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Cadmium Accumulation and Histological Lesion in Mosquitofish (*Gambusia affinis*) tissues Following Acute and Chronic Exposure

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ABSTRACT: In this study, cadmium (Cd) accumulation was studied in an experimental aquatic exposure. Mosquitofish (*Gambusia affinis*) were acutely exposed for 96 h to a high concentration of Cd (12 mg/L) and were chronically exposed to a low concentration of Cd(0.4 mg/L) for 30 days. Cd accumulation profiles differed between the two Cd exposures. The Cd concentation in *G. affinis* tissues increased linearly during acute exposure. In contrast, chronic exposure presented a biphasic pattern of accumulation, with Cd accumulation increasing until 20 days post-exposure then decreasing by the 30th day of the experiment. Histopathological investigations revealed greater changes in gills, kidney and liver tissues after chronic exposure than those recorded during acute exposure. The changes in gill were characterized by epithelial lifting, total and partial lamellar fusion, epithelial necrosis as well as telangiectasis. Necrosis of epithelial cells of renal tubules, glomerular contraction and reduction of Bowman's space were observed in the kidney tissue of exposed fish. The liver hepatocytes showed cytoplasmic vacuolization with lipid droplets and glycogen accumulation. Desquamation of hepatic tissue, congestion of the hepatic central vein and an increase in sinusoidal space were also observed. The result showed that, although Cd accumulation, following acute and chronic exposure, severely affects vital organs in mosquitofish; *G. affinis* adapts to continued metal accumulation. We hypothesise that this adaptation occurs through activation of a metal resistance mechanism.

Key words: *Gambusia affinis,* Cadmium, Acute and chronic exposure, Histopathology

INTRODUCTION

Aquatic organisms such as fish are, in most cases, exposed to multitudes of stressors that are either natural or anthropogenically introduced into environment. Contamination of fish with pollutants might adversely impact exploitation of aquatic resources. Heavy metals occur naturally in the environment and are found, at varying levels, in all ground and surface waters (Martin and Coughtrey, 1982; Vinodhini and Narayanan, 2009; Mehrdadi *et al.*, 2009; Shetty and Rajkumar, 2009; Nasrabadi *et al.*, 2010). Some metals (zinc, iron) are essential elements for normal metabolism while others are non-essential and do not have significant biological roles (Prosi, 1979; Rainbow, 1985; Sanders, 1997; Sahmoune *et al.*, 2009).

According to Mason, (1991) and Sanders, (1997), cadmium (Cd) is one of the five major types of toxic pollutants and causes an ecological problem that tend to be accumulated in living organisms (Jensen and Bio-Rasiriussen, 1992; Alazemi *et al.*, 1996). Natural as well as anthropogenic sources, which include industrial emissions and the application of fertilizer and sewage sludge to farmland, have increased Cd levels in the environment (ATSDR, 2003b). It is a non-essential element (Viarengo, 1985) and it is commonly used in ecotoxicological studies due (Wright and Welborn, 1994; Goering *et al.*, 1995). Low exposure concentrations of Cd can adversely affect organisms (Cope *et al.*, 1994) and leads to pathological conditions in some tissues (Friedman and Gesek, 1994; Yamano *et al.*, 1998; Novelli *et al.*, 1999). As a persistent environmental pollutant, Cd can alter trophic levels for centuries, and freshwater fish are particularly vulnerable to cadmium exposure (Sorensen, 1991).

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As an indicator of exposure to contaminants, histology represents a useful tool to assess the effects of pollution, particularly for sub-lethal and chronic effects (Cengiz and Unlu, 2005). Histopathology has been widely used as a biomarker in the evaluation of the health of fish exposed to cadmium both in laboratory (Wester and Canton, 1991; Randi *et al.*, 1996; Thophon *et al.*, 2003; Au, 2004) and field studies (Schwaiger *et al.*, 1997; Teh et *al.*, 1997). However, the alterations observed in the field cannot be specifically related to individual pollutants. In fish, cadmium accumulates in the liver, gills, kidney and gastrointestinal tract (Norey *et al.*, 1990). The results are histopathological alterations of these organs. In teleost fish, the kidney and gills are responsible for excretion and maintenance of ion homeostasis (Hinton *et al*., 1992; Evans, 1993). Kidney is one of the main targets for cadmium accumulation (Brown *et al.*, 1984; Allen, 1995; De Conto Cinier *et al.*, 1997) while liver is considered as a detoxification organ and essential for both metabolism and excretion of toxic substances in the body (Hinton and Lauren, 1990). Thus, this paper aims to investigate cadmium accumulation in mosquitofish (*Gambusia affinis*) tissues in relation to different concentrations and exposure times, and determine the structural damages induced by this metal in *G. affinis* gills, kidney and liver.

For this purpose, *G. affinis* was selected as the model organism in the present study. It is widespread in small streams in the Tunisian environment, and is a euryhaline organism widely distributed in both freshwater systems and estuaries of temperate regions. It is also amenable to laboratory studies. This fish possesses an important key role in ecosystem and has been considered as a representative of secondary consumers in aquatic ecosystems.

