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Evaluation of Toxicological Effects of *Spondias Mombin* in Adult Male Wistar Rats

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Abstract

Spondias mombin is relied on for various herbal remedies for numerous conditions. This study is to evaluate the acute toxic effect of mombin in adult male Wistar rats. Acute toxicity test was carried out with modified Lorke's method. Twenty-five male rats weighing between 120-180g were used for the sub-chronic study. The rats were divided into five groups A- E (n=5). Group A served as control. Group B and C received respectively 250mgkg^{-1} and 500mgkg^{-1} body weight doses of aqueous leaf extract, groups D and E received 250mgkg^{-1} and 500mgkg^{-1} body weight doses of ethanolic extract. Treatments lasted for twenty eight days. Acute toxicity test carried out showed that leaf extracts of *Spondias mombin* did not produce mortality in rats. Significant ($p<0.05$) reduction in brain and kidney weights was observed in group E treated with 500mgkg-1 of ethanolic extract. Similarly, significant ($p<0.05$) reduction was observed in spleen weights in groups C and E that were treated with 500mgkg-^{1} of both extracts. The levels of LDL ($p<0.001$) and ALP ($p<0.05$) were significantly reduced. Alterations in the histology of the liver and kidney of extracts treated groups were observed. Conclusion: This result suggests that although the use of the leaf extracts of Spondias mombin is relatively safe, hepatic and renal toxicity may occur with prolonged use.

Keywords: Adverse effects, Mortality, Methods, Complication, Utilization

1. Introduction

The use of plant and plant-based products has been a valuable and safe source of medicines for treating different kinds of diseases. This brought about the term herbal medicine, which denotes the use of herbs for their therapeutic or medicinal value (Acharya *et al,* 2008). The World Health Organization estimates that about 75% of the world populations rely on herbs to meet health care needs (WHO, 1991) and many drug classes used today include a prototype from a medicinal plants (Gilani, 2005). Over the past decade, interest in drugs derived from plants, especially the phytotherapeutic ones, has increased expressively (Shu, 1998). It is estimated that about 25 per cent of all modern medicines are directly or indirectly derived from plants (Craig *et al*, 1997). *Spondias mombin* belongs to the family *Anacardiaceae*. It is native to the tropical America and has been naturalized in parts of Africa, India and Indonesia and it is one of the medicinal herbs in Southern Nigeria (Aiyeloja and Bello, 2006). It is called hog plum in English, akika in Yoruba, ijikara in Igbo, tsardarmaser in Hausa, chabbuh in Fulani and nsukakara in Efik (Gill, 1992) . The plant has been traditionally noted for its medicinal and food values. Preliminary results report a wide range of antibacterial and antifungal properties (Okwu, 2001, Urugulaga and Laghton, 2001). In Nigeria, the leaves are used locally by traditional medical practitioners for the treatment of different conditions such as stomach pain, cough, cuts, dizziness, eye ailments, thrush, yaw and as an expectorant.

Scientific investigations have shown that it has anthelmintic, antioxidant,antimicrobial and anti-inflammatory actions (Abad *et al*, 1996; Abo *et al*, 1999; Calderon *et* al, 2000; Kramer *et* al, 2002; Ademola *et al*, 2005;). Leaves of Spondias *mombin* have been reported to be responsible for various actions such as; smooth muscle relaxant (Akubue *et al*, 1983), antispasmodic (Uchendu and Nwankwo, 2005), abortifacient (Offiah and Anyanwu, 1989), sedative and anticonvulsant (Ayoka *et al*, 2006), and anxiolytic (Ayoka *et al*, 2005). Phytochemicals found in leaf extracts of *Spondias mombin* are tannins (3.82%), saponins (7.60%), flavonoids (3.00%), alkaloids (6.00%) and phenols (1%) (Njoku and Akumefula, 2007). The lipid lowering effect of aqueous leaf extract of *Spondias mombin* had been demonstrated (Igwe *et al*, 2008). Recently, its hypoglyceamic effect in combination with *Parinari poliandra* was demonstrated in alloxan-induced diabetic rats (Iweala and Oludare, 2011). The present study was carried out to investigate the toxicological effects of SpM leaf extracts on the morphology, biochemistry and histopathology of Wistar rats since no scientific report on its toxicological effect was found in literature.

