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RESEARCH ARTICLE

Assessing the Beneficial Effects of Ellagic Acid in Mitigating Lead Induced Toxicity in Haematopoietic System

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ABSTRACT

Background: Lead exposure causes oxidative stress, leading to hematological abnormalities and immunotoxicity. Ellagic acid (EA) has shown protective effects against lead-induced toxicity in various organs. **Objective:** This study investigates EA's potential to mitigate lead-induced hematopoietic toxicity. **Methods:** The study consisted of four groups of eight animals each, including a normal control group, a toxic control group receiving lead acetate, and two groups receiving Ellagic acid with lead acetate. The animals were treated for 7 days, after which blood and bone marrow samples were collected for hematological assessments, lead concentration analysis, and bone marrow examination. **Results:** The study found that lead acetate exposure led to significant decreases in erythrocyte count, hemoglobin content, and total leukocyte count, while Ellagic acid treatment effectively restored these counts. Additionally, Ellagic acid treatment reduced plasma lead concentration and alleviated lead-induced hematological alterations, suggesting its potential as a therapeutic agent against lead toxicity. **Conclusion:** This study explores the protective effects of Ellagic acid against lead-induced hematopoietic toxicity, a previously uninvestigated area of research.

Keywords: Lead toxicity; Haematopoietic toxicity; Heavy metal toxicity; Ellagic acid

INTRODUCTION

The hematopoietic system is vulnerable to numerous environmental toxicants, with lead being a prominent example. This widespread pollutant induces oxidative stress, causing dysfunction in multiple cell types.[1] Lead exposure is associated with a range of adverse effects, including neurotoxicity, reproductive toxicity, liver and kidney damage, hematological abnormalities, and immunotoxicity.[2]

The hematological system is a primary site of lead-induced toxicity. Interestingly, nearly all (99%) of the lead present in blood is bound to red blood cells (erythrocytes), which may act as a vehicle for lead transport to other organs and tissues.[3,4]

Lead exposure disrupts normal hematopoiesis, leading to aberrant cell differentiation and impaired hemoglobin production, ultimately resulting in hematological disorders.[5]

Studies have shown that lead salts can cause cytogenetic damage to bone marrow cells in rats and mice.[6]

Research has shown that lead exposure in vitro selectively targets hematopoietic and stromal cells in rat bone marrow. Furthermore, comparative analysis indicates that humans are more vulnerable to lead's toxic effects than mice.[7]

Research has demonstrated that polyphenols can counteract lead-induced inflammation, and their metabolites have been found to possess reducing, metal-chelating, and antioxidant properties.[8] Ellagic acid (EA), a polyphenol with established medicinal value, has been shown to exert protective effects against lead-induced toxicity in multiple organs, including cardiotoxicity, nephrotoxicity, neurotoxicity, hepatotoxicity, and female reproductive toxicity.[9]

Despite the known toxic effects of lead on hematopoiesis, the protective potential of Ellagic acid (EA) against lead-induced hematopoietic toxicity remains unexplored. This study seeks to investigate the ameliorative effects of EA on hematological parameters in the context of lead exposure.

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METHODOLOGY

Chemicals:

Chemicals of analytical grade were used, procured from established manufacturers. Lead acetate, for instance, was sourced from Loba Chemicals in Mumbai.

Phyto-chemicals:

Ellagic acid samples were sourced from Yucca Enterprises, located in Mumbai, India.

Experimental Animals:

The study utilized healthy adult male and female albino rats (150-180 g) housed in polypropylene cages at the institutional Central Animal House. Standardized conditions, including 12 h light-dark cycles and a temperature range of 25° ± 5°C, were maintained, and the animals were provided with standard pellet food and purified drinking water. The study was complying with CPCSEA guidelines and received approval from the IAEC.

