

Effect of Lead Acetate Toxicity on Experimental Chick Embryo

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ABSTRACT

Heavy metals are natural components of the Earth's crust and are important environmental pollutants which are mostly toxic even at very low concentrations. Lead is a common industrial and environment pollutant which is carcinogenic in animals. This study was conducted to understand the toxic effects of lead acetate on antioxidant enzymes and its bioaccumulation in liver and brain using a chick embryo as model. Chick embryos were divided into three groups, first group represented the healthy control and remaining two groups were injected as single dose of 20 μ l and 40 μ l/egg of lead acetate respectively, on 7th day of incubation with 1mg. concentration of lead acetate. On 11th and 14th day of incubation, control and treated chick embryos were sacrificed to collect liver and brain for biochemical assays. Different doses of lead acetate caused an increase or decrease of the activity of all investigated Enzymes, with a variable values, at almost all developmental stages. The lead that entered into the body is accumulated in organs and modifies the function of organ by changing the structure of cells of organs biochemical modifications in cells and functional variations of many of enzymes. Results of the present study clearly suggest that lead acetate induce toxic effects on the chick embryo.

Keywords : Lead Acetate, Antioxidant Enzymes, Liver, Brain

I. INTRODUCTION

Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers. They are a unique class of toxicants, cannot be degraded because of their bioaccumulation. Heavy metal toxicity can result in damage to blood composition, lungs, kidneys, liver and other vital organs and some metals or their compounds may even cause cancer (International Occupational Safety and Health Information Centre 1999). Lead is the most common heavy metal pollutants listed by the Environment Protection Agency (EPA). Important sources of environmental lead contamination include mining, smelting, manufacturing and recycling activities, chemical fertilizers, and canned foods, foods grown around industrial areas, newsprint and colored advertisements. In addition, heavy rains may cause

lead in surface soil to migrate into ground water and eventually into water systems, subsequently transferred to humans through the food chain.

Lead was categorized as toxic element, do not play any metabolic function but can be harmful for humans, even at low concentrations, when ingested over a long time period. Lead is absorbed into the body via inhalation and ingestion and to a limited extent, through the skin. Once absorbed, lead is distributed to blood plasma, the nervous system and soft tissues. Lead is bound to red cells (erythrocytes) in the bloodstream. Absorbed lead is conjugated in the liver and passed to the kidney, where a small quantity is excreted in urine and the rest accumulates in body organs. The available data on the carcinogenicity of lead following ingestion by laboratory animals indicate that lead is carcinogenic. Lead compounds do

not appear to cause genetic damage directly, but may do so through several indirect mechanisms.

The avian embryo is a long-standing model for developmental biology research. Chick embryo has contributed enormously to experimental embryology and there is a vast amount of literature describing the development and their use as model system (Freeman and Vince 1974; Patten 1961).

II. MATERIAL AND METHODS

Materials

Lead Acetate(PbA), Copper Sulphate (CuSO₄), Sodium Carbonate, Sodium potassium carbonate, Phenyl Methyl sulfonyl Fluoride(PMSF), Hydrogen peroxide (H₂O₂), Amino-antipyrine(A.A.P), Tris HCL Buffer, Phosphate Buffer, Acetate Buffer, Triton 100, Formalin, Eosin etc. All other reagents used were of high quality analytical grade and was procured from the local companies.

Methodology

Source of Fertilized Eggs and Incubation Conditions

Freshly laid zero day old fertilized eggs were purchased from Venketashwara hatcheries Pvt. Ltd. Pune, Maharashtra. The eggs were incubated horizontally and rotated (3h intervals) at 37.5±0.5°C with a relative humidity of 65% in an egg incubator.

Experimental Design

Fertile eggs were divided into three groups: group A, Group B and Group C. Two groups A & B were administered as single dose of 20µL and 40µL/egg of lead acetate respectively, on day7 of incubation. Group C received no lead acetate and served as healthy control. Eggs were set-up in an upright position with the blunt end at the top.

