

Adverse Effects of Cadmium on the Biochemical and Histopathological Profiles of Chicken Liver

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Abstract

Background: Cadmium is one of the heavy metals which have toxic effects on the various organs and systems of the body. Estimates demonstrate the increased prevalence of cadmium, particularly in industrial regions. In this study, we examined its harmful effects on the liver of chicken.

Forty chickens

Method: Forty chickens were purchased and were randomly divided into four groups. After collecting liver samples, the microscopic slides were prepared in laboratory. Also, the activities of catalase (CAT), superoxide dismutase (SOD) and the levels of malondialdehyde (MDA) were measured.

Results- The normal structure of liver was observed at control group, but hemorrhage and irregularity in the shape and distribution of hepatocytes in treatment groups were observed. The activities of CAT and SOD decreased significantly at treatment groups compared to the control group. Also, the level of MDA increased significantly at treatment groups compared to the control group.

Conclusion- The results showed the adverse effects of cadmium on the liver that consequently affects the overall function of body. Due to the growing use of cadmium, particularly in industrial regions, this study shows the impacts of cadmium on the birds of industrial regions and provides a warning for preventing its more outbreaks.

Keywords: Cadmium; Chicken; Liver; Oil Red; Catalase; Superoxide Dismutase; Lipid Peroxidation

Introduction

Cadmium is one of the harmful heavy metals and has great motility in soil, high water solubility and superior toxicity, even at low level [1]. As a result, air, soil and water as main environmental sections, are contaminated with cadmium [2]. Studies demonstrate the increased

prevalence of cadmium in industrial regions, resulting in its great accumulation in body, particularly in liver. Furthermore, it has been reported that cadmium leads to the toxic effects in the general population [3-6]. The transportation of cadmium from earth to the environment is caused via mining activity during the extraction of metals such as zinc, lead and copper [7]. The International

Agency for research on cancer has considered cadmium as a class I carcinogen [8]. Due to the resistant of cadmium, the successive release into the environment can lead to entering cadmium into the food chain [9]. It has been shown that the daily consumption of this heavy metal in the U.S is 30 µg and is remarkably higher in the China and Japan [10]. The general population is at risk of exposure and extensive poisoning among wildlife has been reported [11-13].

When the cadmium is absorbed by human body, its biological half-life would be more than 10 years [14]. Osteoporosis, renal dysfunction, diabetes, cancer, blood pressure and reproduction disorders are the results of cadmium exposure [15]. The renal dysfunction in the workers with blood cadmium level even below 10µg/l has been reported [16]. The increase in the excretion of low-molecular-mass proteins was shown as a result of cadmium exposure [17], but the impacts of cadmium on the liver of chicken has been partially studied.

Detoxification, secretion, oxidation and reduction are major functions of liver [18]. Also, liver as an important organ in metabolic pathways, plays a remarkable role in synthesis, metabolism and transport of carbohydrates, proteins and fats [19]. The liver of chicken has two lobes and is placed behind the heart. In this research, we study the adverse effects of cadmium on the histology and biochemical parameters of liver.

Materials and Methods

Animals and Experimental Procedures

Forty chickens were purchased and housed in the laboratory for three weeks which had the conditions like the environment. Then, they divided into four groups. The titles of the groups were Control, Treatment I, Treatment II and Treatment III. They fed as following:

- Control: Libitum feed and the mineral water.
- Treatment I: Libitum feed and 1 ppm cadmium in the water.
- Treatment II: Libitum feed and 5 ppm cadmium in the water.
- Treatment III: Libitum feed and 10 ppm cadmium in the water.

The chickens were kept in according to the instructions of National Institute of health that are applied for the laboratory animals [20]. In order to perform following steps of experiment, 3 chickens from each group were selected for sampling. Also, one repeat was performed for each experiment.

Histopathological Studies

This section was performed similar to our previous work [20]. Briefly, several phases should be performed to prepare microscopic slides. The chickens were anesthetized, and then the femur was separated. All samples were fixed in Bouin solution. Dehydration phases by reducing the level of the ethanol, clearing with xylene, and loading in the paraffin, sectioning and preparing microscopic slides were respectively done, and then the horizontal and perpendicular sections were stained by Hematoxylin and eosin (H&E) and oil red.

