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Histological Alteration in Different Tissues of Freshwater Teleost *Labeo rohita* (Hamilton) Induced by Lambda-cyhalothrin 5% EC and Marshal (Carbosulfan 25%EC)

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ABSTRACT

Though synthetic pyrethroids and carbamates have enormous use as pesticides however here we look for the histological effects of sublethal doses of Lambda-cyhalothrin 5% EC (a Synthetic pyrethroid) and Marshal (Carbosulfan 25%EC) [a Carbamate pesticide] on brain, gill, liver and kidney of *Labeo rohita.* Probit Analysis revels that the LC 50 found to be 0.7µl/L and 10 µl/L for Lambda-cyhalothrin and Marshal respectively. After 10 days of pesticides exposure histopathological studies were performed. Notable changes were observed in brain, liver, gill and kidney.

Key words: Lambda-cyhalothrin 5%EC, Marshal (Carbosulfan 25%EC), Labeo rohita, 96hr LC50, Histopathological studies.

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1. INTRODUCTION

India is an agriculture based country with more than 60-70% of its population dependent on agriculture. For agricultural productivity pest control is essential but application of pesticides causes damage to both terrestrial and aquatic organisms; including fish, bird and humans. Fish accumulate pesticides directly from contaminated water and indirectly through food chain. Pesticides have become an indispensible part in modern agricultural practices and act as one of the vital factors in increased food production, but their indiscriminate use also led to destructions of many plants and animals. Accumulation of pesticide in fish tissues is evident by different physiological and biochemical changes in the fishes (1). Such effects can be noted by changes of several enzymes, metabolites and histopathology and behaviour. Such effects are also observed with both lethal and sublethal doses of pesticides. The magnitude of pesticide contamination was studied in the Indian fishes by many workers (2, 3). Pesticides wherever applied they found their way into water bodies ultimately affecting aquatic fauna in general and fish in particular.Most of the pesticides are hydrophobic in nature, so they could be easily absorbed by soil and could follow their path to rivers, lakes and ponds through run-off causing aquatic pollution. They could enter the food-chain when they become accumulated in aquatic organisms (4). Histological studies have been widely used as biomarkers in the evaluation of fish exposed to pesticides both in laboratory as well as in field. In an understanding of the effect of pesticide in fish histological studies are being used widely. Histological studies are generally recommended as it helps to determine cellular damage (5). Lambda cyhalothrin (LCT) is also used in the vector control such as mosquito control by direct spraying in the water bodies (6). Lambda cyhalothrin is extremely toxic to many aquatic organisms, including fish. Previous research has shown that exposure to lambda cyhalothrin, even at sub-lethal doses, induces behavioural and biochemical changes in fish (7). The current study is aimed to evaluate the effects of sub-lethal doses of Marshal (Carbosulfan 25%EC) on brain, kidney, liver and gill of *Labeo rohita.* Histological tissues are collected from both control and treated groups after 10 days of pesticide exposure. Microtomy is used for histological studies and severe histological changes are found compared to control. Marshal (Carbosulfan 25%EC) is a broad spectrum carbamate pesticide. Carbosulfan is used against mostly caterpillers, green leaf hopper, and brown plant hopper, stem borer of paddy and white aphids of chillies. It is also used for protecting apple, citrus, corn, potato, rice, soybean,

sugar beat, and vegetables. The current study was carried out to evaluate the effect of an environmental pollutant-LCT and Marshal on possible histological changes in the vital organ- gill, liver, kidney and brain of most commonly used edible fish in eastern India –*Labeo rohita.*

