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Histopathological Alterations of Hybrid Walking Catfish (*Clarias macrocephalus* x *Clarias gariepinus*) in Acute and Subacute Cadmium Exposure

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Abstract

Histopathological alterations occur in the gills, livers and kidneys of 3-month old hybrid walking catsfich (*Clarias macrocephalus* x *Clarias gariepinos)* after acute and subacute cadmium exposure in water, and after intraperitoneal injection.The 96-h LC_{50} for cadmium in recirculation open systems was 13.6 mg/l, and the 96-h LD_{50} 1.6 mg/kg of fish. Light microscopic studies were carried out in gills, livers and kidneys. Gill alterations included an increased number of chloride cells, breakdown of the pillar cells and edema of the epithelial cells. In the liver there was blood conjestion in sinusoids and swelling of hepatocytes. The kidneys showed vacuolation and necrosis of proximal tubular cells.

***Keywords*:** Histopathological alterations; cadmium exposure; acute; subacute; catfish

1. Introduction

In the recent years, industrial development and agricultural processes have resulted in increased levels of toxic metals in the environment, although relatively high concentrations can also occur naturally (Lopez Alonso *et al*., 2002). Cadmium is widely used in several industries and is well known as a highly toxic pollutant (Wright and Welbourn, 1994). The major uses of cadmium include electroplating, pigment production, and manufacturing of plastic stabilizers and batteries. In ecotoxicological studies cadmium has been implicated as a cause of numerous human deaths and various deleterious effects in fish and wildlife. Fish have been the focus of many ecotoxicological studies since they are located at the top of the aquatic food chain. Cadmium has been shown to cause pathological changes of varying severity in several organs, such as gill, liver and kidney (Rangsayatorn *et al*., 2004).

The gill epithelium of the fish is the major site of gas exchange, acid-base balance, ionic regulation, and excretion of nitrogenous waste (Thopon *et al.,* 2003). The liver plays a major role in metabolism excretion, excretion, digestion and storage or various substances, including some that are toxic to fish. Histopathological alterations in fish liver and kidney are key indicators of chemical toxicity, and it is a useful way to study the effects of exposure of aquatic animals to toxins present in the aquatic environment (Thopon *et al.,* 2004; Athikesavan *et al.,* 2006; Loganathan *et al.,* 2006).

Hybrid walking catfish (*Clarias macrocephalus* x *Clarias gariepinus*) is an economically important fish, which is commercially cultivated in Thailand. Because of its relatively high market value, it has become an attractive commodity of both large and small-scale aquaculture enterprises. In the present study, hybrid walking catfish was chosen as the target organism for the cadmium toxicity study. The objectives of the present study were to determine the median lethal concentration of cadmium by exposure in water and by intraperitoneal injection, and to study the histopathological alterations in the target organs in acute and subacute cadmium exposure.

2. Materials and methods

2.1. Experimental fish

Three-month old male hybrid walking catfish (length 20-25 cm) were obtained from a commercial fish farm in Chachoengsao Province, Thailand. The fishes were acclimatized for 14 days in well aerated holding polyethylene tanks (~500L). The water in the tanks was passed through a 1 m filter. The animals were fed once daily with granule food and they were starved for 24 h before and during experiment.

2.2. Chemicals

A stock solution of 1000 mg/l cadmium was prepared with 1.792 g $CdCl_2.H_2O$ dissolved in 1000 ml deionized water.

2.3. System design

A recirculation open system was used in a 96-L glass aquarium (40×60×40 cm). The water was circulated continuously at a rate of 4 ml/min and continuous aerated throughout the experiment.

2.4. Experimental procedures

2.4.1. Acute toxicity tests

A static bioassay test was performed to determine the median lethal concentration of cadmium chloride for hybrid walking catfish, following the Standard Methods (APHA, 1995). After the acclimatization period, the animals were transferred from the stock tank to the experimental aquaria. The study was then divided into two parts, the 96-h LC_{50} value was calculated from fish which were exposed to cadmium in water, and the 96-h LD_{50} was calculated from fish which were exposed to cadmium by an intraperitoneal injection.

