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Full Length Research Paper

Toxicological evaluations of ethanolic crude seed extract of *Corchorus olitorius*

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The toxicological effect of the ethanolic seed extract of *Corchorus olitorius* was evaluated in white albino rats as a prelude to further pharmacology assessment of the plant seed extract. The acute, sub acute and chronic effects of the extract following oral administration in the animal were studied. The lethal medium dose (LD_{50}) of the extract was estimated to be higher than 5000 mg/kg. Oral administration of 250, 500 and 1250 mg/mg doses daily for 28 days did not produce any death among the rats. But there was significant ($P < 0.05$) and dose dependent increase in the renal biochemical parameters (sodium, potassium and urea level) and the liver biochemical parameters [the alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), conjugated bilirubin and total bilirubin] supported by histological evidences (tubular necrosis in the kidney and from hepatic fatty change to multifocal hepatocyte necrosis in the liver). In the chronic 90 days study, where even higher doses at 1250, 2500 and 3750 mg/kg were administered orally, in which there were mortalities recorded. Renal biochemical parameters showed a significant ($P < 0.001$) decrease in sodium, increase in potassium and increase in urea while the AST, ALT and albumin were all significantly ($P < 0.05$) increased in the liver. The derangements were all supported by histological evidences, mild medulla lyphiod aggregation, multifocal area of renal lyphiod aggregations, portal vein congestion and multifocal hepatic necrosis. The heamatological system showed an increase in the lymphocyte count ($P < 0.05$) and ($P < 0.001$) in both the 28 and 90 days studies, respectively.

Key words: *Corchorus olitorius* (CO), lethal medium dose (LD_{50}), alkaline phosphatase, alanine aminotransferase.

INTRODUCTION

Herbal medicine or medicinal practice is of great importance to man and his health (Franstisek, 1998). In Africa, hundreds of plants are used traditionally for the management of various diseases. To date, however, only a few of these African medicinal plants have received scientific scrutiny, despite the fact that the World Health Organization (WHO) has recommended that medical and scientific examinations of such plants should be undertaken (WHO, 1980). *Corchorus olitorius* (CO) is an annual, much-branched herb, 90 to 120 cm tall, with

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glabrous stems, leaves 6 to 10 cm long and 3.5 to 5 cm broad, with pale yellow flowers and black trigonous seeds (Kirtikar and Basu, 1975). The leaves of *C. olitorius* was reported to have hypoglycaemic effect (Abo et al., 2008) and high antibacterial activity (Adegoke and Adebayo, 2009).

The seed protein enriched diet was found to increase rats' body weight (Laskar et al., 1986). There was failure to produce adverse effects in young chicken, with levels of seeds (*C. olitorius*) up to 5% of the diet (Johnson and Toleman, 1984). The seeds were found to contain reasonable percentage of biologically active cardiac principals (Sharaf and Negm, 1969). The plant stem is a source of jute fibre, and folkloric uses includes, seeds for purgative, leaves for dysentery, fever, gonorrhea and demulcent (Watt and Breyer-Brandwijk, 1962). The part of the plant targeted in this study is the seed believed to have a greater hypoglycaemic effect (ethanolic extract) as claimed by a traditional medicinal practitioner. Also claimed is its lethality. The safety of the crude seed ethanolic extract of *C. olitorius* was evaluated in white albino rats as a prelude to the assessment of its hypoglycemic effect. The null hypothesis was adopted, that is, no toxicity effect exists, both in short or long term use.

MATERIALS AND METHODS

Plant

The plant seed was purchased in the market, identified by Mshelia H.E in the Department of Pharmacognosy and Ethnopharmacy, Pharmacy School, Usmanu Danfodiyo University, and a herbarium Voucher specimen number (PCG/UDUS/TIL/0002) was obtained.

Preparation of plant extract

The *C. olitorius* seeds were pulverized to powder using a blending machine. The soxhlet extractor was used for extraction of the dried powdered seed using ethanol (99.9%). 10 g of the powdered seed extract was placed inside the thimble made from thick filter paper, which was loaded into the main chamber of the soxhlet extractor. The soxhlet extractor was placed onto a flask containing the extraction solvent (ethanol). The soxhlet was equipped with a condenser. The solvent was heated to reflux and the solvent vapour travelled up the distillation arm and flooded the chamber housing the thimble of solid and around the condenser. The condenser ensured that the solvent vapours cooled and dripped down into the chamber housing the solid material. The chamber containing the solid material slowly filled with warm solvents with some of the desired compound dissolved in the warm solvent. When the soxhlet chamber was almost full, the chamber was emptied by a siphon side arm. This cycle was allowed to repeat many times, over hours and days. During each cycle, a portion of the non-volatile compound (powdered extract) dissolved in the solvent. After many cycles, the desired compound was concentrated in the distillation flask. The advantage of this system was that instead of many portions of warm solvent passed through the sample; just one batch of solvent was recycled. The non-soluble portion of the extract remained in the thimble and was discarded. The extract isolated was kept at −20°C until tested.

Laboratory animals

Albino rats of both sexes from the Biological Sciences Department of Usmanu Danfodiyo University (UDUS) were used for the study. The rats were housed in metal cages in the laboratory at temperature between 35 to 37°C; 12 h/12 h light/dark cycle and maintained with free access to standard rat feeds and water for 7 days before experimentation. 12 h before experimentation, food was withdrawn but water was available *ad libitum*.

Acute oral toxicity studies

The Organisation for Economic Co-operation and Development (OECD) 420 guideline for testing of chemicals (2001) (acute oral toxicity-fixed dose procedure) was used. The seed extract of *C. olitorius* (5000 mg/kg body weight) were administered to five (5) female rats (one after the other at an observation period of 24 h) in a single oral dose using a feeding tube. The dose was chosen following a sighting study conducted using the following doses 5, 50, 300, and 2000 mg/kg. Observations for toxic symptoms was made and recorded systematically, 30 min, 1, 2, 3, 4, 5 and 6 h after administration. Finally the number of survival was noted after 72 h and 14 days for each animal. The toxicological effect was assessed on the basis of mortality, which was expressed as LD_{50}.

Sub acute toxicity

Thirty two (32) rats was randomly selected and divided into four groups of animals labeled A to D, and each group with 8 rats of equal sexes. The initial and weekly weights of the rats were recorded. The animals in group D was left without extract administration to serve as control while those in Groups A, B and C received low (250 mg/kg), medium (500 mg/kg) and high (1250 mg/kg) doses of the herbal extract, respectively daily. Administration of the extract was oral by feeding tube for 28 days. Male rats were separated from female within the groups. The fluid and water consumption of the animals was observed daily. Their various body weights were recorded weekly throughout the period of the study. On the last day of the experiment (29th day), blood samples were collected through cardiac puncture following chloroform anaesthesia. Blood samples for biochemical analysis were collected in non-heparinized bottles and that for hematological studies in ethylenediaminetetraacetic acid (EDTA) bottle. The liver, kidney, pancreas and heart were collected and stored in 10% formalin for histological study. Any rats that died during the test period were analyzed for pathological lesions.

Chronic toxicity

Thirty two (32) rats was randomly selected and divided into four groups of animals labeled A to D. Very high doses were chosen for this study to simulate its local use and because of its safety profile on LD_{50} estimation as well as the knowledge of the results obtained from the sub-acute toxicity not reflecting the lethality warned of by the traditional users. The initial and weekly weights of the rats were recorded. The animals in group D was left without extract administration to serve as control, while those in Groups A, B and C received 1250 mg/kg (0.25 of LD_{50}), 2500 mg/kg (0.5 of LD_{50}) and 3750 mg/kg (0.75 of LD_{50}) doses of the herbal extract, respectively daily. Administration of the extract was oral by feeding tube for 90 days (Loomis, 1996). Male rats were separated from female within the groups. The fluid and water consumption of the animals was observed daily. Their various body weights were recorded weekly throughout the period of the study. On the last day of the experiment (91st day), blood samples were collected through

cardiac puncture following chloroform anaesthesia. Blood samples for biochemical analysis were collected in non-heparinized bottles and that for hematological studies in EDTA bottle. The liver, kidney, pancreas and heart were collected and stored in 10% formalin for histological study. Any rat that died during the test period was tested pathologically.

Haematological studies

The blood sample was collected and inserted in EDTA bottle. Several parameters were determined, the packed cell volume (PCV), red blood cell count (RBC) and white blood cell count (WBC) by a computerized method using the Swelab Alfa Auto.

Biochemical studies

The blood samples collected was centrifuged, using centrifuge model 8000D for 5 min and the sera was collected in plain test tubes and stored in the deep freezer at -17°C until required for the following analysis.

The Randox kit procedure of Richmond (1973) was used for Cholesterol determination.

The Jendanssik and Gof (1997) colorimetric method was used (Randox kit) for bilirubin determination.

The Randox kit method of Doumac et al. (1997) was used for albumin determination.

The Randox kit colorimetric method of Sood (1999) was used for alkaline phosphate determination.

The Randox kit, Reitman et al. (1957) method was used for alanine amino transferase (ALT).

The Randox kit, Reitman et al. (1957) method was used for aspartate amino transferase (AST) determination

Randox kit, Rec (1972) Urease-Bertholot colorimetric method was used for Urea determination while Teco diagnostics kit (1996) method was used for urea determination.

Slyke titration method, Van slyke (1992) was used for bicarbonate determination.

Sodium and potassium concentrations of electrolyte were determined using the flame photometry method; flame photometer model – 6400A manufactured by Bran Science and Instrument Co. Eng. was used. A dilution of 1:100 with distilled water was done by pipetting 0.1 ml of serum into 10 ml of distilled water. The 6400A flame photometer was used only after the liquid of standard concentration was made. To have the comparison determination, absolute value of the concentration of electrolyte sorted (Na or K) were read off the machine following sample introduction and switch to either electrolyte (Na or K) being tested was effected.

