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Effect of *Emblica officinalis* fruits against metallic-lead induced biochemical and hematological alterations in Wistar rats

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Lead toxicity and related health issues have become global concern due to increased use of lead-based products in the modern world. Though attempts are being made to tackle this malady through many ways, the use of naturally occurring materials that are available locally is a subtle approach. In this investigation, the fruits of *E. officinalis* (*EO*) were studied for their potential in overcoming biochemical and hematological alterations caused by metallic lead in rat model. Four groups of rats, each containing six animals, were considered for the study. Group I served as normal control while to other groups (II– IV), metallic lead powder (100 ppm/rat) was orally administrated for 30 consecutive days. From day 31, the animals in groups III and IV were treated with *EO* in doses of 50 and 100 mg/kg body weight (p. o.), respectively, for the following seven days. Group II served as lead-treated control. On day 38, the animals in all groups were sacrificed and the blood was collected and serum separated. The changes in biochemical (aspartate aminotrasferase, alanine aminotrasferase, alkaline phosphatase, gamma glutamyltrasferase, total cholesterol, triglycerides, urea, creatinine and calcium) and hematological (red blood cell count, white blood cell count, hemoglobin, packed cell volume and platelet count) parameters were estimated. Lead treated animals in Group - II showed appreciable changes in hematological and biochemical parameters. Treatment with *EO* (50 and 100 mg/kg) significantly restored the changes in the above parameters to near normal values implying that the fruit of *E. officinalis* is an effective natural material to overcome widespread lead toxicity. This observation is further supported by histopathological studies of liver and kidney tissues wherein the distorted architecture, degeneration and other changes found in lead-treated animals were brought back to near normal stages by the treatment of *EO*.

Keywords: Lead toxicity, Indian gooseberry, Animal study, Biochemistry, Hematology, Histopathology

Lead toxicity is one of the persisting environmental hazards in most parts of the world and the worst affected are the lead-based industrial workers and people who live close by over a long period of time. The evidence of lead poisoning has a long history and dates back to Roman times[1]. Even though lead is present in various forms in the environment, metallic lead plays a vital role in causing morbidity and mortality[2]. As availability of antidotes to overcome this serious and unabated issue is very limited[3], it is logical to turn to natural materials towards finding out a suitable remedy. Among the natural products, plants hold promise as they are available locally, cost-effective and employed to treat and manage diseases in traditional and folklore practices of many countries[4].

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Emblica officinalis Gaertn. (Syn: *Phyllanthus emblica* Linn.; Family: Euphorbiaceae) or Indian gooseberry or *Amla* is regarded as one of the best rejuvenating herbal products in Indian traditional medicines[5]. The fruits contain several antioxidants, minerals, amino acids, tannins and sugars. They possess a wide spectrum of pharmacological activities[6]. In continuation of our earlier study on *E. officinalis* in lead toxicity[7], the present investigation has been undertaken as sequel to find out its effect on lead-exposed rats based on biochemical, haematological and histological profiles.

Materials and Methods

Plant material

E. officinalis fruits (*EO*) were procured from the local market and authenticated by a taxonomist. The seeds were separated and the pulp was shade-dried.

After 7 days, the dried material was ground well and sieved to get a fine powder.

Animals

Necessary approval was obtained from Institutional Animal Ethics Committee for the study (No. BRULAC/SDCH/SIMATS/IAEC/02-2019/012).

Adult male albino rats of Wistar strain (180-190 g) were obtained from Tamil Nadu University of Veterinary and Animal Sciences, Chennai and housed in standard polypropylene laboratory cages containing 5 cm deep layer of sawdust bedding in controlled environmental conditions (temperature: 24±2°C, relative humidity: 50-70 % and 12 h light/dark cycle). They were fed with commercial pelleted feed (supplied by Poultry Research Station, Chennai) and purified water *ad libitum*. The experiments were conducted as per the guidelines of 'Committee for the Purpose of Control and Supervision of Experimental Animals' (CPCSEA).

