Original Article

Histological Changes in the Liver and Biochemical Parameters of Chickens Treated with Lead Acetate II

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ABSTRACT

Background: Lead is one of the heavy metals that is persists in the environment and has destructive effects on various human tissues. We investigated the effects of lead on the histological features of liver and its enzymatic functions.

Methods: Forty chickens were purchased and randomly divided into four groups. In addition to the normal feed, each group received different doses of lead acetate II in the feed. After preparing microscopic slides, the level of liver enzymes was measured.

Results: It was demonstrated that the level of liver enzymes increases and devastating effects on the liver structures occurred.

Conclusion: In according to the adverse effects of lead on the liver and due to the increase use of this harmful agent in the different parts and its increase in the air, especially in Iran, there is a need for considering a comprehensive plan for preventing of more outbreak of this agents and inhibiting the toxic effects of it on the birds.

Key words: Biochemical Parameters, Chicken, Lead, Liver.

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INTRODUTION

Liver is an important body organ with major biochemical functions, such as detoxification, secretion, oxidation and reduction [1]. As a major organ in human metabolic pathways, liver has a significant role in the synthesis, metabolism and transport of carbohydrates, proteins and fats [2]. Chicken liver is located behind the heart and has two lobes.

There have been concerns about the spreading of chemicals in the environment, especially heavy metals. Lead is one of the heavy metals commonly found in the environment and industries [3]. The extensive spread of lead is due to its features, such as softness, low melting point, and its prevalence in the environment. Besides, the toxic properties of lead have been known for centuries. Exposure to lead occurs in different manners, such as ignition of coal, use of paints with lead base, and water pipes which are rich in lead [4]. Lead is present in the atmosphere, which results from the defective combustion of gasoline in vehicles engines. So, the birds are exposed to this toxic heavy metal polluting the air and environment.

Accumulation of lead in the body affects the reproductive, nervous and cardiovascular systems [5]. Also, as a result of lead poisoning, the action of digestion in the digestive tract is disturbed. Further, diarrhea occurs as a result of lead exposure such that the feathers at the *cloaca* turn green [6,7] and the body weight is decreased [8]. However, liver is significantly affected by the adverse effects of lead, since it is initially absorbed by the liver through the portal vein. Lead is then exerts its harmful effects on this vital organ. Also, it has been shown that lead can cause significant histological changes in the liver [9]. Fatty changes, necrosis and nuclear vesiculation are just a few alterations stimulated by lead poisoning [9].

The activity of liver is assessed by the concentration of certain liver enzymes. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are enzymes, involved in the gluconeogenesis and amino acid metabolism. These enzymes function in a pyridoxal phosphate (PLP)-dependent manner. Also, they are accelerators of metabolism of glucose and proteins. Diseases, such as liver metabolic syndrome, atherogenesis and type I and II diabetes can elevate the activity of enzymes [10-14]. Alkaline phosphatase (ALP) is another important liver enzyme, involved in the metabolism of phosphate and catalyzes dephosphorylation of proteins, nucleic acids and various small molecules, and is a well know biomarker of liver diseases [15].

The aim of this study was to investigate the important histological changes in chicken liver and the alterations in the liver enzymes induced by lead poisoning in chickens.

MATERIALS AND METHODS

Animals and Experimental Procedure

Forty chickens at one-day age were purchased and housed in the laboratory for three weeks which had the environment-like conditions. The chickens were cared for and kept in accordance with the guidelines established by the National Institute of Health, applicable laboratory animals. They were separated into four groups assigned as: Controls, Treatment I, Treatment II and Treatment III. These groups were fed differently as follows:

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- Controls: Libitum feed and the mineral water.
- Treatment I: Libitum feed and 50 ppm lead acetate II dissolved in water.
- Treatment II: Libitum feed and 100 ppm lead acetate II dissolved in water.
- Treatment III: Libitum feed and 200 ppm lead acetate II dissolved in water.

Histological Studies

Several laboratory phases were performed to prepare microscopic slides. The chickens were anesthetized, and the abdominal mid line was surgically dissected, and the liver was harvested. All liver samples were fixed in Bouin solution. Sample dehydration phase was performed by reducing the level of ethanol, clearing with xylen, loading in paraffin, followed by sectioning and preparing microscopic slides. The horizontal and perpendicular sections were stained with hematoxylin and eosin (H&E), and with oil red. The effects of lead on the hepatocytes and the resultant fatty accumulation were investigated microscopically.

Biochemical Parameters

Determining the level of serum liver enzymes was performed spectrophotometerically, using the guidelines and laboratory kits supplied by Pars Azemun Company (Tehran, Iran).

