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CURATIVE ROLE OF ASCORBIC ACID ON CADMIUM TOXICITY INDUCED IN GILLS OF THE FRESHWATER CATFISH, HETEROPNUESTES FOSSILIS (BLOCH)

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ABSTRACT

This study reports the protective effect of ascorbic acid on cadmium induced to pathological Change in gills of the freshwater catfish, *Heteropnuestes fossilis*. Exposure of the fish to 16.14 mg/l sub lethal concentration of cadmium for 30 days induced fusion of secondary lamellae, aneurysm, curling of lamellae, hypertrophied secondary lamellae, congestion, increased number of mucous cells, autolysis of epithelial lining and disorganization and disintegration of pillar cell system, extensive curling of lamella and loss of normal gills architecture induced by cadmium in gill of *H. fossilis*. However 16.14mg/l cadmium +1.25mg/l ascorbic acid exposed fish groups revealed signs of recovery of architecture and structure of primary lamella, secondary lamella and pillar cell restoration of normal number of mucous cells after ascorbic acid supplementation. Our study concludes that ascorbic acid have protective influence on cadmium toxicity.

KEYWORD: *Heteropnuestes fossilis*, Cadmium, Gills, Histopathology, Ascorbic acid.

INTRODUCTION

Shortage of pure fresh water is great problem at present and coming future for living organism's existence. Pure fresh water become polluted by various activities of human organism by raw or partially treated sewage of millions of people, different toxic chemicals are used in agriculture industrial effluents mining etc. Drain of heavy metals in freshwater by various activities. Among heavy metals cadmium is considered as one of the most toxic pollutants (Arno *et al*, 2002; Sastry and Shukla, 1994; Reddy, 2012). Cadmium naturally

released into the environment from volcanic sources, along with rain water, different water bodies. On the basis of several studies indicate that heavy metals load including Cadmium in fishes and other consumable fauna (Rani *et al.*, 2015; Jayakumar *et al*., 2016; Reddy *et al*., 2011) and their biomagnifications potential via food web pose serious health risk to fish consumers (Chavan and Muley, 2014). Reports denoting protective influence of some metals, vitamins chelating agents and protein diets on metal toxicity in altering various physio-biochemical and behavioural aspects in fish have also been documented (Rathore and Naik, 1994; Sastry and Shukla, 1994; Girish kumar *et al*., 2014). In consideration of these facts, the present study was undertaken which reports protective influence of ascorbic acid on cadmium toxicity with reference to gill histopathology in the fresh water cat fish, *Heteropnuestes fossilis* (Bloch).

MATERIALS AND METHODS

The fish, *H. fossilis* of an average length 16.5 (± 2.0) cm and weight 40 (± 1.45) g were procured from a local unpolluted fish pond through fishermen to whom the pond was leased out. Their transportation to the laboratory, maintenance, acclimation and feeding procedures are described by Choudhary and Jha, 2014. The water was changed every day during acclimation and chronic experimentation immediately after feeding. Static acute bioassays (APHA, 1998) were performed to determine LC_{50} values of cadmium to the fish *H. fossilis* , which were 69.50; 63.50; 56.50 and 44.0 mg/l for 24,48,72 and 96 hours respectively. The dose for ascorbic acid for ascertaining their curative effects on chronic toxicity of cadmium where selected as 1.25mg/l which are $1/4^{th}$ of the doses at which no mortality of fish took place over a period of 96h. Running tap water (temperature 21 ± 4 °c; pH 7.6; dissolved oxygen 7.8 mg/l ; free CO_2 1.2mg/l; alkalinity 97.40mg/l as CaCo3 and hardness 158.60mg/l) was used during both static acute bioassays and chronic exposure.

For chronic exposure studies, three rectangular glass aquaria (A-C) of 30-litre capacity, each filled with 10 litres of water and 10 number of well acclimatized fishes were taken. Fish of aquarium A were exposed to 16.14mg/l of cadmium whereas those of aquarium B were exposed to 16.14 mg/l cadmium + 1.25mg/l of ascorbic acid. Fishes of aquarium C served as control. The experiment was run for 30 days during which test media were renewed every day immediately after feeding the fishes of each group with chopped goat liver.

