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Research Article

Toxicological Profile of Aqueous Root Extract of *Securidaca longepeduculata* Fresen (Polygalaceae) After 90-day Treatment in Rats

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ABSTRACT

The plant *Securidaca longepedunculata* (SLP) is indigenous to Africa and its bark, roots and seeds have been used in treating a wide spectrum of diseases.The present study explored the tissue/organ toxicity of the root extract after a single and repeated dose exposure of rodents. Doses of 20, 50, 100 and 200 mg/kg were administered intraperitoneally (i.p), while 1000, 3000, 5000 and 7000 mg/kg were given by oral intubation. General behavioural effects and mortality were determined for the initial 24 h, and thereafter for 14 days. In the 90-day toxicity study, 150, 350 and 700 mg/kg extract were given intragastrically to rats and the possible reversibility of the toxic effects due to the highest dose only was explored.Selected biochemical and haematological parameters were determined after 90 days, when the animals were humanely sacrificed and blood samples collected.A significant decrease in body weight was recorded in the SLP treated rats and both liver and kidney toxicities were observed. Median lethal doses via intraperitoneal and oral routes were 44.67 and 3162 mg/kg.SLP extract contained saponins, alkaloids, cardiac glycosides and flavonoids.

Key words: toxicity, liver, kidney, medicinal plant, rats, *Securidaca longepeduculata*

INTRODUCTION

The plant *Securidaca longepeduculata* (SLP), is native to Africa[1] and commonly known as Rhodesian violet, violet tree and also as *Ezeogwu*, *Ipeta* and *Uwar Magunguna* (''mother of drugs'') in Igbo, Yoruba and Hausa languages in Nigeria[2]. It is a slender, small to medium-sized tree that grows up to 6m tall, with characteristic pale grey smooth bark. It is reputed to have over one hundred medicinal uses including purgative, diuretic, emetic and diaphoretic effects. Also it is employed for treating conjunctivitis, malaria, rheumatism, cough, toothache, headache, and inflammation[3-5]. A host of Database reports have documented the toxicity of SLP, however, scientific validation of the latter is still very scanty and the few reports that are available are conflicting.The present study aimed at investigating SLP toxicity both after a single exposure and after repeated dosing over a period.

MATERIALS AND METHODS

Preparation of plant extract: Fresh root of SLP was collected from a farm at Ibadan and authenticated at the Forest Research Institute of Nigeria (FRIN) Ibadan, where a specimen was deposited at the herbarium and voucher No FHI 107126 obtained.The root was chopped into tiny pieces and sun-dried for a week after which 100 g was macerated for 48 h in distilled water at room temperature, with vigorous intermittent shaking, to facilitate extraction, after an initial boiling for 1h. The mixture was sieved though a white muslin cloth, and evaporated to dryness in an oven at 55 °C. Percentage yield was calculated as 5.0 %.

Animals: In-bred mice (15-20g) and Sprague-Dawley rats (150-200g) maintained at standard laboratory conditions and fed with pelleted diet (Neimeth Livestock Feeds, PLC, Ikeja-Lagos, Nigeria), having free access to water *ad-libitum* were used in the study. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC).

Acute Toxicity Test: Both intraperitoneal (i.p) and oral routes were employed to evaluate the toxicity due to a single exposure to the drug and the median lethal dose (LD_{50}) of the herbal preparation extrapolated from probit versus log-dose curve[6].

Phytochemical screening: Simple chemical tests[7] were carried out on SLP extract to identify the phytoconstituents in SPH.

Chronic Toxicity Study: Groups of rats, randomly selected were administered orally with SLP in doses of 150, 350 and 700 mg/kg, while the control received 10 ml/kg distilled water. The group that received 700 mg/kg were divided into two sub-groups A and B for reversibility study.