Histopathological studies

The animals were sacrificed after general anesthesia, with chloroform, a day after the last dose of the drug administration. At autopsy, the liver, heart, kidney and pancreas of each rat were removed and weighed. The tissue samples were then fixed in formalin for histopathological examination. The tissues were fixed in formalin-acetic acid fixative, embedded in paraffin wax, sectioned and stained with haematoxylin and eosin for histological examination.

Statistical analysis

Results were expressed as Mean ± standard deviation (SD). The analysis of variance (ANOVA) was used to compare means using Tukey Kramer test. Statistical significances was considered at $p \leq 0.05$.

RESULTS

Soxhlet extraction yield of ethanolic extract of *C. olitorius*

10 yields were calculated and the mean yield calculated from these.

$$\text{Yield 1} = \frac{\text{Weight loss by thimble } (v_1 - v_2)}{\text{Weight of sample } (v_0)} \times 100$$

v_1 = weight of residue = 49.39 g
v_2 = weight of empty filter paper = 1.56 g
v_0 = weight of sample = 50 g
= 95.66% = % weight loss by thimble
Therefore % yield = 100% - 95.66
Yield 1 = 4.34%

$$\text{Mean yield} = \frac{4.34 + 4.7 + 4.46 + 5.12 + 4.74 + 4.7 + 4.56 + 4.9 + 4.82 + 4.68}{10} = 4.70\%$$

Acute oral toxicity studies

No death was recorded following the single dose administration in either the control or treated groups given 5 g/kg of ethanolic seed extract of *C. olitorius* orally. The animals were however noticed to be hypoactive (slower) in the first 24 h on comparing with the control. The animals did not show any other changes in general behavior or other physiological activities. The LD_{50} of the extract was estimated to be greater than 5000 mg/kg.

Sub acute toxicity

There were no obvious physical changes and no death recorded over the 28 days study in either the control or treated groups. The animals did not show any changes in general behavior or other physiological activities. There were no differences in water and feeds intake in both the control and the treatment groups. There were no significant changes in the weight of the rats in both the control and treated groups (Table 1). There were also no

Table 1. The effect of extract on body weight of rats treated for 28 days.

Week	Control A	250 mg/kg (B)	500 mg/kg (C)	1250 mg/kg (D)	Stat. (one-way ANOVA)
0	126.75±22.52	140.40±14.31	140.0±9.93	137.10±31.32	P=0.544 ($F_{3,28}$=0.7278)
1	140.73±28.0	173.28±17.02	161.0±16.68	156.43±34.39	P=0.1003 ($F_{3,28}$=2.288)
2	149.47±25.05	171.26±29.80	168.64±17.60	160.51±31.51	P=0.3675 ($F_{3,28}$=1.095)
3	153.48±23.47	179.73±31.67	176.48±18.63	167.01±32.42	P=0.2355 ($F_{3,28}$=1.502)
4	160.78±24.19	179.43±29.32	175.36±15.10	170.50±33.45	P=0.5510 $F_{3,28}$=0.7156
Mean wt.	146.29±24.19	168.03±23.56	164.65±14.61	158.29±32.11	P=0.3172 ($F_{3,28}$=1.230)
Mean wt. gain	34.03±10.10	38.98±24.18	35.35±15.20	33.4±12.62	P=0.9163 ($F_{3,28}$=0.1692)

Values are mean ± SD (n=8). *significant difference p<0.05

Table 2. The effect of extract on organ weight of rats treated for 28 days.

Organ	Control A	250 mg/kg (B)	500 mg/kg (C)	1250 mg/kg (D)	Stat. (one-way ANOVA)
Liver	4.98±0.41	5.08±0.77	5.47±0.51	4.68±0.78	P=0.1248 ($F_{3,28}$=2.085)
Pancreas	0.46±0.08	0.57±0.11	0.57±0.11	0.54±0.10	P=0.1067 ($F_{3,28}$=2.230)
Heart	0.51±0.07	0.54±0.07	0.54±0.08	0.53±0.08	P=0.7942 ($F_{3,28}$=0.3434)
Left kidney	0.47±0.07	0.51±0.05	0.54±0.1	0.51±0.09	P=0.5334 ($F_{3,28}$=0.7467)
Right kidney	0.47±0.06	0.51±0.07	0.55±0.09	0.51±0.61	P=0.2035 ($F_{3,28}$=1.636)

Values are mean ± SD (n=8). *significant difference p<0.05.

Table 3. The effect of extract on organ weight relative to body weight of rats treated for 28 days.

Organ	Control (A)	250 mg/kg (B)	500 mg/kg (C)	1250 mg/kg (D)	Stat. (one-way ANOVA)
Liver	3.13E2±2.48E3	2.85E2±3.21E3	3.14E2±3.79E3	2.76E2±2.39E3	P=0.0321* ($F_{3,28}$=3.377)
Pancreas	2.65E3±1.09E3	3.19E3±4.26E4	3.26E3±6.13E4	3.21E3±5.87E4	P=0.3002 ($F_{3,28}$=1.279)
Heart	3.16E3±3.63E4	3.07E3±5.39E4	3.82E3±2.15E3	3.16E3±3.08E4	P=0.5356 ($F_{3,28}$=0.7428)
Left kidney	2.97E3±1.42E4	2.90E3±3.25E4	3.04E3±4.45E4	2.99E3±1.78E4	P=0.8252 (0.2999)
Right kidney	3.77E3±2.38E3	2.84E3±1.93E4	3.11E3±3.55E4	3.08E3±3.60E4	P=0.4820 ($F_{3,28}$=0.8428)

Values are mean ± SD (n=8). *significant difference p<0.05 E=exponential. *In stat column indicate column means is significantly greater than expected by chance.

changes noticed in the organ weights of rats as well as organ weight relative to body weight (Tables 2 and 3). The organs of both control and treated groups were unremarkable and comparable to each sex.

Sub acute toxicity hematological and biochemical observations

The hematological analysis (Table 4) showed no significant differences in some of the parameters examined in either the control or treated groups. But the white blood cell count (WBC) and the lymphocyte count had a dose dependent rise from the control, significantly ($p < 0.05$). There was a rise in the red blood cell count (RBC) and packed cell volume (PCV), though insignificantly in the extract treated groups. There was a significant difference of the treatment groups from the control in all the liver biochemical parameter except for total protein and albumin that showed none (Table 5). Alkaline phosphatase showed a highly significantly dose dependant rise with $p < 0.001$ in the 1250 mg/kg group. The ALT in all the treatment groups showed a highly sig-nificant dose dependant rise with $p < 0.001$ on comparing with control. The AST showed a highly significant dose dependent rise with $p < 0.001$ in the 500 and 1250 mg/kg groups.

Total bilirubin also had a highly significantly dose dependent rise with $p < 0.001$ in all the treatment groups. But the conjugated bilirubin had a significant difference from the control in both the 500 and the 1250 mg/kg groups. The cholesterol also had a significant ($p < 0.001$) dose dependent rise between the treatment groups and the control (Table 5). The electrolyte urea and creatinine results all showed a significant rise, dose dependently from the control (Table 6). Sodium had a rise that was highly significant ($p < 0.001$) in the 500 and 1250 mg/kg groups. Potassium was highly significant ($p < 0.001$) in the 1250 mg/kg group and only significant ($p < 0.05$) in

Table 4. Heamatological parameters of rats treated with extract for 28 days.

Parameter	Control (A)	250 mg/kg (B)	500 mg/kg (C)	1250 mg/kg (D)	Stat. (one-way ANOVA)
RBC×10^{12}/L	5.95±0.57	5.92±1.83	6.78±0.65	7.04±0.43	P=0.2499 ($F_{3,21}$=1.476)
RDW%	21.38±6.68	20.69±4.44	22.33±3.76	19.78±2.06	P=0.8004 ($F_{3,22}$=0.3347)
MCVFl	65.98±8.30	60.21±9.24	60.63±4.64	57.7±9.83	P=0.3904 ($F_{3,22}$=1.050)
PCV%	39.80±2.76	34.41±8.98	41.21±5.66	40.58±7.12	P=0.2207 ($F_{3,22}$=1.888)
HGB	12.40±0.56	11.10±2.83	12.43±1.33	13.5±1.12	P=0.1732 ($F_{3,21}$= 1.827)
WBC×10^{9}/L	2.80±1.63	3.46±2.00	6.51±1.79*	6.66±2.61*	P=0.0029* ($F_{3,22}$=6.351)
Lym×10^{9}/L	2.57±1.44	3.20±1.94	6.00±1.71*	5.82±2.31*	P=0.0046* ($F_{3,22}$=5.765)
Gran×10^{9}/L	0.10±0.2	0.10±0.14	0.24±0.18	0.28±0.13	P=0.1417 ($F_{3,28}$=2.012)
PLT×10^{9}/L	308.33±112.6	325.50±68.48	346.43±99.59	288.00±96.37	P=0.7399 ($F_{3,28}$=0.4208)

Values are mean ± SD (n=8). *significant difference(p<0.05) **significant difference (p<0.01) and ** *significant difference(p<0.001). *In stat column indicate column means is significantly greater than expected by chance.

