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Research Article

Histopathological alterations in Gill, Liver and Kidney of *Cyprinus carpio* (Linn.) exposed to Cypermethrin (25%EC)

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Abstract

The histopathological effects of Cypermethrin on the gill, liver and kidney tissues in *Cyprinus carpio* were determined by light microscopy. The fishes were exposed to 96h lethal and sublethal concentrations of cypermethrin for 4 days with parallel untreated control. No histopathological effects were observed in control group. Hyperplasia, desquamation, and necrosis of epithelial, epithelial lifting, oedema, lamellar fusion, collapsed secondary lamellae, curling of secondary lamellae and aneurism in the secondary lamellae were observed in gill tissue. Hepatic lesions characterized by cloudy swelling of hepatocytes, congestion, vacuolar degeneration, karyolysis, karyohexis, dilation of sinusoids and nuclear hypertrophy were observed in theliver tissue. In kidney, highly degenerative changes were observed in haemopoitic tissue which includes shrinkage of glomerulus, expansion of space inside bowman's capsule, hypertrophied cells and lumen tubules diminished. Besides the above changes severe necrosis, cloudy swelling in renal tubules and granular cytoplasm was also observed.

Keywords: Cypermethrin, Synthetic pyrethroid, *Cyprinus carpio*, Histopathology.

Introduction

Widespread use of various pesticides and their impact on environment are now a worldwide phenomenon (Omitoyin *et al*., 2006). It has been estimated that only about 1 % of applied pesticides land on the target organisms and the rest contaminate the environment (Lawson *et al*., 2011). Synthetic pyrethroids, modified derivatives of pyrethrins, natural substances obtained from flowers of pyrethrum species (Luty *et al*., 2000) have emerged as an alternative for long term ecological problems associated with the use of organochlorine, organophosphate and carbamate pesticides. Pyrethroids have a high rate of gill absorption due to their lipophilic nature, which would be a contributing factor for fish sensitivity to aqueous pyrethroid exposures. Aquatic organisms, including fish, are frequently exposed to a wide variety of environmental pollutants leading to deleterious effects, especially when these contaminants are slightly decomposable, exhibit a high biological effectiveness and possess a high potential for accumulation or synergistic effects (Au, 2004). Fishes, the most diverse group of vertebrate fauna are important component of the food chain and any effect of toxicant may have adverse influence on the nutritional value of fish and on human being through their consumption (Gupta and Srivastava, 2006). They are excellent experimental models for toxicological investigations (Shiekh and Lee, 2008) and are often used as sentinel organisms to assess the biological impacts of contaminants and environmental quality because of their responses to

low concentrations of toxic substances (Ayas *et al*., 2007).

Histopathological alterations have been widely used as biomonitoring tools or biomarkers of health status of fish exposed to chemical compounds both in laboratory experiments (Boran *et al*., 2012) and field studies (Stentiford *et al*., 2003). Histopathological changes in fish organs have been increasingly studied as biomarkers for assessing aquatic contamination in environmental monitoring studies (Fricke *et al*., 2012). Histopathology may therefore prove to be a cost effective tool to determine the health status of fish populations, hence reflecting the health of the entire aquatic ecosystem in the biomonitoring process (Nikalje *et al*., 2012). Considerable interest has been shown in recent years in histopathological study while conducting sub-lethal tests in fish. Tissue changes in test organisms exposed to a sub lethal concentration of toxicant are a functional response of organisms which provides information on the nature of the toxicant. Histological changes associated with pesticides in fish have been studied by many authors. But, very scarce information is available on toxicological research in the field of histopathological changes in fish due to synthetic pyrethroid toxicity in general and cypermethrin in particular. Hence, in the present study, an attempt has been made to observe possible histopathological alterations in certain vital tissues like gill, liver and kidney of the freshwater fish *Cyprinus carpio* exposed to lethal and sublethal concentrations ($1/10^{th}$ of 96h LC_{50}) of cypermethrin (25%EC).