All the rats were daily treated according to the dosing schedule for 90 days[8], and mean body weight determined every two weeks. On day 91, all the animals excluding sub-group B having fasted them overnight, were humanely sacrificed and blood samples (serum) collected for haematological and biochemical assays. The liver and kidney, being the two major organs for drug elimination were excised and prepared for histopathological

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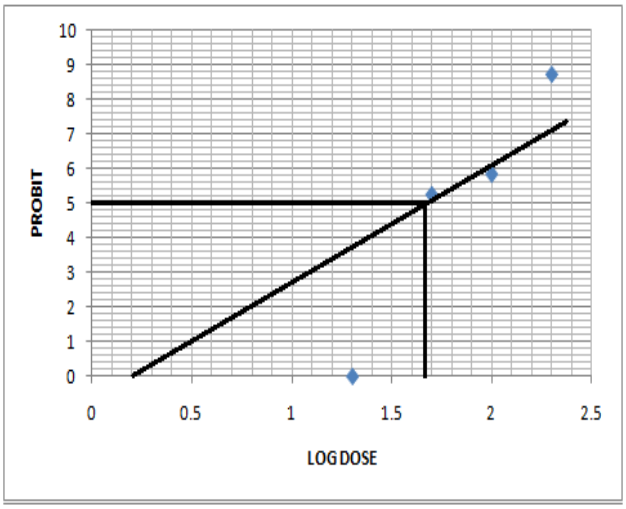

Figure 1: Acute Toxicity of SLP using the intraperitoneal route in mice

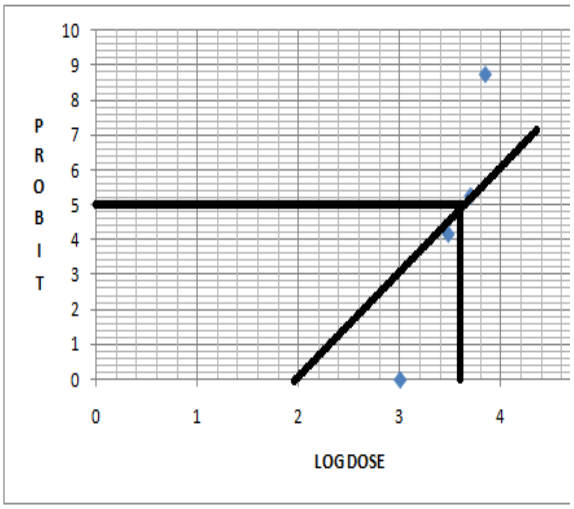

Figure 2: Acute Toxicity of SLP through oral route in mice

Table 1: Effect of SLP on body weight of rats

Test Agent	Mean	Weight (g)/Week					
(mg/kg)	Basal	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Control	200.32±0.13	222.08±0.11	241.16±0.15	264.0±0.09	288.02±0.23	310.5±0.07	328.42±1.25
700	202.14±0.45	*191.20±0.29	*178.22±0.13	*160.02±0.17	*151.04±0.25	*144.13±0.53	*136.30±0.11
350	220.0±1.23	*203.45±1.12	*190.05±0.10	*175.09±0.11	*162.12±0.34	*132.21±1.02	*122.05±0.25
150	200.07±0.86	*193.12±0.33	*170.11±0.32	*164.10±0.14	*154.08±0.76	*140.51±0.43	*120.11±0.29

*****$p<0.05$ when compared with control

investigation. Group B was similarly treated two weeks after dug withdrawal.

Assays: Haematological Analysis-Haematological parameters namely, white blood cell (WBC), red blood cell (RBC), Packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), platelets (PTL) and white blood cell differentials were assayed using Sysmex automated analyzer-model KN-21N[9].

Biochemical Analysis-All biochemical analyses were surveyed using Roche Hitachi 902 automated analyzer.

Histological study-The liver and kidney were excised, fixed in 10% formalin, and stained with haematoxylin-eosin for microscopic examination.

Statistical Analysis: Graph pad prism 3.0 (Graph pad software) was used to compare groups of data and results expressed as mean ±SEM. Results were considered significant when $p< 0.05$.

