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Research Papers

Histochemical analysis of mucosubstsnces in the gill epithelium of Channa punctatus exposed to lethal concentration of malathion

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Abstract

The gill epithelial cells of Channa punctatus (Bloch) exposed to lethal concentration of malathion showed increased production of neutral mucins , sulfomucins and sialomucins in variable amount as compared to only neutral mucins in poor amount in control fish. Increase in the amount of only neutral mucins elaborated by M1 mucous cells and only sulfomucins by M2 mucous cells was also evident. The epithelial cells and mucous cells reaveled the absence of glycogen and any atypical mucosubstances both in control fish as well as in fishes exposed to pesticide.

Key words : *Mucosubstances; Histochemistry; Gills; Epithelial cells; Mucous cells; Channa punctatus.*

INTRODUCTION :

The general structure and functions of the fish gill has become a matter of great interest as it is one of the prime organs of the body of the fish. The fish gill serves a variety of functions to fish such as gaseous exchange, acid-base balance, osmoregulation and ionic regulation Fosket et.al., 1983; Laurent et.al.,1994; and Evans et.al.,1999). The cell types of branchial epithelium has been described in several species of fish (Moris,1957; Cockson, 1975; Munshi,1980; Lewis and Potter,1982 and Usha Kumari et.al.,2008). The mucous cells have been reported by Kies and Wilmer,1932; Laurent and Dunel,1980; Droscher,1982;Gross et.al., 1998; Carmona et.al., 2004 and Diaz et.al.,2005. Epithelial cells and mucous cells have been studied histochemically to understsnd the nature of mucosubstances and their possible role in the life of fish. Carmignnani and Zaccone (1974) reported sulfomucins and neutral mucopolysaccharides in the epithelial cells of gill in young forms of T.ocellata and T.mormorata. Ingale (1981) studied the nature of mucosubstances in the epithelial cells and mucous cells of variety of fishes from different aquatic habitat and found species diversity in having different mucopolysaccharides in them. Porcelli and Novelli (1970) reported the presences of sulfated mucins in the muccoparous cells of developing branchial epithelium of S.fario. Bird and Eble (1979) reported on presence of acidic mucosubstances in the mucous cells of gill filaments. Carmignnani and Zaccone (1974) claimed that the mucous cells in the gills of T.ocellata and T.mormorata contained sulfated mucopolysaccharides.

Lock and Overbeeke (1981) studied the effect of mercuric chloride and methylmercuric chloride on the activity of the mucous cells in the gill epithelium of rainbow trout. Polka and Neef (1969) isolated and characterized the nature of mucosubstances in the gill of twelve brook trout,

S.fontinalis exposed to acidic water and made comparision with that of equal number of control trout. The impact of endosulfan on total sugar and glycogen content in the gills of fishes have been studied by Praveen and Vasantha (1988). They found decrease in total sugar and glycogen and suggested that the carbohydrates which are the ready made source of energy may be utilized under pesticide stress. Careful persue of the existing literature revealed no work has been done on effects of malathion, a widely used organophosphrous pesticide on histochemistry of mucosubstsances in the gill epithelial cells and mucous cells of C.punctatus. Hence, the present investigation is undertaken.

MATERIAL AND METHODS :

For the present investigation live and healthy fishes were collected from river Krishna around Karad. The fishes were then transported to the laboratory and kept in glass aquaria of 100 litre capacity filled with fresh, chlorine free tap water for acclimatization.. A batch of ten ,well acclimatised fishes of uniform size (20 to 25 cm.) were then exposed to different.(4 ppm, 6 ppm, 8 ppm,10 ppm and 12 ppm) concentration of malathion for a definite period in glass aquaria of size 60 X 30 X 25 cm. and about 50 litre capacity and the lethal concentration was calculated for 48 hours.The aquaria were kept open and the fishes were kept starved during experimentation. Control as well as the fishes under experiment (overturned) were taken out of the aquaria. Each fish was sacrificed, its gills were dissected out and immediately fixed in cold (4 0C) 2 % calcium acetate in 10% neutral formalin (CAF fixative) for 24 hours, dehydrated in a graded series of alchohol, cleared in xylene and embedded in paraffin. Serial sections of 4 to 5 µm thickness were obtained some of the sections were stained with hematoxylene-eosin (H.E.) for histological observation and the adjacent sections were subjected to series of well estabilished and recommended histochemical techniques for characterization of mucosubstances.

