WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 8, 203-209 Research Article ISSN 2278 – 4357

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.

Article Received on
15 June 2017,
Revised on 05 July 2017,
Accepted on 25 July 2017
DOI: 10.20959/wjpps20178-9759

***Corresponding Author**
Thyaga Raju Kedam
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.[1] 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.[2] 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.[3] 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.[4] 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.[5, 6, 7, 8]

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.[9] 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.[10, 11] 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.[12] 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).[13] 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.

GROUPS	TREATMENT
Group A	Control
Group B	20µg/egg of lead acetate
Group C	40µg/egg of lead acetate

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 13th 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).

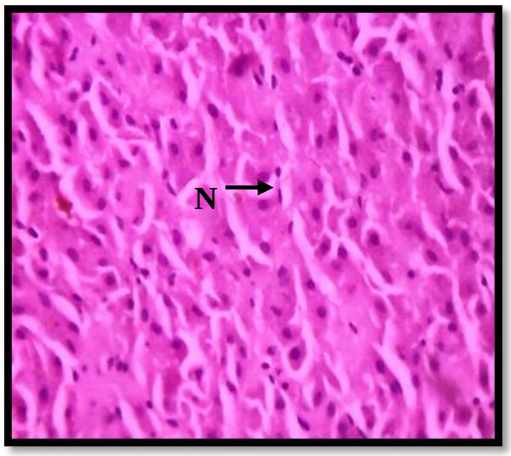

Figure 1: Histological analysis of control liver from 13th day old chick embryo specimen under the light microscope (40X) showing normal hepatocyte with clear nucleus (N).

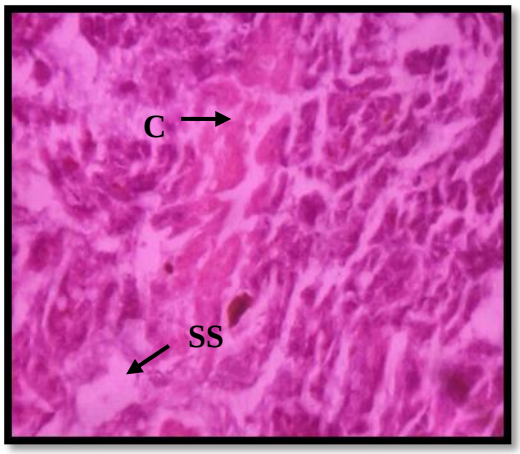

Figure. 2: Histological analysis of lead acetate treated (20µg) liver specimen from 13th day old chick embryo under the light microscope (40X) showing sinusoidal spaces (SS) and congestion (C).

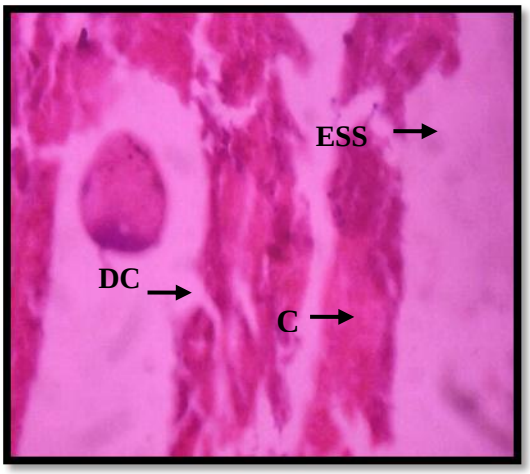

Figure 3: Histological analysis of lead acetate treated (40µg) liver specimen from 13th day old chick embryo under the light microscope (40X) showing degenerative changes (DC), congestion (C) and elongated sinusoidal spaces (ESS).

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.[16] High levels of lead accumulate in tissues where lead-induced pathological changes occur in their structure and function.[17] Specifically, lead is known to cause impairment of liver functions and kidney dysfunction as well.[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 P_{450} content.[19] Also lead induces mitogenic response in the rodent liver.[20] Lead acetate was found to induce glutathione-S-transferase in rat liver.[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.[22]