2. Materials and Methods

2.1 *Preparation of leaf extracts of Spondias mombin*

Fresh leaves of *Spondias mombin* (SpM) plants were obtained from the University of Calabar botanical garden. The leaves were identified and authenticated by Mr Frank Apejoye, the Chief herbarium in the Department of Botany, University of Calabar, Nigeria. A voucher specimen of *Spondias mombin* with voucher number MIA313 was deposited at the Botany's herbarium. The powdered *Spondias mombin* leaves were extracted by the bulk (cold) extraction method using aqueous (100%) and ethanol as solvents for a period of 72 hours. The ethanol extract were concentrated in-vacuo at 40^{o}C, evaporated to dryness and the residues obtained were stored (in a freezer at -80^{o}C) until needed for further test. The given quantities were diluted in distilled water and administered by oral gavage.

2.2 *Procedures*

Acute toxicity test was carried out and median lethal dose was calculated using modified method of Lorke (1983). Twenty-five male rats weighing between 120g-180g were used in this study. The rats were divided into five groups labeled A, B, C, D and E with each group consisting of 5 rats. Group A was the control group, Group B C, D and E was the experimental groups. Group B and C were given 250mg/kg and 500mg/kg body weight doses of ethanolic leaf extract of *Spondias mombin* and those in groups D and E received 250mg/kg and 500mg/kg body weight doses of aqueous leaf extract of *Spondias mombin* respectively with the aid of an orogastric tube.

Administration of extracts was done daily for twenty-eight days during which food consumption and water intake of the groups was measured daily. Body weight of all rats in the groups was recorded weekly. On the 29th day, the rats were anaethesized using chloroform inhalation, and blood was drawn from the heart using a needle and syringe. A portion was transferred immediately into EDTA-containing tubes for measurement of haematological parameters and serum biochemical analysis. The blood was centrifuged and serum analyzed.

2.3 *Organ weight and histopathology*

The liver, heart, lungs, kidneys, stomach, spleen, gonads and brain were excised, and weighed. Relative Organ Weight (ROW) was calculated by expressing absolute organ weight as a percentage of the total body weight. The liver and kidneys were fixed in 10% buffered formalin for 48 hours for routine histology using Haematoxylin and Eosin staining method. Results were expressed as Mean±SD. Statistical analyses was carried out by one-way ANOVA, data was further subjected to post hoc test (Konate *et al*, 2011) and differences between treated groups and control accepted as significant at $p<0.01$ and $p<0.05$.

3. Results

The acute toxicity test carried out did not show any toxicity by extracts on rats. Mortality was not recorded throughout the duration of this study. There was a significant ($p<0.05$) reduction in the body weights of treated rats compared to that of control. Rats in all the groups that received extracts of SpM lost weight during the 4 weeks of administration, but, the weight loss was most significant during the 4th week of treatment (Table 1).

Table 2 shows that the effects of aqueous and ethanolic extract of SpM was insignificant ($p>0.05$) compared to control. Consumption of food decreased in all groups at 3rd and 4th weeks but these decrease was not significant ($p>0.05$) when compared to control. A non significant ($p>0.05$) increase as shown in table 3 in the intake of water in all treated groups was recorded in the first two weeks of treatment. However, a significant ($p<0.05$) increase was observed in all the treated groups during the 3rd and 4th weeks of administration of both extract of SpM. In the 3rd week, the significant increase ranged from 197.9±7.25 -216.90±10.25, and at the 4th week, an increased range of 220.80±4.72 -260.50± 8.21 was recorded in the experimental groups against control at 3 and 4 weeks having a mean water intake of 170.40±5.24 and 164.60±3.55 respectively.