Experimental protocol

Twenty-four animals were randomly segregated into four groups (I-IV) each containing six rats. Group I served as control that received only pelleted feed and water while the remaining groups received metallic lead powder (procured from S.D. Fine Chemicals, Mumbai, India) suspended in coconut oil that was administered orally (100 ppm per animal) using Canula syringe once daily for 30 consecutive days. On day 31, the lead exposed animals in Groups III and IV were treated for seven more days with two doses of *EO* while the animals in Group II served as lead treated control. The above two doses of *EO* were selected based on earlier studies[7].

Group I Normal control, Group II Lead treatment, Group III Lead treatment + *EO* (50 mg/kg), and Group IV Lead treatment + *EO* (100 mg/kg)

Biochemical and hematological studies

On day 38, over-night fasted animals were sacrificed under light ether anesthesia and the blood was collected in tubes with EDTA and clot activators. The blood samples were analyzed for hematological parameters immediately while serum was separated and stored in refrigerator for further biochemical analysis. Hematological parameters such as white blood cell count (WBC), red- blood cell count (RBC), platelet count, hemoglobin (Hb) and packed cell volume (PCV) were analyzed using Beckmann Coulter cell counter (Ac.T 5 Diff), Germany, following standard procedures[8]. The serum samples were analyzed for biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT)[9], total cholesterol (TC), triglycerides (TG)[10], urea, creatinine[11] and calcium[8] by established methods.

Histopathological studies

The liver and kidney tissues were excised immediately after the sacrifice and approximately 5 mm^3 of each tissue was fixed in formal saline. Then, they were dehydrated in tap water and embedded in paraffin wax as blocks. The wax blocks were then sectioned (8 micron thickness), spread on glass slides and after a series of water and ethanol wash, hematoxylin and eosin staining were done. The stained slides were detained and mounted[12].

Statistical analysis

The data obtained in the experiments were subjected to Analysis of Variance followed by Dunnett's *t*-test for multiple comparisons. Values with $P< 0.05$ were considered to be significant.

Results

The biochemical markers in lead induced rats showed an increase in serum AST, ALT, ALP, TC and TG while urea, creatinine and calcium showed a significant decrease. GGT showed insignificant changes compared to control rats. On treatment with *EO* in two doses 50 and 100 mg/kg, no significant difference was observed in 50 mg/kg group in GGT and creatinine values whereas other parameters showed values that were statistically significant. In the higher dose group, all the parameters showed near normal values when compared to lead treated animals (Fig. 1).

The lead exposed rats showed a significant decrease in hematological markers such as RBC, WBC, Hb, PCV and platelets when compared to control rats. Treatment with *EO* in doses of 50 and 100 mg/kg body weight revealed a remarkable reversal in these values in a dose-dependent manner when compared to group II rats (Fig. 2).

In the histopathological studies, normal architecture of liver with radiating hepatocytes and intact sinusoidal space were observed in group I animals while liver architecture distortions with macro vesicular steatosis were noted in lead treated group II rats. Further lipid droplets were also present in the

