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Effects of the Methanol Root Extract of *Cissampelos mucronata* A. Rich on the Kidney and Liver of Rats- a Histological and Biochemical Study

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SHG and TWJ: Project idea, data acquisition, analysis and interpretation; authors SHG and PAO project design; author HAN histopathological interpretations and supervision. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: The aim of this study was to determine the potential toxicologic effects of the methanol root extract of *Cissampelos mucronata* (A. Rich) on liver and kidney tissues.
Methodology: A total of 30 rats were used for the study consisting of 5 female rats for the acute toxicity study and 25 rats for the 28 day repeated toxicity studies, the 25 rats were divided into five groups of 5 rats per group. Group I served as the control, while rats in groups II-IV where administered 100, 200 and 300 $mgkg^{-1}$ body weight of the extract respectively for 28 days. Rats in group V were administered 300 $mgkg^{-1}$ of the extract for 28 days and allowed to stay for 14 days post treatment. At the end of the experimental period the rats were sacrificed, kidney and liver weight taken and fixed for routine histological examinations while blood was obtained for biochemical analysis.
Results: Acute toxicity studies revealed an LD_{50} of >2000$mgkg^{-1}$ following oral

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administration of the extract while 28 days administration of 100, 200 and 300mgkg^{-1} of the extract caused a significant decrease in body weight ($p<0.01$) and significant increases in the serum levels of alanine aminotransferase, alkaline phosphatase, total protein and albumin($P<0.05$-0.001). Histopathological assessment of the liver and kidney tissues revealed moderate lymphocytic infiltration and thrombus formation within the central veins in the liver, moderate epithelial sloughing of the proximal convulated tubules in renal tissues with no remarkable recovery following withdrawal of the extract.
Conclusion: This study suggests that prolonged and constant use of the extract is both hepatotoxic and nephrotoxic.

Keywords: Acute tubular necrosis; herbal toxicity; hepatotoxicity; nephrotoxicity; thrombus.

1. INTRODUCTION

The plant *Cissampelos mucronata* (A. Rich) belongs to the family *Menispermaceae* and is used world wide in traditional medicine to treat varieties of ailments and conditions. Its use as an emmenagogue and diuretic have been reported [1,2]. The root bark are eaten to treat abdominal pains, swollen stomach and gastro-intestinal upset [3-5]. The plant has also been reported in the treatment of leukorrhea [6] , diarrhoea [3,4,7,8], fever and syphilis [9-11], Schistosomiasis [12], venereal diseases [3,11,13] and as a sedative [14]. Powder obtained from the dried root is sprinkled on wounds for healing purposes and in the treatment of coughs [15] and in arrow poisons [9,10]. In Nigeria it has been demonstrated to have antispasmodic activity [16], anti-ulcer activity [17] and hypoglycemic activity in Streptozocin induced diabetic rats [18] .

Its indigenous names in Nigeria include *Jibdar Kasa* or *Damarji* (Hausa) and *abakenwo* in Igbo [2], *Jokoje* (Yoruba), *Barwada* (Kanuri), *Magirahi* (Fulfulde), *Zagaduwa* (Marghi) and *Kwahara* or *Kwahirka* (Babur/Bura) while its English name is Ivy vine. Since the kidney and liver are involved in the excretion and detoxification of many toxic metabolic waste products. It is imperative to evaluate the possible effects of *Cissampelos mucronata* on the histology and biochemical parameters of the kidney and liver using adult Wistar rats as the model.

2. MATERIALS AND METHOD

2.1 Collection and Identification of Plant Materials

This study was conducted from August 2009 to July 2010.The plant was collected in September 2009 around Giwa Military Barracks in Maiduguri metropolis latitude 11°50' 42" North and longitude 13°9' 36" East and identified/authenticated by a botanist with a specimen voucher (CM.01) prepared and deposited at the herbarium of the Department of Veterinary Physiology and Pharmacology, University of Maiduguri, Borno state. The collection, identification and storage of the plant material was carried out according to World Health Organization's standards [19,20]. The root was then sun-dried, pulverised into powdered form using a pestle and mortar and then stored in cellophane bags at room temperature.

