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Pathological Investigations on Galilee Tilapia (*Sarotherodon galilaeus*) Following Chronic Exposure to Cadmium Chloride

Hossam G Tohamy[1] and Ramy M Shourbela[2]*

[1]*Department of Pathology, Faculty of Veterinary Medicine, Alexandria University, Egypt*
[2]*Department of Animal Husbandry and Animal Wealth Development, (Fish Breeding and Production) Faculty of Veterinary Medicine, Alexandria University, Egypt*

Abstract

The morphopathological alterations of chronic cadmium (Cd) toxicity in *Sarotherodon galilaeus* were studied. 126 fish were used to determine 96 hrs LC_{50} of cadmium chloride, the obtained result was 28.3 mg/L. Forty-eight fish were used to induce chronic toxicity, twenty-four fish were exposed to 2.83 mg/L cadmium chloride for 8 weeks and other fish as control. Gills, hepatopancreas, posterior kidney and spleen were the most affected organs during chronic exposure. Eosinophilic granular cells (EGCs) infiltration and goblet cells hyperplasia in tips of primary lamellae, lamellar lifting, lamellar necrosis and Proliferative interlamellar hyperplasia with fusion in gills, hydropic degeneration and necrosis of the hepatic cells, hemorrhage and necrotic renal tubules and splenic necrosis and depletion of lymphocytes were recorded as histopathological changes.

Keywords: Morphopathological; Cadmium; *Sarotherodon galilaeus*; Hyperplasia; Depletion

Introduction

Freshwater acquires contaminated with a large number of pollutants and it has become a matter of major concern allover the world [1,2]. Among pollutants and metals are of particular interest because of their varied effects and the range of concentrations which may cause toxic effects to fish [2]. Many studies are available that demonstrate heavy metal toxicity in fish [3,4]. Cadmium has no biological function in living organisms. It widely used in fertilizer, automotive, dye, plastics and synthetic fiber industries and battery production [5]. Even small amounts of this metal entered into aquatic systems, results in accumulation in various tissues, changes in metabolic, physiologic and biochemical parameters and death in sensitive species. Cadmium exposure may lead to the results of some pathophysiological damages in various tissues including liver [6], brain [7] and kidney [8].

One of the common and commercially important cultured tilapia species is *Sarotherodon galilaeus (S. galilaeus)* [9] but Limited information is available about the morphopathological alterations of chronic cadmium toxicity in this fish. However, some authors are applying some studies of cadmium toxicity on another tilapia species as in *Oreochromis niloticus* [10,11] and *Oreochromis mossambicus* [12]. More as there were no available references concerning the histopathological alterations of chronic cadmium toxicity in this economic species. Ultimately, more research is needed to determine 96 hrs LC_{50} of cadmium chloride in such species and morphopathological alterations of chronic cadmium toxicity of this species. So the aim of this study is to determine the LC_{50} of cadmium chloride and to assess the morphopathological alterations of chronic cadmium toxicity for *S. galilaeus.*

Materials and Methods

Experimental fish

Fish used for the experiments were collected from local lake and were acclimatized for two weeks before beginning of the experiments in glass aquaria (90 × 50 × 35 cm). These aquaria are supplied with chlorine-free tap water. Oxygen supply was maintained in each aquarium using an electric air pumping compressors. Apparently healthy *S. galilaeus* fish were selected for the study having a mean weight 41.4 ± 3.4 gm. The experiments were performed under natural light and ambient temperature (25 ± 1°C) and PH (7.3 ± 0.3). Fish were fed on a commercial fish diet containing 25% crude protein at 3% of body weight daily.

Cadmium chloride

Cadmium chloride (99% purity) was obtained from El-Nasr Chemical Company (Cairo, Egypt) and prepared in aquatic solution to provide the required concentrations of cadmium.

Determination of 96 hrs LC_{50} of cadmium chloride

126 fish (*S. galilaeus*) were used in this experiment. Fish were divided into seven groups having eighteen fish in each. These groups were exposed to 0, 15, 20, 25, 30, 35 and 40 mg/L cadmium chloride up to 96 hrs. Water and $CdCl_2$ were renewed daily. The calculation of LC_{50} is done according to the formula of Stephan [13].

Chronic cadmium chloride toxicity

Forty-eight fish (*S. galilaeus)* were randomly divided into 2 equal groups having twenty-four fish in each; one group as a control group (no cadmium chloride) and the other group exposed to $^1/_{10}$ (2.83 mg/L) of LC_{50} cadmium chloride that determined in the first experiment. The water and cadmium chloride were renewed daily for eight weeks. The clinical signs and mortality were recorded along the experimental period. Weekly necropsy was performed for 3 randomly selected fish from each group up to eight weeks.

