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Effects of cadmium chloride on oxygen consumption and gill morphology of Indian flying barb, *Esomus danricus*

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Abstract

Effects of three sub lethal concentrations of cadmium chloride (0.636, 0.063 and 0.006 mg l^{-1}) on oxygen consumption and gill morphology in Indian flying barb, *Esomus danricus* (Hamilton-Buchanan), a teleost fish, were studied. When compared to control, 0.636 mg l^{-1} of cadmium chloride after 7, 14, 21 and 28 day exposure showed a significant decline in rates of oxygen consumption at 32.98, 28.40, 23.88 and 21.69 ml hr^{-1} $100g^{-1}$ of tissue, respectively; while, 0.063 mg l^{-1} of cadmium chloride for the same exposure durations showed a significant decline in rates of oxygen consumption at 34.28, 29.30, 28.05 and 26.47 ml hr^{-1} $100g^{-1}$ of tissue, respectively. However, significant decline in the rate of oxygen consumption at 0.006 mg l^{-1} of cadmium chloride could be observed from 21st day of exposure. Gill tissue showed various histopathological changes including epithelial lifting, hyperplasia, mucous secretion, marked leucocyte infiltration in the epithelium after 28 days of cadmium chloride exposure.

Key words

Cadmium chloride, Oxygen consumption, Gill histology, *Esomus danricus*

Introduction

Heavy metals are known to act on gill physiology, resulting in a decrease in the oxygen consumption because of ion-regulatory and acid-base disturbances. Oxygen consumption, therefore, could serve as a biomarker in metal toxicity studies in fish and other aquatic animals (Van Aardt and Booysen, 2004). Various heavy metals such as Cd, Cu, Cr^{6+}, Hg and Zn were found to have adverse effects on respiratory capabilities of different fish species (Radhakrishnaiah *et al*., 1993; De Boeck *et al.,* 1995; Jezierska and Sarnowski, 2002; Vutukuru, 2005; Van Aardt and Hough, 2006, 2007). Cd depressed oxygen consumption in crustaceans such as white shrimp, *Litopenaeus vannamei* (Wu and Chen, 2004). Cu was found to increase oxygen consumption and opercular beats in the ornamental fish, *Xiphophorus helleri* (James *et al*., 2003) and oxygen consumption and ammonia excretion in *Catla catla* (Parveen and Javed, 2010). On the contrary, Cu had a depressing effect on oxygen consumption in *Cyprinus carpio* (De Boeck *et al*., 1995) and in *Labeo capensis* and *Micropteris salmoides* (Van Aardt and Hough, 2007). Selenium and selenite were found to reduce oxygen consumption in *Gambusia affinis* at both lethal and sublethal concentrations, while the least toxic selenate decreased it at higher but increased it at lower concentrations (Naik and Patil, 2010). McGeer *et al*. (2000) observed a pattern of disturbance, recovery and stabilization in oxygen consumption in rainbow trout exposed for 100 days to 3 µg l^{-1} Cd. Thus, in spite of the general trend of decreased oxygen consumption under heavy metal exposure, deviations from this pattern could often be observed, especially for essential trace metals like Cu, Fe or Se.

Gill epithelium in fishes is the site for gaseous exchange, ionic regulation, acid-base balance and nitrogenous waste excretion,

and has a great potential to be used as a model system for understanding the various epithelial pathologies resulting from toxic exposure. The branchial epithelium is a versatile tissue that, in spite of the presence of kidneys performs a number of excretory and regulatory functions in fish (Evans *et al*., 2005). Exposure to 2 mg l^{-1} Fe at pH 5 and 6 caused significant thickening of the gill epithelium. Studies on gill histopathology (Poleksic and Mitrovic-Tutundzic, 1994), therefore, could enable us to better understand the mode of action of metals and other toxicants on fish.

Cadmium (Cd) is extremely toxic and provokes adverse effects on the biota in general and particularly on the fish (Wright and Welbourn, 1994). Cd is used in electroplating, pigments and plastic production and this has produced sharp increase in contamination of air, water and soil pollution. Exposure of juveniles and adults of freshwater fishes to water-borne sublethal Cd may lead to many stress symptoms such as disruption of ion and water balance, changes in respiratory function associated with structural damage of the gills, nephrotoxicity, alterations in haematological and biochemical parameters, adverse effects of growth and reproduction, neurological and behavioural changes (Espina *et al.,* 1995, 2000; Suresh *et al.,* 1993; Wendelaar, 1997). Therefore, it would be interesting to see how Cd affects the respiratory function in fish. In the present study, the Indian flying barb, *Esomus danricus* (Hamilton-Buchanan), a common teleost fish species of North India, which is economically important both as ornamental and food fish was used for observing the effects of cadmium chloride on its oxygen consumption and gill morphology.

