Histopathological Study of the Freshwater Fish *Ctenopharyngodon Idella* Exposed to Λ-Cyhalothrin 5% EC

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Abstract

Present study Lambda cyhalothrin is a synthetic pyrethroid widely used for pest management and public health applications to control insects. It is categorized as a restricted use pesticide in Extension Toxicology Network for its toxicity to fish. But its usage for the control of major pest in agriculture is being continued in developing countries like India. The freshwater fish *Ctenopharyngodon idella* were exposed in sub-lethal concentrations (1/10 of lethal concentrations) of lambda cyhalothrin 5%EC for 1 and 12 days along with controls to study histopathological changes under light microscope in the selected tissues viz., liver, kidney and gill were examined. Swelling of the hepatocytes with diffuse necrosis and marked swelling of blood vessels were observed in the liver tissue. Tubules of the kidney were distended, with tubular cells of posterior kidney exhibiting marked necrotic changes. Pathology of the gill tissue showed shortened primary lamellae, loss of secondary lamellae and congestion of blood vessels and hyperplasia of branchial plates and the pathology increased with increasing exposure periods.

Keywords: *Ctenopharyngodon idella, Lambda cyhalothrin 5% EC, Sublethal exposure, histopathology of Gill, Liver and Kidney.*

Introduction

The toxicity of any environmental contaminant is either acute or chronic, and the chronic studies of the organisms; physiological studies alone do not satisfy the complete understanding of pathological conditions of tissues under toxic stress. Hence, it is useful to have consequence of the concentration of the toxicant and it is depend on exposure time. The damage of the particular tissue depends on the toxic potentiality of a particular contaminant accumulated in the tissue[4]. Aquatic vertebrates are susceptible to non-target effects, because of their relatively restricted mobility and also due to pesticide residue dispersion leading to lengthy period of exposure. Most of the histopathological changes can be interpreted as non-specific response to stress and a wide spectrum of pollutants, including pesticides, heavy metals and organic contaminants exposed to fish and other organisms[16].

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Material and Method

Freshwater fish *Ctenopharyngodon idella* (Valenciennes) were acclimatized to laboratory conditions for 10 days. They were exposed to sub-lethal concentrations of lambda cyhalothrin for 1 and 12 days. Fish were randomly selected for histopathological examination. Gill, liver and kidney tissues were isolated from control and experimental fish. Physiological saline solution (0.75% NaCl) was used to rinse and clean the tissue. They were fixed in aqueous Bouin solution for 48 hrs, processed through graded series of alcohols cleared in xylene and embedded in paraffin wax. Gills alone were processed by double embedding technique. Sections were cut of 6 µ (microns) thickness; stained with Enrich haematoxin and Eosin (dissolved in 70% alcohol) [11] and were mounted in Canada balsam. Photo monographs were taken with the help of Intel play microscope attached with computer to analyze.

Results and Discussion

Histopathological studies provide information about the health status and functionality of different organs. Tissue injuries and damages in organs can

result in the reduced survival, growth and fitness, the low reproductive success or increase of susceptibility to pathological agents. The extent severity of tissue damage is a consequence of the concentration of the toxicant and is time dependent. Hence, in the present study, an attempt has been made to observe possible histopathological changes in certain vital tissues like gill, liver and kidney of the Indian major carp *Ctenopharyngodon idella* exposed to sublethal concentration of lambda cyhalothrin 5% EC for 1and 12 days. In the case of 1 day sublethal there was no significant change was observed between control and 1 day sublethal of gill, liver and kidney.

