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Cadmium chloride induced histopathological changes in Air Breathing Organ of Heteropneustes fossilis (Bloch)

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ABSTRACT

Heteropneustes fossilis is an air breathing teleost capable to use atmospheric oxygen in their respiration through air breathing organ also called supra branchial chamber.Toxic effect of cadmium choride on supra branchial chamber of *H.fossilis* has been examined on different concentratios of this heavy metal and remarcable histopathological alterations observed which hazard for survival of the fish.The cadmium chloride is widely used in many industries like paint,ceramics and other from where its indiscriminate flow as industrial waste in warer bodies produce a serious threate for aquatic animals including fish and can also pass to the human body through food chain by consumption of such type of contaiminated fish.

Key Words: *Heteropneustes fossilis*,heavy metal, cadmium choride.

INTRODUCTION

Methods to assess for sign of chronic metal toxicity have included biochemical, histopathological, and physiological deformities of different aquatic animals (Larson and Haux 1985, Gupta 1988 and Banerjee 2007).There has been a great increase in manufucturing of products contains hazardous heavy metal cadmium (Cd) which find their way into the river, lakes and ponds, along with the water run off from the agricultural and forest land where they change the biotic life including fishes. (Verma etal. 1993). When fish fauna are stressed by altered environmental condition, homeostasis is distupted and these animals must adjust behaviourally or physiologically to adapt to the alterd environment.It is known that increased concentration of Cd are often found in some Indian freshwater including river Yamuna, Subarnrekha and Ganga (Singh etal. 1990), as well as in coastal estuarine ecosystem (Kaviraj 1989) due to their widespread use in various industrial and agricultural operations. Toxic effect of non essential metal and chemicals may result from their binding properties **with biologically active** molecules such as protein, lipid, enzymes (Passow etal. 1961). However, the effect of heavy metal interfere with the function of accessory respiratory organ of air breathing teleost is not much known.Bioaccumulation and the relative concentration of this heavy metal in fish body as well as owing to their prolonged life, which posing a threat to the aquatic organisms specially fish fauna. Greater amount of Cd accumulate in fishes, inters in man through food chain.Reports are available on accumulation of this metal in gastrointestenal tract, liver, kidney, gill, gonads, but investigations on ARO (accessory respiratory organ) are inadequate. This however, has created a noteworthy problem in the culture of airbreathing teleost. The effect of heavy metal have been culminating in the reduction of stock, growth, fecundity and viability of the fish population. (Sastry and Subhadra, 1982, Gupta 1988).

Hence, for the valuable development in fisheries, it is important to investigate the effects of Cd on the air breathing organ concerned with uptake of oxygen (respiration) for certain periods without water. This study would help to

accomplish idea regarding the actual mode of actions of this heavy metal with the histopathological structure of the organs concerned.In order to maintain healthy stock of fish in our inland waters,water quality objective have to be determined for each pollutant which insure that the species present are not adversely affected in any way .For this reason, attention has been given to sub lethal response on fish to selected heavy metal (Cd). A large number of authors in the past have surveyed the toxicity of some heavy metal (Cr. Cu, Zn, Hg etc.) in fishes. During recent years, Dallinger etal., (1985) reviewed the toxicity of heavy metal along with other environmental impact in fish biology.The structure and evolution of the air breathing organ of the fish has aroused much excitement, simply because these structures present certain features in their anatomy which resemble that of the gill lying in the branchial cavity and to which these organs are closely associated.