Materials and Methods

Experimental design: Fish of similar length (46.77 ± 4.30 mm) and weight (0.86 ± 0.16 g) were collected from unpolluted, freshwater ponds near Assam University campus, Barak valley, South Assam, India. They were acclimatized under laboratory conditions for 7 days prior to experimentation. Temperature, dissolved oxygen and pH under laboratory condition were 29˚C, 5.5 mg l^{-1} and 6.8 respectively. Test media were renewed every 24 hrs and commercially available fish food was given *ad libitum* twice daily. Stock solution of cadmium chloride was prepared from its salt ($CdCl_2.H_2O$). Serial dilutions of stock solutions were prepared using chlorine free tap water as per dilution techniques (APHA, 2005). Based on 96 hrs LC_{50} value (6.36 mg l^{-1}) three sub-lethal test concentrations viz., 0.636, 0.063 and 0.006 mg l^{-1} were selected.

Oxygen consumption: Oxygen consumption (ml hr^{-1} $100g^{-1}$ of tissue) of fish was studied by close chamber method (Fitch, 1975) at 7 days interval for 28 days by Winkler's iodometric method. Ten fish for each test concentration (0.636, 0.063 and 0.006 mg l^{-1}) were kept separately in 3 l of toxicant treated media for 28 days. Controls were run in dechlorinated tap water. Statistical comparisons among the control and other values were made by one-way ANOVA and Tukey tests using SYSTAT 13 software for Windows.

Histology: Ten fish kept individually in 3 l of test solution for each concentration of 0.636, 0.063 and 0.006 mg l^{-1} cadmium chloride and control for 28 days were sacrificed and gills removed immediately, fixed in 10% formalin for 24 hr, dehydrated and embedded in paraffin. Sections of 5 µm thickness were cut and stained with Harris Haematoxylin and Eosin. Changes induced by cadmium chloride treatment in the gill were photographed and analyzed by light microscope at 10X eye piece magnification and 40X objective magnification (Olympus, model U-CMAD3) with camera attachment of Samsung (model SDC-313B)}.

Results and Discussion

The effects of exposure to cadmium chloride at three sublethal concentrations of 0.636, 0.063 and 0.006 mg l^{-1} on the oxygen consumption rate of *E. danricus* are presented in Table 1. Differences in oxygen consumption up to 14 days were not significant at 0.006 mg l^{-1}, which is $1/1000^{th}$ of the 96 h LC_{50} value of cadmium chloride for *E. danricus* (Das and Gupta, 2010). However, significant differences could be observed on the 21^{st} day, indicating that long-term exposure could produce toxic effects even at extremely low concentrations of cadmium chloride. At higher concentrations of 0.063 and 0.636 mg l^{-1} cadmium chloride ($1/10^{th}$ and 1/100th of 96 h LC_{50} values, respectively), significant reductions in oxygen consumption rates could be observed after 7 days of exposure. Metal toxicants produce a change in the respiration rate of fish, which is mostly a decline in the oxygen consumption rates (Peuranen *et al*., 1994; De Boeck *et al*., 1995; Van Aardt and Hough, 2006, 2007), although in a few studies, elements like Fe and Cu increased oxygen consumption (James *et al*., 2003; Parveen and Javed, 2010), while selenate decreased it at higher but increased it at lower concentrations (Naik and Patil, 2010). It is probable that Cu, Fe or Se that are essential trace elements have a stimulatory effect on oxygen consumption rates at low concentrations, although the process is reversed at higher, xenobiotic levels. On the contrary, Cd being a non-essential element brought about progressive decline in oxygen consumption at all the three concentrations; even at the lowest of 0.006 mg l^{-1}, the differences from control were found to be significant after longer exposure.

