

## Histological and Functional Alteration in the Liver and Kidney and the Response of Antioxidants in Japanese quail Exposed to Dietary Cadmium

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### ABSTRACT

**Background:** The aim of the present study was to find the effects of cadmium (Cd) on Japanese quail on the role of an animal model in order to understand the toxicity of Cd in creatures presented, within the environment.

**Methods:** One hundred 30-day-old male Japanese quails were equally divided into the control group and Cd group. The control group received no Cd while the Cd group was given formula feed comprised of 100 ppm of Cd (CdCl<sub>2</sub>, Merck, Germany) for sixty days. The histological and functional alteration in the liver and kidneys and the response of antioxidants were determined.

**Results:** Histological changes in the liver increased the number of kuppfer cells, vacuolar degeneration, and hepatocytes single cell necrosis in the Cd group. The renal histopathological changes were hyaline cast in renal tubules and necrosis and swelling of tubular epithelial cells. The level of aminotransferases (ALT and AST), blood urea nitrogen (BUN) and creatinine rose significantly in the Cd group toward the control group ( $P < 0.05$ ). The antioxidants (SOD, CAT and GSH) levels considerably diminished, while lipid peroxidation proliferated substantially in the hepatic and renal tissues of Japanese quails in the CD group ( $P < 0.05$ ).

**Conclusion:** Presentation to dietary Cd (100mg/kg diet) for 60 reduces the body weights gain and induces the histological and functional alteration in the liver and kidneys of Japanese quail as well as cellular antioxidant alterations.

**Keywords:** Antioxidant, Cadmium, Function, Histology, Japanese Quail.

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### INTRODUCTION

Cd as a toxic heavy metal is extensively propagated in the ecosystem as a sequel to industrial and farming applications [1, 2]. The introduction of Cd is believed to have harmful impacts on several levels of the food chain, in respects of bioaccumulation. Toxic outcomes of Cd have been observed in the renal system, liver, genital organs, fetus, and the pulmonary and immune systems [1, 3]. The toxic consequences of Cd are interdependent with the deformity of the developing embryo and carcinogenesis [2, 4, 5]. Cd leads to damaging the tissues via oxidative stress and reduces antioxidants, glutathione, and protein-bound sulphhydryl categories [1, 5, 6].

Cd is mainly received from foods, absorbed orally and principally gathered in the Liver and kidneys [2, 6, 7]. Cd first affects the kidneys, then the liver, and the intestines [5]. One of the harmful effects of Cd is the generation of reactive oxygen species and extension in lipid peroxidation in the cells. Free radicals and middle products are

capable of harming and changing the operation of cell membranes, which might result in the improvement of numerous pathological procedures. Various antioxidants that restrict the generation of free-radicals including superoxide dismutase (SOD), catalase (CAT) and glutathione have a critical role in the conservation of cyto-membranes versus oxidative hurt [8-10]. Biochemical changes occur earlier to structural alteration in the body systems. Moreover, the changes in defined activities of enzymes within the body fluids could replicate the expansion of Cd-incited damages in the goal organs [11-13]. Exploring the effects of Cd compounds on birds is a subject extensively studied in eco-toxicological surveys [14]. Experimental studies play a vital role in checking the results of environmental pollutants [15]. The Japanese quail has been nominated by the United States Environmental Protection Agency (EPA) as a passable model organism for surveys that appraise the influence of presentation of environmental contaminants [3].

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In the course of this manner, this is perhaps done to find out the hazard of the disposal of chemical contaminants at completely various levels of the food chain. The aim of our study was to investigate the histopathological and functional effects of dietary Cd on the hepatic and renal systems of male Japanese quail as well as the antioxidant responses to these systems.

## MATERIALS AND METHODS

### *Birds and Survey Plan*

One hundred 30-days-old male Japanese quails (*Coturnix coturnix japonica*) were got from Yazd Agricultural and Natural Resources Research and Education Center, Yazd, Iran and randomly divided into two categories. The control group received no Cd whereas the Cd group received formula feed containing 100 ppm of Cd (CdCl<sub>2</sub>) throughout 60 days. The Cd quantity in the feed (0.006 ppm) and water (0.003 ppm) employed in this study differs under marginal worth. The male quails were held in cages beneath microclimates situation desired for their growth and welfare. The food composition was corn (36.7%), soybean meal (35.4%), wheat grain (15%) corn gluten (8.9%), calcium diphosphate (1%), calcium carbonate, common salt, DL methionine, lysine hydrochloride, L. threonine, B complex and other minerals and vitamins prefix.

