Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(9):698-701

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Transmission electron microscopic study of gills of freshwater fish *Channa punctatus* (Bloch) exposed to the toxicity of cypermethrin

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ABSTRACT

In the present study, an attempt has been made to examine the toxic effect of cypermethrin, a synthetic pyrethroid exposed to freshwater fish Channa punctatus. The fishes were exposed to sublethal concentration of 0.08 mg/L for 15, 30 and 45 days. The gills of the fish plays vital role in respiration and are an important organ with specific functions which are highly sensitive to many factors. The chloride cells present in epithelia of gills plays main role in these functions. Transmission electron microscopic observations in experimental fishes showed accurate alterations in fish gills when compared to control. The pavement cells appeared irregular with a considerable loss of microridges which leads to irregular blood spaces and blood congestion as well as hyperplasia and lamellar fusion. Hyperplasia was often accompanied by extensive proliferation of both mucous and chloride cells. Chloride cells are enlarged and have a large apical surface and come in direct contact with the external medium.

Key words: Cypermethrin, *Channa punctatus*, Gills, TEM studies.

INTRODUCTION

Pesticides usage in the agricultural fields to control pests is extremely toxic to non target organisms like fish and affects fish health through impairment of metabolism, sometimes leading to mortality [3]. Most of pesticides find their way into rivers, lakes and ponds and have been found to be highly toxic not only to fishes but also to the organisms which contribute to the food chain of fishes [1]. Synthetic pyrethroids are one of the wide variety of pesticides contributing to this situation. But these pesticides also tend to affect the biology of non target species along with pests. Cypermethrin is a synthetic pyrethroid broad-spectrum insecticide used to control many pests, such as moth pests attacking cotton, fruit and vegetable crops, including structural pest control, or landscape maintenance [6].

Fish are relatively sensitive to the changes in their surrounding environment. Hence, fish health may reflect the health status of a specific aquatic ecosystem. An early toxic effect of pollution is only evident on cellular or tissue level before significant changes can be identified in fish behaviour or external appearance. Therefore, histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs such as gills, liver, kidney, intestine and gonads. Due to their lipophilicity, pyrethroids are easily permeated through the gills, which is a contributing factor in the sensitivity of the fish to aqueous pyrethroid exposures. These are used to control insect pests in agriculture, household and stored products [8].

Hence the objective of the present study is to know the effects of cypermethrin which is a synthetic pyrethroid on the gills of a fresh water snake headed fish *Channa punctatus* exposed under long term exposure i.e., 15, 30 and 45 days respectively. *Channa punctatus* was selected because it is hardy, readily available and easy to handle and can be kept alive for long duration in the aquaria. This fish is an important species in many water resources and is a common food fish. Thus, the objective of this study is to investigate the acute toxic effect of cypermethrin in *Channa punctatus* with an emphasis on the histopathological changes in gills observed in ultrastuctural changes.

EXPERIMENTAL SECTION

The fish (*Channa punctatus*) were collected from kolathur lake, Chennai and transported to the laboratory in an oxygenated polythene bag. The healthy adult specimens of *Channa punctatus* ranging in length from 13 to 15 cm and weighing about 18 to 20 gm. were selected and acclimatized for the experimental purpose. Water was regularly replaced and the fishes were fed daily. The physio-chemical characteristics of water were analysed as per the methods given in APHA (2005).

Determination of LC_{50}

The pesticide Cypermethrin (10% E.C.) was obtained and the stock solution was prepared by dissolving 10 mg of cypermethrin in 10 ml of analytical grade acetone. A required quantity of cypermethrin was drawn from this stock solution for further experiment. Preliminary tests were carried out to find out the median tolerance limit (LC_{50}) of the fish to cypermethrin for 96 hours by Probit analysis method (Finney, 1971). The concentration of cypermethrin at which 50% mortality occurred was taken as the median lethal concentration (LC_{50}) for 96hrs, which was found to be 0.4 mg/L. One fifth of the LC_{50} value (0.08 mg/L) was selected for sub lethal concentration studies.