RESULTS

SLP extract was a fine brownish powder with aromatic fragrance; it recorded a pHof 5.38 at 200 mg/ml.

Phytochemical screening: Tannins, phlobatannins, alkaloids, flavonoids, saponins and cardiac glycosides were present.

Acute toxicity test: Median lethal dose (LD_{50}) for both i.p. and oral routes were interpolated and recorded as 44.67 mg/kg and 3162.27 mg/kg respectively (Figures 1 & 2). Anxiolysis, sedation and weakness were observed within 30 min post-drug administration and the same persisted for two to three hours.

Repeated doses toxicity assessment: Doses of 150, 350 and 700 mg/kg given intragastrically to groups of rats for 90 days, produced different toxic manifestations in the animals.

Effect of SLP on body weight: Control rats recorded body weight gain, of over 50% during the period of study, whereas, the groups dosed with the herbal preparation featured diminution in body weight, the effect, which was gradual within the first two weeks but became more drastic thereafter, until after drug withdrawal (Table 1). Weight gain resumed in group B animals after cessation of therapy (not shown).

Table 2: Effect of SLP on Organ weight

Test Agent (mg/kg)	Organ Weight (g)	
	Liver	Kidney
Control	4.93±0.41	1.28±0.09
700	*3.81±0.24	*0.98±0.07
350	4.09±1.11	*0.90±0.11
150	4.00±1.22	*0.89±0.06
700 R	4.41±1.55	1.05±0.36

*p<0.05 when compared with control

Table 3: Effect of SLP on Liver Enzymes

Test Agents (mg/kg)	Liver Enzymes		
	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	139.23±13.03	52.75±1.29	140.00±2.50
700	*249.26±42.46	*74.1±4.18	*251.12±15.67
350	*192.31±22.33	*61.35±6.93	*255.01±37.70
150	132.09±4.68	53.48±4.70	*221.85±47.65
700 R	128.46±11.33	*63.58±2.90	*291.11±36.37

*****p<0.05 when compared with control

Table 4: Effect of SLP on Total protein, Total bilirubin and Albumin

Test Agent (mg/kg)	Parameter Total protein (g/dl)	Total bilirubin (µmol/L)	Albumin g/dl)
Control	80.99±3.50	1.56±0.14	39.65±11.33
700	80.62±44.32	*2.78±0.21	38.38±0.74
350	78.65±2.57	*2.44±0.40	29.56±1.33
150	78.48±3.30	*2.29±0.54	32.13±1.78
700 R	82.76±2.67	*3.65±0.27	32.20±2.32

*****p<0.05 when compared with control

Effect of SLP on organ weight: The herbal drug affected the weight of the two organs of elimination as shown (Table2).

Effect of SLP on liver function: While alkaline phosphatase (ALP) concentration was irreversibly increased by SLP, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were reversibly elevated by 350 and 700 mg/kg. Activity of the extract on total protein, albumin and total bilirubin are shown (Tables 3 & 4).

Effect of SLP on Kidney function: SLP especially in large doses, produced a significant ($p<0.05$) increase in uric acid and creatinine levels (Table 5).

Effect of SLP on lipid profile: Total cholesterol and LDL were significantly ($p<0.05$) elevated (Table 6).

Effect of SLP on serum electrolytes: Sodium and chloride ions were significantly ($p<0.05$) reduced (Table 5). While bicarbonate ion showed a non-significant increase at all dose level, potassium ion recorded a significant increase at high doses only.

Effect of SLP on haematological parameters: The erythrocytes, haemoglobin and packed cell volume showed a significant increase with 700 mg/kg extract only; the effect, which was gradually reversible after drug withdrawal.

Total leucocytes (WBC) count reflected a fall in concentration, but an increase in lymphocytes was recorded (Table 8).

Mean corpuscular volume (MCV) and Mean Corpuscular Haemoglobin values were also elevated (Table 7). However, the platelets were not significantly affected.