Table- 1: Effect of different concentrations of cadmium chloride on oxygen consumption of *Esomus danricus*

Treatment (mg l^{-1})	Oxygen consumption (ml hr^{-1} $100g^{-1}$ tissue)			
	7 day	14 day	21 day	28 day
Control (10)	38.94±0.46	39.19±0.29	39.03±0.24	39.06±0.15
0.006 (10)	38.76±0.53^{a}	38.3±0.49^{a}	36.79±0.86*^{a}	34.74±0.88*^{a}
0.063 (10)	34.28±1.04*^{b}	29.30±1.51*^{b}	28.05±0.91*^{b}	26.47±1.19*^{b}
0.636 (10)	32.98±1.11*^{c}	28.40±5.79*^{b}	23.88±1.38*^{c}	21.69±1.51*^{c}

Values are mean of ten fish ± SD; * indicates significant difference from control at $p \leq 0.05$; different alphabets in superscript indicate significant difference among the different values at each interval

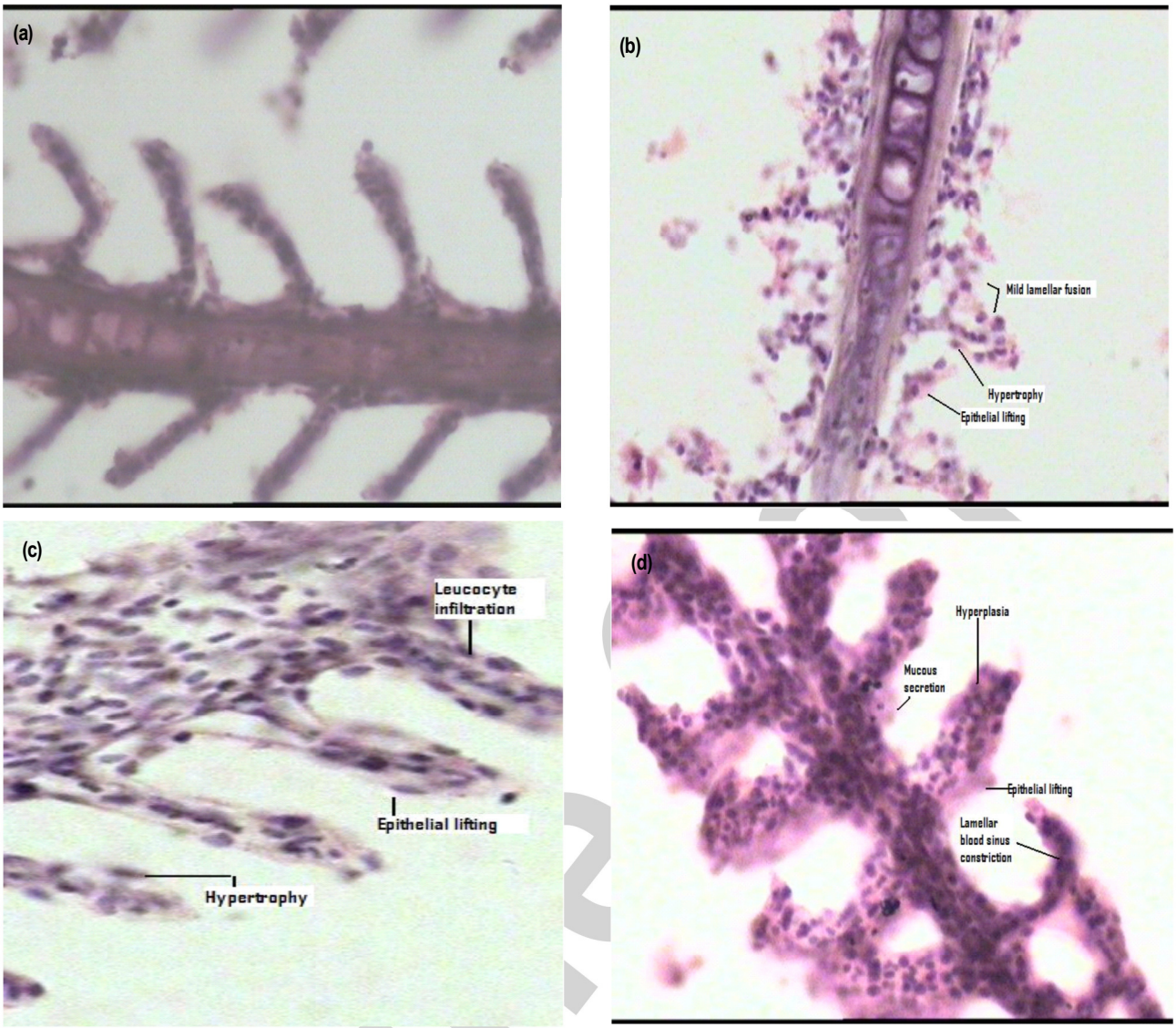

Fig. 1 : (a) T.S of gills of control fish, *Esomus danricus* showing normal gill architecture, (b) T.S. of gills of *Esomus danricus* exposed to 0.006 mg l^{-1} $CdCl_2$ for 28 days, showing mild changes like lamellar fusion, hypertrophy and epithelial lifting, (c) T.S. of gills of *Esomus danricus* exposed to 0.063 mg l^{-1} $CdCl_2$ for 28 days, showing leucocyte infiltration, hypertrophy and epithelial lifting, (d) T.S. of gills of *Esomus danricus* exposed to 0.636 mg l^{-1} $CdCl_2$ for 28 days, showing epithelial lifting, mucous secretion, hyperplasia and epithelial lifting, lamellar fusion and lamellar blood sinus constriction

