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Research Article

TOXICITY EVALUATION ON HYDROALCOHOLIC EXTRACT OF LEAF AND STEM BARK OF *PROSOPIS CINERARIA*

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ABSTRACT

Prosopis cineraria (L) Druce is one of the highly valued plant in the Indigenous System of Medicine. The present study was carried out to evaluate acute and subacute toxicity of a hydroalcoholic extract from leaves and stem bark of *P. cineraria.* Toxicity was evaluated in wistar rats after ingestions of the extract during one day (acute model) and during 28 days (subacute model). The results showed that the LD50 of the extract is higher than 2000 mg/kg. In subacute treatment, there were no statistically significant changes in behavior, body weight, hematological, biochemical parameters and urine analysis. No detectable abnormalities were found in the histopathology of the selected organs. No mortality was recorded in experimental animals treated with the drug orally at a dose of 1000mg/kg. The results suggest that the plant seems to be destituted of toxic effects in mice.

Keywords: *Prosopis cineraria*, Acute toxicity, Subacute toxicity.

INTRODUCTION

Prosopis cineraria (L) Druce syn: *Prosopis spicigera* Linn., and *Mimosa cineraria* Linn[1] belongs to the family *Leguminosae*, sub-family *Mimosoideae* and Class Magnoliopsida – Dicotyledons, is a large shrub up to 10m height with branches prickly, prickles curved and compressed. The species is found throughout India extending to Persia. It is known as *Vanni* or *jambu* in Tamil; *Jand* or *Khejra* in Hindi and *Shami* in Sanskrit[2]. The whole plant is used in Indigenous System of Medicine (ISM) and is called 'Kalpa Plant' in Ayurveda and Siddha literature. The literature survey revealed that the plant has been used in leprosy, dysentery, bronchitis, asthma, leucoderma, muscular tremors and rheumatism. It is also known to possess anthelmintic, antibacterial, antifungal, antiviral and anticancer activities. Water-soluble extract of the residue from methanol extract of the stem bark exhibits anti-inflammatory properties[3,4]. Leaf paste of *P. cineraria* is applied on boils and blisters, including mouth ulcers in livestock and leaf infusion on open sores on the skin. The smoke of the leaves is considered good remedy for ailments of eye. The decoction of the bark in combination with the barks of *Erythrina indica* and *Azadirachta indica* is used in syphilis[5]. The chemical constituents isolated include spicigerine from plant; vitamin K, n-octacosyl acetate, n-hexacosanoic acid and n-octacosanoic acid from stem bark[6]; steroids namely campesterol, cholesterol, sitosterol, stigmasterol; alcohols namely octacosanol and triacontan-1-ol; alkane hentriacontane isolated from leaves**.** The present study is focused to find out the acute and subacute toxicity studies on the hydroalcoholic extract of leaf and stem bark of *P. cineraria* in wistar rats.

MATERIALS AND METHODS

Plant materials

The plant specimens of *P. cineraria* were collected from the Mylapore, Thiruvallur district, Tamil Nadu, India in the month of September 2007. The specimens were identified and authenticated by Prof. P. Jayaraman, Director of Plant Anatomy Research Centre, West Tambaram, Chennai. A voucher specimen (No: A-43/PARC) has been deposited in the same Institution.

Preparation of extracts

The leaves and stem bark were collected, shade dried and coarsely powdered separately by using pulvarizer. These coarse powders were then extracted with 50% alcohol by cold percolation process to yield the respective extracts. The extracts were reduced to a molten mass by rotary vacuum evaporator and the respective yields of PCL and PCB (hydroalcoholic extracts of leaf and stem bark of *Prosopis cineraria*) were 13.46% w/w and 1.16% w/w respectively.

Animals

Adult female and male wistar rats, 125-175g were used. The animals were housed under standard laboratory conditions of 12-hour light/dark cycle, 22 ± 2°C with free access to standard food and water *ad libitum.* The study was approved from the Institutional Animal Ethics committee to carry out in rats (DSCP/M Pharm Col/IAEC/25/09-10).

Methods

Acute toxicity studies

The animals were divided into nine groups, each group consisting of three animals. The control group received saline and each treated group received PCL and PCB separately in a dose of 50, 500, 1000, 2000 mg/kg by oral route. The animals were observed continuously for the first four hours and then they were observed each hour during 24 h after administering PCL and PCB to observe any changes in the behavioral responses and also for tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma and monitored for any mortality[7].