Table 5: Effect of SLP on serum electrolytes, urea and creatinine

Test Agents (mg/kg)	Parameters						
	Na^+ mEq/L	K^+ mEq/L	$HCO3^-$ mEq/L	Cl^- mEq/L	Urea mg/dl	Creatinine mg/dl	Uric acid mg/dl
Control	137.51±1.94	4.25±0.12	19.50±0.96	99.75±0.48	9.10±0.31	72.38±7.41	59.80±0.75
700	*112.40±4.34	*6.49±0.27	20.50±1.04	*94.25±2.46	*10.45±0.10	*84.23±2.72	110.20±6.04
350	*110.57±4.40	*6.18±0.53	*22.25±1.60	*90.75±0.75	8.93±1.03	*54.47±2.12	79.54±11.17
150	*105.22±2.98	*5.33±0.26	*22.25±1.80	*85.53±1.66	10.23±1.17	*53.23±1.32	52.04±10.10
700 R	138.09±0.70	*4.64±0.34	20.75±0.85	100.60±1.08	*5.98±0.79	*58.75±0.25	73.43±18.58

Table 6: Effect of SLP on serum Lipids

Test Agents	Parameter			
	T. Chol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	0.55±0.04	0.72±0.07	1.10±0.18	0.14±0.02
700	*1.33±0.04	0.68±0.73	*1.32±0.02	*0.24±0.01
350	*1.43±0.13	0.66±0.41	1.27±0.10	*0.35±0.06
150	*1.42±0.12	0.63±0.05	*1.35±0.27	*0.20±0.03
700 R	*1.27±0.07	0.76±0.27	*1.35±0.15	*0.42±0.05

*****$p<0.05$ when compared with control

Effect of SLP on histopathological analysis: The two organs of elimination among others (not shown) showed cell damage, the effect which persisted in the kidney only (Table 9).

DISCUSSION

The plant under investigation has been widely used in traditional medicine in both Western and Southern Africa for the treatment of diverse medical conditions such as constipation, conjunctivitis, malaria, venereal diseases, bacterial and protozoal infections, rheumatism, toothache, headache, snakebite among others. Information on the toxicity of SLP has not been robust and the few reports available are conflicting. For example, the plant SLP was reported as relatively safe when administered orally to mice in doses of 300, 900 and 2700 mg/kg orally for 28 days[10]; whereas, its tissue toxicity was documented in another study exposing rats orally for the same period of 28 days to 200, 400 and 800 mg/kg SPH[11] and 2 mg/kg i.p for 14 days[12]. In the present study, aqueous root extract of SLP in doses of 150, 350 and 700 mg/kg given through gastric intubation for 90 days in rats recorded a series of toxic manifestations. A significant reduction in body weight was recorded at all dose levels employed. The latter observation is very important because the toxicity of chemical compounds in experimental animals is often associated with loss of body weight[13-14]. The cause of body weight reduction could not be explained within the scope of the study, however, slow feeding rate was observed in the rats treated with SLP, as compared with the control group given distilled water. Furthermore, the diminution in weight of stomach, testis, ovary (not shown), kidney and liver could have also culminated to the loss in total body weight.

Repeated treatment with the extract significantly increased the levels of serum AST and ALT, which was in consonance with earlier observation[15]. Serum ALT is known to increase when there is liver cell damage and it has been employed as a tool for measuring hepatic necrosis[16]. AST however, is not a liver specific enzyme as high levels of the enzyme can also be found in skeletal and cardiac muscle as well as red blood cells[17]. Increase in serum ALP may be considered as an indicator of cholestasis, which may result from intracellular hepatic canaliculli obstruction associated with inflammation[18]. The latter hepatocellular damage observed could be substantiated by the histopathological report on the liver (Figure 7). The other major organ of elimination was similarly not spared. Focal tubular oedema and congestion were recorded (Figure 4). SLP exerted its toxicity on the liver and kidney by depleting the antioxidant systems[11].Blood is an index of physiological and pathological status in animals and the parameters usually measured are haemoglobin, packed cell volume, white blood cell count, platelets count[19] and their values can be altered by the ingestion of some toxic plants[20-21]. Almost all the haematological parameters explored were reduced except lymphocytes (Tables 7 & 8). The latter finding suggests that SLP would affect innate immunity more than the adaptive. It is possible that the herbal drug contains some compounds that destroy or impair the production of polymophonuclear cells, while that of lymphocytes is boosted[22]. The phytoconstituents identified in SLP were tannins, phlobatannins, alkaloids

