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Amelioration of lead-induced nephrotoxicity by certain adaptogens in broilers

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ABSTRACT

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An experimental study of 6 weeks duration was conducted on 15 groups of broilers to evaluate lead-induced nephrotoxicity and its amelioration by poly herbal formulation (PHF; stressroak), shilajith, amla and vit E + Se. Group 1 was maintained on based diet, 2 on PHF @ 1 g/kg feed, 3 on shilajith @ 100 mg/kg feed, 4 on amla @ 500 mg/kg feed and 5 on vit E (3000 mg/kg feed) + Se (0.3 mg/kg feed). Group 6 was maintained on lead @ 250 mg/kg feed for 42 days (6 weeks) and 7 on lead for 28 days and subsequently on basal diet. Groups 8, 9, 10 and 11 were given lead along with PHF, shilajith, amla and vit E + Se, respectively throughout the experiment for 6 weeks. Groups 12, 13, 14 and 15 were given lead containing diet for the first 4 weeks (28 days) and subsequently treated with PHF, shilajith, amla and vit E + Se, respectively. The activity ALP and the concentration serum creatinine were significantly ($P < 0.05$) increased and there were corresponding pathological lesions in kidney in the toxic control groups on histopathology and electron microscopy at the end of 4th week. However, following treatment, there was a significant ($P<0.05$) reversal in groups 12, 13, 14 and 15. Amongst the drugs in test, PHF (stressroak) was found superior owing to its synergistic antioxidant and adaptogenic herbs, followed by shilajith. Amla and vit E + Se, though reversed the toxicological manifestations to certain extent, followed in order.

Keywords: Amla, broilers, nephrotoxicity, PHF, selenium, shilajith, stressroak, vitamin E

INTRODUCTION

Lead produces acute and chronic poisoning and induces a broad range of physiological, biochemical and behavioral dysfunctions resulting in reduced performance and death in livestock. Lead affects the metabolism of other minerals and has affinity for bone, where it acts by replacing calcium; thus the highest concentrations of lead are usually found in bone, kidney and liver[4] and subsequently may cause damage to these organ systems. Keeping the above points in view, an experimental study was planned to evaluate the lead-induced injury to the kidney and to evaluate the prophylactic and therapeutic potential of poly herbal formulation (PHF; stressroak), shilajith, amla and vit E + Se against experimental lead toxicosis in broilers.

MATERIALS AND METHODS

A total of 225 sexed male broiler chicks (*Cobb* strain) of day old age were randomly divided into 15 groups of fifteen chicks in each group. Feed and water was provided *ad libitum* throughout the experiment. Groups 1, 2 and 3, 4 and 5 were maintained on basal diet control, PHF (stressroak; 100 ppm in feed), shilajith (100 ppm in feed), amla control (500 ppm in feed) and vit E + Se (300 ppm + 0.3 ppm in feed), respectively and groups 6 and 7 were the toxic controls that were kept on lead for 42 days and 28 days, respectively. Groups 8,9,10 and 11 were given lead along with PHF, shilajith, amla and vit E + Se, respectively for 6 weeks (1 - 42 days). Groups 12,13,14 and 15 were maintained on lead for the first 4 weeks and on PHF, shilajith, amla and vit E + Se, respectively for the subsequent 2 weeks.

Blood samples were collected at the end of 4th and 6th week for assay of alkaline phosphatase (ALP) and creatinine by using diagnostic kits (Qualigens Pvt. Ltd., Mumbai, India). The data were subjected to statistical analysis by applying ANOVA as per the standard methods of Snedecor and Cochran[8]. Differences between means were tested using Duncan's multiple comparison test and significance was set at $P<0.05$. Representative pieces of kidney were collected in 10% formal saline for histological studies. The formalin fixed tissues were processed[2] and cut sections were stained with routine H&E stain. For Tranmission electron microscopy, the specimen samples were transferred to vials and fixed in 2.5% gluteraldehyde (EM grade) in 0.5 M phosphate buffer (pH 7.2) for 24 hrs at 4^0C and post fixed in 2% aqueous osmium tetroxide in the same buffer for 2 hrs. After the post fixation, samples were dehydrated in a series of graded alcohol from 50% to 100% for 40 minutes each, infiltrated in 1:1 alcohol and spur and later embedded in Spurr's resin[9]. Both semi-thin and ultra-thin sections were cut with a glass knife on a Leica Ultra cut UCT-GA-D/E-1/00 ultra microtome. Semi-thin sections of 200-300 nm thick were stained with toludine

