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Research Article

Influence of Cypermethrin and Chelating Properties of 2r, 4c-Bis(p-Methoxyphenyl)-7c-(t-Butyl)-3-Azabicyclo (3.3.1) Nonan-9-One Oxime on Ultra structure of Gill and liver

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Abstract

The toxic effects of cypermethrin on the ultra structure of the gills and liver of the freshwater fish Labeo rohita's gills and liver were studied. The purpose was to see if pesticide concentrations and exposure time influenced the degree and nature of ultra structure alterations in exposed fish's gills and liver. Selected fish were exposed to a mixture of 5% concentrations of the LC_{50} of cypermethrin for both short and long periods of time. The gills and liver of specimens subjected to 120 hours experienced similar hyper structural alterations. Clearly indicating a harmful response to both pesticide doses. These ultra structural changes included lesions such as oedema in the primary and secondary lamellae, fusion of neighbouring secondary lamellae, and epithelial layer lifting, hyalinization, hepatocyte vacuolation, cellular swelling, and blood vessel congestion. The amount of the exposure period, however, had an effect on the degree of these hyper structural alterations.

Keywords: *Cypermethrin, Labeo rohita, 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime, Ultra structural characteristics response.*

Introduction

In the aquatic environment, fish are usually regarded as organisms of choice for assessing the effects of environmental pollution on aquatic ecosystems. The freshwater teleost, *Labeo rohita* is a wide spread cyprinoids, generally found in rivers, ponds, reservoirs and lakes. Fish are highly amenable to laboratory conditions and one of the prime cultured species in poly-culture. Cypermethrin is also a commonly and extensively used insecticide for a variety of crops in this region, and the information gathered from the literature is not adequate concerning alterations in the cyto/histopathological and oxidative stress responses to know the integrative impact of this pyrethroid on teleosts. The study aims to correlate selected oxidative stress responses and ultra structure changes in liver, whether or not these can be collectively useful in bio-monitoring programmers. Moreover, biomarkers have proven to be sensitive and short-term indicators of environmental pollution which display little temporal variation and integrate the effects of a variety of different stressors, including environmental contaminants (Triebskorn *et al.*, 1991). Studies on the alterations in the surface ultra structure would reflect the health and physiological status of the fish (Machado, 1999 and Fernandes and Perna-Martins, 2002). Although histological investigation plays an important role in postmortem examination to elucidate the cause and mechanism of death and injury, the Postmortem autolytic process is dependent on various factors such as temperature, air humidity and the type of environment (Janssen,

1984).That is why the ultra structural investigation of fish gills has been used to understand the branchial physiological processes and behavioral aspects of different fishes species (Eiras-Stofella and Charvet-Almeida, 2000).The gill arches, in general, are equipped with gill rakers toward their pharyngeal side and are considered to play an important role in feeding. Munshi et al. (1984), more recently, Kumari. (2005) described surface ultra structure of gill arches and gill rakers in relation to the feeding ecology of a carnivorous catfish rita. The liver can be considered as a target organ and great importance for fish, since it participates in processes such as the biotransformation and excretion of xenobiotics. Therefore, the liver can be studied in environmental monitoring due to its high sensitivity to contaminants (Thophon *et al.*, 2003).Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors.

Hence an attempt has been made to investigation the ultra structural alteration occur in the Cypermethrin (group-2) Cypermethrin along with 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime group-3 and 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime alone (group-4) exposed to fingerlings of *Labeo rohita* fish sub lethal concentration for 24 to 120 hour.

Material and method

Collection and Maintenance of the Experimental Animal

The fresh water fish Labeo rohita were caught at a fish farm in Pinnaloor, Cuddalore district, at Navarathna form. The fish were brought to the laboratory and transferred to rectangular cement tanks (100 175) of 500 liters capacity with chlorine free aerated well water. For the studies, fish of the same size and weight, regardless of sex, were utilized.

Preparation of 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime

It is prepared by the reaction of 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one with the hydroxylamine hydrochloride and sodium acetate in 1:1:3 ratio in ethanol

Experimental design

Group1 (Untreated control)	Fish exposed to tap water observed for 24 to 120 hours
Group2 (Cypermethrin)	Fish exposed to Cypermethrin 30g/l of sub lethal concentration for 24 to 120 hours
Group 3 (CYP+ Oxime)	Fish exposed to Cyperrmethrin 30g/l along with2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime for 2g for 24 to 120 hours
Group 4 (Oxime)	Fish exposed to 2r, 4c Bis (pmethoxyphenyl) -7c-(t-butyl)-3 azabicyclo (3.3.1) nonan- 9-one oxime alone 2g for 24 to 120 hours

Scanning Electron Microscopic (SEM) Studies

For Scanning Electron Microscopy (SEM), the Gill, and Liver of fish tissue were removed and fixed in karnovsky solution (2% par formaldehyde and 2.5 glutaradehyde in 0.2M sodium (cacodylate buffer). The tissues were ten dehydrated in a graded ethanol acetone (1%) solution, followed by four washes in 100% acetone. After during, the samples were assembled on aluminum stubs, coated with gold examined and photographed with Joel Tsm-P15 Scanning Electron Microscope (Wood Ward, 1972).The experiment was carried out following the Regulations of Animal Experimentation of C.M.C. Institute Labe Velur, which is based on the Guidelines of the International Committee on Laboratory Animals.

Result and Discussion

There are four gill arches on each side of the buccal cavity. Each arch is composed of numerous gill filaments which have two rows of secondary lamellae that run perpendicular to each filament. Each lamella is made up of two sheets of epithelium delimited by many pillar cells, which are contractile and separate the capillary channels. One or two erythrocytes are usually recognized within each capillary lumen. Chloride cells are identified as large epithelial cells with light cytoplasm, usually present at the base of lamellae. Mucus cells and pavement cells are also present in the epithelium of the filament and at the base of lamellae, but they lack the light cytoplasm and are smaller than chloride cell.