Table 7: Effect of SLP on haematologic parameters

Test Agents (mg/kg)	Parameter						
	RBC (×106/µl)	Hb (g/dl)	PCV (%)	MCV (fl)	MCHC (g/dl)	MCH (pg)	PLT (×103/µl
Control	6.61±0.13	11.80±0.15	37.20±0.73	53.50±11.75	31.73±0.59	16.18±0.71	803.33±78.67
700	6.88±0.26	*12.88±0.58	*41.58±1.73	60.58±0.99	*26.28±1.03	*18.75±0.60	820.28±70.08
350	*5.48±0.57	*10.05±0.89	*31.20±3.17	58.44±1.39	32.38±0.56	*18.73±0.58	775.43±43.37
150	*5.79±0.52	*10.73±0.94	*34.30±2.56	59.60±2.10	31.18±0.47	*18.55±0.73	760.25±98.56
700 R	*5.55±0.47	*9.73±0.96	*31.60±3.05	58.18c0.41	*30.75±0.57	*17.48±0.38	828.20±55.91

*p<0.05 when compared with control

Table 8: Effect of SLP on Total and Differential WBC

Test Agents (mg/kg)	Parameters			
	LYM (%)	NEUT (%)	MXD (%)	TWBC (×103/µl)
Control	56.10±2.91	21.50±4.19	4.25±1.65	14.10±2.16
700	107.34±6.26	15.00±2.25	5.50±0.87	8.30±2.25
350	91.52±2.60	24.50±5.19	4.50±0.65	6.13±2.75
150	66.50±8.97	31.00±9.04	4.40±0.29	4.83±0.39
700 R	70.75±5.65	19.75±3.15	9.50±2.53	11.26±2.30

Table 9: Histological changes observed in the liver and kidney of rats treated orally with SLP for 90 days

Test Agents mg/kg	Kidney	Liver
Control	Normal	Normal
700	Mild cellular oedema and mild focal tubular necrosis	Acute oedema and congestion
350	Cellular oedema, moderate tubular necrosis	Mild to moderate oedema. Focal reactive changes
150	Cellular oedema, moderate tubular necrosis	Moderate oedema
700 R	Focal interstitial oedema and congestion	Mild to moderate changes; mild congestion.

flavonoids, saponins and cardiac glycosides and some of these could have been responsible for the recorded toxicity[8]; the acidic pH of 5.38 could also contribute to the deleterious effect of SLP observed on the body organs.LD_{50} for both i.p and oral routes were evaluated as 44.67 mg/kg and 3162 mg/kg respectively. The small value obtained via the former route also substantiates the toxicity of SLP in mice after a single exposure. The present study confirms the toxicity of aqueous root extract of SLP after a single exposure and with repeated dosing. It must therefore be used with caution, since it adversely affects the two major organs of drug elimination.

