ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives, 2013; 4(5): 999 - 1006

ORIGINAL RESEARCH ARTICLE

Light and Scanning Electron Microscopic Evaluation and Effects of Cadmium on the Gills of the Freshwater Fish, *Labeo rohita*

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Received 20 Jul 2013; Revised 18 Oct 2013; Accepted 26 Oct 2013

ABSTRACT

The effects of chronic exposure for 10, 20 and 30 days to 10% sub-lethal concentration of cadmium (96 hrs LC_{50} – 3.176 mg/l; 10% SLC 0.3176 mg/L) on the gill histology of the freshwater fish *Labeo rohita* was studied under light and scanning electron microscopy. The gill epithelium of untreated group showed a normal architecture while histological lesions were observed in exposed fishes. The cadmium induced gill damages were fusion of secondary lamellae, hypertrophy, hyperplasia, edema, increased number of mucus opening and necrosis. The severity of the lesions increased with increase in exposure period.

Key words: *Labeo rohita*, Cadmium, LC_{50}, Gill histology, Light and SEM.

1. INTRODUCTION

Aquaculture has made substantial contribution as food to the growing human population. Fish are valuable sources of high grade proteins, mineral salts including calcium, phosphorus and iodine, essential amino acids, omega 3 fatty acids and vitamins A, B, D and E [1]. Fish proteins occupy an important place and it constitutes about 17 - 20%. Moreover, carbohydrate content of the fish flesh is very low and hence, fish can make valuable contribution to any diet [2]. Besides providing food to man, fishes are sources of numerous by products such as fish liver oil, fish flour, fish silage, fish glue, Isinglass etc. which have medical and economic importance. Considering their nutritional value, it is essential for any nation, which is desirous of developing this rich protein source, to develop an efficient aquaculture system, for which maintenance of the environmental water quality is a prerequisite. However, increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment poses problems to humanity, particularly to the aquatic organisms [3].

Among the industrial wastes, heavy metals cause a hazard not only to mankind but also to other plants and animals. Heavy metals are non – degradable and are regarded as hazardous to the aquatic ecosystem for their environmental persistence and their ability for accumulation [4]. Among the non – essential metals, cadmium is one of the most hazardous elements and is recognized carcinogen in mammals [5]. It is also stable like other metal and does not degrade in the environment [6].

Cadmium (atomic weight 112.4 and atomic number 48), a naturally occurring non – essential element found in earth's crust associated with zinc and copper ores was discovered by Fredrich Stromesper in 1817, but was not used commercially until the end of the 19th century. This soft, silver – white metal was first used in paint pigments and as a substitute for tin in World War I. Today, about three – fourths of cadmium is used in battery, electroplating, mining, paints and dye industries [7]. The environmental protection Agency (EPA) has found cadmium to potentially cause a variety of effects from acute exposures in human beings include nausea, vomiting,

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abdominal pain and breathing difficulty. Chronic exposure to cadmium result in kidney dysfunction, lung cancer and prostate cancer [8].

Fish gills are regarded as a major site of respiration, osmoregulation and excretion and remain in close contact with the external environment and particularly sensitive to changes in the quality of water and considered the primary target of the contaminants. For this reason, they are considered excellent indicators of environmental quality [9].

Histopathological studies have been conducted to establish fundamental relationships between contaminant exposure and various biological responses. Histopathological investigation has been increasing recognized as a valuable tool for assessment of the impact of environmental pollutants in fish [10-14]. Over the past two decades, the histopathological changes in gills under acute and chronic exposure to heavy metals have been studied in many fish species [14-29]. Hence the present study aimed to investigate the impact of cadmium on histological structure of gill of Indian major carp *Labeo rohita* in order to understand to mode of action, stress response and organ dysfunction

2. MATERIALS AND METHODS

2.1. Test chemicals

The analytical grade cadmium sulphate ($3Cd.So_4)8H_2O$ was obtained from New India Chemical Enterprises, Cochin, India and used without further purification for the experiment.

2.2. Animal maintenance

The freshwater healthy fingerlings *Labeo rohita* of the weight (10 ±19g) and length (8±0.5 cm) were selected for the experiment and were collected from Katherasan Aquafarm near Thanjavur, Tamil Nadu, India. The collected fish were safely brought to the laboratory and acclimatized for one month in a large cement tank (1000 L capacity). During the acclimatization period, the fish *ad libitum* with rice bran and groundnut oil cake which had no detectable amount of cadmium. Food was provided once a day. The water was renewed daily to avoid accumulation and contamination of excretory materials and feeding was withheld 24 h before the commencement of the experiment.