On day 7, each egg was sterilized with 70% ethanol and egg shell was opened to obtain access to the air cell, where all the test samples were injected directly on to the inner shell membrane. The hole was covered

by a parafine wax to ensure the embryo's health until tissue collection and blood sampling takes place. The eggs were placed back into the humidified incubator. The eggs were further incubated until the date of examination. Eggs were injected by the air sac method according to Blankenship *et al.*, (2003), on day 7 of incubation. On day 11th and 14th of incubation, the egg shell was broken at the air chamber and embryos were pulled out, from which liver and brain were collected. Tissue samples were washed in ice cold normal saline solution to remove blood and fat debris.

Tissue was minced with scissors and homogenized in 50mM Tris-HCl buffer pH 8.0 containing 0.25M sucrose and 1mM PMSF using a potter homogenizer centrifuged (3000xg for 10min). The resulting clear supernatant was used as enzyme source for antioxidant enzyme assays.

A. Alkaline phosphatase assay:

Buffer, 1 ml. (pH 10 for alkaline), is added to 1 ml. substrate(M / 100 Na₂-phenyl phosphate), warmed at 37° C for three min. 0.1 ml. plasma added and mixed. The solution is incubated at 37° C for 15 min. for alkaline phosphatase. Then 0.8 ml. N/2 NaOH is added for alkaline phosphatase and 1.2 ml. M/2 NaHCO₃ added for alkaline phosphatase. Then 1 ml. 0.6% A.A.P. is added and mixed. Finally 1 ml. 2.4% K₃Fe (CN)₆ is added and mixed. Take the readings at 405nm.

B. Acid phosphatase assay:

First add 0.2 ml of the enzyme solutions to 1.2ml of the acetate buffer and 0.1 ml of Triton X-100 and add 0.5ml of the substrate solution. Incubate for 10 min and 20 min and stop the reaction by adding 2ml of the alkaline tris buffer. Read extinction at 405 nm and calculate the enzymatic activity by reference to a standard curve of p-Nitrophenol.

C. Assay of catalase:

Catalase activity was measured by the method of Aebi (1974).

Catalase in tissues with relatively high activity, such as liver and brain, can be determined spectrophotometrically if complete lysis of all organelles and clear (or only slightly colored) solutions or extracts can be obtained. Normally, catalase activity of tissue samples is expressed on a milligram wet weight or milligram total N basis. A convenient method for the measurement of catalase activity in tissue extracts. Assay Conditions Wavelength, 240 nm; light path, 10 mm; final volume, 3.00 ml. Read the sample containing, 2.00 ml enzyme solution or hemolysate and 1 ml H₂O₂ at 20 ° (- room temperature) against a blank containing, 1 ml phosphate buffer instead of substrate and 2 ml enzyme solution or hemolysate. The reaction is started by addition of H₂O₂. The initial absorbance should be approximately A = 0.500. Mix well with a plastic paddle and follow the decrease in absorbance with a recorder for about 30 sec.

III. OBSERVATIONS:

The observations show the various changes in chick embryo. As days pass mortality and change in weight of eggs were observed. Due to treatment of lead acetate change in size of control and treated chick embryo and its tissues were seen. Morphological deformities like blood clot on head region, patches on liver and absence of feathers were observed.

