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The Effect of Lead Chloride on Liver Enzyme Levels in Blood Serum of Japanese Quail Coturnix Coturnix Japonica

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Abstract: The purpose of the current study was to examine the physiological effects of lead chloride (PbCl2) on the Japanese quail's (Coturnix coturnix japonica) liver enzyme levels. Thirty quails of both sexes participated in the study; they were split into three groups of ten birds each at random. For 60 days, distilled water was given to the first group, which acted as the negative control. The second group, which was the initial experimental group, received PbCl2 for 30 days at a dose of 25 mg/kg. The third group, which was the second experimental group, received PbCl2 every day for 30 days at a dose of 50 mg/kg. After 0, 15, 30, and 60 days from the beginning of the experiment, blood samples were collected and the birds were dissected. The birds' serum was tested for the liver enzymes ALT (alanine aminotransferase), AST (aspartate aminotransferase), and ALP (alkaline phosphatase).The experiment's physiological findings showed that,

throughout the course of the three periods, the experimental groups' serum levels of the enzymes ALT, AST, and ALP significantly increased in comparison to the negative control groups. The highest significant increase was found in the second experimental group for all parameters.

Keywords: Lead chloride; Japanese quail ; alanine aminotransferase ; aspartate aminotransferase ; alkaline phosphatase ; liver enzymes

1. Introduction

Birds exposed to lead, a persistent inorganic contaminant, may develop hematological, neurological, immunological, reproductive, and behavioral abnormalities, affecting all bodily systems [1]. One heavy metal that has historical significance is lead (Pb). This metal, which is part of the earth's crust, is considered an environmental hazard because it is found in larger amounts in the atmosphere, soil, water, and food. Although the main source of lead is human activity, other natural sources include volcanic eruptions and soil erosion. [2].

Birds exposed to lead, a persistent inorganic contaminant, may develop hematological, neurological, immunological, reproductive, and behavioral abnormalities, affecting all bodily systems [1]. One heavy metal that has historical significance is lead (Pb). Because it is present in higher proportions in the atmosphere, soil, water, and food, this metal, which is a component of the earth's crust, is regarded as an environmental problem. Soil erosion and volcanic eruptions are examples of natural sources of lead, although human activity is the primary source [3-4].

Because Pb is the heaviest element and Saturn is the heaviest planet, lead poisoning was dubbed Saturnism, or plumbism. A French doctor named Louis Tanquerel des Planches wrote one of the earliest books on lead poisoning, including the symptoms of over 1500 patients. He discussed lead colic and nervous system abnormalities such palsy, regional anesthesia, amaurosis, convulsions, coma, and encephalopathy in his writings. [4].

The neurological, hematologic, skeletal, renal, cardiac, hepatic, reproductive, and gastrointestinal systems are among the organ systems that are impacted by lead. Lead can damage the fetus by passively diffusing over the placental barrier. The transfer of metal ions from the mother in utero and from consuming breast milk are the main causes of the lead burden on infants. [5].

One of the primary organs of the body affected by Pb exposure is the liver, which is involved in the metabolism of xenobiotics. Long-term exposure to lead has been linked to the development of fatty liver disease associated with metabolic dysfunction (MAFLD) and non-alcoholic fatty liver disease (NAFLD), according to numerous studies [6-8] .

1. Objective of the Study

Given the foregoing, the purpose of this study was to determine how lead chloride affected the quail serum levels of the liver enzymes ALT, AST, and ALP.

3. Experimental Method

I. Study birds

The Japanese quail, C. c. japonica, was the subject of the current investigation. They weighed between 200 and 250g and were six weeks old. The Department of Animal Production at the University of Mosul's College of Agriculture and Forestry provided the birds. They were kept in wooden cages and fed constantly while being kept clean and sterile. Throughout the trial, natural lighting, temperature, and ventilation conditions were used. [9-11].

II. Choose the dose used in the study

Based on an experiment to find the LD50, the study dose was chosen. In this investigation, a dose of 25,50 mg/kg of body weight was administered orally using a Gavage needle after being diluted in 0.2 ml of distilled water.

III. Experimental design

In this study, thirty quail birds of both sexes were used, and they were divided equally between three groups. The first group, known as the control group, received distilled water as a dosage for 30 days. The second group, the original experiment group, received a dosage of lead chloride (PbCl2) at a concentration of 25 mg/kg for 30 consecutive days. Lead chloride (PbCl2) at a concentration of 50 mg/kg was administered to the third group, also referred to as the second experiment group, for 30 days.IV. Serum Biochemical Test

After 15, 30, and 60 days following the initiation of the experiment, blood was extracted from the birds. Blood was drawn and processed using [12]'s methodology. [13] was used to estimate the activity of the ALT and AST enzymes, while [13] was used to estimate the activity of the ALP enzyme. [14].

