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Sub-chronic ascorbic acid supplementation accelerates reduction of liver cadmium levels in broiler chicken

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ABSTRACT

Environmental exposure to toxic heavy metals remains one of the main serious global health concerns. The objective of this study was to determine if daily supplementation with ascorbic acid for 4 weeks could lower chicken liver cadmium concentration after intentionally exposing hens to cadmium sulfate for 1 week. Normal mix-breed adult broiler chickens (N = 24) were used in this study. The baseline liver cadmium concentration was determined using a graphite furnace atomic absorption spectrophotometer. Each chicken then received 200 mg/kg/day cadmium sulfate and 500 mg/kg/day ascorbic acid mixed with water and feedstuff for 1 week and liver cadmium concentrations were determined. A group of chicken (n = 6) received 500 mg/kg/day ascorbic acid and another group (n = 6) did not receive ascorbic acid. The baseline of liver cadmium level was 4.63 (± 0.41) µg/g. At the end of week 1, the liver cadmium concentration significantly increased to 60.9 (± 40.1) µg/g (p-value < 0.01). Daily supplementation with ascorbic acid for 4 week lowered the liver cadmium concentration to 20.2 (± 13.7) µg/g (p-value < 0.05). Supplementation with ascorbic acid could accelerate reduction of cadmium concentration in poultry liver after intentional exposure.

Keywords: Ascorbic acid, broiler chicken, cadmium, liver, pollution.

INTRODUCTION

Environmental exposure to toxic heavy metals remains one of the main serious global health concerns. Cadmium is one of the non-essential heavy metals that is widely distributed in our environment. This toxic heavy metal can naturally be found together with lead, copper, and zinc (1). Cadmium is widely utilized in various industrial processes as a coloring pigment, anticorrosive agent, neutron-absorber, and in the production of nickel-cadmium batteries (2). Recent industrialization and urbanization has increased cadmium concentrations in different regions of the world. According to some estimates, cadmium level in soil used in agriculture increases by 0.2% per year in Scandinavia for example (1, 2). Similar to lead, cadmium is believed to accumulate in plants and grains grown on soils contaminated with cadmium (3). In Vietnam for example, rice grown on contaminated soil was reported to contain cadmium in a concentration as high as 0.31 mg/kg (4).

People are increasingly exposed to cadmium as a result of cigarette smoke, welding, and consumption of contaminated foodstuffs and drinks (5). Recently, there has been an increasing interest in assessing foodstuffs for heavy metals and other contaminants like microorganisms (6, 7). Cadmium levels were assessed in poultry meat, beef, pork, and fish products in Eastern Europe, Saudi Arabia, Finland, Russia and Iran (8-13). Absorption from the gastrointestinal tract after consumption of contaminated foodstuffs and beverages is a major site of resorption of cadmium into the human body. Even at low concentrations, cadmium is known to be toxic. Cadmium was shown to have deleterious consequences on the respiratory system, kidneys, reproductive system, bones, in addition to its potential to cause cancer (1,

5).Poultry meat and eggs are major sources of nutrients as proteins, vitamins, and minerals in the diet of the Palestinians (6). The Palestinian Central Bureau of Statistics reported that the average household monthly consumption of poultry meat and eggs was 17.3 kg and 3.7 kg, respectively (14). Beef, pork and chicken livers are widely consumed by different nations and many folk meals contain animal liver. Although poultry liver can be an important source of components necessary for normal body growth and development, cadmium-contaminated livers can be an important source of human exposure to cadmium. Currently, there is no consensus on a standard treatment of cadmium toxicity (5). The Agency for Toxic Substances and Disease Registry reported that there is no evidence of efficacy of EDTA in case of cadmium toxicity (15). The agency recommends supportive treatment like fluid replacement, supplemental oxygen, and mechanical ventilation for victims of cadmium toxicity as specific antidote for acute cadmium exposure does not exist (15). The literature reported conflicting reports on the efficacy of using chelators like Ethylenediaminetetraacetic acid (EDTA), Dimercapto-1-propanesulfonic acid (DMPS), and meso-2,-3-dimercaptosuccinic acid (DMSA) to reduce the body burden in case of cadmium toxicity (5). While EDTA was shown to increase urinary excretion of cadmium, these results were shown in animal models and unfortunately human studies were merely anecdotal (5, 16, 17).Recently, some dietary strategies were suggested as alternatives to chelators which could be effective and affordable, in addition to their less toxic potential (3). Ascorbic acid was previously used as chelating agent with antioxidant activity (18-20). As heavy metals are also known to increase the burden of reactive oxygen species, the use of a chelating agent with antioxidant activity might reduce the deleterious effects of these harmful reactive oxygen species (18, 19). Recently, we have shown that ascorbic acid was able to lower blood lead levels in broiler chicken (6). While previous studies focused on the potential protective effects of ascorbic acid against the histopathological damages related to the oxidative stress caused by cadmium, there is a lack of experimental work on the effects of ascorbic acid on the concentration of cadmium in chicken liver. The objective of this study was to assess the effects of ascorbic acid on the concentration of cadmium in broiler chicken liver after intentional exposure to a concentrated source of cadmium.