On the first day and 60<sup>th</sup> day of experiment, 15 birds were randomly selected from each group, weighed and slaughtered. Furthermore, they underwent post-mortem examination. Blood samples were collected to assay the functions of liver and kidneys. The hepatic and renal specimens were prepared in order to investigate Cd quantity, histological alteration, antioxidants, and lipid peroxidation.

All birds were handled humanely and in consistence with the suggestion of the Ethics Committee of Islamic Azad University.

### *The Dose Selected*

The body weight is a vital indicator of toxic effects. The dosage we utilized was set up as a part of a test contemplates by utilizing body weight. Four groups, each having 10 adult male birds, were exposed to 25, 50, 100 and 150 ppm of CdCl<sub>2</sub> for 60 days. These dosages were computed and adjusted to the previous perceptions in our research center. The period of introduction depended on the increase in birds' body weight. Through this manner, in the course of Cd

introduction, the birds were weighed 72 hours intervals.

Statistical analyses were conducted on the information. The outcomes indicated that 25, 50 and 150 ppm Cd reduced body weight gain throughout the period of the experiment; the 100-ppm dosage alone diminished body weight gain at the termination of the experimental time. Thus, this dose was selected because, at this toxicity rate, it offered Cd cumulative results.

### *Histological Examination*

A segment of liver and kidney was fixed in buffered formalin (10 %), dehydrated in ethyl alcohol and xylene, inserted in paraffin, cut into 5-6 µm sections, and stained with hematoxylin and eosin for microscopic investigation. All histological alterations were recognized.

### *Cd Concentration*

Cd condensation in the hepatic and renal samples was measured by atomic absorption spectrometry (UNICAM939) [6].

### *Function of Liver and Kidneys*

Serum concentration of total protein, albumin, globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) was determined in order to evaluate liver function. The function of kidneys was assessed by measuring the concentration of creatinine and blood urea nitrogen (BUN). The spectrophotometric methods with commercial kits were employed for the analyses.

### *Lipid Peroxidation and Enzymatic and Non-Enzymatic Antioxidant Assessment*

The hepatic and renal tissues were taken, wiped, and promptly perfused with cool saline. The samples were homogenized in cold phosphate buffer saline (pH 7.4, 0.1 Mole). Subsequently, the homogenize was refined and centrifuged (at 2000 g for 20 min); in addition, the supernatant fluid was put away at -80 °C for the purpose of evaluating lipid peroxidation and non-enzymatic and enzymatic antioxidants activities.

The tissue content of malondialdehyde (MDA) was assayed for lipid peroxidation evaluation [16]. The antioxidant condition was estimated via the assessment of the enzymatic antioxidant; catalase (CAT) and superoxide dismutase (SOD), and the non-enzymatic antioxidant; reduced glutathione (GSH) [17, 19].

### Statistical Analysis

The data were subjected to statistical analysis by one-way ANOVA using statistical package for social sciences (SPSS 12th version) (Chicago, IL, USA). By employing Duncan's multiple comparison test, the differences between the means were significant ( $P<0.05$ ).

## RESULTS

### Body Weight Gain

At the onset of the trial, the body weight of

both groups was actually the same. After 60 days, the body weights gain of the quails in Cd group significantly reduced in contrast with the quails in the control group ( $P<0.05$ ) (Table1).

### Cd Concentration

Cd condensation in the liver and kidneys of both groups showed no significant difference on the first day ( $P<0.05$ ). Cd concentrations were much higher in liver and kidneys of the Cd group after 60 days (Table2).