The detailed anatomical pictures of air - breathing organs are available from the work of Munshi (1962) and Munshi and Singh (1968).Particularly in this case, the matter goes back to as early as 1927 whenDas started the study of the air-breathing organ of *H. fossilis* as diverticula of the opercular chamber. However, his work suffered from a serious draw back in the sense that the development of the air breathing organ was followed in the post larval rather than in the larval stages. As such, Das's work (1927) can be said to dwell upon describing the differentiation of the air breathing organ rather than aiming at tracing its development. Major route of entry of heavy metal in the piscine body is either through contaminated food or by direct absorption from water through gills, skin and from atmosphere or dissolved in aquatic medium through accessory respiratory organ (ARO). Therefore the absorptive epithelium of alimentary canal, gill,ARO and skin of the fish are the primary affected site due to metal intoxication. Induced microanatomical structures of those epithelia due to metal toxicity were studied by different authors through light or electron microscopes specially by scanning electron microscope (Ghosh and chakrabarti, 1991, Banerjee 2002, 2007). Ghosh and Chakrabarti (1992) reported the microanatomical shrinkage in epithelialcells of buccopharynx due to cadmium intoxication.

MATERIALS AND METHODS

Experimental design: Healthy specimen of *H. fossilis* of either sex belonging to a single population (Body length 15-20 cm and weight30-42 gm) were collected from the local fisher man.The Healthy fish were acclimatized in laboratory aquaria for one month.The fish were fed with tubifex worm, sliced goat liver and commercial fish food (fishtone). No mortality was recorded among the fish during this period. The experimental fish were pretreated with 1% methylene-blue for 10 minutes to avoid disinfection. Dechlorinated tap water was used as test water. 20 test containers (glass battery jar) used as a test container in which the first container contained no metal and acted as control.

Preparation of heavy metal solution Reagent grade cadmium chloride were obtained from scientific suppliers A calculated amount of the heavy metal were dissolved in 02 test containers in 10 litres of water .Before diluting them with tap water the heavy metal were dissolved in distilled water with 2- 3 drops of HNo, in cadmium and few drops of methanol in the case of . Two series of cadmium chloride (100 ppm for 48 hour exposure and 200 ppm for 72 hour exposure) solution were prepared for experiment

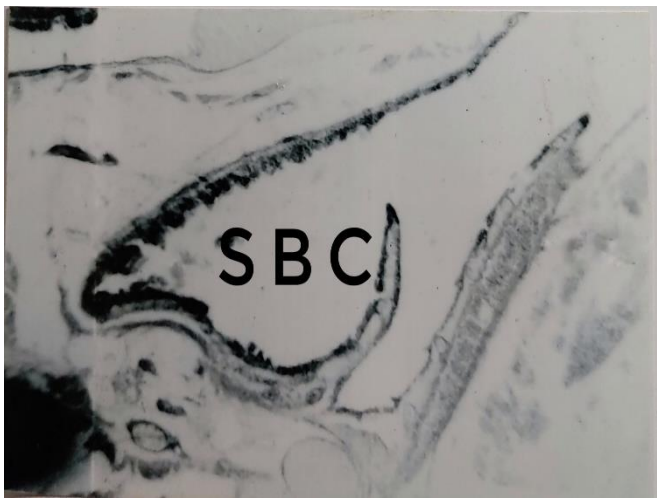
Fixation of tissue for histopathological studies :

After exposure, the fish in different concentrations of heavy metal for a particular period or till opercular immobilization occurred, the control and exposed fishes were removed. They were killed by an abscission of the spinal cord just behind the head and their suprabranchial cavity and air sacs were dissected out carefully. These tissues were fixed in Bouins fixative for different histopathological studies. Paraffin section of 6-7 micron were stained using delafield's haematoxylin-eosin stain. Some selected slides showing different types of histopathological effects were photomicrographed.