MATERIALS AND METHODS

Design

The present study was conducted on 24 normal mix-breed adult broiler chickens. Chickens weighing between 1.8 – 2.2 kg were brought from raisers in Tulkarm region. Chickens were housed in cages separately in a specially designated poultry housing facility of An-Najah National University, Tulkarm campus.

Baseline levels

Chickens were given 1 week for acclimatization. During this period, chickens had access to water and feed *ad libitum*. After 1 week of acclimatization, 6 chickens were slaughtered and their livers were extracted for baseline cadmium determination.

Week 1: cadmium and ascorbic acid treatment

Following the acclimatization period, the remaining chickens were given 200 mg/kg/day cadmium sulfate and 500 mg/kg/day ascorbic acid mixed with water and feed for 1 week. The amounts of cadmium and ascorbic acid used in this study were based on a previous investigation on lead (6). At the end of this period, 6 chickens were slaughtered and their livers were extracted.

Weeks 2-5: with or without ascorbic acid

The remaining chickens were divided into two groups of 6 each. A group continued to receive ascorbic acid 500 mg/kg/day for 4 weeks to investigate if ascorbic acid would help lower the concentration of cadmium in the liver. To investigate if the reduction in cadmium resulted from ascorbic acid treatment or as a result of another detoxification mechanism, the other group of chickens did not receive any treatment during

the 4 weeks period. The decision to assess the effects over a period of 4 weeks was based on a preliminary investigation involving lead acetate (6). Chickens in both groups were slaughtered and their livers were extracted for cadmium level determination.

Ethical considerations

Currently, there is no Institutional Animal Care and Use Committee (IACUC) at An-Najah National University. The Institutional Review Board (IRB) of An-Najah does not review protocols relevant to animal studies. In the US, broiler chickens are exempt from the Humane Slaughter Act. The current study was in compliance with the international regulations on animal care and use. The surgical and tissue collection procedures were performed by a veterinarian who was licensed to practice in Palestine. The vital signs were noted continuously during the study.

Quantification of cadmium

The anodic stripping voltammetry technique was used to determine cadmium concentrations in the liver samples. In this study, we used a polarograph (POL150) coupled with polarographic stand electrode (MDE 150) to quantify cadmium levels. Before use, all glassware and crucibles were incubated in 10% HNO_3 overnight to prevent adsorption of cadmium onto surfaces of the containers. Cadmium concentrations were determined against a calibration curve in a similar matrix. All chemicals used were obtained from Sigma Aldrich and were of analytical grade.

To determine cadmium levels, 10 g wet liver samples were cut and dried at 110 ºC for 5 hr. Then 0.5 g of each dried sample was burned in a crucible with 2 mL of H_2O_2 and HNO_3 (ratio 1:1) at 600 ºC in muffle furnace for 2 hr. The produced ash was grounded and soaked in 10 mL 20% HNO_3 solution for 30 min. The mixture was diluted to 100 mL in volumetric flask. Samples were then filtrated for analysis (21, 22). The limit of detection was in the range of 2.0 to 10.0 µg/L and the limit of quantification was in the range of 5.0 to 10.0 µg/L as previously described (22).

Statistical analysis

Data were entered into IBM SPSS (IBM). Normality of distribution was assessed using the Kolmogorov–Smirnov test. The baseline cadmium concentration, increase in cadmium concentration after 1 week of cadmium sulfate and ascorbic acid treatment, decline in cadmium following 4 weeks of ascorbic acid treatment, and without treatment with ascorbic acid were compared using ANOVA with multiple comparisons. Statistical analysis was done in GraphPad Prism v.6.0 (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was considered * when the *p*-value was < 0.05 and ** when the *p*-value was < 0.01.

RESULTS

Figure 1 shows liver cadmium concentrations at baseline, after 1 week of exposure to cadmium, and after 4 weeks of treatment with ascorbic acid. Following the acclimatization period, the baseline liver cadmium concentration was 4.63 (± 0.41) µg/g. At the end of week 1, the liver cadmium concentration significantly increased to 60.9 (± 40.1) µg/g (*p*-value < 0.01).