Table 4 shows a significant ($p<0.05$) reduction in the mean weight of spleen from a value of 0.52±0.04 in control to 0.41±0.01 and 0.40±0.05 in groups C and E treated with 500mgkg^{-1} of aqueous and ethanolic extracts respectively. Significant ($p<0.05$) reduction (0.64±0.06) in the relative weight of brain was found only in group E that received 500 mgkg^{-1} of ethanolic extract. Extracts of SpM did not cause any significant ($p>0.05$) change in the haematological indices of the treated animals. The changes recorded were within the range of that of control (Table 5). However, the administration of extracts caused a significant ($p<0.01$) reduction in serum LDL levels in groups C and E. These groups recorded a significant reduction of 08.22±0.14 and 06.96± 0.07 respectively compared to control that had a value of 16.34±2.58. Significant ($p<0.01$) reduction was also recorded in ALP levels in groups C and E (5.53±1.61 and 9.34±3.38) compared to control (24.25±9.09). Interestingly, a significant elevation ($p<0.05$) was observed in group D (37.17±20.70) treated with 250mgkg^{-1} of ethanolic extract. Liver biochemical assay also recorded a significant reduction ($p<0.01$) in LDL and ALP levels in groups C and E. Other serum and liver parameters were insignificant ($p>0.05$) compared to control. Tables 6 and 7.

Histologically, the liver of the treated groups were not different from that of control, while mild alterations were observed in the cytoarchitecture of the kidneys of treated animals compared to control. The liver section of the animal in control groups showed a central vein with prominent small-sized nuclei, with the hepatocytes well

separated by sinusoids (Figure 1). Tissue section of group B showed a prominent central vein with relatively large sized nuclei with well preserved cords of hepatocytes and the sinusoids well demarcated (Figure 2). Group C animals treated with 500mgkg^{-1} aqueous extract showed presence of fatty deposits in the central vein, distortion of the radial arrangement of sinusoids and vacuolation of the cytoplasm (Figure 3). Figure 4 shows liver tissues of rats treated with 250mgkg^{-1} characterized by desquamation of the central vein, distortion of the hexagonal arrangement of the hepatocytes and mononuclear cell infiltration. Group E treated with 500mgkg^{-1} of ethanolic extract had great accumulation of fatty deposits in the central vein, severe distortion of arrangement of hepatocytes and area of hepatic necrosis (Figure 5).

The kidneys of the experimental animals treated with extracts of Sp showed varying degrees of derangements compared to control that showed normal renal histology (Figure 6). The observed changes in the treatment groups were distortion of the renal corpuscles, distal and proximal convoluted tubules in group B animals (Figure 7). Group C rats' kidney was totally distorted with presence of vacuoles (Figure 8). Groups D showed marked changes in its renal corpuscles and areas of necrosis (Figure 9). Loss of renal corpuscle was observed in group E characterized by wider capsular spaces. Distal and proximal convoluted tubules were completely eroded. Wider necrotic areas were also present (Figure 10).

4. **Discussion**

The economic and medicinal values of plants cannot be overlooked, since most herbal plants are used for sustaining health and alleviating or treating health issues. Some of these herbal plants have been certified as drugs and are claimed to be safe and effective (Treasure, 2000). Medicinal herbs always have broad actions unlike pharmaceutical drugs that have specific effect on the body. Some investigators however, claim that medicinal plants also act in the therapeutic direction, which are complementary or synergistic and are often non-specific (Shen-nong, 2002). The extracts of SpM increased water intake in rats treated with 500mgkg-1 and may be due to dehydration caused by increased water excretion by the kidney. Hypothalamic stimulation of thirst occurs in the paraventricular nucleus of the hypothalamus, and this has been found to be caused in response to lack of antidiuretic hormone (ADH) or nephrogenic stimulation (Nosiri et al, 2010). The effect of extracts on water intake may be due to disruption in antidiurectic hormone secretion in the hypothalamus or due to disruption in the normal functioning of the kidney. Extracts suppressed body weight, food consumption compared to control. This implies that the appetites of the animals were affected. The extreme lateral part of the ventromedial nucleus of the hypothalamus is responsible for the control of food intake and it has been stipulated that leptin acts on the hypothalamus to decrease food intake(Theologides, 1976), and it has also been stated that body weight is an indicator of drug effect and is used to assess the response to drug therapy (Steyn, 1995). The observed effects on the food consumption and body weights of treated animals may be a response to the effect of SpM mediated through the hypothalamus.

