Original Article

The Effect of Cadmium on the Ultrastructure and Metallothionein Levels in the Liver and Kidneys of Japanese quail

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ABSTRACT

Background: The aim of this study was to use Japanese quail as an animal model to evaluate the effects of cadmium (Cd) on the ultrastructure and the activity of metallothionein (MT) in the liver and kidneys.

Methods: One hundred male Japanese quails were randomly divided into two Cd and control groups in 2015. The first group received 100 ppm Cd for 60 days in their feed. The ultrastructural changes of the liver and kidneys of Japanese quails were examined by the transmission electron microscope and the concentration of MT in these organs was measured.

Results: The ultrastructural alternations of the liver included distension of rough endoplasmic reticulum (RER), mitochondrial swelling and lack of cristae, nuclear chromatin compression, and margination, the increased fat vacuole and damage to intercellular bindings. The kidneys ultrastructural alterations were mitochondrial swelling and damage to the cristae, the increased number of lysosomes, nuclear chromatin compression and margination, the decreased number of microvilli, and cell death. The concentration of MT and Cd in the liver and kidneys of the Cd group was significantly higher than that of the control group *(P*<0.05). A positive correlation was observed between the increased concentration of Cd and MT in the liver and kidney tissues.

Conclusion: The use of oral Cd caused an alternation in the ultrastructure and increased the concentration of Cd and MT in the liver and kidneys of Japanese quail.

Keywords: Cadmium, Japanese quail, Kidneys, Liver, Metallothionein, Ultrastructure.

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INTRODUCTION

Industrial and agricultural activities are actually one cause of environmental cadmium (Cd) pollution. This heavy and toxic metal enters the body through food, water, and air and causes acute cellular and tissue damage to the body [1,2]. Itis mainly absorbed by oral administration mostly accumulated in the kidneys and the liver. Cd has deleterious effects on vital organs such as the liver, kidneys, the immune system and testicles [3, 4]. It also leads to congenital malformation and cancer [5]. Liver and kidneys are organs targeted by Cd toxicity; therefore, it is important to understand the response of these organs to this metal [3,4].

Animal models play an important role in the studies dealing with the effects of environmental pollutants. Birds are increasingly used in studies on the harmful effects of metal toxins [6, 7]. The Environmental Protection Agency of the United States (US EPA) considers the Japanese quail as an appropriate animal model to be used for assessing the effects of ecosystem pollutants [3]. The study of ultrastructural alterations of the liver and kidneys was used for monitoring the environmental contamination by heavy metals [7, 8].

Metallothioneins (MTs) are low molecular weight proteins rich in the amino acid cysteine, which reduce the cell toxicity of heavy metals by binding to them [9,10]. MT is an important biomarker in monitoring the pollution of the environment and living organisms by Cd [11]. The aim of this study was to investigate the ultrastructural alternations of

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the liver and kidneys and the effect of oral Cd on the MT levels in these organs.

MATERIALS AND METHODS

One hundred 30-d old male Japanese quails (*Coturnix coturnix japonica*) were randomly divided into two Cd and control groups in 2015. The birds of the control group were fed a commercial diet. The quails of the Cd group received a commercial diet plus 100 ppm Cd-chloride (Cdcl2, Merck) for 60 d. The selection of Cd dosage was based on the results of our previous study [12].

At the beginning and the end of the experiment (60^{th} d), we dissected 15 quails from each group and collected their liver and kidneys specimens to examine them using the electron microscope and determine the concentration of Cd and MT. The birds were kept under favorable breeding conditions.

The Research Ethics Committee of the Islamic Azad University approved the study.

The liver and kidneys specimens were fixed in glutaraldehyde 2.5%, preparing with common methods, were subsequently examined and photographed using the transmission electron microscope (Philips EM 208). The Cd concentration was measured using the atomic absorption spectrometry (UNICAM939) [6]. The MT levels in the liver and kidneys were measured using a silver-saturation method [13].

The data were analyzed using statistical software SPSS (Chicago, IL, USA).The maximum acceptable error was considered less than 0.5. In this case, two-way ANOVA and Tukey Multiple Comparison Test were used *(P*<0.05).

RESULTS

No significant difference *(P*<0.05) was observed in the concentration of Cd and MT in the liver and kidneys of the Cd and control groups on the first day of the experiment. On the 60th d, Cd and MT concentration was significantly higher (*P*<0.05) in the liver and kidneys of the Cd group than in the control group (Table 1 and 2). A positive correlation was observed between Cd accumulation and MT levels in the liver and kidneys. The concentration of MT was substantially higher in the liver of the Cd group that in the kidneys of that group.