Materials and Methods

Freshwater fish *Cyprinus carpio* was acclimatized to laboratory conditions for 10 days. Prior to conducting the bioassay for histopathlogy, a toxicity bioassay was run to estimate the 96 h LC_{50} value of cypermethrin and the same was found to be 3.31 µg/L. They were exposed to sub lethal (0.331 µg/L) and lethal concentrations of cypermethrin for 96h LC_{50} for 4 days. At the end of the exposure period, fish were randomly selected for histopathological examinations. Gill, liver, and kidney tissues were isolated from normal and experimental fish. Physiological saline solution (0.75% NaCl) was used to rinse and clean the tissue. They were fixed in aqueous Bouins solution for 48 h, 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 at 6 µ thickness and stained with Ehrlich hamatoxillin and Eosin (dissolved in 70% alcohol) (Humason, 1972) and were mounted in *Canada balsam*. Histopathological lesions were examined and photographed with the help of Intel Pentium QX3 computer attached microscope under 400X lens.

Results

Pathology of Gill tissue

Cypermethrin 25% EC exposures have induced marked pathological alterations in fish gills architecture. The changes include epithelial lifting (EL), bulging of tips of primary gill filaments (BTPG), degenerated secondary lamella (DGSL), curling of secondary gill filaments (CSG), atrophy secondary lamella (ASL), fusion of secondary gill filaments (FSG) (Figures A – C). The damage of gills of fish exposed to the higher concentrations of lethal was severe. Shortened and clubbing of ends of the secondary gill lamellae, fusion of adjacent secondary gill lamellae and necrosis in the primary lamellae were well marked. Hyperplasia and hypertrophy of nuclei were also observed. Besides these changes, pyknotic nuclei, vacuolization and degeneration of epithelial cells and pillar cells and lifting of the epithelial layer from the secondary lamellae were also significant.