Table 5. Liver function test of rats treated with extract for 28 days

Parameter	Control (A)	250 mg/kg (B)	500 mg/kg (C)	1250 mg/kg (D)	Stat. (one-way ANOVA)
Alkaline phosphatase	140.76±6.73	144.00±7.42	150.96±6.41	208.05±46.69***	P=0.0001* ($F_{3,28}$=13.847)
SGPT u/L (ALT)	14.22±1.39	18.36±1.90***	20.10±1.39***	21.08±0.87***	P=0.0001* ($F_{3,28}$=35.646)
SGOT u/L (AST)	123.10±5.19	138.46±4.05	306.2±72.48***	295.98±147.76***	P=0.0001* ($F_{3,28}$=16.24)
Conjugated bilirubin (µmol/L)	0.12±0.01	0.149±0.031	0.154±0.018*	0.154±0.019*	P=0.0150* ($F_{3,28}$=4.14)
Total bilirubin (µmol/L)	0.29±0.06	0.44±0.05***	0.46±0.09***	0.52±0.03***	P=0.0001* ($F_{3,28}$=21.215)
Total protein (g/l)	5.19±0.47	5.31±0.36	5.16±0.38	5.30±0.30	P=0.8100 ($F_{3,28}$=0.3211)
Albumin (µmol/L)	2.31±0.29	2.33±0.20	2.41±0.29	2.46±0.18	P=0.5662 ($F_{3,28}$=0.6894)
Cholesterol (mmol/L)	120.50±2.67	127.25±3.92**	129.88±4.39***	131.88±1.73***	P=0.0001* ($F_{3,28}$=17.587)

Values are mean ± SD (n=8). *significant difference (p<0.05) and ***significant difference (p<0.001). *In stat column indicate column means is significantly greater than expected

Table 6. Renal biochemical parameters of rats treated with extract for 28 days.

Parameter	Control (A)	250 mg/kg (B)	500 mg/kg (C)	1250 mg/kg (D)	Stat. (one-way ANOVA)
Sodium (Mmol)	133.25±1.83	138.00±2.27	146.37±5.48***	143.88±±5.41***	P=0.0001* ($F_{3,28}$=16.477)
Potassium (Mmol)	3.56±0.34	4.19±0.48	4.24±0.35*	4.64±0.68***	P=0.00014* ($F_{3,28}$=6.813)
Bicarbonate (Mmol)	20.63±0.52	20.25±0.71	20.00±0.76	19.25±1.04**	P=0.0109* (4.477)
Urea (mg/dl)	5.96±0.61	7.78±0.75	10.81±1.75***	17.44±2.78***	P=0.0001* ($F_{3,28}$=69.270)
Creatinine (mg/dl)	1.10±0.09	1.26±0.11	1.50±0.13*	2.50±0.50***	P=0.0001* ($F_{3,28}$=46.512)

Values are mean ± SD (n=8). *significant difference(p<0.05) **significant difference (p<0.01) and ***significant difference(p<0.001).

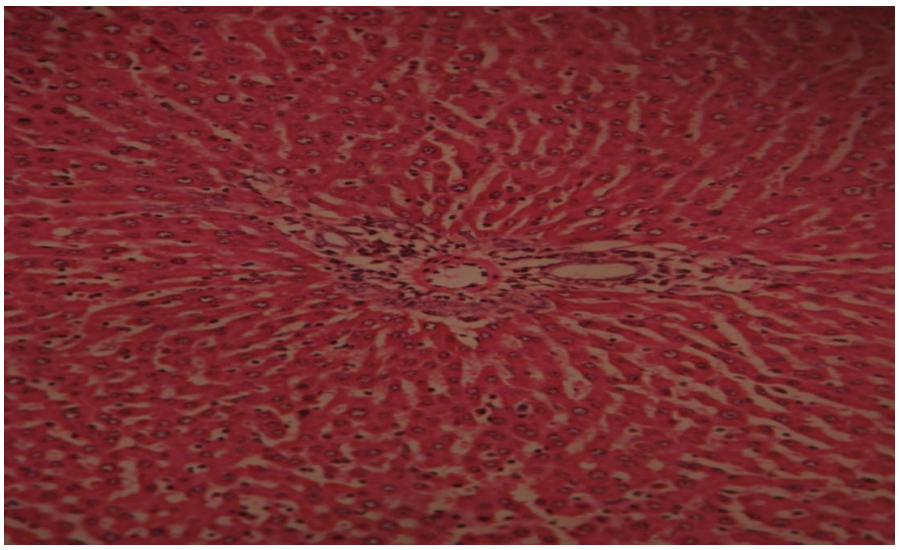

Figure 1. Photomicrograph of liver with portal tract(portal vein, hepatic artery and bile duct. H&E ×20.

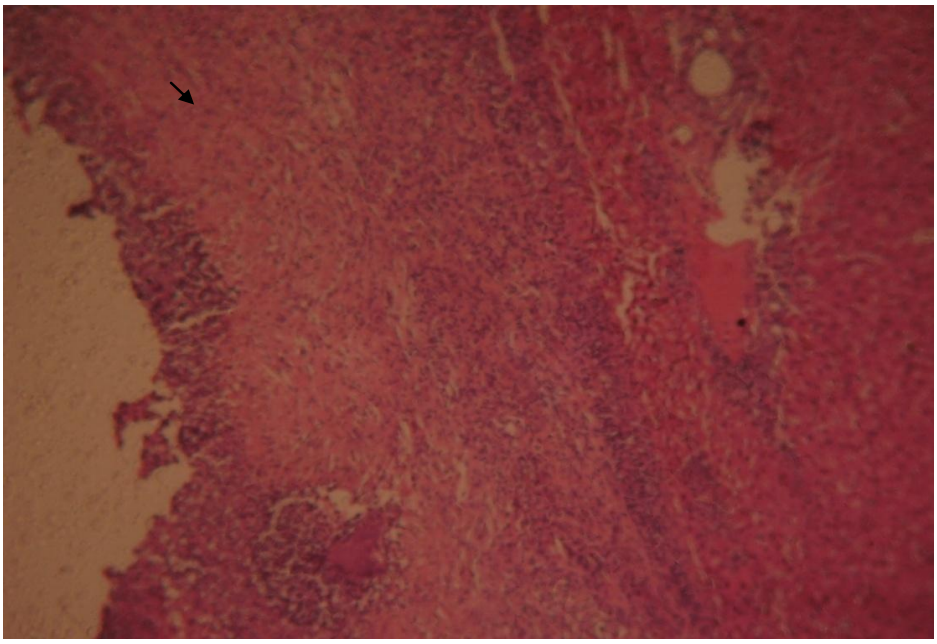

Figure 2. Photomicrograph of liver showing cyst with a mass of actinomycosis. H&E ×20.

the 500 mg/kg group. Bicarbonates had a fall in its level that was very significant ($p < 0.01$) in the 1250 mg/kg group. Urea level showed a highly significant ($p < 0.001$) rise in its 500 and 1250 mg groups. Creatinine was highly significant ($p < 0.001$) in the 1250 mg group and only significant ($p < 0.05$) in the 500 mg group.

Histopathological tissue analysis for the sub acute study

In the liver, the control group had a normal hepatic architecture maintained (Figure 1). The 250 mg/kg dose group was normal as in control. The 500 mg/kg dose group showed a cyst (actinomycosis) and others with mild hepatic necrosis (Figures 2 and 3). The 1250 mg/kg dose group showed hepatic fatty change (Figure 4) and vacuolar degeneration (Figure 5). The kidney of the rats treated with 250 and 500 mg/kg groups both had no pathological changes in the renal tissues (Figure 6). But the 1250 mg/kg group showed acute tubular necrosis (tubulorrhexis) (Figure 7). There were no obvious histopathological changes seen in both the heart and the pancreas of the rats (Figures 8 and 9), respectively.

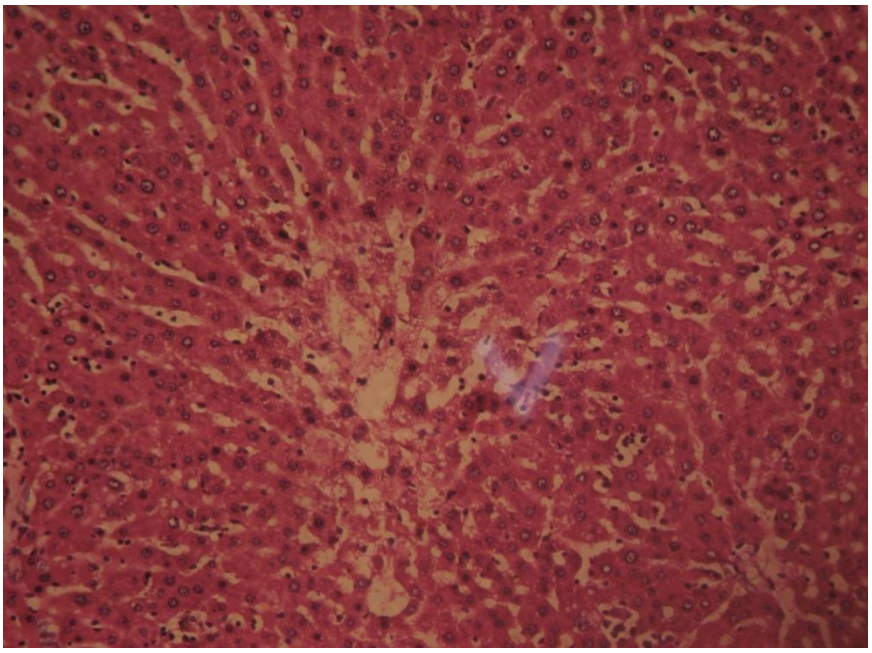

Figure 3. Photomicrograph of liver showing mild hepatocyte necrosis. H&E ×20.