Analysis of the activities of basic liver function enzymes in serum are used to indirectly access the integrity of tissues after exposure to pharmacological agents (Uboh et al, 2010). Serum levels of ALT, AST and ALP were not altered by extracts of SpM, levels of other biochemical parameters were not altered, which suggests that the extracts at 4 weeks is not hepatotoxic. This may be due to the presence of antioxidants in SpM (Calderon et al, 2010). Levels of LDL were significantly reduced in groups treated with 500mgkg-1, although the level of Triglycerides were decreased, it was not significant. The decreased level of LDL suggests that it has a hypolipideamic effect and this supports report on its lipid lowering effect (Igwe et al, 2008).

The mild alterations observed in the liver and kidneys appear to be dose and time dependent. The presence of nephritic necrosis is not unexpected since excretion is mainly carried out in the kidney. The observed found in the kidney of extracts treated rats is in accordance with investigation that noted that tubular necrosis occurred in rats fed with anti-nutritive factors containing feeds (Jovanoic et al, 1991). The presence of alkaloids and tannins in these extracts may be responsible for the observed histological changes, they have been known to exhibit differential biochemical and pharmacological actions that can cause cell toxicity (Konan et al, 2007). This study concludes that the use of leaf extracts of Spondias mombin must be taken with caution to prevent hepatic and renal injury.

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Table 1: Effect of *Spondias mombin* leaf extracts on body weights of rats

Body weights (g)	Treatment				
Day	A	B	C	D	E
0	125.60	124.80	134.88	128.40	136.20
7	130.02	122.47	128.16	126.66	132.40
14	138.10	119.90*	118.90*	126.28	120.00
21	148.48	99.48*	97.71*	94.35*	89.29*
28	148.86	93.86*	96.00*	91.46*	80.60*

Mean ± SEM. * Significantly different from control at p<0.05.

Table 2: Effect of *Spondias mombin* leaf extracts on weekly rats' food consumption.

Treatment	Food consumed (g)			
	Week 1	Week 2	Week 3	Week 4
A	126.52±16.82	120.60±17.28	116.76±7.86	108.65±6.65
B	128.00±18.06	112.22±16.44	107.15±9.64	98.18±5.83
C	124.04±17.28	100.04±14.08	95.46±7.86	42.54±6.22*
D	120.06±12.28	104.06±16.72	98.57±7.68	83.42±7.46
E	124.38±14.76	102.57±12.86	94.55±6.01	56.00±7.96*

Mean± SEM. *Significantly different from control at p<0.05

Table 3: Effect of *Spondias mombin* leaf extracts on weekly water intake in rats.

Treatment	Water intake (mL)			
	Week 1	Week 2	Week 3	Week 4
A	175.60±4.65	158.60±8.05	170.40±5.24	164.65±3.55
B	170.84±6.28	170.40±4.83	174.60±5.22	172.80±4.72
C	172.00±8.02	167.50±3.86	163.98±8.85	234.88±7.12*
D	178.50±7.23	162.22±4.04	164.90±7.25	186.26±8.62
E	175.36±5.44	168.20±3.99	166.26±4.68	258.55±8.21*

Mean ± SEM. *Significantly different from control at p<0.05

Table 4: Effect of *Spondias mombin* leaf extracts on organ weight

Organs	A	B	C	D	E
Brain	1.07 ±0.05	1.02±0.04	1.00±0.05	0.98±0.02	0.64±0.66*
Heart	0.82±0.06	0.74±0.03	0.72±0.04	0.76±0.03	0.69±0.05
Kidneys	0.66±0.01	0.65±0.01	0.58±0.02	0.65±0.02	0.42±0.02*
Spleen	0.52±0.04	0.43±0.03	0.41±0.01*	0.50±0.04	0.40±0.05*
Liver	3.80±0.11	3.56±0.08	3.44±0.06	3.62±0.08	3.48±0.06
Stomach	1.22±0.10	1.10±0.18	1.10±0.10	1.20±0.22	1.07±0.20
Testes	1.75±0.07	1.70±0.03	1.66±0.08	1.74±0.11	1.58±0.04

Mean ± SEM. *Significantly different from control at p<0.05

Table 5: Effect of *Spondias mombin* leaf extracts on haematological indices of rats.