Homogenate Preparation

After separating liver, a section of liver was mixed with 10% NaCl solution with the ratio of 1:9. To remove cell debris, the liver homogenates were centrifuged at 10000 rpm for 10 min. Finally, the supernatant was used for determining the level of malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD).

The levels of MDA, CAT and SOD

The method of Ohkama [21] was used for measuring the level of MDA. Briefly, 1 mL of 10% trichloroacetic acid and 1 mL of 0.67% thiobarbituric acid were used and heated in boiling water for 30 min. The reactive substances of thiobarbituric acid were measured at 532 nm.

The method of Nishkimi, et al. [22] was used for measuring the activity of SOD. Briefly, this method is based on the ability of enzyme to inhibit the phenazine methosulfate-mediated reduction of nitroblue tetrazolium (NBT) dye.

The activity of CAT was measured spectrophotometrically at the wavelength of 405 nm according to the reaction of H₂O₂ with molybdc acid.

Statistical Analysis

ANOVA and Dunnet t-test were applied for statistical analysis. The results were assessed based on mean ± standard error mean (S.E.M). The significant differences were P < 0.05.

Results

Mortality rate: During the conduction of this experiment, no death was happened.

Histopathological examinations: The structure of liver was normal at the control group, but the hemorrhage and alterations in the shape and distribution of hepatocytes were occurred.

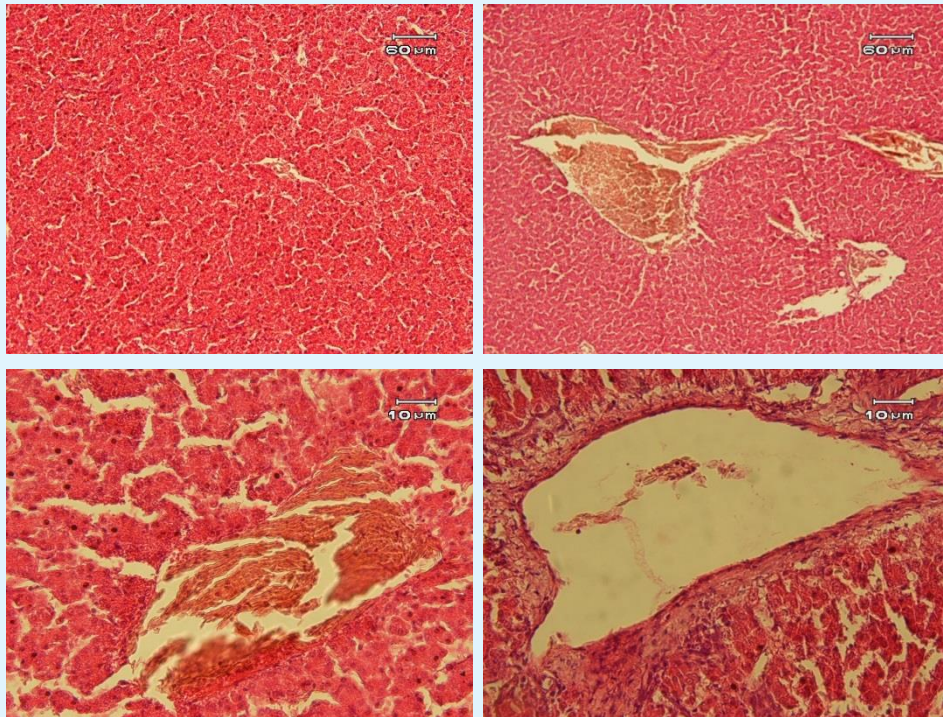


Figure 1: Up left: normal structure of liver; other pictures show the harmful effects of cadmium on the shape of hepatocytes and obvious hemorrhage (H&E).