The gills of the control fish appeared normal at all times. The gills of experimental fish showed extensive edema of the epithelial cells and blood congestion (aneurism) in many areas of secondary lamellae with the breakdown of the pillar cell system. SEM examination showed swelling of secondary lamellae (Fig, a & b). The gills showed extensive aneurism with some ruptures in many secondary lamellae and the breakdown of pillar cell system was seen. In addition, SEM examination also confirmed the severe aneurism, swelling, and enlargement of many secondary lamellae. The gills of many fish showed extensive hypertrophy and hyperplasia of chloride cells and mucus cells at the base of the gill filaments and secondary lamellae.

The gill of cypermethrin along with 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime (group 3) exposed fish shows swollen in comparison to the control fish because of hypertrophy and hyperplasia of the gill epithelial cells. Fusion of secondary lamellae, edema, necrosis and desquamation of lamellar epithelium is observed. The cartilaginous rod at the core of primary lamellae is seen to be disrupted in numerous areas in recover of in treatment in gill. The fish is exposed to 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime (group 4) which showed no changes in gill compared to group 3. The group 4 fish showed Ultra structure (SEM) study pattern to near normalcy. The parenchyma itself is primarily composed of polyhedral hepatocytes typically with central nuclei with densely stained chromatin margins and a prominent nucleolus. Venous blood enters the liver caudally from the intestine *via* the hepatic portal veins and branches into capillaries known as sinusoids. Sinusoids are lined with reticular-endothelial cells which are in turn surrounded by hepatocytes. Liver ultra structural images showed thin cell layers covering dense and irregular connective tissue capsules (Glissons capsules). (Fig, a & b) The cohesion of this cell is supported by desmosomes and peripheral hepatocytes with the presence of fibroblasts. The hepatocytes with in the control fish showed the presence of multiple organelles including mitochondria, rough endoplasmic reticulum (RER), Golgi complex and a rounded nucleus with pronounced found around the nuclei. In contrast, in *Labeo rohita*, the RER is also arranged along and closer to the plasmatic membrane forming an electron-dense thin layer limit among the hepatocytes. Lysosomes and lipid bodies are also sporadically visible in the cytoplasm of hepatocytes. The nucleus presents an abundant euchromation, few condensed heterochromatin at the periphery and a central and evident nucleolus (Fig, c & d). The group 3 recovered in the cytoplasmic vacuolization is prominent and lateralization and condensation of the nuclei and blood are also observed. The fish is exposed to 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime in group 4 ultra structural changes in fish liver cells normal of in control. In the present investigation cypermethrin exposed fish the gill showed extensive edema of the epithelial cell and many areas secondary lamellae with breakdown of pillar cells and presence of mucus protein. Fish gills are the first target of waterborne pollutants such as pesticides because they come into immediate and permanent contact with the environment. The quick binding and accumulation of toxicant in the gills (Wong and Wong, 2000 and Ossana *et al.*, 2009) causes the extrusion of mucoproteins on the epithelium, which in turn may act either as a preventive coat on the gill surface against direct contact with polluted water, restricting the pesticides entry secondary to an increased of the barrier distance for influx of the toxicant (Dutta *et al.*, 1997) or may lead to suffocation due to

interference with the respiratory processes. 1997) or may lead to suffocation due to interference with the respiratory processes. Several authors have reported histopathological abnormalities in gills of other species exposed to environmental pesticides and heavy metals. Battaglini *et al.* (1993); Gargiulo *et al*. (1992) reported for *Carassius auratus* exposed to Cd, the presence of empty mucus cells and large amounts of mucus on the gill surface and noticeable separation of the respiratory epithelium from the capillary one; Domitrovic, (1997) reported hypertrophy and hyperplasia, filament and lamellar fusion in the gills of *Aequidens portalegrensis* exposed to cadmium; he also observed the reversibility of the morphological alterations (except for epithelial hyperplasia) after the depuration period. The gills of the fish showed severe lesions as oedema in the primary and secondary lamellae, fusion of adjacent secondary lamellae, and lifting of the epithelial layer, among others these effects are likely to decrease respiratory efficiency and the reversibility of the changes dependent on the degree of damage.

In the 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime exposed group 3, There was a partial recovery of gill structure, with moderate lesions such as lamellar hypertrophy and hyperplasia.

The liver is known to be one of the major organs that accumulate pesticides. It not only acts as a storage organ but is also the primary site for detoxification mechanisms (Olsson et al., 1989). Toxicants redistributed by circulation to the liver and kidney following uptake through the gills (Glynn et al., 1992). Similarly, ultra structural alterations in the hepatocytes such as mitochondrial condensation of RER (rough endoplasmic reticulum) and numerous large lipoid droplets have been observed in the liver of cypermethrin exposed fish. Liver dysfunction induced by CCl_4 has been reported in rat by Sunita *et al*. (2005). Finally, at ecologically relevant doses, the herbicides cypermethrin (group-2) and 2r, 4c-Bis (p-methoxyphenyl)-7c-(t-butyl)-3azabicyclo (3.3.1) nonan-9-one oxime (group-3) affected ultra structural features of fish gill and liver. These changes, while not fatal, may impair the ability of the gills and liver to deal with xenobiotics and infectious agents. Previously, *Labeo rohita* was used in biomarker studies involving organic pollutants. We demonstrated that it could be used to investigate the effects of a pesticide mixture at low concentrations. The administration of oxime (group-4) results in the maximal normalisation of cypermethrin's toxic effect, emphasising oxime's protective activity.

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