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Biochemical and histopathological alterations of liver and kidney of Japanese quails due to lead toxicity and effects of vitamin C

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ABSTRACT

Lead (Pb) is the most dangerous xenobiotic causing variety of negative impacts on human and animal health. Thus, the current study intended to investigate the effect of different doses of Pb on biochemical and histological abnormalities and the beneficial effects of vitamin C in the kidney and liver of quail. A total of 72 male Japanese quails, aged 12 days were allotted in four groups with four replications. Control group (T_0) received normal diet, group T_1 and T_2 received Pb at a dose of 70 mg and 700 mg/kg body weight (bw), respectively for 21 days, while group T_3 received 700 mg Pb /kg bw for 11 days followed by only vitamin C supplementation at a dose of 70 mg/kg bw without any Pb for next 10 days. After end of treatment, blood serum was collected, and the kidney and liver were taken for a histoarchitecture study. As expected, Pb-induced significant elevations of biochemical markers of liver and kidney injury were observed in different periods of treatment (group T_3 at day 21) and decreased (except creatinine) following vitamin C supplementation in group T_3 at day 21. Also, Pb treatment induced congestion, necrotic foci in different organs, reactive cells in central vein, degeneration of hepatocytes, necrosis in the portal area as well as renal tubular degeneration, all of which were slightly improved by vitamin C in group T_3. Therefore, the findings suggest that the use of vitamin C could be a potential preventive agent against Pb-induced toxicity.

INTRODUCTION

Lead (Pb) is heavy metal, naturally present in the soil, but as a result of global industrialization it is being incorporated into the environment and become hazardous to health [1]. It is now ubiquitous, a big concern to the public health [2]. Animals and humans are exposed to Pb through ingestion and /or inhalation from contaminated sources with the consequence of multisystemic health disorders including hemopoietic, reproductive and cardiovascular disorders [1]. The underlying mechanisms include reactive oxygen species (ROS)-mediated oxidative stress, genotoxicity, and apoptosis [3] and also enhanced encephalopathy [4] and increased lipid peroxidation in brain [5]. Since the previous few decades, environmental pollution has increased [6] and industrial areas are posing risks to all living systems, including birds [7]. Birds are exposed to Pb from a number of different sources, including the general environment, industrial pollution, and agricultural processing-related contamination of water, soil, and food [8]. When taken orally, Pb is absorbed in small amounts but is slowly eliminated, therefore exposure to small amounts over extended periods of time may

result in detrimental effects [9]. When Pb reaches the bloodstream, it is absorbed, with some of it becoming attached to erythrocytes and some of it is conjugated in the liver before passing to the kidney. The remainder remains in plasma to be dispersed throughout the body, where it accumulates in various organs and impairs their functions. Animals have been known to perform poorly and even die after ingesting Pb [10]. In case of poultry it causes muscle paralysis of the digestive tract, weight loss, and even starvation [11]. Additionally, Pb displays certain immunotoxin effects as stated [12]. Birds can sustain high levels of metals in a variety of tissues, including the kidney, liver, meat, and eggs [13, 14]. Such residues can easily be dispatched to human and other organisms through food chain resulting in a wide range of biochemical [14, 15] and physiological abnormalities in different systems [16]. The kidney and liver are dynamic structure in both fitness and illness. A high creatinine level in the Pb treatment indicates the impaired renal function and glomerular filtration [17] while a higher alkaline transaminase (ALT), aspartate aminotransferase (AST), and degenerated hepatocyte also indicate the hepatic malfunction [18]. Chronic nephropathy can result from long-term exposure to Pb in the environment [19]. Further, Pb toxicity has been associated with cancers of the gastrointestinal tract, female genital, renal, respiratory, myeloma, and all forms of leukemia [20].

A few research have concentrated on amelioration of Pb toxicity. Antioxidants have reportedly been shown to significantly reduce Pb toxicity [2]. Scar tissue production and wound healing are both aided by vitamin C. Through the action of electron donation or transfer, vitamin C functions as a free radical scavenger, protecting the cell from oxidative stress caused by ROS [21]. The convenient, safe, and effective dietary administration of vitamins in a wide range of doses makes them the perfect antioxidants and boosts tissue protection against oxidative stress [22]. Beside, the quail's meat with its special qualities such as rapid digestion, minimal lipid and high in protein, is very suitable for infant, older and patients with high blood pressure [23]. Although the Pb induced effects in humans and various animals have been focused, its biochemical and histopathological impact in quail has not been fully elucidated. On the other hand, the potential of vitamin C in regard to revive the oxidative damage of liver and kidney in the quail has not yet known. Thus, the current study aimed to evaluate the properties of Pb on these macro-anatomical changes, biochemical parameters and detect the histological architectural variations in hepatic and renal sample as a result of Pb poisoning, along with the ability to antioxidative nature of vitamin C to reverse the detrimental effect of Pb.