	Treatments				
Blood indices	A	B	C	D	E
RBC	7.44±0.38	7.93±0.22	8.85±0.22	8.11±0.34	7.71±0.33
WBC	19.58±3.50	18.78±1.39	19.32±1.76	23.80±2.45	22.18±1.34
HB	11.68±0.59	12.20±0.26	13.28±0.26	12.26±0.45	11.62±0.62
HCT	45.78±2.69	49.74±1,78	54.30±1.45	50.76±1.41	47.12±3.02
MCV	61.46±0.96	62.66±1.30	62.78±0.77	61.48±1.30	61.48±1.19
MCH	15.70±0.20	15.42±0.22	15.02±0.13	15.14±0.22	15.14±0.21
MCHC	25.58±0.32	24.58±0.46	24.50±0.33	24.14±0.28	24.60±0.27
PLT	907.60±49.14	920.00±51.47	1078.80±78.09	939.00±43.91	1109.20±87.39

Mean ±SEM.

Table 6: Liver biochemical parameters of rats treated with extracts of *Spondias mombin*

Parameters	A	B	C	D	E
Cholesterol($mmolL^{-1}$)	0.18±0.05	0.11±0.01	1.43±0.71	0.11±0.01	0.10±0.02
TP($mmolL^{-1}$)	5.06±0.13	4.28±0.06	4.30±0.03	3.96±0.06	2.70±0.03
HDL($mmolL^{-1}$)	22.84±6.75	15.73±1.65	11.69±1.86	20.76±9.22	7.85±1.87
LDL($mmolL^{-1}$)	1.28±6.75	0.90±0.26	0.06±0.02**	0.89±0.14	0.08±0.01**
TAG($mmolL^{-1}$)	2.02±0.08	2.17±0.20	1.15±0.45	1.91±0.29	1.89±0.25
ALB(gL^{-1})	5.31±0.74	3.50±1.63	1.09±0.34*	3.90±1.58	1.03±0.93**
AST($\mu molL^{-1}$)	373.42±47.45	301.68±12.92	328.35±9.62	294.88±17.07	218.41±50.03
ALT($\mu molL^{-1}$)	170.76±9.66	157.30±3.17	151.82±2.96	154.15±6.31	151.78±4.17
ALP($\mu molL^{-1}$)	05±27. 1.77	27.14±0.55	28.10±0.54	27.70±0.36	29.96±1.06

Mean ± SEM. *Significantly different from control at p<0.05, **Significantly different from control, B & D at p<0.05

Table7: Serum biochemical parameters of rats treated with extracts of *Spondias mombin*

Parameters	A	B|	C	D	E
Cholesterol ($mmolL^{-1}$)	66.08±4.98	78.96±4.38	73.82±4.39	57.20±2.45	61.89±2.39
TAG($mmolL^{-1}$)	58.60±0.10	54.26±0.07	51.60±0.10	53.00±0.09	40.80±0.04
HDL($mmolL^{-1}$)	45.37±5.80	84.61±5.23	54.21±4.55	47.56±5.30	61.47±5.51
LDL($mmolL^{-1}$)	16.34±2.58	11.48±1.03	08.22±0.14**	12.86±1,16	06.96±0.07**
TP($mmolL^{-1}$)	54.36±0.45	49.16±0.19	45.44±0.29	50.58±0.13	48.54±0.14
ALB(gL^{-1})	38.41±1.22	36.47±1.10	44.00±1.61	40.09±1.71	34.49±1.88
AST ($\mu molL^{-1}$)	503.25±16.52	449.70±7.42	475.88±3.09	466.57±10.14	446.88±18.46
ALT($\mu molL^{-1}$)	132.85±3.83	126.10±1.26	127.12±6.12	120.85±3.68	127.48±0.63
ALP($\mu molL^{-1}$)	24.25±9.09	31.13±7.34	35.53±1.61	37.17±20.70	39.34±3.38
Sodium($mmolL^{-1}$)	107.71±10.05	66.24±2.72	69.66±3.72	114.69±20.53	59.95±13.16
Potassium($mmolL^{-1}$)	21.52±3.05	15.19±2.43	9.83±1.67	21.76±4.13	14.56±1.83
Chloride($mmolL^{-1}$)	156.53±7.85	169.96±10.31	179.25±22.73	146.51±2.16	160.30±7.45
Bicarbonate($mmolL^{-1}$)	27.26±0.32	25.02±0.94	22.85±0.39	26.34±0.82	23.38±0.48
Urea($mmolL^{-1}$)	32.08±0.93	35.49±2.77	32.31±2.46	29.72±1.42	34.86±1.87
Creatinine($mmolL^{-1}$)	20.62±0.27	21.68±0.44	26.32±0.19	29.16±0.59	27.38±0.64