At the end of exposure period (days 30) fish of each group were dissected and gills excised out and fixed in aqueous Bouin's and 10% buffered neutral formalin for 24 hrs. After fixation, fixed pieces of gills were decalcified in 5% nitric acid in 70% alcohol after decalcification, gills were washed repeatedly in 70% alcohol and then dehydrated in graded alcoholic series in ascending order and cleared in xylene /benzene followed by their rewashing in methyl benzoate for another 24 hrs. There after the tissues were processed for routine paraffin embedding in paraffin wax. Embedded tissues were sectioned at 6 μ m and stained with haematoxylin and eosin for histological examination under microscope. The selected slides were micro photographed.

RESULT

The gills of control *H. fossilis* revealed normal histology, the primary gill lamellae are flat leaf like structures arranged in double rows, projecting on the lateral sides of which are series of alternately arranged. Secondary lamellae are lined by squamous epithelium supported by pillar cells and chloride cells. They are shown in (fig-1).

In the Section of 30 days cadmium toxicity revealed necrotic lesions of secondary gill lamellae, vascular gills congestion, which leads to clubbing of the lamellar tips, besides hypertrophy of secondary lamellae, there was severe damage to chloride cells, pillar cell system became disorganized, secondary lamellae showed severe congestion, number of mucous cells considerably increased further extensive curling of secondary lamellae and lifting of epithelium were the unique lesions observed in almost all sections of gills.(fig-2 and fig-3).

Section of the gills of *H. fossilis* exposed by co-treatment of cadmium (16.14mg/L) + ascorbic acid(1.25mg/L) showing curling and fusion of secondary lamellae and small degree of edema still persisted but normalcy in architecture of primary and secondary lamellae cartilaginous skeletal structure mucous cells, basement membrane either restored or were in highly improved stage.(fig-4).

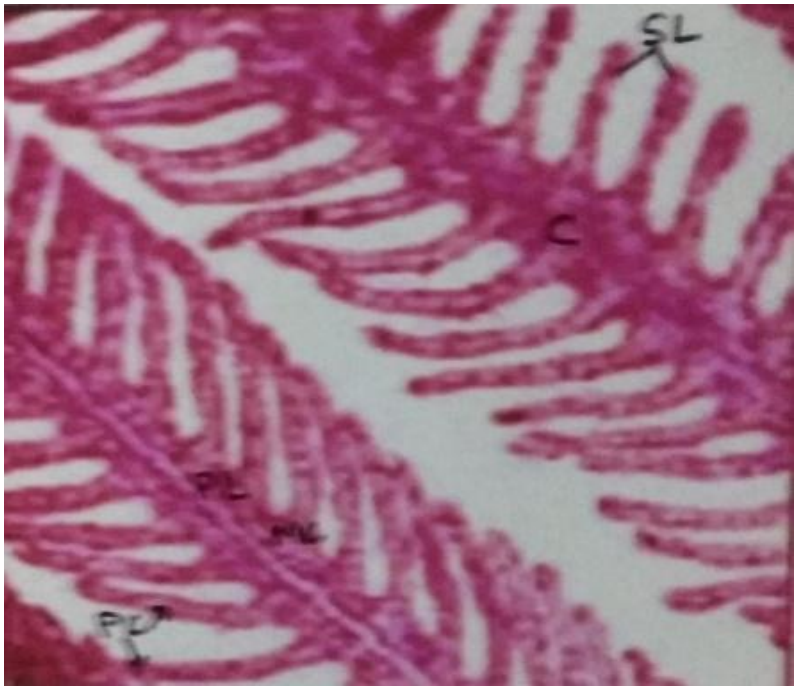

Figure 1: Section passing through gill of Control fish, *H. fossils*.