2.2 Extraction Procedures

A total of one hundred grams (100g) of the pulverised root was subjected to exhaustive soxhlet extraction in methanol (500ml) for 72h at 60°C. The extract obtained was then concentrated in a water bath until a constant dark sticky residue was obtained (11.34gw/w). The extract was further oven dried and maintained in a desiccator until a constant weight was obtained and then stored in a stoppered container in refrigerator at -4°C. Stock solution was prepared by dissolving 2g of the extract in 50ml distilled water in the presence of 1 drop (0.05ml) of dimethylsulfoxide (DMSO).

2.3 Animals and Husbandry

This study was carried out in the Departments of Human Anatomy, University of Maiduguri, Nigeria. Wister strain rats weighing 220-265g and 3-4 months old were used for both the acute and repeated dose toxicity studies. They were purchased from the animal house of the Department of Pharmacology and Pharmaceutical Sciences, University of Jos, Plateau State, Nigeria. Following an acclimatization period of 2 weeks, the rats were individually identified by colour tattoo and weighed. The rats were kept in plastic cages at room temperature with a 12h light/dark cycle. They had access to standard laboratory diet (Pelletised growers Feed by Grand Cereals and oil Mills Limited, Jos) and drinking water *ad libitum*. The rats were cared for according to the Guiding Principles for the Care and Use of Animals based on the Helsinki Declaration as amended by World Medical Assembly, Venice, Italy [21]. Prior ethical approval was obtained from the ethical committee on the use of animals of the College of Medical Sciences University of Maiduguri, Nigeria.

2.4 Experimental Design

2.4.1 Acute toxicity study

A total of five (5) healthy adult nulliparous female rats weighing 220–264g were used for the study [22-24]. The rats were fasted overnight from food but not water prior to dosing and then weighed before the extract was administered. The limit dose of 2000 $mgkg^{-1}$ of the methanolic extract of the root of *Cissampelos mucronata* was given to the first rat orally and observed for mortality and clinical signs, once before, during and every 15 minutes for the first hour, then hourly for three hours and then periodically for 72 hours and then daily for 14 days.

2.4.2 Repeated dose 28-day oral toxicity study

A total of 25 rats weighing 220-253g were used for this study according to standard protocols [25]. The rats were randomly divided into five groups of 5 rats per dosage group (I-V). Group I served as the control group and were administered normal saline equivalent to the volume administered to the highest dosed experimental rats. Rats in Groups II, III and IV were administered with $100mgkg^{-1}$, $200mgkg^{-1}$ and $300mgkg^{-1}$ doses of the extract respectively while rats in Group V served as the satellite group and were administered the highest dose ($300mgkg^{-1}$) of the extract for 28 days and allowed to stay for at least 14 days post treatment to observe for reversibility, persistence or delayed occurrence of toxic effects. At the end of the experimental period, body weights of all rats were taken and recorded. The rats were then sacrificed and the blood obtained was subjected to biochemical investigation. The liver and kidneys obtained were trimmed of any adherent tissue, the wet weight taken and preserved in Bouins fluid for subsequent histopathological examination.

2.4.3 Biochemical analysis

Blood collected from the animals by transection of the jugular vein were put into sterile bottles and centrifuged. The clear serum obtained was analyzed for biochemical parameters using Randox Laboratory kits at the Department of Chemical Pathology, University of Maiduguri Teaching Hospital, Maiduguri.

2.4.4 Histological analysis

The liver and kidney tissue obtained were carefully dissected out, weighed, fixed in Bouins fluid, embedded in paraffin and sectioned at 5µm. Sections were stained with Haematoxylin and Eosin and mounted in Canada balsam. Light microscopic examination of the sections was then carried out.

2.4.5 Statistical analysis

Data obtained from this study were expressed as the mean value±standard error of mean. The data were analysed using one way analysis of variance (ANOVA) and differences between means of control and treated groups were determined using Statistical Package for Social Scientist (SPSS 11.0). p values less than 0.05 or 0.01 were considered statistically significant.

3. RESULT

3.1 Acute Oral Toxicity Study

Administration of the methanolic extract of the root of *Cissampelos mucronata* did not cause any death and the only sign of physical toxicity noticed at the limit dose of 2000mgkg^{-1} oral in the first 48 hours and during the 14 days of observation was loss of apetite. The LD_{50} in rats was therefore taken as above 2000mgkg^{-1} oral.