Dose selection of Ellagic acid:

Based on previous studies, two oral doses of Ellagic acid in a dose of 50 mg/kg (high dose) and 25 mg/kg (low dose), were selected for administration to rats.[10]

Experimental design:

Following a one-week acclimatization period, the animals were allocated into four groups in a random manner, each consisting of eight animals.

Group I: Served as the normal control group and received 2 ml/kg of normal saline orally.

Group II: Served as the toxic control group, where animals received lead acetate (10 mg/kg) via intramuscular injection for a period of 7 days.[11]

Group III: Received Ellagic acid p.o. in 50 mg/kg dose for 7 days, concurrently administered with lead acetate as described for Group II.

Group IV: Received Ellagic acid p.o in 25 mg/kg dose for 7 days,concurrently administered with lead acetate as described for Group II.

After 7 days of treatment, rats were subjected to overnight fasting, followed by sacrifice. Blood samples were collected in heparinized tubes for hematological assessments, including erythrocyte, total leukocyte, differential leukocyte, hemoglobin, and platelet counts.[11]

Plasma samples were separated by centrifugation at 250 × g for 10 minutes at room temperature. The resulting plasma was then analyzed for lead concentration using atomic absorption spectrophotometry.[11]

Following the previous steps, bone marrow examination was conducted. The femurs and tibias were extracted, and the marrow was collected by flushing the bones with 1 ml of phosphate-buffered saline (pH 7.4) using a syringe. This process was repeated until the bones changed color from red to white, confirming complete removal of the marrow contents.[11,12]

Bone marrow smears were stained with Papanicolaou dye and examined under a light microscope using a 100x oil immersion lens. Cell density and megakaryocyte count were assessed, and maturation indices were evaluated.[12]

The parameters estimated will be:

1. Erythrocytes, total leucocytes, differential leucocyte, haemoglobin and platelets counts were taken.
2. Determination of lead level by atomic absorption spectrophotometry.
3. Bone marrow examination stained with papanicolaou dye.

Statistical analysis:

Data are expressed as mean ± SEM. Statistical significance was evaluated using one-way ANOVA, and Tukey-Kramer post-hoc tests. Differences were considered significant at $p < 0.05$.

RESULTS

Peripheral blood analysis: Erythrocytes, haemoglobin and platelets (Table 1)

Lead acetate exposure led to a significant decrease in erythrocyte count and hemoglobin content in rats compared to the normal control group. In contrast, all treatment groups demonstrated a significant rise in erythrocyte count and hemoglobin content when compared with the toxic control.

Table 1: Peripheral blood analysis:Erythrocytes, haemoglobin and platelets

Treatment	Erythrocyte x10^6 (per μl)	Haemoglobin (g/dl)	Platelets x10^3 (per μl)
Normal control	6.37 ± 0.31	11.51±0.23	606±18.5
Toxic control	5.12 ± 0.21*	8.86±0.32*	421±23*
EA 50	6.20±0.32#	11.25±0.28#	593±32#
EA 25	6.16±0.22#	11.21±0.12#	590±25#

Values are mean ± SEM, n=8, *P <0.05 when compared to normal control; #P <0.05 when compared to Toxic control group.

Peripheral blood analysis: total leucocytes and differential leucocyte (Table 2)

The toxic control group exhibited a marked decrease ($p < 0.05$) in total leucocyte and lymphocyte counts in peripheral

blood compared to the normal control group. However, Ellagic acid treatment (EA 50 and EA 25) effectively restored these counts, showing a significant increase ($p < 0.05$) compared to the toxic control group.

Compared to the normal control group, the toxic control group showed a significant ($p < 0.05$) elevation in total granulocyte count in peripheral blood. However, treatment with Ellagic acid (EA 50 and EA 25) effectively reduced total granulocyte count, demonstrating a significant ($p < 0.05$) decrease compared to the toxic control group.