The liver and kidneys of Cd- exposed quails have undergone ultrastructural alternations. The ultrastructural alternations of the liver included distension of RER, mitochondrial swelling and lack of cristae, nuclear chromatin compression and margination, the increased fat vacuole and damage to intercellular bindings (Figures 1-3). The kidneys ultrastructural alterations were mitochondrial swelling and damage to the cristae, the increased number of lysosomes, nuclear chromatin compression and margination, the decreased number of microvilli, and cell death (Figures 4-6).

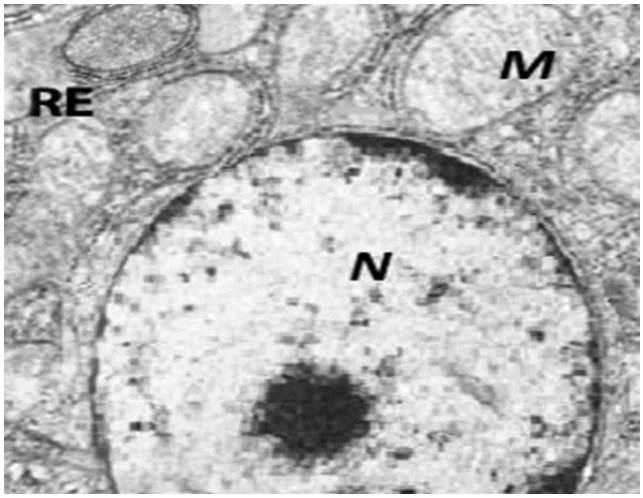

Figure1. TEM micrograph of Japanese quail liver from control group. Nucleus(N), mitochondria(M), RER (RE). (×2000)

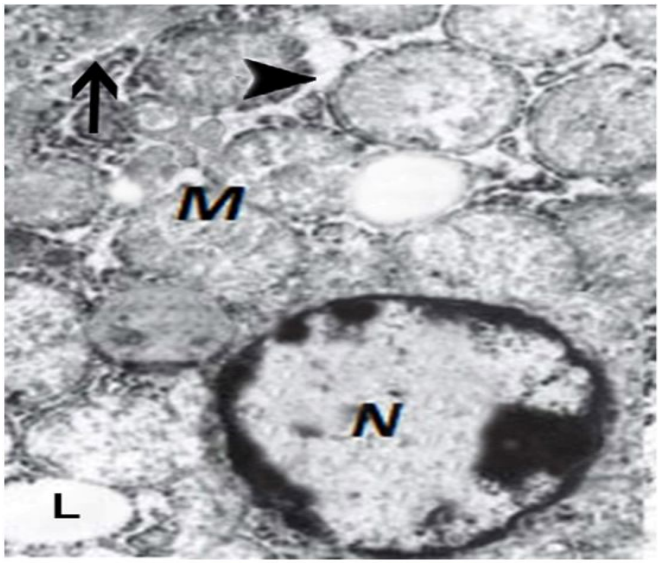

Figure2. TEM micrograph of Japanese quail liver from cadmium group. Nuclear chromatin condensation and margination(N), swollen mitochondria(M), lipid vacuole(L), dilated RER (arrow head), dilated intercellular space(arrow). (×15000)

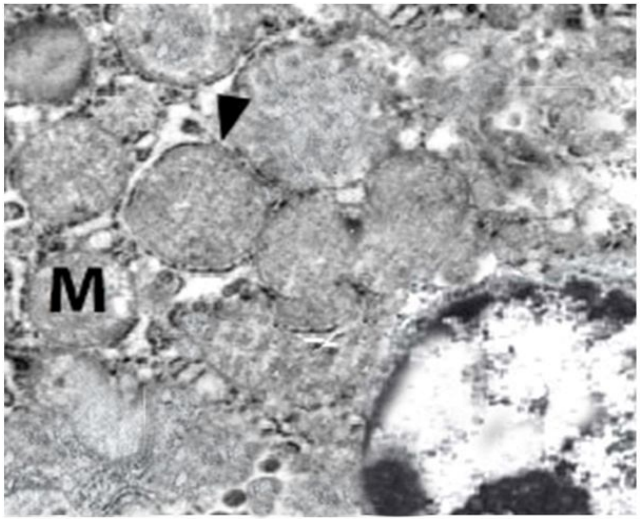

Figure3. TEM micrograph of Japanese quail liver from cadmium group. Nuclear chromatin condensation and margination (N), swollen mitochondria (M), dilated RER (arrow head).(×14000)