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blue, whereas the ultra-thin sections (50-70 nm thickness) were mounted on copper grids. Subsequently, the sections were stained with saturated aqueous uranyl acetate for 30 min and counter stained with 4% lead citrate for 20 min[1] and were later observed at various magnifications under Transmission Electron Microscope (Model: Hitachi, H-7500, Japan).

RESULTS AND DISCUSSION

The activity ALP and the concentration of creatinine were determined to assess the degree of damage to kidney as the levels of these elevated following tissue injury[5]. In this study, the activity of ALP and concentration of creatinine were significantly elevated in the lead toxic control group suggesting the nephrotic insult following administration of lead. The activity of ALP (units/ml) in basal diet control (group 1) was 71.929 ± 0.594, which was significantly ($P < 0.05$) elevated in lead toxic control groups 6,7,10, 11, 12, 13, 14 and 15 at the end of 4th week. Groups 8, 9, 10 and 11 did not reveal any significant difference as compared to group 1 at the end of 6th week. The serum creatinine concentration (mg/dl) in the basal diet control was 0.442 ± 0.011, which was significantly ($P < 0.05$) increased in lead toxic control groups at the end of 4th week. The therapeutic control groups 2 and 3 revealed a significant ($P < 0.05$) decrease in the concentration (0.209 ± 0.012 and 0.206 ± 0.008, respectively) as compared to group 1 at the end of 4th week and a similar trend was noted at the end of 6th week. The groups 8, 9, 10 and 11 revealed a significant ($P < 0.05$) increase in the concentration as compared to group 1 at the end of 4th and 6th week. In groups 12, 13, 14 and 15, there was a significant ($P < 0.05$) decrease in the concentration as compared to groups 6 and 7 (3.544 ± 0.092 and 2.841 ± 0.120, respectively) at the end of 6th week and also their respective 4th week values (Table 1).

The results of the biochemical study revealed nephrotoxicity in lead toxic control groups. Lead has been reported to interact with renal membranes and enzymes, and hence disrupts energy production, calcium metabolism, glucose homeostasis, ion transport processes and the renin-angiotensin system[6]. The biochemical findings of this study are supported by transmission electron microscopy (TEM) of kidney