MATERIALS & METHODS

G. affinis samples were collected from an uncontaminated freshwater source "Oued El Gsil" in the town of Moknine, situated 20 Km away from the city of Monastir (Annabi *et al.*, 2009). A first phase of laboratory maintenance involved a period of quarantine in which the fish samples were acclimated to the laboratory conditions for at least two weeks (15-30 days) prior to the experiment. The samples were kept in glass aquaria (20 × 25 × 40) filled with dechlorinated tap water (pH = 7.09, salinity = 0.9 %), with continuous aeration and temperature of 20 ± 1°C. During the acclimatization period, inspections were conducted twice a day in order to discard wounded, diseased and dead individuals. The photoperiod was 16:8 (16 light hours/8 darkness hours) and fish were fed twice daily with commercially balanced fish food sticks (Tetramine, Hagen, France). The medium was renewed every two days.

The stock solution of Cd was prepared by dissolving analytical grade of cadmium chloride ($CdCl_2.H_2O$) in distilled water. The concentration of cadmium chloride was expressed in term of Cd ion in mg/L. The mosquitofish were exposed during 24, 48, 72 and 96 h to nominal Cd concentration of 12 mg/L. This dose was chosen according to our preliminary results (Annabi *et al.*, 2009), from a toxicity study (to determine LC_{50} values for mosquitofish), but to avoid fish death. Control and treated fish were placed in separate glass aquaria (n=20) filled with dechlorinated tap water at 20 ±1°C, 16:8 photoperiod and continuous aeration. Three replicates were performed for each group. Fish were starved for 24 h prior to and during the experiment.

In the subchronic exposure, 13 fish were removed randomly from each aquarium to prepare for accumulation study. *G. affinis* were exposed to a nominal concentration of 0.4 mg $CdCl_2$/L for 30 days. The dose chosen was theoretically sub-lethal; it was 10 % of the 96 h-LC_{50} value (Annabi *et al.*, 2009). Three replicates per group were performed. Mortality and behaviour were observed during the experiment and fish were fed twice daily with commercially balanced fish food sticks (Tetramine, Hagen, France). Uneaten food was quickly removed from the system. 50 % of the experimental water was changed every 2 days.

To evaluate cadmium accumulation in *G. affinis*, metal determinations were carried out. Fish were killed after 24, 48 and 96 h of exposure to 12 mg $CdCl_2$/L for acute exposure and after 10, 20 and 30 days to 0.4 mg$CdCl_2$/L for chronic exposure.

Fish whole tissues were dried for 48 h at 60°C in Pyrex test tubes. Dried tissues were weighted and digested with concentrated nitric acid (Merck, 65 %) at 120°C. When fumes were white and the solution was completely clear, the samples were cooled to room temperature and the tubes were filled to 10 mL with ultra pure water (Warchalowska-Sliwa *et al.*, 2005; Annabi *et al.*, 2009). Water samples were stabilized at pH 2 with 1 M nitric acid prior to direct determination of total metal concentrations (Bervoets and Blust 2003). To check for possible metal loss during chronic exposure, Cd levels in water were analyzed.

In the case of the control group, Cd concentrations in the acid solutions were measured by Graphite-Furnace atomic absorption spectrophotometry (AAS); while flame AAS was adopted for the exposure group. These were implemented using a ZEEnit 700-Analytik-Jena, Germany (Flame and Graphite-Furnace AAS), equipped with deuterium and Zeeman background

correction, respectively, as recommended by the manufacturer. Detection limits were 0.046 µg/L for Flame AAS and 0.002 µg/L for Graphite-Furnace AAS. The accuracy and precision of the analyses for tissue Cd content were based on the analysis of Cd in a standard reference bovine liver preparation (NIST). We found 0.43 ± 0.02 µgCd/g (n=7) in bovine liver, as compared with the certified level of 0.40 ± 0.03 µg/g. These results show that the analytical results of this study are of satisfactory quality.

Samples were analyzed in triplicate. Concentrations of the metal in tissues were calculated on a dry weight basis and expressed as µg/g dry weight, while that of Cd was expressed as µg/L in water.

Histopathological alterations were evaluated after 48 h and 96 h of exposure to 12 mg $CdCl_2$/L and after 30 days to 0.4 mg $CdCl_2$/L. Fish samples were fixed in Bouin's solution for 24 h, and prepared for histological analysis according to standard procedures (dehydrated in successive grades of ethanol series (70 and 95°) and embedded in paraffin. Serial longitudinal sections (thickness 4–5 µm) were stained with haematoxylin and eosin (H/E) for histological examination under a light microscope. A semi-statistical evaluation of the histopathological findings in the gills, kidney and the liver was done and we used the following symbols to design the degree and the extent of the structural changes: (-) no alteration, (+) mild, (+ +) moderate and (+ + +) severe occurrence.

Statistics

Data related to metal concentrations are given as mean ±S.E. Statistical analyses were performed with unpaired *t*-test using STATVIEW statistical software package. Normality and homogeneity of data were confirmed before unpaired *t*-test. Differences between means were regarded as significant if the *p* value was lower than 0.05.

RESULTS & DISCUSSION

Cadmium level in control fish tissues was found to be 0.678 ±0.12 µg Cd/g of dry weight. After 24, 48 and 96 h of exposure to 12 mg $CdCl_2$/L, these levels became highly significantly elevated and were respectively 6.25±2.74, 30.14±19.18 and 63.24±18.89 µgCd/g of dry weight (Fig.1). During the acute exposure, we noted a positive correlation between Cd levels in *G. affinis* tissues and exposure time (R=0.986).

