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Histological Changes in the Liver and Intestine of Nile Tilapia, *Oreochromis niloticus*, Exposed to Sublethal Concentrations of Cadmium

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Abstract.- Cadmium (Cd) is one of the most harmful heavy metal pollutants in aquatic environments. Fingerlings of Nile tilapia, *Oreochromis niloticus*, were exposed to sublethal concentrations (10, 20 and 30% of the 96-h LC_{50}) $CdCl_2$ which caused significant changes in concentration dependent manner in the hepatic and ileal tissues. The hepatic tissue lost its characteristic architecture, with increased vacoulations in hepatocytes. In addition, abundant eythrocytic infiltration was observed in the 10% of LC_{50} group and clearly increased in the 20% of LC_{50} group. Increase of hemorrhage, vacuolation in hepatocytes and infiltration of sinusoids with leukocytes were observed in the 30% of LC_{50} group. The intestinal tissue in the treated groups was characterized by increased degenerated nuclei and apoptosis in crypts of Lieberkuhn, in addition to abnormally dilated lamina propria infiltrated with a large number of inflammatory leukocytes and disturbance of the longitudinal muscularis. Goblet cells increased in all treated groups, indicating a defense mechanism against the severe pathological changes induced. In conclusion, severe damage to the hepatic and intestinal tissues of the Nile tilapia was observed upon contamination of the fish environment with $CdCl_2$.

Key words: Cadmium, heavy metal contamination, aquatic pollution, *Oreochromis niloticus*

INTRODUCTION

Heavy-metal pollution is one of the five major types of common toxic pollutants in surface waters (Mason, 1991). Heavy metals are among the major contributors to the pollution of South Africa's natural aquatic ecosystems (Sanders, 1997).Because of their chemical stability, heavy metals tend to accumulate into the tissues of different organisms (Hellawell, 1986; Sanders, 1997). Unfortunately, aquatic organisms can be exposed to extremely high levels of these heavy metals. Significant changes inexternal features and behavioral activities can be observed as a result of heavy metalpollution. The liver is one of the most susceptible organs to the harmful effects of heavy metals, because it is a detoxification organ and is essential for the metabolism and the excretion of toxic substances (Hinton and Lauren, 1990).

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Cadmium is one of the most deleterious heavy-metal pollutants in aquatic systems, and exposure leads to severe consequences, such as anemia and emphysema (Nriagu *et al*., 1998; Peraza *et al*., 1998). Various evidence indicates that the toxicity of cadmium may be associated with oxidative damage from the production of reactive oxygen species (ROS) (Bagchi *et al*., 2000; Shi *et al*., 2005). The levels of cadmium in fish are of considerable interest, because fish consumption is a major source of cadmium intake for the general population. It was found that most of the cadmium in fish tissues is highly absorbable, accounting for approximately 3–8% of the ingested cadmium load in the gastrointestinal tract of humans (ATSDR, 2003). Cadmium chloride exposure induced histological changes in kidney and liver of fresh water catfish *Clarias batrachus* (Bilal *et al*., 2011). Considering the 96-h LC_{50} value for $CdCl_2$ in tilapia *Oreochromis niloticus*is indeed cadmium- tolerate species. In fact, that value (14.8 mg/L) was reasonably higher when compared with most of the freshwater species of fish (Garcia-Santos *et al*.,

2006).

The present study was conducted to investigate the effect of Nile tilapia *Oreochromis niloticus* exposure to 10, 20 and 30% of the 96-h LC_{50} of $CdCl_2$. Histological changes in the liver and the intestine were used to assess the toxic effects of this metal.

MATERIALS AND METHODS

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 acclimated to laboratory conditions for two weeks prior to experiments.

Experimental design

One hundred and sixty acclimated fish weighing 28.33± 1.12 g were divided into three groups exposed to 10, 20 or 30% of the $LC_{50}$of $CdCl_2$, which represent 1.68, 3.36 and 5.04 mg/L $CdCl_2$, respectively, for 10 days. An unexposed group served as the controls. Eighty-liter glass aquaria (100×50×40 cm) were used with replicates for each concentration. Fish were fed twice daily at a rate of 2% of body weight with a 32% crude protein diet. Mortalities of each group were recorded daily.