Mean ± SEM. **Significantly different from control at p<0.05

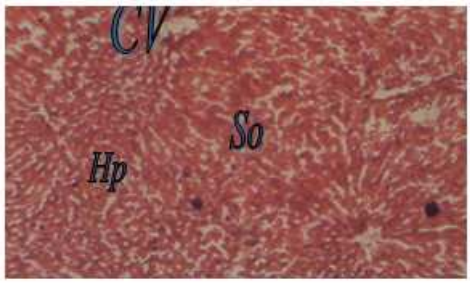

Fig 1: Liver section of control rat showing well defined central vein (CV,) hepatocytes (Hp) and radially arranged sinusoids (So). H and E X 400.

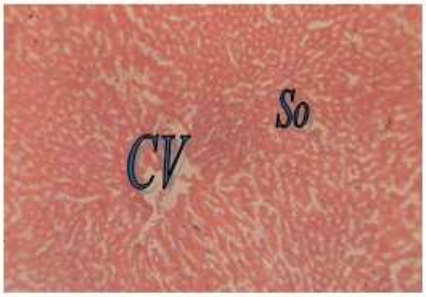

Fig 2: Liver section of rats treated with 250mgkg^{-1} aqueous extract showing distinct central vein (CV) and hepatocytes (Hp). H and E (X 400).

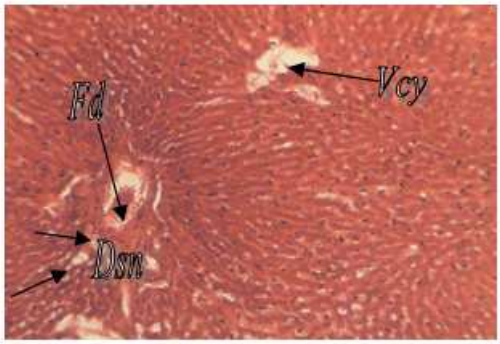

Fig 3: Liver section of rats treated with 500mgkg^{-1} aqueous extract showing presence of fatty deposits (Fd), distortion of the radial arrangement of sinusoids from the central vein (Dsn) and vacuolation of the cytoplasm (Vcy). H & EX 400.

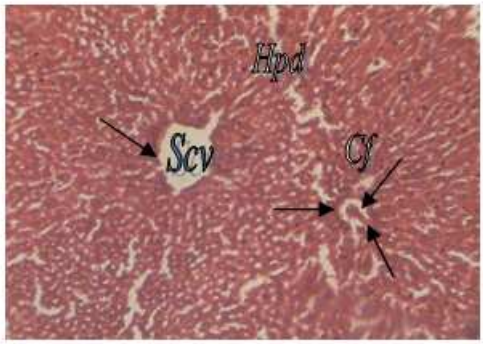

Fig 4: Liver section of rats treated with 250mgkg^{-1} ethanolic extract showing desquamation of the central vein (Scv), distortion of the hepatocytes (Hpd) & mononuclear cell infiltration (Cf). H & E X 400.