3.2 Effects of the Extract on Mean Body and Organ Weights

The rats in the control group had a steady and significant ($p<0.001$) body weight gain while administration of the extract caused body weight loss that was significant ($p<0.01$) in rats administered with 300mgkg^{-1}; this effect was completely reversed after withdrawal of the extract.There was a significant ($p<0.05$) decrease in weights of the liver and concomitant recovery following the withdrawal of the 300mgkg^{-1} of the extract while a slight increase was observed in the weights of the right and left kidneys though the increase was not significant ($P>0.05$) with no recovery even after the withdrawal of the extract for 14 days Table 1.

3.3 Effect of the Extract on Biormakers of the Kidney and Liver

Administration of the extract had no effect on Na^+ and HCO_3^- levels. There were slight increases in K^+ , Cl^- and creatinine levels and decrease in the serum levels of Ca^{2+} and urea. These changes were neither significant nor dose dependent Table 2. Serum levels of total protein, albumin, cholesterol, aspartate aminotransferase(AST),alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were non significantly ($P>0.05$) increased. The extract elicited a significant ($p<0.05$-0.001) increase in the serum levels of alanine aminotransferase and alkaline phosphatase in rats administered with 300mgkg^{-1} of the extract with no significant degree of recovery after withdrawal of the extract Table 3.

Table 1. Effects of 28 days administration of methanol root extract of *cissampelos mucronata* on mean body and organ weights in rats

Doses Administered ($mgkg^{-1}$)	IB Weight (g)	FB Weight (g)	BWD (g)	Liver (g)	Left kidney (g)	Right kidney (g)
0	243.36±1.58	298.98±1.37***	55.6	8.53±0.34	1.00±0.11	1.18±0.20
100	242.90±1.17	237.08±1.79	5.82	7.59±0.41	1.08±0.27	1.12±0.21
200	248.46±1.22	242.09±0.47	6.37	7.14±0.42	1.08±0.28	1.25±0.13
300	238.20±1.44	227.83±2.73**	10.37	7.05±0.41*	1.31±0.26	1.39±0.20
300^{PRG}	240.76±1.59	261.25±3.37***	20.49	8.59±0.17	1.36±0.24	1.39±0.25

*PRG= Post Recovery Group: Sacrificed 14 days after the last administration of the extract, IB=Initial Body, FB=Final Body, BWD=Body Weight Difference. Results are presented as Means ± SEM. Significance relative to initial body weights *** P<0.001,** P<0.01,* P<0.05, N=5.*

Table 2. Effects of 28 days administration of methanol root extract of *cissampelos mucronata* on serum electrolytes, urea and creatinine in rats

	Doses administered($mgkg^{-1}$)				
Parameter (Unit)	**0**	**100**	**200**	**300**	**300^{PRG}**
Na^+(mmol/L)	135.80±2.11	137.60±2.56	145.80±3.25	144.80±5.85	144.20±3.26
K^+(mmol/L)	6.24±0.37	6.94±0.19	6.92±0.17	6.76±0.19	7.84±0.33[a]
Cl^-(mmol/L)	78.80±0.80	87.20±1.93	88.60±1.99	91.60±0.51*	86.00±5.11
Ca^{2+}(mmol/L)	2.70±0.39	2.06±0.20	2.50±0.21	2.04±.28	2.42±0.05
HCO_3^-(mmol/L)	16.80 ±1.20	16.60± 0.81	12.80± 0.49*	15.80±0.86	21.00±1.10[aa]
Urea (mmol/L)	6.32±0.25	6.22±0.37	6.14±0.32	6.68±0.11	5.14±0.34[aa]
CRET(mol/L)	111.60±4.60	121.80±4.49	113.80±4.49	111.80±3.43	124.20±2.01

*PRG= Post Recovery Group: Sacrificed 14 days after the last administration of the extract. Significance relative to control (Group I) *P<0.05, [aa]P<0.01, [a] P<0.05 Significance between $300mgkg^{-1}$ and 300^{PRG} groups. N=5, Results are presented as Means ± SEM. Na^+=Sodium, K^+=Potassium, Cl^- = Choride, Ca^{2+}=Calcium, HCO_3^- = Bicarbonate, Urea= Urea and CRET= Creatinine.*