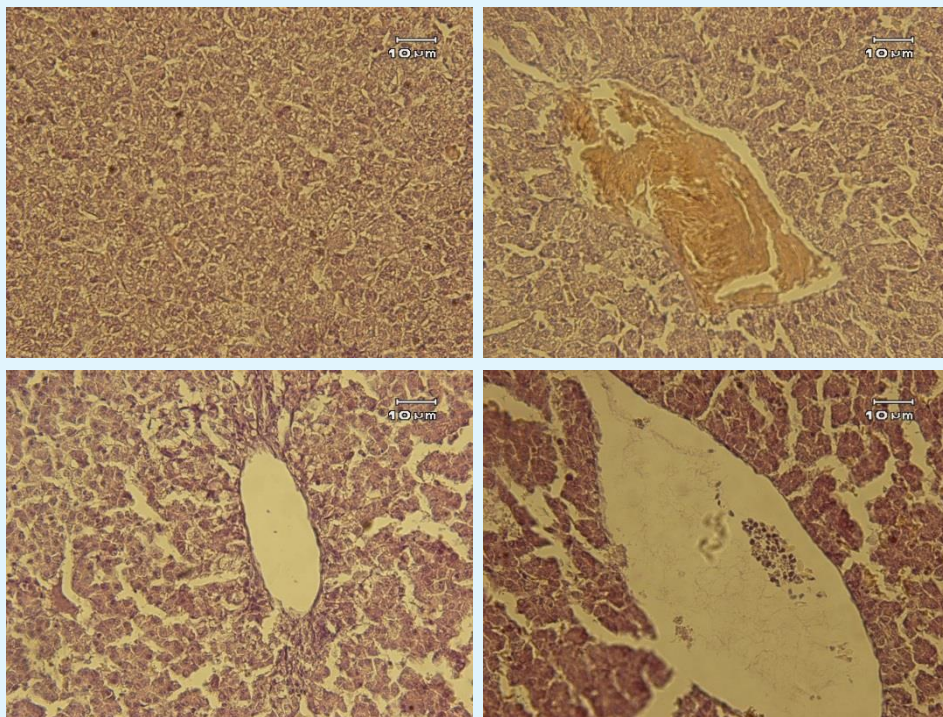
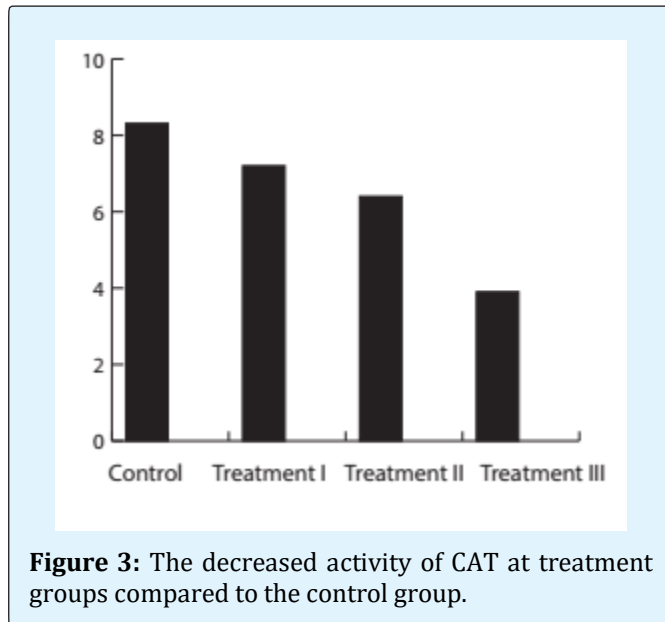


Figure 2: Up left: normal structure of liver; other pictures show the harmful effects of cadmium on the shape of hepatocytes and obvious hemorrhage (Oil red).