A. Normal Vs Affected.



14d developed embryo 20µl PbA treated embryo

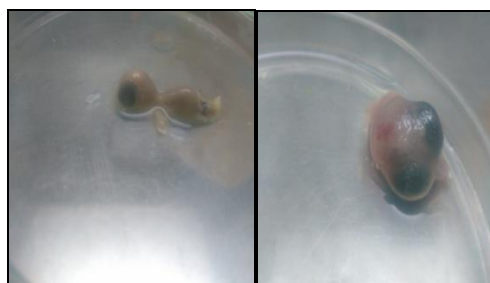


14d developed liver 20µl PbA treated 14d Liver



14d developed Brain 20µl PbA treated 14d Brain

A. Gross Abnormalities:



40µl PbA treated 14d Embryo Blood clot in 40µl PbA Treated embryo



Figure 9 absence of feather 11d

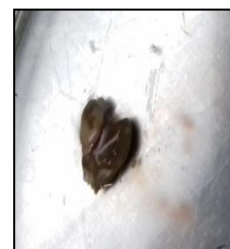


Figure 10. Patches on liver 11 d

IV. RESULT AND DISCUSSION

Tables and Graphs showed changes in the activity of various enzymes in liver and brain tissues of the chicken embryo exposed to lead acetate.

A. Alkaline phosphatase:

The alkaline phosphates content in ($\mu\text{mol/ml}$) brain tissue of chick embryo after treating with lead acetate gradually decreases as days of treatment increases when compare with control.

Table 1&2 and Graph 1&2: Alkaline phosphatase activity in chick embryonic brain and liver exposed to lead acetate

BRAIN

Exposure period (Days)	Control $\pm\text{SD}$	Experimental (20 μL) $\pm\text{SD}$	Experimental (40 μL) $\pm\text{SD}$
12	0.145 \pm 0.008 7	0.211 \pm 0.005 2	0.128 \pm 0.004 6
14	0.205 \pm 0.009 8	0.284 \pm 0.005 2	0.284 \pm 0.004 7

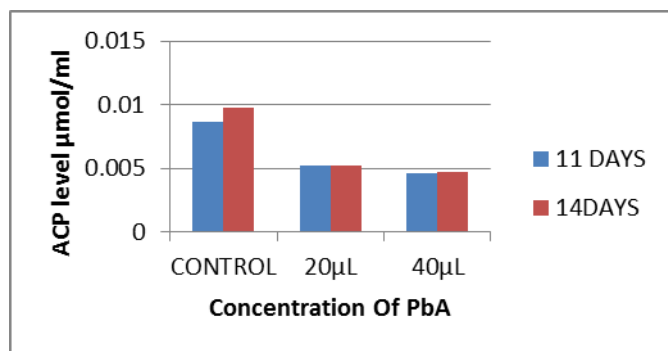


Table 1 and Graph 1: Alkaline phosphatase activity in chick embryonic brain exposed to lead acetate

LIVER

Exposure period (Days)	Control $\pm\text{SD}$	Experimental (20 μL) $\pm\text{SD}$	Experimental (40 μL) $\pm\text{SD}$
12	0.232 \pm 0.0060	0.243 \pm 0.0047	0.257 \pm 0.0051
14	0.208 \pm 0.0085	0.232 \pm 0.0060	0.267 \pm 0.0046

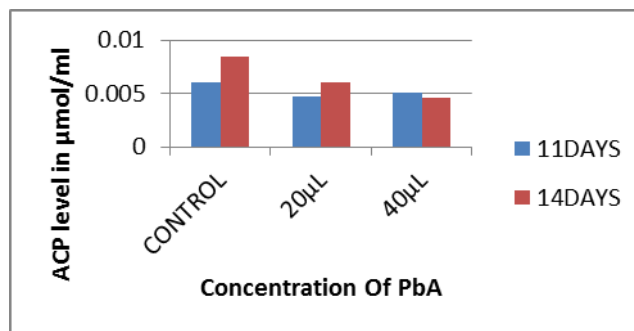


Table 2 and Graph 2: Alkaline phosphatase activity in chick embryonic liver exposed to lead acetate

B. Acid phosphatase:

The acid phosphatase content in ($\mu\text{mol/ml}$) different tissues of chick embryo after treating with lead acetate in comparison with control shows gradually increase as days of treatment increases.

Table 3&4and Graph3&4: Acid phosphatase activity in chick embryonic brain and liver exposed to lead acetate.

