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ROGOR INDUCED HISTOPATHOLOGICAL CHANGES IN THE GILLS OF FRESHWATER FISH *PUNTIUS STIGMA* FROM SUKHANA RIVER, AURANGABAD (M.S) INDIA

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ABSTRACT:

Histological biomarkers of toxicity in fish organs are a useful indicator of environmental pollution. The histological effects of rogor, an organophosphate insecticide, on the gill tissues in *Puntius stigma* were determined. The fishes *Puntius stigma* were exposed to lethal concentrations at 96 hrs LC_{50} and sub lethal concentrations at (1/5, 1/10 and 1/15 ppm) of rogor for 30 days. The fishes shows severe histological changes in the gill lamellae such as bulging, epithelial hypertrophy, fusion of secondary lamellae, hemorrhage, curling of lamellae, swelling of pillar cells, swelling of chloride cells.

Key word: Histopataology, Rogor, LC_{50}, Sub-lethal Concentration, Gills, *Puntius stigma*.

Introduction:

Fish species were recently suggested as environmental biomarkers (Tom *et al.,* 2003). Quantification of fish metallothionein transcript levels in absolute units has only recently been presented (Evans *et al.,* 2000). It also, considered as early warning for degradation of environmental quality, but also specific measures of the toxic, carcinogenic and mutagenic compounds in the biological materials (Verlecar *et al.,* 2006).

Fish are very susceptible to bioaccumulation in their fatty tissues, as they take up linden residues from the water through the gills and skin (Ortiz *et al.,* 2002). The exposure to chemical contaminants can induce a number of lesions and injuries to different fish organs suitable for histopathological examination in searching for damages to tissue and cells (Rabitto *et al.,* 2005).

In fish, gills are critical organs for respiratory and osmoregulatory functions. Respiratory distress is one of the early symptoms of pesticide poisoning. In the gills these toxicants appear to break down the adhesion between epithelial branchial cells and the underlying pillar cells; this is accompanied by a collapse of the structural integrity of the secondary lamellae and subsequent failure of the respiratory functioning of the gills.

A review of literature shows that no much more efforts were made to study the histopathological changes caused by rogor (dimethoate) in the different tissues of the freshwater fishes, *Puntius stigma.* The present investigation was undertaken to study in detail the histopathological changes in the gills of the freshwater fishe, *Puntius stigma* after acute and chronic exposure to the rogor.

MATERIAL AND METHODS

The live specimens of *Puntius stigma* were collected from Sukhana River flowing near Nipani, 25 km away from Aurangabad (M.S.) and brought to the laboratory. The fishes were maintained in glass aquaria and were acclimatized for four weeks. During the acclimatization healthy fishes showing normal activities were selected for histopathological studies.

The fishes were maintained in sufficiently large aquaria so to prevent overcrowding, the acclimatized fishes were given artificial air by aerator. Glass aquaria of size (3* 1* 1* feet) were used as test container.

The *Puntius stigma* ranged from 7.5 to 8.5 cm in length and 4.5 to 5.5 gm in weight were selected for the test. The fish, *Puntius stigma* exposed to lethal concentrations for 96 hr at 7.1 ppm (LC_{50}) of rogor. Simultaneously a control aquarium was also maintained. At the end of acute exposure for 96 hrs the survived fishes were killed by decapitation and gill were removed and fixed in Bouins fluid for 24 hrs, and histopathology was studied.

In the second set of experiment, the test fishes, *Puntius stigma and* were exposed to three sublethal concentrations of rogor for 30 days such as 1/5, 1/10 and1/15 ppm were prepared. Simultaneously, a control aquarium was maintained. At the end of experiment, surviving fishes were utilized for histopathological study. All the tissues were immediately fixed in Bouins fluid for 24 hrs and processed according to standard procedure of routine micro technique.

RESULTS

The remarkable histopathological changes due to exposure to rogor in the gill of *Puntius stigma* are depicted in microphotographs. In the controlled set no histopathological changes were observed.

Histology of Gill (control):

Gills were situated in branchial chamber on either side of the body in fishes. Each gill has a gill arch with double row of elongated, laterally projecting gill filaments. These filaments were flat and leaf like and joint at the base on gill rackers by a gill septum. Numerous semicircular, leaf like projections were lined up along both sides of the primary lamellae (PL) called as secondary gill lamellae (SL). The primary gill lamellae consist of centrally placed rod like supporting axis with blood vessels on either side. The secondary lamellae termed as respiratory lamellae were highly vascularised and covered with thin layer of epithelial cells. Blood vessels were extended into each of the secondary gill filaments provided with pillar cells (PC) and chloride cells (CC).

The secondary lamella was supplied with marginal blood sinus lined by an endothelium. In between the secondary gill lamellae and the primary filament, lined by thick stratified epithelium (ILR). This region between two adjacent secondary gill lamellae was known as interlamellar region (Fig 1a).

