LEAD ACETATE EFFECTS ON HEPATOCYTE DEGENERATION IN CHICK EMBRYO LIVER

Thyaga Raju Kedam* and Pallavi Chittoor

Department of Biochemistry, Sri Venkateswara University, Tirupati 517 502 AP, India.

ABSTRACT

The liver is considered as one of the target organs affected by lead toxicity owing to its site of storage after exposure. Liver is a primary organ involved in the biotransformation and detoxification of toxic substances. Inhalation and ingestion of lead are the most common routes of exposure. Absorbed lead is stored in liver via the portal vein, for this reason the study was conducted to describe the histological changes after lead exposure in developing chick embryo. The lead acetate treated chick embryo liver during development has acquired modification of normal to necrosed structure. The necrosed liver under microscopy showed congestion, elongated sinusoidal spaces and degenerated hepatocytes. Hence the present study concludes that the lead as heavy metal causes damage to liver and hepatocytes.

KEYWORDS: Liver, Chick embryo, Lead acetate, Histology, Detoxification.

INTRODUCTION

Lead is found at low levels in Earth’s crust, mainly as lead sulfide (galena). Small amounts of lead (Pb) reach the surface environment through natural weathering processes and volcanic emissions, thus giving a baseline environmental exposure. However, the abundant and widespread presence of lead in our environment is largely a result of anthropogenic activity. Lead was categorized as toxic element, do not play any metabolic function but can be harmful 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. The most common route of occupational exposure of lead is through inhalation of fumes or dust from ambient air, leading to absorption of Pb through the respiratory system. Lead may
also be ingested and absorbed in the gastrointestinal tract. Once absorbed, lead is distributed to blood plasma, the nervous system and tissues. Lead is bound to red corpuscles (erythrocytes) in the bloodstream. Absorbed lead is conjugated with proteins in the liver and passed to the kidney, where a small quantity is excreted in urine and the rest accumulates in body organs. This affects many biological activities at the molecular, cellular and intercellular levels, which may result in morphological alterations that can remain even after lead level has fallen.

Lead element is one of the most common toxic metal and affects all organs of the body including liver. Liver is the largest organ in the body and it is located in the upper three regions in the abdomen. Hepatocytes are arranged in hexagon-shaped lobules about 2mm in length and 700μm in diameter. The liver performs various vital metabolic functions. Liver plays a central role in biotransformation and disposition of xenobiotics. Smooth endoplasmic reticulum of the liver is the principal ‘metabolic clearing house’ for both endogenous chemicals like cholesterol, steroid hormones, fatty acids and proteins, and exogenous substances like drugs and alcohol.

Most of the foreign substances are lipophilic thus enabling them to cross the membranes of intestinal cells. They are rendered more hydrophilic by biochemical processes in the hepatocyte yielding water soluble products that are exported into plasma or bile by transport proteins located on the hepatocyte membrane and subsequently excreted by the kidney or gastrointestinal tract. Liver may be exposed to large concentrations of exogenous substances and their metabolites. Metabolism of exogenous compounds can modulate the properties of hepatotoxicant either by increasing (toxication or metabolic activation) or decreasing its toxicity (detoxification). The present study was designed to explore the toxic effects of lead acetate on hepatocytes in developing chick embryo using histological examinations.

**METHODOLOGY**

**Source of Fertilized Eggs and Incubation Conditions**

Freshly laid Bobcock strain zero day old fertilized eggs were purchased from Sri Balaji hatcheries, Chittoor, Andhra Pradesh. 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.

Table1: Groups of fertile eggs treatment.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TREATMENT</th>
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<tbody>
<tr>
<td>Group A</td>
<td>Control</td>
</tr>
<tr>
<td>Group B</td>
<td>20μg/egg of lead acetate</td>
</tr>
<tr>
<td>Group C</td>
<td>40μg/egg of lead acetate</td>
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On day 7 of incubation, each egg surface 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.\(^{[14]}\) The hole was covered by a 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 in a horizontal position until the date of examination. On day 13\(^{th}\) of incubation, the eggs shells were broken at the air chamber and embryos were pulled out, from which liver tissue were collected for histological study.