BRAIN

Exposure period (Days)	Control $\pm\text{SD}$	Experimental 1 (20 μL) $\pm\text{SD}$	Experimental 1 (40 μL) $\pm\text{SD}$
12	0.291 \pm 0.011 5	0.358 \pm 0.011 7	0.366 \pm 0.011 8
14	0.299 \pm 0.011 5	0.325 \pm 0.035 3	0.466 \pm 0.023 5

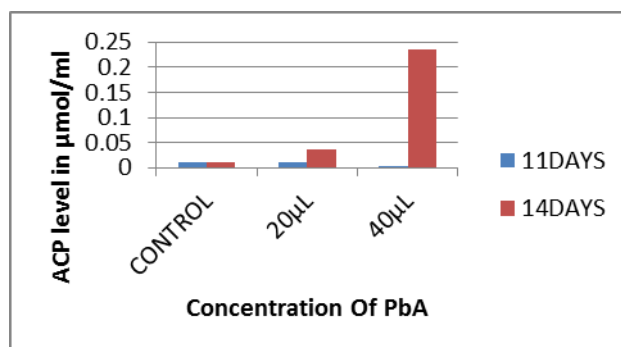
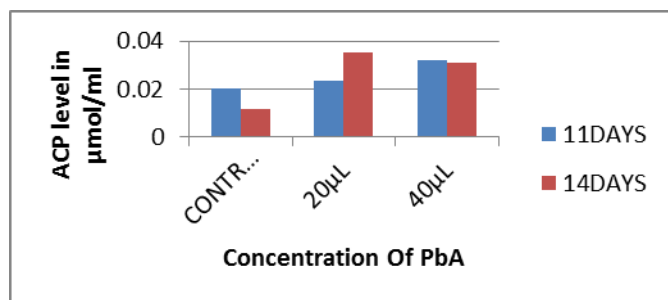


Table 3 and Graph 3: Acid phosphatase activity in chick embryonic brain exposed to lead acetate

LIVER

Exposure period (Days)	Control \pm SD	Experimental (20 μ L) \pm SD	Experimental (40 μ L) \pm SD
12	0.400 \pm 0.020 3	0.408 \pm 0.023 5	0.466 \pm 0.031 8
14	0.491 \pm 0.011 8	0.456 \pm 0.023 5	0.566 \pm 0.031 1



Liver

Table 4 and Graph 4: Acid phosphatase activity in chick embryonic liver exposed to lead acetate

C. CATALASE ACTIVITY:

The effect of lead acetate on catalase activity of chick embryo liver was shown in graph 3. The catalase activity was significantly ($p < 0.05$) decreased in 20 μ L and 40 μ L PbA treatment compared to control group.

BRAIN

Exposure period (Days)	Control \pm SD	Experimental (20 μ L) \pm SD	Experimental (40 μ L) \pm SD
12	0.87325 \pm 0.0075	0.75425 \pm 0.00506	0.5365 \pm 0.00332
14	2.1635 \pm 0.04356	1.90525 \pm 0.0379	0.5345 \pm 0.00785

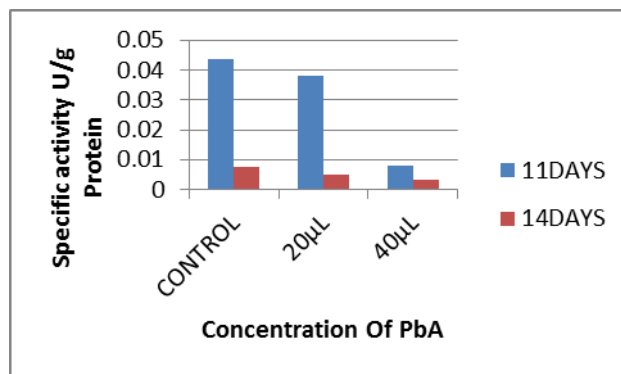


Table 5 and Graph 5: Catalase activity in chick embryonic liver exposed to lead acetate