Subacute toxicity studies

Female rats were divided into seven groups and each group consists of 6 rats. Six groups of rats received PCL and PCB separately in a dose of 200, 500 and 1000 mg/kg orally for 28 days. The group which served as control received equivalent quantity of normal saline orally. Animals were observed for signs and symptoms, behaviour alteration, food and water intake and body weight changes. All animals were observed twice daily for mortality during the 28 days period of study. The weight of each rat was recorded from day one and at weekly intervals throughout the course of the study. At the end of the experimental period, blood was collected by orbital sinus venipunture, under ether anesthesia, for biochemical and haematological analysis. The blood sample was collected after 24h of the last doses of PCL and PCB. Haematological analyses such as white blood cell count (WBC), red blood cell count (RBC), haemoglobin (Hb), erythrocyte sedimentation rate (ESR), platelets, packed cell volume (PCV) and clotting time were performed in total blood using routine method[8]. Biochemical analysis like blood glucose[9], cholesterol[10], Serum glutamate pyruvate transaminase (ALT)[11] and Serum glutamate oxaloacetate transaminase (AST)[12], alkaline phosphatase (ALP), total bilirubin[13], urea and blood urea nitrogen (BUN)[14], total protein and albumin[15] and creatinine[16] were estimated in serum.

Urine samples were analyzed for specific gravity, pH, glucose, proteins, ketones and occult blood. At the end of 28 days, experiment animals were autopsied and vital organs viz. Liver, kidney, spleen, lung, heart and brain were removed and later weighed. Since liver and kidney are organs of metabolism and excretion, potentially toxic agents are likely to affect them. So, portions of these organs were fixed in buffered 10% formalin and 5 μm thick paraffin sections were made and stained with haemotoxylin and eosin and examined for any pathological changes.

Statistical analysis

The results are presented as mean ± SEM, and the statistical analysis was performed by one way analysis of variance (ANOVA), followed by Turkey multiple comparison test. P values of less than 0.05 were considered as indicative of significance.

RESULTS AND DISCUSSION

Acute toxicity studies

In the toxicity study, oral administration of the hydroalcoholic extracts of leaf and stem bark of *Prosopis cineraria* (PCL and PCB) in doses from 50 to 2,000 mg/kg did not produce significant changes in behaviour, breathing, cutaneous effects, sensory nervous system responses and gastrointestinal effects in rats and no mortality occurred within 24h under the tested dose of PCL and PCB.

Subacute toxicity studies

It was observed that the animals show no unusual changes in behavior or in locomotor activity, ataxia and signs of intoxication were observed. No differences were found in growth between the control group and the animals fed with different levels of PCL and PCB (Table-1). No change in fur coating, eyes and respiratory functions. There was no significant difference in the food and water consumption between the treatment and control groups.

According to Onyenyili and co-workers[17], anaemia following administration of an agent can be as a result of lysis of blood cells and/or inhibition of blood cell synthesis by the active constituents of the extract and decrease in hematological parameters in experimental animals has been associated with anemia. It was observed that there was no significant change in haematological parameters such as RBC, WBC, Hb, ESR, platelets, PCV and clotting time in the extracts-treated animals compared to the control (Table - 2). This that there is no lysis of blood cells and/or inhibition in blood cells synthesis by the active constituents of PCL and PCB.

The levels of serum analytes such as glucose, cholesterol, AST and ALT, ALP, total bilirubin, urea, BUN, total protein, albumin and creatinine were not significantly different between the control and the experimental groups of rats when fed with PCL and PCB (Tables-3 & 4). Analysis of the urinary metabolite levels (specific gravity, pH, glucose, proteins, blood cells, and ketones) showed trace or no presence of these in both control and experimental animals fed with extracts (Table - 5).

Table - 6 depicts the organ-to-body mass ratio of animals at the end of 28 days feeding. No abnormal changes were observed in organ mass with respect to body mass of PCL and PCB fed rats when compared with control.

Histopathological examination of the liver and kidneys in the control and the extracts fed groups showed no differences, indicating that feeding of PCL and PCB at these levels to the rats did not found any adverse toxicological effect on these organs (Fig1 to 14). In histopathological studies, the liver of treated animals showed normal histological feature at 200, 500 and 1000mg/kg. No degeneration of hepatocytes, focal steatosis, congestion of central vein and inflammation of portal tract when compared with control animals. The kidney of treated rats showed normal glomeruli and there is no necrosis of tubular epithelium in the kidney. Therefore, gross examination of liver and kidney on rats of all groups were found to be uniformly healthy and lacking in any apparent pathological abnormalities.