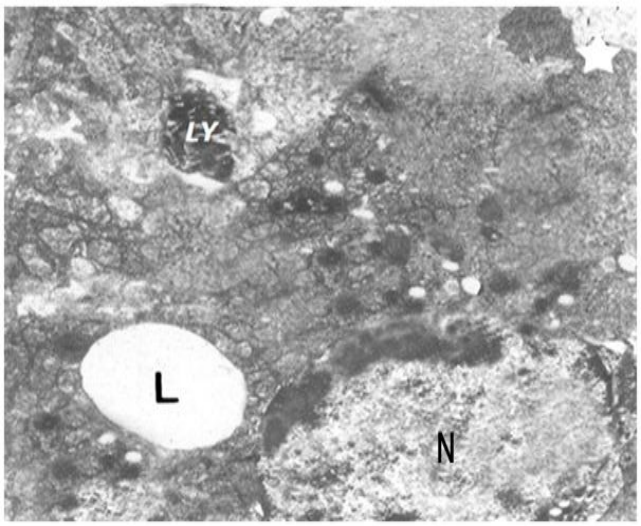

Figure5. TEM micrograph of Japanese quail kidney from Cd group. Nuclear chromatin condensation and margination (N), lysosome(LY), loss of microvilli (asterisk), lipid vacuole (L). (×14000)

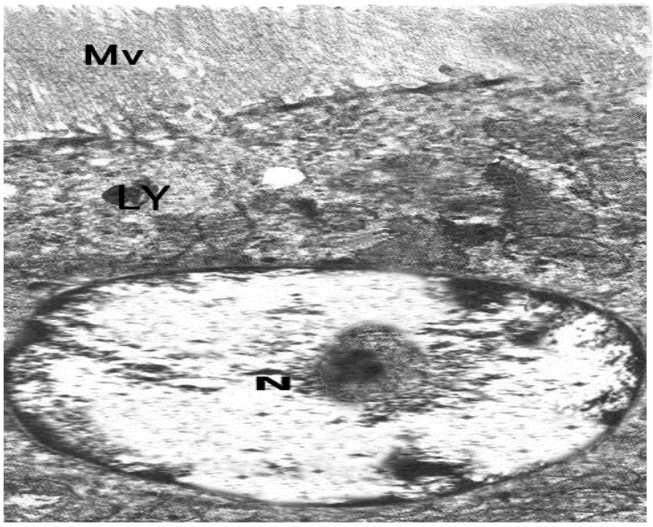

Figure4. TEM micrograph of Japanese quail kidney from control group. Nucleus (N), mitochondria (M), lysosome(LY), microvilli (Mv). (×12000)

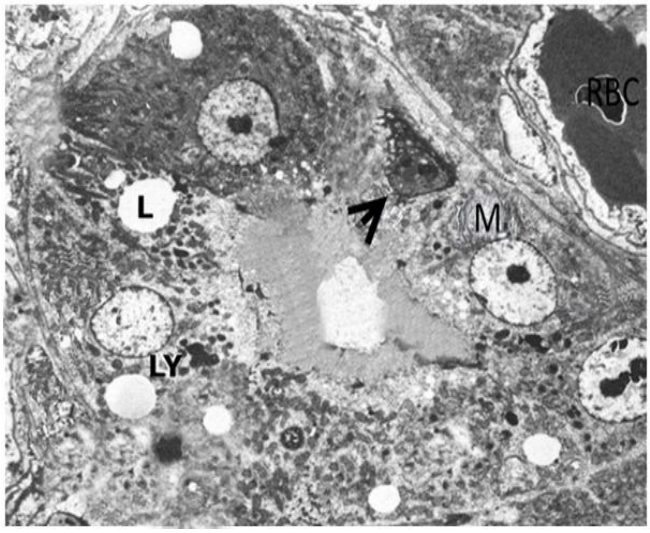

Figure6. TEM micrograph of Japanese quail kidney from Cd group. Lysosome(LY), lipid vacuole (L), dilated mitochondria(M), dead cell(arrow), red blood cell(RBC). (×5000)

Table1. Effect of Cd on Cd and MT concentration (µg/g wet weight) in the liver of Japanese quail.

