Histopathological Study on Induced Eeffects of Cadmium on Liver, Gizzard and Kidney of *Larus argentatus*

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Abstract: The study was carried out on the effects of cadmium (Cd) on liver, gizzard and kidney of *Larus argentatus*. Significant alterations were observed after the treatment of high and low dose of cadmium chloride injected to *Larus argentatus*. Investigation of the effects of Cd on body tissues was done by sectioning of fixed tissue by paraffin section techniques. Major changes were noted at high dose of cadmium chloride in liver, kidney and gizzard. While, under low dose of cadmium chloride, mild to moderate changes in hepatocytes around blood vessels in liver; reduction in thickness of epithelium and muscular layer in gizzard; and slight alterations in arrangement of tubules of loop of henle in the kidney of proximal convoluted tubules with granular cytoplasm were observed. No lesions were found in the mentioned body tissues of *Larus argentatus* of control group.

Keywords: Cadmium, Liver, Gizzard, Kidney, *Larus argentatus*, histopathology.

INTRODUCTION

The pollution of the natural environment by the toxicity of metals is a worldwide concern, as the consequences of metals on the environment can be a critical threat for the security of ecosystems and living organisms. According to the age, sex, mass, weight, and feeding routine, birds at the top of food web, may add a large amount of metals in their organs [1]. Thus there is a serious danger to the fauna and flora due to bioacccumulation, long persistence and biomagnification in the food chains of these pollutants [2]. According to the age, sex, mass, weight, and feeding routine, birds at the top of food web, may get accumulation of a large amount of metals in their organs [1]. The use of biological pointer, birds can provide evidence of the natural environment. Aquatic birds are visible and prominent, widespread, and nourish at different trophic levels, and many birds are long lived [3,4]. Thus, they have extensively been used as biomonitors of environmental contaminants such as metals. The cadmium (Cd) occurs in nature at low concentrations, but human activities have caused Cd contamination in all continents with the exception of Antarctica [5]. In birds, the uptake of metals can lead to a diverse problems such as smaller clutch size and nestling mortality and lesions in kidney, liver or intestinal organs, and induction of reactive oxygen species [6-8]. Histopathological examination of kidneys from environmentally exposed certain species of sea birds have revealed morphological changes of chronic interstitial nephritis [5].

The amount of Cd in animals increase with age even if these animals live in areas that are not contaminated with Cd [9]. It is because of the long biological half life of Cd. After absorption, cadmium (Cd) is circulated in blood. It primarily distributed to the liver and then redistributes progressively to the kidney as Cd-metallothionein (Cd-MT). After distribution, 50% of the total body burden is reported to be found in the liver and kidney [10]. In living organisms Cd was shown to be toxic to all tissues and toxic effects of Cd include morphological and functional damage in hepatic and renal tissues [11, 12].

The work that was reported earlier on marine birds in Pakistan, was restricted to the population studies and very little on pesticides determination but with reference to present study it is the first attempt. The present study was conducted to determine the effects of Cd on the body tissues (liver, gizzard and kidney) of herring gulls (*Larus argentatus*) that were collected from selected coastal area of Hawks bay, Karachi, Pakistan. It is expected that this research will provide the information about the effects of heavy metals on birds.

MATERIALS AND METHODS

The experimental work was carried out on adult herring gulls (*Larus argentatus*). Alive gulls were

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caught with the help of local people from selected areas of Hawks bay, coast of Karachi, Pakistan. Collected gulls were kept in cages in the Lab, Department of Zoology, University of Karachi, Karachi. The gulls were fed with tap water and bread pieces. Nine herring gulls (*Larus argentatus*) were kept in cages in three groups' i.e. Group A, B and C for 15 days before the treatment. Each group had three birds for high and low dose, respectively. Whereas, three birds were kept in group C as control.

After 15 days, stock solution was prepared by liquefy 1 g cadmium chloride in 100 ml distilled water. To study the different types of effect, two concentrations of cadmium chloride were applied, i.e. 0.0004 g Cd/0.04 ml and 0.0002 g Cd/0.02 ml were injected in the subcutaneous abdominal region of gulls by using insulin syringe. The effects of Cd were observed after 8 hours of the treatment because because life span of all treated birds was not more than 8 hours.

To observe the effects of Cd on treated gulls the liver, gizzard and kidney tissues were fixed in 10% Formalin. Histological study of liver, gizzard and kidney was carried out by sectioning of fixed tissue by paraffin section technique.