Table 1: Changes in body weight of rats

S. No.	Treatment	Body weight (g)	
		Initial	After 28 days treatment
1.	Control	165 ± 1.5	188 ± 1.7
2.	PCL (200mg/kg)	168 ± 1.4	186 ± 1.1
3.	PCL (500mg/Kg)	162 ± 0.5	183 ± 1.5
4.	PCL (1000mg/kg)	167 ± 2.2	190 ± 1.2
5.	PCB (200mg/kg)	170 ± 0.7	186 ± 0.9
6.	PCB (500mg/kg)	160 ± 1.4	183 ± 1.2
7.	PCB (1000mg/kg)	165 ± 1.06	187 ± 1.3

n = 6; values are expressed as mean ± SEM

NS= statistically not significant

Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test.

Table 2: Effect of PCL & PCB on haematological changes in rats

S. No.	Treatment	Hb gm %	RBC 10^6/cu.mm	Total WBC 10^3/cu.mm	ESR mm/1st hr	Platelets (K/μL)	Clotting time (Sec)	PCV %
1.	Control	14.75 ± 0.46	4.92 ± 0.58	8.10 ± 0.24	3.14 ± 0.07	576±18.68	109.33±0.49	42.20±2.83
2.	PCL (200mg/kg)	14.02 ± 0.35	4.65 ± 0.25	7.94 ± 0.17	3.05 ±0.09	550±13.24	107.83±0.47	44.46±1.10
3.	PCL (500mg/kg)	14.52 ± 0.62	4.77 ± 0.32	8.46 ± 0.26	3.18 ± 0.10	575±36.58	111.33±0.88	43.50±2.96
4.	PCL(1000mg/kg)	13.78 ± 0.24	4.58 ± 0.25	7.86± 0.14	3.20 ± 0.6	617±28.59	108.17±0.47	46.25±3.56
5.	PCB (200mg/kg)	15.01 ± 0.29	4.78 ± 0.23	8.01 ± 0.33	3.3 ± 0.09	607±44.10	111.33±0.49	45.46±2.15
6.	PCB (500mg/kg)	14.86 ± 0.33	4.90 ± 0.17	8.2 ± 0.20	3.62 ± 0.07	613±21.67	108.17±0.47	47.50±2.06
7.	PCB(1000mg/kg)	15.23 ± 0.29	4.83 ± 0.15	7.8 ± 0.12	3.45 ± 0.08	637±64.21	111.33±0.49	48.25±1.36

n = 6; values are expressed as mean ± SEM

NS = statistically not significant.

Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test.

Table 3: Effect of PCL & PCB on biochemical parameters in rats

S. No.	Treatment	Glucose mg%	Cholesterol mg%	AST U/L	ALT U/L	ALP U/L	Total Bilirubin Mg %
1.	Control	98 ± 1.7	108 ± 1.70	116 ± 2.0	46.8 ± 1.1	182 ± 2.10	0.65 ± 0.002
2.	PCL (200mg/kg)	90 ± 1.5	96 ± 1.10	124.6 ± 1.5	52.0 ± 1.7	186 ± 1.96	0.52 ± 0.003
3.	PCL (500mg/kg)	84 ± 1.9	106 ± 1.98	108.5 ± 1.7	43.0 ± 2.6	178 ± 1.75	0.66 ± 0.004
4.	PCL (1000mg/kg)	89 ± 2.0	103 ± 2.86	121.0 ± 1.3	50.6 ± 2.0	180 ± 2.0	0.74 ± 0.006
5.	PCB (200mg/kg)	95 ± 2.1	97 ± 1.03	117.6 ± 2.2	49.0 ± 1.1	190 ± 1.45	0.55 ± 0.009
6.	PCB (500mg/kg)	92 ± 1.3	106 ± 1.16	126.5 ± 2.3	45.0 ± 2.6	172 ± 1.24	0.68 ± 0.008
7.	PCB (1000mg/kg)	88 ± 1.6	110 ± 2.10	114.0 ± 2.1	51.2 ± 1.3	176 ± 2.83	0.71 ± 0.006

n = 10; values are expressed as mean ± SEM.

NS = statistically not significant

Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test.