Fish showing and abnormal behavior was removed as soon as possible. In the present study tap water free from chlorine was used which had the following physico – chemical characteristics [30]; temperature 28±0.13 pH 7.6±0.04, salinity 1.2±0.13 ppt, D.O.5.6±0.2 mg / l and total hardness 35±0.5 mg / L. Before the start of the experiment suitable numbers of fish were transferred into two glass aquaria which were continuously aerated.

2.3.Preparation of stock solution and determination of 96 h LC_{50} value of cadmium

Stock solution of cadmium was prepared by dissolving 1 g of cadmium in an appropriate amount of water. For the determination of median tolerance limits or LC_{50} different concentrations of cadmium (1,2,3,4 and 5mg/L) were prepared from the stock and added in separate glass aquaria containing 50 L of water. Three replicates were maintained for each concentration and 10 fishes of equal size and weight were introduced. The test water was renewed at the end 24 h and freshly prepared cadmium was added to maintain the concentration of cadmium at a constant level. A concurrent control of 30 fish in three different glass aquaria was maintained under identical conditions. The mortality was recorded after 24, 48, 72 and 96 h, and median lethal concentration (LC_{50}) values were calculated by the Finney method [31]. $1/10^{th}$ value of the LC_{50} value for 96 hrs was taken as the sub-lethal concentration [32].

2.4. Sub-lethal studies

For sub-lethal toxicity tests 200 fingerlings were selected and divided into four groups (one control and three experimental) with 50 fish in each aquarium filled with water. The desired concentration (1/10 of 96 h LC_{50}) of the toxicant was added directly in order to maintain constant concentration of the toxicant. The experiment was conducted for 30 days and sampled at 10 days interval and no mortality was observed during the above treatment period. At the end of the stipulated periods (10^{th}, 20^{th} and 30^{th} day) of exposures fish were randomly selected and sacrificed for histological studies.

2.5. Histology

2.5.1. Light Microscopic Studies

On 10, 20 and 30^{th} day fish were taken out, sacrificed and the gill was excised out. The gill tissue was fixed in Bouin's fluid and then they were processed [33] and embedded in paraffin wax ($58 – 60^0C$). Serial sections of 8 µm thickness were cut and deparafinshed sections were stained in haematoxylin and counterstained with aqueous eosin.

2.5.2. SEM studies

The gills were dissected, washed in 1% phosphate buffer and fixed in 3% glutaraldehyde. Then gill tissues were dehydrated in a graded alcohol – acetone mixture series dried using the critical point technique. The dried gills were mounted on the stub and were coated with gold in a gold coating unit. The morphology of the gills was examined under a JOEL JSM 6360 SEM Japan [34].

3. RESULTS

3.1. LC_{50} value – 96 hrs

The results of the acute toxicity test are presented in (**Table 1**). The LC_{50} value based on probit analysis was found to be 3.176 mg/l for 96 h of exposure to cadmium (Fig a - d). During this study the behavior of the control fish was normal, while the fish introduced into the sublethal concentration of the cadmium showed different abnormal behavior. Abnormal behavior such as erratic swimming, increase in surface activity, spreading of excess of mucus of the body and restlessness were observed in fish exposed to the cadmium

3.2. Histological study

3.2.1. Control gill – light microscopic observation

The structure of gill in *Labeo rohita* consist of highly vascular plate like process called primary and secondary lamellae. The secondary lamellae of the gill appeared as finger- like structure and are covered with thin layer of epithelial cells. They are very thin, slender and attached on either side of the primary lamellae (**Fig 1**).

3.2.2. Histological changes of gill tissue induced by Cd under light microscopic observation

In sub-lethal exposure of cd, the gill of *Labeo rohita* showed marked histological changes. Appreciable changes were noticed in the histology of gill after 10 days treatment including fusion of secondary lamellae, degeneration of epithelium and vaculation (**Fig 2**)

The damage was more severe and progressive after 20 days of exposure. The primary and secondary gill lamellae were damaged to a great extent. Hypertrophy, hyperplasia, necrosis and lamellar fusion were observed (**Fig 3**). However such changes were drastic to the extent that disintegration of lamellar epithelium fusion of secondary lamellae and degenerated secondary lamellae were found in the 30 days treated fish (**Fig 4**).

3.2.3. SEM study of control gills

In gills of control *Labeo rohita*, the primary gill lamellae appeared normal architecture and mucous free and uniform branching of secondary lamellae from primary lamellae (**Fig 5**). The gill filaments bear micro ridges on the surface epithelium (**Fig 6**).