Experimental exposure

After acclimatization, 20 fish were transferred to experimental tanks (80 L) containing dechlorinated tap water. Duplicate cultures were established for each concentration tested, adding calculated amounts of a 1000 mg/L stock solution of $CdCl_2$ prepared in deionized water, with an unexposed group serving as the control fish. The cadmium treatment level was based on the 96-h LC_{50} of $CdCl_2$in *O. niloticus*, which was previously determined to be 16.8 mg/L by Zirong and Shijun (2007).

Histological examination

The livers and the intestines of the control and the treated fish were fixed in 10% neutral-buffered formalin, and the samples were then processed for routine wax histological evaluation (dehydrated and embedded in paraffin). Sections of 5µm were prepared and stained with hematoxylin and eosin stains as described by Luna (1968) and Bernet *et al*. (1999).

RESULTS

This study was designed to investigate the effects of cadmium on the Nile tilapia, *Oreochromis niloticus*. Hepatic and ileal tissues from this fish were observed after treatment with three different concentrations of $CdCl_2$ (10, 20 and 30% of the 96-h LC_{50}), revealing that the histopathological changes occurred in a concentration-dependent manner.

Histopathological changes in the hepatic tissues

The liver sections from the group exposed to 10% of the LC_{50} of $CdCl_2$showed clear hepatic tissue damage. The examination of the liver sections of this group showed that it had lost its characteristic architecture, with markedly increased vacuolation in hepatocytes (Fig. 1A). In addition, the cytoplasm of the hepatocytes in this group was characterized by coarse, pink and darkly stained granules and vacuoles. Abundant eythrocytic infiltration was also observed in this group (Fig. 1B). The eythrocytic infiltration was clearly increased in the group of Nile tilapia exposed to 20% of the LC_{50} of $CdCl_2$, as shown in figure 1C. Sections of the group exposed to 30% of the LC_{50} of $CdCl_2$are characterized by erythrocyte infiltration into blood sinusoids, increased hemorrhage, vacuolation in hepatocytes and leukocyte infiltration into sinusoids, which indicates increased inflammation in hepatic tissues (Fig. 1D).

Histopathological changes in the intestinal tissues

Damage to intestinal tissue, particularly to enterocytes and villi structures, was detected histologically. The sections of the 10% of LC_{50} group (Fig. 2 A,B and C) showed increased degenerated nuclei and apoptosis in the crypts of Lieberkuhn, in addition to abnormally dilated lamina propria infiltrated with a large number of inflammatory leukocytes. A disturbed longitudinal muscularis was also observed. Mucous-secreting goblet cells proliferated and multiplied in all treated