Table 2: Peripheral blood analysis:total leucocytes and differential leucocyte

Treatment	Leucocyte x10^3 (per μl)	Granulocyte %	Lymphocyte %
Normal control	14.23 ± 0.21	28.4±2.11	73.21±1.9
Toxic control	7.92 ± 0.15*	64.26±0.21*	49.43±2.2*
EA 50	12.23±0.62$^{\#}$	35.17±0.23$^{\#}$	64.32±2.4$^{\#}$
EA 25	12.10±0.31$^{\#}$	35.56±0.28$^{\#}$	63.19±3.1$^{\#}$

Values are mean ± SEM, n=8, *P <0.05 when compared to normal control; $^{\#}$P <0.05 when compared to Toxic control group.

Determination of lead levels (Table 3)

Compared to the normal control group, the toxic control group exhibited an extremely significant ($p < 0.001$) elevation in plasma lead levels in peripheral blood. However, treatment with Ellagic acid (EA 50 and EA 25) resulted in a significant ($p < 0.05$) reduction in plasma lead concentration compared to the toxic control group.

Table 3: Determination of lead levels

Treatment	Lead (μg/dl)
Normal control	0.87±0.10
Toxic control	40.1±1.7***
EA 50	27.7±2.1$^{\#\#}$
EA 25	28.2±1.3$^{\#\#}$

Values are mean ± SEM, n=8, ***P <0.001 when compared to normal control; $^{\#\#}$P <0.01 when compared to Toxic control group.

DISCUSSION

This investigation examined the potential of Ellagic acid (EA) to protect against hematological toxicity induced by lead exposure. The study's findings showed that EA provided dose-dependent protection, counteracting the adverse effects of lead on the hematopoietic system and promoting overall hematological health. As a pervasive environmental pollutant, lead poses a significant threat to human health, causing damage to multiple organ systems, including the nervous, renal, reproductive, and hematological systems, primarily through oxidative stress.[2,13]

Lead exposure in rats resulted in a significant reduction in red blood cell (RBC) count and hemoglobin (Hb) levels, characteristic of normocytic normochromic anemia, a condition marked by decreased RBC production.[10]

Photograph of bone marrow aspirate:

Lead's hematotoxicity can be explained by two primary mechanisms: firstly, lead's direct inhibitory effect on hematopoietic organs, impeding erythropoiesis, and secondly, lead's binding to RBCs, which increases membrane fragility and results in rapid RBC destruction. Lead exposure may also impair hemoglobin production through multiple mechanisms, including inhibition of the ALAD (aminolevulinic acid dehydratase) enzyme and disruption of iron metabolism, as lead competes with iron for essential binding sites.[14–16]

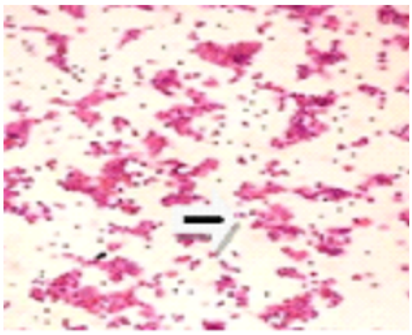
Lead exposure generates oxidative stress in blood cells, causing them to deteriorate, reducing their lifespan, and disrupting their production. Moreover, lead initiates the Fenton reaction, a process that leads to lipid peroxidation and oxidative damage in multiple tissues.[17]

Lead exposure in rats resulted in alterations to blood cells in both peripheral blood and bone marrow, suggesting the activation of immune and oxidative stress pathways. Lead exposure in rats displayed alterations in their immune cell profiles, characterized by elevated granulocyte counts and reduced lymphocyte counts, indicative of enhanced immune activation and oxidative stress. Typically lead exposure-induced lymphopenia implies that lead may exert its immunotoxic effects by targeting macrophages, key immune cells responsible for recognizing and eliminating pathogens.[18] Granulocytes play a crucial role in responding to acute inflammation and toxins by generating and utilizing free radicals to combat infection and promote healing.[19]

