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HISTOPATHOLGICAL CHANGES IN THE GILLS OF *PUNTIUS TICTO* (HAM) UNDER DIMETHOATE TOXICITY

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ABSTRACT
Puntius ticto (Ham), freshwater fish exposed to lethal and two sublethal concentrations of dimethoate (5.012ppm, 2.506ppm and 1.253ppm) for 96hrs and 60 days respectively. Histopathological changes in the gills were observed after exposure period. Cells of gill lamellae *i.e.* epithelial, chloride cells, pillar cells shows clowdy swelling after acute toxicity; while marked degenerative changes *i.e.* curling andfusion of secondary gill lamellae, necrosis hypertrophic conditions observed during chronic exposure.

INTRODUCTION

Wide use of pesticides in agriculture to control the pests has indirectly created the problem of pollution of aquatic environment. These chemicals/pesticides enter freshwater resources like ponds, river and lakes by various means and ultimately contaminate the water. These pesticides find their way into the body of aquatic fauna by means of gills, oral membrane, and gastrointestinal mucosa and from general body surface. These are deposited in the tissues and produces toxic effects. Therefore it is necessary to study in detail on the histopathological alterations in different organs of fishes and thoroughly investigate them in order to assess the extent of damage.

The pervious histopathological studies of fish exposed to pollutants revealed that fish organs are efficient indicators of water quality (Cardoso *et al.,* 1996 and Cengiz *et al.,* 2001). The gills are important organ in fish to perform respiration, osmoregulation, acid base balance and nitrogenous waste excretion (Heath, 1987). Fish gills are also vulnerable to pollutants in water because of their large surface area and external location. For this region, fish gills are considered to be most appropriate indicator of water pollution levels (Alazemi *et al.,* 1996). Many investigators have reported the histopathological changes in the gills of different fish species exposed to pesticides (Cengiz andUnlu, 2002; Vermurugan *et al.,* 2007; Butchiram *et al.,* 2009). However there has been little information on the histopathological impact of dimethoate on gills of *Puntius* ticto. Therefore, the present investigation was undertaken with a view to study in detail about histopathological changes in the gills of *Puntius ticto*, to dimethoate toxicity.

MATERIALS AND METHODS

The freshwater fish *P.ticto* were selected from the freshwater sources around Aurangabad city. They were acclimatized in aged, dechlorinated and well aerated water for two weeks in the laboratory. During acclimatization they were fed on alternate days with pieces of live earthworms. The LC_{50} values are determined by following the guidelines given by committee of toxicity tests with aquatic organism (Annon, 1975) and Probit Analysis Method (Finney, 1971). The acclimated fish were exposed to lethal concentration (5.012 ppm) for four days and sublethal concentrations (2.506ppm and 1.253ppm) for sixty days. Simultaneously a control group of healthy fishes were maintained under identical conditions. The twenty healthy fishes showing normal activity were exposed for chronic study. After commencement of exposure period fishes were killed by decapitation and gills are removed and fixed in Bouins fluid for 24h and processed according to standard procedure of routine microtechnique. For staining double stain method was followed by using Haematoxylin and Eosin and mounting was done in DPX.

RESULTS

The gill of *P.ticto* is of the typical teleostean type. Each gill has a gill arch with double row of elongated laterally projecting gill filaments or primary gill filaments. These filaments are flat and leaf like and join at the base by a gill septum. Numerous circular, leaf like projections are lined up along both side of the primary gill lamellae, called secondary gill lamellae. The primary gill lamellae consist of centrally placed rod like supporting axis (SA) with blood vessels on either side. The secondary gill lamellae also termed as respiratory lamallae

(RL) are highly vascularised and covered with thin layer of epithelial cells (EC). Blood vessels are extended into each of the secondary gill lamellae.

