

Ameliorative effect of *Plumbago zeylanica* root extract against lead toxicity in rats

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Received: 21.9.2017; Accepted: 3.3.2018

ABSTRACT

Kadam, R.G., Panda, S., Hingade, S.S., Pagrut, S., Ingole, R.S. and Joshi, M.V. (2018). Ameliorative effect of *Plumbago zeylanica* root extract against lead toxicity in rats. Indian J. Vet. Pathol., 42(1) : 36-42.

Being a toxic heavy metal, lead can induce many annihilating effects on different systems of the body in all living entity. To combat this toxicity, traditional herbal treatment is gaining importance now-a-days due to their various advantages. The present study was undertaken to evaluate the ameliorative effect of ethanolic root extract of *Plumbago zeylanica* against lead toxicity in rats. The extract was given at the rate of 100 mg/kg body weight, orally to Wistar rats along with the lead acetate administered orally at the rate of 500 ppm for 24 days. The results were compared with control group receiving normal water and feed, and toxicity group receiving 500 ppm of lead acetate in drinking water. *P. zeylanica* treated group showed better feed consumption and improved blood parameters with no clinical abnormalities. On histopathological examination, the organs of plant extract treated group showed relatively less degenerative changes in liver, kidneys, lungs, brain and testes, with promising results of serum analysis showing decreased enzymatic levels of ALT, AST, creatinine and blood glucose. Blood calcium was increased upon *P. zeylanica* treatment indicating its protective effect on bone, whereas the phosphorous level showed opposite trend probably be due to its beneficial effect on kidneys restricting its elimination. These results were encouraging for the use of have created an ardency to use this plant in amelioration of lead toxicity.

Keywords: Ethanolic root extract, lead toxicity, pathology, *Plumbago zeylanica*, Wister rats

INTRODUCTION

The modern world cannot give up use of metals albeit many of them have created toxicity havoc¹. Among them, lead toxicity through occupational or environmental exposure is a serious issue across the globe². Due to the non-biodegradable nature and accrued use, lead accumulates in the environment day by day with increasing the risk of hazards³. The deleterious effects are mainly because of its pharmacological property to remain bound to mammalian tissues for a long duration in various species of animals⁴. Lead toxicity can affect haematopoietic system, central nervous system, liver and kidneys². It is reported that erythrocytes are more vulnerable to lead induced oxidative damage and simultaneously spreading to other body organs⁵. High dose of lead may induce carcinogenicity either through DNA damage or by inhibition of DNA synthesis and repair. Lead can substitute zinc in proteins like transcriptional regulators and protamines. This mechanism suggests an epigenetic involvement of this heavy metal in altered gene expression resulting into carcinogenicity⁶.

In recent years, herbal drugs supplementation has received considerable clinical attention in case of tissue injury⁷⁻⁹. In spite of availability of many ameliorating agents against lead toxicity, herbal agents cannot be

abnegated because of their biologically active components, effectiveness, fewer side effects and relatively low cost¹⁰. Various workers have used several herbal extracts to reduce the deleterious effect of lead toxicity^{7,11,12}. One such traditionally used plant, *Plumbago zeylanica*, commonly known as Ceylon leadwort (English) and Chitra (Hindi), is a perennial, subscandant shrub found wild in South India and West Bengal. The root of this plant contains several bioactive chemical constituents. Plumbagin, a naphthoquinone, is the major component constituting about 0.03% of dry weight of the roots and is considered as an active ingredient responsible for its multipurpose therapeutic effects¹³. The root powder of *Plumbago zeylanica* has beneficial effects against lead induced changes¹⁴. Hence the present experimental design was set up to evaluate the efficacy of root extract of *Plumbago zeylanica* against lead induced toxicity in male Wistar rats.

