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Histopathological changes in the suprabranchial chamber of fresh water fish *Channa gachua* exposed to a fungicide sedaxane

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Abstract: The present investigation is conducted to assess histopathological alternations caused in suprabranchial chamber of an air breathing teleost *Channa gachua* (Bloch) after exposure of sedaxane (sublethal concentration of sedaxane for 96 hours is 0.045 ppm). Light microscopic studies showed many alternations in the suprabranchial chamber of fish. These alternations are observed as pathological biomarkers indicating the load of pollution of toxic stress. The effect of sedaxane can be observed in the form of hyperplasia of mucous cells found in the epithelial lining of SBC. The higher degree of wear and tear caused sloughing off of epithelial cells and thus the non-tissue space is formed in the respiratory epithelium. Breakdown of vascular papalliae caused disintegration of vascular system. The blood capillaries of SBC also bulged out extensively. These observations are thus indicative of the toxic affect caused by sedaxane at histopathological level in suprabranchial chamber of *Channa gachua* (Bloch).

Keywords: Histopathology, suprabranchial chamber, *Channa gachua*, sedaxane, toxicity

INTRODUCTION

Pesticides encompass a wide range of chemicals used to kill insects, fungi or weeds for massive production in agriculture. Pesticides are extensively used in world for pest control. Pesticides are used as important component in agricultural development and protection of public health in India, as the climate of India is very suitable for plant breeding.[1] Use of Pesticide in agricultural field to control pests is very toxic to non-target organisms like fish and affects fish health through impairment of metabolism, sometimes leading to mortality.[2] Among all pesticides, sedaxane is widely used to control pests. It inhibits respiration by binding to the succinate dehydrogenise complex of fungi. These insecticides enter into the aquatic ecosystem and then food chain and are responsible for adverse effect in aquatic ecosystem and finally human.

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The accessory respiratory organ in some fishes has developed to fulfill the high oxygen demand of the body even in the adverse conditions. The main respiratory organ in fishes is no doubt the gill but some fishes have evolved air- breathing organs as an adaptive feature to supplement optimum oxygen from the atmospheric air. The air breathing organs helps the fish in surviving even in very low oxygen containing water bodies as its enables the fish to absorb oxygen from the atmospheric air and helps in sustaining their life for certain period of time out of water. It is an adaptive feature associated with life in hypoxic and hypercarbanic ambient water have be well documented by Carter (1957)[3]. Thus the air breathing fishes differ from others in having accessory respiratory organs, blood physiology feeding habits and in their breaking water behavior.

The accessory respiratory organs in fishes are usually newly evolved which are accommodated within the limits

of buccophyrax or even beyond it in some cases. The major problem related with neomorpic air-breathing organ is to develop a respiratory epithelium suitable for gas exchange with air. Once it is achieved the immediate task before a fish is to increase the respiratory surface area which can be accommodated in a minimum of space .Some common Indian air- breathing fresh water teleost that have neomorphic air-breathing organs are *Anabas testudineus*, *Colisa fasciatus* and *Osphronemus nobilis* (Anabantidae); *Heteropneustes fossilis* (silurooidae); *Clarias batrachus* (Claridae); *Amblyceps mangois* and *Amblyceps murray stuarti* (Amblycepatidae); *Channa marulius* and *Channa gachua* (Channidae) and *Monopterus cuchia* and *Monopterus albus* (symbranchidae). Besides, the fresh water forms a number of estuarine genera and species belonging to the family gobiidae are air breathers like *Psudapocryptes lanceolatus*, *Perioptha*, *Imodanseholosseri* and *Boleophthamus boddarti*.

The histopathological study on different fish part are quite common but the work on SBC is scanty and mainly restricted to a limited number of air-breathing fishes. The origin structure and function of accessory respiratory organs differs in various species of air breathing fishes hence the degree of impact on them also varies the main target of various toxicants in the SBC is their epithelial lining and thus it undergoes histopathological alterations after getting their exposure. The severity of damage depends upon the variety and concentration of hazardous material along with some ambient physico chemical factors. Hence, the efforts have been made to know the degree of hazardous effect of Sedaxane on the air breathing organs of *Channa gachua*.