Table 4: Effect of PCL & PCB on biochemical parameters in rats

S. No.	Treatment	Urea mg %	BUN mg%	Creatinine mg %	Total Protein gm%	Albumin gm%
1.	Control	37.5 ± 1.14	25.65 ± 2.03	0.8 ± 0.10	8.3 ± 1.10	4.67 ± 0.16
2.	PCL (200mg/kg)	32.7 ± 1.33	22.65 ± 1.07	0.6 ± 0.02	7.4 ± 1.06	4.13 ± 0.19
3.	PCL (500mg/kg)	35.6 ± 1.47	26.24 ± 1.98	0.7 ± 0.07	8.7 ± 1.20	4.27 ± 0.17
4.	PCL (1000mg/kg)	37.2 ± 2.0	27.16 ± 2.66	0.9 ± 0.06	6.8 ± 1.28	4.83 ± 0.13
5.	PCB (200mg/kg)	39.7 ± 1.96	28.65 ± 2.0	0.7 ± 0.07	9.1 ± 1.12	4.10 ± 0.10
6.	PCB (500mg/kg)	38.5 ± 2.47	26.95 ± 1.15	1.0 ± 0.03	7.9 ± 1.07	4.18 ± 0.14
7.	PCB (1000mg/kg)	40.2 ± 2.16	29.78 ± 1.26	0.9 ± 0.10	8.8 ± 1.32	4.36 ± 0.10

n = 6; values are expressed as mean ± SEM.

NS = statistically not significant

Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test.

Table 5: Effect of PCL & PCB on urine analysis in rats

S. No.	Treatment	Specific gravity	pH	Glucose	Protein	Ketone bodies	Blood cells
1.	Control	1.03 ± 0.04	6.42 ± 0.05	NIL	NIL	NIL	NIL
2.	PCL (200mg/kg)	1.05 ± 0.06	6.53 ± 0.08	NIL	NIL	NIL	NIL
3.	PCL (500mg/kg)	1.08 ± 0.03	6.46 ± 0.02	NIL	NIL	NIL	NIL
4.	PCL (1000mg/kg)	1.02 ± 0.02	6.87 ± 0.02	NIL	NIL	NIL	NIL
5.	PCB (200mg/kg)	1.03 ± 0.01	6.40 ± 0.03	NIL	NIL	NIL	NIL
6.	PCB (500mg/kg)	1.07 ± 0.04	6.39 ± 0.04	NIL	NIL	NIL	NIL
7.	PCB (1000mg/kg)	1.06 ± 0.02	7.01 ± 0.03	NIL	NIL	NIL	NIL

n=6; values were expressed as Mean ± SEM

NS- Not significant

Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test.

Table 6: Effect of PCL & PCB on weight (g) of vital organs of rats

S. No.	Treatment	Liver	Kidney	Heart	Lungs	Spleen	Brain
1.	Control	4.32 ± 0.22	1.38 ± 0.27	0.75 ± 0.03	1.35 ± 0.14	0.98 ± 0.07	1.86 ± 0.38
2.	PCL (200mg/kg)	4.45 ± 0.30	1.45 ± 0.32	0.71 ± 0.04	1.30 ± 0.12	0.87 ± 0.06	1.94 ± 0.16
3.	PCL (500mg/kg)	4.37 ± 0.28	1.42 ± 0.21	0.69 ± 0.02	1.29 ± 0.10	1.0 ± 0.10	1.93 ± 0.13
4.	PCL (1000mg/kg)	4.54 ± 0.23	1.52 ± 0.30	0.78 ± 0.05	1.34 ± 0.16	1.1 ± 0.18	2.0 ± 0.05
5.	PCB (200mg/kg)	4.35 ± 0.60	1.43 ± 0.16	0.74 ± 0.09	1.32 ± 0.12	0.9 ± 0.07	1.96 ± 0.16
6.	PCB (500mg/kg)	4.42 ± 0.58	1.46 ± 0.14	0.80 ± 0.02	1.47 ± 0.10	1.0 ± 0.10	1.88 ± 0.13
7.	PCB (1000mg/kg)	4.34 ± 0.26	1.41 ± 0.10	0.70 ± 0.10	1.32 ± 0.06	1.2 ± 0.18	2.0 ± 0.25