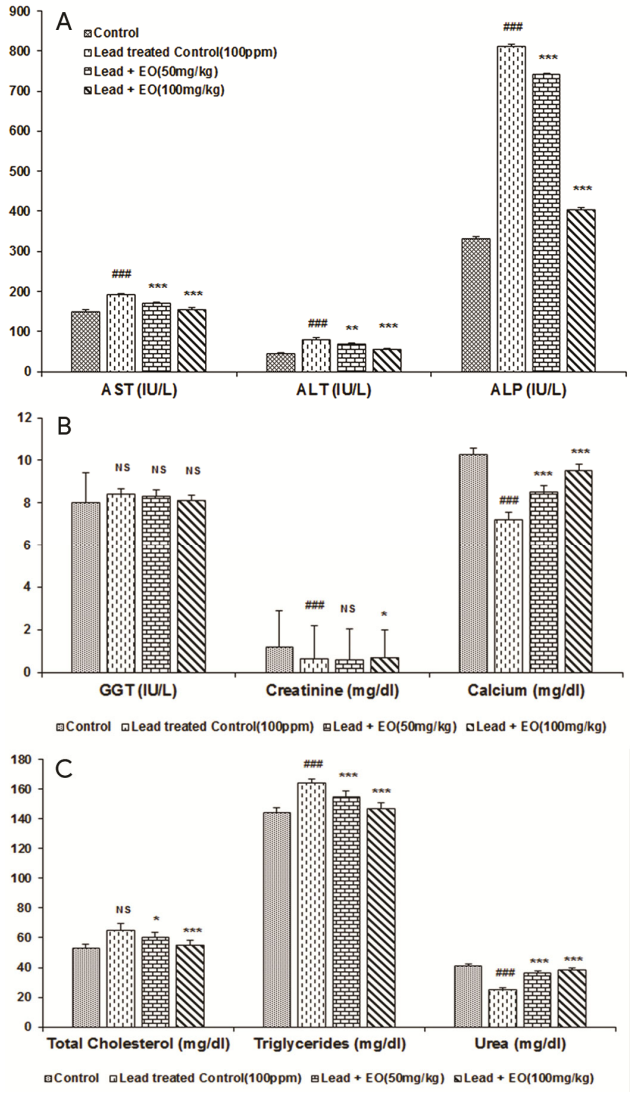

Fig. 1 — Effect of *E. officinalis* fruit powder (*EO*) on biochemical markers in lead-treated rats. Values represent mean ± SD of six animals. $^{##}P< 0.01$; $^{###}P< 0.001$ compared with control rats. $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$; ^{NS}-Non-significant compared with lead treated rats

hepatocytes that caused liver degeneration. In the low dose *EO* treated animals (group III), the liver tissues showed small lipid droplets besides mild liver degeneration whereas in group IV rat tissues, the lipid droplets were absent and there was regeneration of liver. Similarly, the kidney tissue in group I rats showed normal Bowman's capsule and renal tubules while lead treated rats revealed congested, degenerated glomeruli with significant renal tubule distortion. The low and high doses of *EO* treatments significantly ameliorated nephrotoxic potential of lead as evidenced by the normal renal tubule and its architecture (Fig. 3).

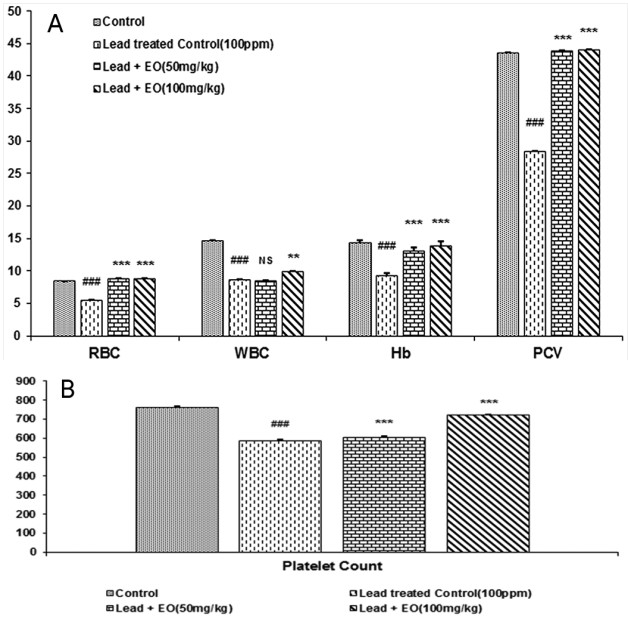

Fig. 2 — Effect of *E. officinalis* fruit powder (*EO*) on hematological markers in lead-treated rats. WBC - $10^3/\mu L$; RBC - $10^6/\mu L$; Hb - g/dl; PCV - %; Platelet count - $10^3/mm^3$. Values represent mean ± SD of six animals. $^{##}P< 0.01$; $^{###}P< 0.001$ compared with control rats. $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$; ^{NS} -Non-significant compared with lead treated rats