3.2.4. Histological alterations of gill in Cd treated fish under SEM observation

The damages, fusion of secondary lamellae and edema of primary lamellae, were observed after 10 days of exposure (**Fig 7**). On exposure to cd for 20 days, epithelial hypertrophy, fusion of secondary lamellae and necrosis were observed (**Fig 8**). In fish, treated upto 30 days, the changes observed in the gill of *Labeo rohita* were deformation and edema of primary and secondary lamellae, fusion adjacent lamellae (**Fig 9**) and degenerating microridges with mucous opening (**Fig 10**).

4. DISCUSSION

Fish gills are considered that most vulnerable organ for the toxicants [35] because they are in direct contact with the surrounding water. Alterations in gill structure affect the normal functioning of vital physiological processes such as gas and ion exchanges, osmoregulation, excretion of nitrogenous wastes and acid – base equilibrium [36,9].

Pathological lesions induced by cadmium were characterized by hypertrophy, hyperplasia, fusion of secondary lamellae and necrosis. Several authors have reported histopathological anomalies in gills of other species exposed to heavy metals.

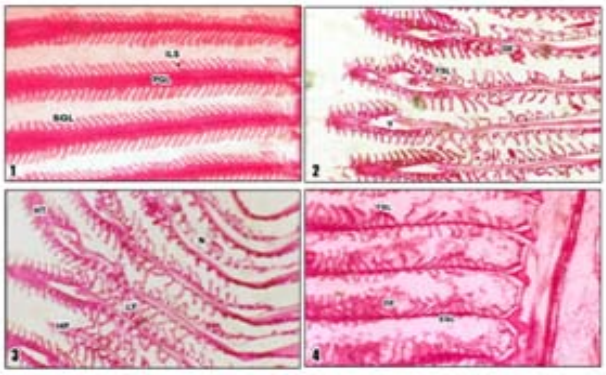
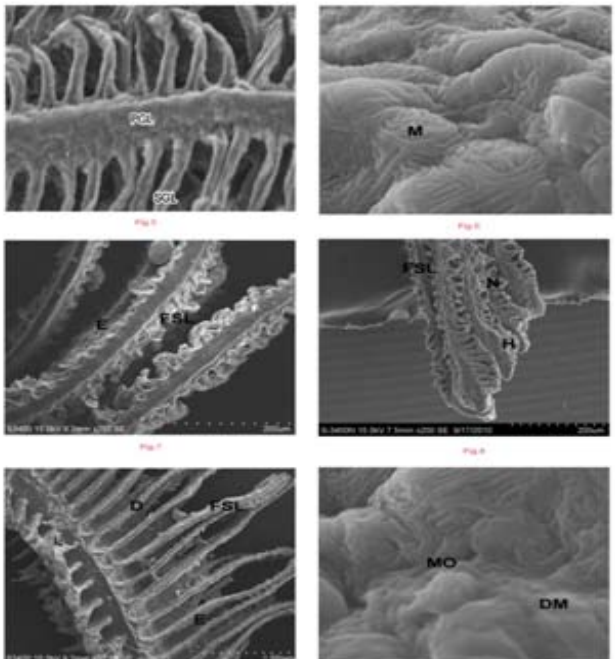


Fig.1. Light micrographs of the gills of Labeo rohita (control). Primary lamella (PGL) with uniform interlamellar space (ILS) and secondary gill lamellae (SGL) (H&E X200). Fig.2. Fusion of secondary lamellae (FSL), degeneration of epithelium (DE) and Vaculation (V) of the gill of 10 days Cd treated fish. (H&E X200).Fig.3. Hypertrophy (HT), hyperplasia (HP), necrosis (N) and lamellar fusion (LF) of the gill of 20 days treated fish. Fig.4. Disintegration of epithelium (DE), fusion of secondary lamellae (FSL), erosion of secondary lamellae (ESL) of the gill of 30 days Cd treated fish (H&E X200).



5. Scanning electron microscopes of the gill of *L.rohita* (control). Normal architecture of gill. Primary gill lamella (PGL), secondary gill lamellae (SGL). 6. Microridges (M) on the normal gill epithelium. 7. Fusion of secondary lamellae (FSL), edema (E) of the gill of 10 days cadmium treated fish. 8. Hypertrophy (H), fusion of secondary lamellae (FSL) and necrosis (N) of the gill of 20 days cadmium treated fish. 9. Deformation (D), edema (E) and fusion of secondary lamellae (FSL) of the gill of 30 days cadmium treated fish.10.Degeneration microridges (GM) and mucous opening (MO) of the gill of 30 days cadmium treated fish.