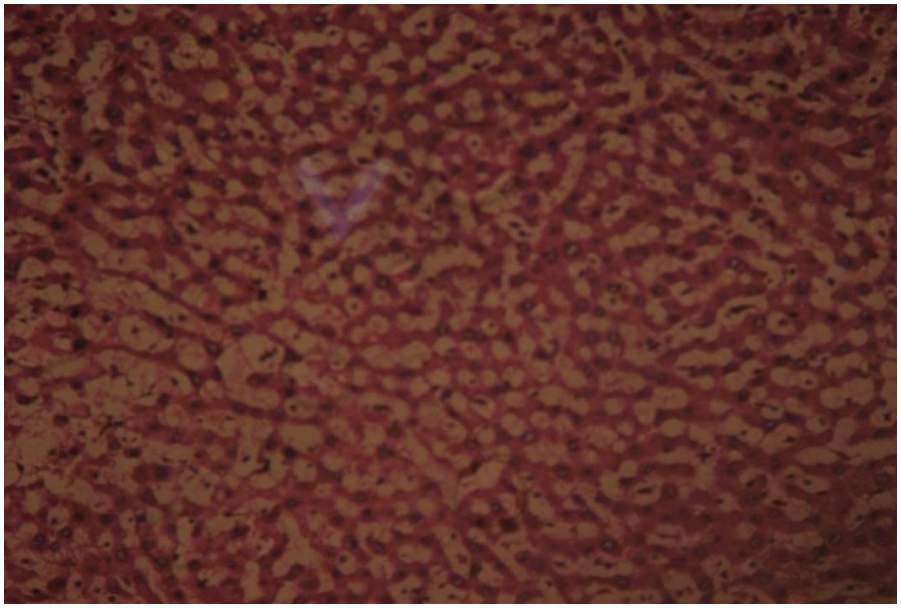

Figure 4. Photomicrograph of liver showing fatty change. H&E ×20.

Chronic toxicity study

There were changes and deaths in the treatment groups recorded over the 90 days study. The rats in the highest dose (3750 mg/kg) group had diarrhea/soft stool as from the 6th week of treatment and they were less active than the other groups. The rats that died in the low dose (1250 mg/kg) group did not show any sign of illness, and the deaths occured from the 20th day of study while those that died in the highest dose group (3750 mg/kg) were inactive for several days before dying (death occurring from the 34th day of study). There was no death recorded in the 2500 mg/kg dose and control groups. The weekly weight of the rats did not show any significant difference but the mean weight gain of rats over the period of study showed that there was a significant

Figure 5. Photomicrograph of liver showing vacuolar degeneration. H&E ×40.

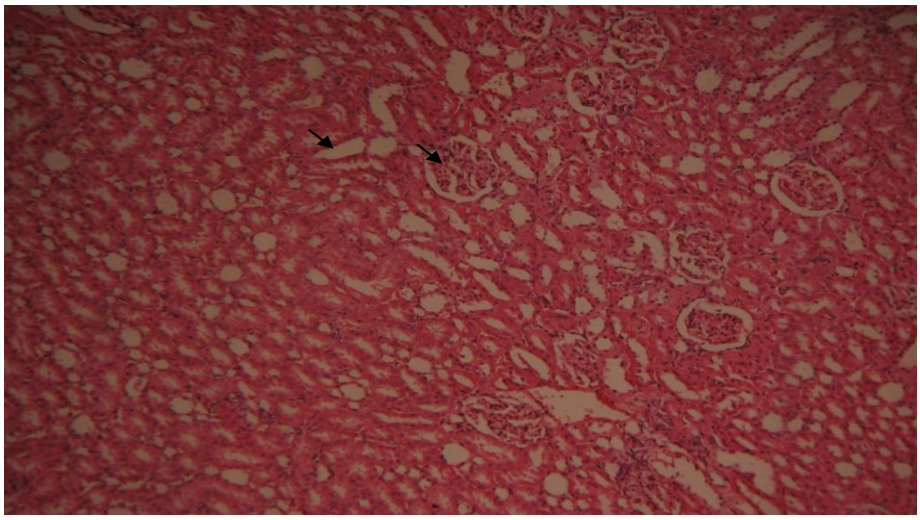

Figure 6. Photomicrograph of kidney showing normal tubules and glomeruli. H&E ×20.

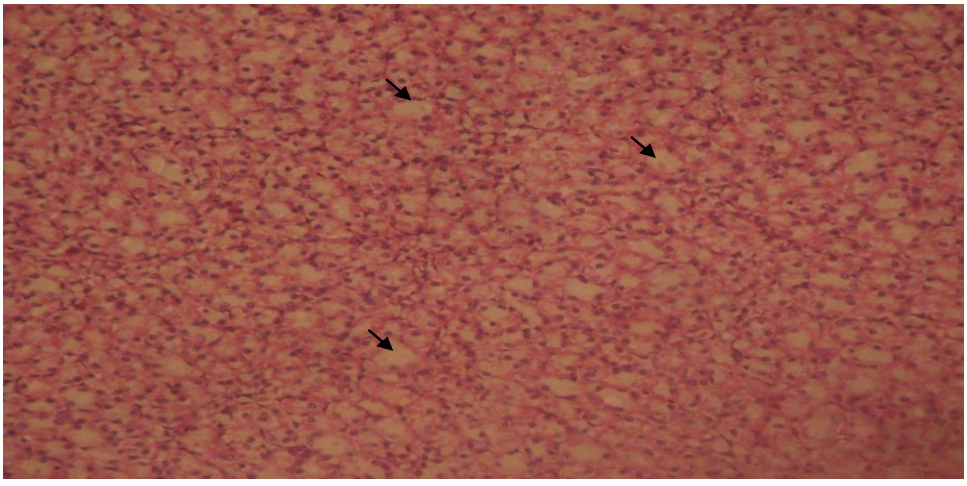

Figure 7. Photomicrograph of kidney showing tubular necrosis (tubulorrhexis). H&E ×20.

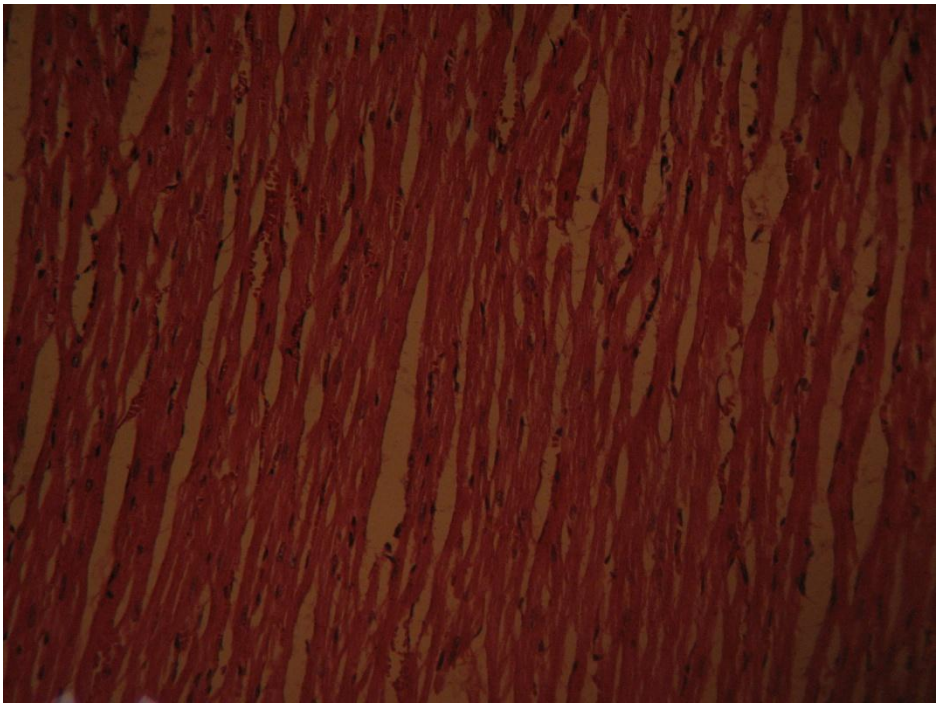

Figure 8. Normal cardiac muscle. H&E ×20.

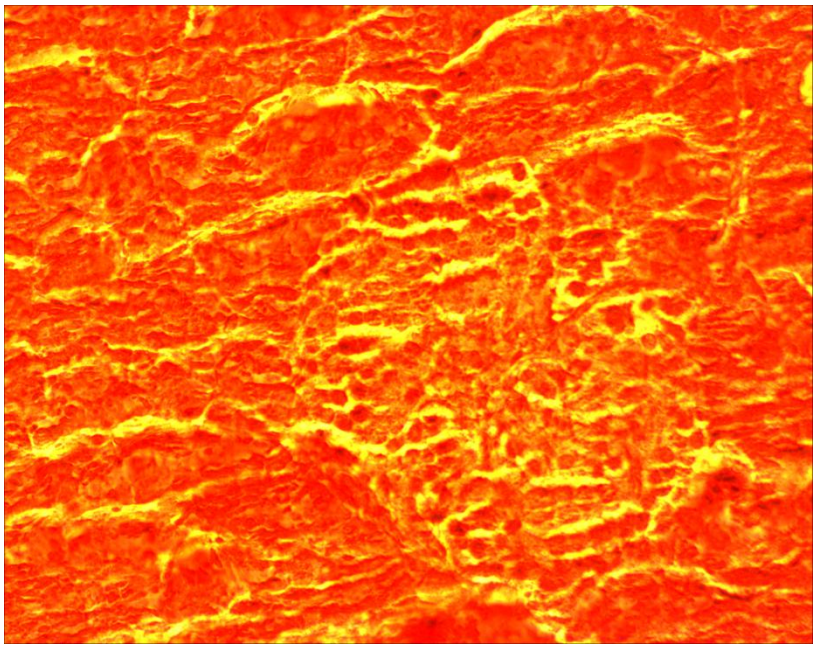

Figure 9. Normal pancreas. H&E ×20.

difference within the groups (Table 7). The rats in the medium dose group had the lowest weight gain that was of statistical significance. The control group had the highest weight gain. The heart of the rats was noticed to have reduced in weight significantly dose dependently (Table 8); the smallest being the 3750 mg/kg group and next being the 2500 mg/kg group significantly. The control group's heart was noticed to be larger than that of the treated groups. Other organ weights did not have any significant change within the groups. The trend was the same in the organ weight relative to body weight. The heart being of significance in organ weight relative to body weight in both the 1250 and 2500 mg/kg groups dose dependently (Table 9).