**Table 1.** Effect of Cd on body weight gain in male Japanese quail.

Groups	Initial body weight(gr)	Final body weight(gr)	Change(gr)
Control	234.3±3.9 <sup>a</sup>	276.1±4.8 <sup>b</sup>	42.5±1.1 <sup>b</sup>
Cd	240±3.1 <sup>a</sup>	245.6±5.2 <sup>a</sup>	5.2±2.1 <sup>a</sup>

Distinctive letters in the similar column demonstrate statistically significant difference ( $P<0.05$ )

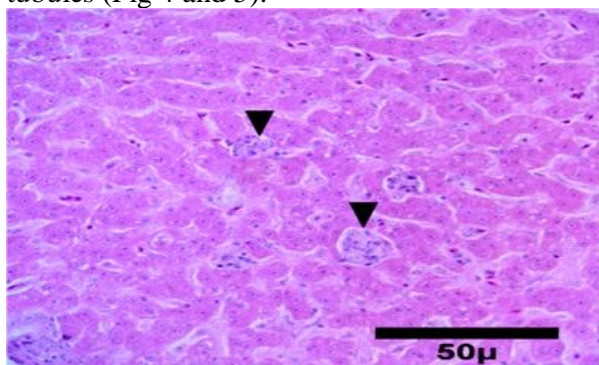
**Table 2.** Cd concentration (ppm) in the liver and kidneys of male Japanese quail.

Groups	Initial liver concentration	Final liver concentration	Initial kidneys concentration	Final kidneys concentration
Control	0.31±0.01 <sup>a</sup>	0.36±0.11 <sup>b</sup>	0.41±0.012 <sup>a</sup>	0.51±0.03 <sup>b</sup>
Cd	0.39±0.02 <sup>a</sup>	5.11±0.08 <sup>a</sup>	0.36±0.02 <sup>a</sup>	7.9±0.2 <sup>a</sup>

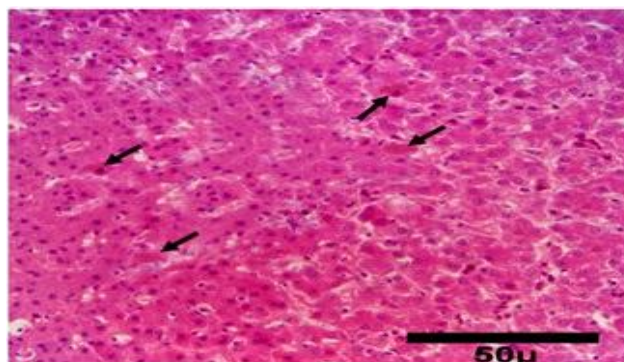
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### Histological Changes

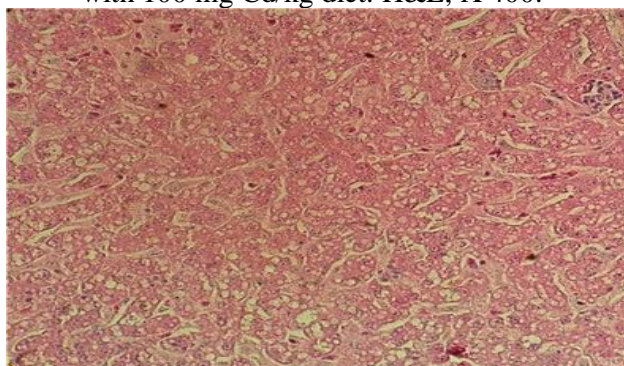
The hepatic of control group indicated a normal histology. In the Cd group, Cd lead to an increase in the number of kupffer cells, vacuolar degeneration, and single hepatocyte necrosis (Fig 1-3). The normal histology of the kidneys was noticed in the control group. The renal histological alteration was observed in quails of the Cd group. The renal histopathological changes were swelling and necrosis of renal tubules epithelial cells, and hyaline casts in the renal tubules (Fig 4 and 5).



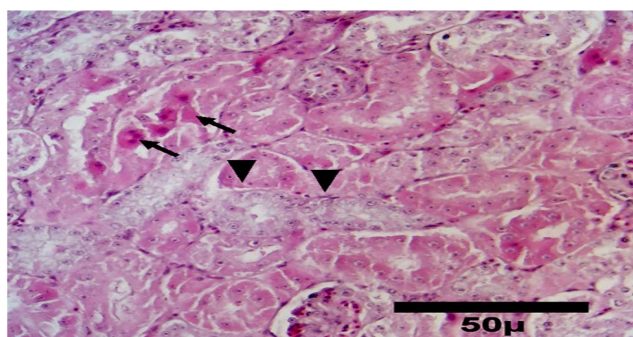
**Figure 1.** Increased number of kupffer cells (head arrows) in the liver of male Japanese quail fed with 100 mg Cd/kg diet. H&E, X 400.