RESULTS

During the experimental process, no chicken death happened in any of the groups. The histological changes observed in the microscopic slides were as follows:

1. Histological Results

1-1. Hepatocyte Features

In the treatment groups, the borderlines between the hepatocytes were destroyed and cells had irregular shape and atypical position within the tissue (Fig. 1& 2).

1-2. Liver Hemorrhage

There was no liver hemorrhage seen in the treatment I and II groups; however, in the treatment III group, liver hemorrhagic areas were observed such that hepatocytes were completely destroyed in the hemorrhagic areas (Figure 3).

1-3. Hepatocytes and Hyperemia

In all treatment groups, the hyperemia in the liver observed (Figure 4& 5).

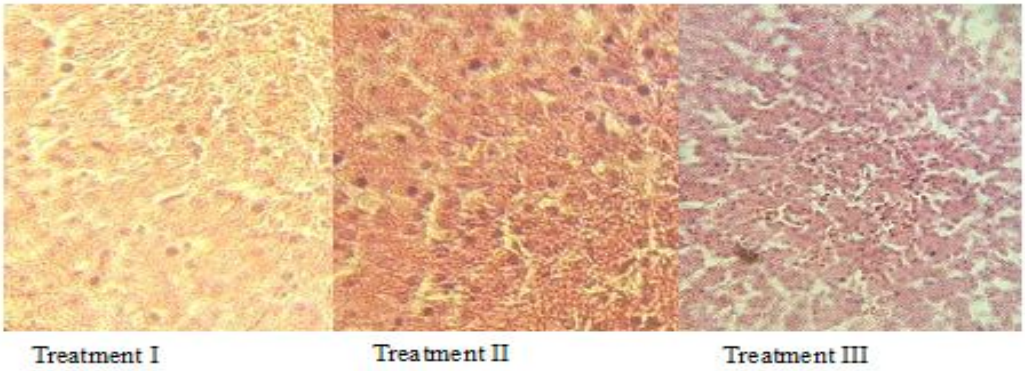

Figure1. The histological changes in the hepatocytes (irregular shape and position; H&E × 400).

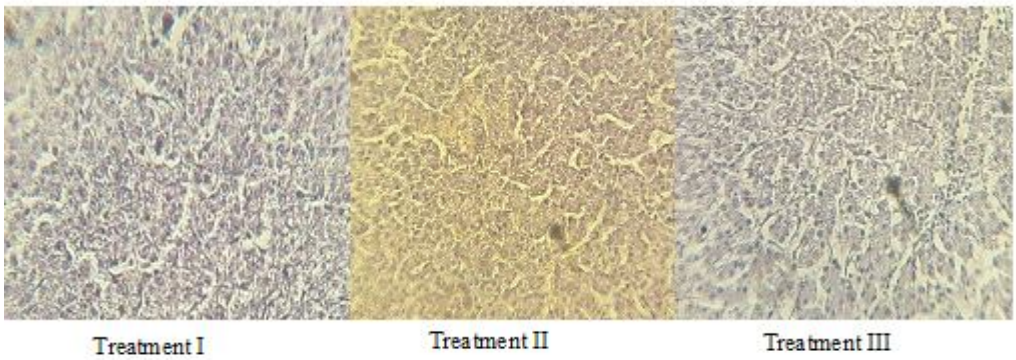

Figure2. The histological changes in the hepatocytes (irregular shape and position) (Oil red × 400).

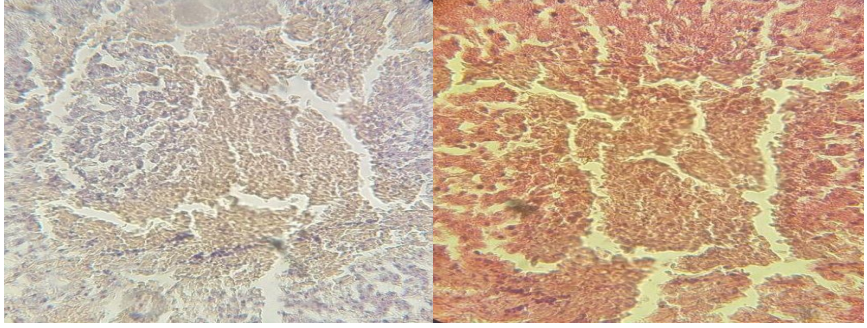

Figure 3. The obvious hemorrhage (H&E×400, left; Oilred × 400, right).