Following 10 and 20 days of exposure to 0.4 mg $CdCl_2$/L (10% of LC_{50} for 96 h), Cd levels were significantly increased (P<0.01) and were 46.14±7.03 µgCd/g and 155.24±27.56 µgCd/g dry weight, respectively (Fig. 2). In contrast, Cd levels dropped significantly (P<0.01) after 30 days of exposure to a concentration of 55.48±11.93 µgCd/g dry weight (Fig. 2). During chronic exposure, Cd concentration in aquaria water is 0.36 ± 0.007 mgCd/L.

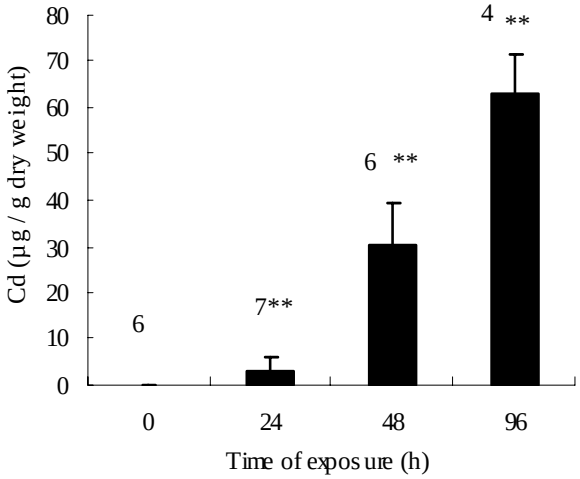

Fig. 1. Cadmium uptake during acute exposure to 12 mg $CdCl_2$/L

Asterisks (**) denote significant difference between control and application method (P< 0.01).

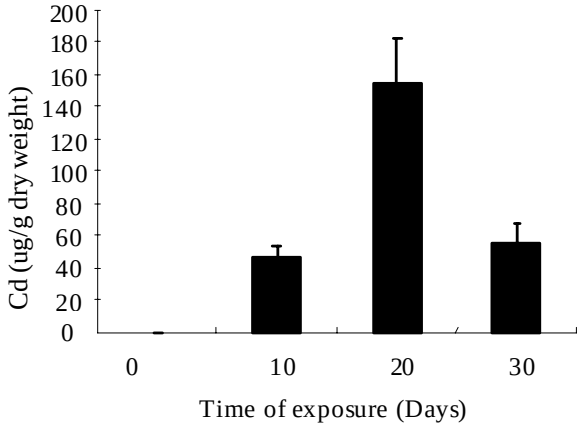

Fig. 2. Cadmium uptake during chronic exposure of 0.4 mg $CdCl_2$/L (n=7)

Asterisks (**) denote significant difference between control and application method (P< 0.01)

The gills of the control fish were normal at all times. They were made up of primary lamellae and secondary lamellae (Fig. 3a). The secondary lamellae were regularly lined up along both sides of the primary lamellae. Secondary lamellar were formed by an epithelium and blood capillaries kept aside by pillar cells. Spaces between this pillar cells were the blood spaces of the secondary lamellae (Fig. 3a).

After 48-h of exposure, we observed histological alterations consisting mainly of epithelial cells and disorganization of many areas, causing congestion of pillar cells of blood capillaries (Fig. 3b). Alterations of the gill structure became more pronounced after 96 h of exposure. These changes were reflected by the hypertrophy and hyperplasia of the secondary lamellar epithelium, incipient telangiectasis and fusion of adjacent secondary lamellae (Fig. 3c; Table 1). After 30 days of exposure, gills showed a very pronounced separation of respiratory epithelium, oedema and complete fusion and shortening of adjacent secondary lamellae (Fig.3d). Oedema of epithelial cells and telangiectasis detected in chronically exposed fish were more severe than those observed in acutely exposed fish (Fig. 3d; Table 1).

The kidney is made up of renal corpuscles and renal tubules. The glomerulus is a tuft of capillaries (Fig. 4a). Proximal tubules were characterized by

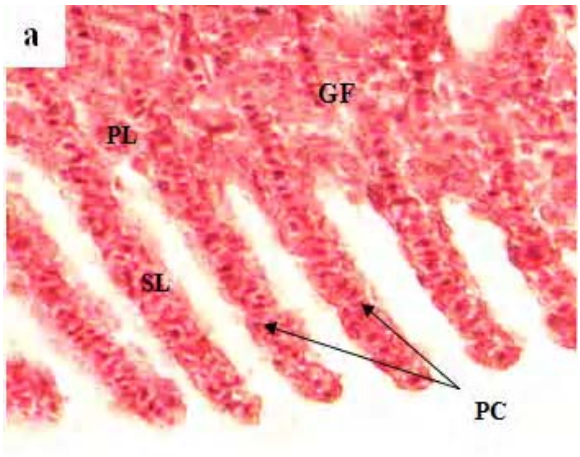

Fig. 3. a) Gill structure of control *G. affinis* (x 40).
GF: gill filament
PC: pillar cells
PL: primary lamellae
SL: secondary lamellae