Table 1: Per cent mortality of *Labeo rohita* exposed to different concentrations of cadmium for different periods

Hours of Exposure	$LC_{50(ppm)}$	L.C.L	U.C.L	Regression Equation	Calculated χ^2 value	Table χ^2 value
24	4.68942	5.273744	4.169836	Y= 2.315143 + 4000568 X	20.61743	14.07
48	4.103295	4.658179	3.61451	Y= 2.638352 + 3.851774 X	27.77094	14.07
72	3.622016	4.023861	3.2603	Y= 2.951044 + 3.665722 X	22.79392	15.51
96	3.176811	3.624564	2.78437	Y= 3.519341 + 2.949571 X	18.27809	14.07

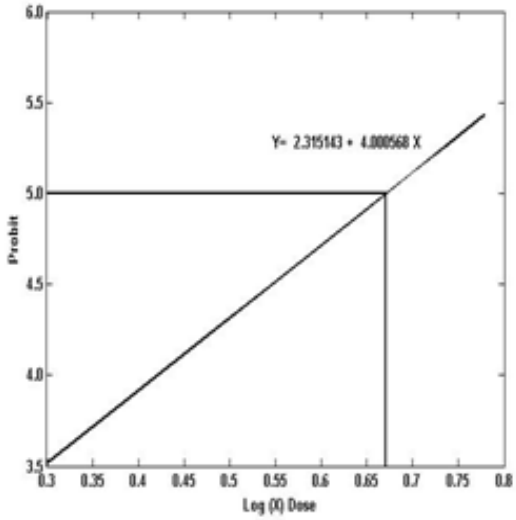


Fig a: Linear relationship between probit response and log concentration of cadmium exposure to *Labeo rohita* fingerlings on 24 hrs

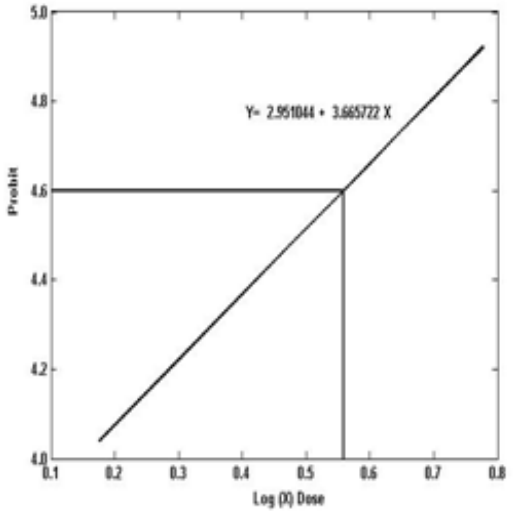


Fig b: Linear relationship between probit response and log concentration of cadmium exposure to *Labeo rohita* fingerlings on 72

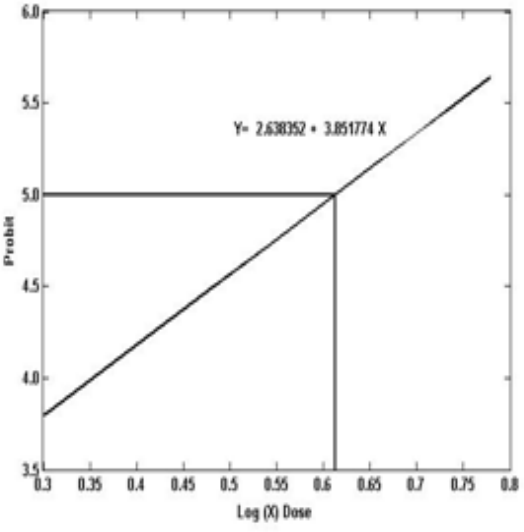


Fig c: Linear relationship between probit response and log concentration of cadmium exposure to *Labeo rohita* fingerlings on 48 hrs