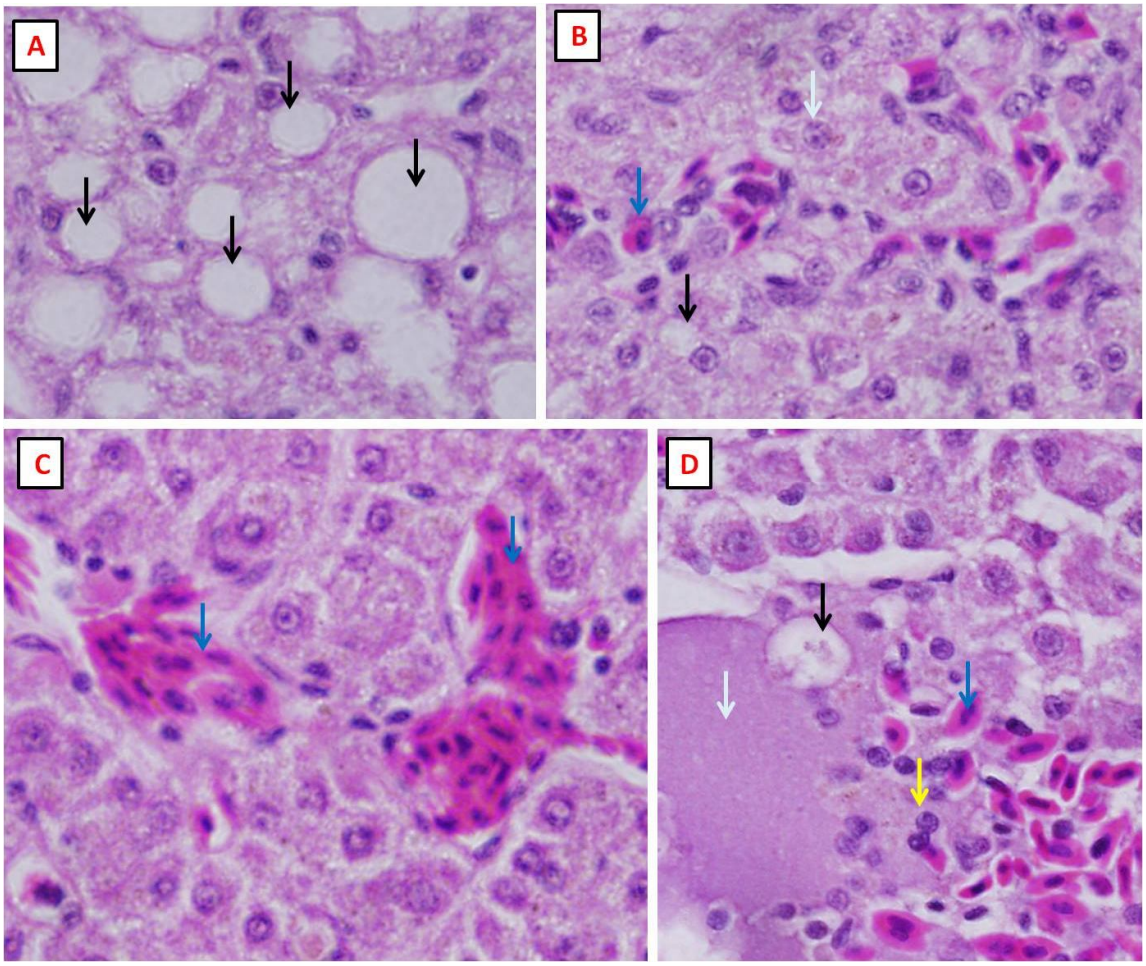

Fig. 1. Histological structure of liver of Nile tilapia showing histopathological alterations due to 10-day $CdCl_2$ exposure at different concentrations. A: Liver section from a fish exposed to 10% of the 96-h LC_{50} of $CdCl_2$, showing the increased vacuolation in hepatocytes (black arrows); B, A liver section from the 10% of LC_{50} group shows degenerated nuclei (white arrows), vacuolation (black arrows) and erythrocyte infiltration into blood sinusoids (blue arrow);C, Erythrocyte infiltrationinto blood sinusoids and degenerated nuclei are observed in this representative sectionfrom fish exposed to 20% of the 96-h LC_{50} of $CdCl_2$; D, Sections from fish exposed to 30% of the 96-h LC_{50} of $CdCl_2$ are characterized by erythrocyte infiltration into blood sinusoids (blue arrows), increased hemorrhage (white arrow), vacuolation in hepatocytes (black arrow) and infiltration of sinusoids with leukocytes (yellow arrow).
Stain: H & E; Magnification ×1000.

groups, indicating a defense mechanism against the severe pathological changes induced by $CdCl_2$ contamination.

The 20% of LC_{50} group (Fig. 2 D,E) showed pathologies similar to the 10% of LC_{50} group, in addition to increased levels of inflammatory cells and characteristic dilated blood vessels. Thickening in the circular muscularis was clearly identified in this group.

In the 30% of LC_{50} group (Fig. 2F,G), the serosa and muscularis were thickened, and some lymphocyte nodules were observed. The examination of the ileal sections of all treated groups indicated many pathological changes compared to the control group, which showed normal intestinal epithelial cells (Fig. 2 H).

The mortality of control group and fish exposed to 10, 20 and 30% of the LC_{50} $CdCl_2$ were