Histopathological studies

Tissue specimens (gills, hepatopancreas, posterior kidney and

***Corresponding author:** Ramy M Shourbela, Department of Animal Husbandry and Animal Wealth Development, (Fish Breeding and Production) Faculty of Veterinary Medicine, Alexandria University, Egypt, Tel: +20 3 5921675; E-mail: ramy_aqua@yahoo.com

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Group	$CdCl_2$ mg/L	No. of fish	Number of dead fish					
			24 hrs	48 hrs	72 hrs	96 hrs	Overall death within 96 hrs	Mortality %
GP1	0	18	0	0	0	0	0	0
GP2	15	18	0	0	0	0	0	0
GP3	20	18	0	0	0	0	0	0
GP4	25	18	0	4	2	2	8	44.44
GP5	30	18	2	3	4	2	11	61.11
GP6	35	18	5	4	4	3	16	88.89
GP7	40	18	7	6	5	0	18	100

$LC_{50} = (AB)^{1/2}$
A = the minimum test material concentration that cause 100% death.
B = the maximum test material concentration that cause 100% viability

Table 1: Mortality pattern during the estimation of 96 hrs LC_{50}.

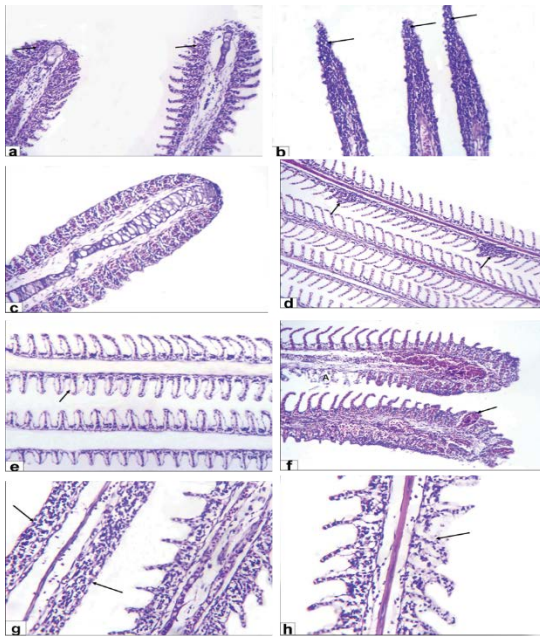

Figure 1: Photomicrograph of *S. galilaeus* gills exposed to 2.83 mg/L $CdCl_2$ and stained with HE. (a): Eosinophilic granular cells (EGCs) infiltration in tips of primary lamellae (arrows, X200) during 1st week. (b): Hyperplasia of the goblet cells in the tips of primary lamellae (arrows, X200) during 2nd week. (c): Filamentous clubbing (X200) during 3rd week. (d): Multifocal fusion of secondary lamellae (arrows, X200) during 4th week. (e): Lamellar lifting (arrow, X200) during 5th week. (f): Lamellar necrosis (A) and telangiectasis (arrow, X200) during 6th week. (g): Interlamellar epithelial hyperplasia with lamellar fusion (arrows, X400) 7th week. (h): Lamellar necrosis (arrow, X400) during 8th week.

spleen) were rapidly fixed in 10% formalin-saline solution. The fixed specimens were processed through the conventional paraffin embedding technique. Paraffin blocks were prepared, from which 5 microns thick sections were obtained. These sections were stained by Hematoxyline and Eosin (HE) [14].

Results

Determination of 96 hrs LC_{50} of cadmium chloride in *S. galilaeus*

The 96 hrs-LC_{50} of cadmium chloride toxicosis in *S. galilaeus* is summarized in Table 1. The result showed that the 96 hrs-LC_{50} of cadmium chloride in *S. galilaeus* was 28.3 mg/L.

Chronic cadmium chloride toxicity

Clinical signs and post-mortem findings: The most obvious signs in all toxicated fish were respiratory manifestation in the form of gasping, rapid operculum movements and collecting at the oxygen source began during the first week and extended to the end of an experiment with more obvious signs at the last three weeks. No mortalities were recorded during the chronic experiment. Internally, congestion of the gills, hepatopancreas, kidney and spleen was evident. The gills exhibited dark red spots alternative with another pale area.

Histopathological lesions

Gills: The noticeable lesions during 1st and 2nd week were EGCs infiltration and the goblet cells hyperplasia in the tips of primary lamellae (Figures 1a and 1b). interlamellar epithelial hyperplasia at the tips of gill filaments lead to filamentous clubbing (Figure 1c) and at the secondary lamellae lead to multifocal fusion of the secondary lamellae (Figure 1d) were noticed during 3rd and 4th week. Lamellar lifting where separation of the surface epithelium of secondary lamellae from capillary beds by edema (Figure 1e) and lamellar telangiectasis due to rupture of retaining pillar cells (Figure 1f) were appeared during 5th and 6th week. Lamellar fusion (Figure 1g) and lamellar necrosis (Figure 1h) were detectable during 7th and 8th week.