LIVER

Exposure period (Days)	Control \pm SD	Experimental (20 μ L) \pm SD	Experimental (40 μ L) \pm SD
12	1.33825 \pm 0.2 521	0.98275 \pm 0.0 1176	0.59175 \pm 0.0 0665
14	0.895 \pm 0.027 68	0.82425 \pm 0.0 1628	0.26425 \pm 0.0 0723

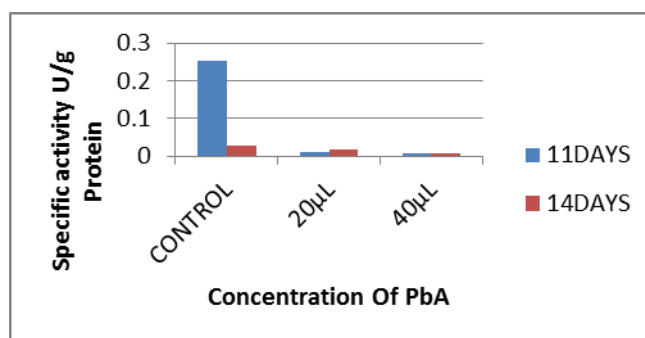


Table 6 and Graph 6: Effect of lead acetate on catalase activity of 12d and 14 day old chick Embryonic Brain.

V. DISCUSSION

Lead toxicity is a particularly insidious hazard with the potential of causing irreversible health effects. It is known to interfere with a number of body functions and is primarily affecting the central nervous, hematopoietic, hepatic and renal system producing serious disorders.

Phosphatases are nonspecific phosphomonoesterases having pH specificity, which hydrolyze various phosphate esters and liberate phosphate from the substrate. Acid phosphatase (ACP) hydrolyses the phosphorous ester in acidic medium and autolysis process of the cell after its death. The employment of biochemical markers in monitoring pollution resulting from heavy metals has significantly shown the sensitivity of the tools and the potential risks facing man at the upper trophic level along the food chain. The present investigation shows toxicity of heavy metals on biosystems. Acid and alkaline phosphatase activities of control embryos are gradually changes as development proceeds from the 11th to 14th day of incubation. This findings are in accord with those of Romanoff (1988) and King and Liu (1974 a and b).

Catalase is one of the important antioxidant enzymes which help in converting the hydrogen peroxide to water and oxygen. Catalase is an efficient decomposer of H₂O₂ and is known to be susceptible to lead toxicity. (Sandhir and Gill 1995). The obtained results have displayed that treatment of chick embryo with lead acetate caused a reduction of liver catalase (Graph:4 and 5).Reduction in CAT impairs scavenging of hydrogen peroxide radicals. The reduction of catalase in liver and brain by Pb must be due to Pb induced inhibition of heme biosynthesis and since heme is a basic prosthetic molecule available with catalase for catalysis of peroxy radicals. Therefore the present results are in accordance with the earlier reports (Sandhir and Gill 1995).

VI. CONCLUSION

The environmental contamination by lead generated from human activities has become an evident problem during the last decades. Lead can penetrate to the human or animal organisms by inhalation, ingestion and by skin (Ryan and Terry, 1988;El-Fekiet al., 2000). The adverse effects of lead on health and productivity have been studied relatively little in poultry, particularly in layers, in comparison with other farm

animals. Berget al.,(1980) stated that contamination of the environment with lead has reached such a level that can affect the growth, productivity, and health of poultry as the toxicity of these metals (cadmium, lead) relies on binding the metallic cations with sulphhydryl, amino and carboxylic groups of enzymes thus inhibiting enzymatic activities and disturbing energy metabolism.

In the present study, treatment with Pb-acetate has resulted in altered activity of alkaline phosphatase and acid phosphatase and the significant decrease of CAT levels. It shows changes in proteins content. It leads to excessive liver and brain damage and shows the tendency of bioaccumulation in the chick embryo. According to the results obtained in the present study, it appears that lead acetate administration to chick embryo would increase the toxicity and causing damage to vital tissue.

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