2. MATERIALS AND METHODS

Experiments were conducted in laboratory condition. Healthy and active species of Labeo *rohita* (Hamilton) fish (20±1.4gm in weight and 12.7±0.75cm in length) were collected from local market, disinfected with 0.05% $KMnO_4$ solution. The fishes were kept in glass aquarium containing dechlorinated tap water and with sufficient oxygen supply. Fishes were acclimatized to laboratory condition for at least 14 days under equal exposure of light and dark. Fishes were not fed during the experimental period. Water quality characteristics were maintained by following the guidelines of APHA, EPA (8). The measured characteristics of water qualities were such as temperature: 26^0±2^0C, pH: 7.2±0.4 dissolved oxygen: 7.3±0.2 ppm, total hardness: 233±1.78 ppm. Experiments were run to determine the LC_{50} (through SPSS 16.0) which was 0.7 µl/L and 10µl/L (through SPSS 16.0) for LCT and Marshal respectively. The sub-lethal concentrations under study were 0.3 µl/L, 0.4 µl/L, 0.5 µl/L and 0.6 µl/L for LCT and 1µl/L, 2µl/L, 4µl/L and 8µl/L for Marshal. After 10 days, fish tissues were fixed in 10% Neutral Buffered Formalin for one week, Gill tissues were fixed in Davidson's Fixative for 24 hours and then in Neutral Buffered Formalin. Microtomy was done for fixed tissue sectioning of about 5µm thickness followed by staining with haematoxylin and eosin. Light microscopic studies were done for histopathological effects.

3. RESULTS AND DISCUSSION

3.1. Behavioural Changes

After administration of Lambda-cyhalothrin and Marshal in fishes with increasing dose exposure (all are sub-lethal doses) in aquariums following behavioural changes are observed-

- The fishes secreted lots of mucous during the total exposure period. The amount of secretion was increased with increased dose.
- During initial exposure their movements were very fast and they were hyperactive, increasing gulping movement with fast opercular movements was also seen.
- Pale gills were seen as compared to control in high doses.
- Irregular, erratic swimming behaviour, loss of balance, drowning and change 4s in body pigmentation become more prominent with increase concentration of pesticides.
- Blood patch was also seen on operculum.

3.2. Brain

Histological observation of control brain (Figure 1a and Figure 2 a) showed normal architecture i.e. the intact molecular, granular region and purkinje cell layer. In the experimented group both of the pesticides show drastically affected molecular and granular region causing necrosis which is evidenced by the appearance of white patches, pycnotic nuclei (Figure 1b, 1c, 1d, 1e and Figure 2b, 2c, 2e) and vacuolation in the molecular region (Figure 1b and Figure 2d).

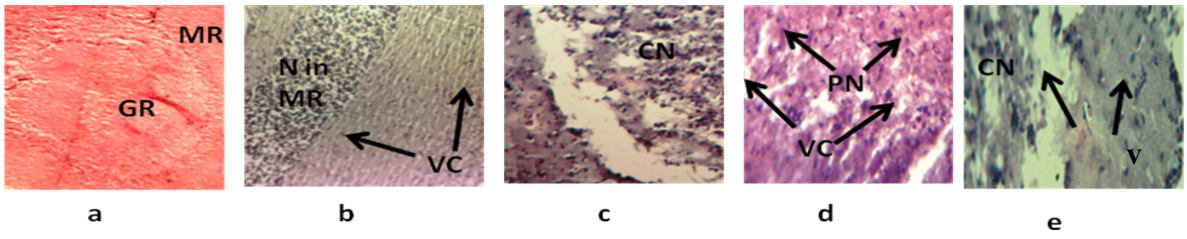

Figure 1. Histology of Lambda- Cyhalothrin exposed fish: a-e Brain sections (H and E stained, X400)

Control (a) and b, c, d and e are LCT exposed for 0.3 µl/L, 0.4 µl/L, 0.5 µl/L and 0.6 µl/L respectively;
BC- Bowman's capsule, CN- Complete necrosis, DGL – Degenerated Glomerulus, DRT- Degenerated Renal Tubule, DT- Degenerated tissue, MR- Molecular region, PN- Pycnotic nuclei, RT- Renal Tubule, VRT- Vacuolated Renal Tubule, BS-Blood Spillage, DGE- Degenerated Gill Epithelium, DHP- Degenerated Hepatocytes, PGL- Primary Gill Lamellae H- Hepatocytes, LT- Lamellar Telangiectasia, PGL- Primary Gill Lamellae, SGL- Secondary Gill Lamellae, DBS- Degenerated Blood Sinus, DCV- Degenerated Central Vein, PNPC-Primary Necrosis and Pycnotic Cell.