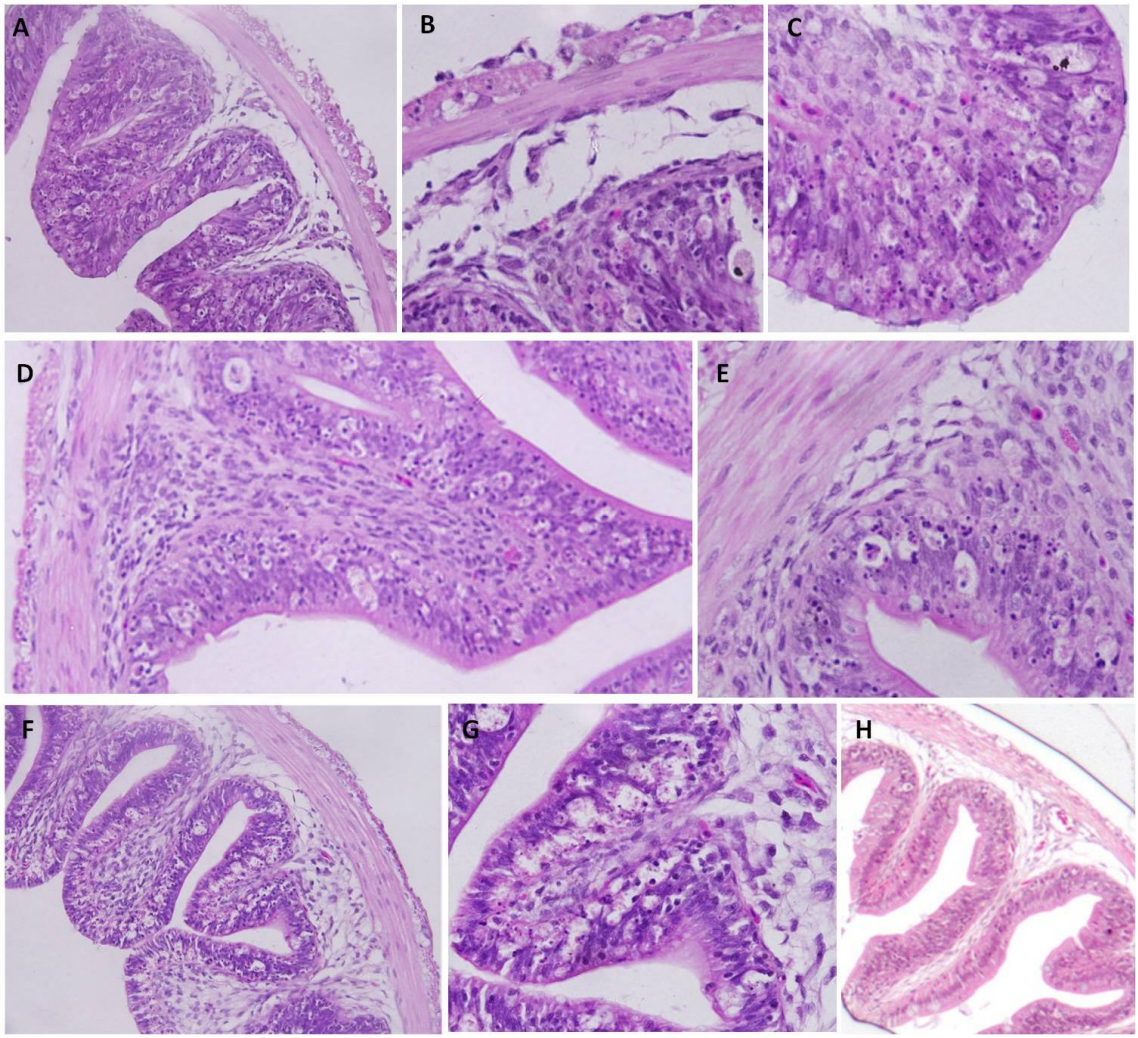

Fig. 2. Histological structure of intestine of 10-day Nile tilapia exposed to 10% of the 96-h LC_{50} of $CdCl_2$. A, Histological changes of the ileum from the treated group; B, a magnified section from the same group showing the muscularis and the lamina propria; and C, a magnified section from the same group showing the villus tip and changes attributed with enterocytes; D, A representative section from a fish exposed to 20% of the 96-h LC_{50} of $CdCl_2$; E, and a magnified section from the same group showing the muscularis and the lamina propria; F, A representative section from a fish exposed to 30% of the 96-h LC_{50} of $CdCl_2$ and G, a magnified section from the same group showing the muscularis and the lamina propria; H, A, representative section of a control fish (showing normal intestinal epithelial cells) for comparison with the $CdCl_2$-treated fish samples.
Stain: H & E; Magnification A, D, F, H ×200; B, C, E, G, ×1000.

Table I.- Mortality of Nile tilapia, *Oreochromis niloticus*, exposed to different concentrations of cadmium chloride for 10 days.

No. of fish	Total	10	9	8	7	6	5	4	3	2	1	Days/Treatment
40	0	0	0	0	0	0	0	0	0	0	0	Control
40	6	2	2	1	1	0	0	0	0	0	0	T1 10% of LC_{50} (1.68 mg/L)
40	10	3	2	2	1	1	1	0	0	0	0	T2 20% of LC_{50} (3.36 mg/L)
40	14	4	3	2	2	1	1	1	0	0	0	T3 30% of LC_{50} (5.04 mg/L)

increased with increasing concentration and time that recorded 0, 6, 10 and 14 fish, respectively during 10 days exposed (Table I).