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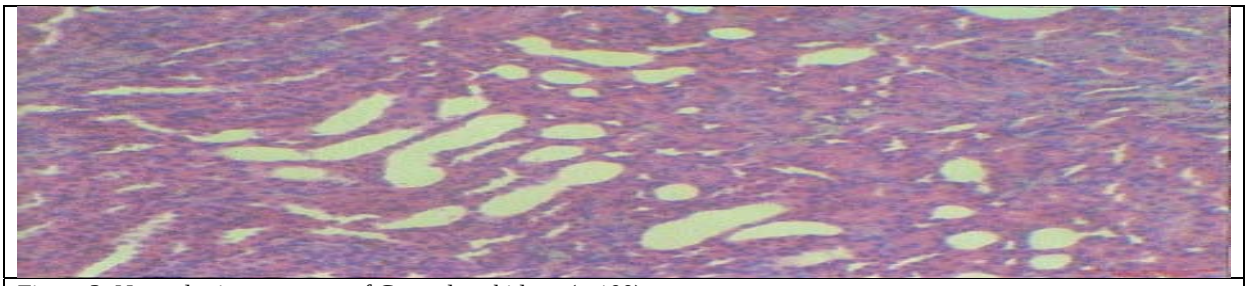

Figure 3: Normal microstructure of Control rat kidney (× 100)

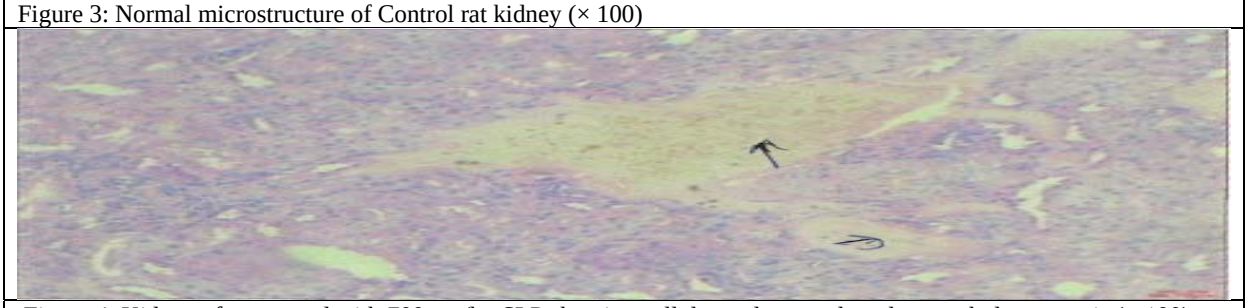

Figure 4: Kidney of rat treated with 700 mg/kg SLP showing cellular oedema and moderate tubular necrosis (× 100).

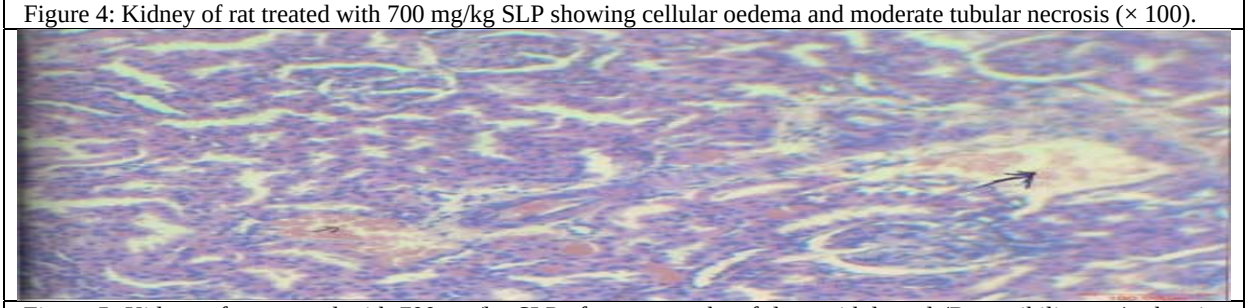

Figure 5: Kidney of rat treated with 700 mg/kg SLP after two weeks of drug withdrawal (Reversibility test), showing mild subcapsular oedema and congestion with focal necrosis (× 100).