Histopathology of gill:

The fish exposed to lethal concentration for 96 hrs at 7.1 ppm (LC_{50} of 96 hrs) of rogor showed noticeable degenerative changes in the architecture of gill, fusion of secondary lamellae (FSL), curling of secondary lamellae (CSL), swelling of chloride cells (SCC), swelling of pillar cells (SPC) and degeneration of secondary lamellae (DSL) have been noticed (Fig.2 b).

Fish was exposed to sublethal concentration at 1.41 ppm (1/5) of rogor (dimethoate), for 30 days displayed marked histopathological changes. In the gills, the most common symptoms of toxic exposure were haemorrhage (HR), curling of secondary lamellae (CSL), swelling of chloride cells (SCC) and swelling of pillar cells (SPC). (Fig.3 a).

Fish was exposed to sublethal concentration at 0.70 ppm (1/10) of rogor for 30days exhibited noticeable pathological changes. The most common symptom was hemorrhage (HR), curling of secondary lamellae (CSL), swelling of chloride cells (SCC) and widening of primary gill lamellae (WPL). (Fig.4 b).

The fish exposed to sublethal concentration at 0.46 ppm (1/15) of rogor for 30 days showed pathological changes such as bulging tip of primary lamellae (BPL), fusion of secondary lamellae (FSL) hemorrhage (HR) and reduction in secondary lamellae (RSL) have been noticed (Fig.5 a).

DISCUSSION

In the present study it has been observed that increased exposure period, though exposed to a lower concentration, leads to increased damage to the tissue of the freshwater fishe *Puntius stigma* respectively.

The main objective of the histological assessment of the gill is to verify the possible damages caused to the organism by rogor, evidencing alterations resulted from the acute and chronic toxicity. The gills have a large superficial area through which gaseous exchanges between the blood and the external medium take place (Newstead, 1987). Beside the respiratory function, this organ performs other vital functions such as osmoregulation and excretion (Mallat, 1985). The direct contact between this organ and water promotes the interaction with toxic substances present in the water as they are sites of ionic link to perform normal functions. Adsorption of metal and other pollutant with charges may eventually occur; bring about toxic effect on the organism (Hollis and Playle, 1997).

Many investigators have reported the histopathological changes in gills of different fish species exposed due to pesticides. Mucus extrusion, lamellar swelling, fused and reduced microridges were observed in bluegill sunfish, *Lepomis macrochirus* to different sublethal concentrations of diazinon (Dutta *et al.,* 1997).

In another study, cloudy swelling, bile stagnation, focal necrosis, atropy and vacuolization have been reported in the *Corydoras paleatus* exposed to methyl parathion (Fanta *et al.,* 2003). Hyperplasia, vacuolation, disintegrated blood vessels, disrupted hepatocytes, focal coagulative necrosis, disorganized hepatic canaliculi were observed by (Sarkar *et al.,* 2005) in *Labeo rohita* exposed to cypermethrin.

Herbicide atrazine was administered to *Labeo rohita* fingerlings in 120 hours. The used dose of atrazine was 0.18 mg/l for 120 hours .The histopathological changes in the gill tissue like epithelial hyperplasia, curling of secondary lamellae and changes in chloride cells, besides these changes pyknotic nuclei, vacuolization, degradation of epithelial cells and pillar cells, were noticed (Jayachandran and Pugazhend 2009).

The present study reveals extensive damage to the gill architecture of treated fishes compared to gill of control fish. In the present investigation, *Puntius stigma* subjected to rogor showed marked histopathological changes in gill like, bulging tip of primary lamellae, Fusion and curling of secondary gill lamellae, widening of interlamellae distance, swelling in pillar and chloride cells and their nuclei appear swollen and pyknotic. Hemorrhage at primary in the rogor treated fishes in contract to control fish. The pathological changes in the gills might have resulted due to shifting from aerobic to anaerobic pathway in tissue respiration of fish under stress.

Histopathological evidences in the present study have been correlated to some extent with the work of (Paithane, 2010; Butachiram,*et al.*, 2009; Daksh and Capoor; 2011 and Subburaj A *et al.*,2018).

In the present study an attempts have been made to evaluate the intensity of the damage done to different organs of fishe *Puntius stigma* subjected to its lethal and sublethal concentrations of rogor. Histological changes induced due to the rogor in the gills of the freshwater fishes *Puntius stigma* were studied.