Exposure in water: The test consisted of a control and a series of five concentrations of cadmium (5, 10, 20, 25 and 30 mg/l). There were three replicates, with ten fishes in each replicate.

Exposure by intraperitoneal injection: The tests consisted of a control and a series of five doses of cadmium (0.5, 1.0, 2.0, 3.0 and 4.0 mg/kg fish). There were three replicates with ten fish in each replicate.

The criteria for death were no gill movement and no reaction to gentle prodding. Fish mortality in each aquarium was recorded at the intervals 24, 48, 72 and 96 h. Dead fish were immediately removed.

2.4.2. Acute exposure (1-4 days)

Exposure in water: Fish were exposed to nominal 6.8 mg/l Cd as $CdCl_2.H_2O$ from stock solution. The dose chosen was 50% of the 96-h LC_{50} value obtained from the acute toxicity test, which was 13.61 ± 0.58 mg/l (n=10). Two fish from each aquarium were sampled for histopathological studies after 24, 48, 72 and 96 h of exposure.

Exposure by intraperitoneal injection: The dose of cadmium chosen, 0.8 mg/kg of fish was 50% of the 96-h LC_{50} value obtained from the acute toxicity test. Two fish from each aquarium were sampled 24, 48, 72 and 96 h after injection.

2.4.3. Subacute exposure (10-30 days)

The chosen concentration was the lowest observed effect level (LOEL) which was calculated from the acute toxicity test. The fish were fed once daily with granule fish food at 9 am. Uneaten food was quickly removed from the system.

Exposure in water: The chosen concentration of 3.0 mg/l was the lowest observed effect level (LOEL) which was calculated from the acute toxicity test by exposure in water. Six fishes were kept in the aquarium with cadmium, and two of these fishes were sampled after 10, 20 and 30 days, respectively. Two other fishes served as controls and were kept in an aquarium without any cadmium.

Exposure by intraperitoneal injection: The chosen concentration was 0.02 mg/kg of fish, which was calculated from the acute toxicity test by intraperitoneal injection. Six fishes were injected with cadmium, and two of these fishes were sampled at 10, 20 and 30 days, respectively. Two other fishes served as controls and were not given any injection.

2.5. Data analysis

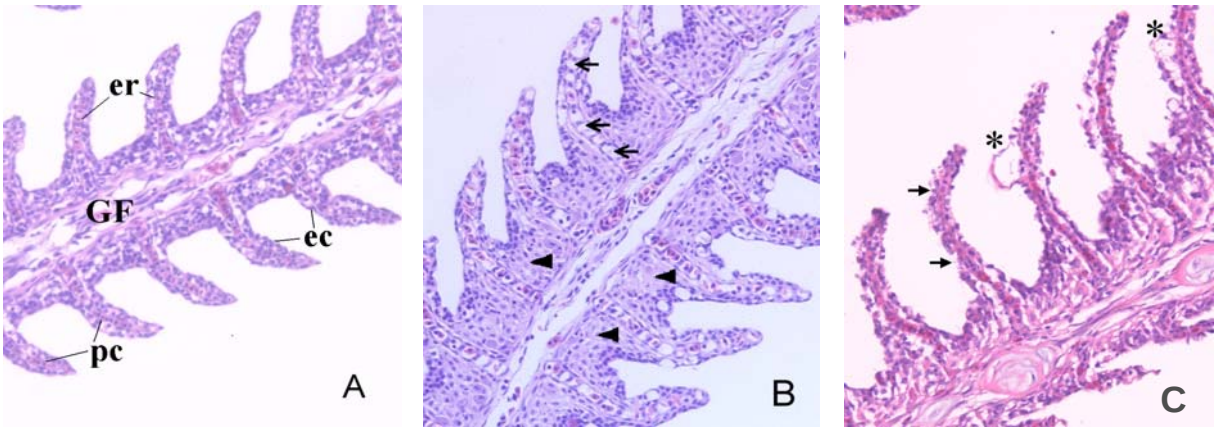

Figure 1. (A) Gill from control group: gill filaments showing normal appearance of erythrocytes (er), pillar cells (pc) and epithelial cells (ec). x250. (B) Gill from fish exposed to cadmium (6.8 mg/l) in water for 1 day. Marked increase of chloride cells (▶) and breakdown of pillar cell (➜). x250. (C) Gill from fish 30 days after intraperitoneal injection of cadmium (0.02 mg/kg). Marked edema (✱) and breakdown (➜) of epithelial cells. x250.