DISCUSSION

Cadmium is considered to be highly toxic to marine and freshwater aquatic life (DWAF, 1996). In the present study, the cumulative effects of low and high concentrations (10, 20 and 30% of the 96-h LC_{50} of $CdCl_2$ for 10 days) on liver histology were investigated. The results included common histological characteristics, such as hyalinization of hepatocytes, increased vacuolation associated with lipid accumulation, congestion of blood vessels, and cellular swelling. These changes are generally associated with the response of hepatocytes to toxicants (Van Dyk *et al*., 2007; Hinton and Laure´n, 1990). Therefore, the histological changes identified in hepatocytes in this study may have been the result of various biochemical disruptions. These pathologies also may be related to the vacuolation of hepatocytes that is associated with inhibited protein synthesis, energy depletion, disaggregation of microtubules, or shifts in substrate utilization, as described by Hinton and Laure (1990) and Ajani and Akpoilih (2010). Hyalinization is reportedly the result of protein synthesis malfunction (Cheville, 1994). Moreover, protein inclusion bodies are commonly associated with metal toxicity. Rabitto *et al*. (2005) and Oliveira Ribeiro *et al* (2006) reported that histopathological biomarkers have been primarily used in fish to identify and evaluate the toxic effects of exposure to contaminants. In agreement with these results, Kaoud *et al*. (2011) reported that the liver of *Oreochromis niloticus* treated with cadmium showed hepatocyte degeneration, with nuclear pyknosis in the majority of the cells and the accumulation of metal binding proteins in their nuclei. In addition, Abdel-Warith *et al*. (2011) noted that the degree and the nature of histological changes in the liver of fish exposed to a sublethal concentration of zinc were affected by the exposure period. They reported that histological changes were primarily observed in fish exposed over short-term periods, while regenerative responses were noted in fish exposed over a long-term period.

The histological responses that have previously been reported in the liver of various fish species exposed to cadmium include the following: atrophy and necrosis of hepatic cells, decrease in the size of the nuclei and nucleoli, and indistinguishable cell membranes *Cyprinus carpio* (Morsey and Protasowicki, 1990); formation of macrophage granulomas *Carassius auratus* (Tafanelli and Summerfeldt, 1975); and increase in connective tissue and hepatocyte nuclei *Halobatrachus didactylus* (Gutierrez *et al*., 1978).

The liver is associated with detoxification and biotransformation processes, and due to these functions combined with its location and access to the blood supply, it is one of the organs most affected by water contaminants (Camargo and Martinez, 2007; Mohamed, 2009). In this work, liver tissues showed increased vacuolation in hepatocytes and degenerated nuclei in the 10% of LC_{50} group. Erythrocyte infiltration into blood sinusoids in addition to degenerated nuclei were observed in the 20% of LC_{50} group. The 30% of the LC_{50} group is characterized with erythrocyte and leukocyte infiltration into blood sinusoids, increased hemorrhage, and vacuolation in hepatocytes. These results are in agreement with data obtained by Soufy *et al*. (2007), who reported that the liver showed increased vacuolar degeneration in hepatocytes, necrotic foci, thrombosis formation in central veins, dilation and congestion in blood sinusoids, and fibrosis. These changes may be attributed to the direct toxic effects of pollutants on hepatocytes. The vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the circulatory system (Gingerich, 1982). Oxygen deficiency as a result of gill degeneration is the most common cause of cellular degeneration in the liver. The vascular dilation, intravascular hemolysis and thrombosis formation observed in the blood vessels with subsequent stasis of blood may also be responsible for the cellular degeneration and necrosis in the liver (Mohamed, 2001). Furthermore, our findings are in agreement with those observed in many previous studies that have investigated the effects of different pollutants on fish liver (Mohamed, 2001; Ptashynski *et al*., 2002; Fanta *et al*., 2003; Abdel-Warith *et al*., 2011).

Olojo *et al*. (2005) also observed degeneration of the hepatocytes and focal necrosis in the liver of *Clarias gariepinus* exposed to lead. Exposure of *Oncorhynchus mykiss* to copper sulfate was found to induce degeneration of hepatocytes, sinusoidal dilation and congestion in the blood vessels of the liver (Atamanalp *et al.*, 2008).