For histopathological study, the techniques of [13] were implemented. Then photomicrographs were obtained at X4, X10 and X40 by Olympus BX41 microscope with assessment of Olympus DP12 Camera.

RESULTS

In this study the experimental work was performed on adult *Larus argentatus*. The gulls were treated by two concentrations of Cd. (0.0004 g Cd/0.04 ml and 0.0002 g Cd/0.02 ml). After 8 hours of the treatment samples from liver, gizzard and kidney of *Larus argentatus* were examined.

It was found that under high dose of cadmium chloride (0.0004 g Cd/0.04 ml) the rigorous degenerative alterations in the hepatic cords and disruption in the sequence of cells around the blood vessels was observed in liver of *Larus argentatus*. The cells were so much deranged that they were difficult to recognize and their nuclei cannot be seen (Figure **1**).

The histopathological variations were observed as a result of low dose of cadmium chloride (0.0002 g Cd/0.02 ml) in the liver of *Larus argentatus*. Mild to moderate alterations in hepatocytes around the blood vessels were noted. Although the cells were moderately deranged but all of them contain nuclei and were seem to be radiating away from the central vein (Figure **2**). The untreated *Larus argentatus* of group-c showed no remarkable changes in liver cells (Figure **3**).

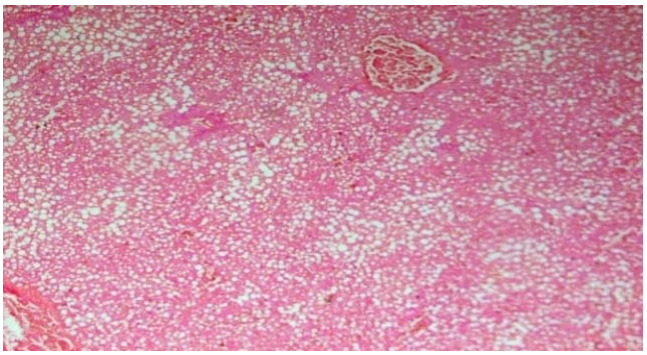

Figure 1: The liver section of treated *L. argentatus* showing rigorous degenerative alterations in the hepatic cords, deranged cells with unseen nuclei (X10).

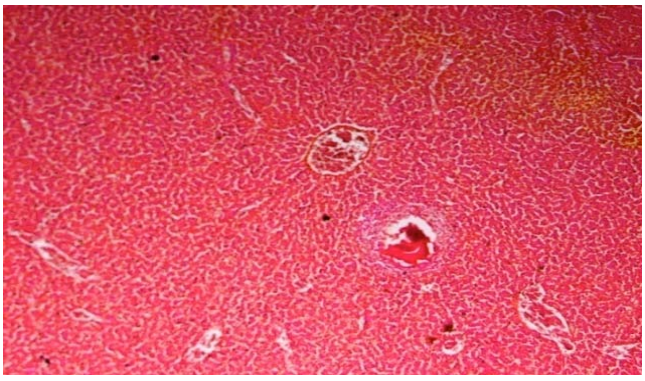

Figure 2: The The liver section of treated *L. argentatus* showing variations in hepatocytes around blood vessels and unbalanced cells (X10).

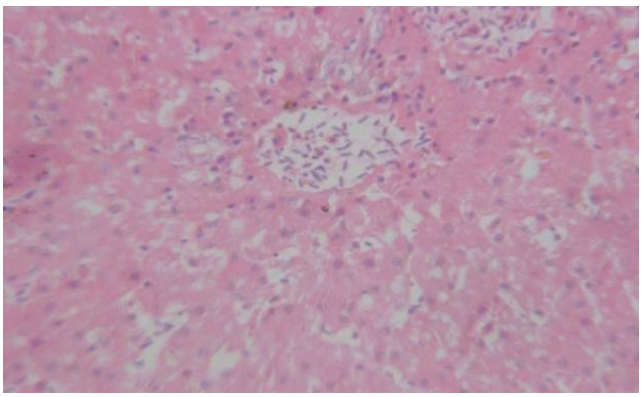

Figure 3: The liver section of control *L. argentatus* showing normal structure of cells (X40).

The effect of high dose of cadmium chloride (0.0004 g Cd/0.04 ml) on gizzard of *Larus argentatus* showed

severe damage to mucosa with deranged epithelium and most of cells were observed without nuclei. The muscular layer was also noted thin (Figure **4**).