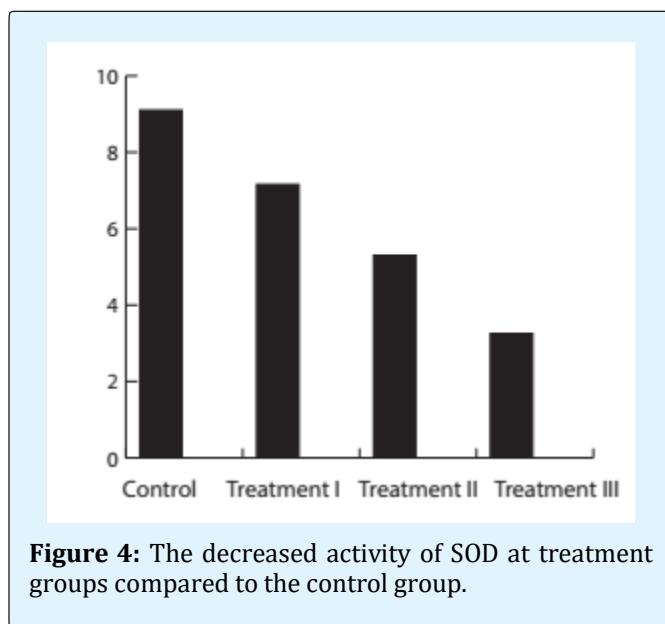
Activity of CAT

The activity of CAT (U/mg protein) significantly decreased at treatment groups compared to the control group.



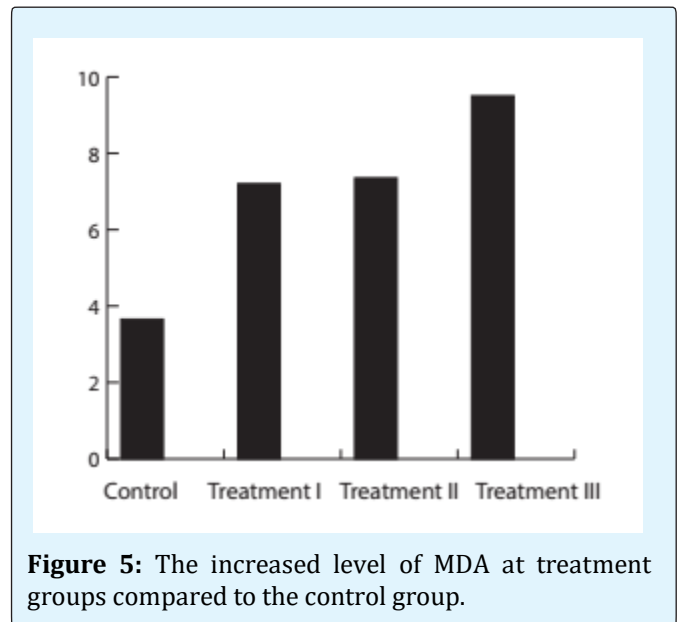
Activity of SOD

The activity of SOD (U/mg protein) remarkably decreased at treatment groups compared to the control group.



The Level of MDA

The level of MDA (nmol/mg) significantly increased at treatment groups compared to the control group.



Discussion

In the present study, we revealed that cadmium negatively affects liver of chicken as we demonstrated by hemorrhage and irregular shapes of hepatocytes. During recent years, much attention has been made towards the toxicity of agents and cadmium is one of them. Besides, there are much efforts to decrease or even inhibit the cytotoxic effects of cadmium or other potential toxic agents using natural or synthetic compounds [20,23-33]. In fact, this study provides a notice about the consumption of liver of chicken. Because of the frequency of cadmium in the water, chickens are exposed to the high levels of this toxic agent. Accumulation of high concentration of cadmium in the liver results in destructive effects on the hepatocytes which would harmfully affect the people who consume these livers. Zeinali and colleagues assessed the level of cadmium in the liver, meat and kidney of cow and sheep [34]. Their results showed that the concentration of cadmium in the meat, liver and kidney samples of sheep and cow collected from Birjand, a city located at southeast of Iran, is high and notably, cadmium level in cow kidney had the highest carcinogenic rate, requiring warning about their consumptions, particularly by children. Hence, researchers have focused on finding and developing

