Biochem. Cell. Arch. Vol. 7, No. 2, pp. 211-216, 2007 *ISSN 0972-5075*

RESPONSE OF 2, 4-DICHLOROPHENOXY ACETIC ACID (2, 4-D) ON ACIDOPHIL CELLS IN GILLS OF A CAT FISH, *HETEROPNEUSTES FOSSILIS*

Anurag Singh, Anil Kumar*, H. S. Singh and J. P. N. Singh*

Department of Zoology, T. D. (P. G.) College, Jaunpur - 222 002, India
*Department of Zoology, R. H. S. (P. G.) College, Singramau, Jaunpur - 222 175, India

ABSTRACT – Increase in density and dimension of acidophil cells has been observed in gills of *H. fossilis* exposed to sublethal concentration of herbicide 2, 4-D at different duration of treatment. The change in number and size of acidophil cells has been correlated to cope with the poisoned aquatic environment.

Key Words : Acidophil cells, catfish, *Heteropneustes fossilis.*

INTRODUCTION

The main respiratory organ of *Heteropneustes fossilis* possesses five pairs of gills. A complete gill consists of a gill arch supported by cartilage. Each arch bears gill rakers towards the inner side and vascular plate like gill filaments projecting towards the out side and bears a series of secondary gill lamellae arranged on both sides alternately. The acidophil cells are found mainly in interlamellar region and gill tip region in addition to other cellular architecture viz. epithelial cells, mucous cells and pillar cells system. Most of the studies related to effect of different pollutants on gill of different fish species are confined to epithelial cells and mucous cells. Christie and Battle (1963) studied the histological effect on larval lamprey and trout. Munshi and Singh (1971) investigated effect of insecticides and other chemicals on the respiratory epithelium of predatory and weed fishes. Smith and Piper (1977) studies pathological effects on formalin treated rainbow trout (*Salmo gairdneri*). Daye and Garside (1976) studied the histopathological changes in superficial tissues of brook trout, *Salvelinus fontinalis* from acidified lakes, exposed to acute and chronic levels of pH. Huges and Perry (1976) evaluated the pollutant action by implying the morphometric study of gill damage on swimming performance of rainbow trout. Chevalier *et al* (1985) investigated the histopathological and electron microscopic structure of gills of brook trout *Salvelinus fontinalis* from acidified lakes. Mallat (1985) presented a statistical review on fish gill structural changes induced by toxicants and other irritants. Leino *et al* (1987) studied histophatholgical changes on Pearl dale, *Semotilus margarita* and fat head minnows, *Pimephales promelas* exposed to experimentally acidified lakes. Ojha *et al* (1989) enumerated effect of biocidal plant sap on the gills of a hill stream fish *Garra lamta* (Ham.). Roy *et al* (1990) studied the effect of saponin extracts on morphology and respiratory physiology of an air breathing fish, *H. fossilis*. Prasad (1989, 1991) described the SEM study on effects of crude

oil on the gills and air breathing organs of stripped gourami, *Colisa fasciatus* and climbing perch, *Anabas testudineus* respectively. Munshi and Singh (1962) evaluated effect of Low pH on gill of *Channa punctatus*. Dutta *et al* (1996) studied the ultrastructural changes in the respiratory lamellae of *H. fossilis* after sublethal exposure to malathione. Dutta *et al* (1997) studied effect of Diazinon on blue gill sunfish, *Leponis microchirus*. Park and Kim (2001) reported histology and mucin histochemistry of the gastrointestinal tract of the mud loach, in relation to respiration. Ojha and Huges (2001) studied effect of branchial parasites on efficiency of *Wallage attu*.

Recently, Singh *et al* (2006) enumerated impact of malachite green on chloride cells in gills of *Anabas testudineus*, Singh *et al* (2007) studied effect of Cadmium on secretion of branchial mucous cells of *Lepidocephalichthys guntea*. Daya Shankar *et al* (2007) investigated effect of chlordecone on acidophil cells of gill arch region of *Channa striatus*.

Present investigation aims to the study the effect of 2, 4-D, a herbicide on acidophil cells of gill filament and gill tip region of the cat fish, *Heteropneustes fossilis*.