MATERIALS AND METHODS

Plant material and hydroethanolic extraction

Root powder of *Plumbago zeylanica* was procured from authenticated Ayurvedic supplier, Nagpur, Maharashtra and subjected to cold ethanolic extract. The powder was immersed in the hydro-alcoholic solvent (40% distilled water + 60% ethanol), and kept on the orbital shaker at 150 rpm for 48 h at room temperature. Then the extract was filtered through the muslin cloth

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and Whatman filter paper No. 1. The extract was transferred to the Petri dish and kept in hot air oven at 40°C for evaporation of solvent.

Experimental animals

The experimental protocol met followed the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and was approved by the Institutional Animal Ethics Committee (IAEC). Twenty-four albino Wistar male rats of 6-7 weeks age were procured from the recognized Laboratory Animal Breeding Center, Shree Farm, Bhandara, Maharashtra and maintained under the standard managerial conditions.

Experimental design

Animals were randomly divided into four groups, each group comprising of six rats. All the experimental groups were given *ad lib* feed and drinking water throughout the experimental period of 28 days. Group I animals served as control group which were given normal feed and water, whereas group II, and IV rats were given 500 ppm lead acetate ($C_4H_6Pb_3H_2O$; 397.33) (Hi-Media Laboratories Pvt. Ltd., Mumbai) in drinking water. Group III and IV were given ethanolic extract of dry root powder of *Plumbago zeylanica* at 100 mg/Kg body weight orally (Table 1).

Clinical observation

Animals of each group were observed for any clinical symptoms and abnormalities in general body performance (feed consumption and average weekly body weight) and mortality if any was recorded.

Haematology

On completion of the experimental period of 28 days, blood was collected from heart for hematology after proper chloroform ($CHCl_3$) induced anaesthesia. Blood was collected in dry sterilized vials containing as an EDTA (ethylene diamine tetra acetic acid) @ 1 mg/ml and used for estimation of haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC), differential leukocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular

hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Serum biochemistry

Blood was collected in sterile vial without anticoagulant for serum separation. Sera samples were analyzed for calcium, phosphorus, alanine aminotransferases (ALT/SGPT), aspartate aminotransferases (AST/SGOT), glucose and creatinine using standard commercial kits (Span diagnostics, India).

Histopathological studies

Appropriate representative tissue samples like liver, lungs, heart, kidneys, brain and testes were collected in 10% neutral buffer formal for histopathological examination. Sections of 4-6-micron thickness were cut and stained with routine haematoxylin and eosin technique¹⁴.

RESULTS

Clinical signs and general performance

In the present study, no mortality was observed among the animals of any group during the experimental period. However, after 3 weeks of experiment, animals of group II showed variable signs of excitement i.e. running, climbing on each other and fighting with pasty dark brown colored feces and diarrhea. Group IV animals did not reveal any abnormal signs of nonsocial behavior and excitement. General performance was evaluated on the basis of feed consumption and average weekly body weight. Average highest feed consumption (734.46 ± 8.95) was recorded in group III, followed by group I (732.83 ± 8.37). However, the average feed consumption was significantly lower in group II (651.0 ± 3.89) rats receiving 500 ppm lead acetate. Animals of group IV showed higher feed consumption (686.76 ± 8.71) than when compared with group II, suggesting the beneficial effect of *Plumbago zeylanica* during lead toxicity (Table 2).

Average weekly body weight of the rats in different groups from 0 to 4 weeks of experiment is depicted in Table 3. There is a significant decrease in weekly body weight in group II (211.5 ± 7.12) as compared to the control group I (246.66 ± 4.93). But group IV animals showed increased body weight than group II.

Table 1. Experimental design of this study

Sr.No.	Group	No. of Rats	Treatment	Period
1	Group I	6	Normal feed and water	28 days
2	Group II	6	500 ppm lead acetate in drinking water	28 days
3	Group III	6	<i>Plumbago zeylanica</i> (root extract)@ 100 mg/kg body weight, orally	28 days
4	Group IV	6	500 ppm lead acetate in drinking water and <i>Plumbago zeylanica</i> (root extract)@ 100 mg/kg body weight, orally	28 days

Table 2. Average weekly feed consumption (g) in different groups.