MATERIALS AND METHODS

Adult healthy specimen of *Channa gachua* (Bloch) of length 7-10 cm and weight 10-12.5 gm were taken without any sex differentiation from paddy field of my home town Kanti, Muzaffarpur, Bihar. After that these fishes were transported to the laboratory in aerated containers and then these fishes were treated with 0.5% $KMnO_4$ solution for 10 minutes to remove dermal infection. After that these fishes were acclimatized in the tank for 15 days and provided with chopped earthworm or fish tone. To maintain dissolve O_2 concentration water was changed on alternate day. During experiment no food was given to them. Important parameters to maintain the fish were strictly followed by as par condition recommended by APHA (2005)[4]. After that fishes were exposed to sedaxane. After exposure kidney was isolated from the control and experimental fish and then they were cleaned and fixed by using saline solution for 24 hours. This vital organ was kept in formaldehyde and then dehydrated, embedded in paraffin wax and then sliced by using microtome of thickness 5 micrometre. They were stained by using double staining technique (Haematoxylin and Eosin) and viewed in compound microscope.

RESULTS

SBC of control fish:-

It was found that the lining of suprabranchial chamber (SBC) is formed by the active participation of ectoderm, mesoderm and endoderm during the embryonic development. The SBC develop dorsal to the gill arches in the pharyngeal roof and lateral to the prootic bone of the auditory capsule. On the dorsal side it is protected by the skull bone while on the ventral side it is lined by bronchial epithelial and the opposite wall with supra bronchial line. Each suprabranchial chamber is divided into an interior small and a posterior large compartment by a self like transverse outgrowth. The pharynx open into the interior compartment and there is no shutter present to guard this opening. The interior compartment is freely connected with posterior compartment. The pharyngeal epithelium covers interoventrally with taste buds and supra bronchial lining posteriorly the suprabranchial chamber from below the original pharyngeal epithelium thus bucco pharynx become able to draw oxygen from engulf air. The bucco pharyngeal lining or epithelial lining consist numerous taste buds. The excellent opening serves as an exit for expired air to pass from SBC to opercula chamber which is located at the dorsal part of first gill silt. An inhalant opening is found at the anterior ventral position which allow the posterior compartment of SBC to communicate with pharyngeal chamber and it is guarded by a shutter. The supra bronchial chamber is formed by many layer of ectodermal cells and basophilic cells derive from gill mass and becomes folded posteriorly and remain buldges out into opercula chamber. The constrictor (suprabranchialis) muscle fiber is present in the wall of SBC consisting vascular (islets) consisting of a number of rosettes and non-vascular (lanes) areas. Each rosett is form by a group of blood capillaries projecting on the surface epithelium. The two blood capillaries in vascular papillae gets separated like structures and in these vascular papillae mainly the

gaseous exchange take place. The lanes are covered by stratified epithelium consist polygonal mucous secreting cells (Figure 1, 2).

Figure 1.T.S of SBC of *Channa gachua* control showing blood vessels and respiratory epithelium.H/E X 150

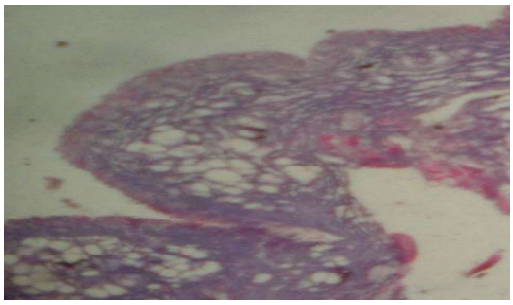
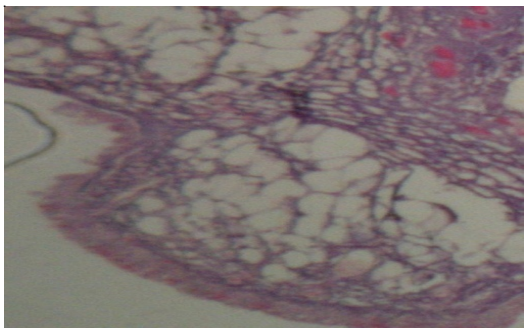