V. Statical Analysis

The data were analyzed using the simple and factorial experiment system Complete random design. Transactions have been tested Dunken's New Multiple Rang Test, where the significantly different coefficients were distinguished by different alphabets and under 5% probability level.

4. Results and Discussion

Effect of Lead Chloride ($PbCl_2$) Concentrations on Liver Enzyme Activity in Quail Birds

I. Activity of Alanine Aminotransferase (ALT)

Statistical results indicated that there were no significant differences in ALT levels in the serum among the study groups at the beginning of the experiment at a significance level of ($P \leq 0.05$) (Table 1). Table 1 demonstrates a noteworthy rise in ALT levels in the serum of birds in the experimental groups relative to the control group after 15 days from the beginning of the trial. In addition, the second experimental group had the highest serum ALT level when compared to the first experimental group.

The statistical results at one and two months from the beginning of the trial were comparable to those at 15 days, indicating that the second experimental group's blood ALT levels were higher than those of the control and first experimental groups (Table 1). When comparing ALT levels within the same group, (Table 1) indicates that there were no significant differences in ALT concentration among the control group during the three experimental periods, although there was an increase from the start of the experiment to the other periods; however, this increase was not significant. In the first experimental group, (Table 1) shows a significant positive increase among the three study periods, although this increase was not significant after one month and two months from the start of the experiment.

In the second experimental group, it is observed from (Table 1), that the level of ALT significantly increased after one month and two months compared to the 15-day period from the start of the

experiment, which itself had significantly increased from the baseline period. Additionally, it is noted that at the two-month, the level decreased compared to the one-month period, although this decrease was not statistically significant.

Table 1. Activity of Alanine Aminotransferase (ALT)

Period Groups	0 days	15 days	30 days	60 days
Control group	B 11.26 a	AB 11.90 e	AB 11.75 e	AB 11.70 e
First experimental group	C 11.69 a	B 20.32 c	A 32.77 b	A 33.18 b
Second experimental group	C 11.24 a	B 54.39 a	A 65.71 a	A 64.88 a

- According to Duncan's test, a significant difference between experimental groups at a probability level ($p \leq 0.05$) is indicated by different lowercase letters, and vice versa.
- According to Duncan's test, different capital letters denote a significant difference within the same group at a probability level ($p \leq 0.05$), and vice versa.

The enzyme ALT is responsible for protein metabolism and the breakdown of food to produce the necessary energy, and it is found in the highest concentration in liver cells [15]. The results of the current study align with those of a previous study conducted on mice exposed to lead for 35 days, where an increase in the levels of liver enzymes ALT and AST was observed, along with an elevation in levels of SOD (superoxide dismutase), LDH (lactate dehydrogenase), and MDA (malondialdehyde). Lead exposure also led to liver enlargement and disturbances in lipid metabolism by affecting the regulation of linoleic acid metabolism, sphingolipids, and glycerolipids. Studies indicate that exposure to lead chloride results in increased levels of ALT, reflecting liver damage, and that the level of liver enzymes, including ALT, is an important marker for detecting the toxic effects on the liver [16].

The results of the current study showed an increase in the activity of alkaline phosphatase in the intestines of birds, which is consistent with the findings of a study conducted by [17] on birds, as a biological indicator of heavy metal contamination, including cobalt. Cobalt causes significant changes in enzyme activity, including its effect on metabolic processes in the intestines, leading to an increase in enzyme levels. Furthermore, exposure to cobalt induces oxidative stress, stimulating the production of reactive oxygen species, which causes cell death and tissue damage in the intestines and muscles of birds. This was also observed in other studies that utilized heavy metals other than lead, such as the study by [18], which addressed the use of cobalt chloride on quail.

II. Activity of Aspartate Aminotransferase (AST)

Table (2) indicates that the level of AST in the serum of Japanese quail in both the control and experimental groups shows no significant differences among the groups at the start of the experiment at a significance level of ($P \leq 0.05$).

However, after 15 days from the start of the experiment, there was a significant increase in AST levels among the experimental groups compared to the control group. The second experimental group showed higher levels than the first experimental group.

When compared to the control group, the experimental groups' considerable increase persisted one month after the experiment began. When comparing the control group and the experimental groups two months after the experiment began, the results were comparable to those shown at the one-month point. (Table 2).

When comparing the AST concentration levels in the serum of the birds within the same group across the three research periods, it was noted that there was no significant difference in AST concentration levels in the control group between the start of the experiment and the three research

periods. In the first experimental group, Table (2) shows a significant upward trend in AST levels; however, there was no significant difference in concentration between the 30 and 60 day periods. In the second experimental group, there was an increase in AST levels during all three study periods compared to the start of the experiment, with the 30 day period being significantly higher than the other periods, and the 60 day period being significantly higher than the 15 day period in terms of AST concentration (Table 2).