Discussion

Lead toxicity has become a global concern as it has been found to be the cause for many diseases. A report on lead's role in cardiovascular disease stresses the need for combating the malady through a cheap and readily available natural material[13]. As of now, Chelation therapy is the main line of treatment but this causes side effects. The use of plant products has come to stay as they are cost effective and easily available. India is blessed with numerous varieties of plants and most of them are within the reach of the common man. Newer applications of plant products are attempted to benefit the society as a result of research outcomes. In this study, the fruits of Indian gooseberry have been considered as an antidote for lead toxicity by investigating its effect on hematological and biochemical parameters in rat model.

Continuous administration of metallic lead to rats for 30 days significantly decreased the hematological parameters (RBC, WBC, Hb, PCV and platelets). One of the known toxic effects of lead is its interference with heme biosynthesis[14]. Lead has also been reported to possess high affinity to bind to red blood cell causing of hypochromic microcytic anemia due to bone marrow depression[15].

Administration of *EO* attenuated the lead induced action on hematological parameters which might be

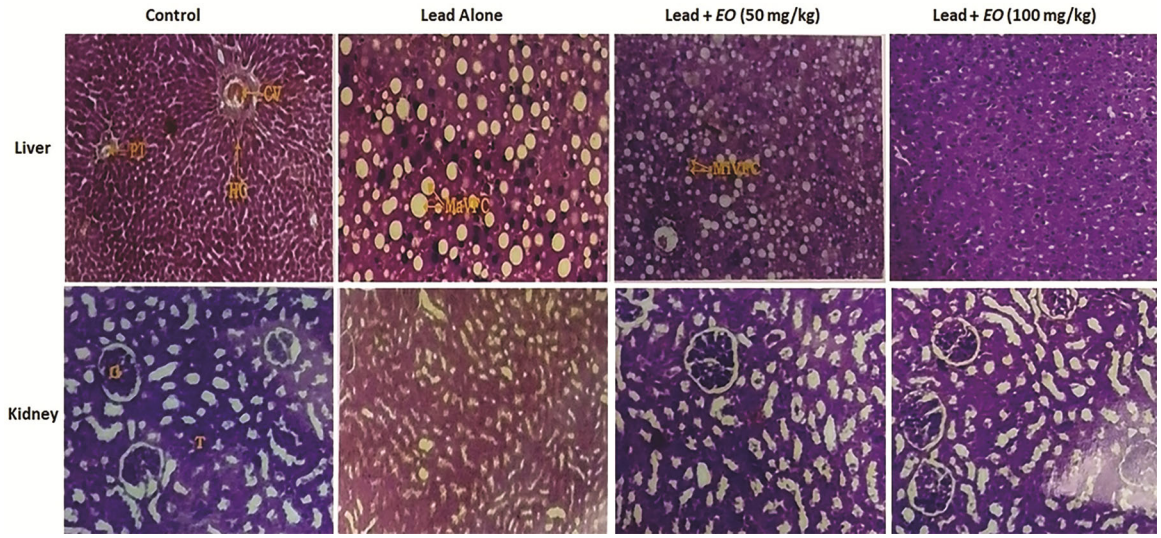

Fig. 3 — Histopathology of liver and kidney tissues(10X). Treatments were done as described in the Materials and Methods. PT- Portal triad, CV - Central vein, MaVF-Macrovesicular fat droplets, MiVF-Mirovesicular, fat droplets, B-Bowman's capsule, T- Urinary tubules

due to the rich content of ascorbic acid that primarily acts to modify excretion of lead from the chelatable bone pool or bound in red blood cells[16]. White blood cells produced by the immune system to defend the body against infection have increased with doses of *EO* indicating non-alteration of the defense mechanism. A decrease in PCV showed the extent of shrinking cell size due to lead intoxication[17] while its dose-dependent increase indicated a positive effect of *EO* treatment. The platelet count also revealed a reasonable increase.