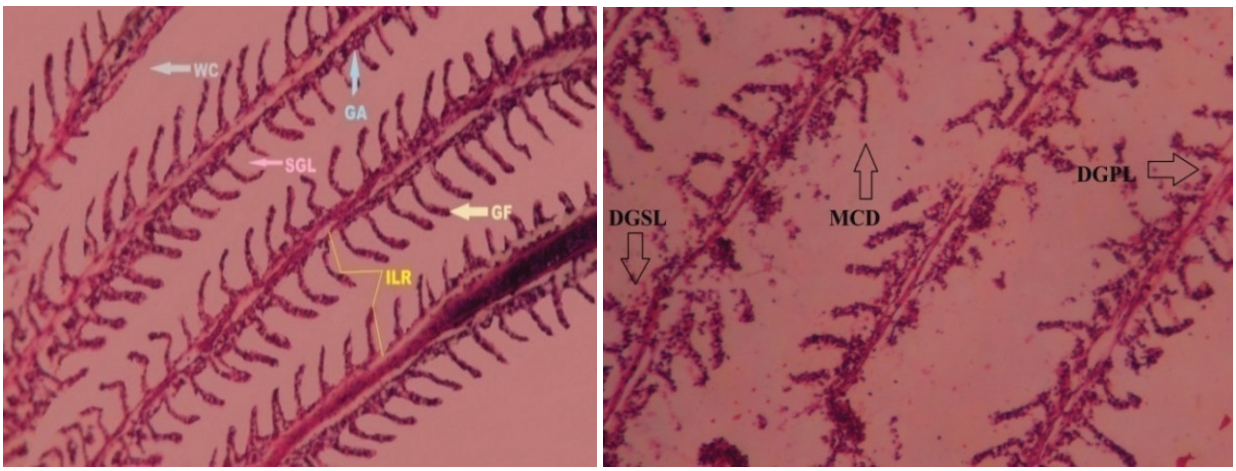
Pathology of Gill tissue under Lambda Cyhalothrin toxicity:

Gills apart from being the primary respiratory organ in fishes, are also responsible for other vital physiological functions like excretion of nitrogenous wastes, acid base balance and ion regulation. So when fish are exposed to environmental pollutants, these vital functions are deleteriously affected and the functional impairment of gills can significantly damage the health of fish. The biological function of the inflammatory response is to destroy "WALL OFF" Irritating substance so that damaged tissue might heal. So the pesticide exposure causes severe alterations in the tissue biochemistry and histology of fish[18].

Present study Lambda Cyhalothrin exposed to the fish *Ctenopharyngodon idella* and the pathological changes in fish gills includes bulging in tips of primary gill filaments. The secondary gill filaments lost their original shape and curling of gill filaments was observed. The pillar cell nucleus showed necrosis and development of vacuoles in the secondary gill epithelium, and a tendency in fusion of disorganized secondary gill filaments. Shortened and clubbing ends of the secondary gill lamellae, fusion of adjacent secondary gill lamellae and necrosis in the primary lamellae were identified. Hyperplasia and hypertrophy of nuclei were also seen. The epithelial layer of secondary lamellae of gills forms a barrier between the fish blood and surrounding water. In fish, gills are the main organs to contact with the pollutant. Hence, it is more vulnerable to damage than any other tissue and the proliferation of gill lesions observed in some places after exposure to water soluble toxicants (Plate1.B).

PLATE- 1 (GILL)

Fig.A .Control Fig.B.12 days sublethal



LEGEND FOR FIGURES: PGL: Primary gill lamella, SGL: Secondary gill lamella, PC: Pillar cell, HGL: Hyperplasia in gill lamella, EC: Erythrocyte and DGSL: Degenerated Secondary lamella, MC: Mucus cell and ASL: Atrophy of Secondary lamella.

Degenerative changes in lamellar hyperaemia, lamellar oedemas, clumping, cellular, lamellar atrophy in gill of rainbow trout exposed to chlorpyrifos for 24, 48, 72 and 96 hrs[22]. It also observed in the secondary lamellae and respiratory epithelium of the fresh water teleost *Channa punctatus* under exposure to Alachlor and states that the degeneration of respiratory epithelium and damages of gills tissue causes a decrease in energy metabolism[7]. Toxicity of Formalin cause of pathological damage in the gill, gill dysfunction, osmoregulatory

and respiratory imbalance in ornamental fish Amazon blue spotted corydora (*Corydoras melanistius*) [17]. Present study lamellar fusion caused by the filamentary epithelium proliferation and some lamellar aneurisms were also found. Hypertrophy; hyperplasia, lifting of epithelial cells and fusion of secondary gill lamellae were pronounced in the treatment of λ-cyhalothrin exposed to *Ctenopharygodon idella.* The main histopathological changes in gills were showed edema, lifting of lamellar epithelia and an intense vasodilation of the lamellar vascular axis[20].