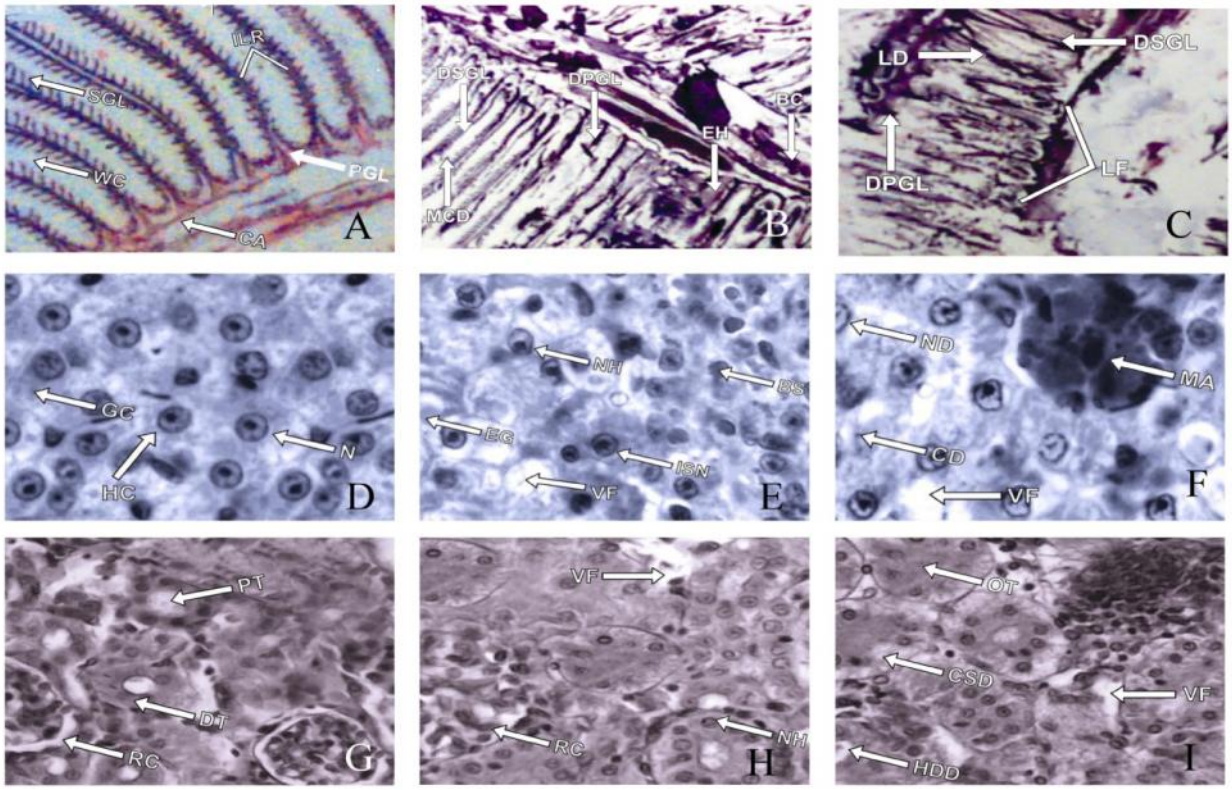
Pathology of Liver tissue

Cypermethrin 25% EC exposures have induced discrete pathological alterations in the liver tissue of the fish *Cyprinus carpio.* These changes include degenerated hepato pancreatic tissue (DGHP), blood cells among hepatocytes (BC), appearance of blood streaks among hepatocytes (ABS), formation of vacuoles (FV), along with atrophy, necrosis and disappearance of hepatocytic cell wall and disposition of hepatic cords (Figures D – F). The degenerative changes are intensified in lethal exposures.

Pathology of Kidney tissue

Renal tissues of the fish exposed cypermethrin 25% EC showed some common pathological changes. Highly degenerative changes were observed in haemopoitic tissue which includes shrinkage of glomerulus (SG), expansion of space inside Bowman's capsule (ESBC), hypertrophied cells (HTC) and lumen

tubules diminished (LTD). In 25 % EC Intra cytoplasmic vacuoles in epithelial cells of renal tubules (ICV), Degenerating haemopoietic tissue with erythrocytes (DGHTE) more prominently observed (Figures G – I). Besides the above changes severe necrosis, cloudy swelling in renal tubules and granular cytoplasm was also observed.



Figures (A-I): Histopathological alterations in Gill, Liver, and Kidney of *Cyprinus carpio* exposed to lethal and sublethal concentrations of Cypermethrin

A-C (Gill): A=Control: PGL-Primary Gill Lamella; SGL-Secondary Gill Lamella; CA-Central Axis; ILR- Inter Lamellar Region; WC-Water Channel. B=Sublethal: DPGL-Degenerated Primary Gill Lamella; DSGL- Degenerated Secondary Gill Lamella; MCD- Marginal Channel Dilation; EH- Epithelial Hyperplasia; BC- Blood Congestion. C=Lethal: DPGL-Degenerated Primary Gill Lamella; DSGL-Degenerated Secondary Gill Lamella; LD-Lamellar Disorganization; LF-Lamellar Fusion.

D-F (Liver): D=Control: N-Nucleus; GC-Granular Cytoplasm; HC-Hepatic Cell. E=Sublethal: NH-Nuclear Hypertrophy; BS-Bile Stagnation; VF-Vacuole Formation; EG-Eosinophilic Granules; ISN- Irregular Shaped Nucleus. F=Lethal: ND-Nuclear Degeneration; CD-Cytoplasmic Degeneration; MA-Melanomacrophages Aggregate; VF-Vacuole Formation.

G-I (Kidney): G=Control: RC- Renal Corpuscles (Showing glomerulus and bowman's capsule); PT-Proximal Tubule; DT-Distal Tubule. H=Sublethal: RC- Renal Corpuscles (Showing glomerulus expansion and absence of bowman's space); NH-Nuclear; VF-Vacuole Formation. I=Lethal: OT-Occlusion of Tubular Lumen; CSD-Cloudy Swelling Degeneration; HDD-Hyaline Droplets Degeneration; VF-Vacuole Formation.