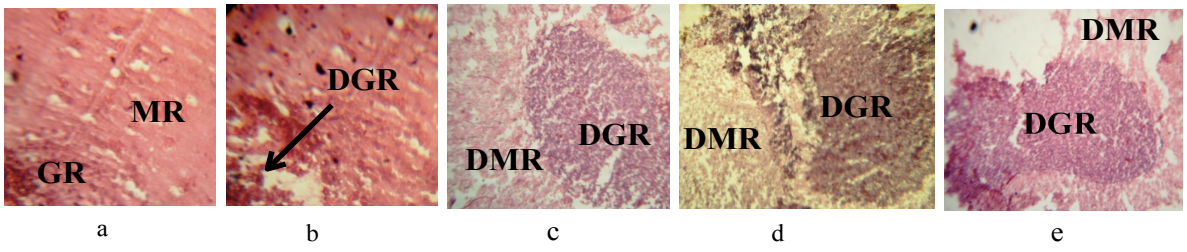

Figure 2. Histology of Marshal exposed fish: a-e Brain sections (H and E stained, X400)

Control (a) and b, c, d and e are Marshal exposed for 1 µl/L, 2µl/L, 4µl/L and 8µl/L respectively;
BC- Bowman's capsule, DGL – Degenerated Glomerulus, DT- Degenerated tissue, DMR- Degenerated Molecular region, DGR- Degenerated Granular region, PYC-Pycnosis, CC-Chloride cell, DHC- Degenerated Hepatic Chords, DPGL- Degenerated Primary Gill Lamellae, FPC-Fused Primary Chloride cell, PGL- Primary Gill Lamellae, DGL- Degenerated Gill Lamellae, DPC- Degenerated Primary Chloride cell,DBC- Degenerated Bowman's Capsule, HC-Hepatic Chord, CV-Central Vein, DCV- Degenerated Central Vein, PCT-Proximal Convoluted Tubule, DCT-Distal Convoluted Tubule, SGC-Shruken Glomerulus,VT-Vacuolated Tubule.

3.3. Kidney

Kidney of control group shows normal and systematically arranged tubules, compact renal mass and haematopoietic cells (Figure 3 a and Figure 4 a). Kidney from experimental group showed large vacuum between tubules, necrotic tubular epithelium,shrunken glomerulus, dilatedtubular lumen, atrophied collecting tubules, degenerated Bowman's Capsule enlarged lumen and disintegrated, vacuolated renal tubules (Figure 3 b-e and Figure 4 b-e).

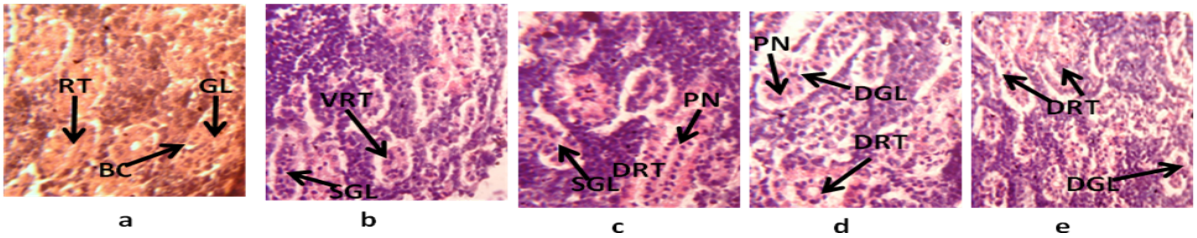

Figure 3. Histology of Lambda- Cyhalothrin exposed fish: a-e Kidney sections (H and E stained, X400)

Control (a) and b, c, d and e are LCT exposed for 0.3 µl/L, 0.4 µl/L, 0.5 µl/L and 0.6 µl/L respectively;
BC- Bowman's capsule, CN- Complete necrosis, DGL – Degenerated Glomerulus, DRT- Degenerated Renal Tubule, DT- Degenerated tissue, MR- Molecular region, PN- Pycnotic nuclei, RT- Renal Tubule, VRT- Vacuolated Renal Tubule, BS-Blood Spillage, DGE- Degenerated Gill Epithelium, DHP- Degenerated Hepatocytes, PGL- Primary Gill Lamellae H- Hepatocytes, LT- Lamellar Telangiectasia, PGL- Primary Gill Lamellae, SGL- Secondary Gill Lamellae, DBS- Degenerated Blood Sinus, DCV- Degenerated Central Vein, PNPC-Primary Necrosis and Pycnotic Cell.