The secondary gill lamella is lined by a squamous epithelium. Inside this epithelium are lamellar blood sinuses separated by pillar cells. At the tip of primary lamellae is a marginal blood sinus lined by an endothelium. In between the secondary gill lamellae and the primary filament is lined by a thick stratified epithelium. This region contains the mucous cells and chloride cells.

The fish exposed to dimethoate shows marked histological changes after four days exposure to 5.012 ppm dimethoate. Some mild degenerative changes were observed in the inter-lamellar region. Epithelial lining of the secondary gill lamellae, chloride and pillar cells showed cloudy swelling and nuclei appeared swollen and pycnotic. The lifting up of the epithelium, some necrosis and bulging at the tips of primary filaments were noticed. The gill damage is marked by curling/ kinking and fusion of some secondary lamellae increased mucous cell was also observed.

More degenerative changes were observed after sixty days exposure (chronic). Dimethoate at 1.253 ppm exposure results in cloudy swelling, pycnotic nuclei in epithelial, chloride and pillar cells. The lifting of epithelium and bulging at the tips of primary gill filaments were observed. Curling and fusion of secondary gill lamellae, some necrosis and hypertrophy

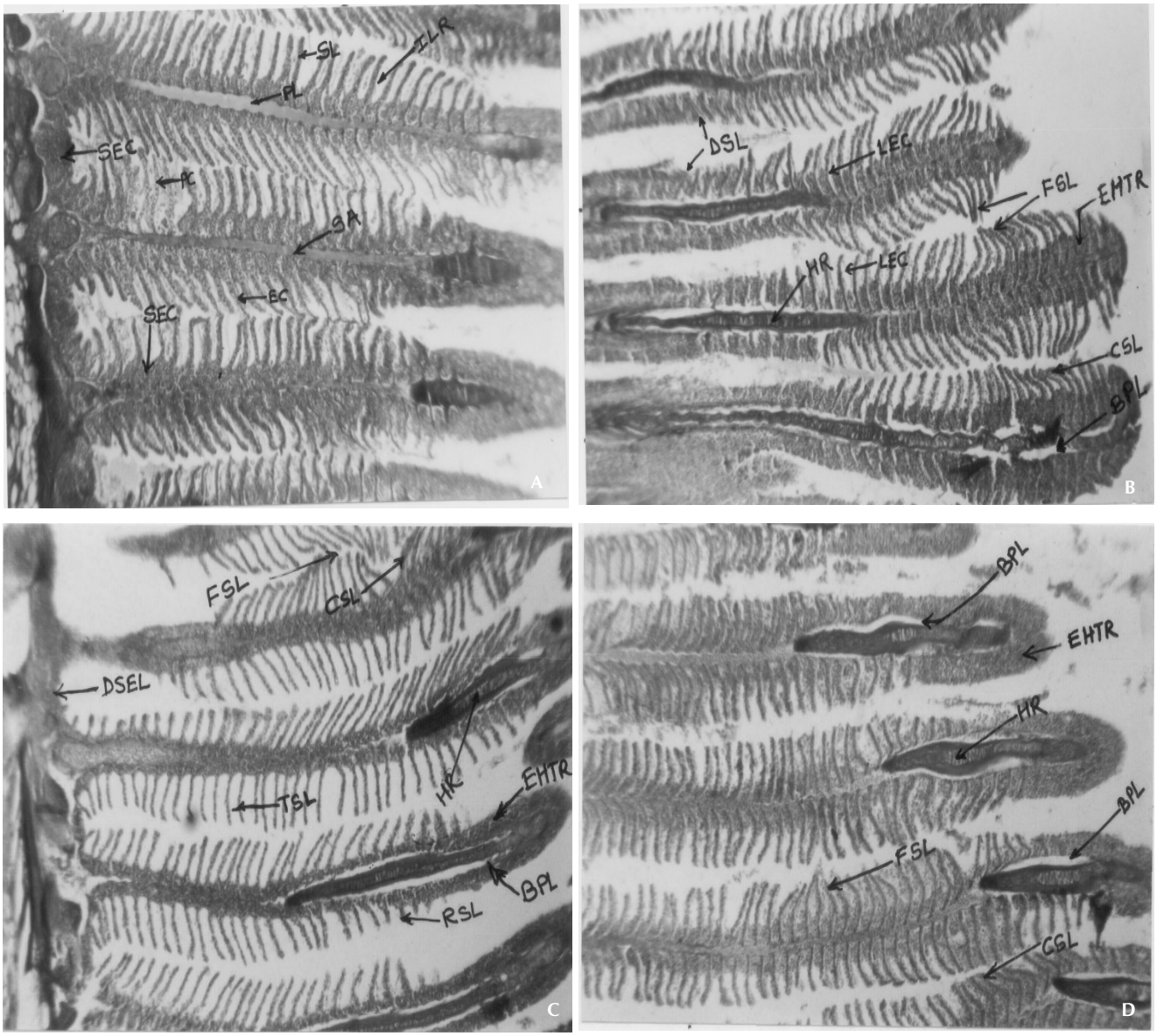

Figure 1: (A) L.S. of gills of *Puntius ticto* (Control) Haematoxylin/Eosin 100X; (B) L.S. of gills of *Puntius ticto* after 5.012 ppmexposure to dimethoate ,Haematoxylin/Eosin 100X; (C) L.S. of gills of *Puntius ticto* after 2.506 ppmexposure to dimethoate, H/E 100X; (D) L.S. of gills of *Puntius ticto* after 1.253 ppm exposure to dimethoate, H/E 100X