The median lethal concentration and lowest observed effect level of cadmium were determined by using the Probit analysis method (Finney, 1971).

2.6. Light microscopic study

The fish were killed and tissue specimens of liver, kidney and gill (second arch) were cut into small pieces of 3 mm thickness and fixed in 4% neutral buffered fomaldehyde for 24 h. Tissue specimens from gill were decalcified in formic acid-sodium citrate solution for 3 h. Fixed tissues were rinsed in tap water, dehydrated through a graded series of ethanol, infiltrated with xylene and then embedded in paraffin. Four-micron thick sections were cut in the microtome from the tissue blocks and picked up on glass slides. The sections were deparaffinized in xylene, rehydrated through decreasing concentrations of ethanol, stained with hematoxylin and eosin (Humason, 1966), and then examined by light microscopy.

2.7. Semiquantitative scoring

Histopathological alterations were assessed using a score ranging from - to +++ depending on the degree and extent of the alteration: (-) none, (+) less than 30% of occurrence, (++) 30% -70% of occurrence, (+++) more than 70% of occurrence.

3. Results

3.1. Water quality

The water characteristics of the aquaria are described in Table 1.

Table 1. Water quality characteristics during the experiment (acute toxicity test, acute exposure and subacute exposure)

Parameter	Range
Temperature (^{o}C)	25.0 – 28.2
Dissolved oxygen (mg/l)	7.0 – 7.2
pH	6.9 – 7.2

3.2. Visual observations

The control fish swam normally. The fish exposed to high doses of cadmium in water swam erratically with an increased open-close rate of the opercula, and had an increased amount of mucus on the body. Exposure to cadmium by intraperitoneal injection also resulted in an increased open-close rate of the opercula; in addition the abdomen was swollen but there was a decreased amount of mucus on the body.

3.3. Histopathological alterations

3.3.1. Gills

In catfish exposed to cadmium in water the proportion of chloride cells was increased during the first four days. There was some breakdown of pillar cells during the entire period of cadmium exposure. The epithelial cells displayed considerable edema and some breakdown from the third day and onwards.

Also in the catfish given cadmium by intraperitoneal injection there was a hyperplasia of chloride cells during the first four days. Breakdown of pillar cells occurred later. Epithelial cell edema was marked from the third day after injection, and there was considerable breakdown of these cells in the subacute period (Fig. 1, Tables 2 and 3).

Table 2**.** Overview of microscopical observations in catfish after exposure to cadmium in water.

		Acute exposure(day)				Subacute exposure(day)		
		1	2	3	4	10	20	30
gill	Hyperplasia of chloride cells	++	++	++	+	-	-	+
	Breakdown of pillar cells	++	+	+	+	++	+	+
	Epithelial cells							
	- Edema	-	+	+++	++	++	+	+++
	- Breakdown	-	-	+	+	+	+	++
liver	Blood congestion in sinusoids	-	-	++	++	+	+	++
	Cell swelling	+	++	+	-	-	+	-
kidney	Vacuolation of proximal tubule epithelium	+	++	++	++	-	-	+
	Necrosis of proximal tubule epithelium	-	-	+	+	-	-	+