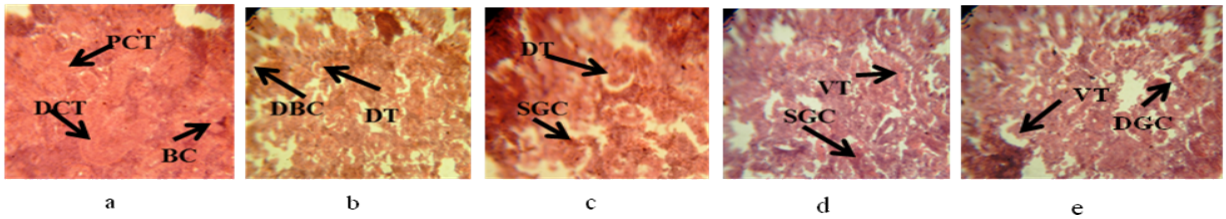

Figure 4. Histology of Marshal exposed fish: a-e Kidney sections (H and E stained, X400)

Control (a) and b, c, d and e are Marshal exposed for 1 µl/L, 2µl/L, 4µl/L and 8µl/L respectively;
BC- Bowman's capsule, DGL – Degenerated Glomerulus, DT- Degenerated tissue, DMR- Degenerated Molecular region, DGR- Degenerated Granular region, PYC-Pycnosis, CC-Chloride cell, DHC- Degenerated Hepatic Chords, DPGL- Degenerated Primary Gill Lamellae, FPC-Fused Primary Chloride cell, PGL- Primary Gill Lamellae, DGL- Degenerated Gill Lamellae, DPC- Degenerated Primary Chloride cell,DBC- Degenerated Bowman's Capsule, HC-Hepatic Chord, CV-Central Vein, DCV- Degenerated Central Vein, PCT-Proximal Convoluted Tubule, DCT-Distal Convoluted Tubule, SGC-Shruken Glomerulus,VT-Vacuolated Tubule.

At the first dose kidney lesions were not so marked/ mild changes. In some tubules, lumen diameter was increased. Degenerative changes were enormous, epithelium desquamated & vacuolisation in some tubules.

3.4. Liver

Both LCT and Marshal exposure at different doses caused severe pathological lesions in liver tissue when compared to control- loss of parenchymatous structure i.e. ruptured central vein (Figure 5 c, d and Figure 6 b), swollen and ruptured parenchymal cells, loss of parenchymal cord structure (Figure 5 b-e, Figure 6 b-e), vacuoles filled with normal cellular debris that means the whole normal cellular structure of the hepatic cells of liver is diminished. With increasing dose of exposure of pesticides, experimental fish liver showed loss of parenchymatous structure i.e. hepatic chord like structure, dissociated swollen hepatocytes, vacuolisation, extensively degenerated and granular cytoplasm. Karyolysis and pycnosis of nuclei (Figure 5 d) were also profound. Blood capillary endothelium ruptured and blood was spilled into the liver tissues (Figure 5 b). The cells outline becoming indistinguishable (Figure 5 c, d, e and Figure 6 e).

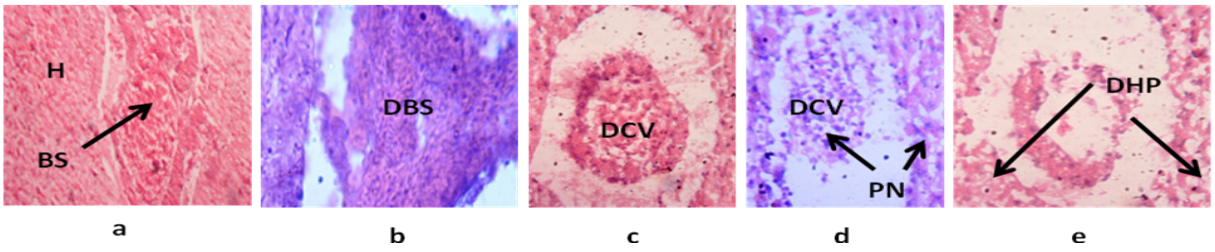

Figure 5. Histology of Lambda- Cyhalothrin exposed fish: a-e Liver sections (H and E stained, X400)