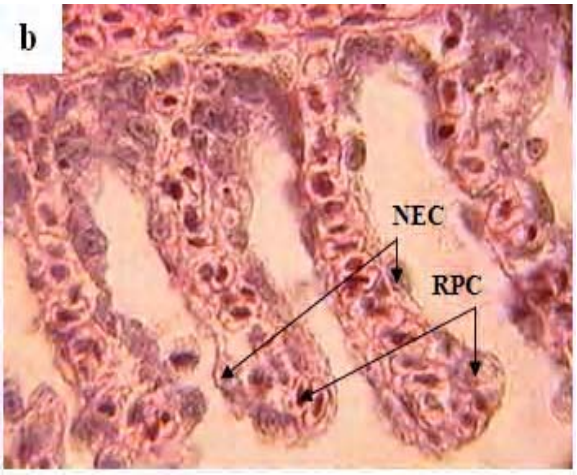

Fig. 3. b) Gill structure after 48 h of exposure to 12 mg$CdCl_2$/L (x 100).
NEC: necrosis of epithelial cells
RPC: rupture of pillar cells

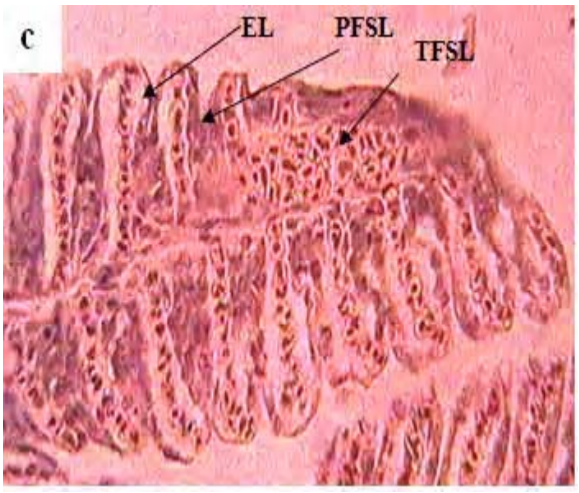

Fig. 3. c) Gill structure after 96 h of exposure to 12 mg$CdCl_2$/L (x 10).
EL: epithelium lifting
PFSL: partial fusion of secondary lamellae
TFSL: total fusion of secondary lamellae

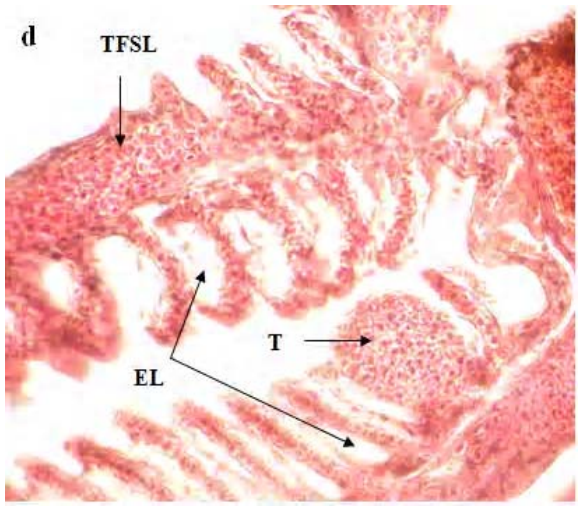

Fig. 3. d) Gill structure after 30 days of exposure to 0.4 mg $CdCl_2$/L (x 10).
EL: epithelium lifting
TFSL: total fusion of secondary lamellae
T: telangiectasis

Table 1. Semiquantitative scoring of gills lesions during acute and chronic exposure to 12 mg $CdCl_2$/L and 0.4 mg $CdCl_2$/L, respectively, in *G. affinis*

Lesions	Acute exposure		Chronic exposure
	48-h	96-h	30 (days)
Epithelial lifting	+	+ +	+ + +
Total fusion of secondary lamellae	-	+ +	+ + +
Partial fusion of secondary lamellae	-	-	+ +
Shortening of secondary lamellae	-	-	+ +
Telangiectasis	-	-	+ + +

(-) none, (+) mild, (++) moderate and (+ + +) severe occurrence

columnar cells with brush border located along the apices of the cells while distal tubules were low columnar epithelium cells with basally round nucleus. Most of the changes that occurred in the histological structure of kidney as a result of acute exposure were related to areas involved in excretory function, including clusters, proximal and distal tubules, and the haematopoitic tissue. These histological alterations consisted mainly of clusters alteration and vacuolation of tubular epithelial cells, noted after 48 h of exposure to cadmium (Fig. 4b). However, after 96 h of experimentation, alteration of clusters and vacuolation of epithelial cells became very pronounced. Tubular necrosis and the accumulation of fat inclusions in the epithelial cells were also observed (Fig. 4c; Table 2). The effect of chronic exposure on the histological structure of the kidney yielded mainly glomerular distortion and swelling of Bowman's space (Fig. 4d; Table 2).

The liver tissue is formed from parenchyma called hepatocytes. These were located among the sinusoids forming cordlike structures known as hepatic cell cords. The hepatocyte has a polyhedral cell body with a central core containing generally one spherical nucleolus (Fig. 5a).

The main histological alterations of this tissue, noted during the acute contamination, were hypertrophy of hepatocytes and liver tissue necrosis, observed after 48 h (Fig. 5b) and a significant desquamation of the liver tissue was noted after 96 h of exposure (Fig. 5c; Table 3). After 30 days of exposure the liver of treated fish showed a congestion of the central hepatic vein (Fig. 5d) with a notable hypertrophy of hepatocytes and accumulation of lipid droplets (Fig. 5d; Table 3).