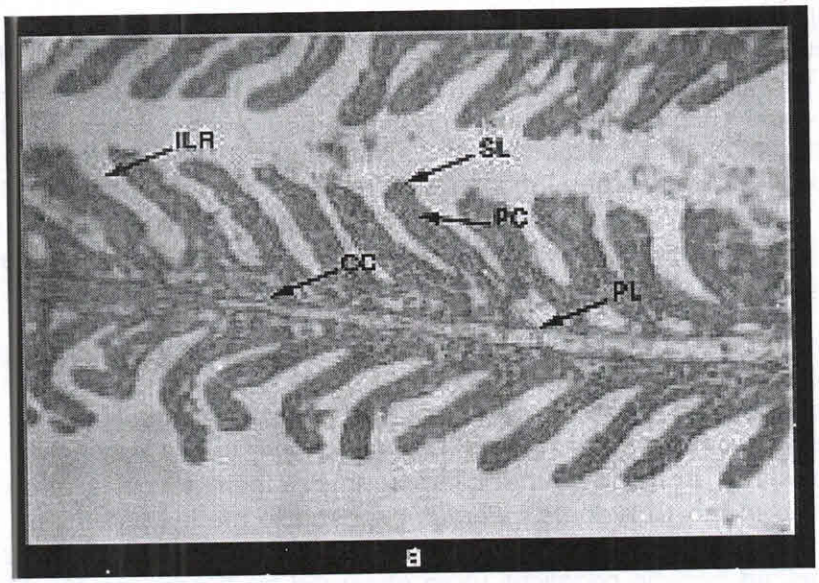

Fig 1(a) L.S. of Gill of Puntius stigma (Control). H/E 100X: ILR (Inter Lamellar Region), P (Primary gill Lamellae), CC (Chloride Cell), SL (Secondary Gill Lamellae), PC (Pillar Cell)

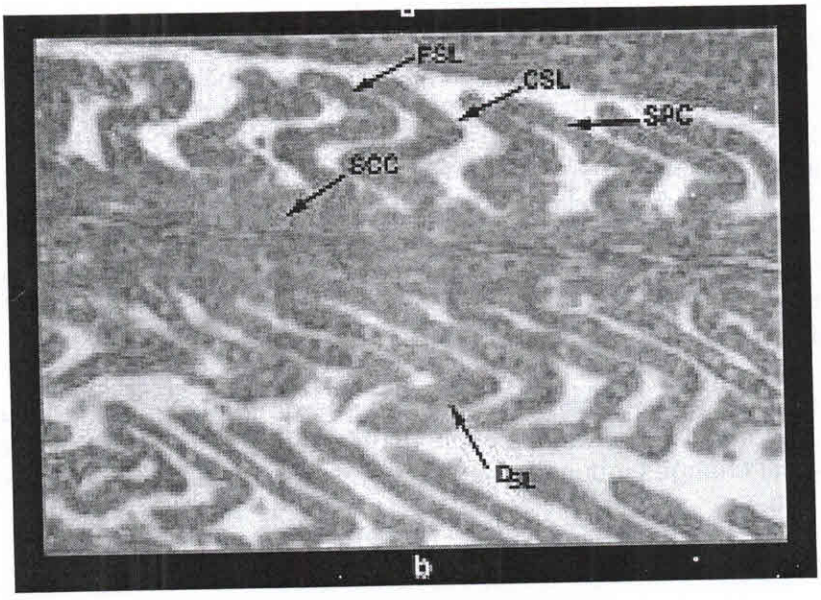

Fig 2 b) L.S. of gill of *Puntius stigma* after 7.1 ppm (LC_{50} of 96 hrs) exposure to rogor. H/E 400 x: DSL (Degenerated Secondary Lamellae), SPC (Swelling of Pillar Cell), CSL (Curling of Secondary Lamellae), SCC (Swelling of Chloride Cells), FSL (Fusion of Secondary Lamellae)