The teleost liver is one of the most sensitive organs to the biochemical disruption caused by various types of environmental pollutants (Hinton and Couch, 1998, Van Dyk *et al*., 2007; Younis *et al*., 2012). The deterioration of the regular compartmentalization of the cytoplasm is a very early and nonspecific signal of disturbance of hepatocellular homeostasis (Braunbeck, 1998).

The primary mechanism of heavy metal cytotoxicity is the alteration of ion and nonelectrolyte transport and cell volume regulation, which finally lead to cell swelling (Ballatori and Boyer, 1996). An increase in lipid droplets observed by other researchers after lindane, cadmium and terbuthylazine exposures (Sylvie *et al*., 1996; Thophon *et al*., 2004; Dezfuli *et al*., 2006) could be due to the decline of protein synthesis and the consequent non-utilization of lipids for lipid-protein conjugation (Cheville, 1994). The manifestation of cytopathologic changes reported here suggests a severe hepatic dysfunction and the impairment of the physio-metabolic process in *D. labrax* liver.

Toxic pollutants will enter the digestive tract of fish via the food and water that they consume, causing a deterioration of structures and functions in the gut (Bano and Hasan, 1990; Banerjee and Bhattacharya, 1995). Force feeding *D. labrax* with Benzo [a] pyrene leads to a high increase in the number of vacuoles and lysosomes and degenerative modifications of mitochondria in enterocytes (Lemaire *et al*., 1992). The formation of autophagolysosomes and myelinoid bodies indicates an increased turnover of cellular components following cell degeneration and can be induced by a variety of drugs and xenobiotics (Ghadially, 1997).

The present study revealed that *Oreochromis niloticus* exposed to three different concentrations of $CdCl_2$ for 10 days showed severe hepatic degeneration, in addition tonecrotic changes in the intestinal mucosa and submucosa, atrophy in the muscularis and submucosa and aggregations of inflammatory cells in the mucosa and submucosa. This was in agreement with data obtained by Bilal *et al*. (2011) who reported that the liver of catfish exposed to 4 and 8 ppm $CdCl_2$ for 30 and 60 days effected several histological alterations such as deshaping of hepatocytes, eccentric position of nuclei, enucleation, development of vacuoles in cell cytoplasm and necrosis of hepatic tissue. However, these authors studied on low concentration of $CdCl_2$ than our work but for long term exposure (60 days). Bhatnagar *et al*. (2007) and Mohamed (2009) observed irritation and destruction of the mucosa membrane of the intestine, hampering absorption. Epithelial degeneration, inflammatory cell infiltration in the submucosa and submucosal edema were observed in the intestine of tilapia exposed to carbofuran (Soufy *et al*. 2007). Mohamed (2008) reported that uptake of metals occurs mainly through gills but may also occur *via* intestinal epithelium. The histopathological alterations observed in the intestine of both *Oreochromis niloticus* and *Lates niloticus* revealed severe degenerative and necrotic changes in the intestinal mucosa. Edema between submucosa and mucosa may be a result of the absorption of toxic metals (Hanna *et al*., 2005). The present results are in agreement with those observed by many investigators studying the effects of metals on fish intestine (Giari *et al.*, 2007 and Hanna *et al*., 2005).

Kaoud *et al*. (2011) reported that the pathological findings in the intestine of *Oreochromis niloticus* treated with cadmium included atrophy in the muscularis, degenerative and necrotic changes in the intestinal mucosa and submucosa with necrotized cells aggregated in the intestinal lumen, and edema and atrophy in the submucosa. These findings are similar to *Chana punctatus* exposed to cadmium (Stromberg *et al*., 1983) and lead (Sastri and Gupta, 1978).

Fish mortality increased in a dose dependent manner, this was in agreement with Abdel-tawwab *et al*. (2011) who reported that large increases in fish mortality are associated with the increases in exposure concentrations of Zn on Nile tilapia fingerlings. Likewise, De Schamphelaere and Janssen (2004) reported that fish mortality might be a more sensitive endpoint for assessing effect of Zn exposure. Also, Shetty *et al*. (2007) reported that the

determination of acute toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds.

In conclusion, this study confirmed a toxic effect of cadmium introduced as a water pollutant to the tilapia fish, *Oreochromis niloticus*.At all concentrations tested cadmium will enter the digestive tract of fish via the food and water that they consume, causing the deterioration of structures in the gut and in the hepatic tissues.

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