The main goal of this study was to assess cadmium accumulation in *G. affinis* tissues after acute and chronic exposure and determine the histological lesions following this exposure. A growing number of evidence has shown that several factors influence Cd accumulation in fish tissues. These factors include the environmental metal concentration and time of exposure. Indeed, several authors showed that animals tissues, contaminated in the laboratory, accumulate heavy metals in a concentration and contamination period dependent manner (Allen, 1995; Kraal *et al.*, 1995; Roméo *et al.*, 1999; McGeer *et al.*, 2000; Francis *et al.*, 2004). Fish have the ability to accumulate heavy metal in their tissues by the absorption along the gill surface and gut tract wall to higher levels than the toxic concentration in their environment (Chevreuil *et al.*, 1995). Moreover, it was reported that Cd is rarely distributed uniformly within the fish body tissues, and it is nevertheless accumulated by particular target organs (Surech *et al.*, 1993). During an acute exposure, we observed a continuous accumulation of Cd throughout the experimental period and there were significantly higher Cd levels after 96 h of exposure than after 24 and 48 h. During chronic exposure, two major patterns of accumulation were observed: Cd accumulation increased significantly until 20 days post-exposure and then decreased by the 30th day of exposure. De Conto Cinier *et al.*, (1999) stated on cadmium uptake in fish liver and kidney can be divided into two groups according to the presence or absence of a plateau in the cadmium accumulation kinetic curves. Plateaus in cadmium accumulation after 2 or 3 months have been reported in liver and kidney of zebrafish (*Danio rerio*) (Rehwoldt and Karimian-Teherani, 1976) and in one-summer-old carp (*Cyprinus carpio*) exposed to 446 µgCd/l (De Conto Cinier *et al.*, 1997). Continuing accumulation has been observed in rainbow trout (*Oncorhynchus mykiss*) exposed to 3.6 and 6.4 µgCd/L for 178 days (Giles, 1988). Entry of heavy metals into the organs of a fish mainly takes place by the adsorption and absorption and the rate of accumulation is a function of uptake and depuration rates (Sreedevi *et al.*, 1992). McDonald and Wood, (1993), suggest that, after the initial shock phase of metal exposure, fish physiologically adapts to compensate for ion losses by secreting mucus and altering gill structure at the cellular and subcellular level. A reduction of accumulation has been reported in many other taxonomic groups as a physiological mechanism for metal resistance and adaptation (Hall *et al.*, 1979; Bariaud *et al.*, 1985; Tsuchiya and Ochi, 1994; Yanagiya *et al.*, 1999).

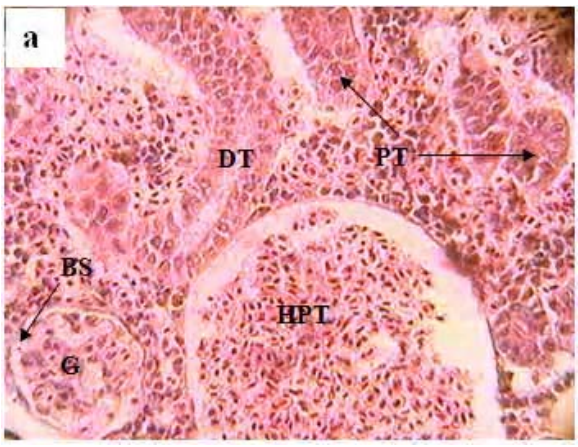

Fig. 4. a) Kidney structure of control *G. affinis* (x 40).
BS: Bowman's space
DT: distal tubule
G: glomerule
HPT: hematopoitic tissue
PT: proximal tubule

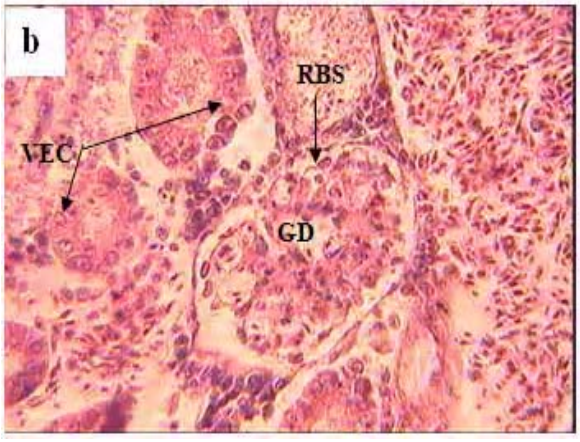

Fig. 4. b) Kidney tissue structure after 48 h of exposure to 12 mg $CdCl_2$/L (x 40).
GD: Glomerular distortion
RBS: reduction of Bowman's space
VEC: vacuolization of epithelial cells