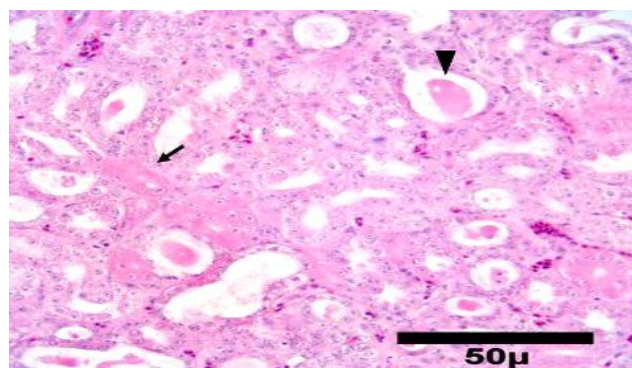
**Figure 2.** Single cells necrosis of hepatocytes (arrows) in the liver of male Japanese quail fed with 100 mg Cd/kg diet. H&E, X 400.



**Figure 3.** Vacuolar degeneration in the liver of male Japanese quail fed with 100 mg Cd/kg diet. H&E, X 400.



**Figure 4.** Necrosis (arrows) and swelling (head arrows) in the renal tubular epithelial cells in kidneys of male Japanese quail fed with 100 mg Cd/kg diet. H&E. X400.



**Figure 5.** Hyaline cast in the renal tubules (head arrow) and renal tubule necrosis (arrow) in kidneys of male Japanese quail fed with 100 mg Cd/kg diet. H&E. X400.

### Alteration in Function of Liver and Kidneys

The level of total protein, globulin, AST and ALT increased while albumin concentration decreased (Table 3).

### Antioxidants Activity and Lipid Peroxidation

The activity of enzymatic antioxidants

(SOD and CAT) and non-enzymatic antioxidant (GSH) significantly decreased ( $P < 0.05$ ), while lipid peroxidation increased notably in the liver and renal tissues of Japanese quail in the Cd group ( $P < 0.05$ ) (Table 4 and 5).

**Table 3.** Impact of Cd on function of the liver and kidneys of male Japanese quail.

Groups	Total protein(g/dl)	Albumin (g/dl)	Globulin (g/dl)	Alb/Glob	AST (U/L)	ALT (U/L)	ALP (U/L)	BUN (mg/dl)	Creatinine (mg/dl)
Control	3.18 ± 0.04 <sup>b</sup>	1.17 ± 0.03 <sup>b</sup>	2.01 ± 0.2 <sup>b</sup>	0.58 ± 0.3 <sup>b</sup>	12.66 ± 1.05 <sup>b</sup>	20.46 ± 2.33 <sup>b</sup>	1303.3 ± 30 <sup>a</sup>	3.05 ± 0.05 <sup>b</sup>	66 ± 2 <sup>b</sup>
Cd	3.81 ± 0.26 <sup>a</sup>	0.85 ± 0.02 <sup>a</sup>	2.96 ± 0.15 <sup>a</sup>	0.28 ± 0.5 <sup>a</sup>	16.24 ± 8.28 <sup>a</sup>	25.88 ± 2.8 <sup>a</sup>	1452.04 ± 40.02 <sup>a</sup>	4.14 ± 0.25 <sup>a</sup>	1.53 ± 0.03 <sup>a</sup>

Distinctive letters in the similar column demonstrate statistically significant difference ( $P < 0.05$ )

**Table 4.** Effect of Cd on levels of SOD, CAT and GSH and lipid peroxidation in the hepatic tissue of male Japanese quail.

Group	SOD u/gr tissue	CAT u/gr tissue	GSH mgr./gr tissue	Lipid peroxidation nmol/gr tissue
Control	4.62 ± 0.02 <sup>b</sup>	5.63 ± 0.02 <sup>b</sup>	56.32 ± 2.26 <sup>b</sup>	36.6 ± 0.23 <sup>b</sup>
Cd	3.52 ± 0.12 <sup>a</sup>	3.53 ± 3.69 <sup>a</sup>	42.34 ± 1.08 <sup>a</sup>	54.03 ± 2.3 <sup>a</sup>

Distinctive letters in the similar column demonstrate statistically significant difference ( $P < 0.05$ )

**Table 5.** Effect of Cd on levels of SOD, CAT and GSH and lipid peroxidation in the renal tissue of male Japanese quail.