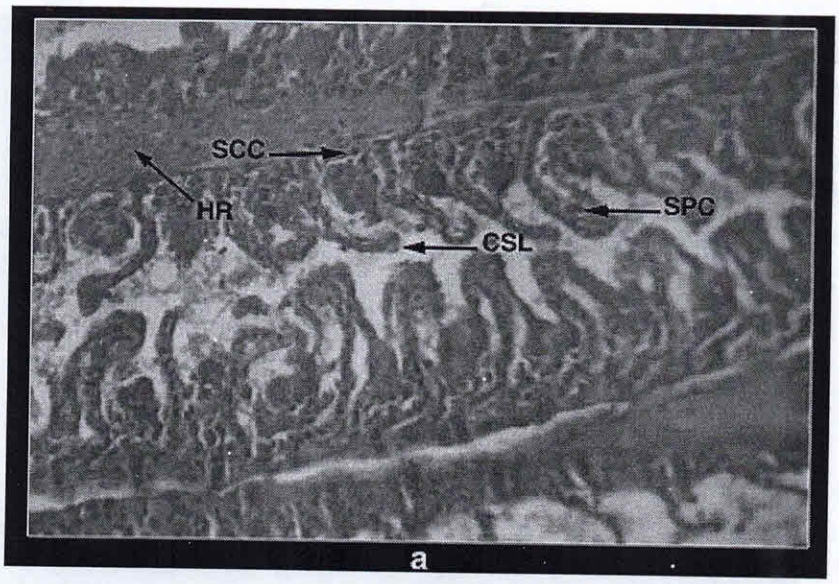

Fig 3 a) L.S. of gill of *Puntius stigma* after 1.70 ppm (1/5) exposure to rogor. H/E 400 x. HR (Haemorrhage), CSL (Curling of Secondary Lamellae), SCC (Swelling of Chloride Cells) and SPC (Swelling of Pillar Cells).

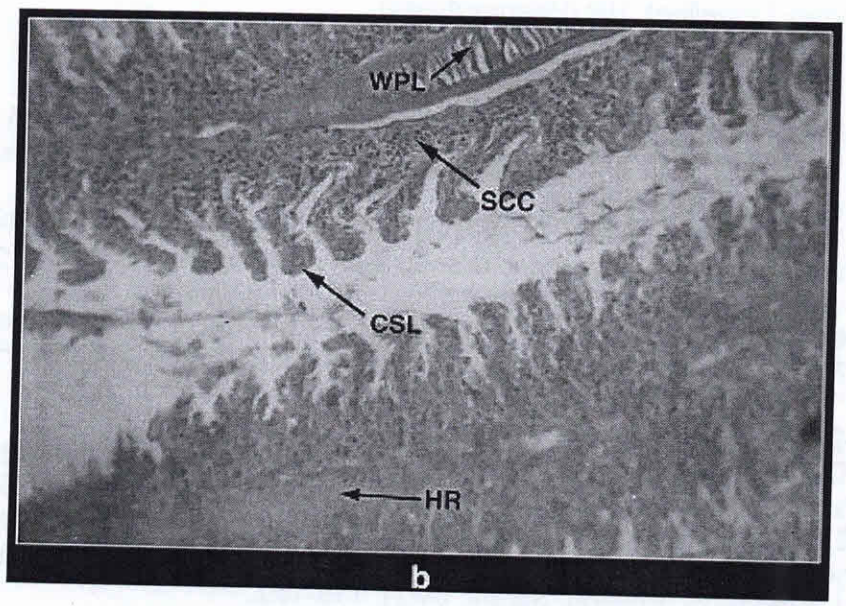

Fig 4 b) L.S. of gill of Puntius stigma after 0.70 ppm (1/10) exposure to rogor. H/E 400 x. SCC (Swelling of Chloride Cells), WPL (Widening of Primary Lamellae), HR (Haemorrhage), CSL (Curling of Secondary Lamellae).

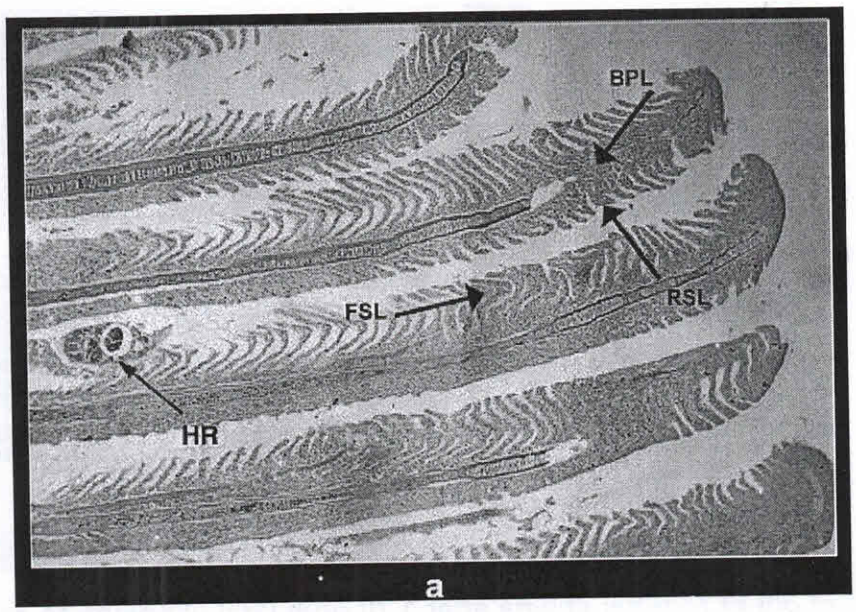

Fig 5 a) L.S. of gill of Puntius stigma after 0.46 ppm (1/15) exposure to rogor. H/E 100 x. BPL (Bulging tip at the Primary Lamellae), FSL (Fusion of Secondary Lamellae), RSL (Reduced Secondary Lamellae), HR (Haemorrhage).

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