Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

ISSN: 2347-8314

Int. J. Modn. Res. Revs.

Volume 4, Issue 2, pp 1098-1102, February, 2016

ORIGINAL ARTICLE

PROTECTIVE ROLE OF ATROPINE ON THE HISTOPATHOLOGY OF GILL AND LIVER IN THE FRESH WATER FISH *LABEO ROHITA* (HAMILTON) EXPOSED TO CYPERMETHRIN

[1]A. Vijayakumar, [*2]N. Thirnavukkarasu, [3]K.Jayachandran and [4]M.Susiladevi

[*2]Assistant Professor in Zoology, PG Department of Advanced Zoology &Biotechnology, Dr.Ambetkar Government Arts College Vysasarpadi, Chennai-39

[1]Research scholar, Department of Zoology, Bharathiar University, Coimbatore, Tamil Nadu.

[3]PG.Research and Department of Biotechnology, EGS.Pillay Arts and Science College, Nagapattinam, Tamil Nadu.

[4]Government Arts College Cuddalore, PG Research Department of Zoology, Tamil Nadu.

Article History: *Received 5th February ,2015, Accepted 27th February, 2016, Published 28th February ,2016*

ABSTRACT

Herbicides Cypermethrin environmentally relevant concentrations alter ultra structural characteristics of fish gill and liver. These changes although not leading to lethal outcome, might compromise gill and liver ability to handle the xenobiotics and infectious agents. *Labeo rohita* is previously used in biomarker studies generally involving organic pollutants. Here it is shown that it could be used in the study of effects of mixture of herbicides at low concentrations. Gills are the first organs which come in contact with environmental pollutants. Paradoxically, they are highly vulnerable to toxic chemicals because firstly, their large surface area facilitates greater toxicant interaction and absorption and secondly.

Keywords: Cypermethrin, Atropine, *Labeo rohita*, Histopathological characteristics response, Gill and Liver

1.INTRODUCTION

Studies on the alterations in the surface histopathological 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 ultrastructural 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 histopathological of gill arches and gill rakers in relation to the feeding ecology of a carnivorous catfish Rita 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 histopathological alteration occur in the Cypermethrin (group-2) Cypermethrin along with atropine group-3 and atropine alone (group-4) exposed to of *Labeo rohita* fish sublethal concentration for 24 to 120 hours.

2.MATERIALS AND METHOD

COLLECTION AND MAINTENANCE OF THE EXPERIMENTAL ANIMAL

The fish Labeo rohita of size 14 to 16 cm and 50 to 70g weight were brought from a local fish farm in Pinnaloor, and Navarathna form. Fish collected and acclimatized at 28°C in the large sized aquarium for acclimatization in the laboratory condition for 15 days. During laboratory condition fishes

Corresponding* author: ***Dr. N. Thirnavukkarasu,* *Assistant Professor in Zoology, PG Department of Advanced Zoology &Biotechnology, Dr.Ambetkar Government Arts College Vysasarpadi, Chennai-39*

were feed with artificial feed, water was renewed on every day to maintain water quality. The excess amount of feed and fecal matter was removed from the water and was provided the healthy environment before experimentation, to find out it's suitability for fish rearing. The LC50 concentration of Cypermethrin was noted at 120 hrs.

EXPERIMENTAL DESIGN

Fishes were exposed in 4 groups.

Group-1 fish exposed to tap water
Group-2 fish exposed to cypermethrin
Group-3 fish exposed to cypermethrin along with atropine
Group-4 Fish exposed to atropine alone

HISTOLOGICAL STUDIES

To examine the extent of cellular damage caused by cypermethrin, the gill, and liver of the control and treated tissues were fixed in Bouin's fluid. After 24 hrs, the standard histological technique was followed by the method of Gurr (1959). The tissues were dehydrated in ascending grades of alcohol. After dehydration in graded series of alcohol, they were cleared in acetone and xylol. Then, they were treated with cold and hot infiltration and embedded in paraffin wax (58-60°C). Serial sections were cut at 6 to 8 μm thickness, deparaffinized in xylol, passed through descending grades of alcoholic series, hydrated in water, stained in Heidenhain's iron haematoxylin and counterstained with aqueous eosin. Stained sections were mounted in DPX for microscopic observations.

3.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. Histopathological 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, histopathological 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 ypermethrin along with atropine (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 atropine (group 4) which showed no changes in gill compared to group 3. The group 4 fish showed Histopathological 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 ultrastructural 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 organells 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 heterochromation 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 atropine in group 4 histopathological changes in fish liver cells normal of in control. (Fig.e&f,g,h)

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.