n = 6; Values are expressed as mean ± SEM

NS = statistically not significant

Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test

Histopathological Studies of Liver - Subacute toxicity Studies

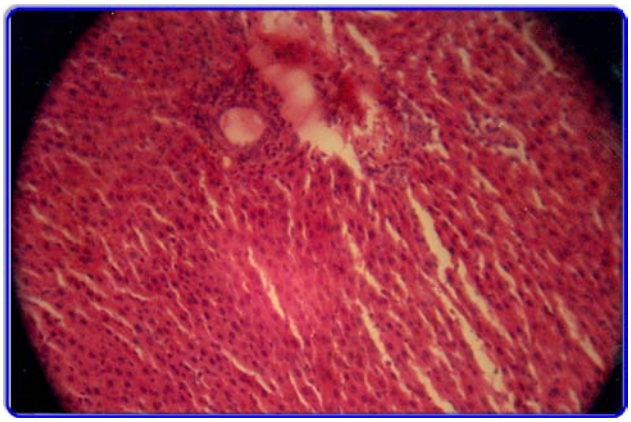

Fig. 1: Section of liver treated with normal saline

Fig. 2: Section of liver treated with PCL (200mg/kg)

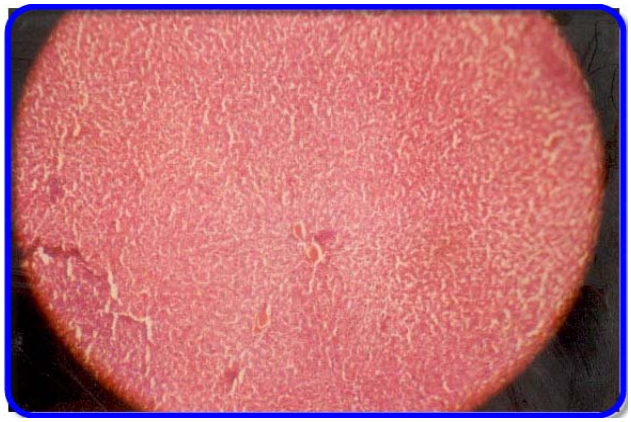

Fig. 3: Section of liver treated with PCL (500mg/kg)

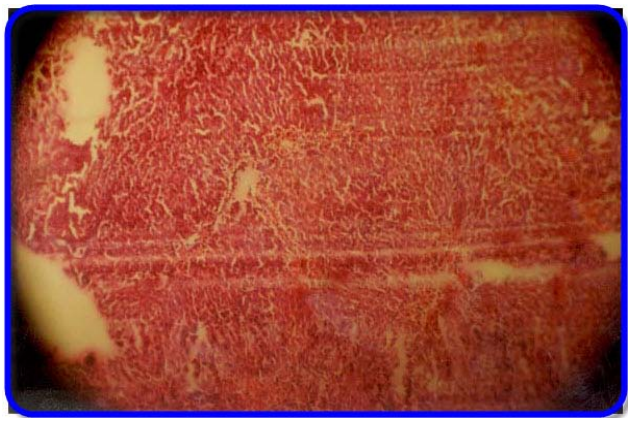

Fig. 4: Section of liver treated with PCL (1000mg/kg)

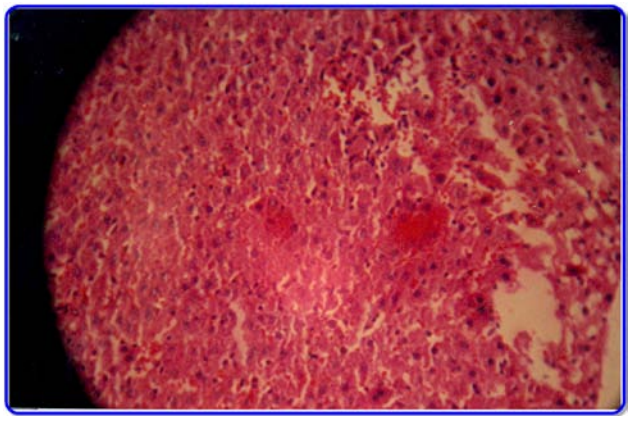

Fig. 5: Section of liver treated with PCB (200mg/kg)