CONCLUSSION

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

1. IARC. Summaries & evaluations: Inorganic and organic lead compounds. Lyon, International Agency for Research on Cancer. IARC Monographs for the Evaluation of Carcinogenic Risks to Humans, 2006; 87.
2. Somers E. The toxic potential of trace metals in foods. A review, *J. Food Sci*, 1974; 39: 215–217.
3. Bress WC, Bidanset JH. Percutaneous *in vivo* and *in vitro* absorption of lead. *Vet Hum Toxicol*, 1991; 33(3): 212-214.
4. Pallavi C (2016). Ph. D thesis submitted to Sri Venkateswara University, Tirupati, A.P. India.
5. Sidhu, P. and B. Nehru. Lead intoxication: Histological and oxidative damage in rat cerebrum and cerebellum. J. Trace Elem. Exp. Med, 17(1): 45-53. Simons T. 1993. Lead-calcium interactions in cellular lead toxicity. *Neurotoxicology*, 2004; 14:77-86.

6. Flora SJS, Flora G, Saxena G. Environmental occurrence, health effects and management of lead poisoning" In: Cascas SB, Sordo J, editors. *Lead chemistry, analytical aspects, environmental impacts and health effects*. Netherlands: Elsevier Publication, 2006; 158-228.
7. Ibrahim NM, Eweis EA, El-Beltagi HS and Abdel-Mobdy YE. Effect of lead acetate toxicity on experimental male albino rat. *Asian Pac. J. Trop. Biomed*, 2012; 2: 41–46.
8. Pallavi. C and Thyaga Raju K. Lead acetate induced genotoxicity in developing chick embryo. *International Journal of Advanced Scientific and Technical Research*, 2014; 4(4): 538-544.
9. Gartner LP and Hiatt JL. Color textbook of histology. *Saunders Elsevier*, 2007; 3.
10. Miyai K, Meeks RG, Harrison SD, Bull RJ. Structural organization of the liver. In: *Hepatotoxicology*. 1991; CRC Press, Boca Raton, FL.
11. Haseenabhanu S, Meenabai M and Thyaga Raju K. Histopathological and DNA Damage studies of Sting the Mice Liver treated with Paracetamol and Active Principle of Hybanthus Enneaspermus extract. *Journal of pharma research*, 2014; 3(5): 88-91.
12. Tostmann A, Boeree MJ, Aarnoutse RE, de Lange WCM, van der Ven AJ, *et al*. Antituberculosis drug-induced hepatotoxicity: Concise up-to-date review. *J Gastroenterol Hepatol*, 2008; 23: 192-202.
13. Kedderis GL. Biochemical basis of hepatocellular injury. *Toxicol Pathol*, 1996; 24: 77-83.
14. Blankenship, A.L., K.N.M. Hilscherova, K.K. Coady, S.A. Villalobos, K. Kannan, D.C. Powell, S.J. Bursian, and J.P. Giesy. "Mechanisms of TCDD-induced Abnormalities and Embryo Lethality in White Leghorn Chickens. *Comparative Biochemistry and Physiology Part C-Toxicology Pharmacology*, 2003; 136: 47–62.
15. Harris H.F. A rapid conversion of haematoxylin into haematin in staining reaction. *J. Appl. Microsc. Lab. Method*, 1900; 3: 777.
16. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact*, 2006; 160: 1-40.
17. Khalil-Manesh, F., Gonick, H. C, Cohen, A. H., Alinovi, R., Bergamaschi, E., Mutti, A., and Rosen, V. J. Experimental model of lead nephropathy. I. Continuous high dose lead administration. *Kidney Int*, 1992; 41: 1192-1203.
18. Patrick, L. Lead Toxicity Part II: The Role of Free Radical Damage and the Use of Antioxidants in the Pathology and Treatment of Lead Toxicity. *Alternative Medicine Review*, 2006; 11(2): 114-128.

19. US-EPA. Air quality criteria document for lead (Pb), Vol. 4. Washington DC: US *Environmental Protection Agency*, 1986; 264-7.
20. Calabrese, E. J., Baldwin, L. A. Lead-induced cell proliferation and organ-specific tumorigenicity. *Drug Metabolism Reviews*, 1992; 24(3): 409-16.
21. Suzuki, T., Morimura, S., Diccianni, M. B., Yamada, R., Hochi, S.-i., Hirabayashi, M. *et al*. Activation of glutathione transferase P gene by lead requires glutathione transferase P enhancer I. *Journal of Biological Chemistry*, 1996; 271(3): 1626-32.
22. Mudipalli A. Lead hepatotoxicity and potential health effects. *Indian J Med Res*, 2007; 126: 518-527.