EFFECT OF LEAD ON ERYTHROPOIETIC SYSTEM OF
INTACT AND SPLENECTOMIZED RATS

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Summary: Erythropoietic alterations in normal and splenectomized mature male rats treated with aqueous lead acetate intraperitoneally at dosages of 4 mg/kg and 6 mg/kg body weight were observed over a period of 30 days. Significant retardation in growth might be due to gradual increases in lead toxicity. The elevated blood lead level, increased urinary \( \Delta \)-aminolevulinic acid (ALA-U) excretion, depletion in RBC and haemoglobin content and more number of reticulocytes in peripheral blood indicated the increased intensity of lead toxicity and inhibitory effect on haem biosynthesis. The accelerating action of lead on erythropoietic cellular series i.e. pronormoblast, early and intermediate normoblast and late normoblast was evident by the significant increase in number of cellular count both in intact and splenectomized rats after treatment with lead.

Key words: splenectomy, reticulocyte, lead acetate, bone marrow cells

INTRODUCTION

Exposure of lead in industry caused different systemic disorders in human (6, 14). Chronic lead exposure induces acute haemolytic crisis, inhibited haem biosynthesis and ultimately resulted in anaemia in humans (1, 15). Few studies also confirmed the similar effects after chronic lead exposure in experimental animals (4). Animal studies have also been reported \( \Delta \)-aminolevulinic acid dehydratase inhibition in the tissues concurrently with the inhibition in the circulating erythrocytes after 30-40 days treatment of lead acetate. Similar experiment showed the ultrastructural changes of mitochondria in rat bone marrow cells in early stages of poisoning (10).

Several pathological disorders were observed after splenectomy, like deficiency in plasma cells, megakaryocytes and bone marrow precursor cells (9). But little is known regarding the effect of lead on erythropoietic processes in mammalian system. Therefore,
the present investigation was undertaken to determine the relative influence of lead on erythropoiesis in intact and splenectomized rats.

**MATERIAL AND METHODS**

Sixty male albino rats, weighing 135±5 gm were equally divided into six groups (n=10). All groups (Gr. I-VI) were maintained on standard diet and water was supplied ad libitum. Gr. I served as control. Animals of groups II, IV and VI were anaesthetized by sodium pentobarbitone at a dose of 30 mg/kg/rat (ip) and operated for splenectomy. The rest groups (Gr.III and V) were considered for sham operation. Following that animals of all groups were kept for seven days in post operative care. Aqueous solution of lead acetate was administered at dosages of 4 mg/kg to Gr.III and IV and 6 mg/kg to Gr.V and VI intraperitoneally daily over a period of 30 days. Animals were observed carefully throughout the experimental period.

Body weight was recorded on every alternate day. Blood samples were collected on initial day, 15th and 30th day for erythrocyte (RBC) count, haemoglobin (Hb) content and lead estimation by Atomic Absorption Spectrophotometer (AAS) (13, 5). Blood smears were analysed for reticulocyte count after staining with 1% brilliant cresyl blue (13). Twenty four hour urine samples were collected as per above experimental schedule and processed for δ-aminolevulinic acid (ALA) estimation (8). After completion of lead acetate treatment on the 38th day, animals were sacrificed by cervical dislocation. The marrow samples collected from femur, were processed for bone marrow lead content by AAS and for smear preparation (11). Different developing stages of erythopoietic cells were counted at 1000x after staining the smears by 1% leishman (13). Student t-test was employed to determine the level of significance.

**RESULTS**

Significant decrease in body weight was observed in all experimental groups (Gr. II-VI) in comparison to control over a period of 30 days. Decrement is more significant in both the intact and splenectomized rats treated with 6 mg/kg lead acetate (Gr. V, VI) (Fig.1). Lead content in blood and bone marrow was significantly high in all the treated groups (Gr.III—VI) (Figs. 2 and 3). Specially Gr.V and VI exhibited fifty fold increase in blood lead content (P<0.001) whereas five times increase were noted in bone marrow in the same groups over a period of 30 days (P<0.001). Intermittent observations (0, 15, 30 days) of urinary ALA excretion indicated gradual increase in all lead treated groups both in intact and splenectomized rats (Gr. III-VI). Highly significant increase (P<0.001) was observed in Gr. V and VI on 15th and 30th day (Fig.4).
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The relative influence of lead on erythropoiesis was evaluated by dividing the animals into six groups. The diet and water were supplied ad libitum and provided for splenectomy operation. Following the anesthetic agent, aqueous solution of lead acetate at 4 and 6 mg/kg to the animals. Animals were observed for more than 30 days. Blood samples were collected and haemoglobin (Hb) content determined by the method of Folin-Wu (13, 5). Blood was collected with 1% brilliant cresyl blue solution (13). After completion of the experiment, the animals were sacrificed and the bone marrow lead content by the method of erythropoietic cells (13). Student t-test was used for the calculation of significance. The decrement of erythropoiesis was more significant in lead acetate-treated groups (Gr. V, VI) than in the controls. A significant increase (P<0.001) was noted in bone marrow of all lead treated groups compared to the control group.