Group	SOD u/gr tissue	CAT u/gr tissue	GSH mgr./gr tissue	Lipid peroxidation nmol/gr tissue
Control	3.53 ± 0.22 <sup>b</sup>	4.56 ± 0.03 <sup>b</sup>	46.24 ± 4.04 <sup>b</sup>	46.6 ± 1.33 <sup>b</sup>
Cd	2.62 ± 0.33 <sup>a</sup>	2.22 ± 0.4 <sup>a</sup>	33.45 ± 2.08 <sup>a</sup>	66.02 ± 3.34 <sup>a</sup>

Distinctive letters in the similar column demonstrate statistically significant difference ( $P < 0.05$ )

## DISCUSSION

Cd is introduced for the most part by means of utilization of polluted water and food. The biological half-life of the metal is lengthy (twenty years) and has an impact on the renal system and liver [5]. We observed that the low body weight increased in the quails of the Cd group. Lisunora et al. [1] and Sant Ana et al. [3] observed weight loss in quails exposed orally to 2ppm Cd in feed for 60 days and 100 ppm Cd in purified water for 28 days, respectively. Alteration in the intestinal mucosa and weight loss in chickens exposed to dietary Cd reported by Teshfam et al. [20]. Cigankova et al. [15] observed a harmful effect of Cd on histology and electron microscopy structure of the small intestine epithelium of Japanese quail. The damaging effects of Cd on weight gain were possibly related to its toxic effects on nearly every system in the animal's body [21, 22].

Our result indicated a significant increase ( $P<0.05$ ) in Cd condensation in the kidneys and liver of the Cd group compared with the control group. The kidney, prior to liver, is the first organ where Cd is accumulated [2, 6, 7]. Cd is accumulated and deposited in different organs of Japanese quail when administered orally [1, 23]. Cd has a higher concentration in kidney than in the liver in broilers chicken when fed with dietary Cd [24]. Holoveska et al. [25] observed a considerable growth in Cd levels in the liver of turkeys exposed to Cd.

"Cd accumulation in the liver and kidneys of quail dietary exposed to this metal resulted histological changes of these two organs " [26]. Holovska et al. [25] observed purported alterations in the liver of turkeys in Cd groups, for example, hyperemia, dilation of the sinusoid, the gathering of inflammatory cells, and sporadic necrotizing hepatocytes. Hesaraki et al. [27] reported swelling in epithelial cells, necrosis in renal tubules, and hyperemia in kidneys of broiler chicken fed with a diet containing Cd. Binkowski et al. [28] observed congestion, fatty change in hepatocytes, necrosis of single hepatocytes, leukocyte infiltration in the liver, swelling and necrosis in renal tubules and epithelial cells, and congestion in the kidneys of wild living mallards and coots with the notable concentration of lead and Cd. Uyanik et al. [13] reported degeneration in hepatocytes and proliferation of kupffer cells in the liver as well as degeneration of the epithelium of renal tubules and hyaline casts in the lumen of

the kidneys tubules of broiler chicken exposed to dietary Cd.

"During chronic exposure, Cd-induced toxicity in liver and kidneys is dependent on hepatic and renal Cd concentration". "The source of Cd intake is mostly food, and most of the metal that is absorbed after oral exposure mainly accumulates in the liver and kidney, where it induces production of metallothionein a low molecular-weight protein that binds Cd with high affinity"[2]. The metallothionein shaping a compound with Cd to decrease the toxic possibility of Cd. Once the binding capacity of metallothionein is saturated, the multiplied level of free Cd ions begins procedures that may lead to cellular injury [29]. Cd operates kupffer cells that enforce the inflammation and ancillary hurt in the hepatic [30]. "It is thought that injured hepatocytes release a Cd-MT complex in to the blood, which is then filtered in the kidneys through glomeruli and reabsorbed by proximal tubule epithelial cells, which are the target for extracellular Cd-MT"[2].