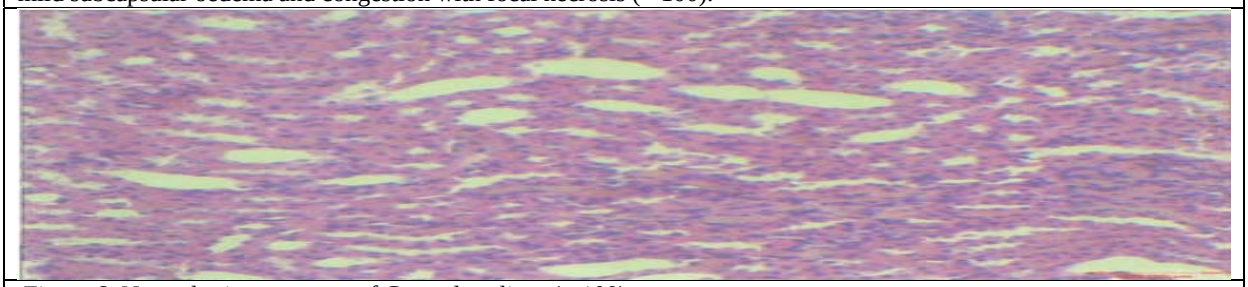

Figure 6: Normal microstructure of Control rat liver (× 100)

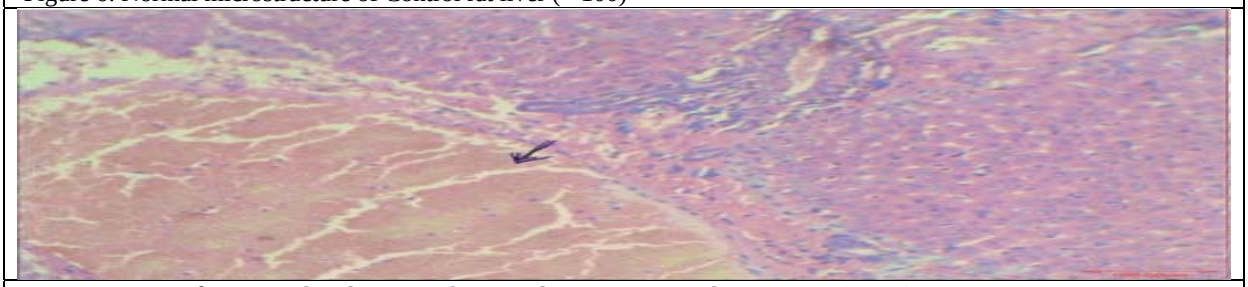

Figure 7: Liver of rat treated with 700 mg/kg SLP showing acute and congestion (× 100)

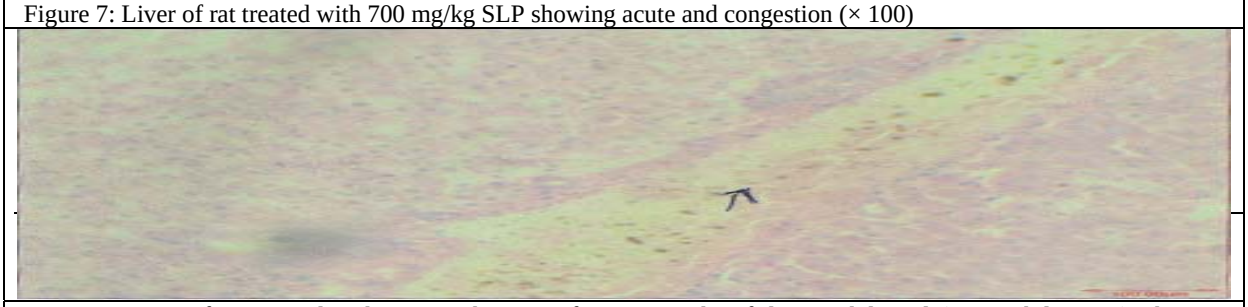

Figure 8: Liver of rat treated with 700 mg/kg SLP after two weeks of drug withdrawal (Reversibility test), showing mild to moderate changes with congestion (× 100).

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