MATERIALS AND METHODS

Live specimens of *Heteropneustes fossilis* (approx. length 15±3 cm and wt. 50±10g) were collected from local ponds and river Gomati at Jaunpur city (U.P.) and were acclimated to optimum lab conditions for 15 days. Fish during acclimatization were fed with minced goat liver on alternate days. Fishes were exposed to sublethal concentration (0.3mg/L) of 2, 4-D. Fishes were cold anaesthetized following Mittal and Whitear (1978) and gills from both sides of fishes were excised, rinsed in saline water and were fixed in 10% neutral formalin and Bouin's aqueous at the 2h, 4h, 6h, 12h, 1d, 2d, 4d, 5d, 6d, 8d, 9d and 10d of 2, 4-D treatment. Standard method of dehydration clearing and embedding were used. Paraffin sections were cut at 5µm thick and stained with Ehrlich's haematoxylin/ Eosin (H/E) to study structure of acidophil cells. Sections were subjected to various histochemical tests for carbohydrate and proteins following, Lillie (1954), Gurr (1958), Bancroft and Stevens (1982) and Pearse (1985).

RESULT AND OBSERVATION

Control

In control, acidophil cells of gill filament region at interlamellar position and on gill tip region are rounded, or oval and elliptical in shape with homogeneous cytoplasm having strong reaction for eosin. (Plate Ia, b). The small nucleus is not clearly distinguished due to dense cytoplasm. These cell display moderately magenta with PAS with and without prior diastase/saliva treatment and do not stain with AB at pH 2.5 and at pH 1.0, whereas moderately magenta with AB/ PAS technique. These reactions suggest that acidophil cells contain neutral glycoproteins. These cells display strong blue colour reaction with mercury bromophenol blue method for general protein and solochrome cyanin –R method for basic proteins and Ninhydrin/Schiff method for protein bound $-NH_2$ groups.

Herbicide 2, 4-D Treatment :

At 3h, 6h and 12h treatment these cells of gill filament and gills tip region do not show significant changes and appear polygonal shape.