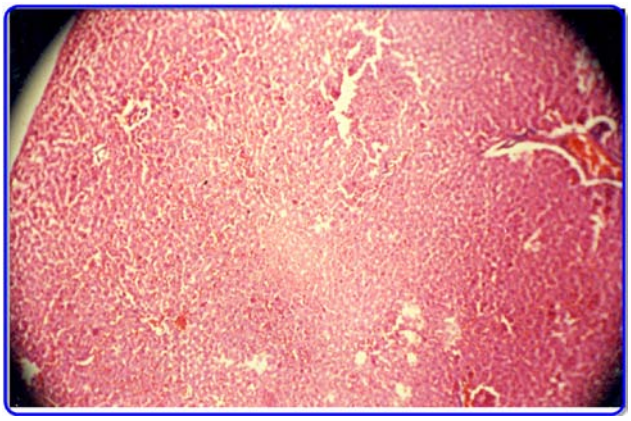

Fig. 6: Section of liver treated with PCB (500mg/kg)

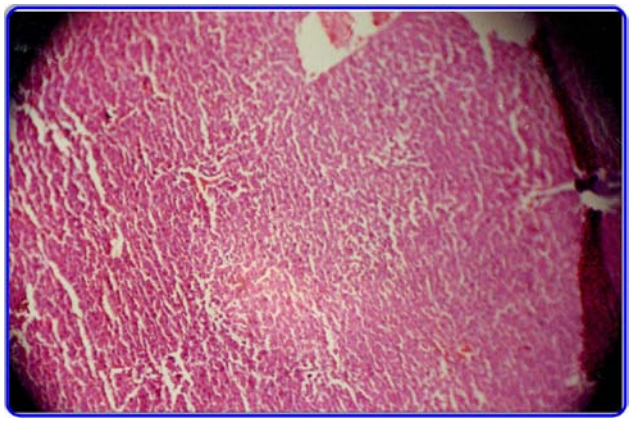

Fig. 7: Section of liver treated with PCB (1000mg/kg)

Histopathological Studies of Kidney – Subacute toxicity studies

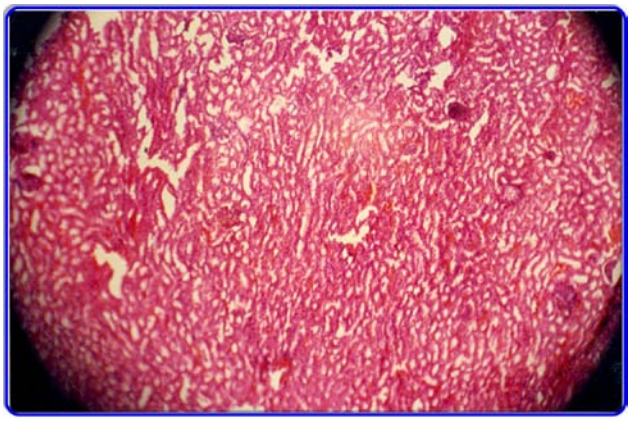

Fig. 8: Section of kidney treated with normal saline

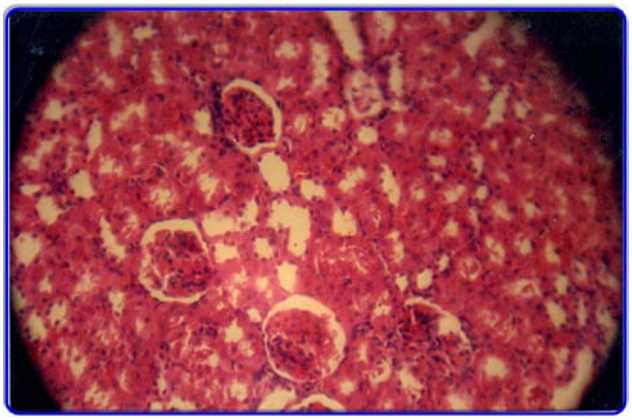

Fig. 9: Section of kidney treated with PCL (200mg/kg)

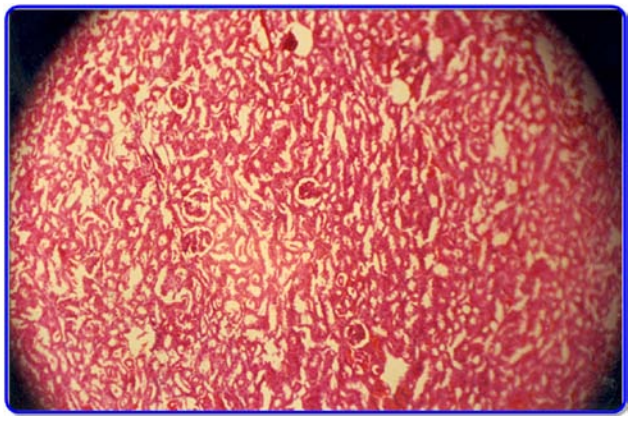

Fig. 10: Section of kidney treated with PCL (500mg/kg)