Groups	Weeks				Average feed consumption
	1	2	3	4	
I	713.14	725.14	710.07	665.04	732.83±8.37 ^a
II	658.8	649.44	732	684.32	651.00±3.89 ^c
III	946.76	654.96	747.3	690.41	734.46 ±8.95 ^a
IV	886.73	640.83	748.5	707.28	686.76 ±8.71 ^b

Mean values with common alphabet as superscript do not differ significantly

Table 3. Average weekly body weight (g) of rats in different groups.

Groups	Experimental weeks				Average weekly body weight
	0	1	2	3	
I	201.92±3.48	213.74±4.91	222.53±4.67	234±4.29	223.77±7.773 ^a
II	202.58±7.13	204.01±7.42	205.88±7.45	207.83±7.29	206.36±1.558 ^b
III	202.83±2.63	214±3.45	223.72±3.09	235.67±4.07	224.63±7.77 ^a
IV	201.67±2.37	207.33±1.52	210.17±1.32	217.17±1.60	212.07±3.88 ^c

Mean values with common alphabet as superscript do not differ significantly

Table 4. Effect of *P. zeylanica* on haematological values of different experimental groups.

Groups	Hb (gm/dl)	PCV (%)	TEC (10 ⁶ /cu mm)	MCV (fL)	MCH (pg)	MCHC (%)
I	15.1±0.23 ^a	43.66±1.58 ^a	8.71±0.45 ^a	51.27±4.52 ^a	17.32±1.19	34.58±0.89 ^b
II	10.76±0.36	24.66±2.23 ^c	6.69±0.25 ^b	36.97±3.19 ^b	16.08±0.45	43.64±3.05 ^a
III	15.23±0.40 ^a	44.66±1.60 ^b	8.77±0.23 ^a	51.02±2.14 ^a	17.35±0.51	34.10±1.68 ^b
IV	12.53±0.56 ^b	32.66±1.97 ^b	7.84±0.30 ^a	41.64±1.51 ^b	15.97±1.18	38.36±3.32 ^{ab}

Mean values with common alphabet as superscript do not differ significantly

Table 5. Absolute leucocyte count (10³/cu mm) in different groups.

Groups	Total leucocyte count	Absolute neutrophil count	Absolute lymphocyte count	Absolute eosinophil count	Absolute basophil count	Absolute monocyte count
I	11.72±0.46 ^a	2715±210.44 ^b	9006.16±350.34 ^a	5.33±0.66	0	2±0.77
II	9.46±0.19 ^b	3700.167±96.73 ^a	5753.17±152.4 ^c	7.16± 0.47	0	1.5±0.56
III	11.22±0.46 ^a	2731.5±254.24 ^b	8491±341.15 ^a	5.83±0.74	0	1±0.44
IV	10.97±0.47 ^a	3370.33±354.39 ^{ab}	7596.66±253.11 ^b	5.83±0.60	0	1.5±0.42

Mean values with common alphabet as superscript do not differ significantly

Table 6. Effect of *P. zeylanica* on serum biochemical parameters in different experimental groups.

Groups	Creatinine (mg/dl)	AST (IU/L)	ALT (IU/L)	Glucose (mg/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)
I	0.74±0.05 ^b	136.33±1.26 ^b	37.55±0.71 ^c	57.23±2.49 ^c	10.785±0.41 ^a	7.206±0.86 ^c
II	1.35±0.09 ^a	216.17±4.22 ^a	57.73±2.58 ^a	101.79±5.19 ^a	5.86±0.31 ^c	13.17±0.33 ^a
III	0.73±0.07 ^b	136.43±2.26 ^b	38.7±1.27 ^c	87.87±4.52 ^b	10.82±0.50 ^a	7.64±0.50 ^c
IV	1.15±0.09 ^a	208.88±7.41 ^a	44.1±2.08 ^b	92.83±5.90 ^{ab}	7.89±0.21 ^b	11.39±0.48 ^b

Mean values with common alphabet as superscript do not differ significantly

Haematological parameters

Haematological parameters were studied to evaluate the functional status of haematopoietic system. There was a significant improvement in the mean values of Hb, PCV, TEC, TLC and MCV in group IV in comparison

to group II (Table 4). Low absolute neutrophil count was also noticed in group IV when compared to group II animals. Similarly, lymphocyte count was significantly lowered in group II, where as restoration of the value was seen in group IV (Table 5).

