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Effect of Curcumin on Lead Induced Haematological, Oxidative and Bone Marrow Damage in Wistar Rats

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ABSTRACT

Background: Lead is a well-recognized environmental pollutant and multisystemic toxin, which imposes a serious health concern in animals. Present study was conducted with the objectives to assess toxic effect of lead on mature and precursor blood cells and its amelioration with curcumin.

Methods: Haematology and bone marrow cytology and histopathology were performed in rats for all the experimental groups after administration of lead acetate and curcumin in respective groups for 30 consecutive days.

Result: Significant decrease in haemoglobin level, packed cell volume, erythrocytic, leukocytic and lymphocytic counts were observed in rats exposed to lead acetate on 15th and 30th day of experiment. Significant decrease in relative erythroid cell count and significantly increased myeloid to erythroid ratio were recorded in cytology along with histopathological alterations in bone marrow of lead exposed rats as compared to control rats. Oxidative stress marker malondialdehyde level was found to be significantly increased in association with significantly increased blood lead levels. Significant protection against these toxic changes was observed in rats administered with curcumin along with lead.

Key words: Blood cells, Bone marrow, Erythroid cells, Lead, Oxidative stress, Rats.

INTRODUCTION

Lead (Pb) is a hazardous heavy metal, whose concentration in environment and food chain is increasing day by day due to industrialization, automobiles and ammunition. Its ubiquitous presence has caused increased concentration not only in air, soil, water, plants, feed, fish, vertebrates, but even in higher animals and human beings. Longer half-life, bioaccumulation and biomagnification qualities have made lead as an integral part of food chain (Patra, 2006).

Lead toxicity has accounted for high mortality and disabilities in bovine of industrial areas and has made a negative impact on growth and production worldwide (Dash *et al*., 2019 and Elrasoul *et al*., 2020). It is a multi-systemic toxin, mainly affecting vital systems of the body like hematopoietic, nervous, hepato-renal system and is also found to be teratogenic and carcinogenic in various experimental studies (Gomaa *et al*., 2019 and Abdelhamid *et al*., 2020).

There are various ameliorative strategies explored by previous researchers to reduce the toxic effect of lead on animals. Amongst them, phytochemicals with antioxidant and chelating properties can protect living cells from the lead-induced damage (Dewanjee *et al*., 2013). Curcumin, chemically known as diferuloylmethane, is such a phytochemical obtained as active fraction of turmeric (*Curcuma longa*) which has been used in traditional Indian herbal medicine since ancient times. Curcumin scavenges reactive oxygen and nitrogen species, prevents lipid peroxidation and chelates heavy metals (Abdel-Moneim *et al*., 2015 and Veni *et al*., 2022).

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MATERIALS AND METHODS

Animals

Thirty two wistar rats of either sex aged 6-8 weeks and weighing 180-200 g were housed in central laboratory animal house, Co.V.ScandA.H., Jabalpur. These animals were provided with standard environmental conditions as described in CPCSEA guidelines including 22±3°C temperature, 50-60% humidity and 12 hrs dark and light cycles along with pelleted feed and water.

Experimental design

The experimental study was conducted at the Department of Veterinary Pathology, Co.V.Sc., Jabalpur in the year 2022. The rats were administered with lead acetate @ 50/100/ 150 mg/kg b.wt. in a dose dependent manner during a pilot

study for 30 consecutive days to select dose producing moderate toxicity. In the main study, three treatment groups and a control group of rats having eight animals each were included, along with a control group of 6 animals were treated orally in the following manner for 30 consecutive days. Group I rats served as control animals since they were maintained in a similar environment as the rats of other groups. Group II rats were administered with lead acetate (Pb) @150 mg/kg b.wt. Whereas, group III rats were administered with curcumin (Cur) @ 400 mg/kg b.wt. Group IV rats were administered with lead acetate and curcumin (Pb+Cur) in the above-mentioned doses.

Methodology

Blood for haematology, lead level and malondialdehyde (MDA) level estimations was collected from the rats of all the groups on 15th and 30th day. Complete blood count including erythrocytic (Hemoglobin, Total Erythrocyte Count and Packed Cell Volume) and leukocytic parameters (Total Leukocyte Count and Differential Leukocyte Count) were analyzed using automatic blood cell analyzer (Make: Abacus). Blood smears were prepared and stained with Giemsa stain according to the method described by Bolliger (2004).