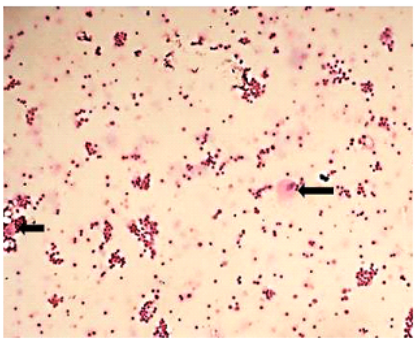
Exposure to toxic agents like lead can induce the overproduction of reactive molecules, leading to impaired lymphocyte-mediated immune responses and increased oxidative stress.[20]

Microscopic examination of the bone marrow smear from the control group revealed well-preserved cellularity and an adequate number of megakaryocytes. Lead exposure induced significant changes in the bone marrow, characterized by severe hypocellularity, decreased megakaryocyte numbers, and myelosuppression, reflecting impaired hematopoiesis. In contrast, Ellagic acid treatment promoted hyperplastic changes in the bone marrow.

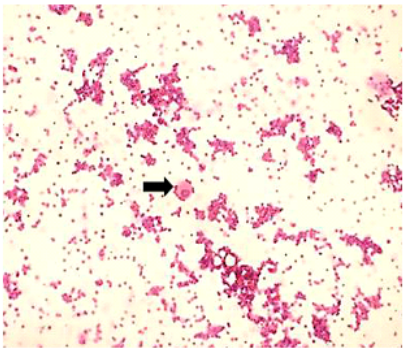
Research has shown that specific natural antioxidants possess immunoprotective effects by virtue of their antioxidant properties. By neutralizing free radical oxidants, these antioxidants can prevent cell membrane damage, maintain immune cell viability, and support overall immune function.[21,22]



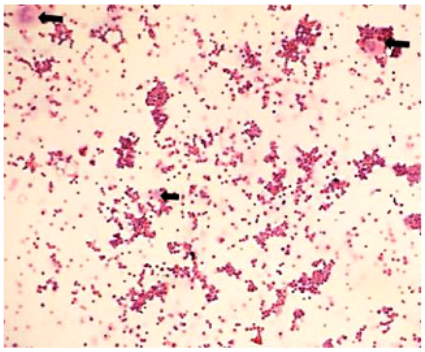
a: Papanicolaou dye stained (100X) photograph of bone marrow aspirate of normal control rat Black arrows: Megakaryocytes



b: Papanicolaou dye stained (100X) photograph of bone marrow aspirate of toxic control rat Black arrows: Megakaryocytes



c: Papanicolaou dye stained (100X) photograph of bone marrow aspirate of EA50 treated rat Black arrows: Megakaryocytes



d: Papanicolaou dye stained (100X) photograph of bone marrow aspirate of EA25 treated rat Black arrows: Megakaryocytes

Fig. 1: Papanicolaou dye stained photograph of bone marrow aspirate. Photographed at 100X

The co-administration of Ellagic acid with lead exposure has been shown to effectively reverse the toxic effects of lead on blood cells, indicating that Ellagic acid may have a therapeutic application in the treatment of lead poisoning and related hematological disorders.

Ellagic acid's ability to protect against oxidative stress is probably attributed to its potent free radical scavenging activity, which neutralizes harmful reactive oxygen species.[23,24]

The protective mechanism of EA against lead-induced hemotoxicity may involve the prevention of protein damage and oxidation, which helps to maintain the stability and function of blood cell membranes, ultimately mitigating the toxic effects of lead.[25,26]

CONCLUSION

This study concludes that EA offers dose-dependent protection against lead-induced hematotoxicity by mitigating oxidative stress through its antioxidant properties. These results are particularly relevant for individuals with prolonged lead exposure. Incorporating EA-rich foods or supplements into their diet may help safeguard their hematopoietic system. Further clinical research is needed to validate these findings.

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