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HISTOPATHOLOGICAL CHANGES IN THE GILLS OF *CHANNA GACHUA*, AN AIR BREATHING TELEOST AFTER SHORT TERM EXPOSURE OF HOSTATHION

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ABSTRACT
Gills are the prime organs for gaseous exchange and perform several other physiological functions including osmoregulation and excretion. Harmful effects of several insecticide on the gill histomorphology are well known. In this paper the effects of a commonly used insecticides on the gills of *C.gachua* is demonstrated. *Channa gachua*, a freshwater teleost was exposed to a broad spectrum organophosphate insecticide hostathion for 24, 48, 72 and 96h at 0.25, 0.30, 0.35 and 0.40ppm. 96h LC_{50} value was determined. The histological sections of gills of snake headed air breathing teleost examined under light microscope showed severe structural alterations like mucus cells hyperplasia, buldging of taste buds, formation of interlamellar bridge and sub epithelial spaces in the primary and secondary gill lamellae.

INTRODUCTION

Fish live in intimate contact with the surrounding water through their gills (Pratap *et al*., 1989) whose surface comprises over half of the body surface area (Wood and Soivio, 1991). Only a few micron of delicate gill epithelium separate the internal environment of the fish from a continuously flowing external environment (Wood and Soivio 1991). Thus, it makes the fish very suceptible to aquatic pollutants.

The gradual accumulation of toxic material in water due to run-off from the agricultural land containing insecticides, pesticides, herbicides etc. Dutta *et al*., 1996 have demonstrated the effect of several heavy metals seriously damage the gills of teleostean fish. Hemalatha and Banerjee, 1997 (a), 1997 (b) have studied the toxic impact of the trace element zinc chloride ($ZnCl_2$) on the gills and acessory respiratory organs of *Heteropneustes fossilis*. Parashar and Banerjee, 2002, have studied the toxico-pathological impact of lead nitrate on the gills of air breathing catfish *Heteropneustes fossilis*. Organophosphates are considered highly toxic to many acquatic species (Qin and Dong, 2004 and Velmurugan *et al*., 2009). Prashanth *et al*., 2011, studied the effect of sodium cyanide on behaviour and respiratory surveillance in *Labeo rohita* (Ham). Pandey Govind *et al*., 2011 have studied the detergent toxicity on the gill of *Puntius ticto*. In 2012, Rani and Venkataraman reported the effect of malathion on the gills of *G.giuris* (Ham). However, almost no studies are available on the toxic impact of hostathion, an organophosphate compound on the gills of an air breathing teleost *Channa gachua*. Generally this compound is used to control pests but knowledge about its impact on the fish gill is still scanty. Therefore, in the present study efforts have been made to examine the toxicity of hostathion on the gills of *Channa gachua*.

MATERIALS AND METHODS

Healthy specimens of *Channa gachua* (32.0 to 41.0g body weight, 13.0 - 16.0 cm in length) were collected from the water resources in the vicinity of district Samastipur (Bihar) with the help of fisherman and brought to fish laboratory B. R. A. Bihar University, Muzaffarpur in earthen pots where, fish were washed with $KMnO_4$ (0.05mg/L) to remove dermal infections. After proper washing with several changes of water the fish were acclimated in clean tap water under normal laboratory condition between 27ºC to 30ºC for 15 days. They were fed with commercial fish food (fish tone) and chopped earthworm on alternate days and water was renewed after every 24hrs, to eliminate faecal matter and unconsumed food. However, feeding was stopped 24hrs. Prior to the commencement of the experiment.

Prior to the bio-assay test, 96hr median lethal concentration (96h LC_{50}) of hostathion, an organophosphate compound (Bayer crop science limited factory, Gujrat, India; 40% EC) was estimated by standard log-probit method (Sprague, 1976).

Five groups of 10 fish each were exposed to 0.4 ppm (96h LC_{50} value) of hostathion. Each group was exposed separately to 20 L of hostathion solution, prepared in tap water (having

dissolved O_2 7.2 + 0.66 mL/L, pH 7.51 + 0.20, water hardness 34.2 mg/L and water temperature 25ºC + 2ºC). Five separate battery jars containing 20 L of fresh tap water having 6 fish in each were used as control.

After the exposure period of 24h, 48h, 72h and 96h, five fish each from the respective experimental, as well as control jars were dissected and the entire gill from both the sides of the fish were taken out and were fixed in 10% neutral formalin, aqueous bouin's fluid and zenker helly's fluid. 6 μm paraffin sections were stained with Harris haemotoxylin and Eosin (H&E) for routine histopathological analysis.