Blood samples were acid digested and the amount of lead was estimated by ICP-MS as per the standard procedure (Welna *et al*., 2011). Membrane peroxidative damage in erythrocytes was determined in terms of malondialdehyde (MDA) production according to the method suggested by Rehman (1984) with slight modifications. Briefly, erythrocytic palate was separated from centrifuged blood, washed thrice with 0.15 M NaCl and diluted to 33% suspension using phosphate buffer saline. One ml of 10% w/v TCA was added to 1 ml of 33% of erythrocyte suspension followed by addition of 1 ml of 0.67% w/v TBA. The absorbance of samples was read at wavelength 535 nm using biospectrophotometer. The bone marrow specimens were collected from the femur of rats immediately after euthanasia. Paint brush smears were prepared and stained using Giemsa staining as per the method described by Bolliger (2004). The other femur was fixed 10% buffered formalin and histopathological sections were prepared as per the procedure described by Gridley (1960).

RESULTS AND DISCUSSION

Haematology

A significant decrease ($p \leq 0.005$) in the mean values of haemoglobin, TEC and PCV were recorded in rats of group II administered with lead acetate as compared to the control rats of group I on the 15th (Table 1) and 30th day (Table 2). The total leukocyte count (TLC) was found to be significantly increased ($p \leq 0.005$) along with a decrease in lymphocyte count and increase in neutrophil count in lead acetate administered rats (group II) as compared to the group I rats on day 15 and 30. Significant improvement ($p \leq 0.005$) in these parameters was observed in rats administered with curcumin along with lead acetate (group IV) as compared to group II rats, however the mean values of these parameters were significantly lower than that of control rats

Table 1: Haematological parameters of rats of control and treatment groups on day 15.

Parameters	Group I (Control)	Group II (Pb)	Group III (Cur)	Group IV (Pb+ Cur)
Hb (g/dl)	14.36^{a}±0.14	12.93^{c}±0.06	14.22^{a}±0.11	13.86^{b}±0.05
TEC (× 10^{6}/µl)	07.41^{a}±0.14	05.44^{c}±0.15	07.63^{a}±0.11	06.48^{b}±0.11
PCV (%)	42.93^{a}±0.41	38.70^{c}±0.24	42.28^{ab}±0.37	41.62^{b}±0.18
TLC (× 10^{3}/µl)	07.65^{c}±0.37	09.10^{a}±0.07	07.72^{bc}±0.09	07.98^{b}±0.03
Lymphocytes (%)	76.75^{a}±0.73	66.62^{b}±0.53	75.62^{a}±0.63	77.25^{a}±0.59
Neutrophils (%)	20.87^{b}±0.61	30.50^{a}±0.54	21.37^{b}±0.37	20.87^{b}±0.47
Monocytes (%)	01.47^{a}±0.18	01.51^{a}±0.64	01.50^{a}±0.18	01.50^{a}±0.18
Eosinophils (%)	01.00^{a}±0.26	01.37^{a}±0.32	01.50^{a}±0.26	00.50^{a}±0.18

Means with different superscripts in rows differ significantly ($p \leq 0.005$).

Table 2: Haematological parameters of rats of control and treatment groups on day 30.

Parameters	Group I (Control)	Group II (Pb)	Group III (Cur)	Group IV (Pb+ Cur)
Hb (g/dl)	15.00^{a}±0.10	10.15^{c}±0.34	14.87^{a}±0.28	14.07^{ab}±0.18
TEC (× 10^{6}/µl)	07.71^{a}±0.22	03.98^{b}±0.35	07.08^{a}±0.26	06.83^{a}±0.14
PCV (%)	44.18^{a}±0.85	30.11^{c}±0.52	42.51^{ab}±0.59	40.51^{b}±0.64
TLC (× 10^{3}/µl)	07.08^{c}±0.40	11.30^{a}±0.28	07.34^{c}±0.15	10.16^{ab}± 0.16
Lymphocytes (%)	75.00^{a}±1.15	54.75^{d}±0.85	61.90^{c}±0.66	72.60^{b}±0.76
Neutrophils (%)	22.83^{c}±1.30	43.75^{a}±1.03	36.90^{b}±0.77	26.80^{c}±0.68
Monocytes (%)	01.16±0.16	01.50±0.29	01.22±0.22	01.10±0.18
Eosinophils (%)	00.83 ±0.31	01.00±0.41	01.10 ±0.23	00.90 ±0.18