In the biochemical studies, serum AST, ALT and ALP showed a significant elevation. These intracellular enzymes are regarded as markers of liver injury and get released into blood stream unequally depending on the pathological conditions[18]. The ALP alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites. In the present study, it was found that lead intoxication caused a significant increase in the activities of AST and ALT that could be due to severe damage of the membrane of hepatocytes. The GGT levels which measure hepatic cholangiocytic activity[19] did not change significantly. If the liver is injured, the liver cells spill the enzymes into blood thereby raising the enzyme levels in the latter. Previous studies have shown that lead intoxication induced a significant elevation of serum AST, ALT, ALP and GGT levels[20]. Treatment with *EO* revealed a significant hepato-protective activity. It was observed that scavenging of oxygen free radicals and inhibition of lipid peroxidation are the desirable properties of an antidote against toxicity[21]. Since *EO* contains antioxidants, the protective effect of the fruits may be attributed to this property.

A slight increase has been noted in TC and TG levels in animals that were chronically exposed to lead[22]. The rise in the lipid content of lead treated animals indicated enhanced accumulation of fatty mass that might be due to serum phospholipids forming lead phosphate[23]. This interaction may be partly responsible for the increase and treatment with *EO* restored the same to near normal values.

The efficacy of *EO* has been further supported by histopathological studies. The observed degeneration of liver tissues and presence of lipid droplets in the lead treated animals were counteracted by the administration of *EO* especially in higher dose.

Lead is also known to cause sub clinical renal damage depending upon the extent of exposure[24]. In the present study, urea and creatinine were significantly decreased in lead treated rats. An association observed in this study with increased lead content resulting in lower urea and creatinine levels is in accordance with the earlier observation among Korean lead workers[25]. The reduced levels of urea might indicate cell destruction leading to the release of large quantities of protein and purine bases, which are metabolized to urea and uric acid. On treatment with *EO*, the urea content has been restored to normal value while that of creatinine showed a great improvement.

Histopathological studies of kidney tissues further lend support to *EO*'s effectiveness. The distorted tissue architecture and other ultra-structural changes

brought out by chronic lead exposure were significantly restored by *EO* treatment.

Calcium homeostatis is the mechanism by which the body maintains adequate calcium levels. It has been found to decrease on 30 days lead treatment that might be due to the chemical resemblance of Ca^{2+} to Pb^{2+} ions. The latter competitively inhibits the uptake of calcium in mitochondrial calcium transport of proteins. Lead may also compete with calcium for binding into the triphosphate chain of ATP[26]. Studies on laboratory animals have demonstrated that diets low in calcium increases lead retention, leading to enhanced lead toxicity[27]. This increased toxicity has been attributed to the increase in the gastrointestinal absorption of lead. On treatment with *EO*, serum calcium levels were restored to near normal values which are in agreement with the reported study[28]. This observation clearly suggests a direct relation between lead and calcium that compete with each other for a biochemical matrix.

Conclusion

The efficacy of any protective material mainly depends on its capacity to either reduce the harmful effects or maintain normal physiology of cells and tissues that have been damaged by toxins. The phytochemicals present in the fruits of *E. officinalis* might have contributed to its restorative effects that are consumed in different forms by the people for many years. Thus the present study revealed a new utility for Indian gooseberry fruits in combating lead toxicity. Studies of this kind on the readily accessible antidotes that are cost effective as well shall open up new avenues in finding remedies for a wide variety of toxicity problems in future.

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Conflict of interest

The authors declare no conflict of interest.

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