Hepatopancreas: The encountered lesions in the hepatopancreas were congestion of the pancreatic acini and the hepatic sinusoids (Figure 2a) during 1st week beside sever hydropic degeneration of the hepatocytes where the cytoplasm is replaced by clear fluids and the nucleus not affected either in shape or location (Figure 2b), and activation of melanomacrophage centers (Figure 2c) during 3rd and 4th week. In late stage of the experiment the observed lesion was necrosis of the pancreatic acini (Figure 2d) and necrosis of the hepatocytes (Figures 2e and 2f).

Posterior kidney: histopathological findings in posterior kidney during first two weeks of cadmium chloride toxicity were congestion of blood vessels and activation of MMCs (Figure 3a). During 4th week the posterior kidney exhibited extravasations of erythrocytes from the blood vessel (Figure 3b). The posterior kidney showed intraepithelial hyaline droplets in proximal convoluted tubules replaced the necrotic lining epithelium (Figure 3c) during 6th week beside tubular necrosis (Figure 3d) during late stage of the experiment.

Spleen: The microscopic lesions of the spleen consisted of enlargement and activation in MMCs (Figure 4a) and the melanophores appeared heavily loaded with dark brown melanin pigment (Figure 4b) that was encountered all over the experiment. Multifocal lymphocytic cell necrosis and depletion was the most detected lesion in late period of the experiment (Figure 4c).

Discussion

Cadmium is one of the most toxic heavy metals in water environment

which affects aquaculture and fish health [11]. Cadmium is toxic at low concentrations to all life, including plants, fish, birds, mammals [15-17]. In this study, the LC_{50} of $CdCl_2$ was 28.9 mg/L, showing that *S. galilaeus* is more sensitive to acute cadmium toxicity than other tilapia species as showing in previous studies, LC_{50} of cadmium chloride in *O. niloticus* was 40.5 mg/L [10] and 80 mg/L in *O. mossambicus* [18]. Therefore, histopathological lesions in fish tissue can be used as a tool in reviling the direct toxic effects of chemicals in target organs [19], because they reflect the damage caused by period and severity of exposure to toxic element and the tissue adaptive capacity [20]. In the 10% of LC_{50} group the histopathological changes were existed mainly in the gills, hepatopancreas, posterior kidney and spleen.

Gill epithelium is the primary target organ for aqueous exposure, which suffers an acute edema and epithelial lifting during exposure. Edema with lifting of lamellar epithelium could be serve as a mechanism of defense, because separation epithelial of the lamellae

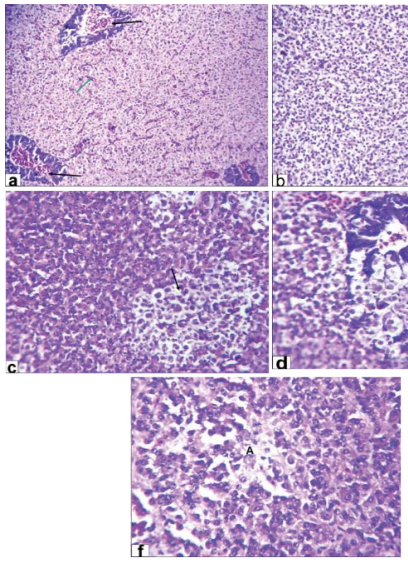

Figure 2: Photomicrograph of *S. galilaeus* Hepatopancreas exposed to 2.83 mg/L $CdCl_2$ staining by HE (a): Congestion of pancreatic acini (black arrows) and hepatic sinusoids (green arrow, X200) during 1st week. (b): Sever hydropic degeneration of hepatocytes and congestion of blood vessel (arrow, X200) during 3rd week. (c): Activation of MMCs (X200) during 4th week. (d): Necrosis of pancreatic acini (X400) during 6th week. (f): Necrosis of the hepatocytes (A, X400) during 7th week.