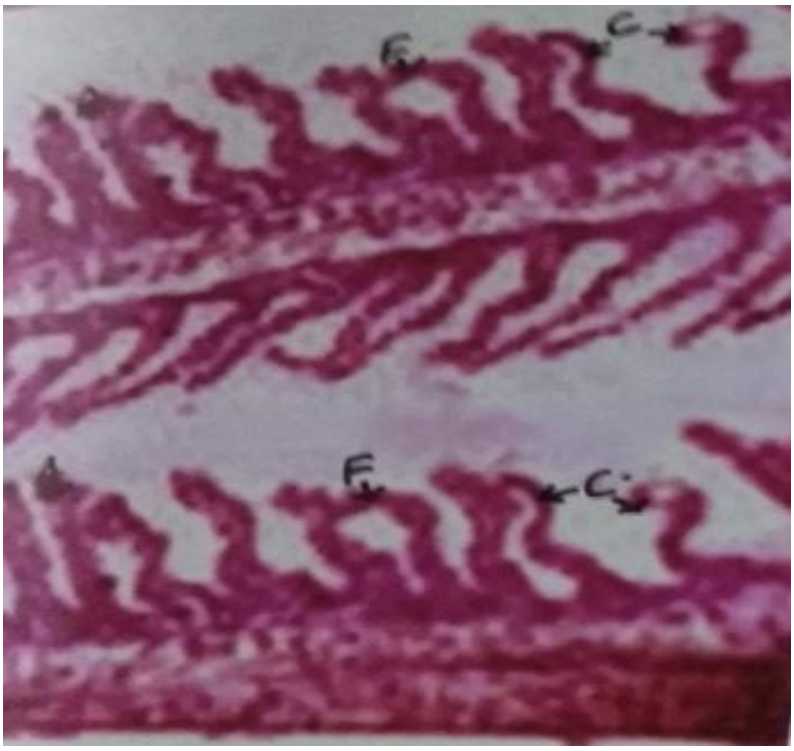

Figure 2: Section passing through gills of *H.fossils* exposed to Cadmium for 30 days.

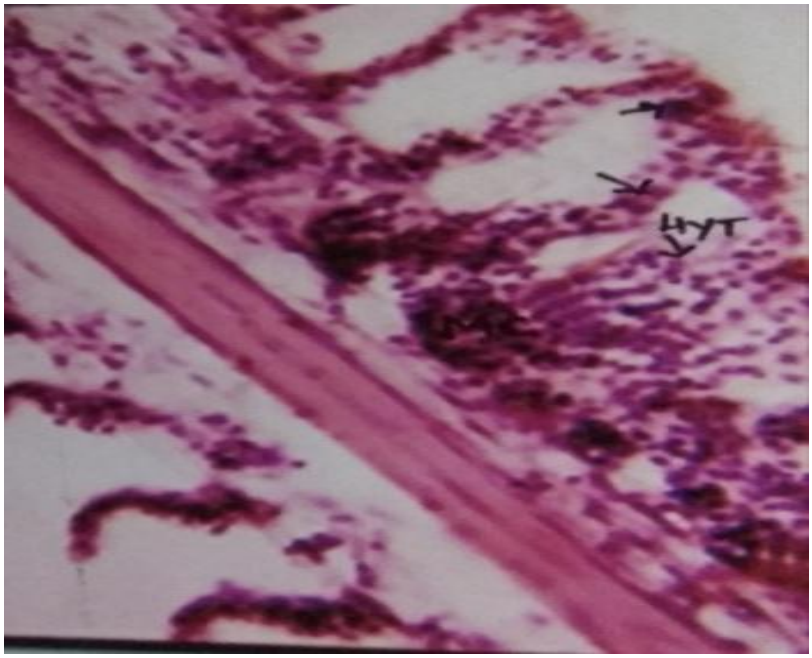

Figure 3: Section passing through gills of *H. fossilis* exposed to Cadmium for 30 days revealed Hypertrophied secondary lamellae.

