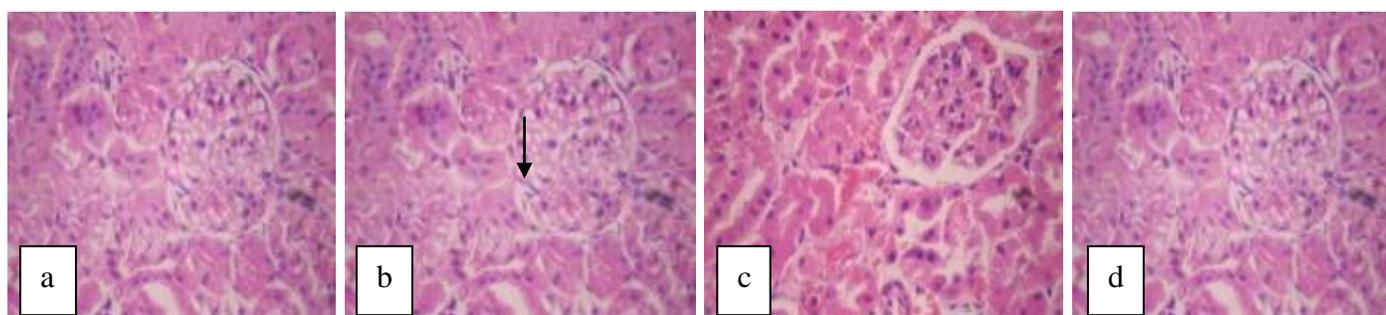


Figure - 4
Histological section of kidney of rats administered with varied doses of infected extract (a)250mgkg⁻¹ b)500mgkg⁻¹ c)750mgkg⁻¹ d)control(no extract) (H and E stain)mag.x400