Serum biochemical parameters

Serum was analyzed to determine the functional status of other vital organs. Serum calcium, phosphorus, creatinine, AST, ALT and glucose were estimated and the values were depicted in Table 6. Group II animals showed significant decrease in calcium level but marked increase in the serum phosphorous, creatinine, AST, ALT and blood glucose values when compared with group IV.

Gross and histopathology

Grossly, only group II animals showed mildly congested brain and lungs, pale discoloration of liver and haemorrhages. Histologically, liver of group II animals showed marked sinusoidal congestion, and hepatocytes showed atrophy and fatty changes (Fig. 1), hyperplasia of bile duct, round cell infiltration and marked congestion in the portal vein (Fig. 2), whereas hepatic parenchyma was normal with mild congestion

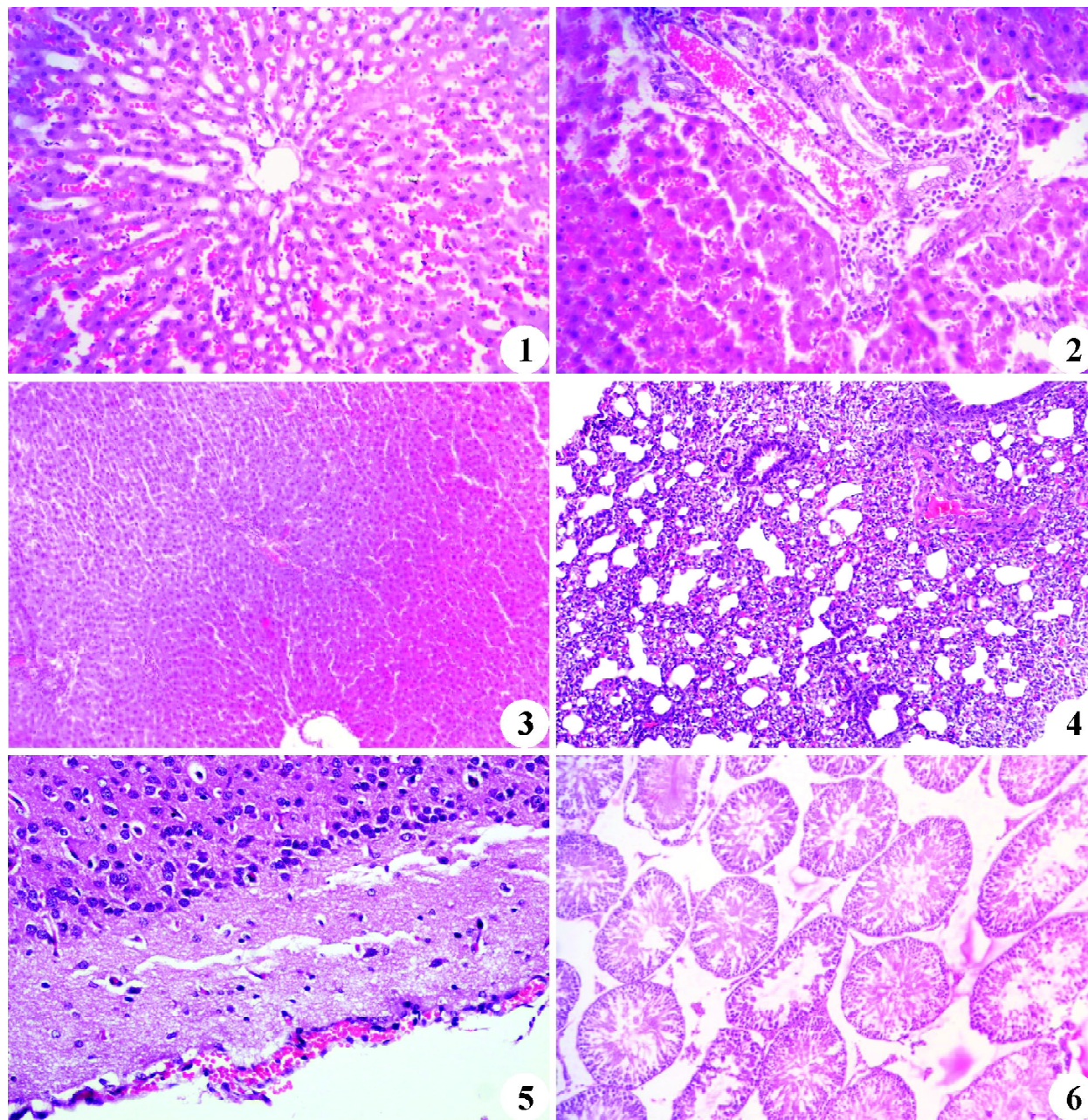


Fig. 1. Section of liver from group II rat showing sinusoidal congestion, atrophic parenchyma and fatty changes. H&E x200; **Fig. 2.** Liver from group II rat showing hyperplasia of bile duct, round cell infiltration and marked congestion in the portal vein. H&E x200; **Fig. 3.** Liver from group IV rat showing Mild congestion. H&E x100; **Fig. 4.** Lung from group II showing congestion, thickening of inter alveolar septa with lymphocytic infiltration and haemorrhages. H&E x100; **Fig. 5.** Brain from group II showing meningeal congestion, edema and neuronal degeneration. H&E x200; **Fig. 6.** Testis from group II showing hypoplasia, scanty spermatozoa and degenerative changes. H&E x100.