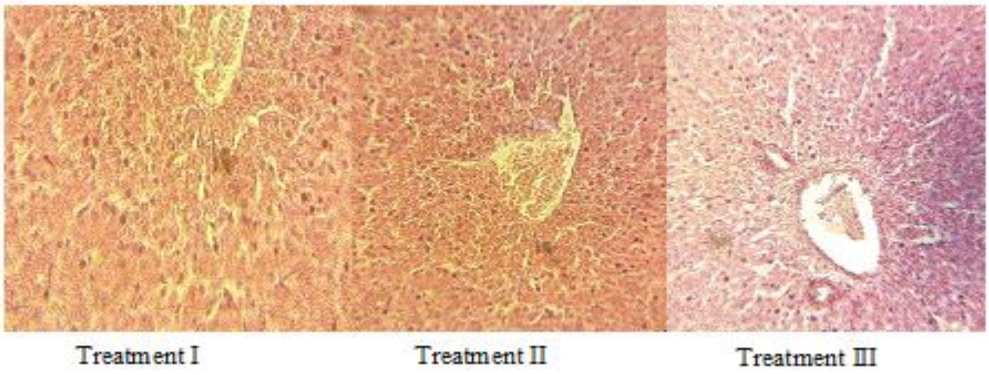

Figure 4. Irregular hepatocytes and hyperemia (H&E × 400).

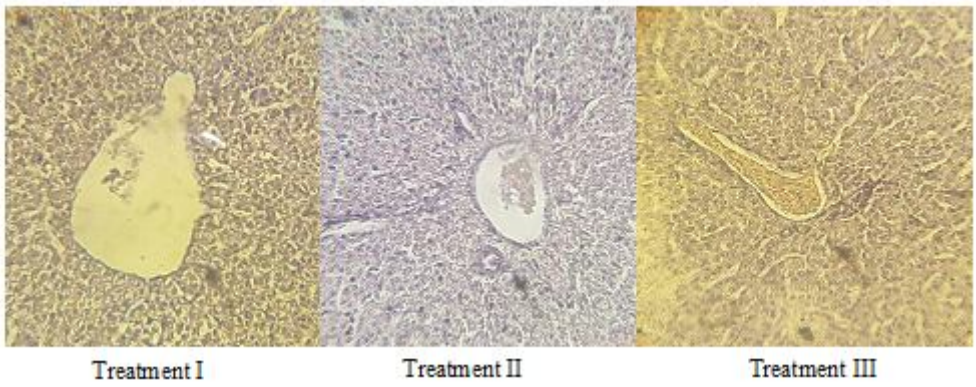

Figure 5. Irregular hepatocytes and hyperemia (Oil red × 400).

2.Biochemical Results

The levels of ALT, AST and ALP are shown in figures 1-3.

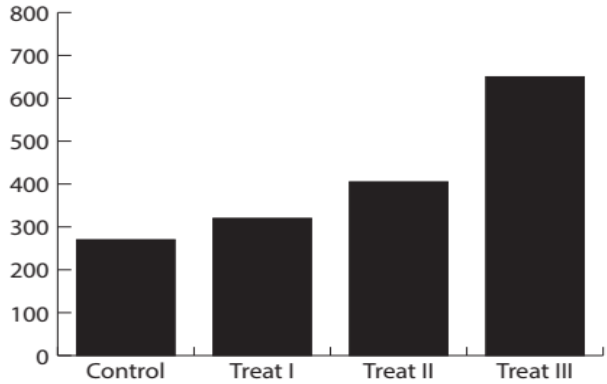

Figure 6. The concentrations of AST in different groups (IU/L).

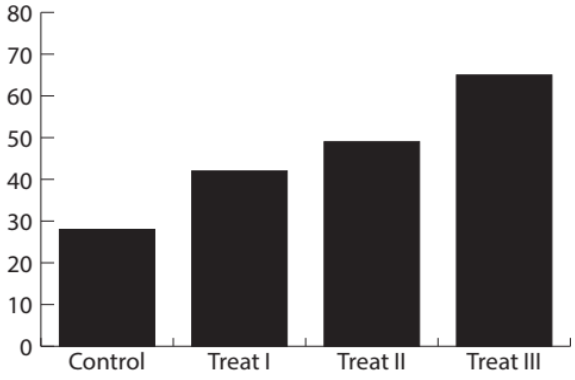

Figure 7. The concentrations of ALT in different groups (IU/L).