R E S U L T S :

Histomorphologically the gill of C. punctatus revealed identical structure to that of many fresh water teleosts (fig.1) The histochemical analysis of mucosubstances was carried out in the epithelial cells and mucous cells in the gills of control fish and in fishes exposed to lethal concentration of malathion. The mucous cells were found distributed in the epithelium of gill arch, primary gill lamellae and secondary gill lamellae. These were more numerous at the tip of primary gill lamellae (Fig.1). On the basis of results obtained they could be divided in to M1 and M2 mucous cells (figs. 5,7).

The histochemical reactivities of mucosubstsances in the gilll epithelial cells and mucous cells of control fish atre illustrated in microphotographs (figs.2-4) and of fishes under experimentation are illustrated in microphotographs (figs.5-12) The histochemical reactivities of mucosubstances in the gill epithelial cells and mucous cells control fish and fishes under experiments are recorded in table No. 1 and table No.2 respectively according to staining intensities (: ++++ intense,+++ moderate, ++ weak,+ poor ± trace and – negative) and shades.The results obtained are given in the table No. 3.

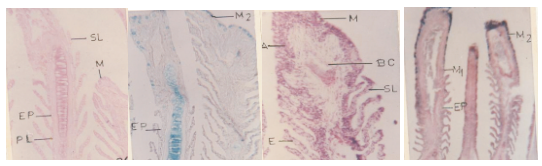

Fig. 1 : L.S.of gill of control fish H.E.staining X 3oo.
Fig. 2 : L.S.of gill of control fish PAS. staining X 3oo.
Fig. 3 : L.S.of gill of control fish AB pH 2.5 staining X 3oo.
Fig. 4 : L.S.of gill of control fish AB pH 2.5 staining X 3oo.

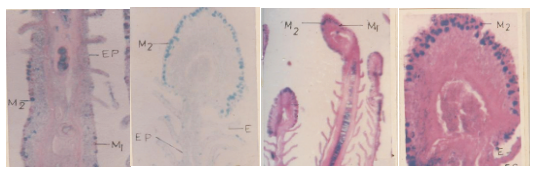

Fig. 5 : L.S.of gill fish exposed to 8 ppm malathion for 72 hrs C.I.-PAS staining X 3oo.
Fig 6 : L.S.of gill fish exposed to 8 ppm malathion for 72 hrs. AB pH 2.5 staining X 3oo.
Fig. 7 : L.S.of gill fish exposed to 8 ppm malathion for 72 hrs.AB pH 2.5.-PAS staining X 150.
Fig. 8 : L.S.of gill fish exposed to 8 ppm malathion for 72 hrs.C.I.-PAS staining X 450.

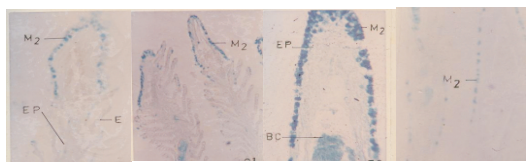

Fig. 9 : L.S.of gill fish exposed to 8 ppm malathion for 72 hrs.Acid hydrolysis AB pH 2.5
Fig. 10 : L.S.of gill fish exposed to 10 ppm malathion for 48 hrs.AB pH 2.5 staining X 150.
Fig. 11 : L.S.of gill fish exposed to 12 ppm malathion for 12 hrs.C.I.-PAS staining X 3oo.
Fig. 12 : L.S.of gill fish exposed to 12 ppm malathion for 12 hrs.DM 37-AB pH 2.5 staining X 3oo.