Table 3. Effects of 28 days administration of methanol root extract of *cissampelos mucronata* on total protein, albumin, cholesterol and liver enzymes in rats

	Doses administered($mgkg^{-1}$)				
Parameter (Unit)	**0**	**100**	**200**	**300**	**300^{PRG}**
T/P (g/L)	65.80±2.27	67.20±2.56	68.00±1.41	68.80±0.97	76.00±3.15
ALB(g/L)	26.20±1.36	28.40±0.87	28.60±0.75	29.00±0.32	31.08±0.52
TCHO(mmol/L)	1.74±0.05	1.94±0.05	1.68±0.06	1.98±0.09	1.90±0.35
AST(iu/L)	114.80±5.28	115.60± 1.44	120.00±5.38	133.80±8.94	140.20±2.54
ALT(iu/L)	42.00±3.36	43.60±1.86	45.20±4.52	54.80±1.63*	44.20±3.56
ALP(iu/L)	269.4±8.96	304.2±14.20	308.2±14.08	349.2±5.78***	350.4±0.93

PRG= Post Recovery Group: Sacrificed 14 days after the last administration of the extract. Significance relative to control (Group I). N=5, Results are presented as Means ± SEM.T/P= Total Protein, ALB= Albumin, TCHO=Total Cholesterol,AST=Aspartate Aminotransferase ,ALT = Alanine Aminotransferase and ALP = Alkaline Phosphatase .

3.4 Histopathologic Findings

Light photomicrographs of the paraffin section obtained from the liver of control rats showed a normal liver parenchyma with preserved central veins, portal veins , hepatic arteries, bile duct and hepatocytes arranged in form of cords which are round to polyhedral in shape and radiating peripherally with the cords separated by sinusoids Fig. 1. Administration of 100, 200 and 300 $mgkg^{-1}$of the extract showed liver tissues with portal tract moderately infiltrated by lymphocytes with occasional thrombus observed within the central veins with the severity being dose- dependent Fig. 2. Withdrawal of the extract for 14 days did not show any remarkable recovery because liver tissues were still characterized by lymphocytic infiltration Fig. 3. Light photomicrographs of the paraffin section obtained from the kidney of control rats showed a renal tissue with normal arrangements within the renal cortex and medulla which was composed of renal corpuscle, renal tubules and connecting tubules Fig. 4. Administration of 100, 200 and 300 $mgkg^{-1}$of the extract showed renal tissues characterized by moderate sloughing of the proximal convulated tubules in most areas Fig. 5. Withdrawal of the extract for 14 days did not show any remarkable recovery from the injury caused by the extract because kidney tissues were still characterised by sloughing of the proximal convulated tubules in most areas Fig. 6.