Means with different superscripts in rows differ significantly ($p \leq 0.005$).

of group I. However, the haematological observations in rats of group IV were comparable to control rats on day 15 and 30. Basophilic stippling was observed in peripheral erythrocytes of group II rats only.

Lead inhibits various heme-synthase enzymes including δ-aminoleuvulinic acid synthase, δ-ALA dehydratase and ferrochelatase contributing to reduction in haemoglobin level. It also degrades the integrity of erythrocytic cell membrane resulting into increased destruction and anemia (Oyem *et al*., 2021). Furthermore, it binds and inhibits pyruvate kinase and pyrimidine-5 nucleotidase enzyme essential for erythrocytic maturation resulting in presence of immature erythrocytes in peripherial blood (Abdelhamid *et al*., 2020). Lead exposure causes release of various inflammatory cytokines, resulting in leukocytosis with neutrophilia (Shaban *et al.,* 2021).

Bone marrow cytology

In the present study, bone marrow cellularity was estimated as a measure of toxic effects of lead on precursor blood cells (Fig 1 and 2). A significant decrease in erythroid cells count along with significantly increased in the myeloid cells count and myeloid-erythroid ratio was observed in the lead exposed rats of group II as compared to the control rats. A significant improvement in these parameters was observed in group IV rats administered with antioxidants along with lead acetate as compared to the lead treated rats (group II) such that no significant difference could be observed in these parameters of rats of groups I, III and IV. Similar results were reported by Schlick and Friedberg (1982); Othman *et al.* (2004); Sharifi *et al*. (2011); Queiroz *et al.* (2011) and Owolabi *et al*. (2012).

Bone marrow is a collection of rapidly dividing haematopoietic precursor cells present in the marrow cavity of bones. It also contains some mature blood cells, fibroblast-like reticular cells, adipocytes, macrophages and extracellular matrix. The reduction in erythroid cells of bone marrow is suggested due to lead induced activation of intrinsic pathway of apoptosis in haemopoetic and messenchymal stems cells of bone marrow (Sharifi *et al.,* 2011). Reduced biosynthesis of the cellular DNA, RNA and protein and inhibition of bone marrow ALA enzyme activity. ALA enzyme provides porphyrin-molecules to mitochondrial

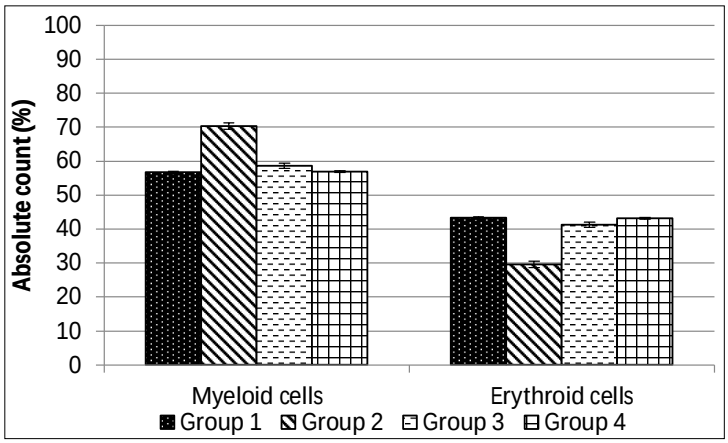
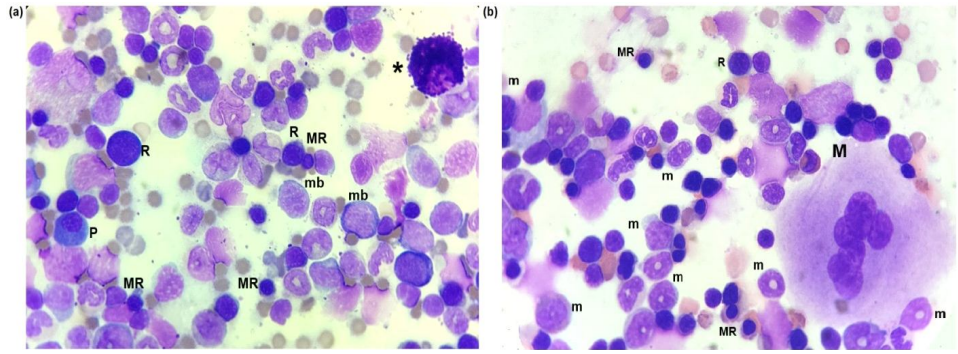