Control (a) and b, c, d and e are LCT exposed for 0.3 µl/L, 0.4 µl/L, 0.5 µl/L and 0.6 µl/L respectively;
BC- Bowman's capsule, CN- Complete necrosis, DGL – Degenerated Glomerulus, DRT- Degenerated Renal Tubule, DT- Degenerated tissue, MR- Molecular region, PN- Pycnotic nuclei, RT- Renal Tubule, VRT- Vacuolated Renal Tubule, BS-Blood Spillage, DGE- Degenerated Gill Epithelium, DHP- Degenerated Hepatocytes, PGL- Primary Gill Lamellae H- Hepatocytes, LT- Lamellar Telangiectasia, PGL- Primary Gill Lamellae, SGL- Secondary Gill Lamellae, DBS- Degenerated Blood Sinus, DCV- Degenerated Central Vein, PNPC-Primary Necrosis and Pycnotic Cell.

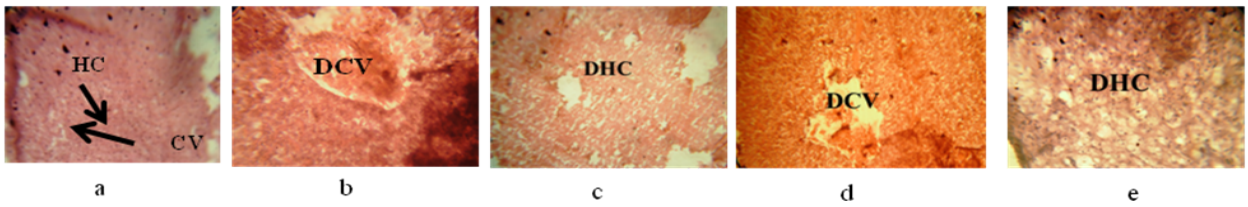

Figure 6. Histology of Marshal exposed fish: a-e Liver sections (H and E stained, X400)

Control (a) and b, c, d and e are Marshal exposed for 1 µl/L, 2µl/L, 4µl/L and 8µl/L respectively;
BC- Bowman's capsule, DGL – Degenerated Glomerulus, DT- Degenerated tissue, DMR- Degenerated Molecular region, DGR- Degenerated Granular region, PYC-Pycnosis, CC-Chloride cell, DHC- Degenerated Hepatic Chords, DPGL- Degenerated Primary Gill Lamellae, FPC-Fused Primary Chloride cell, PGL- Primary Gill Lamellae, DGL- Degenerated Gill Lamellae, DPC- Degenerated Primary Chloride cell,DBC- Degenerated Bowman's Capsule, HC-Hepatic Chord, CV-Central Vein, DCV- Degenerated Central Vein, PCT-Proximal Convoluted Tubule, DCT-Distal Convoluted Tubule, SGC-Shruken Glomerulus,VT-Vacuolated Tubule.

3.5. Gill

Histological changes also observed in pesticides treated fish gills (Figure 7 a and Figure 8 a). Degenerative changes were noticed in the inter-lamellar region. Detachment of epithelial surface in primary gill lamella from secondary lamella was observed. Swelling at the tips of the secondary gill lamellae followed by hypertrophy (Figure 7 b) and marked hyperplasia. This was followed by the separation of the basement membrane, curling and fusion of adjacent gill lamellae and epithelium (Figure 7 b, c, d and e, Figure 8 b, c). They showed reduced central axis i.e. necrotic condition which leads into interlamellar space formation (Figure 7 b, c, d and e). Gill, in general, showed marked pathological changes such as bulging in the tips of primary gill lamellae (Figure 8 b-e), proliferation of interlamellar cells, separation of epithelial layer from the central sinus of the filament of primary gill lamellae (Figure 7 e, Figure 8 b and c), lamellar telangiectasia (Figure 7 b) and hypertrophy of chloride cells. Fusion of piller cells (Figure 8 d), degeneration of piller cells (Figure 8 e) was also observed.