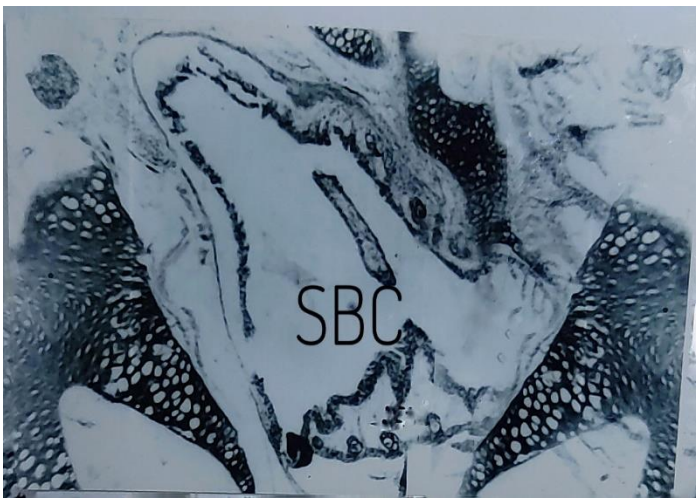
OBSERVATION AND DISCUSSION

Histopathological and Microanatomical changes:-

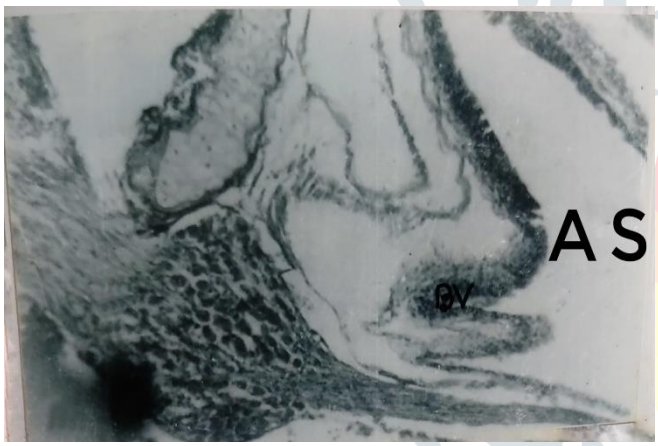
Heteropneustes fossilis (Bloch) commonly called "singhi is an active air breather and according to Munshi (1962) it meets around 40% of its oxygen demand by aerial respiration through ARO(accessory respiratory organ) indicating the vital role played by this organ for the sustenance of the species. In present study, the increased gulping activity and opercular movement by the exposed fish may be the reflection of an attempt by the fish to extract more oxygen to meet the increased energy demand to withstand the and cadmium toxicity.On the other hand it may also be correlated to the formation of hypoxic condition due to the interference in gaseous exchange caused by the accummulation of mucous on the gill epithelium In order to overcome this, the fish might also be actively resorting to the aerial mode of respiration through increased gulping activity.The prominent bulging of lamellar cells into the lumen of the ARO at various stages of present study could also be correlated to the attempt on the part of the organism to facilitate maximum oxygen absorption to overcome the cadmium stress..As a resultthe pillar cell system of the secondary lamellae become hyperemic and it causes stretching and dilation of the blood channels.The extreme load on these thin walled tubular structures along with the memberane damaging necrotic effect of cadmium might be responsible for rupture of the lamellar system ing to haemorrhage into the lumen of ARO in the exposed fish.Seepage of blood materials into the lumen could not only chock it but may also result in anaemia ing to the reduced oxygen carrying capacity of the blood.Mucous cell hyperplasia was quite distinctat some places of respiratory epithelium when trated with 200 ppm used toxicant. Blood capillaries dilation was also observed in the form of swollen epithelium as seen in the figure(D) which leads to its rupture. Thopon etal..(2003) have also noticed aneurism with rupture of the respiratory epithelium of secondary lamellae and break down of pillar cell system in the gills of cadmium exposed *Lates calcarifer*.The absence of such histopothological and microanatomical alteration in the control ARO confirm the deleterious effect of cadmium exposure .Even though the ARO never comes in direct contact with the toxicant medium as the case of gill (Munshi 1962)the hyperplasia of epithelial cells and mucous cells at various stages of the present study are some what similar to the alteration reported by Thopon etal(2003) in the gill of cadmium exposed *L.calcarifer*.The primary consequences of epithelial cells and mucous cells hyperplasia is the increased thickness of the respiratory epithelium. More or less same effects are also produced by the epithelial lining development of non-tissue spaces or intracellular vacuolization.All these pathological changes result in the increased barrier distance between the blood in the pillar cell system and the air in the lumen of ARO to impaired respiration.The fusion & clubbing of secondary lamellae of the ARO in the present study might be the result of the combined effect of hyperplasia of epithelial cells and mucous cellsnecrotic lesion and compositional changes in the mucous layerErosion of epithelial lining and alteration in microanatomy of mucous cells occur due to cadmium intoxicationThese events could disturb the normal ability of the cells to recognise different cell types resulting in the fusion of the secondary lamellae.The immediate physiological consequences occurs as reduction in the respiratory epithelial lining.In suprabranchial chamber, mucous cells showing marked hyperplasia than air sac in H. fissilis. Like supra branchial chamber, The air sac is not closely associated with the gills. Entrance of water in to the supra branchial chamber and air sac is checked by the presence of some valves formed by fan like structures borne by the 4th gill arch (Singh etal., 1981) in case of H. fosillis. The operation of this valve is seems to be affected by non functioning of the appropriate branchial muscles due to the paralytic action of the heavy mtals (and Cadmium) particularly at high conentration. At this stage, the heavy metal pollutants make their entrance into supra branchial chamber and air sacs filling up the entire space and causing a series of histopathological and micro anatomical alterations. Mucous cells of the gill epithelium are an obvious site of pathogenic interactions between the fish and the environment, Since, the air breathing organ of *H. fossilis* is embryologically derived from the gill materials (Singh etal. 1981) and remain in close vicinity of the gills throughout life, their responses to the environmental agents may be expected to be similar to that of the gill. Pathological changes observed here in the air breathing organ of *H. fossilis* strongly favour this