Histopathological and electron microscopic examination

For transmission electron microscope investigations, the gill tissues of newly sacrificed fishes of 15, 30 and 45 days of exposure to cypermethrin were dissected out and fixed in 4% Glutaraldehyde and were washed in buffer. Again it was fixed by 1% osmium tetroxide and washed in buffer. This double fixation gives stability during dehydration. Dehydration was carried out in ascending series graded alcohol (50% to 100%), cleared by propylene oxide and then was embedded in siliconized rubber mould with epoxy resin. Then the mould was kept in incubator at 60^{o}C for 48 hrs, cool down. The blocks were then ready for sectioning. Ultrathin section (below 100 nm) were cut through ultramicrotome (Leica) with diamond knife (Diatome). Ultrathin section are taken on copper grid and stained (Double metallic) uranyl acetate and Reynold's solution (sodium citrate +Lead mitrate) which gives contrast. Then the sections were transmitted in EM (Phillins Tecnai TR spirit by Netherland) and were photographed.

RESULTS

Histological observations of gills – control

The control fish, *Channa punctatus* showed the normal ultrastructure of the gill. The gill arch is a curved osseous or bony structure from which radiate, double rows of paired primary gill lamellae or filaments. Each of these primary gill lamellae (PGL) has a series of secondary gill lamellae (SGL) located perpendicular to the basal lamina (BL) and the epithelial layer surrounded by micro ridges (MR). The primary gill lamella is covered by a mucoid epidermis which may have within it pale-staining saline, or salt secreting chloride cells (CC). These chloride cells are numerous at the basal (proximal) part of the lamellae. They are surrounded by the pavement cells (PC) and accessory cells. The chloride cells are filled with numerous mitochondria and apical part forming deep pit with microvilli. Mucous cells (MC) are apically located in the primary filament. They are characterized by the presence of large number of mucous containing vacuoles with variable electron density and basal nucleus (Fig.1).

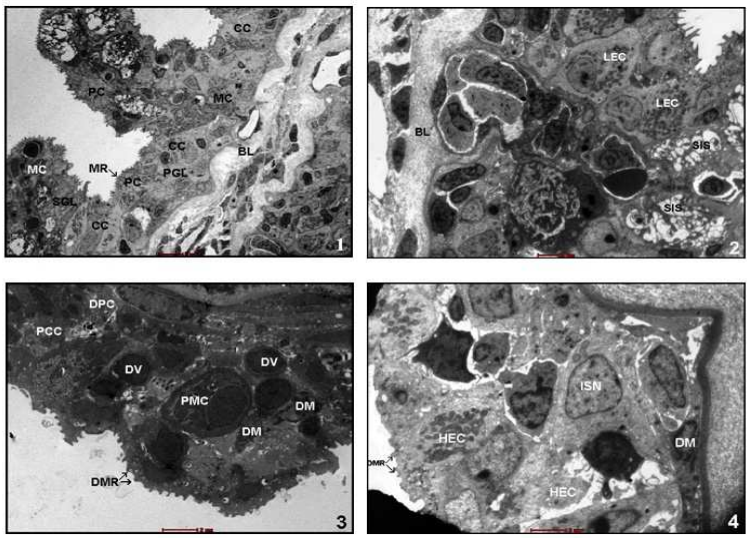

Fig. 1 Ultra structure of gill of control fish *Channa punctatus*.
Figs. 2-4 Ultra structure of gill of the fish, *Channa punctatus* exposed to cypermethrin for 15, 30 and 45 days.