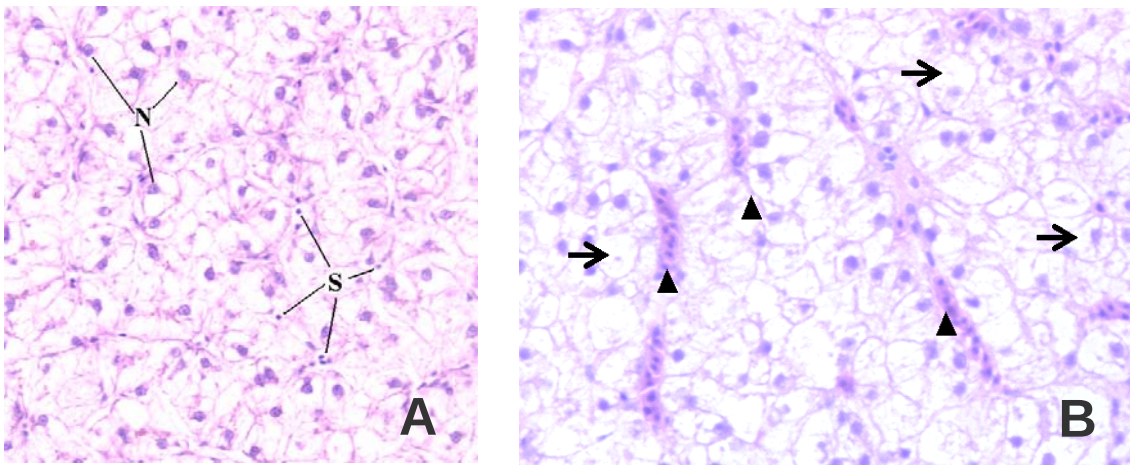

Figure 2. (A) Liver of control group, hepatocytes showing normal appearance of nucleus (N) and sinusoids (S). x250. (B) Liver after exposure to cadmium in water (6.8 mg/l) for 3 days. Marked cell swelling (➜) and blood congestion in sinusoid (►). X250.

3.3.2. Liver

In fish exposed to cadmium in water there was hepatocyte swelling mainly during the first 3 days of exposure. Blood congestion in sinusoids was observed from the third day and onwards.

After intraperitoneal injection of cadmium hepatocyte swelling was observed at the beginning and at the end of the study period. Blood congestion in sinusoids was moderate (Fig. 2, Tables 2 and 3).

3.3.3. Kidney

Fishes exposed to cadmium in water displayed vacuoles mainly in the epithelium of the proximal tubules, starting on the first day of exposure. It became more pronounced up to four days, and then less marked during the subacute stage. Necrosis of proximal tubules was evident after three and four days.

Intraperitoneal injection of cadmium produced vacuolization of the epithelium of the proximal tubules at most of the time intervals studied. Marked necrosis was observed four days after injection (Fig. 3, Tables 2 and 3).

4. Discussion

4.1. Visual observations

In comparison with previous investigations, the results of the present study on catfish shows some differences. It should be borne in mind, however, that several factors could explain these differences, *i.e.*, the water characteristics, the test organs, the age of the animals, and the exposure method. By visual observation it was noted that the amount of mucus on the fish was much larger when they were

Table 3**.** Overview of microscopical observations in catfish after exposure to cadmium by intraperitoneal injection.

		Acute exposure (day)				Subacute exposure (day)		
		1	2	3	4	10	20	30
gill	Hyperplasia of Chloride cells	+	+	++	++	-	-	-
	Breakdown of pillar cells	-	-	+	+	+	+	++
	Epithelial cells	-	+	++	++	-	++	+++
	- Edema							
	- Breakdown	-	-	+	+	++	+++	+++
liver	Blood congestion in sinusoids	-	+	+	++	-	-	+
	Cell swelling	++	-	-	-	-	+	++
kidney	Vacuolation of proximal tubule epithelium	+	+	++	+	-	++	+
	Necrosis of proximal tubule epithelium	-	-	-	++	-	+	+