Histochemistry

- Tissue samples were washed in ice cold normal saline solution to remove blood and fat debris. They were fixed in Bouin’s solution until processing.
- The tissues were washed with running tap water, overnight to remove Bouin’s solution.
- After dehydrating through a graded series of alcohols, the tissues were cleared in methyl benzoate and embedded in paraffin wax.
- Sections were cut at 4-6μ thickness and stained with haematoxylin\(^{[15]}\) and counter stained with Eosin dissolved in 95% alcohol.
- After dehydration and cleaning, sections were mounted in Canada balsam.
  Histological examinations of the tissues were followed according to Humason 1972 and the specimens were observed under the light microscope. Photomicrographs were taken by Ricoh 35mm SLR camera.

RESULTS

Histology is the study of the microscopic anatomy of tissues. An accurate diagnosis of cancer and other diseases usually requires histopathological examination of biological samples. Histological examinations of the chick embryo liver tissues were sectioned and stained with haematoxylin and eosin, the specimens of tissue were observed under light microscope.

Hepatic specimens obtained from the control group showed normal architecture of hepatic tissue with normal hepatocytes (Figure. 1). Specimens examined from liver sections taken
from chick embryo exposed to 20μg lead acetate showed loss of hepatic architecture with sinusoidal spaces and congested hepatic cells (Figure. 2). Specimens taken from chick embryo liver treated with 40μg lead acetate showed loss of hepatic architecture with degenerative changes and elongated sinusoidal spaces (Figure. 3).

**Figure 1:** Histological analysis of control liver from 13th day old chick embryo specimen under the light microscope (40X) showing normal hepatocyte with clear nucleus (N).

**Figure 2:** Histological analysis of lead acetate treated (20μg) liver specimen from 13th day old chick embryo under the light microscope (40X) showing sinusoidal spaces (SS) and congestion (C).

**Figure 3:** Histological analysis of lead acetate treated (40μg) liver specimen from 13th day old chick embryo under the light microscope (40X) showing degenerative changes (DC), congestion (C) and elongated sinusoidal spaces (ESS).

**DISCUSSION**

Lead is an environmental pollutant and its toxicity was associated with health hazards. The liver plays a major role in the detoxification process and is one of the target organs affected by lead toxicity owing to its storage in the liver. Lead induces a wide range of structural
alterations to hepatic cells; this is an indicative of liver toxicity.\textsuperscript{[16]} High levels of lead accumulate in tissues where lead-induced pathological changes occur in their structure and function.\textsuperscript{[17]} Specifically, lead is known to cause impairment of liver functions and kidney dysfunction as well.\textsuperscript{[18]}

The present study supports that lead acetate treated chick embryo liver showed significant toxic changes which are manifested as hepatic damage (Figure: 2&3). These changes may be because of that lead acetate was exhibited to decline cytochrome P450 content.\textsuperscript{[19]} Also lead induces mitogenic response in the rodent liver.\textsuperscript{[20]} Lead acetate was found to induce glutathione-S-transferase in rat liver.\textsuperscript{[21]} Damage to hepatic structure integrity induced by Pb-acetate is further supported by our histological examination, where severe hepatocyte damage, congestion, dilation of blood sinusoids and loss of architecture were seen in chick embryo after lead acetate treatment. Histopathological alterations in hepatic tissue due to lead exposure were also reported earlier.\textsuperscript{[22]}

**CONCLUSION**

The microscopic examination of embryo groups has revealed the disturbed hepatic architecture and hepatocyte degeneracy. So, the results of the present experiment clearly indicate that exposure to lead acetate causes significant structural changes in developing chick embryo liver.

**REFERENCES**