Abbreviations used in figures and tables.

PAS = Periodic Acid Schiff, P-PAS = Phenylhydazine-PAS, D-PAS = Diastase-PAS, AB = Alcian blue, C.I.:Colloidal iron, AF = Aldehyde Fuschin, CEC = Critical electrolyte concentration, M37 = Mild methylation, DM37 = Mild methylation saponification, M60 = Active methylation, DM60 = Active methylation saponification, A = Acidophils, M, M1, M2 = Mucous Cells, BC = Blood Cells, EP, Epithelium, PL = Primary gill lamellae, SL = Secondary gill lamellae.

Table No.1 :
Comparative histochemical reactivities of mucosubstances in the gill epithelial cells of control fish and fishes exposed to different concentrations of malathion.

Sr. No.	Histochemical Reactions	Control	Fishes expose to different concentration of malation		
			8 ppm	10 ppm	12 ppm
1	PAS	+P	++±P	++±P	+±P
2	P-PAS	-	+P	+P	+P
3	D-PAS	+P	++±P	++±P	+±P
4	AB pH 1.0	-	±B	+B	±B
5	AB pH 1.0- PAS	+P	++±BP	++±BP	+±BP
6	AB pH 2.5	-	±B	+B	±B
7	AB pH 2.5- PAS	+P	++±BP	++±BP	+±BP
8	C.I.	-	±B	+B	±B
9	C.I.-PAS	+P	++±BP	++±BP	+±BP
10	AF	-	±P	+P	±P
11	AF- AB pH 2.5	-	+PB	+P	±P
12	Azure A pH 1.5	-	±M	+M	±M
13	Azure A pH 3.0	±O	±M	+M	±M
14	Azure A pH 4.5	+O	±M	+M	±M
15	Sulfation Azure A pH 1.5	+M	++±M	++±M	+±M
16	CEC + 0.1 M Mg++	-	±B	+B	±B
17	CEC + 0.2 M Mg++	-	±B	+B	±B
18	CEC + 0.4 M Mg++	-	-	-	-
19	CEC + 0.6 M Mg++	-	-	-	-
20	M 37 AB pH 2.5	-	±B	+B	±B
21	DM 37 AB pH 2.5	-	+B	+B	±B
22	M 60 AB pH 2.5	-	-	-	-
23	DM 60 AB pH 2.5	-	±B	-	-
24	Acid hydrolysis-AB pH 2.5	-	±B	+B	±B
25	Sialidase- AB pH 2.5	-	±B	+B	±B
26	Hyaluronidase- AB pH 2.5	-	+B	+B	±B
27	Pepsin AB pH 2.5	-	+B	+B	±B

Table No.2
Comparative histochemical reactivities of mucosubstances in the gill epithelial cells of control fish and fishes exposed to different concentrations of malathion.