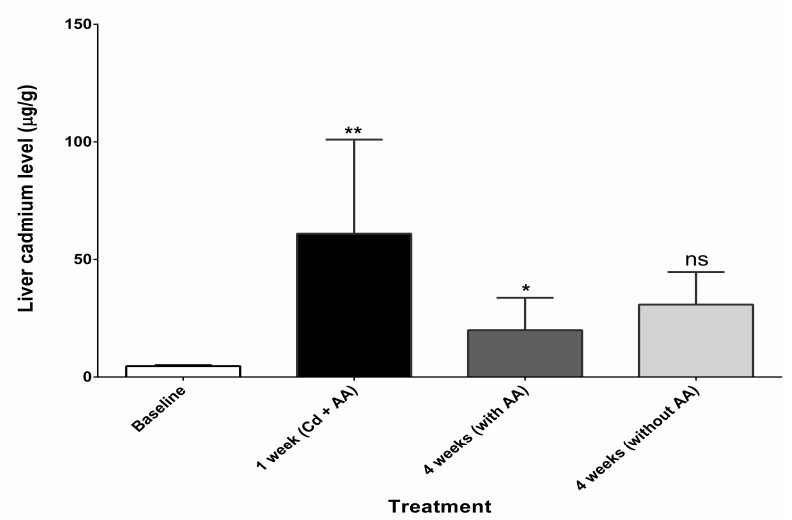

Figure (1): Baseline liver cadmium concentration, following exposure to cadmium sulfate (200mg/kg/day) and ascorbic acid (500 gm/kg/day) for 1 week, and 4 weeks with supplementation with or without daily ascorbic acid (500 mg/kg/day). Statistical significance was considered * when the *p*-value was ≤ 0.05, ** when the *p*-value was ≤ 0.01, and ns when the *p*-value was > 0.05.

DISCUSSION

In this study, we have shown that daily supplementation with high dose ascorbic acid lowers liver cadmium levels in broiler chicken after intentional exposure to a concentrated source of cadmium. The findings extend those previously reported on the effects of daily supplementation of ascorbic acid on lead levels in broiler chicken (6). These findings are particularly interesting as currently there is no specific antidote for cadmium to be administered in case of acute exposure. Although still controversial, the use of EDTA and other classic chelators was not shown to be effective (15).

In this study, we have used ascorbic acid which is a vital nutrient that can easily be obtained from diet. Additionally, this agent is available in pharmacies as supplements. Our findings are unique as we are reporting for the first time that ascorbic acid can significantly lower cadmium levels in the liver of broiler chicken. In congruence with our results, it was reported that ascorbic acid deficiency is associated with increased risk of cadmium and lead toxicity (23, 24). Ascorbic acid is an antioxidant that is capable of scavenging free radicals which decrease lipid peroxidation. In rats, ascorbic acid attenuated damages caused by oxidative stress and reduced the histopathological alterations caused by cadmium in the lungs and brains of the tested animals (20). Ascorbic acid was also reported to possess chelating activities which were effective to chelate and lower lead levels in similar way to EDTA (6, 25, 26). Combing ascorbic acid with vitamin E was also shown to reduce spermatogenesis damages related to oxidative stress in mice exposed to cadmium (18). Similarly, this combination was shown to protect steroid production in rats exposed to cadmium (19). The present findings thus confirm the protective effects of ascorbic acid against cadmium induced damages.

In this study, we have used a group of mixed-breed adult broiler chickens. These chickens were similar to those raised by farmers and owned for domestication by families (6). We have shown that liver cadmium levels increased significantly from the baseline levels after 1 week of exposure (Figure 1). These findings suggest that cadmium was absorbed from the intestine of the hens. It is highly likely that chickens grazing on grains contaminated with cadmium will absorb cadmium which will then accumulate in their livers. Consumption of such livers would expose the human consumers to cadmium. In a study conducted in Finland, livers of cow, heifer, and pig contained cadmium in

concentrations of 66 µg/kg, 36 µg/kg, and 21 µg/kg, respectively (13). The same study showed that beef, pork and chicken contained cadmium below the detection limit of the analytical method used. In Germany, the average blood cadmium level was reported as 0.5 µg/l and the maximum permissible value for workers was 15 µg/l (1). On average, a German citizen has a daily intake of about 30-35 µg of cadmium, of which, about 95% are taken up from contaminated foods and drinks. It has been reported that an average smoker would have an additional 30 µg of cadmium per day. Taken together, our results suggest that supplementation with ascorbic acid might lower the burden of cadmium following exposure. This was in line with previous studies which reported that daily supplementation with ascorbic acid lowered blood lead levels in smokers (26).

The new findings should be explained considering a number of limitations. First, the number of chickens used in each treatment group was relatively small. Using a larger sample number would have provided more robust results. Second, in this study a graphite furnace atomic absorption spectrophotometer was used to quantify cadmium levels. Using other methods, such as coupled plasma mass spectrometry, could have provided more precise concentrations. Finally, cadmium has not been quantified in tissues other than liver. Future studies are needed to quantify cadmium levels in different poultry tissues.

CONCLUSION

In conclusion, poultry liver contaminated with cadmium could present a potential source of exposure. In this study, daily supplementation with ascorbic acid lowered cadmium levels in poultry liver. Supplementing feedstuff with ascorbic acid could be a strategy to consider to lower cadmium in environmentally exposed flocks. Further studies are still needed to determine if residuals of citrus fruits containing ascorbic acid can lower cadmium levels in environmentally or intentionally exposed flocks.

CONFLICT OF INTRESTS

The authors report no conflicts of interest in this manuscript

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