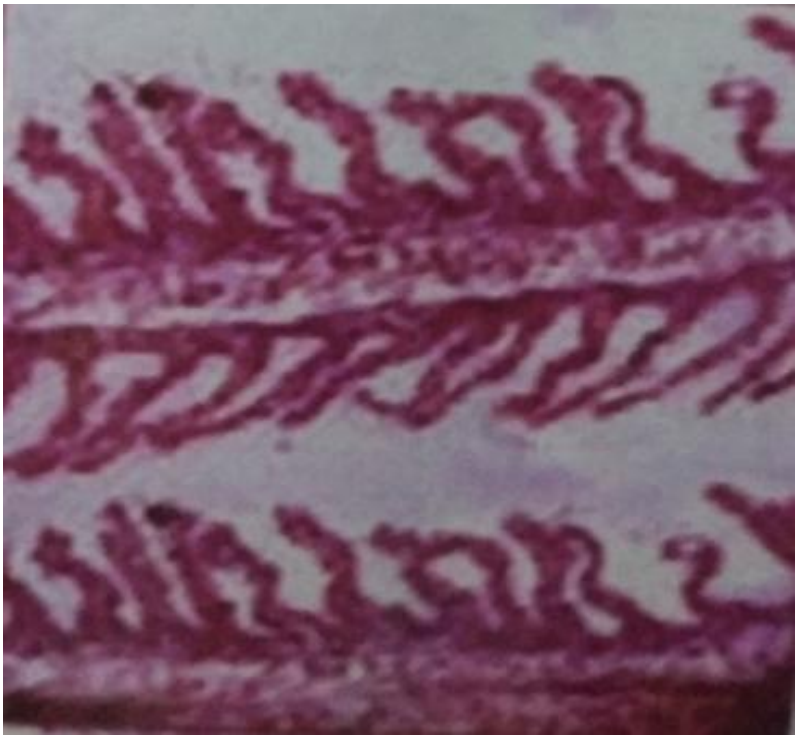

Figure 4: Co-treatment of cadmium+ ascorbic acid in *H. fossilis* revealed signs of recovery.

Abbreviations: PL= Primary gill lamellae; SL= Secondary gill lamellae; C=Cartilaginous core; PC=Pillar cells; MC=Mucous cells; (F)=aneurysm; (HYT),congestion(→)increased number of mucous cells (MC) recovery of architecture.

DISCUSSION

Gills of fishes are almost always exposed to aquatic pollutants due to their large surface area and external location, gills are very sensitive and respond extremely fast to water pollution (Mishra and Mohanty, 2008) in the present investigation it was observed that cadmium exposure induced fusion of secondary lamellae, vascular congestion leading to clubbing of the lamellar tips hypertrophy and curling of the secondary gill lamellae along with disorganization of pillar cell system, increase in the population of mucus cells epithelial lifting, disintegration and/or autolysis of the epithelial lining of the secondary lamellae as well as sub epithelial oedema the above findings are in close agreement with some earlier studies (Usha Rani 1999; Prabhakar *et al*., 2012; Mekkawy *et al*., 2013; Selvanathan *et al*., 2013).the observed histopathological changes most likely hindered the respiratory function of the gill (Bais and Lokhande, 2012). The observed hyperplasia, hypertrophy and fusion of adjacent lamellae appear to be a protective response to cadmium toxicity giving less load of the heavy metal to the blood as opined by Hughes *et al*.,(2009) and Singhdach *et al*.,(2009) reported that lamellar damaging and clubbing could be for the protection. It reduces the amount of entry of heavy metal pesticides. Further, It has been found that secondary lamellae has capillary congestion and aneurism similar findings are reported by Mekkawy *et al*., (2013) in *Oreochromis niloticus* exposed to cadmium. This may be attributed to observed the most of the pillar cell are collapsed, damaged vascular integrity with bleeding in lamellar epithelium, Garcia-Santos *et al*.,(2006). The epithelial lifting noticed in this study appears as an initial reaction of gill activity as a protective measure by increasing the cadmium – blood diffusion distance and such responses have been advocated as typical inflammatory response (Schwaiger *et al*., 2004). As regards oedematous change observed in the present study, the possible explanation given by Thophon *et al*.,(2003) seem to be very logical that edema is a consequence of lifting of the secondary lamellae from pillar cell system leading to increased distance from water to blood. The observed increase in the number of mucous cells may be attributed to defensive mechanism against cadmium toxicity.

CONCLUSION

Gill lesions induced by cadmium toxicity, reduced the oxygen diffusing capacity of the gills which may ultimately cause death of fish primarily from insufficient oxygen uptake. Again some of the observed gill lesions (e.g. epithelial lifting, hyperplasia, hypertrophy, lamellar fusion & curling etc.) may be categorized as adaptive ones, since these protect the fish by slowing the rate of entry of heavy metal, cadmium. Whereas others (e.g. aneurysm, collapse of pillar cell system etc.) may be considered deleterious.

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