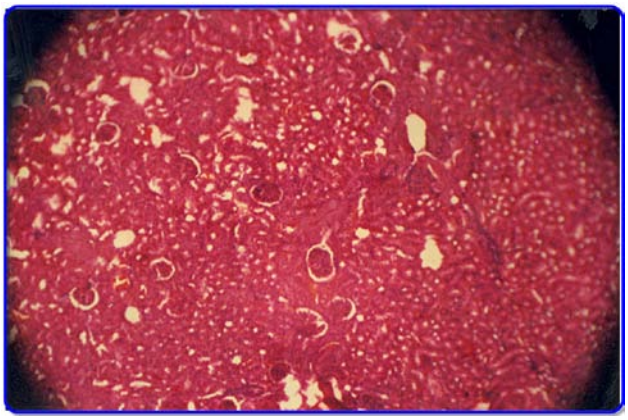

Fig. 11: Section of kidney treated with PCL (1000mg/kg)

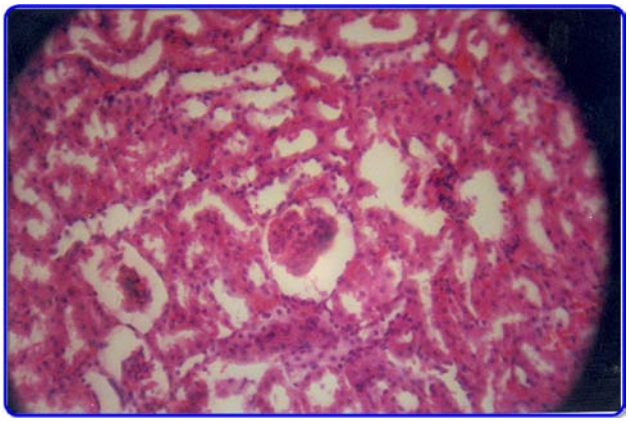

Fig. 12: Section of kidney treated with PCB (200mg/kg)

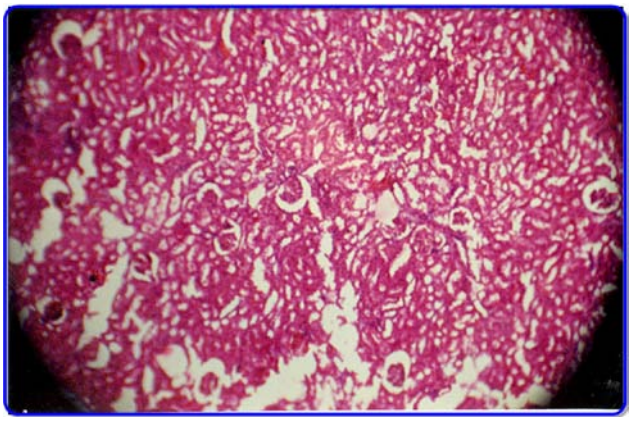

Fig. 13: Section of kidney treated with PCB (500mg/kg)

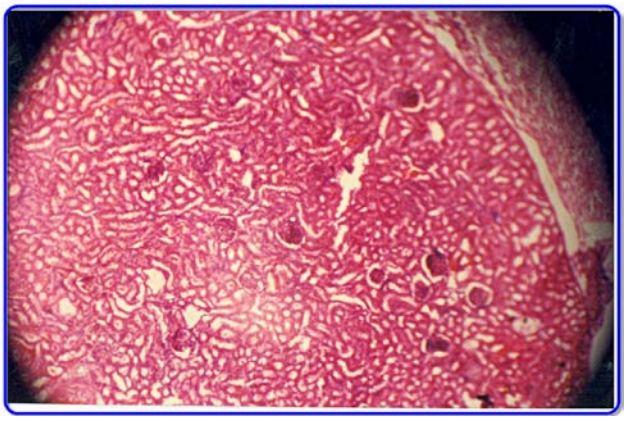

Fig. 14: Section of kidney treated with PCB (1000mg/kg)

CONCLUSION

In conclusion, the results indicated that PCL and PCB given orally at dose of 1000mg/kg/day for 28 days did not show any evidence of toxicity in rats.

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