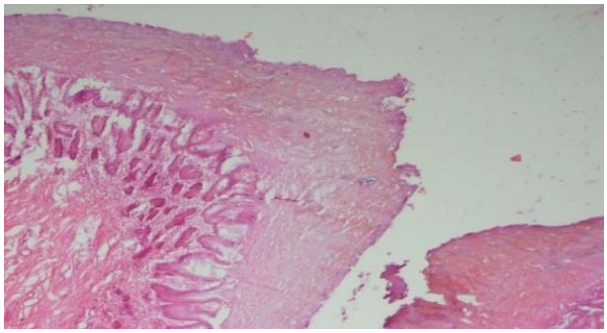

Figure 4: The gizzard section of treated *L. argentatus* showing severe damage to mucosa with deranged epithelium and most of the cells are without nuclei (X10).

In the case of low concentration of cadmium chloride (0.0002 gm Cd/0.02 ml) on gizzard of *Larus argentatus* the histopathological study confirmed the severe disruption of lining epithelium of gizzard with thinness of lining cells. The thickness of epithelium was reduced as well as the muscular layer was also reduced (Figure **5**). While untreated *Larus argentatus* gizzard exhibited normal morphology of mucosa and lining epithelium. The normal arrangement of different muscular layers was observed (Figure **6**).

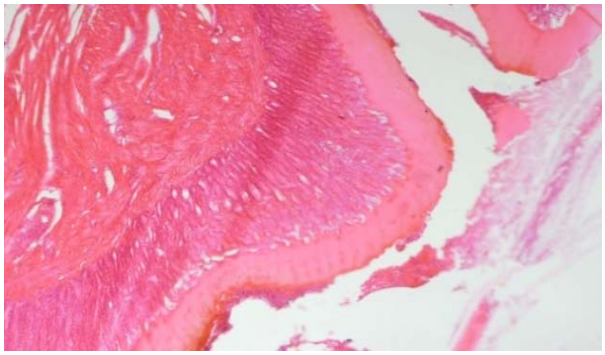

Figure 5: The gizzard section of treated *L. argentatus* showing severe disruption of lining of epithelial cells (X10).

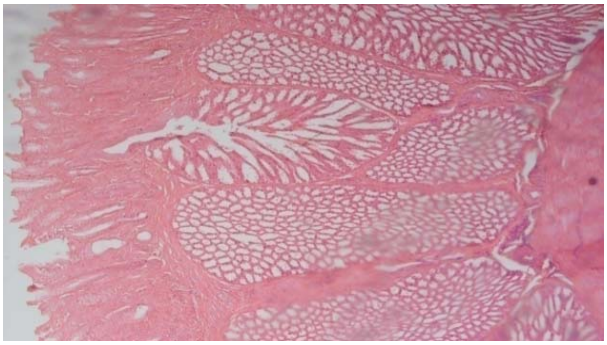

Figure 6: The gizzard section of control *L. argentatus* showing normal structure of cells (X4).

The effect of high dose of cadmium chloride (0.0004 g Cd/0.04 ml) proved obvious architecture of tubules with marked alterations in loop of henle and reduction in tubular cells around glomeruli in kidney of *Larus argentatus*. A number of vacuolated spaces in between the tubules were appeared (Figure **7**).

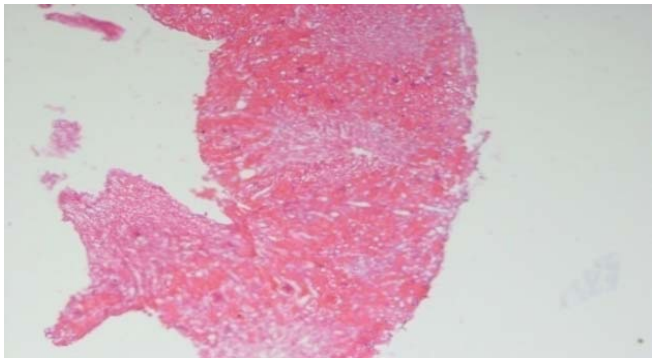

Figure 7: The kidney section of treated *L. argentatus* showing marked alterations in the loop of henle and reduction in tubular cells around glomeruli (X10).

The effect of low dose of cadmium chloride (0.0002 gm Cd/0.02 ml) explained slight alterations in the arrangement of tubules of loop of henle in kidney of proximal convoluted tubules with granular and swollen cytoplasm (Figure **8**) whereas, untreated kidney cells of *Larus argentatus,* were appeared to be as normal cells (Figure **9**).