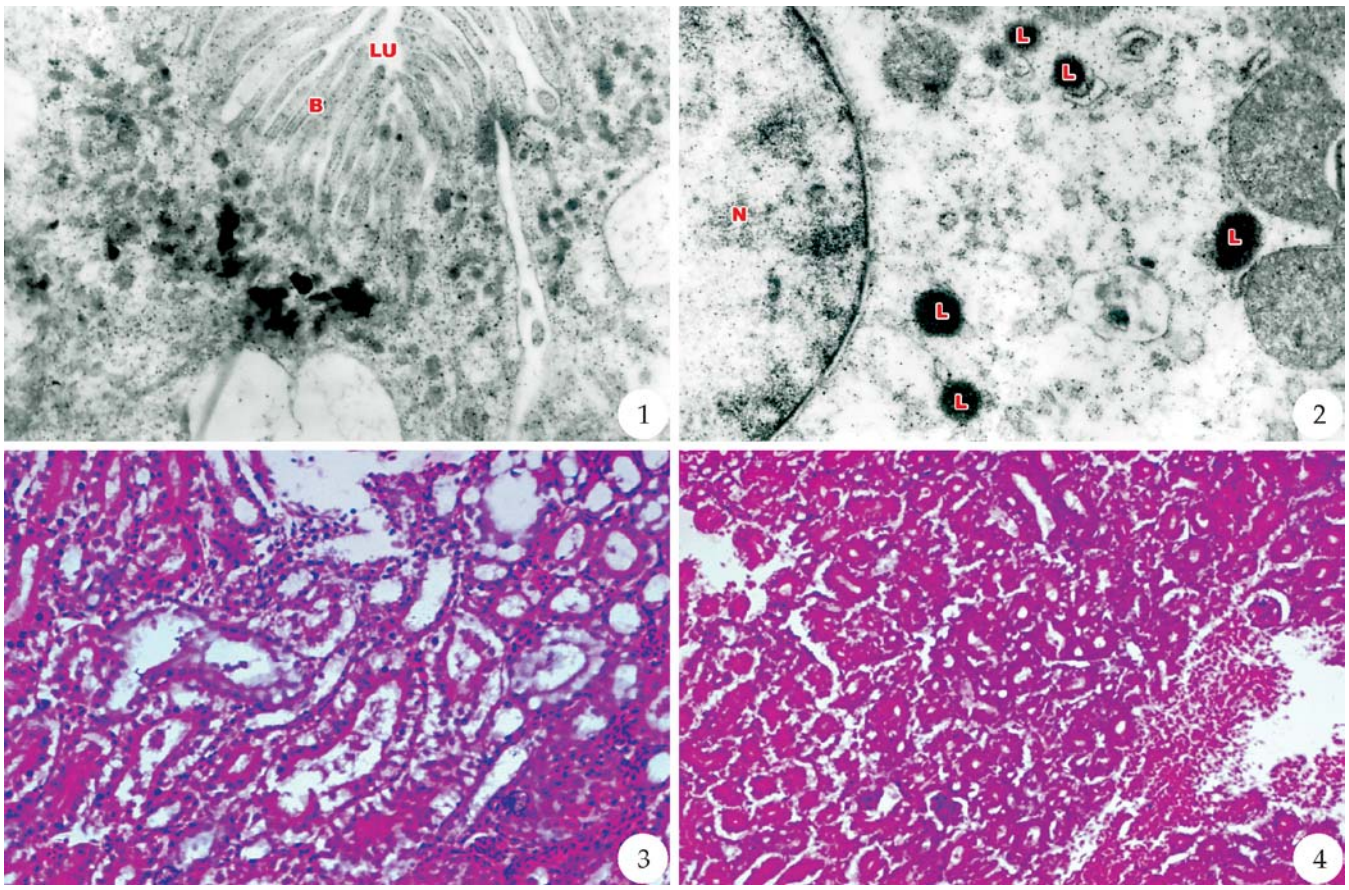

Fig. 1. TEM – PCT showing destruction of brush border (B) towards narrowed lumen (LU) x26850. (group-6). **Fig. 2.** TEM – PCT with prominent lysosomes (L) x21480. (group-7). **Fig. 3.** Kidney showing moderate degenerative changes in the tubular epithelium. HE x200 (Group - 6). **Fig. 4.** kidney showing marked inter tubular haemorrhages HE x100 (Group - 7)

Table 1: Activity of alkaline phosphatase (ALP) and concentration of creatinine in serum of broilers.