In this study, the increase in ALT, AST, BUN and creatinine could result in damages to the liver and kidney as upheld by the pathological detection. The alteration in biomarkers of liver and kidneys indicate abortive function of liver and kidneys [13, 31, 32]. "Nephro-hepatotoxicity is a widely recognized effect of Cd that has been attributed to accumulation of the metal and formation of superoxide radicals in these organs "[21]. Hence, the albumin amounts decreased, higher levels of globulin can raise the total protein. Uyanik et al.[13] noted a higher serum total protein and higher levels of globulin, AST and ALT in the broiler chicken orally exposed to Cd. Swapana et al. [32] and Bharavi et al.[24] observed a higher activity in the serum ALT, BUN, and creatinine in broiler chicken exposed to dietary Cd. San Ana et al.[3] noted higher serum levels of ALT and AST and no change in ALP and creatinine in quails orally exposed to a dilution of 100 mg CdCl<sub>2</sub>/L distilled water for 28 days.

Our results showed a diminishing in the activities of SOD, CAT, and glutathione in the hepatic and kidneys of the Cd group compared to the control group. The quantity of MDA, a marker of tissue lipid peroxidation, markedly increased in the liver and kidneys of quails exposed to Cd. This suggests increased production of reactive oxygen species. The reactive oxygen species

assault the cell membrane and lead to destabilization and disintegration of the cell membrane as an outcome of lipid membrane peroxidation [33,34]. The production of reactive oxygen species and the ability to repair and detoxify the mechanisms of the body determine the level of cell damage in cells under stress and heavy metals [35,36]. Antioxidant enzymes decay the oxidative radical-prompted response and protectively affect the adjustment and metabolic process in cells that keep the advance of oxidation damage and hypoxia. Superoxide dismutase is noticed as the primary line guard against the harmful impacts of oxygen radicals within the cells. The suppressor effects of CdCl<sub>2</sub> on SOD result perhaps from the contest between Cd, Zn, or Cu, which is the requirement for SOD activation [37]. Catalase, as a hem protein, protects the cell against oxidative stress by catalyzing the degradation of H<sub>2</sub>O<sub>2</sub> to water and O<sub>2</sub> [34]. The decline in catalase function by Cd might be ascribed to the diminished absorption of iron, an essential trace element required for the action of catalase. The concentration of GSH was reduced in the Cd group, which indicates the attachment of Cd to SH groups of glutathione [24].

Alteration in the level of antioxidants (SOD, CAT, and GSH) in the hepatic and renal tissues of quails in the Cd group indicates the discharge of cellular antioxidants and enhances the generation of oxygen free radicals, agitating lipid peroxidation. This is demonstrated by enhancing the condensation of MDA in tissues of liver and kidneys in quails exposed to Cd [9, 38, 39].

Bharavi et al. [24] reported an increase in SOD and CAT levels in erythrocytes, a decrease in GSH levels in liver and kidneys tissues, and an increase in lipid peroxidation in hepatic and kidneys of broiler chicken exposed to dietary Cd at 100 mg/kg feed for 28 days. Kant et al. [40] observed a higher amount of SOD and CAT, higher lipid peroxidation, and reduction in GSH in red blood cells of poultry orally exposed to 400mg CdCl<sub>2</sub>/L for 20 days. Erdogan et al.[21] reported an increase in the plasma levels of MDA as an indicator of lipid peroxidation and lower activity of blood SOD in broiler chicken exposed to oral Cd at 200mg/L for 6 weeks. Holoveska et al. [25] reported no changes in lipid peroxidation and no increase in SOD activity in the liver of turkeys (after 71 days of oral exposure to Cd, aqueous solution, 0.5 mg/kg feed). Jihen et al. [38]

reported higher MDA level and lower SOD and CAT activities in the liver of rat (after 35 days of oral exposure to Cd at 200mg/L). The difference between the results of this study and those of other studies could be related to dose, duration, and route of Cd exposure as well as different animal species.

It is realized that the interaction of Cd with organelles of cell, including mitochondria, endoplasmic reticulum, and microsomes brings about the generation of free radicals and lipid peroxidation in the cell membranes [41,42]. An increase in lipid peroxidation and other consequences caused by free radicals damage liver and kidney tissues as revealed by changes in the function and histology of liver and kidneys.

## CONCLUSION

Cd induces histological and functional changes in the liver and kidneys of Japanese quails. Our study also demonstrated that oral exposure to Cd leads to lipid peroxidation and exhaustion in enzymatic and non-enzymatic antioxidant activities. We finally found that the weakening of cellular antioxidant following the exposure to Cd plays an important role in nephro-hepatotoxicity in Japanese quail.

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