MATERIALS AND METHODS

Chemicals

Lead acetate (II) trihydrate (316512, ≥99.999% purity) and vitamin C (A4544, 98% purity) were purchased from Sigma-Aldrich, St. Louis, MI, USA, unless otherwise stated.

Animals

Total 72 quails (12 days of old male quail) were obtained from the Dinajpur Quail Farm, Birol, Dinajpur, Bangladesh. The temperature of the farm was kept at 28±2ºC with a relative humidity of 40–70% and provided the birds with complete access to feed and water. The quail were housed under-regulated lighting (12-hour light/dark cycle). They were kept in an animal house with plenty of ventilation. For seven days, under lab

conditions, birds were cared for and adapted. Following the acclimation phase, birds were randomly chosen to form 4 groups, each containing 18 birds. Animals in all groups were also weighed at intervals of three days and then divided into four treatment groups with three replicates.

All the experiments were conducted by ensuing the ethics and guidelines, including animal care followed by the Department of Anatomy and Histology of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur-5200, Bangladesh. The approval number was HSTU/VAS/ANH-1070 dated: 01-06-2020 (Resolution No: 10).

Experimental design

There were four experimental groups such as T_0, T_1, T_2, and T_3. The control group (T_0), which received a basal diet and distilled water. Pb was administered orally to second group T_1 for 21 days at a dose rate of 70 mg/kg body weight (bw) with basal diet and adlibitum distilled water. The third group (T_2) received Pb orally for 21 days at a rate of 700 mg /kg bw. The last group, T_3 had 700 mg Pb /kg bw for 11 days followed by only vitamin C supplementation at a dose of 70 mg/kg bw without any Pb for next 10 days (Table 1).

Table 1. Experimental treatment.

Groups	Diets+ compositions	Duration or Period of treatment		
		Treatment starting day (d_0) to 10th day of treatment (d_{10})	11th days of treatment (d_{10}) to 21 days of treatment(d_{21})	Treatment starting day (d_0) to 21 days of treatment (d_{21})
T_0	Basal diet+ distilled water (Control)	×	×	√
T_1	Basal diet+ distilled water +70mg Pb /kg bw	×	×	√
T_2	Basal diet+ distilled water +700mg Pb /kg bw	×	×	√
T_3	Basal diet+ distilled water +700mg Pb/kg bw	√	×	×
	Basal diet+ distilled water +70mg vitamin C/kg bw	×	√	×

Collection of samples

Blood collection was performed from randomly selected three birds from each group for 3 time periods such as at 0 day (d_0), 10th day (d_{10}), and 21st day (d_{21}). The jugular vein was suitable for taking blood from quail. After the end of the experimental period (21 days) and 12 h of circumvention of diet, quails from each treatment group were sacrificed to obtain viscera (Hepatic and renal organs, muscular stomach, intestinal tract, and lungs) as well as to determine the macroscopic and histological alterations of those organs. Blood was then preserved in a lithium heparinized collecting tube (BD 366667; Thermo Fisher, Waltham, Massachusetts, USA, clot activator tube) for biochemical examinations.

Histopathological analysis

The samples were maintained for fixation in 10% formalin for a 24 h period to conduct a histopathological analysis. The tissues were then left in each grade of alcohol (70, 80, 90, 95, 100%, and 100%) for 1 h to dry. The tissues were then submerged for 90 minutes

each in xylene-1 and xylene-2 (214736., Sigma-Aldrich, St. Louis, MI, USA). The procedure was then repeated with the samples submerged in melted paraffin at 55-60 ^{o} C for 90 minutes. Paraffin blocks were made after the tissues had been embedded in paraffin (76242., Sigma-Aldrich, St. Louis, MI, USA). Using a microtome machine, the paraffin blocks were sliced at a thickness of 5–6 micrometers. (Leica RM2135 Manual Rotary Microtome, Germany). Sectioning was followed by floating the paraffin block's slices in warm water in a water bath. After cutting the paraffin block into slices, the slices were floated in warm water and heated to 45°C to lengthen. Hematoxylin and Eosin's (H & E) stain (H3136 and 45260., Sigma-Aldrich, St. Louis, MI, USA) was used to color the slices on glass slides for general histology study. With a light microscope, the slides were examined, and pictures were taken with Camera headed microscope (AmScope B100-E 1000X Digital Compound Binocular Microscope, USA).