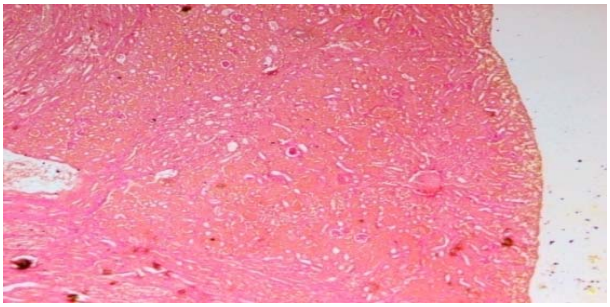

Figure 8: The kidney section of treated *L. argentatus* showing alterations in arrangement of tubules of loop of henle and granular cytoplasm (X10).

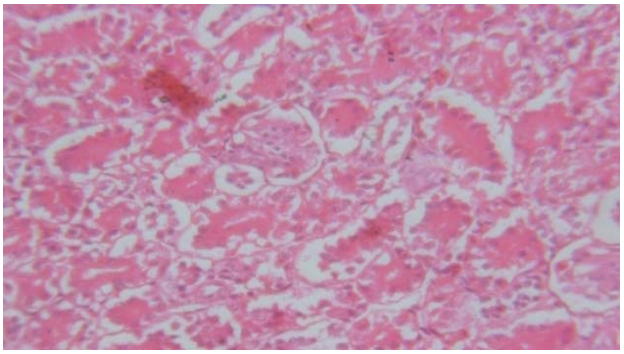

Figure 9: The kidney section of control *L. argentatus* showing normal structure of cells (X40).

DISCUSSION

Various toxic effects of Cd include morphological and functional changes in hepatic and renal tissues, testicular necrosis and changes in gastrointestinal tract [11,14-16]. In present study, untreated Herring gulls did not show remarkable changes in hepatic cords and cells around the blood vessels in liver of *Larus argentatus* (Figure **3**) but in high dose 0.0004 g Cd/0.04 ml (Figure **1**) and low dose 0.0002 g Cd/0.02 ml (Figure **2**) showed severe lesions and mild to moderate variations in hepatocyte around blood vessels, respectively.

The effects of dietary Cd on development of young chickens were observed. 700 ppm Cd treated birds showed a low basophilia and cloudy swelling in liver cells [17].

The histopathological effects of Cd on liver of broiler chickens, Pekin ducks and growing Pheasant (*Phasianus colchicus*) were also observed [18-22]. Mild to severe dystrophy in the form of hepatic necrosis was found in the H and E stained sections of liver of embryo of mallard [23]. Present findings show the same results in liver tissue (Figures **1** and **2**).

The epithelial cells of gizzard of 700 ppm Cd treated young chickens were hypertrophied and contained enlarged nuclei and mucosa was thickened with mononuclear cells [17]. In present work, the histopathological consequences of high dose of cadmium chloride (0.0004 g Cd/0.04 ml) showed severe damage to the mucosa of gizzard with deranged epithelium and most of the cells came in to view without nuclei (Figure **4**). The severe disruption of lining of epithelium of gizzard with thinness of lining cells was observed. The thickness of epithelium and muscular layer was found reduced (Figure **5**).

The pathological variations including comprised swollen granular cytoplasm, cloudy swelling and degenerating nuclei in epithelial cells of renal tubules were observed in Japanese quail (*Coturnix coturnix Japanica*) and in young chickens after the introduction of 75 mg/Kg Cd and 700 ppm Cd, respectively [17,24]. Slight to severe kidney lesions were found in mallard ducks (*Anas platyrhynchos*) fed 200 ppm Cd and mallard ducklings fed 20 ppm of Cd [25-27]. In present findings, high dose of 0.0004 g Cd/0.04 ml (Figure **7**) showed arrangement of tubules with marked alterations in the loop of henle and reduction in the tubular cells around glomeruli. Large number of vacuolated spaces in between the tubules was also found. Marked ultra structural changes contained swollen mitochondria and damaged nuclei in kidney of cockerels after treating with cadmium chloride [18]. Sub lethal effects of Cd intake (50 and 450 ppm Cd) on kidney of mallard ducks were studied [24]. Kidneys were relapsed in broiler chickens after Cd administration and severe kidney lesions were also found in Cd treated Pekin ducks (*Anas platyrhynchos domestica*) [19-21]. In present work, low dose 0.0002 g salt/0.02 ml of cadmium chloride (Figure **8**) in *Larus argentatus* illustrated slight alterations in the architecture of the tubules of the loop of henle. The ultra structure of kidney of Pekin ducks exposed to Cd and degenerative changes in proximal tubules were found [28]. The present study showed scattered groups of proximal convoluted tubules with granular cytoplasm and with swollen granular cytoplasm in *Larus argentatus* (Figure **8**). The effects of ascorbic acid on Cd induced oxidative stress and performance of broiler and toxicity of cadmium, evaluation of body weight, hepatic and renal functions, and cellular immune response in Japanese quail was investigated**.** The results showed reduced body weight and induced hepatic toxicity while kidney functions were not affected by Cd exposure [7, 29]. Cd induced enteropathy in domestic cocks: a biochemical and histological study after sub chronic exposure was investigated and histological data indicated that cadmium chloride causes an increase in number of goblet cells and granular lymphocytes in intestinal mucosa [8]. The relationship between Cd accumulation and histopathological changes were examined in the kidney and liver of magpies (*Pica pica*) from Zinc smelter area. There was positive correlation between the concentration of Cd (2.2-17.9 µg/g) and histopatholo-gical alterations (interstitial inflammation and tubular cell degeneration) in the kidneys. Contrary, liver accumulation of Cd was low (0.88-3.38 µg/g) and no association between hepatic Cd and histopathology was observed [30]. In present study histopathological variations were observed as a result of low dose of cadmium chloride mild to moderate alterations in hepatocytes around the blood vessels were noted whereas, under high dose of cadmium chloride the rigorous degenerative alterations in the hepatic cords and disruption in the sequence of cells around the blood vessels was observed in the liver of *Larus argentatus*.