RESULTS

The gill is composed of two parts gill head and gill filaments. The gill head is covered over by a thick tissue composed of mucus glands, taste buds, and connective tissue. The gill head contains bony part of gill arch and gill rackers. The surface epithelium of gill arch showing parallel arrangement of epithelial cells having mucus pores. Examination of thin sections of gill arch of *Channa gachua* (Control) showing four pairs of typical teleostean gill arches bearing two rows of primary gill filaments. Each gill filament bears a series of alternately arranged semicircular secondary lamellae on both sides Fig. 1. The surface of gill lamella is lined by a thin layer of simple squamous epithelium which rests on basement membrane covering the pillar Cell-blood channel system and which constitutes the main vascular area of the gills Fig. 2.

Gills of *Channa gachua* exposed to hostathion solution exhibited varying degree of damage in sublethal concentration (< 0.4 ppm) after 48 hrs. Mucus cell hyperplasia was generally more pronounced towards the proximal end of the filament. After 96h of exposure, hyperplasia of epithelial cells resulted in the fusion of many lamellae Fig. 3.

In 0.35 ppm exposure up to 96h the mucus cells buldged outward and become enlarged. Some lamellae appeared thickened and retracted while some get reduced and sub-epithelial space developed Fig. 4.

After 96h of exposure, in < 0.4 ppm (sublethal concentration), buldging of taste bud Fig. 5 and 6, gill racker, formation of interlamellar space, fusion of secondary lamellae, breakage of lamellar blood capillaries, swollen tip, telangiectatic secondary lamellae and clotting of blood were observed.

DISCUSSION

Gills have an extensive surface area and minimal diffusion distance between dissolved O_2 and blood capillary for efficient gaseous exchange. However, fish gills are marvelously equipped with a defense mechanism working against the environmental irritants which essentially is the mucus cell. The mucus cells react instantaneously to the pollutants and secrete copious mucus to form a thick protective layer over the entire exposed surface. According to Part and Lock; 1983, the mucus layer creates a microenvironment that may act as an ion trap, concentrating trace elements in the water. The histomorphological response of the gills of fish exposed to ambient insecticides (including metal salts) is often manifested by a prominent increase in the density of its mucus cells (Baker, 1969; Cardeilhac *et al*., 1979; Matey, 1984; wise *et al*., 1987; Dutta, 1997). The large amount of mucous secretion acts as a defence mechanism against several toxic substances (Mc. Donald, 1983; Handy and Eddy, 1991; Mazon *etal*., 1999). The regular elimination of mucous layer from the gill surface into aquatic media helps to remove the bound pathogens, toxicants and foreign matters (Powell *et al*., 1992) which remain stick to the gills. Peuranen *et al*., 1994 noticed that the gill microenvironment differs considerably from that of the surrounding body and water causing deposition of metals on the gill surface (Playle and Wood, 1989).

Due to hostathion intoxication the gill epithelium was completely separated from the basement membrane and pillar cells and there was a swelling of the secondary lamellae and dilation of the vessels. The pillar cell nucleus showed necrosis and vacuolation in the secondary gill epithelium. The disorganised fusion in secondary gill epithelium was prominently observed after exposure of the toxicants.

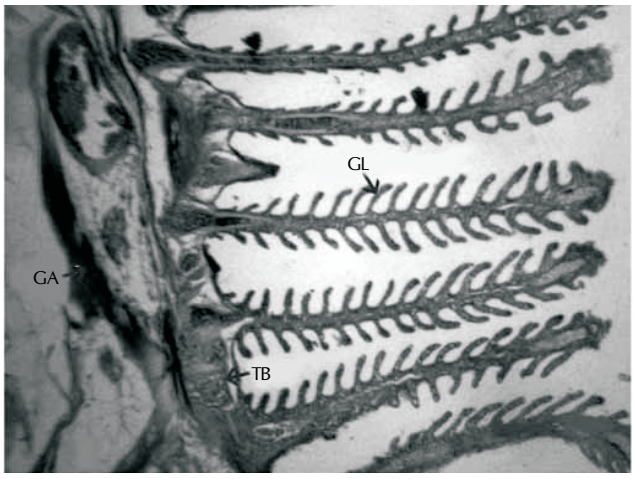

Figure 1: part of control gill showing structural or gananization. Note the gill lamella (GL), trastebud (TB) and gill arch (GA). H/E x 100