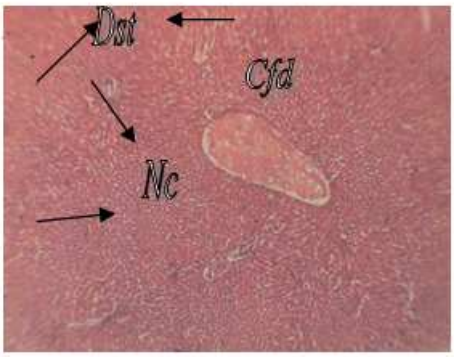

Fig 5: Liver section of rats treated with 500mgkg^{-1} ethanolic extract showing accumulation of fatty deposits in the central vein (Cfd), severe distortion of hepatocytes (Dst) and area of hepatic necrosis (Nc). H & E (X 400).

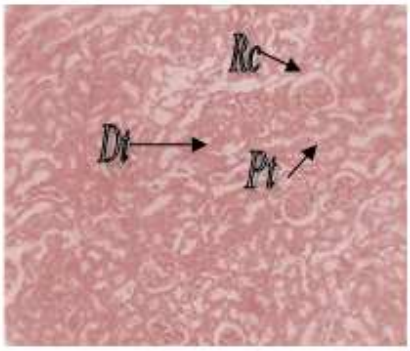

Fig 6: Kidney section of control rats showing normal renal corpuscules (Rc), well defined distal (Dt) and proximal tubules (Pt). (H & E X 400).

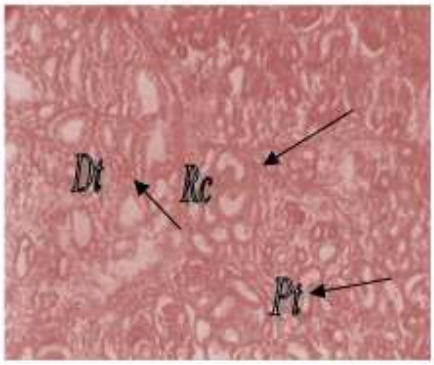

Fig 7: Kidney section of rats treated with 250mgkg^{-1} aqueous extract showing distortion of the renal corpuscles (Rc), distal and proximal convoluted tubules.(H & E X 400)

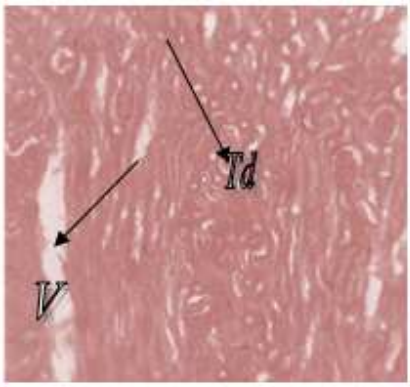

Fig 8: Kidney section animals treated with 500mgkg^{-1} aqueous extract showing total distortion(Td) and vacuolation(V) (arrows). H and E (X 400).

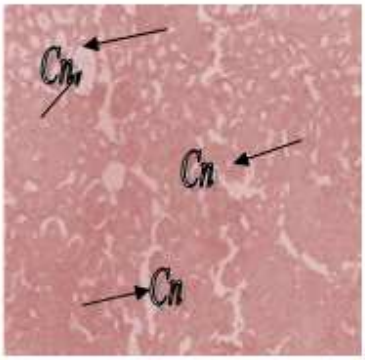

Fig 9: Kidney section of rats treated with 250mgkg^{-1} ethanolic extract of showing distortion of renal corpuscles (Drc)and areas of cell necrosis (Cn). H and E (X 400).

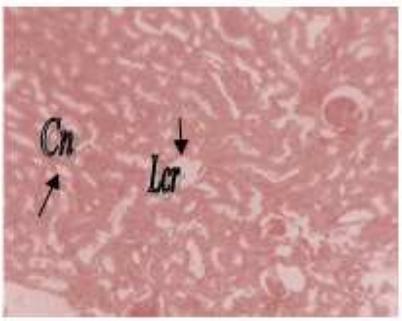

Fig 10: Kidney section of rats treated with 500mgkg^{-1} ethanolic extract showing loss of renal corpuscles (Lrc) and wider areas of cell necrosis (Cn). H and E (X 400)