Sr. No.	Histochemical Reactions	Control		Fishes expose to different concentration of malation			
				8 ppm		10 ppm	12 ppm
		M1-Cells	M2-Cells	M1-Cells	M2-Cells	M2-Cells	M2-Cells
1	PAS	++P	++±P	+++P	+++±P	+++P	+++P
2	P-PAS	-	++±P	-	+++±P	+++P	+++P
3	D-PAS	++P	++±P	+++P	+++±P	+++P	+++P
4	AB pH 1.0	-	++±B	-	+++±B	+++B	+++B
5	AB pH 1.0- PAS	++P	++±B	+++P	+++±B	+++B	+++B
6	AB pH 2.5	-	++±B	-	+++±B	+++B	+++B
7	AB pH 2.5- PAS	++P	++±B	+++P	+++±B	+++B	+++B
8	C.I.	-	++±B	-	+++±B	+++±B	+++±B
9	C.I.-PAS	++P	++±B	+++P	+++±B	+++±B	+++±B
10	AF	-	++±P	-	+++±P	+++P	+++P
11	AF- AB pH 2.5	-	++±P	-	+++±P	+++P	+++P
12	Azure A pH 1.5	-	++±M	+O	+++±M	+++M	+++M
13	Azure A pH 3.0	+O	++±M	++O	+++±M	+++M	+++M
14	Azure A pH 4.5	++O	++±B	+++O	+++±M	+++M	+++M
15	Sulfation Azure A pH 1.5	++M	++±B	+++M	+++±M	+++M	+++M
16	CEC + 0.1 M Mg++	-	++±B	-	+++±B	+++B	+++B
17	CEC + 0.2 M Mg++	-	++±B	-	+++±B	+++B	+++B
18	CEC + 0.4 M Mg++	-	++±B	-	+++±B	+++B	+++B
19	CEC + 0.6 M Mg++	-	+±B	-	++±B	++B	++B
20	M 37 AB pH 2.5	-	++±B	-	+++±B	+++B	+++B
21	DM 37 AB pH 2.5	-	++±B	-	+++±B	+++B	+++B
22	M 60 AB pH 2.5	-	-	-	-	-	-
23	DM 60 AB pH 2.5	-	-	-	-	-	-
24	Acid hydrolysis-AB pH 2.5	-	++±B	-	+++±B	+++B	+++B
25	Sialidase- AB pH 2.5	-	++±B	-	+++±B	+++B	+++B
26	Hyaluronidase- AB pH 2.5	-	++±B	-	+++±B	+++B	+++B
27	Pepsin AB pH 2.5	-	++±B	-	+++±B	+++B	+++B

Table No. 3 :
Nature of mucosubstances in the gill epithelium and mucous cell of of control fish and fishes exposed to different concentration of malathion.

Sr. No.	Gill component	Control fish	Fishes exposed to different concentration of malathion		
			8 ppm	10 ppm	12 ppm
1	Epithelial cells	Presence of neutral mucosubsta-nces (poor)	Neutral mucins (poor toweak), sulphomucins (Trace) and sialomucins (trace)	Neutral mucosubstan-ces (Poor to weak) and sulfomucins (Poor)	Neutral mucoubstan-ces (poor) and sulfomucins (trace)
2	M1 Mucous cells	Neutral mucosubstan cces (weak)	Neutral mucosubstancces (moderate)	No mucous cells	No mucous cells
3	M2 Mucous cells	Sulfomucins (weak to Moderate)	Sulfomucins (moderate to intentse)	Sulfomucins (moderate)	Sulfomucins (moderate)

DISCUSSION :

The histochemical results obtained in the present investigation reaveled the absence of glycogen both in the epithelial cells and mucous cells in control fish. The absence of glycogen has also been reported by Carmignnani and Zaccone (1974) in the branchial epithelium of young individuals of T.mormorata and T.ocellata. Praveen and Vasant (1988) reported decrease in glycogen content in the gills of fishes exposed to endosulfan. According to them the glycogen which is the source of energy may be utilized under pesticide stress. However, in the present study absence of glycogen was noticed in fishes even exposed to the pesticide.

Cuparao (1967) reported the presence of sulfated mucoplysaccarides in the branchial epithelial cells of T.Shirana Chilwae. Carmignnani and Zaccone (1974) found considerable quantity of sulfated mucosubstances in the epithelial cell of the gill of adult specimen of T.mormorata and T.ocellata. Yamada and Yaokete (1975) reported neuraminic acid containing mucopolysaccharides with vicinal hydroxyl sulphate and carboxyl grouping and glycoprotein in the epithelial cells of eel, A.japonica. Ingale (1981) reported that the epithelial cells in fresh water fish like kharpa,katarna and shinggti elaborate only neutral polysaccharides. However, these cells in other fresh water fishes like kolshi, murungi,etc. contains neutral mucopolysaccharides and sialic acid fraction. In esturine and marine fishes these cells elaborate additional mucopolysaccarides i.e. sulfated polyanions. The present histochemical study demonstrated the presence of only neutral mucosubstances in the gill epithelial cells of control fish.