Biochemical analysis

Blood samples were given a minimum of 30 minutes to clot (maximum of 60 min). Following clotting, the samples were centrifuged for 10 minutes at 6000 rpm. Then the serum was taken by pipette and mixed with available reagents (RANDOX, Crumlin, Country Antrim, United Kingdom) for analyzing ALT and AST by using biochemical analyzer (18200; HUMAN, Wiesbaden, Germany) and renal function marker (Serum Creatinine) in the blood serum were determined using a modified version of Larsen's kinetic Jaffé reaction.

Statistical analysis

Experiments were carried out at least three times. The results of biochemical parameters analyzed as a ratio against control and Pb exposed group were expressed as Mean ± Standard Error of Mean (SEM). Single factor analysis of variance (ANOVA) was done by IBM SPSS version 25 software to analyze the statistical differences between the groups and t-test was done for differences between the period of treatment. Differences were considered significant at the level of $p<0.05$.

RESULTS

Postmortem findings of quails with lead toxicity

Pb-treated birds showed mucosal erosion of the gizzard (Figure 1A), intestinal gas buildup (Figure 1B), change in the color, and caecal extension (Figure 1C) and pinpoint hemorrhage in the caecal tonsil (Figure 1D). Pb toxicity induced hepatomegaly (Figure 1E), as well as renal congestion and hemorrhage (Figure 1F) in quails. In addition, hemorrhage was found in lung of Pb-intoxicated quails (Figure 1F).

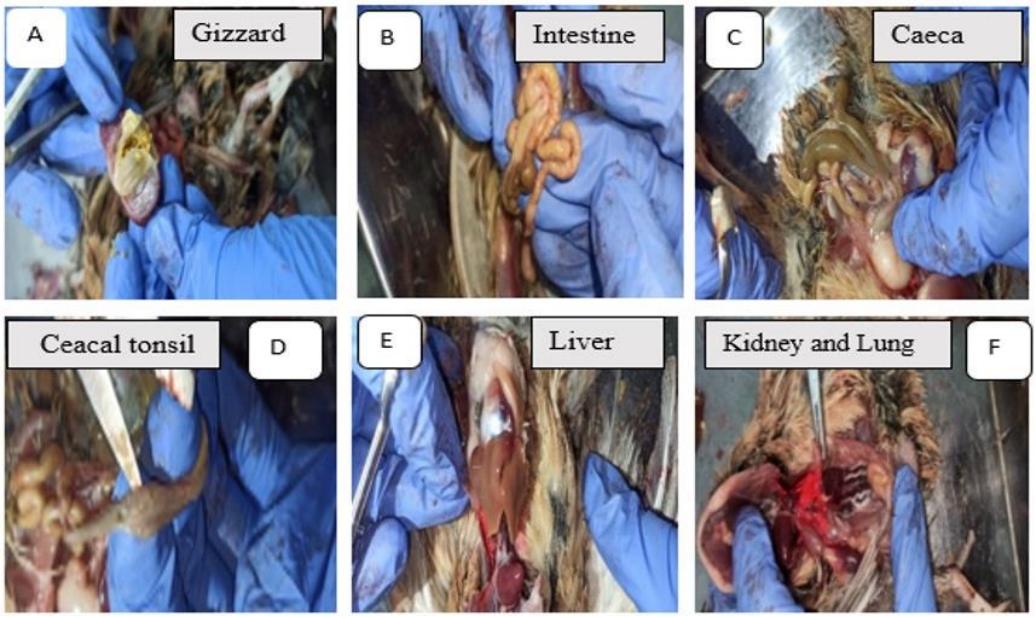

Figure 1. Some gross changes due to the treatment effect of Pb on different internal organs showing Mucosal erosion in Gizzard (A), accumulation of gas (B), enlargement of caeca (C), Pinpoint haemorrage in caecal tonsil (D), enlargement of liver (E) and lastly congestion in kidney and lung (F).