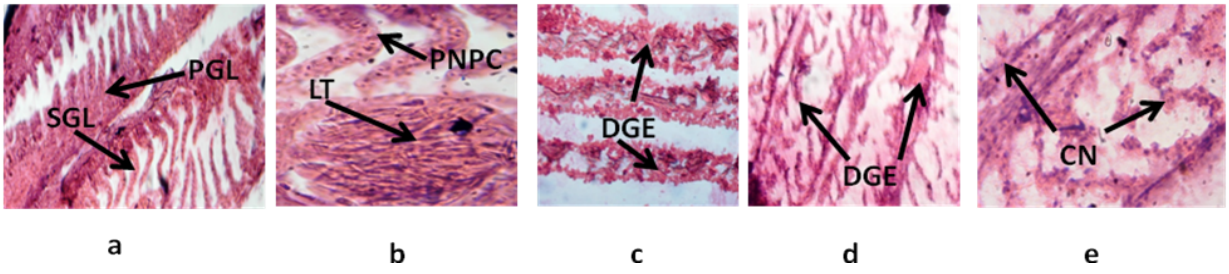

Figure 7. Histology of Lambda- Cyhalothrin exposed fish: a-e Gill sections (H and E stained, X400)

Control (a) and b, c, d and e are LCT exposed for 0.3 µl/L, 0.4 µl/L, 0.5 µl/L and 0.6 µl/L respectively;
BC- Bowman's capsule, CN- Complete necrosis, DGL – Degenerated Glomerulus, DRT- Degenerated Renal Tubule, DT- Degenerated tissue, MR- Molecular region, PN- Pycnotic nuclei, RT- Renal Tubule, VRT- Vacuolated Renal Tubule, BS-Blood Spillage, DGE- Degenerated Gill Epithelium, DHP- Degenerated Hepatocytes, PGL- Primary Gill Lamellae H- Hepatocytes, LT- Lamellar Telangiectasia, PGL- Primary Gill Lamellae, SGL- Secondary Gill Lamellae, DBS- Degenerated Blood Sinus, DCV- Degenerated Central Vein, PNPC-Primary Necrosis and Pycnotic Cell.

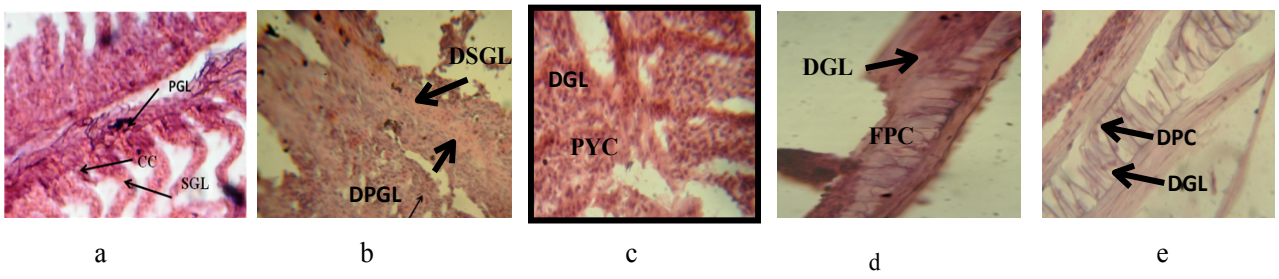

Figure 8. Histology of Marshal exposed fish: a-e Gill sections (H and E stained, X400)

Control (a) and b, c, d and e are Marshal exposed for 1 µl/L, 2µl/L, 4µl/L and 8µl/L respectively;
BC- Bowman's capsule, DGL – Degenerated Glomerulus, DT- Degenerated tissue, DMR- Degenerated Molecular region, DGR- Degenerated Granular region, PYC-Pycnosis, CC-Chloride cell, DHC- Degenerated Hepatic Chords, DPGL- Degenerated Primary Gill Lamellae, FPC-Fused Primary Chloride cell, PGL- Primary Gill Lamellae, DGL- Degenerated Gill Lamellae, DPC- Degenerated Primary Chloride cell,DBC- Degenerated Bowman's Capsule, HC-Hepatic Chord, CV-Central Vein, DCV- Degenerated Central Vein, PCT-Proximal Convoluted Tubule, DCT-Distal Convoluted Tubule, SGC-Shruken Glomerulus,VT-Vacuolated Tubule.