ILR = Inter lamellar region; EC = Epithelial cells; PL = Primary gill lamellae; PC = Pillar cells; SA = Supporting axis; SL = Secondary gill lamellae; SEC = Stratified epithelial cells; BPL = Bulging tip of primary gill lamellae; CSL = Curling of secondary gill lamellae; DSL = Degenerated secondary gill lamellae; EHTR = Epithelial hypertrophy; FSL = Fusion of secondary gill lamellae; LEC = Lifting of epithelial cell lining; DSEL = Degenerated stratified epithelial lining; HR = Hemorrhage; RSL = Reduced secondary gill lamellae; TSL = Thinning of secondary gill lamellae

condition in epithelial cells were noticed.

The second sublethal exposure (2.506ppm) resulted in marked degenerative changes in the architecture of gills. They showed reduced central axis, reduction in number of mucous cells *i.e.* necrotic condition which leads into interlamellar space formation. Swelling and pycnotic conditions were also observed in epithelial cells, chloride cells and pillar cells. Thining, reduction in size and rupture was noticed in some places of secondary gill lamellae. KinKing, fusion of secondary gill lamellae, lifting of epithelium, necrosis and bulging at the tip of primary gill filament were observed dominantly. Thus, chronic toxicity at higher dose exposure shows severe damage than the lower dose

DISCUSSION

Under toxicity stress hypertrophic response of mucus glands observed in gills which covers all the surface of gill which is conformity with the findings of Richmonds and Dutta (1989) and Cardoso *et al*. (1996).The mucous cells secrete excess amount of mucus substance over the epithelial cells in order to protect and hamper the erosion and damage of the respiratory epithelium.

The epithelial cloudy swelling, lamellar fusion and kinking or curling of lamellae could be seen as a defensive response (Morgan and Tovell, 1973) against prolonged exposure. Epithelial lifting and lamellar fusion were also suggested as a protective measure by decreasing the vulnerable surface area of the gills to maintain its osmoregulatory function (Abel, 1976). Such reactions that help to slow down toxicant uptake could result in dysfunctional or even non-functional gills. Similar results were observed by Mallatt (1985), Erkmen *et al*. (2000).