Group	ALP (Units/ml)		Creatinine (mg/dl)	
	4th Week	6th Week	4th Week	6th Week
1. Basal Diet (1-42 d)	71.929±0.594^{deA}	76.007±0.838^{cdefB}	0.442±0.011^{bA}	0.543±0.011^{bB}
2. PHF (Stressroak) (1-42 d)	68.298±0.601^{aA}	71.341±0.762^{aB}	0.209±0.012^{aA}	0.298±0.006^{aB}
3. Shilajith (1-42 d)	68.234±0.750^{abA}	71.917±0.472^{aB}	0.206±0.008^{aA}	0.292±0.006^{aB}
4. Amla (1-42 d)	69.295±0.968^{bcA}	74.255±1.170^{cdB}	0.294±0.020^{abA}	0.394±0.017^{abB}
5. Vit E + Se (1-42 d)	70.329±0.645^{cdA}	75.293±1.331^{cdB}	0.294±0.018^{abA}	0.406±0.008^{abB}
6. Lead (1-42 d)	78.098±0.392^{hA}	82.240±0.692^{gB}	2.900±0.041^{ghA}	3.544±0.092^{iB}
7. Lead (1-28 d) ; Basal Diet (29-42 d)	77.146±0.640^{ghA}	75.137±0.778^{cdB}	3.064±0.187^{hA}	2.841±0.120^{hB}
8. Lead + PHF (Stressroak) (1-42 d)	71.090±0.876^{cdeA}	74.126±1.6409^{bcB}	1.935±0.060^{cA}	2.174±0.096^{defB}
9. Lead + Shilajith (1-42 d)	73.008±2.069^{eA}	76.042±1.034^{cdefB}	2.098±0.044^{dA}	1.914±0.015^{cB}
10. Lead +Amla (1-42 d)	75.036±0.982^{fA}	77.275±1.804^{efB}	2.338±0.119^{eA}	2.199±0.089^{efB}
11. Lead +Vit E + Se (1-42 d)	75.962±0.763^{fgA}	77.967±1.192^{fB}	2.291±0.108^{eA}	2.223±0.074^{efB}
12. Lead (1-28 d) ; PHF (Stressroak) (29-42 d)	78.003±1.174^{hA}	72.186±1.071^{abB}	2.810±0.058^{fgA}	2.018±0.06^{cdB}
13. Lead (1-28 d) ; Shilajith (29-42 d)	78.206±1.093^{hA}	74.167±1.122^{cdB}	2.913±0.191^{ghA}	2.104±0.080^{deB}
14. Lead (1-28 d) ; Amla (29-42 d)	77.150±1.853^{ghA}	75.955±1.292^{cdeB}	2.706±0.154^{fA}	2.332±0.129^{fB}
15. Lead (1-28 d) ; Vit E + Se (29-42 d)	76.972±0.623^{fghA}	76.093±0.383^{defB}	2.904±0.175^{ghA}	2.500±0.074^{gB}

Values are mean ± SE of 8 observations; Means with different alphabets as superscripts differ significantly (P<0.05) ANOVA; Capital alphabets (Horizontal comparison) Small Alphabets (Vertical comparison)

conducted at the end of 6th week, which revealed ruptured mitochondria and vacuolation in the proximal convoluted tubules along with destruction of brush border and narrowing of lumen (Fig. 1) in the toxic control group 6. Prominent lysosomal bodies were observed in group 7 (Fig. 2). Few lysosomal bodies were found in the groups 13 and 14, while groups 12 and 13 exhibited no specific cellular changes on electron microscopy. Semi-thin sections of kidney (group 6) showed swollen glomerular tuft with mild degenerative changes in the tubular epithelium. Similar findings on ultrastructural studies of kidney of ducks exposed to lead were reported by Rao *et al.*[7]. Further, the histopathological sections of the kidney from the lead toxic control (group 6) showed moderate degenerate changes in the tubular epithelium (Fig. 3) and marked intertubular congestion. The group 7, where lead was discontinued after 28 days, showed marked intertubular hemorrhages (Fig. 4), which were in accordance with the report of Del Bono and Braca[3]. Groups treated with PHF (stressroak), shilajith, amla and vit E + Se, following discontinuation of lead, resulted in significant decrease in serum creatinine and ALP as compared to toxic control groups, which confirms the therapeutic potential of the drugs in test. The beneficial renal protective actions of drugs in test may be attributed to their antioxidant / free radical scavenging actions and protection of protein thiols from deleterious actions of lead in kidney.

In conclusion, the study revealed that supplementation of adaptogens in test could counter the nephrotoxic potential of lead. Amongst the drugs in test, PHF (stressroak) was found superior owing to its synergistic antioxidant and adaptogenic herbs (*Withania somnifera, Ocimum sanctum, Phyllanthus emblica* and *Mangifera indica* along with shilajith), followed by shilajith, amla and vit E + Se.

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