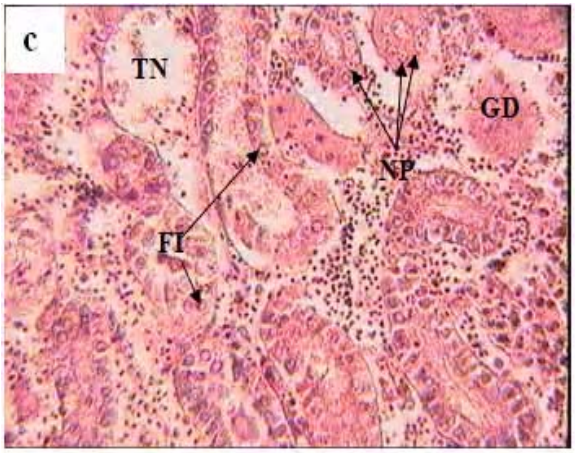

Fig. 4. c) Kidney tissue structure after 96 h of exposure to 12 mg $CdCl_2$/L (x 40).
FI: fat inclusions
GD: glomerular distortion
PN: pyknotic nucleus
TN: tubule necrosis

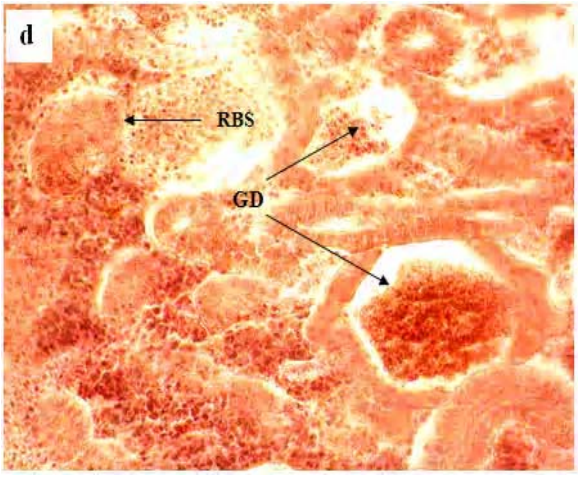

Fig. 4. d) Kidney structure after 30 days of exposure to 0.4 mg $CdCl_2$/L (x 40).
GD: glomerular distortion
RBS: reduction of Bowman's space

Table 2. Semiquantitative scoring of kidney lesions during acute and chronic exposure to 12 mg $CdCl_2$/L and 0.4 mg $CdCl_2$/L, respectively, in *G. affinis*

Lesions	Acute exposure		Chronic exposure
	48-h	96-h	30 (days)
Pyknotic nuclei	+	+ +	+ + +
Hyaline droplet	-	+ +	+ + +
Tubular necrosis	-	+ +	+ + +
Glomerular alteration	-	-	+ +
Reduction of Bowman's spaces	+	+	+ +

(-) none, (+) mild, (++) moderate and (+ + +) severe occurrence

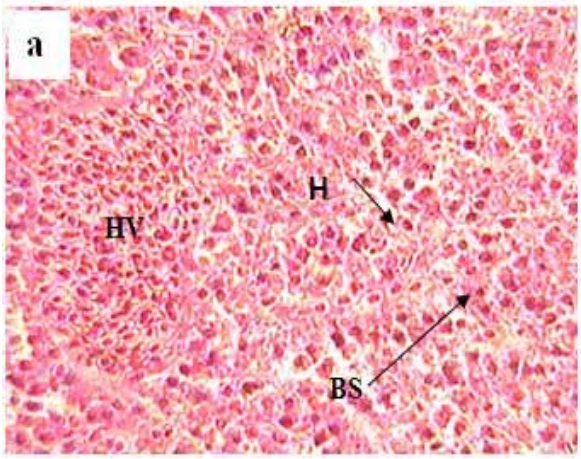

Fig. 5. a) Liver structure of control *G. affinis* (x 40).
BS: Blood sinusoid
H: hepatocyte
VH: hepatic vein

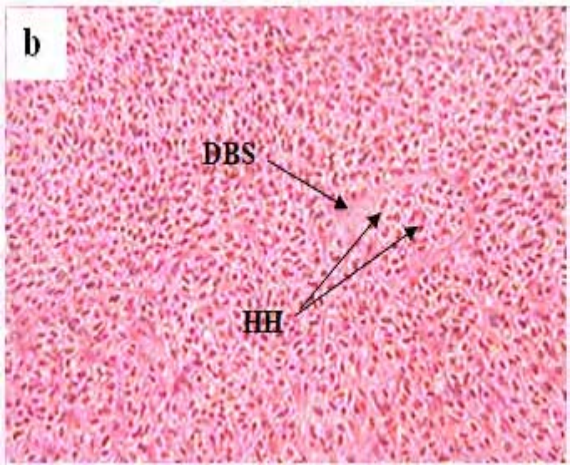

Fig. 5. b) Liver structure after 48 h of exposure to 12 mg $CdCl_2$/L (x 40).
DBS: dilatation of blood sinusoid
HH: hypertrophy of hepatocyte

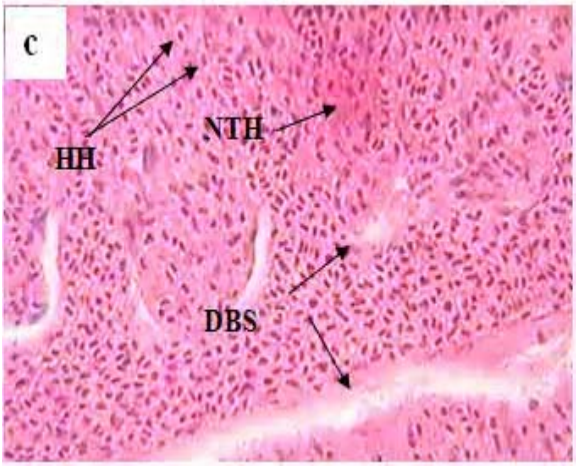

Fig. 5. c) Liver structure after 96 h of exposure to 12 mg $CdCl_2$/L (x 40).
DBS: dilatation of blood sinusoid
HH: hypertrophy of hepatocyte
NTH: necrosis of hepatic tissue