Table 7. The Effect of extract on the body weight (g) of rats treated for 90 days.

Week	Control A	Low dose (1250 mg) B	Medium dose (2500 mg) C	High dose (3750 mg) D	Stat. (one-way ANOVA)
0	168.41±38.41	180.35±35.54	198.88±43.02	179.15±28.99	P=0.4333 ($F_{3,28}$=0.9426)
1	183.11±35.65	182.41±42.54	194.78±47.88	173.20±27.81	P=0.7492 ($F_{3,28}$=0.4069)
2	197.40±34.33	193.88±43.39	198.46±50.60	176.74±31.89	P=0.6900 ($F_{3,28}$=0.4930)
3	199.01±35.17	205.22±52.41	202.96±53.42	177.89±35.24	P=0.6073 ($F_{3,28}$=0.6211)
4	214.33±38.90	221.81±40.90	207.55±57.02	172.71±35.48	P=0.1540 ($F_{3,28}$=1.897)
5	218.06±34.38	219.91±37.04	210.31±55.51	181.43±29.69	P=0.2779 ($F_{3,26}$=1.357)
6	232.84±30.03	225.07±36.71	217.84±64.88	182.24±28.64	P=0.1505 ($F_{3,26}$=1.924)
7	232.23±26.85	228.77±41.08	211.83±59.17	189.24±29.822	P=0.2096 ($F_{3,26}$=1.617)
8	228.94±31.85	234.73±41.19	216.91±61.59	188.9±36.87	P=0.2443 ($F_{3,26}$=1.476)
9	234.05±31.01	244.34±49.41	224.59±73.08	190.87±41.23	P=0.2563 ($F_{3,26}$=1.431)
10	236.11±30.77	250.54±52.44	229.58±71.57	205.12±42.49	P=0.4811 ($F_{3,25}$=0.8472)
11	239.38±29.71	257.43±56.53	228.35±71.94	207.58±43.44	P=0.4376 ($F_{3,24}$=0.9382)
12	241.75±31.73	258.88±58.03	226.35±70.74	212.05±44.54	P=0.4670 ($F_{3,24}$=0.8766)
13	248.37±33.04	258.52±54.47	228.53±67.42	215.80±49.03	P=0.4796 ($F_{3,24}$=0.8514)
Mean weight	219.69±31.28	228.40±45.92	214.03±59.60	193.72±35.47	P=0.5884 ($F_{3,24}$=0.6538)
Mean weight gain	83.00±26.68	74.65±34.55	29.57±39.98*	32.85±34.28	P=0.0096* ($F_{3,24}$=4.759)

Values are mean ± SD (n=8). *significant difference (p<0.05).

Table 8. Effect of extract on organ weight (g) of rats treated for 90 days.

Organ	Control A	Low dose (1250 mg) B	Medium dose (2500 mg) C	High dose (3750 mg) D	Stat. (one-way ANOVA)
Pancreas	0.56±0.09	0.64±0.16	0.61±0.13	0.67±0.08	P=0.3586 ($F_{3,24}$=1.125)
Right kidney	0.78±0.07	0.83±0.17	0.76±0.19	0.67±013	P=0.3252 ($F_{3,24}$=1.217)
Left kidney	0.79±0.05	0.80±0.17	0.72±0.20	0.64±0.13	P=0.2601 ($F_{3,24}$=1.425)
Liver	7.09±0.94	7.55±1.76	6.89±1.80	7.08±1.61	P=0.8815 ($F_{3,24}$=0.2200)
Heart	0.92±0.09	0.81±0.19	0.71±0.15*	0.68±0.08*	P=0.0086* ($F_{3,24}$=4.883)

Values are mean ± SD (n=8). *significant difference(p<0.05)* In stat column indicate column means is significantly greater than expected by chance.

Table 9. Effect of extract on organ weight relative to body weight of rats treated for 90 days.

Organ	Control A	Low dose (1250 mg) B	Medium dose (2500 mg) C	High dose (3750 mg) D	Stat. (one-way ANOVA)
Pancreas	2.33 3±7.36E4	2.48 E 3±2.8E4	2.78 E3±7.52E4	3.18 E3±5.25E4	P=0.09 ($F_{3,24}$=2.372)
Right kidney	3.06 E±5.15E4	3.22E3±3.40E4	3.36 E3±2.15E4	3.11 E3±1.85E4	P=0.3803 ($F_{3,24}$=1.07)
Left kidney	3.22E3±4.37E4	3.10E3±3.02E4	3.18 E3±3.05E4	2.96 E3±2.23E4	P=0.5194 ($F_{3,24}$=0.7750)
Liver	0.0286±0.0025	0.0292±0.0036	0.0286±0.0040	0.0337±0.0100	P=0.2964 ($F_{3,24}$=1.303)
Heart	3.75E3±4.79E4	3.11E3±2.91E4*	3.15 E3±3.25*	3.24 E3±5.39E4	P=0.0237* ($F_{3,24}$=3.778)

Values are mean ± SD (n=8). *significant difference(p<0.05). *In stat column indicate column means is significantly greater than expected by chance.

The WBC showed a highly significant rise in its level in the 3750 mg/kg dose group. All other haematological parameters were of no significant difference (Table 10). The liver, SGPT (ALT), showed a dose dependent rise in its level that was significant in the 3750 mg/kg dose group (Table 11). So did SGOT (AST) have a dose dependent rise in its levels that was significant in the 2500 and 3750 mg/kg groups. Albumin was severely raised raised significantly in the 3750 mg/kg group (Table 11). Cholesterol rather had a dose dependent fall in its level that was highly significant in the low dose (1250 mg/kg) group (Table 11). Renal biochemical parameter showed that sodium, potassium and urea level all had a significant change in their levels. The sodium had a drop while potassium and urea had rather a rise in their levels (Table 12).

Table 10. Heamatological parameters of rats treated with extract for 90 days.

Parameter	Control (A)	Low dose B	Medium dose C	High dose D	Stat. (one-way ANOVA)
RBC×10^{12}/L	7.17±0.64	6.65±0.28	6.73±0.40	6.99±0.92	P=0.3855 ($F_{3,21}$=1.064)
RDW%	16.71±0.77	15.34±0.28	16.43±0.98	17.60±1.80	P=0.0219* ($F_{3,21}$=3.966)
MCVFl	49.03±1.13	47.22±1.33	49.65±1.41	47.42±4.82	P=0.2478 ($F_{3,21}$=1.484)
PCV%	35.11±2.85	31.56±2.03	33.43±2.20	33.00±3.87	P=0.1844 ($F_{3,21}$=1.766)
HGB	12.91±1.00	11.68±0.84	12.33±0.80	12.26±1.53	P=0.2539 ($F_{3,21}$=1.460)
WBC×10^{9}/L	7.43±2.40	7.04±0.67	7.31±3.41	19.00±8.46***	P=0.0003* ($F_{3,21}$=9.650)
Lym×10^{9}/L	5.31±2.38	5.40±0.85	5.07±2.05	10.63±7.07	P=0.0521 ($F_{3,21}$=3.03)
Gran×10^{9}/L	1.29±0.69	0.90±0.37	1.43±0.37	3.74±4.45	P=0.1453 ($F_{3,21}$=1.997)
PLT×10^{9}/L	462.88±54.61	431.00±39.45	402.00± 57.70	475.60± 43.25	P=0.0727 ($F_{3,21}$=2.686)

Values are mean ± SD (n=8). *significant difference(p<0.05). ***significant difference(p<0.001). *In stat column indicate column means is significantly greater than expected by chance.

Table 11. Liver function test of rats treated for 90 days with extract.

Parameter	Control A	Low dose (1250 mg) B	Medium dose (2500 mg) C	High dose (3750 mg) D	Stat (one-way ANOVA)
Alkaline phosphatase (u/L)	48.63±13.04	25.20±25.94	42.60±16.16	54.17± 25.06	P=0.1453 ($F_{3,24}$=1.987)
SGPT u/L (ALT)	15.00±5.90	28.00±12.59	22.00±11.99	39.83± 24.78*	P=0.0328* ($F_{3,24}$=3.462)
SGOT u/L (AST)	32.50± 9.70	64.80± 28.39	73.63± 30.13*	73.83±36.02*	P=0.0192* ($F_{3,24}$=4.034)
Conjugated bilirubin (µmol/L)	0.68±0.25	0.14±0.00	0.39±0.53	0.38±0.28	P=0.1149 ($F_{3,24}$=2.215)
Total bilirubin (µmol/L)	1.27±0.31	0.72±0.42	1.44±0.41	3.32±4.41	P=0.1884 ($F_{3,23}$=1.732)
Total protein (g/L)	6.78±0.93	6.48±1.27	6.62±0.27	6.17±1.11	P=0.6651 ($F_{3,24}$=0.5314)
Albumin (µmol/L)	2.42±0.30	2.36±0.63	2.67±0.51	4.17±1.32**	P=0.0006* ($F_{3,22}$=8.293)
Cholesterol (mmol/L)	43.75±10.61	107.5±15.00***	56.25± 10.61	40.00±10.95	P=0.0001* ($F_{3,22}$=34.321)

Values are mean ± SD (n=8). *significant difference (p<0.05). **significant difference (p<0.01).

Table 12. Renal biochemical parameters of rats treated with extract for 90 days.