Discussion

Gills are very important in respiration, acid-base balance, osmoregulation and excretion of nitrogenous wastes in fish (Evans *et al*., 2005). They remain in close contact with the external environment and particularly sensitive to change in the quality of water are considered the primary target organ of the contaminant (Camargo and Martinez, 2006). They absorb even minute concentrations of the pyrethroids pesticides and are the first site where pyrethroid induced lesions may occur. The changes in gill tissues found in the present experiments were mild to moderate congestion of the primary lamellae and hyperplasia of branchial plates. The changes were indicative of lowered oxygen supply to the test fish, resulting in hypoxic respiratory responses. Although not lethal, gill damage caused by the pollutant is important from the aspect of morbidity as it retards growth and affects reproduction (Das and Mukherjee, 2000). Damages of the gills indicated that impairment in gaseous exchange efficiency of the gills oedematous of the lamella and hyperplasia were observed and this is similar to the observation of Omoniyi *et al*., (2002). Observed edematous changes in gill filaments are probably due to increased capillary permeability (Olojo *et al*., 2005). Alterations like fusion of some secondary lamellae are examples of defense mechanisms, since; in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Camargo and Martinez, 2007). As a consequence of the increased distance between water and blood due to epithelial lifting, impaired oxygen uptake is evident. However, fishes have the capacity to increase their ventilation rate, to compensate low oxygen uptake (Fernandes and Mazon, 2003).

Many authors reported the histopathological changes in gills of different fishes exposed to various pesticides. Tilak *et al*., (2001a) observed hydropsy, vascular degeneration and bulging and severe necrotic changes in the secondary in the gill tissues of fish *Labeo rohita* exposed to the sublethal concentrations of technical as well as 20% EC of chlorpyrifos. The damaged pillar cells can result in an increased blood flow inside the lamellae, causing dilation of the marginal channel, blood congestion or even an aneurysm (Rodriguez *et al.*, 2002). Shorter gill lamellae, fusion, complete destruction of lamella, increased vacuolation, irregular appearance of gill lamellae were observed in guppy *Poecilia reticulata* exposed to chlorpyrifos (De Silva and Samayawardhena, 2002). Cengiz and Unlu (2003) observed a variety of histopathological effects due to sublethal concentrations of malathion in gill tissues of *Gambusia affinis* such as gill lesions, necrosis and desquamation of secondary lamellar epithelium, epithelial lifting, intra-epithelial oedema, fusion of adjacent secondary lamellae, haemorrhage at primary lamellae, disorganization and rupture in secondary lamellae, hypertrophy and hyperplasia of epithelial cells and concluded that the alterations were time and dose dependent. The formation of aneurysm is related to the rupture of the pillar cells (Martinez *et al.*, 2004) due to a bigger flow of blood or even because of the direct effects of contaminants on these cells. Degenerative changes in gill, such as intraepithelial edema in the secondary lamellae, thick coating of mucus covering the entire gill filaments and lamellae, erosion of secondary lamellae, thickening of lamellae, inflammation of epithelial cells, breakages in primary lamellae, degeneration of secondary lamellae, necrosis, rupture of epithelium were noticed during exposure of sublethal concentrations of monocrotophos by Rao *et al*., (2005). Cengiz (2006) observed histopathological effects of deltamethrin on the gill (desquamation, necrosis, aneurysm in secondary lamellae, lifting of the lamellar epithelium, oedema, epithelial hyperplasia and fusion of the secondary lamellae) of common carp after acute exposure at a concentration of 0.029 and 0.041 mg/l.

Liver is an established organ and plays a fundamental role in the uptake, biotransformation and detoxification of foreign compounds (Gernhöfer *et al*., 2001) in the body and is thus a target organ of xenobiotics. It is also one of the most affected organs by contaminants in water (Camargo and Martinez, 2007) and as a consequence it undergoes different levels of damage. Liver of the cypermethrin exposed fish showed degeneration in the hepatocytes, necrosis and aggregation of inflammatory cells, dilatation and congestion in blood sinusoid and fibrosis. These changes may be attributed to the direct toxic effects of pollutants on hepatocytes, since the liver is the principal organ responsible for detoxification in vertebrates generally and in fish particularly. The liver of the exposed fish had vacuolated cells showing evidence of fatty degeneration. Vacuolations of hepatocytes is a common response associated with