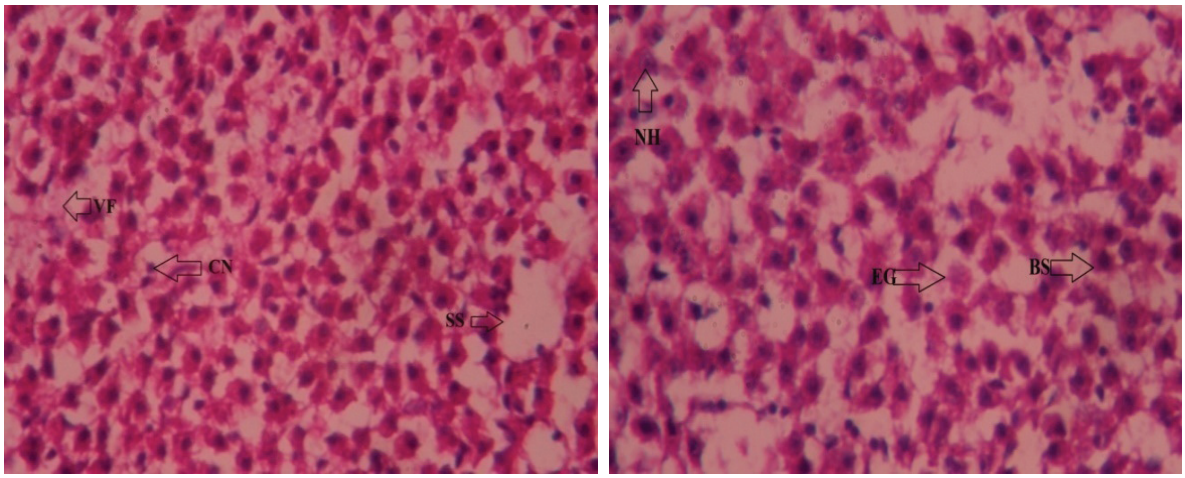
Pathology of Liver tissue under Lambda Cyhalothrin toxicity

Hepatic cells are roundish polygonal, containing clear spherical nucleus and they are located among sinusoids forming cord like structures known as hepatic cell cords. In fish, these structures are generally obscure. Bile canaliculi, is centrally located in each cord and large quantities of lipid glycogen granules are also observed in the cytoplasm of fish hepatic cell cords (Plate.2 & Fig. A). Toxicity discrete pathological changes were observed in the liver tissue of the test fish *Ctenopharyngodon idella* (Valenciennes). These changes were degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, rupture of blood vessels, necrosis and disappearance of hepatocyte cell wall and disposition of hepatic cords. Formation of vacuoles cytoplasm in hepatocytes, atrophy, blood streaks among hepatocytes, intercellular empty space and blood cognation were observed and there was decrease in the size of nucleus of the hepatocytes also observed. (Plate: 2. & Fig: B).

Necrosis with inflammatory infiltration, vacuoles in the hepatocytes and hepatic sinusoids congestion in albino rats treated with dimethoate [10, 2]. The changes may be attributed to direct toxic effects of pollutants on hepatic cells, since the liver is the site of detoxification of all types of toxic substances[19]. The sublethal effects of pesticide lambda cyhalothrin affect the liver histology of *Oreochromis mossambicus* under long term exposure to 0.0025 ppm of sublethal concentration the liver was comprised of polygonal hepatocytes with centrally placed nucleus[15].