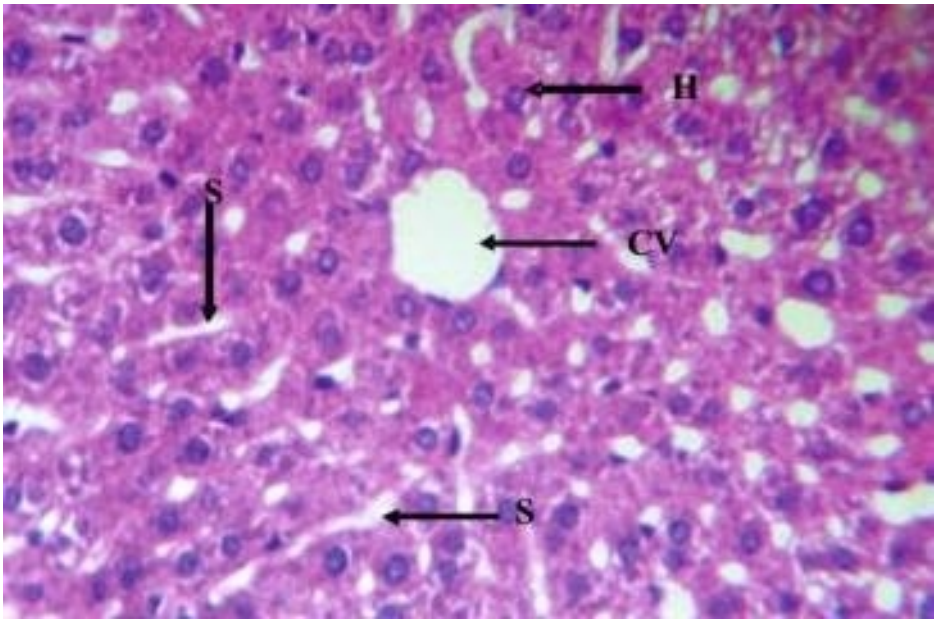

Fig. 1. Photomicrograph of the paraffin section of the liver of a control rat showing normal liver parenchyma with central vein (CV) and hepatocytes (H) arranged in form of cords which are round to polyhedral in shape and radiating peripherally with the cords separated by sinusoids (S). H and E stain (magnification x 400)

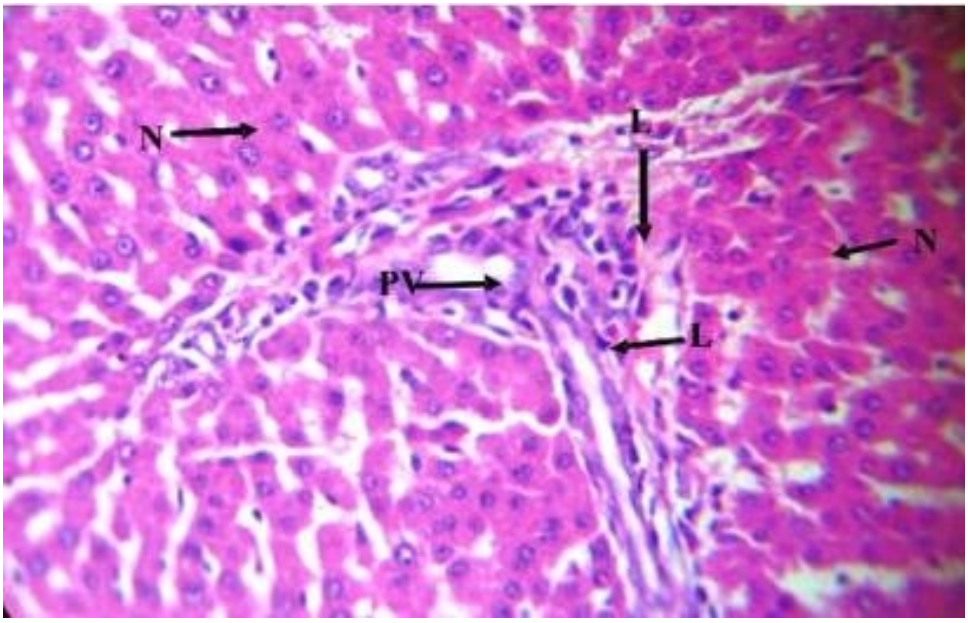

Fig. 2. Photomicrograph of the paraffin section of the liver of a rat treated with 300 mgkg^{-1} of the extract showing moderate infiltration of the portal vein (PV) by lymphocytes (L), mild and focal necrosis (N) . H and E stain (magnificationx400)