Ingale (1981) studied histochemicaly the gill epithelium of variety of species of fishes from different habitat and described six types of mucous cells on the basis of nature of polysaccharides elaborated by the particular cell. He pointed out that these mucous cell showed distinct variation with regard to their mucopolysaccharide. According to him this distinct difference in the nature of mucopolysaccharide can be correlated with the type of habitat the fish is inhabiting. The present histochemical studies revealed only two types of mucous cells (M1 and M2) in control fish and fishes exposed to 8 ppm malathion while only one type (M2 mucous cells) in fishes exposed to 10 ppm and 12 ppm malathion distributed throughout the epithelium of the gill arch, primary gill lamellae, and secondary gill lamellae.These were

more numerous at the tip of primary gill lamellae. Dilck DILER and Kenan CINAR (2009) identified the presence of mucous cells distributed in the primary gill filament epithelium of sea bass, D.labrax elaborating neutral glycoconjugates. Mucous cells in secondaru gill lamellae containing different glyoconjugates have been reported by Calabro et.al.,2005; Diazz, et.al., 2005 and Cinar et.al.,2008.The present histochemical study revealed the presence of only neutral mucins in M1 type of mucous cells and only sulfomucins in the M2 type of mucous cells in control fish

Some of the studies are concerned with the effects of some toxicants or change of habitat on mucin secretion in the gill of fishes. Planka and Neff (1969) found an increase in the mucin content and proliferation of mucus cells in the gill of the brook trout, S.fontinalis exposed to acidic water.Mucus accumulation on the gills of fishes has been observed in the gold fish following exposure to lead nitrate (Westfall,1945) or mercuric chloride (Mckone et.al.,1971; Lock,1975; Varanasi et.al.,1975) in cat fish treated with copper or zink sulphate (Lewis and Lewis, 1971) and in rainbow trout exposed to methyl chloride (Olson et.al.,1973; Lock,1975). Lock and Van Overbeek (1981) studied the effects of mercuric chloride and methyl mercuric chloride on the activity of mucous cells in the gill epithelium of rainbow trout, S,gairdneri. They found increased number of mucous cells and release of mucus from them in water in case of both the toxicants.The results obtained in the present investigation also revealed increased amount of mucins secretion by epithelial cells and mucous cells in the gill of fishes exposed to different lethal concentration of malathion. The change in the nature of mucosubstances secreted by the epithelial cells in the gill of fishes exposed to pesticide was also noticed.

The mucosubstances secreted by the gill epithelial cells and mucous cells perform some functions in the life of fishes. According to Jakowaska (1963) continuous production and release of mucus could prevent the settling of pathogenic organisms on the gill surface . Fletcher and Grant (1969) stated that the presence of bacteriolytic enzymes,antibodies and lysosomes activity in surface mucus indicates its protective function. Yamazaki (1972) stated that mucus might be involved in coagulation and precipitation of particles in suspension thus providing protection to delicate tissue such as the gill filaments. One of the more important functions of the mucus is its role in osmoregulation. Pickford et.al.,1966; Wittouck,1975; Marshall,1976; Hentschel and Miller,1979 suggested the role played by mucus in osmoregulation. Cockson (1971) attributed the osmoregulatory role for carboxymucins in the gill epithelium of T.Shirana Chilwae. It has been suggested that the layer of mucus covering the gill may facilitate ion uptake by its ion binding capacity (Kirschner, 1977; Marshall, 1978). The auther is in aggrement with that which has been suggested by earlier workers in this connection. The present investigation reaveled change in the nature of mucus from only neutral in the epithelial cells of control fish to neutral, sulfomucins and sialomucins in the gill epithelial cells of fish exposed to 8 ppm malathion, neutral and sulfomucins in fishes exposed to remaining concentration of malathion. However, there was no change in the nature of mucosubstances secreted by the mucous cells even after the exposure of fishes to pesticide.The problem why there is change in the nature of epithelial secretion is not understood. However, it is assumed that by doing this the fishes may tried to protect themselves from the dangerous effecs of the pesticide.

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