Parameter	Control A	Low dose B	Medium dose C	High dose D	Stat. (one-way ANOVA)
Sodium (Mmol)	148.50± 7.93	136.80±5.40**	131.38± 1.51***	136.00± 2.45***	P=0.0001* ($F_{3,23}$=16.004)
Potassium (Mmol)	4.35±1.00	6.66± 2.35	5.03±1.25	7.50±2.29*	P=0.009* ($F_{3,23}$=4.886)
Biocarbonate (Mmol)	21.00±1.77	21.17±2.99	22.00±2.88	22.17±1.72	P=0.7460 ($F_{3,24}$=0.4119)
Urea (mg/dl)	79.11±29.26	88.30±42.63	69.56±29.77	152.33±68.10*	P=0.0086* ($F_{3,24}$=4.887)
Creatinine (mg/dl)	0.84±0.42	0.75±0.29	0.70±0.26	0.55±0.05	P=0.3695 ($F_{3,24}$=0.3695)

Values are mean ± SD (n=8). *significant difference (p<0.05) **significant difference (p<0.01) and ***significant difference (p<0.001).

Histopathological tissue analysis for chronic toxicity

In the liver, the control group had a normal maintained hepatic architecture (Figure 10). The treatment groups all showed congestion of portal vein (Figure 11), portal triaditis (Figure 12) and multifocal hepatocyte necrosis (Figure 13). In the kidney, the control group showed a normal cortex and medulla with normal glomeruli and tubules (Figure 14). The 1250 and 2500 mg/kg groups showed mild focal lyphoid aggregation within the medulla (Figure 15). The 3750 mg/kg group showed multifocal multifocal areas of lyphoid aggregations (Figure 16)

The heart was normal histologically

In the pancreas, the control group displayed a normal endocrine and exocrine pancreatic architecture (Figure 17) rich with large islet cell clusters. The 1250 mg/kg group pancreatic tissue was as in the control. The 2500 mg/kg group showed a normal architecture but with fewer islet cell clusters noticed (Figure 18). These islet cells clusters were far much fewer in the 3750 mg/kg group

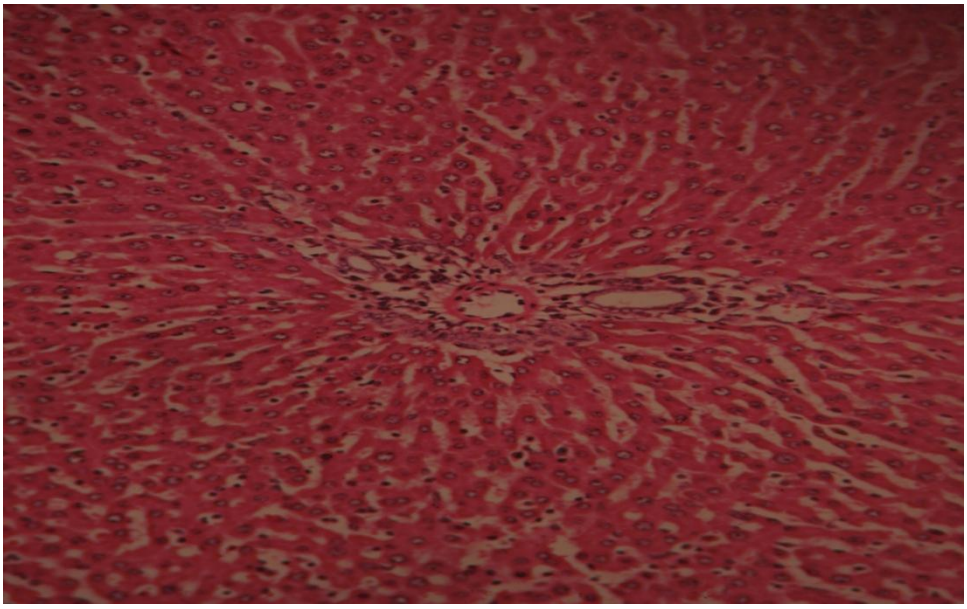

Figure 10. Normal hepatic tissue. H&E ×20.

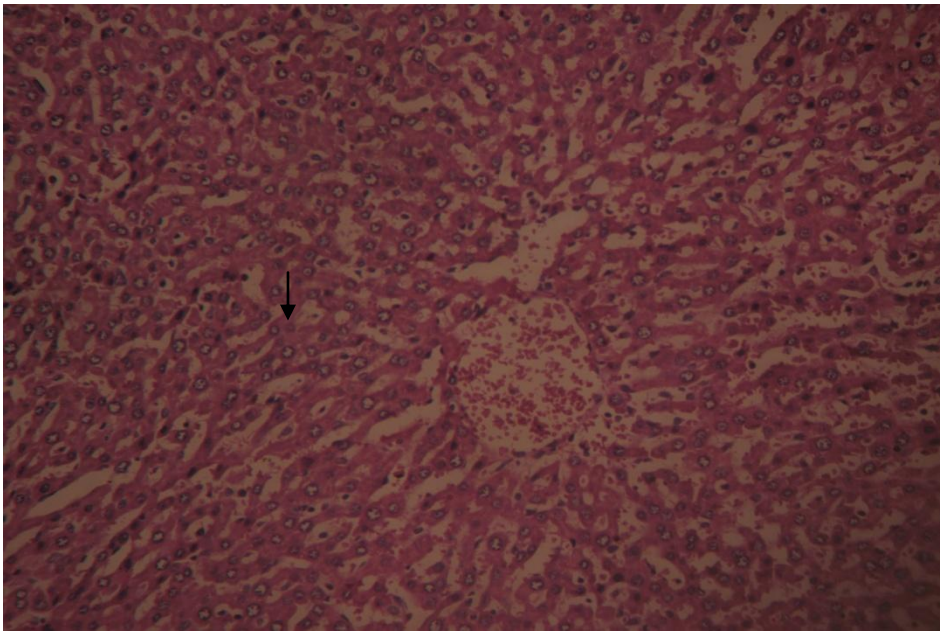

Figure 11. Photomicrograph of liver showing mild congestion of portal vein and background sinusoidal congestion. H&E ×20.

(Figures 19 and 20).

DISCUSSION

Previous toxicity studies gave much reference to the adverse effect of *C. olitorius* seeds on the heart. In this study, there were no deaths recorded following a single oral administration of 5 g/kg of ethanolic seed extract of *C. olitorius*. The LD_{50} of the extract was therefore estimated to be greater than 5000 mg/kg in albino rats. In earlier studies, the LD_{50} of a 10% alcoholic extract of the seeds in water were 0.75 g/kg BW in mice and 6 g/kg BW in toads (Sharaf and Negm, 1969). The test limit of 5000

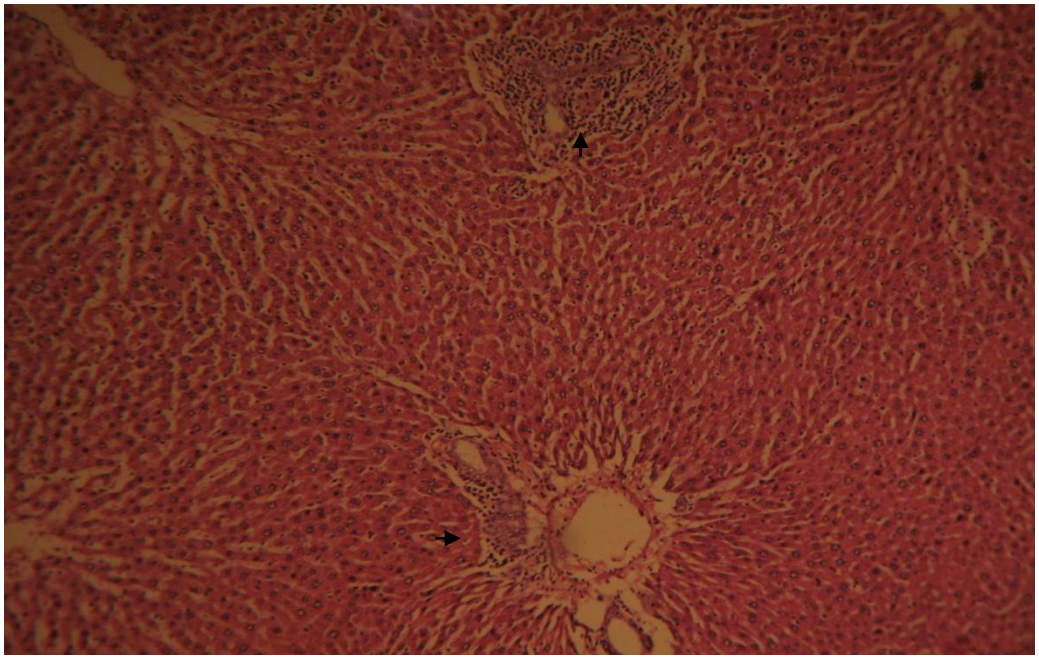

Figure 12. Photomicrograph of liver showing portal triaditis. H&E ×20.