Histopathological and ultra structural changes of kidney were investigated in response to cadmium chloride toxicity in broiler chickens. Renal histopathology explained swelling, degenerative changes, necrosis and apoptosis in tubular epithelium [31]. Present study showed the same results in kidney

of herring gulls (Figures **7** and **8**). Internal organs such as liver, kidney, lungs and gizzard were severely affected at 750 mg/Kg dose of cadmium chloride in broiler chicks [32]. The effects of different levels of Cd on histological alterations and the rate of lymphoid cells apoptosis of bursa of Fabricius in broiler chickens were determined. Male Ross broiler were divided into four groups i.e Group 1,2,3 and administered with 25, 50, 100 ppm Cd, respectively while control group-C did not get Cd in histological findings, tissues were observed to be hypo cellular and some of them were edematous and in group-2 and 3, intra epithelial cysts were also found as compared to control group [33].

Histopathology of liver and kidney of wild living mallard (*Anas platyrhyncos*) and coots (*Fulica atra*) with significant concentration of Cd was observed. Histological modifications were noted in liver and kidney such as retrogressive changes and inflammation to leucocytes infiltration. The control group of birds was revealed by very small number of mentioned lesions [34]. However, in present study, the control group of *Larus argentatus* did not show any modification or lesions in liver, gizzard and kidney (Figures **3**, **6** and **9**, respectively). Cd induced hepatotoxicity was studied in chicken (*Gallus domesticus*). 150 mg cadmium chloride /Kg of diet was introduced to 100 days old chickens for 600 days. Histological and ultra structural changes in liver were found. Cd also reduced the activities of antioxidants super oxide dismutase (SOD) and increased lipid per oxidation (LPO) and nitric oxide (NO) production [35]. Path morphological changes were evaluated in various organs of broiler chicks administered with Cadmium Chloride at 250, 500, and 100 ppm, respectively in drinking water in group II, III, IV for six weeks while group-I was served as control. The lesions were dominated by degeneration, necrosis, cellular and vascular alterations. The main target organs were kidney and liver. Cd is hepatotoxic as well as nephrotoxic. Severe lesions were observed in group IV followed by group III and II. However, group-I disclosed normal appearance of kidney and liver [36]. The induced effects of lead, chromium, and cadmium on poultry bird *Gallus domesticus* were investigated. The histo-pathological study revealed that the induction of a high dose of these heavy metals (Pb, Cr and Cd) showed abnormalities of cells size and function, damage to cells and tissue of liver, kidney, intestine and brain [37].

In present study the results indicated that major changes were noted at high dose of cadmium chloride in liver, kidney and gizzard of *Larus argentatus*. While, under low dose of cadmium chloride, mild to moderate changes in hepatocytes around blood vessels in liver, reduction in thickness of epithelium and muscular layer in gizzard; and slight alterations in arrangement of tubules of loop of henle in the kidney of proximal convoluted tubules with granular cytoplasm were observed. No lesions were found in the mentioned body tissues of *Larus argentatus* of control group. The present results are in agreement with the earlier reports. On the basis of present findings it is concluded that effects of heavy metals on birds are harmful.

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