Fig 1: Bone marrow cellularity of rats of control and treatment groups.



M: Megakaryocyte, MR: Metarubricyte, mb: Myeloblast, m: Myelocyte, P: Plasma cell, R: Rubricyte. (Giemsa × 1000).

Fig 2: Photomicrograph of bone marrow smear of (a) control rat showing myeloid and erythroid cell lines along with mast cell (asterisk); (b) Pb treated rat showing reduced erythroid precursor cells.

enzymes for maintenance of electron transport chain and cellular respiration. The reduction in ALA level of the bone marrow cells results in alterated cellular metabolism triggering cellular degeneration (Owolabi *et al*., 2012).

Blood lead level

Blood lead level was analysed at the end of the experiment in all studied groups. Group II rats, receiving 150 mg/kg b.wt. lead acetate, showed a significant increase in blood lead level as compared to the control rats. Significant ($P \leq 0.005$) reduction in blood lead level was found when rats were administered with curcumin along with lead intoxication (Group IV). Group III rats receiving curcumin only and control group (I) rats revealed negligible traces of lead in blood (Table 3).

In our study, 30 consecutive days of lead exposure @ 150 mg/kg b.wt. yielded approximately 20 times higher blood lead levels in rats than the maximum permitted toxic limit. Oral administration of curcumin along with lead acetate resulted in lesser lead level in blood which is suggested by reduced intestinal absorption of lead by its direct chelating effects. Administration of curcumin also reduces lead burden in erythrocytes which may be due to its lipophilic nature that helps it in crossing the cellular membranes. Intracellularly, it chelates heavy metal ions leading to further reduction in blood lead level. Ameliorative effect of curcumin on blood lead level was in agreement with the finding of Abdel-Moneim *et al*. (2015).

Erythrocytic MDA level

Erythrocytic membrane lipid peroxidative damage was assessed as concentration of thiobarbituric acid reactive product, malondialdehyde (MDA) in blood samples of all rats under study. There was significant ($p \leq 0.005$) increase in blood MDA level in lead exposed rats (group II) when compared to the control rats. Marked decrease in MDA level was noticed in rats receiving curcumin along with lead acetate (group IV) as compared to lead exposed rats. There was no statistical difference in blood MDA level of rats receiving curcumin alone (Group III) and control rats (Table 3).

Several studies conducted during the past decade have suggested that oxidative damage is the main mechanism involved in Pb induced cytotoxicity (Apostoli *et al*., 1988; Aykin-Burns *et al*., 2003; El-Reheem and Zaahkcuk 2007; Abdel-Moneim *et al*., 2015; El-Magd *et al.,* 2016; Adetunji *et al.,* 2019 and Alfwuaires *et al.,* 2023). MDA or thiobarbituric acid-reactive species is the end product of lipid peroxidation that plays a vital role in lipid membrane damage in cells by increasing production of reactive oxygen species (ROS). Lead is also known to have high affinity for sulfhydryl (-SH) groups. Thus, it binds with functional SH groups of several enzymes degrading antioxidant defence systems of cells (Flora *et al.,* 2008 and Wang *et al.,* 2007). Curcumin is a potent antioxidant, which diminishes free radical generation, scavenges ROS and activates the antioxidant defense system. Hence, it successfully ameliorates degeneration and cell damage induced by administration of lead (Sudjarwo *et al*., 2017).

