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Histopathological alterations in the liver and intestine of Nile tilapia *Oreochromis niloticus* exposed to long-term sublethal concentrations of cadmium chloride*

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Abstract Fingerlings of Nile tilapia *Oreochromis niloticus* were exposed to 1.68, 3.36, and 5.04 mg/L cadmium (as $CdCl_2$), which represent 10%, 20%, and 30% of their previously determined 96-h LC_{50}. After exposure for 20 days, sections of the liver and intestine of treated fish were examined histologically. Histopathological changes varied from slight to severe structural modification, depending on the exposure concentration. The hepatic tissues of fish exposed to 10% LC_{50} showed markedly increased vacuolation of the hepatocytes and coarse granulation of their cytoplasm. Abundant erythrocytic infiltration among the hepatocytes was observed in fish exposed to 20% LC_{50}. In the intestinal tissues of fish exposed to all doses, goblet cells proliferated and were greatly increased in size, the longitudinal muscularis mucosa was disturbed and, in the crypts of the sub-mucosal layer, apoptosis increased, indicated by large numbers of degenerated nuclei. Large numbers of inflammatory cells and dilated blood vessels were observed in the intestine of the group treated with 30% LC_{50}.

Keyword: fish; tilapia; pollutant; cadmium; liver; intestine

1 INTRODUCTION

Heavy metal pollutants have been reported in many aquatic organisms (Olojo et al., 2005). These contaminants build up in the food chain and are responsible for adverse effects including the death of organisms (Farkas et al., 2002). Fish are widely used to evaluate the health of aquatic ecosystems because their physiological changes can serve as biomarkers of environmental pollution (Younis et al., 2012). The Nile tilapia *Oreochromis niloticus* is one of the most frequently used freshwater fish in these kinds of toxicological studies (Figueiredo-Fernandes et al., 2006a, b; Abdel-Warith et al., 2011; Younis et al., 2013).

Cadmium (Cd) is non-essential for the normal biological functions of aquatic organisms although they may sometimes be exposed to high levels of this metal in the environment. Chronic sublethal exposure of fish to waterborne cadmium is known to lead to accumulation of the metal, especially in the kidneys, liver, and gills (McGeer et al., 2000). In addition, cadmium toxicity is reflected in effects on growth, reproduction, respiratory functions and osmoregulation (Pratap and Bonga, 1990).

When aquatic animals are exposed to toxic concentrations of cadmium, their organs may accumulate the element (Jalaludeen et al., 2012; Omer et al., 2012), which may then cause biochemical and morphological alterations, particularly in the liver, intestine, gills and kidney (Abdel-Warith et al., 2011; Jalaludeen et al., 2012; Younis et al., 2013).

The liver plays an important role in the metabolism and excretion of xenobiotic compounds and it is known that some toxic conditions can cause morphological alterations in the organ (Van Dyk et al., 2007, 2012; Abdel-Warith et al., 2011; Younis et

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al., 2013). Depending on the element and its concentration, the fish species, the period of exposure, and other factors, heavy metal exposures have been associated with both increases and decreases in the activities of hepatic enzymes, and with histopathological changes in hepatic tissues (Paris-Palacios et al., 2000; Van Dyk et al., 2007, 2012; Younis et al., 2012).

Monitoring histological changes in fish liver is a highly sensitive method of assessing the effects of xenobiotic compounds in field and experimental studies. However, the uptake of metals occurs mainly through the gills and intestinal epithelium (Mohamed, 2008) and histopathological alterations may also occur in these organs, related to the absorption of toxic metals (Hanna et al., 2005). The present study was carried out to investigate the harmful effects on the liver and intestine following long-term exposure of Nile tilapia *Oreochromis niloticus* to sublethal concentrations of cadmium chloride.

2 MATERIAL AND METHOD

2.1 Experimental fish

Fingerlings of Nile tilapia *O. niloticus* were collected from the fish-seed hatchery of King Abdulaziz City for Sciences and Technology, Mozahmiya, Riyadh, Saudi Arabia. Fish were acclimatized to laboratory conditions for 2 weeks prior to the commencement of the experiments. Water temperature was maintained at 28±1°C by thermostatically controlled heaters, and other water quality parameters were: pH 7.1–8.0, ammonia-N 0.07–0.20 mg/L, nitrite-N 0.15–0.35 mg/L, nitrate-N 4.35–5.77 mg/L, and dissolved oxygen 5.3–6.7 mg/L.

2.2 Experimental design

One hundred and sixty acclimatized fish weighing 28.33±1.12 g were divided into an unexposed control group and three treatment groups exposed to either 1.68, 3.36 or 5.04 mg/L Cd (as $CdCl_2$, purity 98%, Aldrich Chemical Company Inc., USA) for 20 days. These concentrations correspond to 10%, 20%, and 30% of the 96-h LC_{50} for Cd for juvenile *O. niloticus* (16.8 mg/L; Xu and Bai, 2007). Duplicate 80-L glass aquaria (100 cm×50 cm×40 cm) were established for each of the treatment and control groups. Fish were fed twice daily at a rate of 2% of body weight with a 32% crude protein diet. The physiochemical parameters of the water were similar to those during the acclimatization period. Mortalities in each group were recorded daily.

2.3 Histological examination

The livers and intestines of the control and treated fish were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin wax, and processed for routine histological evaluation. Sections (5 μm) were prepared and stained with hematoxylin and eosin, as described by Luna (1968) and Bernet et al. (1999). Histopathological changes were scored according to Dommels et al. (2007). A value of either (-) no change, (+) slight structural changes, (++) moderate structural changes and (+++) severe structural changes was assigned to each investigated section, and then converted to a numerical value for statistical analysis (0, 1, 2, 3, respectively).

2.4 Statistical analysis

Differences among the treatment groups in the scores of histopathological change were analyzed using one-way analysis of variance (ANOVA). Mean values are stated ± their standard deviations and significant differences (5% level) among means tested using Fisher's least significant difference test as described by Snedecor and Cochran (1989).

3 RESULT

3.1 Histopathological changes in the liver

Histopathological changes occurred in a concentration-dependent manner. Clear evidence of hepatic tissue damage was observed in tilapia exposed to 10% and 20% of the LC_{50} of Cd. The liver of these groups had lost its characteristic architecture and clearly exhibited an increased level of vacuolation of the hepatocytes, relative to that of the control fish (Fig.1a, b, d). Erythrocytic infiltration of the liver was more extensive in the 20% LC_{50} group (Fig.1b). In addition, the cytoplasm of the hepatocytes in this group was characterized by densely stained coarse pink granules, and vacuoles. Abundant erythrocytic infiltration was observed in the group exposed to 30% LC_{50} (Fig.1c). Table 1 shows the scores of the histopathological changes in the hepatic tissues of the different groups.

3.2 Histopathological alterations in the intestinal tissues

Cytological damage to the intestinal tissue,