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,

FIG.1. GILL

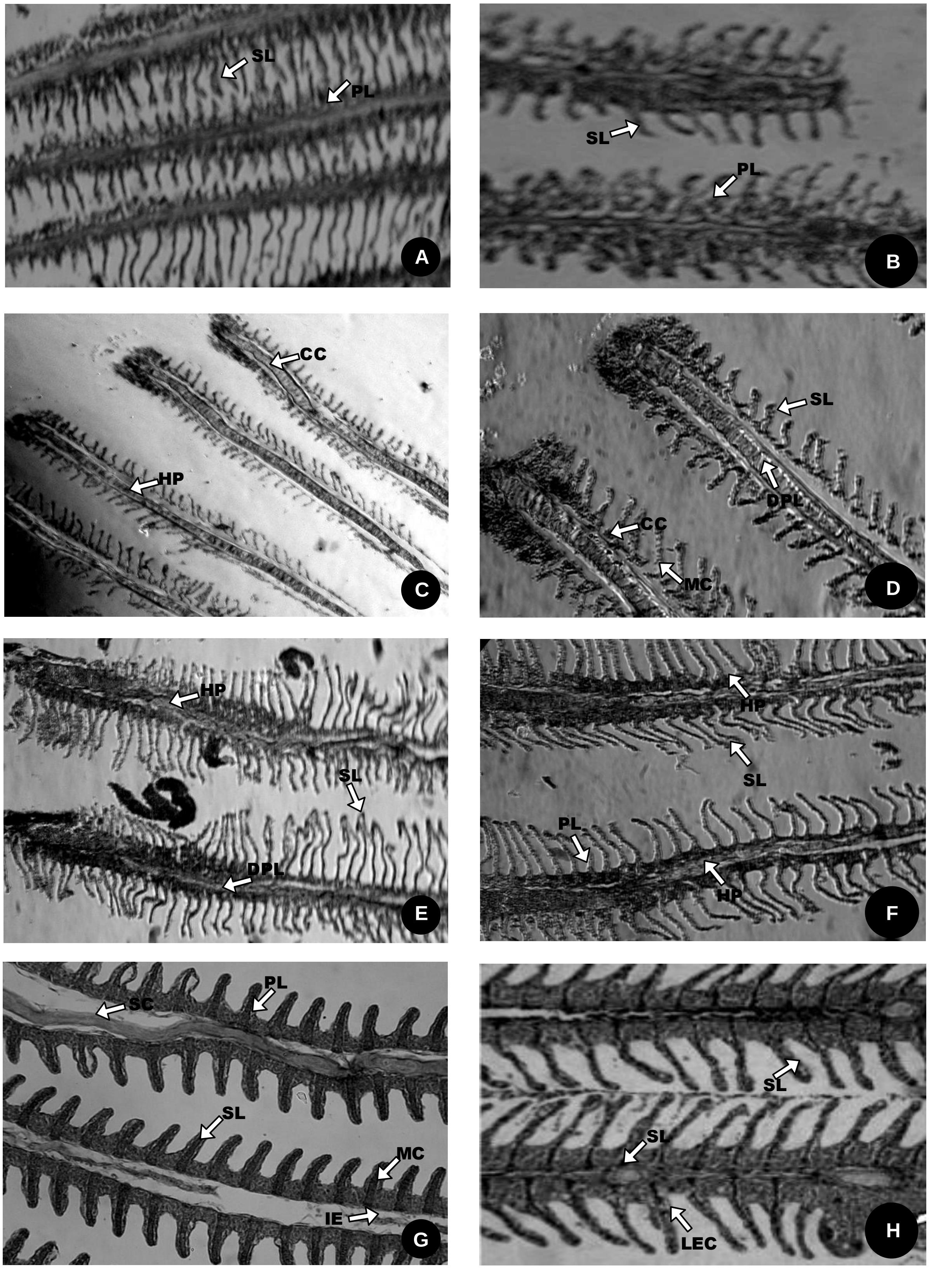

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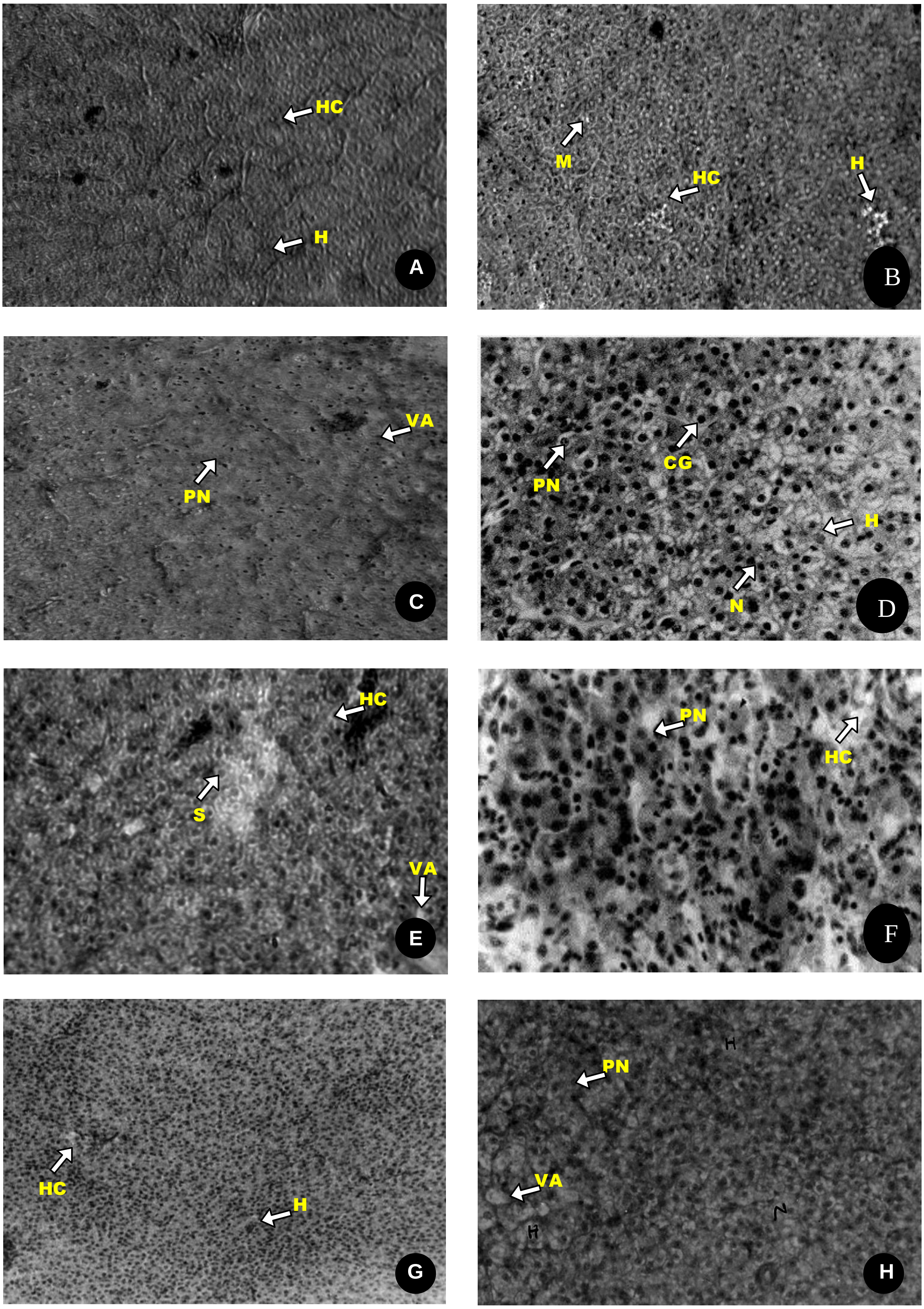
FIG. 2. LIVER



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 atropine 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, ultrastructural 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). They have reported the changes mainly to nuclei, mitochondria, rough and smooth endoplasmic reticulam, sinussoida and reversible nature in the changes of glycogencontent. Exposure to cypermethrin resulted in a moderate and transient dilation of the interhepatocytic space. Previous studies have showed similar perturbations associated with necrosis of fish hepatocytes exposed to chemical hepatotoxicants (Biagianti-Risbourg, 1990).

In conclusion, herbicides cypermethrin (group-2) and along with atropine (group-4) at environmentally relevant concentrations altered histopathological characteristics of fish gill and liver. These changes although not leading to lethal outcome, might compromise gill and liver ability to handle the xenobiotics and infectious agents. *Labeo rohita* was previously used in biomarker studies generally involving organic pollutants. Here we showed that it could be used in study of effects of mixture of herbicides at low concentrations.

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