Histopathology

The histopathological sections of the femur of lead-exposed rats revealed an increased accumulation of adipose tissue with reduced cellularity (Fig 3). A reduction in the marrow cavity was also observed in some of the sections of the femur of group II rats as compared to group I rats. Random regenerating areas were observed in between the less cellular areas in the sections from the femur of the group IV rat treated with curcumin along with lead exposure. These regenerating areas consisted of higher comparative cellularity and an increased number of megakaryocytes and other haemopoietic precursor cells. Normal histological

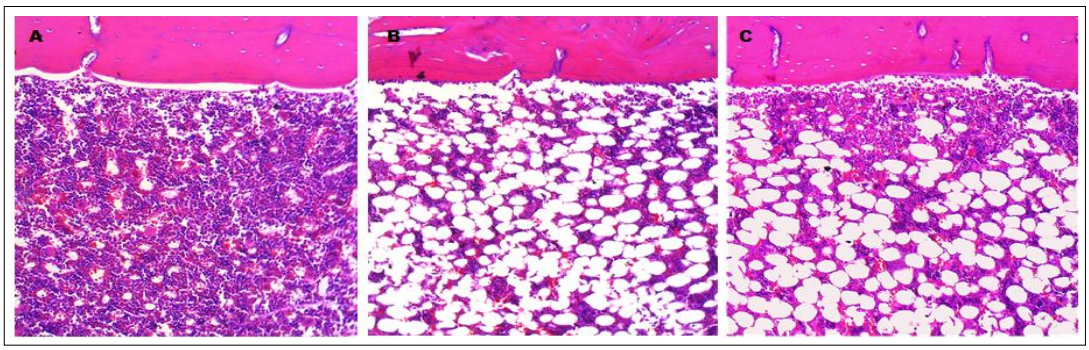


Fig 3: Photomicrograph of histopathological sections of bone marrow showing increased adiposity and reduced cellularity in group II (B) and increased adiposity with regeneration in group IV (C) as compared to normal cellularity in group I (A). (H and E ×100).

Table 3: Erythrocytic MDA levels with respective lead levels in the blood of control and treatment groups.

Parameters	Group I (Control)	Group II (Pb)	Group III (Cur)	Group IV (Pb+Cur)
Blood Pb level (ppm)	$0.001^{c} \pm 0.003$	$2.459^{a} \pm 0.213$	$0.002^{c} \pm 0.001$	$0.095^{bc} \pm 0.002$
Erythrocytic MDA level (nmol/ml RBCs)	$03.89^{d} \pm 0.11$	$33.48^{a} \pm 1.73$	$05.54^{cd} \pm 0.41$	$09.21^{bc} \pm 1.17$

Means with different superscripts in rows differ significantly ($p \leq 0.005$).

observations were recorded in the tissue sections of rats of groups I (control) and III.

The microscopic evaluation of bone marrow, together with haematological analysis, can provide a thorough understanding of the status of the haemtopoietic system. The observations recorded in the present study were in coordination with (Tham *et al*., 2013; Lu *et al*., 2014; Ramesh *et al*., 2018 and Qi *et al*., 2019). Lead toxicity induces oxidative stress in variety cells throughout the body, including haematopoietic cells, by impairing the activity of enzymes such as glutathione peroxidase, catalase and superoxide dismutase (Tham *et al*., 2013). This results in free radical-induced cell death ultimately, reducing cellularity in bone marrow, as observed in the present study. Further, accumulation of adipose tissue in bone marrow is another indication of reduced bone marrow density (Ramesh *et al*., 2018). These toxic changes were found to be ameliorated in the bone marrow of rats after the administration of curcumin along with lead acetate, attributed to the antioxidant and metal chelating properties of curcumin.

CONCLUSION

In the present study, thirty consecutive days administration of lead acetate @150 mg/kg b.wt. orally resulted in marked subacute toxicity in haematopoietic system evident as prominent duration dependent reduction in haemoglobin, erythrocytes and PCV parameters along with increased MDA level in peripheral blood. These alterations were traced in rat bone marrow as reduced erythroid precursor cells. Curcumin, a herbal product, co-administered with lead showed promising ameliorative effects on lead induced toxic alterations in haematopoietic system and oxidative stress, which could be an easier available alternatives to combat with adverse effects of lead pollution in animals and human beings.

Conflict of interest

There is no conflict of interest observed by the authors of the manuscript.

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