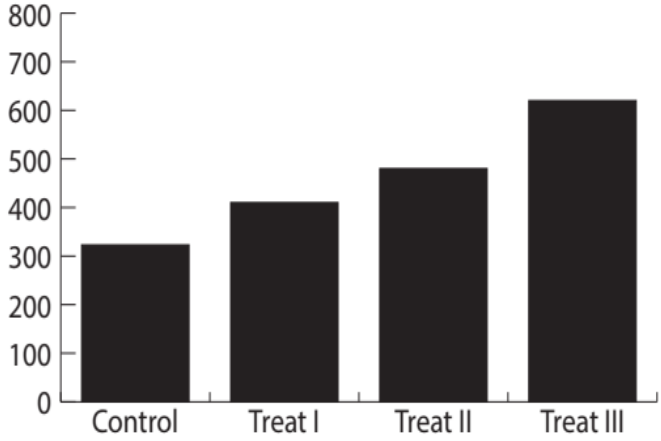

Figure 8. The concentrations of ALP in different groups (IU/L).

DISCUSSION

The aim of this study was to investigate the harmful effects of lead acetate II on the histological structures of liver and its enzymatic activity. The liver plays a significant role in major detoxification processes and its functions are affected by lead toxicity. It has been shown that fatty changes in the liver parenchyma degenerate hepatocytes and nuclear pyknosis are the result of exposure to lead [16]. It has been reported that after feeding chickens contaminated with lead, the number of apoptotic cells increases in the liver. Also, cellular swelling, rupture and vacuolization of mitochondria and wrinkling of the cell body are caused

by lead toxicity [17]. Further, moderate focal lymphoid integrations are created after chickens are given foods contaminated with lead [18].

There are ample reports on the harmful effects of lead on other organ tissues. Kidney is one of the organs which is affected by this heavy metal. It has been reported that lead causes production of intracellular inclusions, focal atrophy and intra-cytoplasmic calcification in proximal tubules. Also, the infiltration of lymphocytes occurs in the interstitial tissue of kidney and focal fibrosis occurs [19]. Babiker et al [2018] showed that exposure to lead increased the level of cellular apoptosis in the cardiomyocytes of rats [20]. Also, studies have reported that lung macrophages become sensitive to lead exposure in the short-term [21]. Besides, it has been reported that young, male animals are more sensitive to lead poisoning than the female counterparts [22].

It has been shown that the adverse effects of lead on tissues are caused primarily by the stress from oxidative processes, interfering with the lipid peroxidation, cell membrane integrity, and widespread damage in tissues [23]. It has been reported that lead destroys the morphologic structures of hepatocytes, due to cellular interruptions, necrosis and infiltration of inflammatory cells in the liver [24].

There are also reports about the dilation of sinusoids and reservoir of red blood cells in the liver as a result of lead poisoning [16]. Further, it has been reported that lead stimulates interruptions in the scaffolding of the hepatic lobules and pyknotic areas found in the degenerated hepatocytes, and dilation of liver portal vein is often observed [25].

In addition, it has been shown that after 40 days of feeding mice with lead nitrate, the general structure of hepatic lobules is interrupted. Also, the hepatocytes' alignment alters and the dilation of central and portal veins happen. The vacuolization in the cytoplasm of hepatocytes was prominent and their special form changes. Accumulation of fat was obvious and some leukocytes infiltrate in this important organ [26]. It has been reported that after two weeks of exposure to lead, the liver was thickened, because of the mild proliferation of hepatocytes. Also, distention of central and portal veins happened [27].

The level of liver enzymes showed that lead acetate II increased the concentration of ALT, AST and ALP [28]. These enzymes are biomarkers, representing liver tissue damages. ALT and AST are enzymes involved in the metabolism of carbohydrates and amino acids, linking between substrates of citric acid cycle. Further, ALP is an enzyme that attaches to the cell membrane at varying affinity, influences the membrane permeability, and disrupts the metabolite transport. Also, the increased level of ALP has been repeatedly shown in animals that were exposed to lead. Besides, ALP serves as a biomarker of cellular adjustment to the various injuring factors [28-31].

CONCLUSION

This study demonstrated that feeding chickens with various doses of lead acetate II negatively affected the structure and general organization of liver cells. Subsequently, alterations in the shape and position of hepatocytes, hemorrhage and hyperemia were observed. Besides, changes in the levels of liver enzymes after exposure to lead causes certain adaptive conditions. Because of the increase of lead in the air that occurs, e.g., from the defective combustion of gasoline in the vehicles engines, the governments, especially in Iran, should pay particular attention to this important subject for controlling the harmful level of lead in the environment.

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CONFLICT OF INTERESTS

Authors declare that there was no conflict of interests in the course of conducting this research.

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