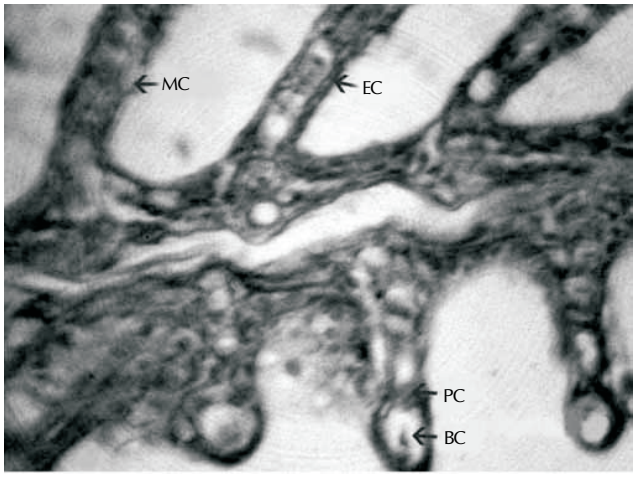

Figure 2: Magnifed view of the gill of control fish showing mucous cell (PC), blood channel (BC) and epithelial cell (EC). H/E x400

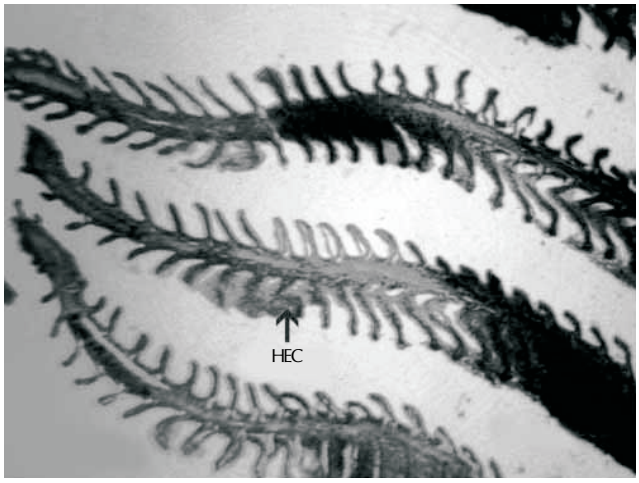

Figure 3: A part of hostathion treated gill showing hyperplasia of epithelial cell (HEC) lining the secondary lamella. H/E x150

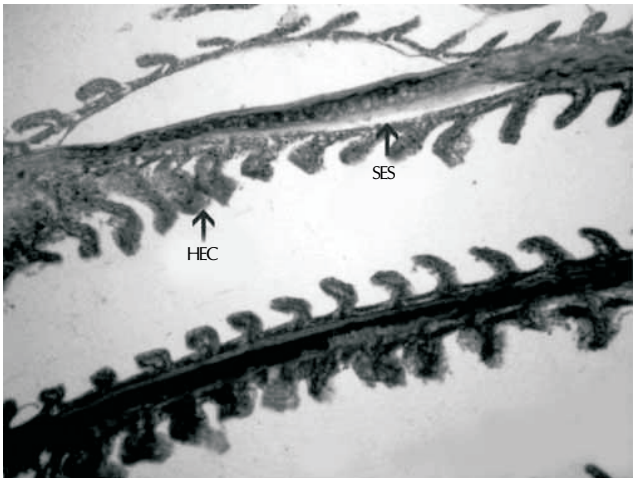

Figure 4: Gill showing sub epithelial space (SES) and hyperplasia of epithelial cells (HEC) after 96 hrs exposure of hostathion. H/E x150

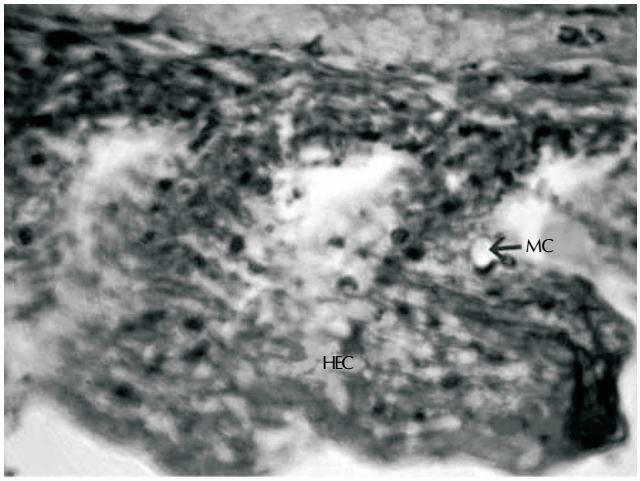

Figure 5: Epithelial cells showing fusion and hyperplasia (HEC) along with enlarged mucous cell (MC) of secondary lamella. H/E x 520