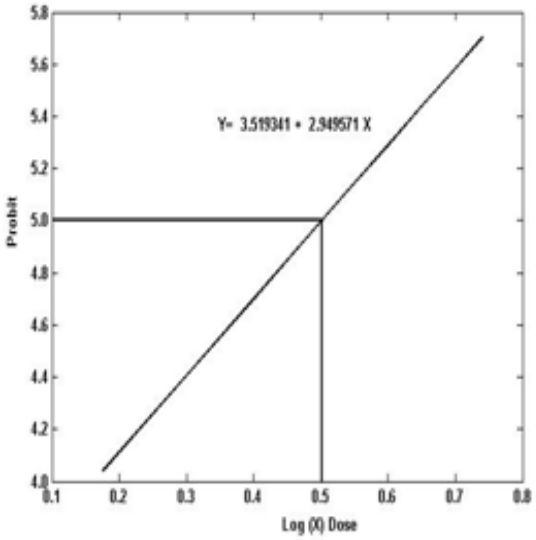


Fig d: Linear relationship between probit response and log concentration of cadmium exposure to *Labeo rohita* fingerlings on 96 hrs

Gupta and Rajbanshi [20] observed fusion and clumping of gill lamellae in mercury treated *Rasbora daniconius*. [37], Venkatesan and Subramanian [38] and Campagna *et al*., [39] noted similar types of gill lesions in copper treated *Danio rerio*, *Oreochromis mossambicus* and *Brochilodus scrofa*. Gupta and Kumar [23] also noted serve gill lesions in mercury treated *Cirrhinus mrigala*. In the present study, degeneration of gill lamellae necrosis and edema of secondary lamellae were apparent in *Labeo rohita* exposed to cadmium (Fig.) These observations are quite comparable to pathological lesions induced in gills by nickel in *Hypophthalmichthys molitrix* [40], by inorganic mercury treatment in *Salvelinus alpines* [22] and by treatment with cadmium in grass carp *Ctenophanyngodon idella* [41]. Radhika and Krishnamoorthy, [27] also noted alterations in the copper induced gill tissues of *Oreochromis mossambicus*. The present study showed that the gills of *Labeo rohita* exposed to Cd during 30 days presented a higher occurrence of histopathological lesions such as hypertrophy, hyperplasia and fusion of gill lamellae. These observations are in good agreement with the results reported by Gupta and Dua [42] in the air breathing freshwater fish *Channa punctatu* treated with mercury; Thophan *et al*. [43] and Rangsayatron *et al*. [22] in *Lates calcarifer* and *Puntius gonionotus* treated with cadmium Pane *et al*. [44] in nickel administrated *Oncorhynchus mykiss*. Further Al – Attar [25] also observed such gill damages in nickel treated *Oreochromis niloticus*.

The SEM is a technique that allows the study of the damage of surface ultrastructure of the gill epithelium that cannot be revealved by light or TEM [45,46]. The scanning electron micrographs of the gill epithelium also revealved that rohu of untreated group showed normal architecture. In contrast the present study showed that the gills of *Labeo rohita* exposed to cadmium during thirty days presented a higher occurrence of histopathological lesions such as hypertrophy, fusion of secondary lamellae, edema and mucus openings. These pathological changes may be a reaction to toxicants intake or an adaptive response to present the entry of the pollutants through the gill surface [47]. The damages observed in the gills in terms of hypertrophy, fusion of secondary lamellae and necrosis could cause a decrease in free gas exchange, thus affecting the general health of fish [48]. Similar of these changes in gill epithelia of *Oreochromis niloticus* were ultrastructurally observed by Nath and Kumar [49]. Crespo [18] in the dog fish, *Scyliorhinus canicula* subjected to zinc sulphate; Temmink *et al* [50] in rainbow trout, *Salmo gairdneri* exposed to chromate; Gupta and Dua [42] in the *Channa punctatus* intoxicated with mercury. Pane *et al*., [44] in *Oncorhynchus mykiss* treated with nickel. Acharya *et al*. [51] in *Labeo rohita* treated with sublethal acidic (HCl) and alkaline (NaoH) pH. In the study of Muthukumaravel *et al*.[26], copper exposure resulted in marked ultrastructural damage to the respiratory epithelium of gill in *Oreochromis mossambicus* including swelling and fusion of secondary lamellae. Palaniappan *et al*. [52] observed hypertrophy, hyperplasia, alteration of lamellar surface and fused lamellae in pb exposed *Catla catla*.

5. CONCLUSION

In the present study, it can be stated that cadmium exposure during sublethal treatment produces severe toxic effects on the respiratory organ of the freshwater fish *Labeo rohita*. The finding of the present study indicate that ultrastructural changes observer serve as “biomarkers” for assing heavy metal toxicity in aquatic environment.

ACKNOWLEDGEMENTS

The authors are thankful to the Principal, Khadir Mohideen College, Adirampattinam and Dr. S.

Sriman Narayanan, University of Madras for providing necessary laboratory facilities.

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