Jagoae and Haines (1983) found swelling of primary and secondary lamellae, fusion of secondary lamellae, increased mucus production and secondary lamellae appeared thickened and shortened in brook trout, *Salvelinus fontinalis* exposed to pH 4.5 and 5.0 for 456h. Ramesh (1994) reported that separation and lifting up of the epithelium might be a defense response of the fish in response to toxicants.

Nowak and Barbara (1992) studied effects of residues of endosulfan in gills of catfish, found odema with lifting of lamellar epithelium and hyperplasia of epithelium. Vijayalakshmi and Tilak (1996) studied effect of monocrotophos and fenevalerate on the gills of *Labeo rohita* and found bulging at tips of primary gill filaments, curling of secondary filaments, necrotic pillar cells and fusion of gill filaments to monocrotophos intoxication. These observations are also observed during present investigation. Pfeiffer *et al*. (1997) also observed secondary lamellar fusion, distortion, thining, mucus release, detached pillar cells and chloride cells in *Carassius auratus* under carbaryl toxicity stress. Similar results were also observed by Erkman *et al*. (2000) and Cengiz and Unlu (2002).

Vermurugan *et al*. (2007) found epithelium hyperplasia, aneurism, curling and fusion of secondary lamellae in *Cirrhinus mrigala*, after exposure to monocrotophos. Ayoola and Ajani (2008) observed cellular infiltration, swollen tip of the gill filament, congestion, severe gill damage and heterophilic infiltration in Jwenile African Catfish *Clarius gariepinus* under cypermethrin toxicity. Cristina *et al*. (2008) found epithelial rupture, secondary lamellae fusion and hyperplasia of branchial epithelium in *Carassius auratus gibelio*, exposed to 0.05mg/lit. Malathion. Butchiram *et al*. (2009) observed bulging of tip of primary gill filaments, necrosis in nucleus of pillar cells, fusion of secondary gill lamellae and vacuoles in the secondary gill lamellae of *Channa punctatus* (Bloch) under Alachlor toxicity. Jayachandran and Pugazhendy (2009) observed excessive mucus secretion, lifting up of the epithelium and lamellar fusion, hyperplasia of gill filaments, fusion of gill filaments, necrosis of gill epithelium, shortened and clubbing ends of the secondary gill lamellae, degeneration of pillar cells and the development of vacuoles in the epithelium of freshwater fish *Labeo rohita* (Hamilton) exposed to sublethal concentrations of atrazine Zaki *et al*. (2009).

Observed necrosis of both primary and secondary gill lamellae, hyperplasia of primary and secondary lamellar epithelium with shortening and fusion of gill lamellae in *Tilapia zilli* exposed to climate change and cadmium chloride. Chezhian *et al*. (2010) observed swelling, hyperplasia and hypertrophy, proliferation of chloride cells, lifting up of the epithelium, degenerative changes of epithelial cells, fused lamellar filaments, necrosis and disintegration of epithelial cells, desquamated epithelium and haemorrhage in effluent treated estuarine fish *Lates calcarifer*. Belicheva and Sharova (2011) observed epithelial cells hyperplasia, marked proliferation epithelium on the tips of the secondary lamellae and interlamellar zones, epithelial lifting accompanied by the enlargement of the intertissue space between the epithelium and underlying pillar cells, necrosis and destruction of respiratory lamellae in gills of *Abramis brama* L, *Rutilus rutilus* L, *Perca fluviatilis* L and *Stizostedion lucioperca* L fish from polluted Vygozero reservoir.

The rupture of the branchial epithelium is considered as a direct, dose dependent, while hyperplasia, lamellar fusion and mucous hyper secretion could be signs of the branchial defense responses (Datta *et al.,* 1996). Lamellar fusion could be protective behavior as it diminishes the amount of vulnerable gill surface area in fish (Mallatt, 1985). Fusion of secondary lamellae and swelling of primary and secondary lamellae increases the diffusion distance (Tietge *et al.,* 1988) and reduced surface area (Smith and Haines, 1995).

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