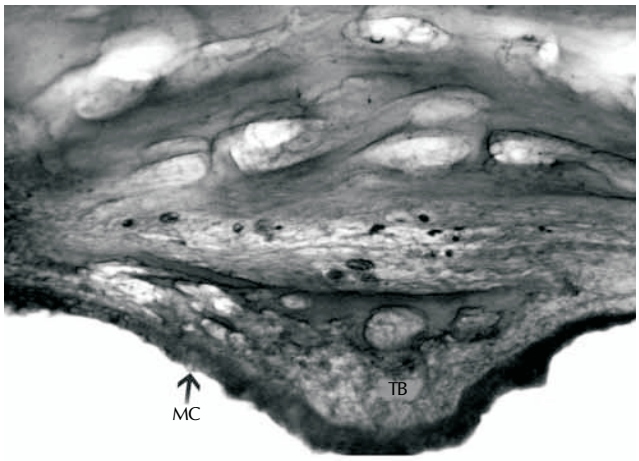

Figure 6: Section of gill arch showing bulged taste bud (TB) and Mucous cell (MC) after 96 hrs of exposure. H/E x 300

Histopathological change in the gill of *Labeo rohita* was reported by Vijaya Lakashmi and Tilak, 1996 after exposure of the fish to organophosphate pesticide monocrotophos. According to their investigation epithelial proliferation, congestion of blood vessel and hyperplasia of mucus cells was reported in the gills. The similar changes were also observed by Kumaraguru *et al.,* 1982; Velmurugan *et al*., 2009; Rani and Venkataramana, 2012. The structural alterations in the gill morphology had been categorised by Dutta *et al*. (1996) into two groups: (1) the insecticides causing necrosis and rupture of the branchial epithelium. These changes are dose dependent and often reported under lethal conditions. The death of branchial cells and their rupture usually develops either by autolysis or by rapid lysis caused by the direct action of toxicants on the cells' constituents (Abel, 1976) and (2) branchial defence response achieved by mucus hyper secretion, epithelial lifting, swelling, hyperplasia and lamellar fusion.

It is well established that secondary gill lamellae play an important role in the transport of respiratory gases. The damage done to the lamellae might have reduced the O_2 transport which in turn would have influence the metabolic system of the fish. The accumulation of the pesticide on gill imitated the elevation of mucus secretion and decreased ventilation which ultimately decreased the O_2 uptake through gills. The similar observation was reported by Bradbury *et al*., 1987; Bradbury and Coats, 1989 and Prashanth *et al*., 2011. Wannee *et al.*, 2002 was observed the epithelial lifting and aneurysn in the *Nile tilapia*, *Oreochromis niloticus* under exposure to glyphosate for 96h. The enlargement of chloride secreting cells and their nuclei supports the above assumption.

The periodic secretion of mucus might be one of the important means for elimination of toxicants from the surface of gills. This finding also match with the observation of Pandey Govind *et al*., 2011. Witters *et al*., 1990 observed that complexion with organic material lowers the harmful effect of toxicants on the gill surface. Arillo and Melodia, 1990 stated that some components of mucus probably the protein bound sulphydryl groups, have a deep toxifying function against ambient toxins. One of the prominent phenomenon that has been observed

in present investigation was the fusion of secondary lamella. This was due to counter stress and may also be due to transformation of electrically charged properties of the epithelial cells which favour adhesion between the cells of two neighbouring secondary lamellae. The fusion of secondary lamellae causing a drastic reduction in the respiratory surface area. Several other toxicants are also known to induce fusion of secondary lamella of gills (Leino *et al*., 1987; Dutta *et al*., 1996; Parashar and Banerjee, 2002, Wendelaar Bonga, 1997). Hence it is reasonable to conclude that hostathion intoxication caused severe aerobic stress in *Channa gachua*. Collapsing of slimy protective covering that fails to prevent the penetration of insecticide. This leads to various degrees of wear and tear, which causes damage to thin protective device of the gill epithelium of *Channa gachua*. The other changes in the gill epithelium that occurred after the exposure to hostathion includes the separation of respiratory epithelium from basement membrane that increases the thickness of secondary lamella as similar to the findings of Karlsson-Norrgren *et al*., 1985; Parashar and Banerjee, 2002. The increasing thickness of SL decrease the diffusion capacity, it might be to form an additional barrier to prevent the entering of insecticides dissolved in water. Identical lifting of the respiratory epithelium of the SL of the gills and/or ARO has also been observed in *H. fossilis* subjected to desiccation stress (Parashar and Banerjee, 1999b; 1999c) and Lead nitrate exposure (Parashar and Banerjee, 1999a).

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