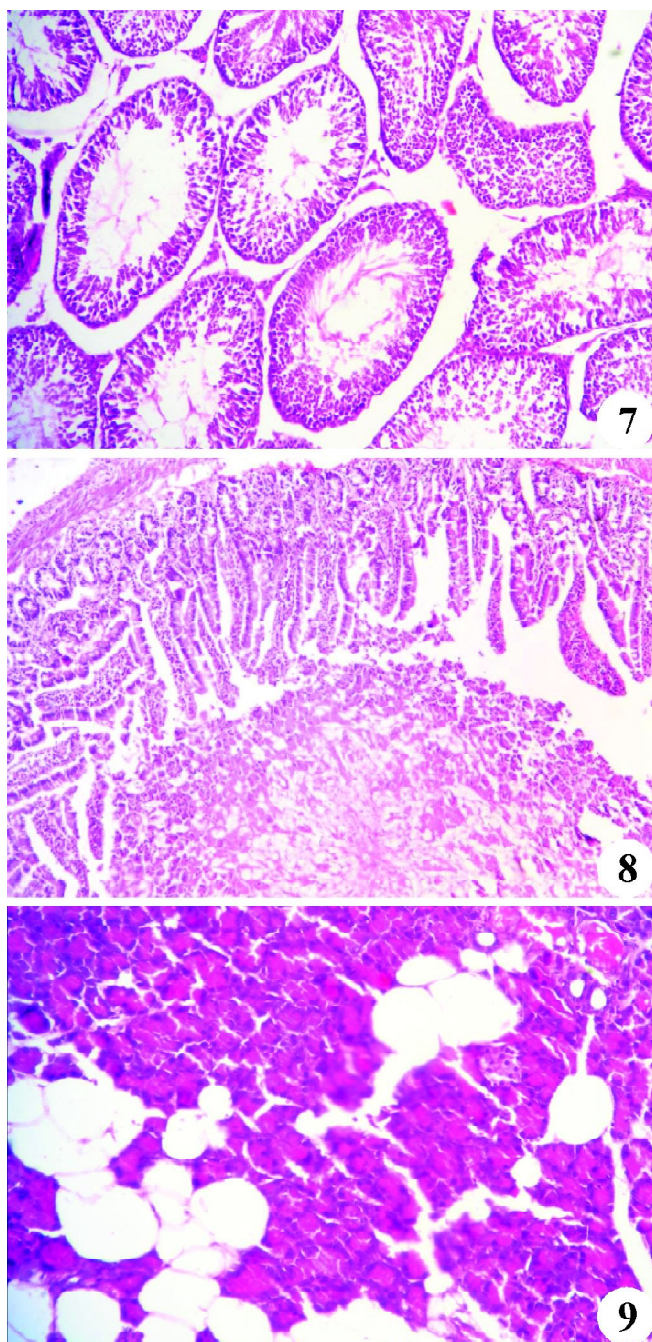


Fig. 7. Testis from group IV showing scanty spermatozoa. H&E x100; **Fig. 8.** Intestine from group II animals showing loss of intestinal villi and desquamated epithelium in the intestinal lumen. H&E x100; **Fig. 9.** Pancreas from group II animals revealed vacuolation, disruption of pancreatic acini and atrophy of islets of Langerhans. H&E x100.

in group IV (Fig. 3). Section of lung from group II showed congestion, thickening of inter alveolar septa with round cell infiltration specially lymphocytes and hemorrhages (Fig. 4), whereas in group IV animals there was mild pulmonary venous congestion with normal bronchiolar epithelium. Heart of group II animals revealed myocardial degeneration with marked congestion and

hemorrhages when compared to group IV, which showed congestion and hemorrhages but in decreased intensity. In brain of group II animals meningeal congestion, edema, neuronal degeneration and satellitosis were observed (Fig. 5). Group IV animals also showed mild congestion and edema in brain. Group II, III and IV animals revealed testicular degenerative changes which were more severe in group II causing distortion of seminiferous tubule (Fig. 6), however normal architecture of the seminiferous tubules was noticed in group IV (Fig. 7).

Sections of intestine from group I, III and IV animals showed normal intestinal epithelium whereas group II animals showed loss of intestinal villi and desquamated epithelium in the intestinal lumen (Fig. 8). Sections of pancreas from group I and III revealed normal endocrine structure characterized by normal islets of Langerhans however, sections from group II animals revealed vacuolation, disruption of pancreatic acini and atrophy of islets of Langerhans (Fig. 9)