Groups	Initial Cd concentration	Initial MT concentration	Final Cd concentration	Final MT concentration
control	0.31±0.01[a]	13.07 ± 3.1[a]	0.36±0.11[b]	14.02±2.08[b]
cadmium	0.39±0.02[a]	14.26± 2.96[a]	5.11±0.08[a]	115.36±10.35[a]

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

Table2. Effect of Cd on Cd and MT concentration (µg/g wet weight) in the kidney of Japanese quail.

Groups	Initial Cd concentration	Initial MT concentration	Final Cd concentration	Final MT concentration
control	0.41±0.012[a]	10.07±2.3[a]	0.51±0.03[b]	12.01±1.45[b]
cadmium	0.36±0.02[a]	9.8±1.65[a]	7.9±0.02[a]	75.22±8.06[a]

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

DISCUSSION

Our results showed that Cd and MT concentrations in the liver and kidneys of the Cd group was significantly higher *(P*<0.05) than the control group. The increased concentrations of Cd and MT in the liver and kidneys of oral Cd-exposed Ringed Turtle Doves [14] and Japanese quail [15] have been reported. A positive correlation between the concentration of Cd and MT in the kidney of Greater Flamingo [16] and the liver and kidney of Black-backed gulls [17] and Magpie [18] has been reported. MT leads to the accumulation of Cd in the liver and kidney. This protein has a capacity for binding to high molecular weight metal cations and protects the cell against the toxic effects of Cd [9,19]. The activity of MT in the liver and kidneys increases when the bird is exposed to heavy metals [14-17]. In our study, the Cd group had a lower rate of production of MT and higher accumulation of Cd in the kidneys than the liver. The lower production of MT in the kidneys is a potential reason why they are targeted when exposed to Cd [14, 20].

Ultrastructural alternations were observed in the liver and kidneys of Cd-exposed quails in the present study. Similar ultrastructural alternations have been reported in the studies conducted on birds [21-23]. Swelling of the mitochondria and damage to cristae, nuclear chromatin concentration, and margination, RER dilatation, the increased fatvacuole and destruction of intercellular bindings have been reported in the liver of oral Cd-exposed cockerels [21] and turkeys [22]. RER dilatation, the increased number of lysosomes, the increased fat vacuole, mitochondrial swelling, damage to cristae, and nuclear chromatin condensation and margination have been observed in the kidneys of dietary exposed cockerels [21] and broilers [23].

Oxidative stress plays a major role in the Cd toxicity. Cd causes oxidative stress by increasing the reactive oxygen species and interfering with the cell defense [12, 24, 25]. This metal crosses the cell membrane and combines with biomolecules, especially MT. After the MT binding capacity is saturated, a large amount of Cd ion is released into the cell so the metabolism and cellular activity is disrupted [18, 26]. Mitochondria are the target cellular organelle of Cd. The reactive oxygen species cause changes in the permeability of the mitochondrial membrane, destroy the respiratory tract and subsequently lead to the production of more reactive oxygen species [27]. Disruption of oxygen production and uptake of water and salts into the mitochondrial matrix cause mitochondrial swelling and the loss of cristae. The uptake of water into the RER leads to its swelling and destruction. This change is one of the symptoms of cellular damage and leads to cellular lysis by stopping the cellular activity [8,26].

Through the prohibition of DNA repair and methylation, Cd leads to the continuous inactivation of nuclear components and the consequent accumulation and margination of chromatin [5, 8]. Cd substitutes calcium in Cadherin-Eextracellular bindings and increases the space between hepatocytes by disrupting the intercellular bindings [18, 22]. The reduced production of protein in damaged cells leads to an increase in intracytoplasmic fat droplets. The MT-Cd complex of damaged hepatocytes is released into the blood stream and is absorbed into the proximal tubules after glomerular purification [9, 25]. The reduced microvilli of the renal tubule cells were observed in the present study. The accumulation of Cd in the urinary tract cells causes general impairment in renal reabsorption. Cd blocks the ion channels of the cell membrane, such as the calcium channel and the renal outer medullary potassium channel. It also blocks the activity of sodium-hydrogen exchanger in the toothbrush of the renal tubules. The interference of Cd with the channels and ion exchanges disrupts the regulation of cellular osmolarity [25,28]. The increased number of lysosomes is one common response to the exposure of cells to heavy metals. This change is indicative of the cell's efforts to digest Cd and is a general sign of cellular damage [18,26].

CONCLUSION

Oral Cd causes an alteration in the ultrastructure and the concentration of Cd and MT in the liver and kidneys of Japanese quail. These changes can be used to monitor the Cd environmental pollution.

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