![Graph](image)

**Fig. 1**: Changes in body weight over a period of 3 days in lead acetate (i.p.) exposed normal and splenectomized rats.

![Graph](image)

**Fig. 2**: Elevated blood lead level in lead exposed (i.p.) normal and splenectomized rats.
Fig. 3: Accumulation of lead in bone marrow of lead exposed normal and splenectomized rats.

Fig. 4: Effect of lead acetate (i.p.) treatment on urinary ALA excretion in normal and splenectomized rats.

Fig. 5: Haemoglobin levels in lead exposed normal and splenectomized rats.

Fig. 6: Haemoglobin levels in lead exposed normal and splenectomized rats.
Effect of Lead on Erythropoietic System

**Fig. 5:** Effect of lead acetate (i.p.) exposure on erythrocyte count in normal and splenectomized rats.

**Fig. 6:** Haemoglobin in normal and splenectomized rats exposed to lead acetate (i.p.).
Hematological studies were carried out on 0, 15 and 30 day. Significant decrease in RBC numbers and haemoglobin content ('Hb') was observed after lead acetate treatment in intact (Gr.III, V) and splenectomized groups (Gr. IV, VI) on 15th and 30th day. Decrement was significant at a level of \( P < 0.05 \) in RBC count on 30th day in Gr. V and VI (Fig. 5). Highly significant (\( P < 0.001 \)) diminution of Hb was observed in Gr.V and VI (Fig. 6). Gradual increase in reticulocyte numbers in peripheral blood was observed in all treated groups (Gr. III-VI) on 15 and 30 day in comparison to control and splenectomized control (Fig. 7). Erythropoietic cellular count in bone marrow observed after 30 days treatment indicated graded increase (\( P < 0.05 \)) in number of different cells i.e. pronormoblast (PNB), early and intermediate normoblast (ENB and INB) and late normoblast (LNB) in all experimental groups (Gr. II-VI) (Fig. 8).

**DISCUSSION**

Retardation in growth was due to lead toxicity as it was evident by the significant increase in blood lead content and urinary ALA excretion (12). Higher accumulation of lead in bone marrow in all lead treated groups revealed the deposition tendency of the metal in bone. Heavy metals conjugate with specific proteins in blood and form a stable bonds which are active in RBC numbers and 'Hb' numbers in peripheral blood. In turn inhibited the haem synthesis in both intact and splenectomized rats causing low 'Hb' concentration.

In the present investigation, spleen did not have any difference from the regular reticulocyte development, though it may be suggested that the spleen did not have any difference from
Effect of Lead on Erythropoietic System

Fig. 8: Effect of lead acetate exposure (i.p.) on bone marrow erythropoietic cell count in normal and splenectomized rats.

stable bonds which are actively get associated within the tissues (2). Gradual decrease in RBC numbers and 'Hb' content on 15th and 30th day along with elevated reticulocyte numbers in peripheral blood in lead treated rats indicated the potential lead toxicity which in turn inhibited the haem biosynthesis after lead treatment (7). These changes are similar in both intact and splenectomized rats treated with different dosages of lead. Splenectomy causes low 'Hb' concentration and induces the hyper function of bone marrow cells (3). In the present investigation, the mitotic accelerating action of lead on erythropoiesis up to the stages of late normoblast was well evident in all experimental groups. The haematological disturbances and erythropoietic changes induced by lead in rats, suggested that spleen did not have an evident role in the process of lead toxicity. Therefore, it may be suggested that the intensity of lead toxicity in intact and splenectomized rats did not have any difference from the present experimental conditions.
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REFERENCES


Summary: Five of the various CNS attributes of analgesics in various animal models were not pronounced in MES were not pronounced ED50 and LD50 in mice. MD50 and LD50 in mice.

Key words: ethylenediamine diamine.