These results are consistent with other studies that examined the exposure of mice to lead for 12 weeks, where an increase in the activity of liver enzymes AST and ALT was observed. Additionally, high levels of AST production indicate oxidative stress caused by the presence of the toxic substance, lead [19]. Similar findings were reported in a study conducted on fish [20].

Table 2. Activity of Aspartate Aminotransferase (AST)

Period Groups	0 days	15 days	30 days	60 days
Control group	A 92.38 a	A 91.40 d	A 92.20 e	A 92.49 e
First experimental group	C 92.30 a	B 114.95 b	A 175.37 c	A 175.89 c
Second experimental group	D 92.22 a	C 132.69 a	A 241.38 a	B 237.33 a

- According to Duncan's test, a significant difference between experimental groups at a probability level ($p \leq 0.05$) is indicated by different lowercase letters, and vice versa.
- According to Duncan's test, different capital letters denote a significant difference within the same group at a probability level ($p \leq 0.05$), and vice versa.

The results of the current study also align with a study on mice exposed to lead concentrations of 50, 70, and 90 mg/kg for four to eight weeks, which showed a decrease in AST and ALP enzymes, along with an increase in creatinine levels. This is attributed to lead toxicity, which leads to a reduction in the radius and number of red blood cells, as well as an increase in the number of white blood cells and platelets, accompanied by damage to liver and kidney tissues, and necrosis of both the white and red pulp in the spleen [21]. However, this study does not agree with the results of another study that investigated other heavy metals, as the study by [22] did not show any effect of cobalt on AST enzyme levels in vertebrates.

III. Activity of Alkaline Phosphatase (ALP)

Table (3) shows the results of the statistical analysis of ALP levels in the serum of birds from the control group and the experimental groups at a significance level ($P \leq 0.05$). The Table indicates that there were no statistically significant differences in ALP levels at the start of the experiment between the control group birds and the other experimental groups.

However, after 15 days from the start of the experiment, a significant increase in ALP concentration was observed in the experimental groups compared to the control group, with the highest concentration noted in the serum of the second experimental group.

After one month and two months from the start of the study, the results were similar to those observed at the 15 day. The experimental groups were superior to the control group in both periods, with the second experimental group also showing the highest concentration during both timeframes. When comparing the ALP concentration levels in the serum of birds from the same group across the three research periods, there was no significant difference in the control group birds during the three periods compared to the start of the experiment (Table 3).

In the first experimental group, a significant increase was observed between the start of the research and the subsequent periods, while a significant decrease was noted after two months compared to

the one month period, with both latter periods being significantly higher than the 15 day period. In contrast, in the second experimental group, there was a significant progressive increase in ALP levels across the three research periods compared to the start of the experiment (Table 3).

The cytoplasm of liver cells and the canalicular membrane of hepatocytes, which is situated on the cell membrane's outer layer, contain the ALP enzyme [23]. The current study's findings are consistent with those of a study on quail, which found that lead toxicity at a level of 0.4 mg/kg resulted in histological changes in the liver as well as an increase in the liver enzymes ALP, AST, ALT, creatinine, and uric acid. [24].

Table 3. Activity of Alkaline Phosphatase (ALP)

Period Groups	0 days	15 days	30 days	60 days
Control group	A 109.04 a	A 109.43 e	A 108.56 e	A 109.88 e
First experimental group	D 109.69 a	C 154.71 b	A 196.63 b	B 191.74 b
Second experimental group	C 109.36 a	B 191.29 a	A 243.52 a	A 244.66 a

- According to Duncan's test, a significant difference between experimental groups at a probability level ($p \leq 0.05$) is indicated by different lowercase letters, and vice versa.
- According to Duncan's test, different capital letters denote a significant difference within the same group at a probability level ($p \leq 0.05$), and vice versa.

Consequently, the observed increase in liver enzymes is a result of liver toxicity induced by lead chloride. These findings are consistent with a study by [25], which reported an elevation in ALP levels attributed to its strong impact on liver function indicators, formation of reactive oxygen species (ROS) and oxidative stress. The findings of this study also concur with a study by [26] that found that mosquito fish exposed to lead chloride had higher levels of ALP, as well as previous studies that looked at the effects of PbCl2 on different fish species. (Sherif et al., 2020).

5. Conclusion and Future Scope

The study concluded that lead has harmful pathological effects that are physiological, macroscopic, histopathological, and immunohistochemical on various body organs. Therefore, exposure to lead should be avoided for both humans and economic animals, as well as animals in general, to maintain their safety in the environment.

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Data Availability- None

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