Effect of vitamin C on histopathological changes in liver of Pb-intoxicated quails

In the present study, the liver was found with normal histological architecture in the control group T_0 of quails (Figure 2A). The liver of the Pb-treated quail showed somewhat congestion in the central vein (CV), increased lymphocytic infiltration, and increased von Kupper cells in Group T_1 (Figure 2B). Whereas necrosis (black arrow) was found in both Group T_2 and T_3 of Pb-treated quail (Figure 2C-D). Also, reactive cells were found in both Group T_2 and T_3 (Figure 2C-D) but the amount was less in case of Group T_3 (Figure 2D), and tubule was observed in Group T_3 (Figure 2D).

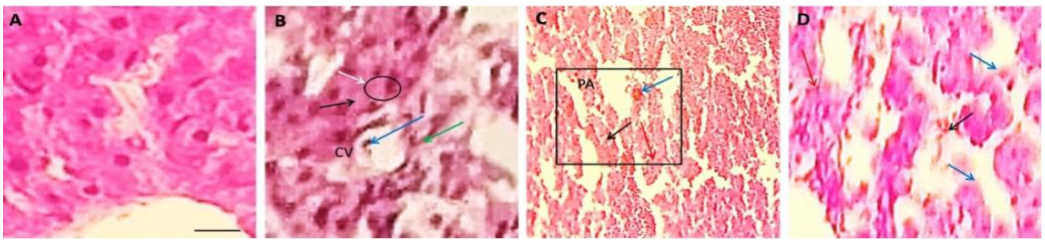

Figure 2. Histological observation of liver in quail using H&E staining. A) Section of liver of control quail (Group T_0) is showing normal histotexture of liver (40X). B) Section of liver of Pb exposed quail (Group T_1) showing lymphocytic infiltration (black arrow), slightly congestion in central vein (blue arrow) with increased number of Kupffer cells (green arrow), and hepatic cell hypertrophy (white arrow) was found in the liver of Group T_1 of quail (40X). C) Sections of the liver of quail fed with Pb (Group T_2) showing huge Lymphocytic cell infiltration (black arrow) and necrosis in the portal area (PA) (black box), Cytolysis of hepatocyte (blue arrow), dilatation of bile canaliculi (red arrow) (40X). D) Sections of liver of quail of (Group T_3) showing Less amount of reactive cells infiltrations (blue arrow) and slightly necrosis formation (black arrow), less hepatic cells disarrangement (green arrow), and preservation of normal histotexture of liver cell (40X). Scale bar: Scale bar: 1 μm(40X).

Effect of vitamin C on histopathological changes in kidney of Pb-intoxicated quails

In the present study, kidney was found normal morphological appearance such as regular glomerulus was observed in Group T_0 (Figure 3A). Screening reactive cell infiltration and necrosis on renal tubules, shrinkage of glomerulus was discovered in Group T_2 (Figure 3C) and increased reactive cell infiltration and necrosis on renal tubules were detected in Group T_1 (Figure 3B). Less reactive cell infiltration and less necrosis on renal tubule was observed in Group T_3 (Figure 3D).

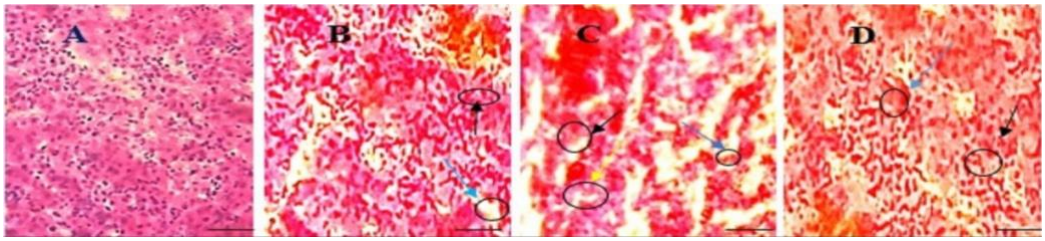

Figure 3. Histological observation in the kidney of quails using H&E staining. Normal appearance of kidney was found in the control group, T_0 (A, 40X), Whereas increase reactive cell infiltration (black arrow) and necrosis on renal tubule (blue arrow) was found in T_1 group (B, 40X). On the other hand, screening huge reactive cell infiltration (black arrow) and necrosis on renal tubule (blue arrow), shrinkage of glomerulus (yellow arrow) was found in T_2 group (C, 40X) whereas less reactive cell infiltration (black arrow) and less necrosis on renal tubule (blue arrow) was observed in T_3 group (D, 40X). Scale bar: 1 µm (40X).