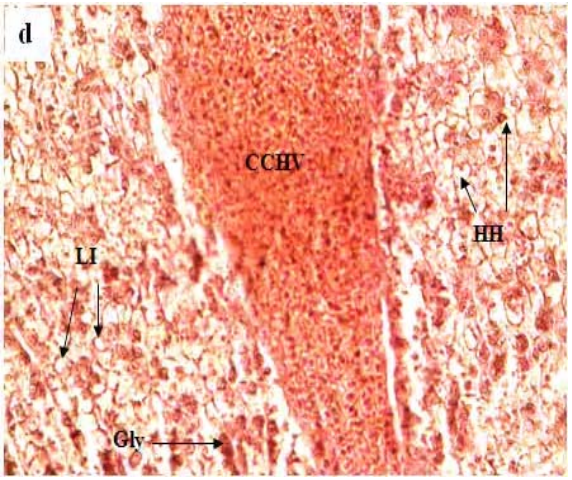

Fig. 5. d) Liver structure after 30 days of exposure to 0.4 mg $CdCl_2$/L (x 40).
CCHV: congestion of central hepatic vein
Gly: Glycogen
HH: hypertrophy of hepatocyte
LI: lipid inclusions

Table 3. Semiquantitative scoring of Liver lesions during acute and chronic exposure to 12 mg $CdCl_2$/L and 0.4 mg $CdCl_2$/L, respectively, in *G. affinis*

Lesions	Acute exposure		Chronic exposure
	48-h	96-h	30 (days)
Dilatation of blood sinusoid	-	+ +	+ + +
Lipid droplet accumulation	-	+ +	+ + +
Hepatic central vein congestion	-	-	+ + +
Hypertrophy of hepatocyte	+	+	+ +
Glycogen content	-	-	+ +

(-) none, (+) mild, (++) moderate, and (+ + +) severe occurrence

As gills come in direct contact with ionic cadmium, failure of gill function during acute exposure to this pollutant can lead to the death of fish (Laurent and Perry, 1991). Gills play an important role in the capture, accumulation and transfer of metal toward internal compartments via blood transport. As noted herein, lamellae structure of gills was severely damaged by cadmium exposure. The gills are the site of respiration and transport system involved in osmoregulation, and it has been confirmed that accumulation of metal ions within them may have an effect on these functions (Thurberg *et al.*, 1973; Jones, 1975). After thirty days of exposure, a total and partial fusion of secondary lamellae, mainly epithelial oedema and telangiectasis were observed. The changes in appearance of the secondary lamellae result from the collapse of the pillar cell system and breakdown of vascular integrity with release of large quantities of blood that push the lamellar epithelium outward (Alazemi *et al.*, 1996). Cengiz and Unlu, (2005) confirmed that epithelial oedema increases distance between the contaminant and the bloodstream, while secondary lamellae fusion significantly reduces the gill surface and thus decreases the contact between the pollutant and gill epithelium. Gill structural alteration found in *G. affinis* during an acute as well as chronic exposure may be classified into two groups: the first constitutes the lesions linked to the direct effect of the toxic element and the other constitutes the defence responses of gills. Necrosis and cell desquamation of the gill epithelium, observed during the acute contamination, were due to the direct toxic effect of cadmium, while epithelial oedema and partial and total fusion of the lamellae represent a defence response.

The teleostean kidney is one of the first organs to be affected by contaminants in the water (Thophon *et al.*, 2003) and is considered as preferential site for Cd accumulation in fish (Brown *et al.*, 1984; Allen, 1995). Alterations of kidney tissue during the acute exposure were severe. They were composed principally of tubule necrosis, glomerular alteration and lipid inclusion accumulation in epithelial cells. Following the chronic contamination, severe glomerular alteration was noted in this tissue. These findings confirm those noted in *Salmo gairdneri* (Forlin *et al.*, 1986) and *Lates calcalifer* (Thophon *et al.*, 2003). Hawkins *et al.*, (1980) found renal tubule necrosis and degeneration in *Leiostomus xanthorus* exposed to 10, 15 and 25 mg $CdCl_2$/L during 96 hours. Hypertrophy and degeneration of renal tubule have also been observed in *Puntius conchonius* following cadmium exposure (Gill *et al.*, 1988). Handy and Penrice, (1993) found swollen Bowman's capsule cells in the kidney of trout (*Salmo trutta*) and tilapia (*Oreochromis mossambicus*) exposed to mercuric chloride. Cattani *et al.*, (1996) showed that Cd is accumulated first in kidney, second in liver and third in gills of *Dicentrarchus labrax* living in contaminated waters. Weber *et al.*, (2003) showed that pyknotic and fragmented nuclei, as indicators of apoptotic and necrotic cell death, were mostly observed in the epithelial cells of proximal and distal convoluted tubules and were rarely associated with other renal cells. Moreover, they added that the occurrence of dilated tubules appears to be a consequence of dead and dying epithelial cells, while a thickening of Bowman's capsule can arise as a result of fibrosis.