PLATE-2 (LIVER)

Fig.A Control Fig.E.12 days sublethal



LEGEND FOR FILGURES- HC: Hepatic Cell, N: Nucleus, BC: Bile canaliculus. FV: Formation of vacuoles, BS: Blood streaks among hepatocytes, BC: Bile canaliculus.

Cytoplasm with nuclear degradation; cellular degradation and damaged hepatocytes were observed in fish *Cyprinus carpio* exposed to cadmium[23]. The necrosis of hepatocytes with enlarged sinusoid in freshwater fish *Cirrhinus mrigala* exposed to acute and chronic levels of cythion and also reported that significant alterations in the hepatocytes, pyknotic nuclei and necrosis in the liver of *Clarias gariepinus* exposed to cypermethrin[24]. Moderate focal necrosis, granular glycogen, nuclei piknosis, loss of architecture structure; onion-like cells were observed in fish *Cyprinus carpio* due to the presence of microcystins[1]. Vacuolization and presence of sinusoid spaces were reported

in liver tissue of *Danio rerio* exposed to 200µg/L of chlorpyrifos for 24, 48, 72 and 96h[14]. Hyperaemia and degenerative changes in liver and lamellar hyperaemia, lamellar oedemas, clumping, cellular degeneration, hyperplasia and lamellar atrophy in gill were noted in the fish rainbow trout exposed to 2.25, 4.5 and 6.75 µg/L of chlorpyrifos for 24, 48, 72 and 96h[22].

In the present study there is a strong link between liver damage and toxicants. Vacuolar degeneration, hemorrhage and necrosis noticed. It might be due to the presence of toxicants. Necrosis, haemorrhage, degeneration of hepatocytes and pyknosis in the liver tissue were witnessed in *Labeo rohita* exposed to zinc[9]. Vacuole formation, degeneration of hepatic cells, haemorrhage and necrotic lesions were noted in *Heteropneustes fossilis* exposed to sewage for 180 days[13]. In the present study degenerative changes are intensified in liver to sublethal exposures. They include degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, rupture in blood vessels, necrosis and disappearance of hepatocyte wall disposition of hepatic cords decrease in size of nucleus pyknotic and vacuolar degeneration within the nucleus was evident (Plate: 2, Fig B). The same degenerative changes in *Catla catla, Labeo rohita, Ctenopharyngodon idella* and *Channa punctatus* under Chloropyrifos, fenvelarate and butachlor toxicants[21].

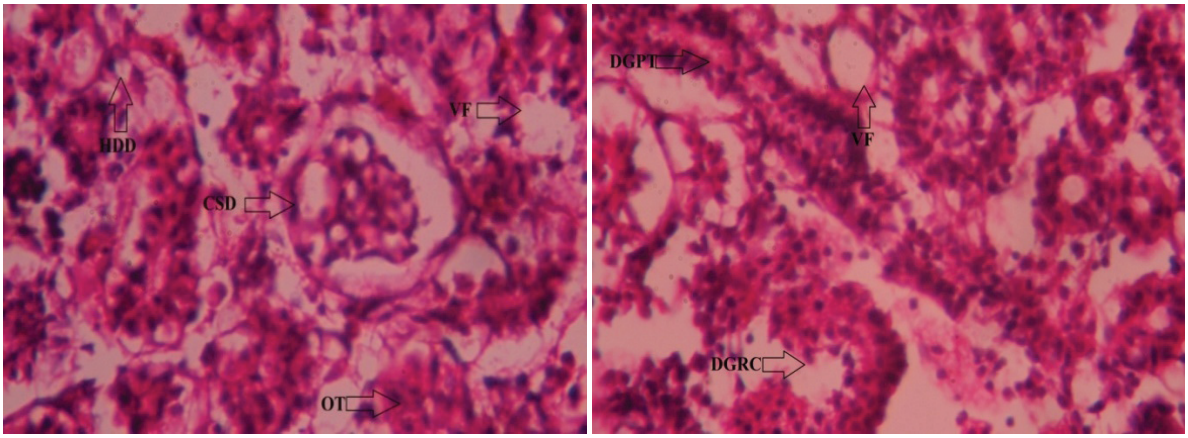
Pathology of Kidney tissue under Cyhalothrin toxicity:

The renal tubules are composed of cuboidal epithelial cells with densely arranged microvilli in the tubular lumen. In segment II, renal tubules are composed of cuboidal epithelial cells. Cilia and microvilli are found in the tubular lumen. In the distal convoluted segment, epithelial cells have no microvilli. The cells of this segment are stained with eosin more faintly than those of proximal convoluted segment (Plate. 3& Fig. A). Present study indicates the freshwater fish *Ctenopharyngodon idella* (Valenciennes) under cyhalothrin toxicity evidenced marked pathological changes in renal tissues. Highly degenerative changes were observed in haemopoitic tissue which include severe necrosis, cloudy swelling in renal tubules, cellular hypertrophy and granular cytoplasm. The epithelial cells of the distal convoluted tubule decreased in size. The interstitial renal tissue was less affected. It showed formation of vacuoles and cellular contours were not clearly distinguished (Plate.3 & Fig. B).

Lesions in the kidney tissues of fish exposed to deltamethrin, Tubular degenerations were noted in catfish, *Ictalurus punctatus* upon exposure to methyl mercury [8]. Diethyl phthalate induce changes in fish *Clarias gariepinus* had necrosis of epithelial cells of renal tubule, pyknotic in haemopoietic tissues and degeneration of glomerulus[12]. Degenerative changes such as necrosis in renal epithelium, Cloudy swelling in renal tubules, cellular hypertrophy granular cytoplasm and swelling of mitochondria in the renal tubules were observed in animals administered Pyraclostrobin[3].

PLATE -3(KIDNEY)

Fig.A.Control Fig: B.12 days sublethal



LEGEND FOR FIGURES: PCS: proximal Convoluted Segment, DCS: Distal Convoluted tubule. DART: Degeneration and atrophy in renal tubules, DL: Decreased Lumen in tubules, DGHTE: Degenerating haemopoietic tissue with erythrocytes and FVRIT: Formation of Vacuoles in the renal interstitial tissue.

Reduced glomerular filtration rate, glomerular lesions, degeneration of cellular boundaries and clumping of glomeruli were reported at some places in the kidney of rainbow trout (*Onchorhynchus mykiss*) exposed to fungicide captanin[6]. Disorientation in glomerular structure, cloudy swelling, dilation in the inter space urinary tubular. Necrosis in the haematopoietic tissue, appearance of vacuoles indicates the effects of toxicity in fish rainbow trout (*Onchorhynchus mykiss*) [5]. The present observations are in agreement with the reports of all the above reports. Thus, when fish is exposed to pesticide, they suffer reparable architectural changes in various vital organs making the fish less fit for better survival. These histopathological changes can alter various physiological activities of the fish.

Conclusion

The present study title proved pyrethroid compounds damaged the fish active organs like gill, liver and kidney and the susceptibility of the fish in the context of environmental monitoring and the biomarker approach. The histopathological studies showed that λ-Cyhalothrin 5% EC at different sublethal concentration causes significant morphological and pathological changes in the selected tissues of *Ctenopharyngodon idella* at higher dose of toxicant than the lower dose level. In gill progressive degeneration, severe necrotic changes were noticed in the epithelial cells of secondary gill lamellae and it also caused profound pathological changes in liver tissue of the test fish such as degeneration of cytoplasm in hepatocytes, atrophy, and formation of vacuoles, necrosis. It showed severe cloudy swelling of renal tubules, disintegration of interstitial tissue, pyknotic nucleic, etc., in kidney.

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Ethical Clearance: The test fish *Ctenopharyngodon idella* is an edible fish so there is no need to take ethical clearance.

Source of Funding: Self

Conflict of Interest: No competing Interests

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