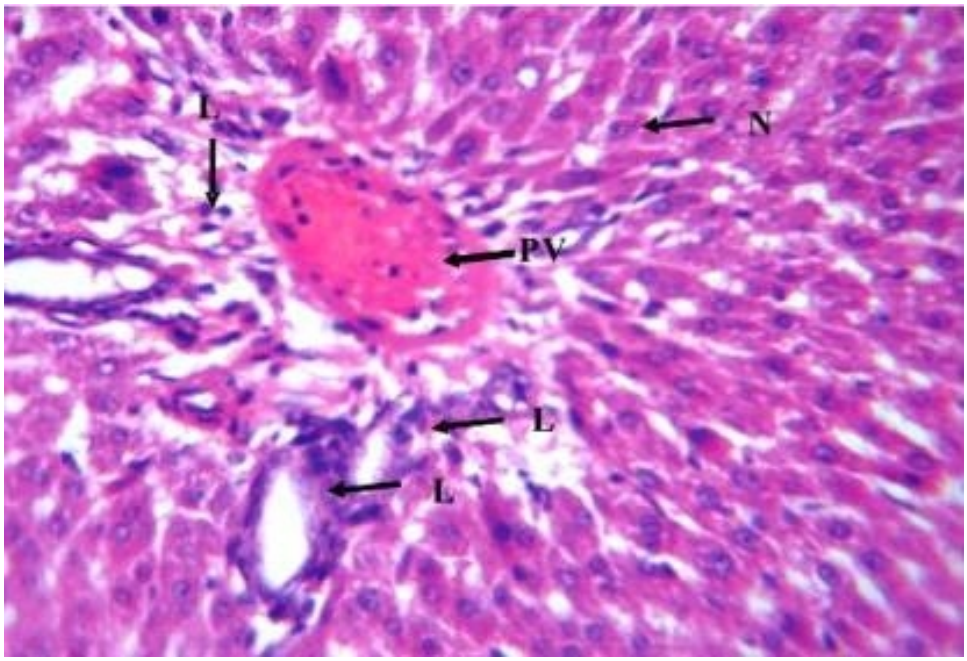

Fig. 3. Photomicrograph of the paraffin section of the liver of a rat treated with 300 mgkg^{-1} of the extract and allowed a recovery period of 14 days showing no sign of recovery because tissue was still characterized by moderate infiltration of the portal vein (PV) by lymphocytes (L). H and E stain (magnificationx400)

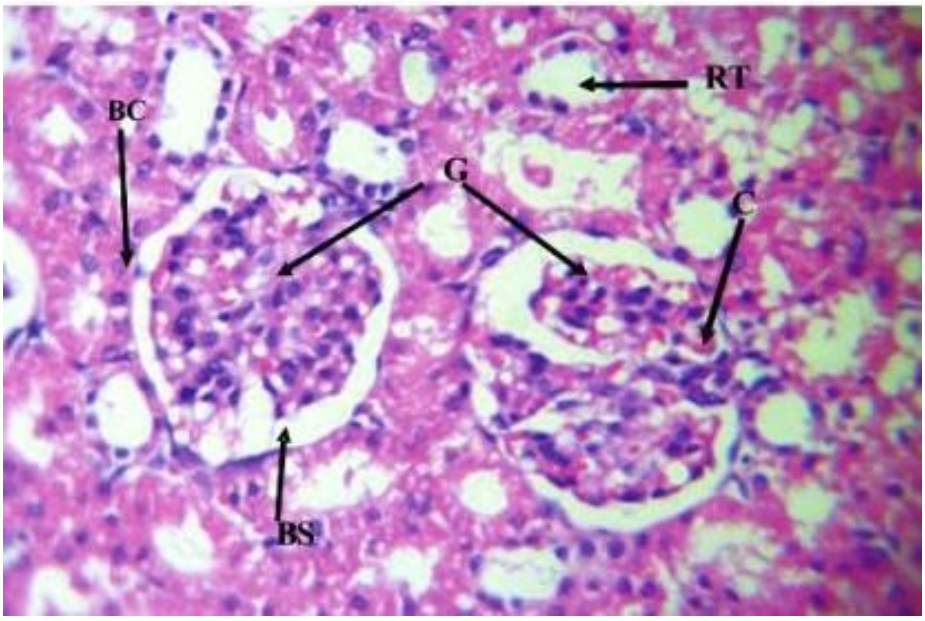

Fig. 4. Photomicrograph of the paraffin section of the kidney of a control rat showing the renal cortex composed of Glomeruli (G), Bowman's capsule (BC), Bowman's space(BS), Renal tubles (RT) and capillary(C) . H and E stain (magnificationx400)