Liver is one of the secondary site of cadmium accumulation, and the first site of detoxification (Brown *et al.*, 1984; Olsson *et al.*, 1996; Thophon *et al.*, 2003). In hepatic tissue, the histological alterations noted in *G. affinis* during the acute exposure to Cd were hepatocyte atrophy, desquamation and necrosis of hepatic tissue. During the chronic exposure, central hepatic vein congestion, hepatocyte hypertrophy and the presence of lipid inclusions were recognized. These findings are consistent with cadmium inducing greater hepatic alteration in fish after chronic exposure than after acute exposure (Wani and Latey, 1983; Brown *et al.*, 1984; Tophon *et al.*, 2003; Van Dyk *et al.*, 2007). The histological alterations of hepatocytes identified in this study may be the result of various biochemical lesions and act as a signal of degenerative processes that suggests metabolic damage (Pacheco and Santos, 2002). Hinton and Lauren, (1990) verified that vacuolation of hepatocytes is associated with the inhibition of protein synthesis, energy depletion or a shift in substrate utilization. Many authors (Kohler, 1990; Teinen-Moslen, 2001; Teh *et al.*, 2004) have confirmed that hepatocyte vacuolation and abnormal accumulation of neutral lipids such as triglycerides are common responses of the liver to perturbations in lipid metabolism that arise from contaminant exposure.

The structural lesions of gills during chronic exposure, including epithelial oedema and total and partial fusion of secondary lamellae, may impair blood-water exchange by reducing distances between lamellae, leading to the reduction of the contact surface area available for cadmium uptake (Wood, 2001). These responses, whether adaptive or pathological, invariably affect homeostatic regulation of the internal environment (Laurent and Perry, 1991), in particular decreasing the efficiency of gas exchange (Jagoe *et al.*, 1996; De Oliveira Ribeiro *et al.*, 2002). Lipids inclusion accumulation noted in the *G. affinis* kidney may be the result of glomerular alteration and an increase in permeability (Bucher and Hofer, 1993). After chronic and acute exposure, some of the histological alterations in different tissues were specific to cadmium. The fusion of secondary lamellae and telangiectasis in gills tissue may represent a cadmium-specific damage.

However, secondary lamellae hyperplasia may be non-specific, indeed Richmonds and Dutta, (1989) detected secondary lamellae hyperplasia after 96 h of exposure to malathion. Accumulation patterns of contaminants in fish depend both on uptake and on elimination rates (Hakanson, 1984) and a low rate of elimination observed during acute exposure could also lead to greater accumulation (Albert, 1973). In gill epithelium, Cd readily enters via the lanthanum (La)-sensitive apical voltage independent calcium (Ca) channels (Verbost et *al.,* 1987; Verbost *et al.,* 1989; Wicklund Glynn *et al.*, 1994). Numerous authors found that competitive interaction between Cd and Ca lead to reduce Cd accumulation in fish (Hollis *et al.,* 2000; Wu *et al.,* 2007; Zhang and Wang, 2007). A reduction in gill epithelial Ca-Channels expression, observed when fish are fed a Ca-enriched diet, would limit uptake of both Cd and Ca (Galvez *et al.,* 2007). Like other teleosts belonging to the Cyprinidae family, *G. affinis* has cellular bone tissue (Moss, 1961; 1965). Therefore, a Cd-induced disturbance of the Ca balance would probably lead to a situation, in which hypocalcaemia is compensated by an increased release of Ca from skeletal bone. In the hepatic tissue, cadmium not bound to metallothionein, wich represents the main transport and storage protein for cadmium (Jin *et al.,* 1998), induces synthesis of the protein as a protective mechanism (Nordberg and Nordberg, 2000). However, Cd bound to metallothionein re-enters the blood stream and is filtered at the glomeruli of the kidney and reabsorbed by the proximal tubule cells. Excess of free cadmium in the kidney, damage the proximal tubules resulting in renal dysfunction (Brzoska *et al.,* 2003) and apoptosis of kidney cells (Ishido *et al.,* 1995). Many study of gene expression following acute and chronic Cd exposure found that heat shock protein (hsp70 family) was a short term adaptation to Cd exposure while metallothionein was likely used for long-term detoxification and sequestring of Cd (Blechinger *et al.,* 2002; Chen *et al.,* 2007). Indeed, Gonzalez *et al.,* (2006) found that strong hsp70 gene expression in the gill of *Danio rerio* was present after 7 day of Cd exposure while after 21 days, hsp70 expression had returned to normal levels and metallothionein expression was induced.

CONCLUSION

The present study showed that cadmium uptake was both time- and dose-dependent. Indeed, we observed a different pattern of metal accumulation following acute and chronic exposure. The histological changes observed in the gills, kidney and liver of the *G. affinis*, after exposure, were characteristic of direct damage by cadmium and the secondary effects caused by a stress response. The findings of the present histological investigations demonstrate a direct correlation between cadmium accumulation and histopathological damage in gill, kidney and liver. Such results support that mosquitofish has developed cadmium-sequestering detoxifying systems and may enhance further elucidation of physiological and biochemical mechanisms of resistance to metal toxicity. *G. affinis* is a species that was shown to be appropriate for *in situ* tests and for metal environmental monitoring.

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