DISCUSSION

During decades, lead concentration has been increased in the environment. This heavy metal has shown many divesting effects on different body systems. Lead toxicity is a comparatively more challenging issue in developing countries than the developed countries³. Use of herbal agents for this can be an alternative solution to avoid costly treatment regimen. Therefore, the present experimental design was set up to evaluate the efficacy of *Plumbago zeylanica* plant in lead toxicity. In the present study, animals fed solely with lead showed neurological symptoms clinically which was confirmed histologically. This could be possibly due to the adverse effect of lead on central nervous system as it can cross the blood brain barrier^{3,16}. Similarly lead can induce diarrhea observed in the present study was due to its action on intestinal mucosa⁴. Group IV animals neither reveal any abnormal signs of nonsocial behavior like excitement nor any type of diarrhea. Similar findings have been reported in earlier studies. In the present study, lower feed consumption in solely lead fed group may be due to the direct toxic effect on digestive system¹⁷. Group IV rats showed increased feed consumption than group II rats suggesting the beneficial effect of *Plumbago zeylanica* during lead toxicity¹⁸. Digestive disturbance and decreased feed intake might be the cause of significant decrease in weekly body weight in group II which was not encountered in group IV as there was improvement in appetite due to inclusion of *Plumbago zeylanica* in diet¹⁹.