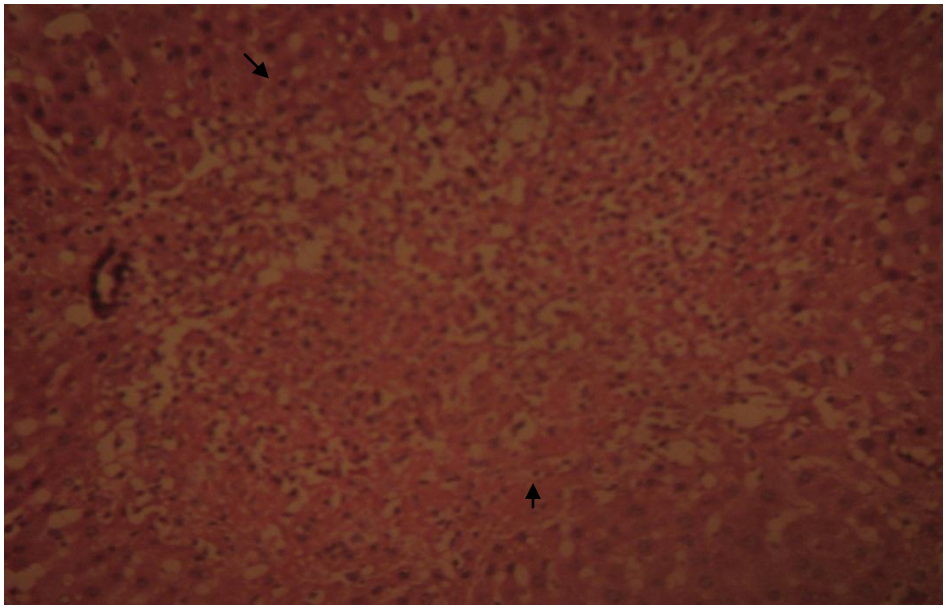

Figure 13. Photomichrograph of liver showing hepatocyte necrosis. H&E ×20.

mg/kg for acute oral toxicity is generally considered to be the point, at which it can be concluded that a test substance is practically non-toxic or non-lethal after an acute exposure (OECD, 2001).

In the sub acute toxicity study, in which rats were treated orally with 250, 500 and 1250 mg/kg of ethanolic seed extract of *C. olitorius* for 28 days, there were no deaths recorded also, indicating further the safety profile at these doses. But there were deaths recorded in the chronic toxicity study where even higher doses were employed (in the bid to exclude toxicity) over 90 days, at 1250, 2500 and 3750 mg/kg levels. The rats that died in the low dose (1250 mg/kg) group were believed to have died from fighting, since they were apparently healthy the previous day. While those that died in the highest dose group (3750 mg/kg) obviously resulted from the effect of

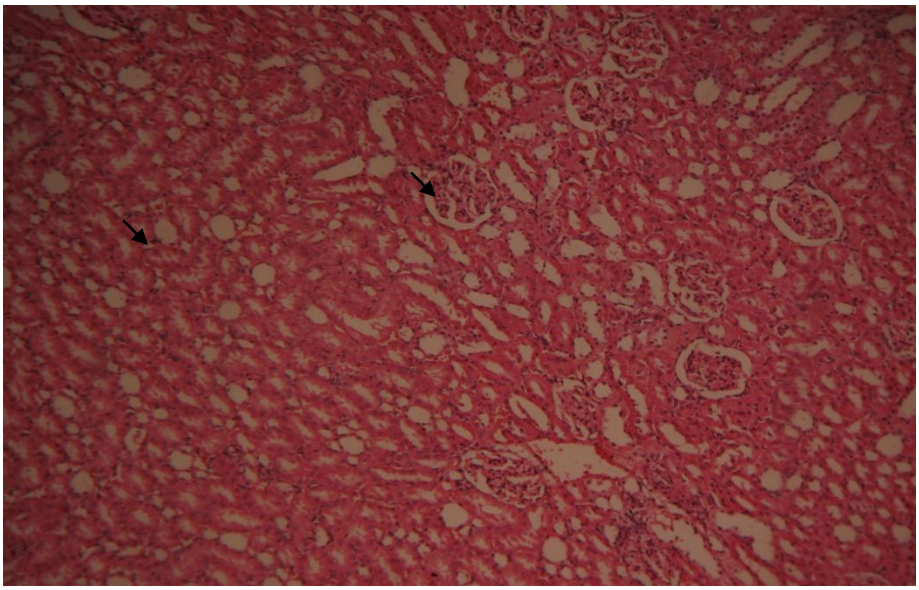

Figure 14. Photomicrograph of kidney showing normal glomeruli and tubules. H&E ×20.

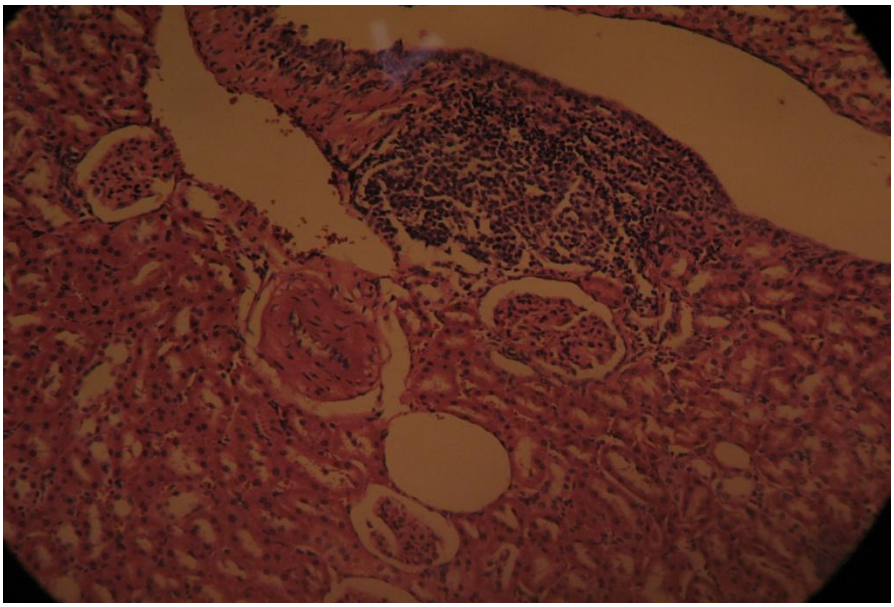

Figure 15. Photomicrograph showing lymphocytic infiltration of the kidney parenchyma. H&E ×20.

the seed extract of *C. olitorius* being inactive for several days before dying. The probable cause of deaths could be from the cardiotoxic effect of *C. olitorius* seed (Sharaf and Negm, 1969). The extract seems to produce a reduction in weight gained by the animals as it was noticed in the 90 days study that rats in the highest dose (3750 mg) and medium dose (2500 mg) group had a lower weight gain than the low dose (1250 mg) group. The control group had the highest weight gain.

A healthy weight loss is known to improve health in many ways (reduce blood pressure, lower cholesterol level and reduce risk of developing type 2 diabetes) (Stephen, 2006). Generally there were no obvious changes in the organ weight and organ weight relative to body weight of the rats in the 28 days study. In the chronic 90 days toxicity, only the heart was noticed to have reduced in weight significantly and dose dependently. The heart weight relative to body weight in both the 1250 and the 2500 mg/kg

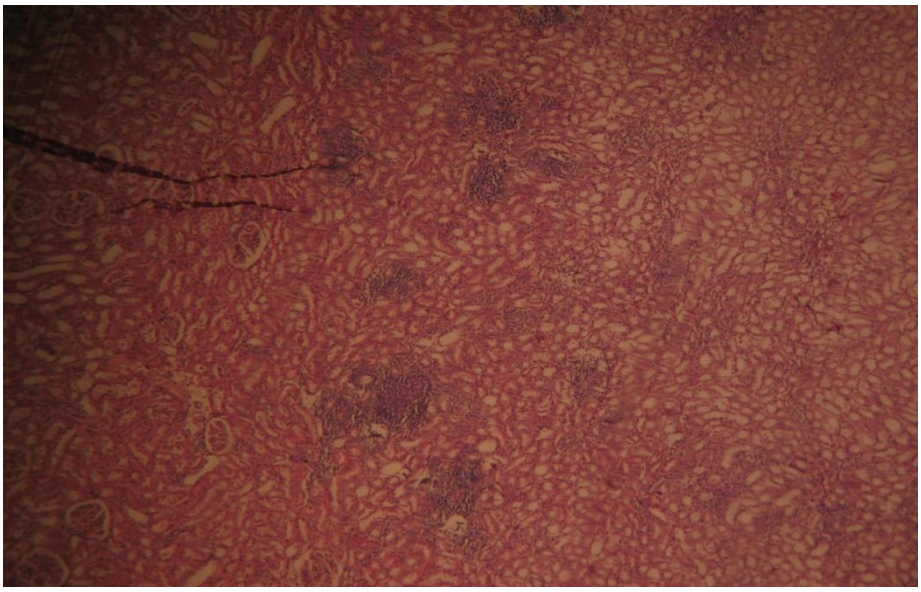

Figure 16. Photomicrograph showing multifocal lymphocytic infiltration of the kidney parenchyma. H&E ×20.