strategies to reduce the adverse effects of cadmium. In a study conducted by Ren and coworkers, the impact of glutathione against cadmium-induced liver oxidative stress was examined [35]. It was found that exposing to cadmium remarkably enhances the levels of aspartate aminotransferase, xanthine oxidase, γ -glutamyl transpeptidase and α -smooth muscle actin, whereas it decreases the activities of antioxidant enzymes including superoxide dismutase, glutathione peroxidase and catalase. Glutathione administration exerted potential therapeutic effects in terms of reducing cadmium uptake, restoring the activities of oxidative enzymes, activating NF- κ B and preventing excessive reactive oxygen species [3] production. Interestingly, exposing to a combination of harmful agents may lead to more destructive effects. In a study, the effect of a combination of lead and cadmium on the rat blood, liver and kidney was examined [36]. Their results revealed that exposure to a combination of cadmium and lead produces more pronounced effects compared to the single metals. Dubey and colleagues investigated the protective effects of probiotic *Lactobacillus pentosaceus* GS4 on the liver toxicity caused by chronic cadmium exposure [37]. Biochemically, it was shown that GS4 remarkably binds to the cadmium, resulting in its decreased accumulation in liver and enhanced fecal evacuation. Histopathologically, GS4 effectively reduced cadmium-induced toxicity in gut by decreasing lymphocyte infiltration and reduction of hyperplasia. Duan and colleagues examined the ameliorative impact of vitamin E and metallothionein (MT) in cadmium-induced liver injury in *Ctenopharyngodon idellus* [38]. It was shown that cadmium exposure results in liver poisoning, so that cadmium enhances hepatocyte apoptosis, the concentration of malondialdehyde, and apoptosis-related gene mRNA transcription expression. Importantly, VE and MT administration effectively exerted hepatoprotective impacts through reducing cadmium contents, lipid peroxidation and histopathological damage as well as decreasing the percentage of hepatocyte apoptosis.

High generation of oxygen radicals or dysfunction of antioxidant enzymes lead to the oxidative stress [22]. SOD is one of the components of antioxidant defense which responds to the oxidative stress. Its role is the conversion of highly toxic superoxide anions into H_2O_2 . Our results revealed that cadmium at the doses of 1, 5 and 10 ppm results in the decreased activity of SOD.

CAT is considered as a primary enzyme that catalyzes the conversion of H_2O_2 into H_2O [39]. Our results showed the decreased activity of CAT at the doses of 1, 5 and 10 ppm. This decreased activity is maybe due to releasing free iron which catalyzes ROS generation, starts lipid

peroxidation and elevates redox imbalance in the liver samples.

Lipid peroxidation is an important contributor of the loss of cell function under oxidative stress conditions [40]. Lipid peroxidation is a marker of oxidative damage. Lipid peroxidation leads to the ROS production and impaired antioxidant defense cannot effectively scavenge them. Our results showed that exposing to cadmium increases the level of MDA.

There are concerns about the outbreak of heavy metals. Ageing, cigarette smoking and high exposure to environmental pollution increase the levels of cadmium in the blood [41]. It has been shown that intraperitoneal injection of cadmium at a large dose of 3.9 mg/kg results in severe damage in liver of rats [42]. In a study, after culturing rat hepatocytes in vitro, inhibitory impacts of cadmium were observed on the albumin secretion [43]. We showed that cadmium results in hemorrhage, hyperemia and irregular shapes of hepatocytes. Our results are in agreement with other investigations that demonstrated similar effects on the liver tissue. Cadmium causes impairments in the integrity of membranes, generates ROS and involves cytotoxic and inflammatory mediators. Cadmium exerts its destructive effects via two pathways: direct and indirect. In direct pathway, it causes injury in the hepatocytes that is called primary injury. This injury is produced by binding of cadmium to sulfhydryl groups on critical molecules in mitochondria. Oxidative stress, alterations in mitochondrial permeability and mitochondrial dysfunctions are results of inactivation of thiol groups. In the indirect pathway, it acts through inflammation. This injury is the result of ischemia induced by endothelial cells damage.

Conclusion

In summary, exposing to cadmium results in adverse effects on the liver tissue, as we demonstrated by increased level of MDA (increased level of oxidative stress), decreased activities of CAT and SOD, and destructive effects on the structure of liver tissue.

Conflict of Interest: The authors declared no potential conflict of interest with respect to the research, authorship and/or publication of this article.

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