Effect of vitamin C on liver and kideny injury biomarkers in Pb-intoxicated quails

There was a significant difference in concentration of ALT (Figure 4A) and AST (Figure 4B) between d10 and d21 treatment periods in Pb-treated groups (T_1, T_2, and T_3). Interestingly, d21 period of group T_3 showed significant lower levels of ALT than d10 period which caused due to the supplementaion of vitamin C in T_3 group (Figure 4A). Thus, vitamin C-treated period (from day 11 to day 21) showed significant lower ALT level than non vitamin C treated period at d10 of T_3 group (Figure 4A).

In case of AST, there was no significant difference among all Pb-treated groups (Figure 4B). Further, d10 period of group T_3 showed statistically higher levels of AST than the period d0. Interestingly, there was significant lower AST levels in d21 period as compared to d10 period of T_3 group beacuse of vitamin C treatment in d21 (Figure 4B).

There was an increased trend of creatinine levels in all Pb-treated groups but there was no significant difference among all Pb-treated groups. Interestingly, there was remarkable significance difference in creatinine levels between the period of treatment (d0 and d10) of Pb-treated group. In addition, the value of creatinine at d21 period of T_1 and T_2 group shown the non-significantly increasing trend it might be due to the sex, body weight, physiological, and environmental factor. Interestingly, lower value of creatinine was due to vitamin C supplementation in T_3 group (d21) as shown in Figure 4C.

Considering the data above, it is stated that Pb significantly increased the serum ALT, AST, and serum creatinine levels in different periods of treatment except d21 period of T_3 group. Treatment of Pb-exposed birds with vitamin C showed marked improvement of biochemical findings at d21 period of T_3.

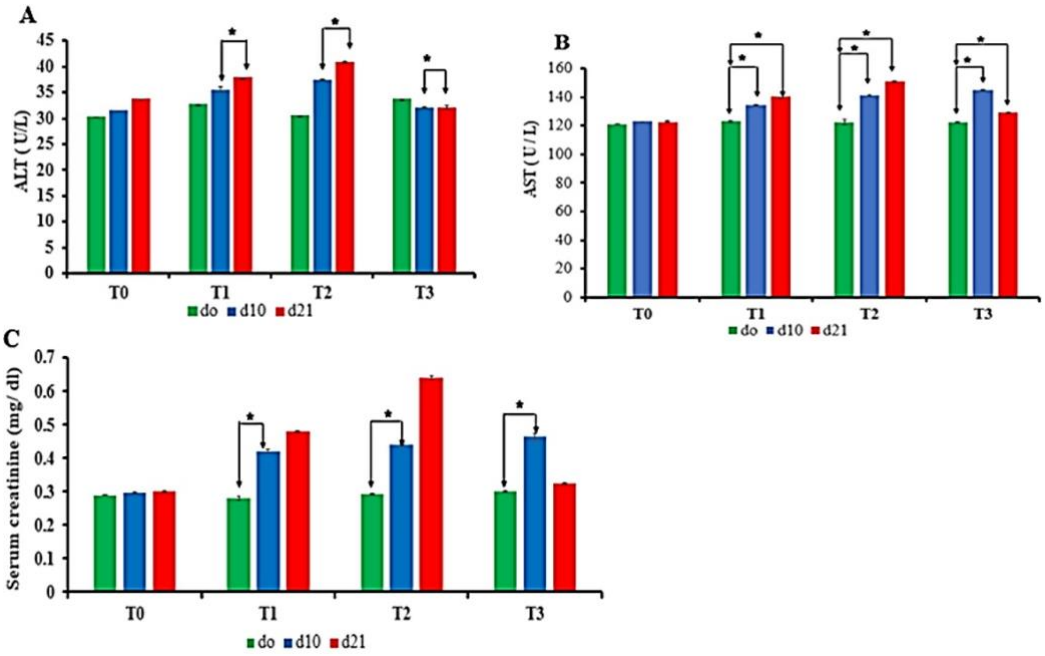

Figure 4. Effect of different doses of Pb and antioxidative effects of vitamin C supplementation on liver and kidney function biomarkers in quail. Quail kept untreated with Pb (Group T_0), Group T_1 (70mg Pb /kg bw), GroupT_2 (700mg Pb /kg bw), Group T_3 (700 mg Pb /kg bw) for 10 days followed by only vitamin C supplementation at a dose of 70 mg/kg bw without any Pb for next 10 days and the values of (A) ALT, (B) AST, and (C) Serum creatinine of four treatment groups and three different period (d0,d10 and d21) were measured. Data indicate mean ±SEM of 4 replicates. Single factor analysis of variance was done to analyze the statistical differences between treatment groups and t-test was done for differences between the period of treatment, here, *statistically significant at 5% level (P≤0.05). Each group contains 18 (eighteen) quails.