Histopathological observations of gills

The freshwater fish, *Channa punctatus* exposed to sub lethal concentration (0.08 mg/L) of cypermethrin have shown the swelling of the inter-cellular spaces (SIS) between the epithelial lining and the basal lamina of the respiratory gill lamellae leading to the appearance of wide spaces from the base towards the tip of the gill lamellae, the epithelial cells of gill filament have shrunken resulting degeneration of microridges and lifting of epithelial cells (LEC) after 15 days exposure (Fig.2). The proliferation of chloride cells (PCC) and mucous cells (PMC) were identified in the secondary lamella. The chloride cells have appeared with dilated vesicles (DV) within the cytoplasm and damaged mitochondria (DM). Dilation of the blood vessel walls and degeneration of the pillar cells (DPC) are shown in the ultrastructure of the gill in the fish exposed to sub lethal concentration of cypermethrin for 30 days exposure (Fig.3). The chloride cells of fish exposed to cypermethrin after 45 days of exposure showed the apical surface slightly invaginated, the cytoplasm more electro dense, the nucleus surface somewhat irregular (ISN), and the more numerous damaged mitochondria (DM) were elongated with a highly electro-dense matrix and were localized in close association with the tubular system. The primary and secondary gill lamellae exhibited hypertrophy and hyperplasia of the epithelial cells (HEC) and disintegration of micro ridges (DMR) (Fig.4).

DISCUSSION

Gill is the main osmoregulating organ in fishes, and it is highly sensitive to many factors, including stress, pollution and changes in the salinity of environment [7]. In the present study, the gills of *Channa punctatus* showed marked histopathological alterations. These alterations included severe degenerative, necrotic and proliferative changes in gill filaments and secondary lamellae and dilation in the blood vessels of gill filaments. Some of the structural changes may serve as a defence mechanism in protecting the fish from the pesticides-contaminated water by increasing the diffusion distance. Similar results were recorded by Tkatchehca *et al*., (2004) who reported toxic effects of mining effluents on fish gills in a subarctic lake system in NW Russia. The lamellar and interlamellar epithelial necrosis observed in the present study indicates a direct toxic effect of the pesticides on the gill tissue.

The histopathological changes observed in the gills of the studied fish are in agreement with those observed in other fish species under the influence of different pesticides [7], [10], [9] and [19]. Also, Yildirim *et al.*, (2006) reported that exposure of *O. niloticus* to 5µg/L of deltamethrin resulted in lamellar fusion in the gills. Exposure of *Danio rerio* to aldrin and heptachlor was found to induce cell proliferation between secondary lamellae, lifting of respiratory epithelial cells, fusion of several secondary lamellae and dilation of blood vessels in the gills [5]. Sublethal concentrations of fenvalerate resulted in epithelial hyperplasia, epithelial necrosis, desquamation, lamellar fusion, epithelial lifting, edema, swelling at the tips of secondary lamellae in the gills of *Cirrinus mrigala* [18].

The present investigation showed the severe branchial lesions like lifting of lamellar epithelium, necrosis and cellular sloulghing in the *Channa punctatus* exposed to cypermethrin. Similar observations were reported by Lqbal, *et al*., (1992). Mucus secretion is one of the common responses of aquatic organism to irritants. Rounding off and swelling of mucosal cells and their organelles should be considered as a first reaction to membrane damage. Damages of the gills indicated an impairment in gaseous exchange efficiency of the gills, Oedematous of the lamella and hyperplasia [15]. Mallat (1985) reported that the microridges are related with the retention of mucous on the epithelium as a way to protect it against environmental alterations.

The present study showed a reduction in the quantity of the microridges of the pavement cells in the gills of *Channa punctatus* induced by cypermethrin. Similar findings were also observed by Wong & Wong (2000), Mazon *et al*., (2002) and Biafini *et al*., (2009). Gill hyperplasia might serve as a defensive mechanism leading to a decrease in the respiratory surface and an increase in the toxicant-blood diffusion distance. Increased mucus production and fusion of lamellae were obvious on exposure to both the phenolic compounds. Mucus cells contain mucins, poly anions composed of glycoproteins that can be effective in trapping toxicants and aid in the prevention of toxicant entry into the gill epithelium [16]. Extensive epithelial desquamation was also observed in the phenol treated group. It is well known that changes in fish gill are among the most commonly recognized responses to environmental pollutants [13]. The increase of cellular layers of lamellar epithelium may be due to an increase in the mitotic divisions of the lamellar epithelium. The lamellar and inter-lamellar epithelial necrosis observed in the present study suggests the direct toxic effect of the pesticides on the gill tissue.

Acknowledgement

The authors thank to the Department of Zoology, Pachaiyappa's College, Chennai, India for providing the laboratory facilities for carrying out the present work.

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