Significant decrease in Hb, PCV, TEC, TLC and MCV values in group II could be possibly due to the influence

of lead on synthesis of heme by inhibiting δ -amino levulinic acid (δ -ALA) dehydrates enzyme, conversion of coproporphyrinogen III to protoporphyrin IX and inhibition of heme synthetase which is required to incorporate iron heme molecule^{3,20}. Lead also prevents the entry of iron from cytosol to mitochondria²¹. Addition of *Plumbago zeylanica* in diet of group IV animals showed improvement indicating its hematinic property as reported in the literature¹⁴. Increase in absolute neutrophil with significant decrease in lymphocyte count in lead fed group II possibly due to lead induced injury resulting inflammation along with reduction in peripherally circulated T and B lymphocyte as reported by other researchers¹⁶. The restoration of values in group IV indicates the immunomodulatory effect of *Plumbago zeylanica* suggesting its beneficial effect on lymphopoiesis or preventing lead induced lymphoid damage. Serum analysis showed significantly low level of calcium in group II than group IV. This can be attributed to the toxic effects of lead on bone or disturbances in calcium metabolism but protective effect of the plant under study¹⁹. Interestingly, unlike serum calcium phosphorus levels showed opposite trend may be due to lead induced kidney damage causing lameness during experimental lead toxicity. In the present study, increase in creatinine, AST and ALT values in group II and their restoration in group IV was observed. This may be due to lead caused renal, cardiac and hepatic impairment that can be correlated with the present histopathological findings²². Blood glucose level was also found to be increased significantly in group-II could be due to enhanced enzyme activity, stimulation of sympathetic component causing release of adrenaline from adrenal glands, secretion of glucocorticoids from adrenal cortex under stress conditions and destruction of beta cells in islets of Langerhans of pancreas in lead toxicity as stated by earlier workers²³. However, group IV animals showed non-significant decrease in blood glucose level due to presence of some principles in root extract when compared with group II. It suggests that *Plumbago zeylanica* increases the blood glucose level physiologically whereas lead does the same through oxidative damage of pancreas and allied organs²⁴. Hence comparative reduction in glucose level of group IV when compared to group II might be due to protective effect and anti-oxidant property of *Plumbago zeylanica*.

In the present study, grossly, pale discoloration, mild venous congestion and hemorrhage observed in liver of group II, which corroborates with earlier workers¹⁶. Microscopically, lead induced hepatotoxic lesions in group II may be possibly due to the action of lead on hepatic glycogen and DNA content^{4,25}. But hepatic lesions were mild in group IV supports the hepatoprotective effect of *Plumbago zeylanica*¹⁹. Occurrence of severe

pulmonary lesions in lungs particularly hyperplasia of bronchial epithelium in group II animals but lesions were mild in group IV is also corroborated in earlier publications⁴. Similarly, the intensity of degenerative changes in heart as observed in group II was not so prominent in group IV, which depicts the cardiotoxic effects of *Plumbago zeylanica*¹⁹. Protective effect of the plant extract on lead induced neurotoxicity can be explained due to mild pathomorphological changes in brain of group IV animals, but neuronal degeneration was severe in group II. These changes could be possibly due to hypoxia as a result of anemia observed during present study or direct toxic action of lead on central nervous system²⁶. On the contrary, *Plumbago zeylanica* was found to have limited protective effect on reproductive organs in case of lead toxicity which is evident from the presence of intact architecture of the seminiferous tubules in group IV than group II animals. This could be because of disruption of signaling mechanism by exposure to lead, altering thereby the gonadotropin-releasing hormone system in case of male rat and subsequent degenerative changes²⁷.

In conclusion, lead being a highly toxic metal can bring out several degenerative changes in many vital organs of the intoxicated rats. But the irony is that efficacy of its treatment cannot be ascertained hence preventive measures are always preferred over treatment regimens to nullify its toxic effect. Medicinal plants like *Plumbago zeylanica* has shown promising results which can be used in the preventive medicine diaspora against lead induced toxicity.

ACKNOWLEDGMENTS

The authors are very grateful to the staff of PGIVAS, Akola and MAFSU for the financial assistance for research programme to the first author.

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