The gill of unexposed fish showed clear unaffected gill architecture with intact gill lamellae and gill filaments (Fig. 1a). Gills of fish exposed for 28 days to 0.006 mg l^{-1} cadmium chloride showed slight epithelial lifting, hypertrophy and lamellar blood sinus constriction (Fig. 1b). These effects were more pronounced at 0.063 and 0.636 mg l^{-1} cadmium chloride with additional symptoms like mucous secretion and marked leucocyte infiltration in the epithelium (Fig. 1c, d). More than 80 % of the test fish showed distinct histological changes. *E. danricus* gill is covered by a complex epithelium whose function is controlled by perfusion through a rather intricate vascular system (Vutukuru *et al*., 2005). The gill epithelium is the dominant site of gas exchange, ionic regulation, acid-base balance, and nitrogenous wastes excretion by fishes (Evans *et al*., 2005), thereby serving a multitude of vital functions in these aquatic animals. Gills are also the major entry sites of metals and act as a transient store for accumulated metals (Soegianto *et al*., 1999). Our study shows that exposure to cadmium chloride even at low concentrations such as one-thousandth of the 96 h LC_{50} value can induce alterations in gill morphology and histology which in turn significantly depress oxygen consumption if the exposure is continued for a relatively longer period. Since symptoms of metal toxicity normally involve respiratory distress (Sorensen, 1991), the decreased oxygen consumption of cadmium chloride exposed fish is probably due to the absorbance of more metal through the gills which are in direct contact with the toxic test medium. At higher, though sublethal (one hundredth and one tenth of 96 h LC_{50}) cadmium chloride concentrations, more pronounced effects

in terms of edema, mucous secretion and marked leucocyte infiltration in the epithelium were found to set in. Direct damage to gill structure and function of fish caused by exposure to higher levels of different heavy metals has been reported in several studies, including one with different fish species exposed to Cu at concentrations ranging from 0.1-10 mg l^{-1} that showed edema, lifting of epithelium, hyperplasia, lamellar aneurysm, mucous secretion and structural damage to gill lamellae (Van Aardt and Hough, 2007; Figueiredo-Fernandes *et al*., 2007; Georgieva *et al*., 2010). Similar effects were produced by lead at 24 and 71 mg l^{-1} (Martinez *et al.,* 2004); nickle at 5.7 mg l^{-1} in *Hypophthalmichthys molitrix* (Athikesavan *et al*., 2006); zinc at 0.1 mg l^{-1} (Velcheva *et al*., 2010); and in the case of cadmium, at concentrations ranging from 0.3-10 mg l^{-1} in different fishes (Susithra *et al*., 2007; Pantung *et al*., 2008; Kaoud *et al.,* 2011). In the context of the above findings, initiation of epithelial lifting at 0.006 mg l^{-1}, and leucocyte infliltration, lamellar blood sinus constriction, hyperplasia and mucous secretion beginning at 0.063 and becoming pronounced at 0.636 mg l^{-1} of cadmium chloride reflect the vulnerability of small fish species like *E. danricus* to toxic metals like cadmium.

It has been suggested that edema resulting in lifting of lamellar epithelium could serve as a mechanism of defence, because separation of epithelia from the lamellae increases the distance across which waterborne pollutants must diffuse to reach the bloodstream (Arellano *et al.,* 1999). This mechanism also increases the diffusion barrier to the pollutant. In a study, the distance from water to blood was significantly elevated after exposure to copper (Van Heerden *et al.,* 2004). The proliferate thickening (hyperplasia) of gill epithelium is a histological change that appears to be a general safety measure against toxicants as has also been reported by Pandey *et al.* (1993). Our study, therefore, reveals that the histopathological changes brought about by cadmium chloride exposure in the gill epithelium of *E. danricus* is in accordance with the depression in rates of oxygen consumption, which in turn might be associated with osmoregulatory, acid base, or haemodynamic malfunctions.

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