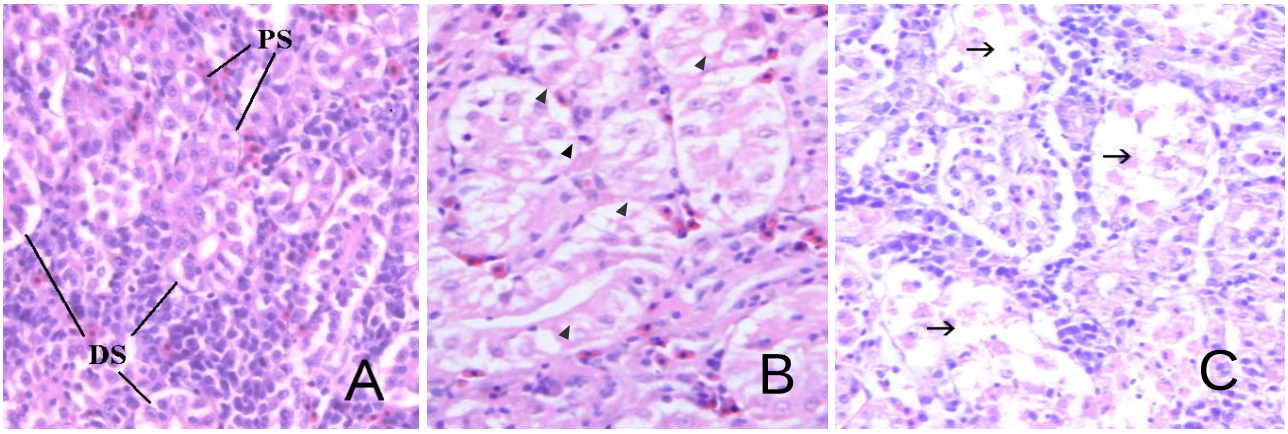

Figure 3. (A) Kidney from control showing normal appearance of proximal segment (PS) and distal segment (DS) of tubules. X250. (B) Kidney 1 day after intraperitoneal injection of cadmium (0.8 mg/kg). Marked vacuolation (►) of proximal tubule epithelium. X250. (C) Kidney 4 days after intraperitoneal injection of cadmium (0.8 mg/kg). Marked necrosis (➜) of proximal tubule epithelium. X250.

exposed to cadmium in water than when cadmium was given by intraperitoneal injection. This is in agreement with previous studies on *Lates calcarifer* (Chaiyamong, 2004; Thopon, 2003) and *Notopterus notopterus* (Ghosh and Chakrabarit, 1992). The source of the mucus is mucous cells, including goblet cells, in the epidermis of the fish. It is concluded that exposure to cadmium in water stimulated secretion from the cells, while an intraperitoneal injection did not.

4.2. Histopathological alterations

4.2.1. Gills

In the catfish exposed to cadmium in water there was an increased proportion of chloride cells, an edema of the epithelial cells, and a breakdown of both pillar cells and epithelial cells. These findings confirm previous observations in *Lates calcarifer* (Thopon, 2003).

In this context it is of interest to note that cadmium uptake by the gills takes place through calcium channels in the epithelial layer (Verbost *et al*., 1987; Verbost *et al*., 1989). The cadmium appears to bind to the active site of baso-lateral calcium pumps. Since calcium controls, to some extent, the permeability of the gills, displacement of calcium could stimulate loss and water uptake (Reid and McDonald, 1991), causing edema and hyperplasia of chloride cells.

4.2.2. Liver

As in the present study, Van Dyk *et al.*, (2005) observed congestion of blood vessels and cell swelling in *Cichlidae* 1 and 28 days after exposure to cadmium. In addition, they also found vacuolization of hepatocytes, which was not a prominent feature in the present study. Hinton *et al*. pointed out the swelling of hepatocytes as a direct effect of exposure to toxins; for instance a direct effect on cellular ATPases would severely upset ion transport mechanisms (Hinton and Lauren, 1990).

4.2.3. Kidney

Perhaps the most severe effects of cadmium intoxication were found in the kidneys of the catfish, with vacuolation and necrosis of the proximal tubule epithelium. These changes occur after exposure to cadmium in water, and even more severely after an intraperitoneal injection. Previous studies in *Leiostomus xanthurus* (Hawkins *et al.*, 1980) and in Rainbow trout (Forlin *et al.*, 1986) revealed similar type of damage to the proximal tubules. For this reason, it is expected that the fishes will suffer from severe disturbances in water and electrolyte balance.

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