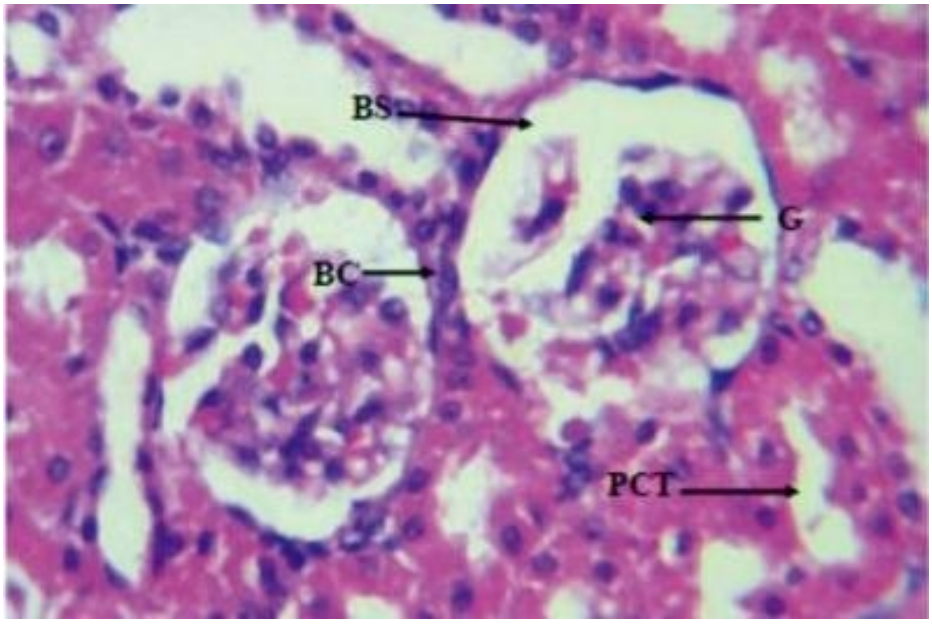

Fig. 5. Photomicrograph of the paraffin section of the kidney of a rat administered with 300 mgkg^{-1} of the extract showing a Glomerulus (G), Bowman's space (BS) , moderate thickening of the Bowman's capsule (BC) and sloughing of the proximal convulated tubule (PCT) in most areas.H and E stain (magnificationx400)

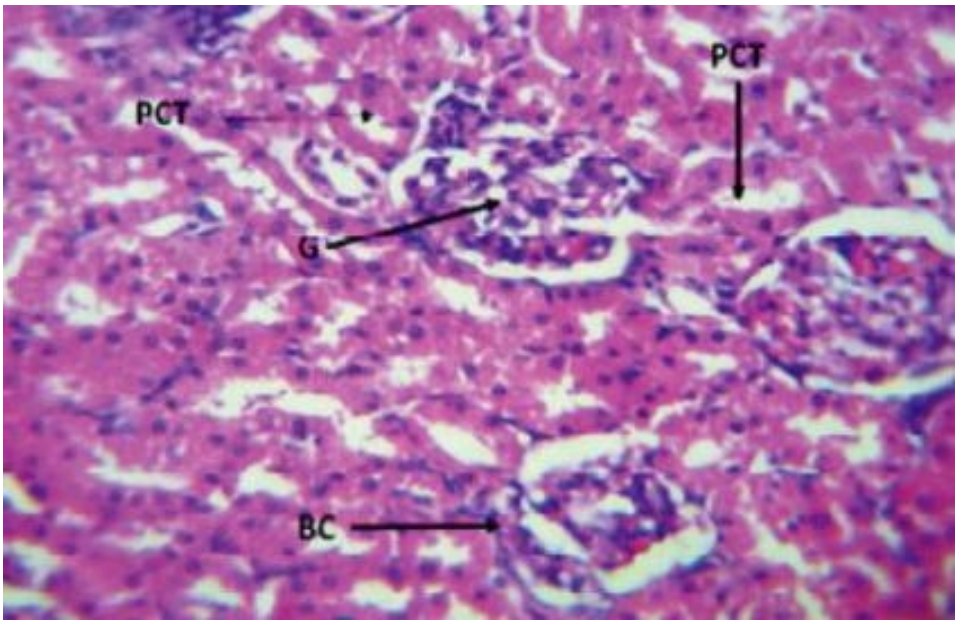

Fig. 6. Photomicrograph of the paraffin section of the kidney of a rat administered with 300mgkg^{-1} of the extract and allowed a recovery period of 14 days showing no sign of recovery because tissue was still characterized by moderate sloughing of the proximal convulated tubules (PT) in most areas. Bowman's capsule (BC) . H and E stain (magnificationx400)

4. DISCUSSION

Preliminary safety pharmacological evaluation of the safety of *Cissampelos mucronata* from previous work have showed an LD_{50} of 5000mgkg^{-1} in mice [18] indicating that the extract is of low toxicity as evidenced by the value of >2000mg kg^{-1} body weight during oral administration obtained in this study which also lacks the classic first signs associated with acute toxicity [26-30].