Figure 2. Same is enlarged to show blood vessels. H/E X 400



Histopathlogical changes in SBC due to Effect of Sedaxane:-

Though the structure and physiology of SBCs varies in different species of air breathing fishes and thus the toxic impact of stressors are also different. The SBC was lined by a stratified epithelium situated above a layer of connective tissues which were highly vascularised. Numerous fine sub-epithelial blood capillaries reaches to the surface of SBC. Minute blood channels (BCs) forming the vascular papillae (VP). The projected aerial surface of papillae was covered with a thin respiratory epithelium. Numerous mucous cells (MCs) were present towards the outer surface of the epithelium probably to protect them by forming a slimy layer over it. After getting the exposure of Sedaxane for about 2-6 hours the blood capillaries of the vascular papallie get swollen and bulged out due to accumulation of RBCs. The blood capillaries get congested and forms finger like structure at some places. Enlargement of mucous cells had been seen when the exposure time gets enlongated. At some places blood spots were also seen on the surface of SBC probably due to the rupture of highly stressed blood capillaries. At some places detachment or lifting of the respiratory epithelia from the underline vascular region. A large number of wear and tear of the epithelial lining and severe necrosis of epithelial cells can also be observed (Figure 3). However the SBC of the fish do not lie in the direct contact of toxicant mixed in their surrounding water but it harms the SBC by reaching there through the blood. The effect of sedaxane can be observed in the form of hyperplasia of mucous cells found in the epithelial lining of SBC. The higher degree of wear and tear caused sloughing off of epithelial cells and thus the non-tissue space is formed in the respiratory epithelium. Breakdown of vascular papalliae caused disintegration of vascular system. The blood capillaries of SBC also bulged out extensively, probably to compensate the damage caused by the toxicants to the gills by taking part in the process of gaseous exchange. It has reduced the blood atmospheric air barrier distance (Figure 4).

Figure 3. SBC Treated with sedaxane showing breakdown of blood vessels in *Channa gachua* after 96 hours. H/E X 600

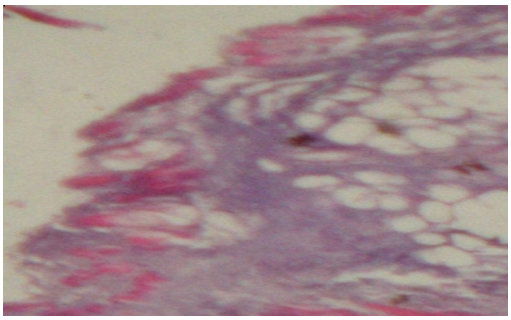
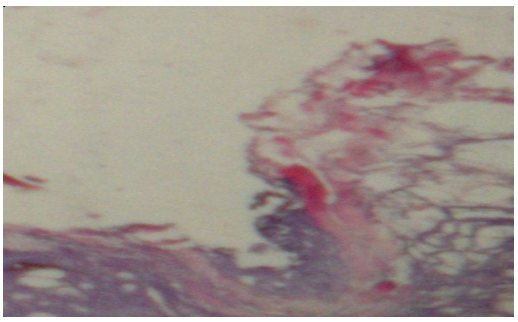


Figure 4. Total breakdown of epithelial layer of sbc and blood capillary in *Channa gachua* after the sedaxane exposure. H/EX800



DISCUSSION

Channa gachua regularly visits the water surface to engulf air for aerial respiration but the exposure to toxic water hampered the bronchial respiration and thus the fish

were compelled to take oxygen from the air. At the initial stage the surface visiting behaviour increases but as the toxic material reaches to blood or come in direct contact to the body part induced the mucous secretion. The inner epithelial lining of the air sac often gets stick together causing a decrease in respiratory area and their volume. The similar effect in *H.fossilis* was also observed by Johansen (1970)[5]. The accumulation of mucous and blood materials in the air sac also lead to the disturbance in the gaseous exchange. The mucous cells show the quantitative as well as qualitative alterations. After getting the exposure as the saline secreted contains a mixture of neutral acidic and sulphated glycol proteins, the finding matches with the work of others.[6-10] During the earlier stage of exposure fish showed restlessness and respiratory distress the opercula activity gets enhanced. Haemorrhage due to the rupture of lamellar system leads to seepage of blood materials would not only chock it but my also result in anaemia leading to reduced oxygen carrying capacity of the blood. A wide range of functions has been attributed to the fish mucous including protection against environmental contaminants and UV radiation while some are having the opinion that the mucous consists a metal binding property while the many more have observed the hyperplasia of mucous cells in the suprabranchial chamber, the dendritic plates is also closely associated with gills the operation of the shutter seems to be effected by the pesticides as the respiratory failure is the main cause of fish dead however the failure of nervous system also assists in it.[11] The available data on the toxic impact of different stessors on the air breathing organs indicates that the effect was not vary serve however the alterations noticed include immense congestion of the blood cells which project out from the respiratory surface.[12]

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