DISCUSSION

Birds treated with Pb causes different gastrointestinal, renal, and hepatic changes as well as significantly alters the hematological parameters [1]. Pb is a hazardous substances which may cause renal failure and hepatic damage [23]**.** It has been reported that short term exposure to Pb causes hemorrhages in the tonsils of the caecum, liver hypertrophy [24] and accumulation of gas in intestine [25]. In addition, Pb toxicity causes condense movement of the proximal gastrointestinal tract and it is associated with hematological dysfunction [26].

Repeated exposure of Pb causes significant elevation of ALT and AST, which is consistent with a previous finding [27]. Also, Pb-induced elevations in serum ALT and AST were observed in different periods of treatment that was agreed with previous findings [28]. In the present study, the ALT and AST levels were found significantly lower in vitamin C treated group (T_3 group) at d21. Because vitamin C prevent or lessen the oxidative stress [22]. Additionally, Pb exposure increased the level of creatinine which was effectively decreased after vitamin C supplementation. This was little bit contradict with another group of researchers [29]. It might be due to huge tissue damage by Pb that could not revive fully by vitamin C supplementation and also may be dose factor. It is also reported that the elevation of creatinine values caused by severe renal parenchymal injury, which may have prevented glomerular infiltration [30]. However, this damage was not significantly reduced when the birds were given a vitamin C that was not in agreement with previous report [31].

An increase in lymphatic infiltration and Kupffer cells in the liver of Pb-treated group was observed. Likewise, liver necrosis was found in the Pb-treated group, but fewer

Kupffer cells, less reactive cell infiltration, and less necrosis in liver of vitamin C-treated group were detected, which is consistent with previous results [32]. Several histopathological conditions and increased ALT and AST level indicate the damaging of hepatic cells that was similar with other report [33]. Consistently, reduced histopathological conditions and decreased ALT and AST level indicate the antioxidative effect of vitamin C [34]. According to the presence of responsive cells in the liver caused by connections between amino acid and stimulant of the interstitial tissue of liver and these were inhibited by the antioxidative defense mechanism [35, 36]. According to the another group of researchers [37], the higher AST and ALT levels may be due to liver injury, as evidenced by different histological alterations [38] as well as an increase in the number of Kupffer cells [39]. Recent research showed that the variations in enzyme activity are linked to the result of hepatotoxicity [40, 41]. These variations are linked to liver damage caused by metallothionein, a protein generated within the liver with a high affinity for heavy metals resulting in hepatotoxicity [42]. Pb accumulated in the kidney of quails in a dose dependent way [43, 44]**.** Consequently, Pb exposure increased biochemical markers, which may have been responsible for the structural, functional, and metabolic alterations by Pb toxicity in the kidney and liver [45, 46]. Thus, our findings demonstrated that vitamin C supplementation partially mitigated the harmful effects of lead on the kidney and liver of quail by suppressing Pb-induced the activity of free radicals, and the presence of Pb, glutathione, superoxide dismutase, and catalase enzyme form a complex with the Pb ,and they are important to execute normal antioxidant functions causing irreversible damage to cell membranes leading to cell death [23, 43, 47, 48].

CONCLUSIONS

In conclusion, different doses of Pb cause biochemical and histopathological alterations in liver and kidney. These alterations are due to the oxidative stress caused by ROS. Interestingly, the supplementation of vitamin C in Pb-induced toxicity in quail mitigates oxidative stress as well as the histopathological and biochemical alterations in the liver and kidney. Due to the high doses of Pb and for a short time of experiment, the vitamin C couldn't fully reverse the anatomical, and histopathological alterations in the liver and kidney. Significant histological changes were observed in vitamin C-treated group, suggesting vitamin C as a potential preventive agent against Pb-induced toxicity. Further experimentation on low doses and long-term use of Pb and vitamin C with more population, bioaccumulation rate of Pb on different organs and transgenerational effect of Pb will be needed to get for better result of vitamin C against Pb intoxication.

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AUTHOR CONTRIBUTIONS

Conceptualization and design of the research, MAH and MTH; methodology, MAH; experimental investigation, MAH, MTH, NHP, and KAF; sample resources, MAH; writing and original draft preparation, MAH, SS and KAF; writing-review and editing,

MAH, MTH, NHP, and KAF; supervision, MAH; project administration, MAH. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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