exposure of fish to a variety of toxic chemicals which might be an indication of imbalance between the rate of synthesis of substances in the parenchyma cells and the rate of their release into the circulation (Gingerich, 1982). Cytoplasmic vaculaization observed in the present study are in agreement with Indirabai *et al*., (2010). Necrosis of some portions of the liver tissue that were observed probably resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification. The inability of fish to regenerate new liver cells may also have led to necrosis. These findings are in accordance with the observation of Rahman *et al*., (2002). Significant changes such as hyperplasia, disintegration of hepatic mass, disorganized hepatic canaliculi and focal coagulative necrosis were found in *Labeo rohita* exposed to cypermethrin (Sarkar *et al*., 2005). Velisek *et al*., 2006b observed teleangioectasiae of secondary lamellae of the gills and degeneration of hepatocytes in periportal zones in rainbow trout after cypermethrin exposure. Teleangioectasiae indicates acute respiratory distress. Hepatic lesions in the liver tissues of fish *Gambusia affinis* exposed to deltamethrin were reported such as hypertrophy of hepatocytes, increase of kupffer cells, circulatory disturbances, focal necrosis, fatty degeneration, nuclear pycnosis, narrowing of sinusoids (Cengiz and Unlu, 2006). The cypermethrin induced hepatocyte pathologies are same with those reported by earlier workers under influence of different pesticides (Singh, 2013). Hydropic swelling of hepatocytes with pyknotic nuclei were observed in the present investigation is in accordance with the findings of Deka and Mahanta (2012) in malathion treated *Heteropneustes fossilis*.

Cypermethrin exposure induced marked abnormalities in the kidney initiated with disruption of tubular organization. Thereafter degeneration of tubular epithelial cells and lympocytic infiltration was evident. In the present study, cloudy swelling of renal tubules in acute exposure was evident which can be identified by the hypertrophy of the cells and the presence of small granules in the cytoplasm. Initial stage in the degeneration process can progress to hyaline degeneration, characterized by the presence of large eosinophilic granules inside the cells. These granules may be formed inside the cells or by the reabsorption of plasma proteins lost in the urine, indicating damage in the corpuscle (Hinton and Lauren, 1990). In more severe cases, the degenerative process can lead to tissue necrosis (Takashima and Hibiya, 1995). The presence of tubule degeneration, coupled with the absence of necrosis in the kidney in the present study indicates that the kidney suffered damage after exposure to lethal or sublethal doses of cypermethrin. The kidney cells were observed to have been massively destroyed. The renal corpuscles of the kidney were scattered resulting in their disorganisation and consequently obstruction to their physiological functions. Some of the kidney cells were found clogging together while they were disintegrated in some tissues of the organ. This also agreed with the findings of Rahman *et al*., (2002). Cengiz (2006) observed histopathological effects of deltamethrin on the kidney (degeneration in the epithelial cells of renal tubule, pycnotic nuclei in the haematopoietic tissue, dilatation of glomerular capillaries, degeneration of glomeruli, intracytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen) of common carp after acute exposure at a concentration of 0.029 and 0.041 mg/l. Histopathological changes in the kidney tissue such as severe necrosis, cloudy swelling, cellular hypertrophy and granular cytoplasm were reported by Tilak *et al*., (2007) in *Channa punctata* exposed to sublethal concentration of Butachlor and Machete, an Herbicide.

The appearance of atrophic or pyknotic nuclei in fish kidney increases with the increase of time course. It has been suggested that a nuclear and nucleolar changes are induced preceding a trophy and necrosis of cells in other animals. At the beginning, the change may probably form part of a defuse mechanism, leading to defuse an activation of synthetic or other activities in the cell. However, during prolonged treatment, further accumulation of cypermethrin causes a condensation of nuclear material to form darvely stained pyknotic nuclei. Leucocytes are common in the interstitial space of control fish kidney, but they are rarely aggregated so densely and abundantly as in cypermethrin treated renal tissue. The increase of leucocytes may have been an inflammatory response to cypermethrin. The dilation of the lumen of the kidney tubules, degeneration in the hemopoitic tissue rupture in the collecting tubules and necrosis as observed in the present investigation after cypermethrin treatment have also been reported in various fish species (Vardhani and Gowri, 2002).

Conclusion

It is concluded that, when fishes are exposed to pesticides, they suffer irreparable 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. Thus, the histological changes observed in the gills, liver and kidney of the freshwater fish *Cyprinus carpio* exposed to cypermethrin 25% EC indicated that the fish were responding to the direct effects of the toxicants as much as to the secondary effects caused by stress.

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