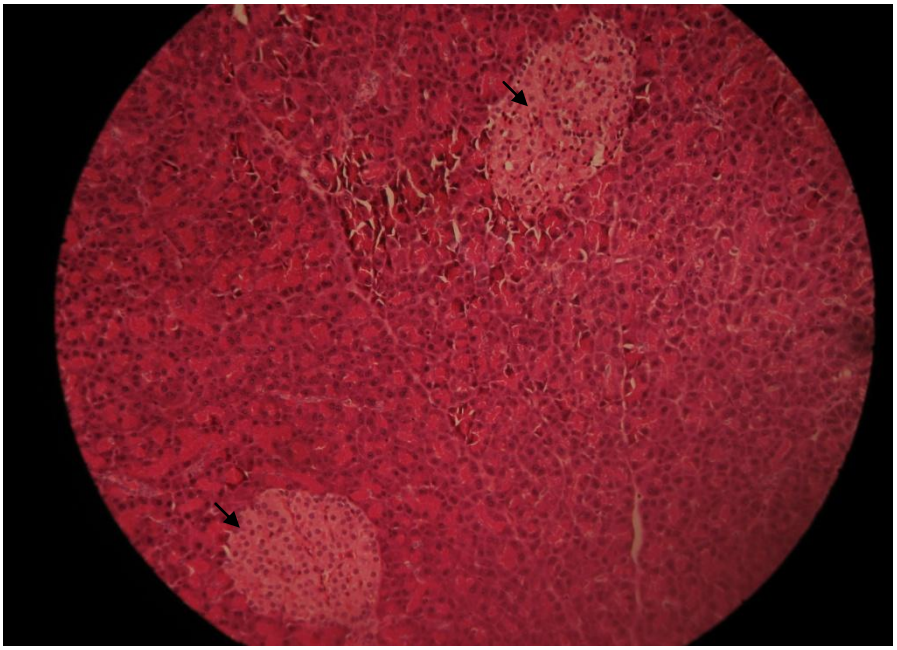

Figure 17. Photomicrograph of pancreas showing endocrine(2 cluster or islet cells) and exocrine pancreas. H&E ×20.

groups were of significance, dose dependently. Toxic activity of *C. olitorius* have been attributed to olitorisides and corchoroside which are found to possess a strophanthin-like action on the heart (Sharaf and Negm, 1969). The histological report showed the heart as being normal in both the 28 and 90 days study. The cholesterol in the 28 days toxicity study had a significant dose dependent rise between the treatment groups and the control. But in the 90 days study, the cholesterol rather had a dose dependent fall in its level that was highly significant in the low dose (1250 mg/kg) group. These cholesterol results were difficult to explain.

The white blood cell count (WBC) and the lymphocyte count had a dose dependent rise from the control significantly, in the 28 days sub acute toxicity study, indicating an effect of the ethanolic seed extract of *C. olitorius*; this

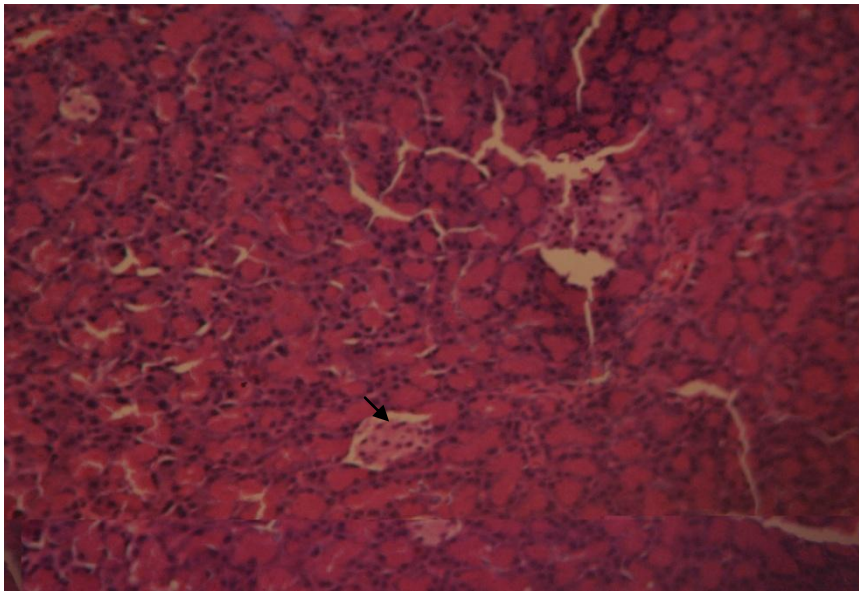

Figure 18. Photomicrograph of pancreas showing smaller cluster of islet cells. H&E ×20.

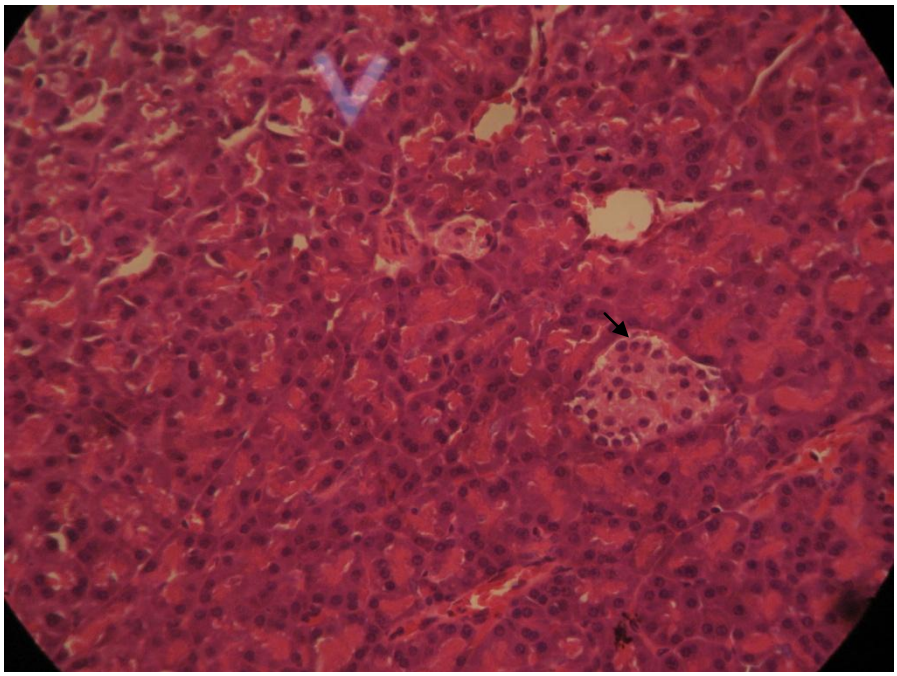

Figure 19. Photomicrograph of pancreas showing fewer clusters of islet cells. H&E ×20.

was supported by the fact that the WBC showed a highly significant rise in its level in the 3750 mg/kg dose group, in the 90 days toxicity study. There was a rise in the red blood cell count (RBC) and packed cell volume (PCV), though insignificantly. It would be in place to conclude that the extract has boosted haemopoitic cells production and may alleviate aneamic condition and raised immune response. This inference may support report of folklore use of *C. olitorius* in the treatment of gonorrhea, chronic cystitis, fever and tumours (Zeghichi et al., 2003) and Zakaria et al. (2006) report of *C. olitorius* anti inflammatory and anti pyretic activity in rats. The increase WBC and RBC may be due to the high content of iron and folate in *C. olitorius*, useful for prevention of aneamia

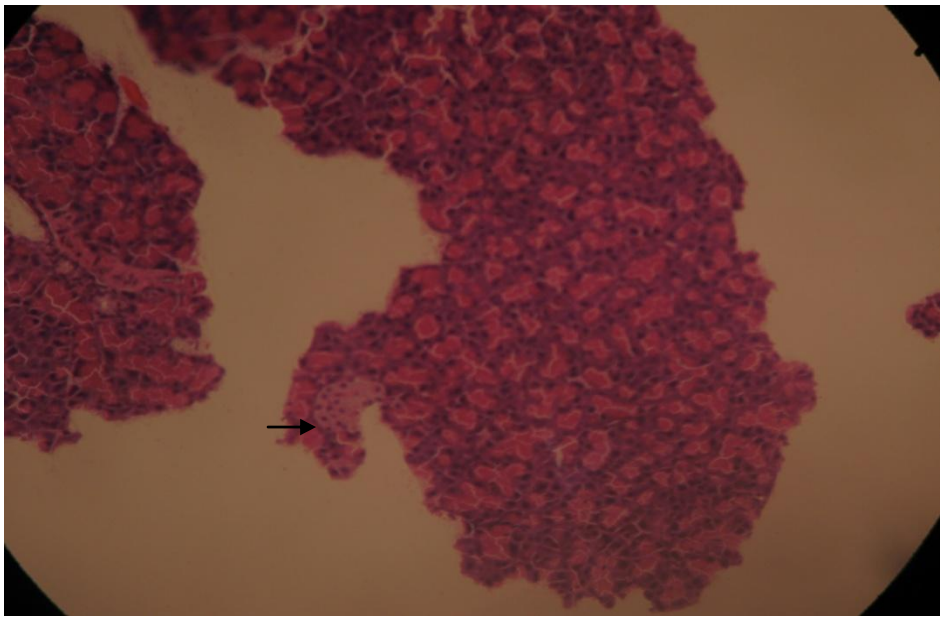

Figure 20. Photomicrograph of pancreas showing fewer clusters of islet cells. H&E ×20.

(Oyedele et al., 2006).

In the 28 days toxicity, there was a significant difference of the treatment groups from the control in all the liver biochemical parameter except for total protein and albumin that showed none. This was also the case in the 90 days study. These findings all indicates a dose dependent compromised hepatic milieu that was supported by the histological reports. Likewise, when *C. olitorius* seed protein enriched diet were fed to albino rats, slight fatty infiltration in the liver of test animals was seen, also observed was that AST, ALT and total lipid of liver increased significantly (Laskar et al., 1986).

The renal biochemical parameter in both the 28 days and the 90 days studies showed that sodium, potassium and urea level all had a significant change in their levels. The imbalance in the electrolyte may be due to the mineral elements content of the extract (Fe, Mg, Cu, Zn, Ca Na and K) found to be present in the plant leaf (Amanabo, 2012) and raised urea is evidence of renal affectation of the ethanolic seed extract of *C. olitorius* which was supported by the histological findings already mentioned.

The evaluation has shown that the ethanolic crude extract of *C. olitorius* seed may be safe following oral administration in albino rats especially at low doses (less than 1000 mg/kg). The evidences of compromised renal and hepatic milieu, supported by histological results at repeated high dose usage suffices for caution and selection of a proper dose in its use locally. The extract improved both the white and red cell count in rats, an indication that it may possess the potential to boost immunity and treat aneamia.

ABBREVIATIONS

AP, Alkaline phosphatase; **ALT,** alanine amino transferase; **AST,** aspartate amino transferase; **H&E,** heamatoxylin and eosin stain.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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