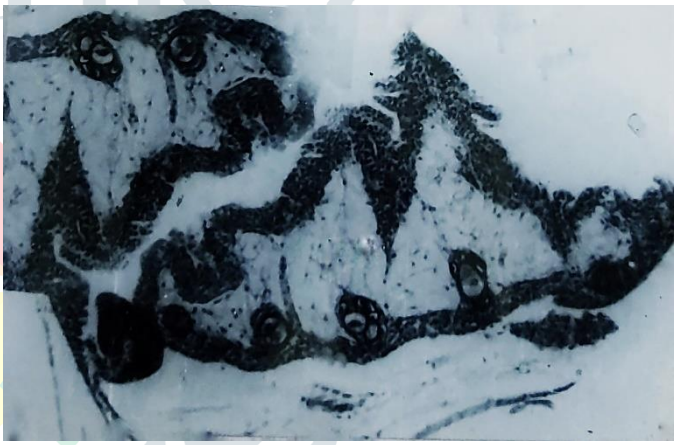
(A) T.S. of suprabranchial chamber of H fossilis (H/E × 350) control



(B) T.S.of portion of suprabranchial chamber of H.fossilis showing lamellar structure for respiration in air (H/E × 850) control



(C) T.S.of suprabranchial chamber of H.fossilis treatrd with 100 ppm toxicant for 48 hour exposure showing deformity in epithelial lining (H/E x 350)



(D) T.S.of suprabranchial chamber and air sac showing enlargement and rupturing of blood vessels in air sac treated with 200 ppm toxicant for 72 hour exposure (H/E x850)

SBC- Suprabranchial chamber

AS - Air Sac

similar to that of the gill. Pathological changes observed here in the air breathing organ of *H. fossilis* strongly favour this hypothesis.Hyperplasia of mucous cells was more pronounced in the suprabranchial chamber of *H. fossilis* than the air sac epithelium.The result obtained through these investigation have revealed that cadmium chloride which are usually released into water bodies from various industries are very much toxic to the fish.Even a trace amount of this heavy metal have enough potentiality to cause different histopathological alterations impairing the vital system of the fish. The toxicity of this heavy metal not only affect the fish itself but also exert negative impact on the health of those people who consume the fish from the stock of polluted water bodies.To maintain a healthy stock of fishes, it is necessary to treat the industrial effluents containing heavy metal properly before drain out them into water system. The criteria laid down by different natioal and international organizations for environmental protection must be followed to maintain the safe disposal range for industrial effluents containing heavy metal, to free the fish for human consumption from heavy metal contamination.

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