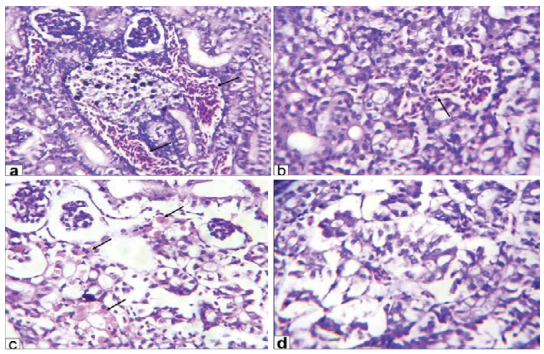

Figure 3: Photomicrograph of *S. galilaeus* posterior kidney exposed to 2.83 mg/L $CdCl_2$ and staining by HE. (X250) (a): Congestion of blood vessels (arrows) and activation of melanomacrophage centers (MMCs) during 2nd week. (b): Hemorrhage (arrow) during 4th week. (c): Homogenous structure-less esinophilic material replaced necrotic lining epithelium of the proximal convoluted tubules (arrows) during 6th week. (d): Necrotic renal tubules during 8th week.

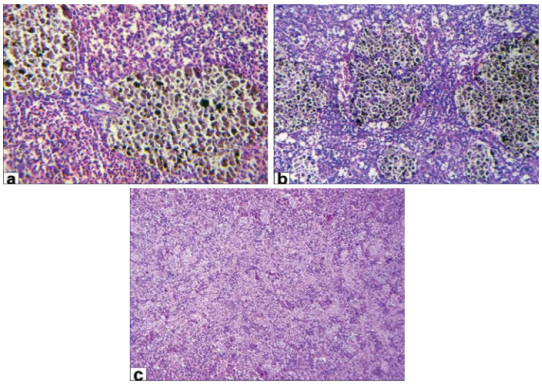

Figure 4: Photomicrograph of Spleen of *S. galilaeus* exposed to 2.83 mg/L $CdCl_2$ and staining by HE. (a): Enlargement and activation of MMCs (X250) during 2nd week. (b): Enlargement and activation of MMCs where the melanophores appeared heavily loaded with dark brown melanin pigment (X250) during 4th week. (c): Necrosis and depletion of lymphocytes in white pulp (X160) during 6th week.

increases the distance across which waterborne pollutants must diffuse to reach the bloodstream [21]. These alterations have been reported for other species exposed to cadmium chloride [22,23]. The epithelial proliferation of secondary lamellae is one histological change found in fish exposed to cadmium and other pollutants [24,25]. The hyperplasia induced by any pollutant may be due to the simple response to cellular necrosis [23,26]. Moreover, Shaker et al. [27] reported that the epithelial hyperplasia is known as a protective and defense mechanism of fish gills. Necrosis of lamellar epithelial cells was evident. The exposure to pollutants leads to rupture of pilaster cells, which normally join the dorsal surface of secondary lamellae to the ventral one. The result will be dilatation of the lamellar capillary and pooling of the blood leading to the telangiectasis which is the characteristic pathological lesion of the gills associated with physical or chemical causes [28,29].

The hepatopancreas is the site of detoxification of all types of toxins and chemicals. It is one of the organs most affected by water contaminants [30]. Microscopically, the detectable lesions in the hepatopancreas were congestion of the hepatic blood vessels, hepatic sinusoid and pancreatic acini. Moreover, there was acute cellular swelling of the hepatocytes where the primary mechanism of heavy metal cytotoxicity is the alteration of ion and non electrolyte transport and cell volume regulation, which finally lead to cell swelling [21,31,32]. In advanced cases, there was activation of MMCs. The circulating macrophage replete with particulate matter, home selectively on the melanomacrophage centers, hence the activation of the MMCs considered as a line of defense [28,33]. Moreover, there was necrosis of the hepatocytes and pancreatic acini [34]. Several studies had shown a variety of changes in the liver of O. niloticus, resulting from exposure to different toxic chemicals [35,36].

Upon the microscopical examination of the posterior kidney were in the form of congestion of large blood vessels and hemorrhage. Moreover, there was activation of melanomacrophage centre (MMCs) that play a role in the defense mechanism besides necrosis of renal tubular cells [33,37]. Eosinophilic staining hyaline droplets deposition within the cells of proximal tubules can often appear to replace necrotic renal epithelial and represent protein which has been reabsorbed from the glomerular filtrate. Since the renal tubular epithelium has its major function in the excretion of divalent ions, pollution with heavy metals such as cadmium is highly likely to affect these cells [38]. The histopathological changes observed due to cadmium toxicity were similar to other fishes due to heavy metal toxicity [39].

Herein, the spleen showed enlargement and activation in melanomacrophage centre; the melanophores appeared heavily loaded with dark brown melanin pigment and lymphocytic cells necrosis that may be attributed to direct cytotoxic effect of cadmium chloride on lymphopoietic tissue, that may correlated with immune depressed [28,33].

Conclusion

The histopathological changes on fish are a useful biomarker for understand the changes that occurring in different organs due to environmental pollution as well as constitutes a potential risk concern on human consumer's health so we recommend the authorities not to dispose the sewage and industrial wastes into surface water or at least to treat these effluents before its dumping.

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