The body weight loss observed in the treated groups might be attributed to the loss of appetite or the effect of the phytochemical constituents such as polyphenols that have been linked with antiobesity effects [31-36]. It is pertinent to note that withdrawal of the extract caused slight recovery in body weight they lost.

The decrease observed in liver weights reflects retardation in body weight gain, which is a consequence of body weight lost during the course of the study or the effect of some phytochemicals that have been linked to decrease in liver weights. Administration of the extract was observed to have significantly elevated the serum levels of alanine aminotransferase and alkaline phosphatase in the rats administered with 300 mgkg^{-1} of the extract. The elevated levels are an indication that the extract is hepatotoxic since the most commonly used markers of hepatocellular injury; aspartate aminotransferase(AST) and alanine aminotransferase (ALT) have been elevated following administration of the extract. These injuries to the liver may affect the integrity of hepatocyte leading to the release of membrane bound enzymes (e.g. ALT and AST) causing damage to the hepatobiliary system with concomitant release of essential enzymes in hepatobiliary systems (e.g. ALP). The

biochemical damages is in conformity with the histopathological changes observed on liver tissues that were chracterised by narrowing of the sinusoids and infiltration by lymphocytes with occasional thrombus. The occasional infiltration of inflammatory cells seen in the portal triads of the extract treated rats which were mainly lymphocytes and plasma cells was more in rats that were administered with 300mgkg^{-1} suggesting that the extract could interact with proteins and enzymes of the hepatic interstitial tissue interfering with the antioxidant defense mechanism and leading to reactive oxygen species (ROS) generation which in turn may initiate an inflammatory response [37,38].

The kidney functioning capacity was assessed in this study by measuring the levels of electrolytes, creatinine and urea in the serum of the animals. The absence of any significant effect of the extract on serum concentrations of sodium, chloride ions and creatinine of the animals suggest that the normal functioning of the organ in relation to these electrolytes were unaffected. While some authors have demonstrated that estimation of urea and creatinine levels are not sensitive enough in detecting low level of renal toxicity or damage due to the great functional reserve of the kidney, others have reported that absolute kidney weight to be a relatively sensitive indicator of nephrotoxicity for known toxicants [39-41]. Nephrotoxicity has, therefore, been defined as increased kidney weight (either absolute or relative) coupled with a significant alteration in at least one serum parameter [39]. The slight increase in absolute weights of the kidney is an indication of the extract's nephrotoxic potentials which is supported by the fact that the serum levels of potassium seen in this study usually arises as a result of excessive destruction of cells, with redistribution of potassium from the intra- to extracellular compartement, as a result of massive haemolysis [39]. Histopathological evaluations of the tissues from treated groups showed features consistent with renal epithelial injury from toxins. Many herbal preparations have been found to exhibit renal tubular necrosis showing extensive interstitial fibrosis and severe tubular loss most prominent in the outer cortex [42,43].

5. CONCLUSION

In conclusion this study has demonstrated that the methanol root extract of *Cissampelos mucronata* significantly lowers body weight and was also shown to elicite a significant increase in the serum levels of alanine aminotransferase, alkaline phosphatase , aspartate aminotransferase, total protein, albumin with moderate infiltration of lymphocytes and thrombus within the central veins with moderate sloughing of the proximal convulated tubules in renal tissues of rats with no remarkable recovery following withdrawal of the extract suggesting that prolonged and constant use of the extract is both hepatotoxic and nephrotoxic.

CONSENT

Not applicable.

ETHICAL APPROVAL

Animal procedures followed international standards and the project was approved by the local ethics committee of the College of Medical Sciences University of Maiduguri, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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