At 1d and 2d, treatment acidophil cells

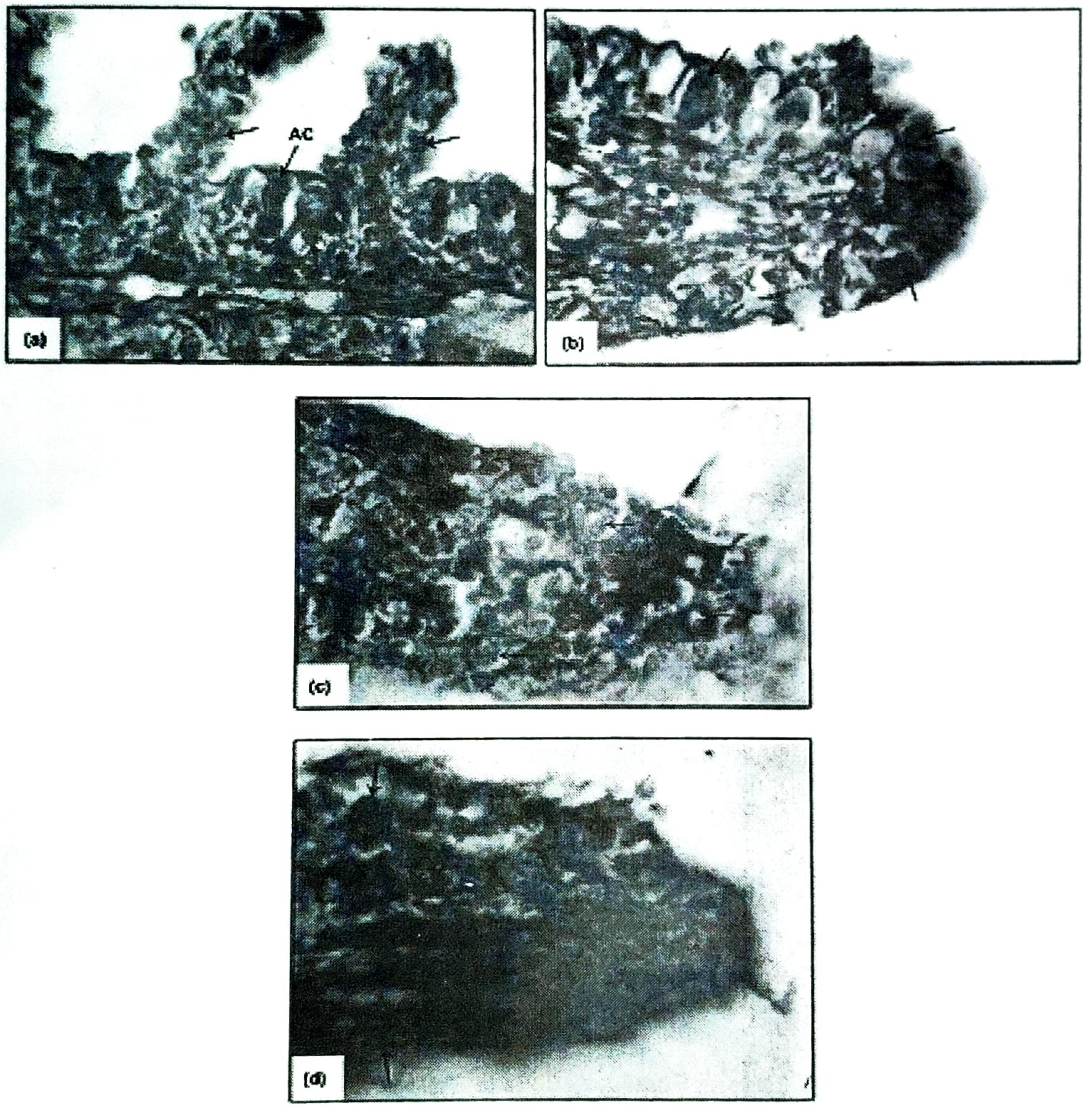

Plate (a-d) : Photomicrographs of the cross section of gill tip and gill lamellar region of *Heteropneustes fossillis* at control and at sublethal concentration of 2, 4-D herbicide treatment. **(a)** Showing acidophill cells (AC) at inter lamellar region strongly eosionophilic (pink in original) in nature. Note strongly stained gill filament epithelial cells (arrow) [Control, HE x 1000]. **(b)** Showing large, rounded acidophill cells at gill tip region (arrow), strongly eosinophilic in nature [Control, HE x 1000]. **(c)** Showing increase in density and number of acidophill cells at gill tip region (arrow) [2d, HE x 1000]. **(d)** Showing spherical or rounded shaped acidophill cells strongly stained (blue in original) in superficial layer in gill tip region (arrow) [8d, MBPB x 1000].

are found below mucous cells in deeper layer. There is appreciable increase in size and are somewhat flattened in shape.

At 3d, 4d, these cells are not clearly distinguished but few cells reappeared at 5d treatment.

At 6d, exposure numerous rounded acidophil cells were visible in superficial layers.

At 8d and 9d, acidophil cells display

more numerous occupying superficial and middle layer. They are voluminous and mostly spherical in shape.

At 10d, these cells secrete their cytoplasmic contents at the surface.

Histochemistry :

At earlier duration no characteristic histochemical changes occur within acidophil cells.

At 4d, 5d and 6d the acidophil cells show moderate PAS positive and their intercellular secretion are also magenta in colour.

At 7d and 8d, the acidophil cells display moderate reaction for glycoproteins.

At 9d and onward, these cells stain strongly for neutral glycoproteins.

No marked change in protein content is observed in acidophil cells at whole duration of 2,4-D treatment.

DISCUSSION

Increase in number and size of acidophil cells from 12h to 2d is due to intensive rate of synthesis of secretory contents in these cells. Decrease in number and disappearance at 3d and 4d, suggest that after secreting their cytoplasmic contents in the intercellular spaces at different duration get exhausted at 3d and 4d. These cells reappear at 5d treatment possibly after reviving their capacity for excessive synthesis of their secretory contents. In subsequent treatment from 6d to 10d either there is increase in density or dimension concluding more secretory activity of these cells.

The position, size and histochemical properties of the acidophil cells tally with chloride cells (Keys and Willmer, 1932; Liu, 1942; Morris, 1957; Copeland, 1950; Petengill and Copeland, 1948). However, they differ from negative reaction to chloride test. It means that these are not involved in chloride regulation when fish is in its natural habitat. But, when the fish is treated to intraperitoneal injection of NaCl of different concentration varying from isotonic to sea water salinity, these cells give strong positive reactions for chloride. This is indication their active participation in the extension of ions as that of the chloride cells in marine habitat, supported by Singh *et al* (2006).

Increase in size and number of acidophil cells may be correlated to cope with the influx of herbicide 2, 4-D, which is supported by Daya Shanker *et al* (2007). Further these cells at 3d and 4d treatment could not sustain the toxic environment and therefore due to heavy lysis of cellular constituents these cells also degenerate, however again arise at onward duration. The number increase at 6d and latter treatment as per according to the need of fish kept in toxic solution supported by Daya Shanker *et al* (2007).

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