Histopathological changes are important to identify the environmental effects of pesticides (9-11). The cause behind this type of behaviour is due to that the organs are affected when exposed to foreign bodies or toxic materials causing loss of equilibrium, irregular movements, and increase in opercular movements, imbalance and finally leading to death. In the present study, histological alterations were found in different tissues (gill, kidney, brain and liver) of Lambda-cyhalothrin and Marshal exposed fish (*Labeo rohita*). It is evident from the above findings that both Lambda-cyhalothrin and Marshal are highly toxic to fish. The histological changes in the organs of experimental fish increased with increasing dose exposure. Anithakumari and Sreeram Kumar (12) reported several histopathological changes in kidney, liver and gills, due to impact of industrial effluents in the fish, *Channa punctatus* and *Hetropneustes fossils*. Brain is the organ which serves as the centre of nervous system. Both of the pesticide treatment drastically affected the molecular and granular region of brain which causes necrosis, appearance of pycnotic nuclei and vacuolation of molecular region resulting in severe damage of nervous system. The brain also indicated severe congestion and generalised spongiosis that indicate severe brain damage. This agreed with the findings of Omitoyin et al (13). Kidney is one of the very important organs which are affected by contaminants in water as it acts as a filter in animal body. Elsan treatment in *Channa punctatus* also revealed significant decrease in the dimension of Bowman's capsule and glomerulus, and the tubules lost their regular shape due to precipitation of cytoplasm and karyolysis (14). Hypertrophy of renal cells, changes in the nuclear structure, formation of vacuoles, necrosis and degeneration of renal components were noticed on the renal cells of *Cyprinus carpio* exposed to malathion and sevin (15). Cengiz (16) demonstrate lesions in the kidney tissues of fish exposed to deltamethrin, Tubular degeneration observed in catfish, *Ictalurus punctatus* upon exposure to methyl mercury (17). Sub-lethal concentration of phenolic compounds exhibited degeneration and dissolution of epithelial cells of renal tubules, hypertrophy and necrosis in *Notopterus notopterus* (18). In another vital organ liver abnormal changes were also found. Fish liver can be regarded as the body's detoxification center and hence a target organ for various xenobiotic substances.Necrosis, which is a passive mode of cell death, shows that the capacity to maintain homeostasis was affected. Thus occurrence of necrosis may be one of the important reasons for decreased lysosomal membrane stability observed leading to the leakage of lysosomal marker enzyme acid phosphatase to the soluble fraction. Pycnotic nuclei observed indicate that the cells became hypofunctional. Pycnosis results in irreversible condensation of chromatin in the nucleus of a cell. Acute toxic injury usually includes cloudy swelling or hydropic degenerations and pycnosis, karyorrhexis and karyolysis of nuclei (19, 20). Cloudy swelling, bile stagnation, focal necrosis, atrophy and vacuolization have been reported in the *Corydora spaleatus* exposed to methyl parathion (2). Cengiz and Unlu (16) also reported hypertrophy of hepatocytes, increase of kupffer cells, circulatory disturbance, narrowing of sinusoids, pycnotic nuclei, fatty degeneration and focal necrosis in the liver of *Gambusia affinis* exposed to deltamethrin. The cellular degeneration in the liver may be also due to oxygen deficiency as a result of gill degeneration and/or to the vascular dilation and intravascular haemolysis with subsequent stasis of blood (21). In teleost gills are critical organs which perform respiratory, osmoregulatory and excretory functions. Due to their lipophilicity, pyrethroids have a high rate of gill absorption, (22). The gills of the fish exposed to both the pesticides exhibited marked histopathological changes. The main features observed in gills exposed to sub-lethal concentration of pesticides were partial degeneration of epithelium ofsecondary gill lamellae. In some place adjacent secondarygill lamellae appeared to adhere each other. Fusion of secondary gill lamellae resulting in reductionof respiratory surface and vacuolization was alsoobserved. The similar results were reported by Rao et al. (23), Cengiz and Unlu (16); Vermuruganet al., (6); Butchiram et al., (24).Choudhan and pandy (25) while studiedon toxicity of various pesticide on freshwater fish. Srivastava and Shrivastva (26), studied effect of sub-lethal concentration of malthion chloride on the histopathology of the gills of *Channa gachua* and observed hyperplasia, hypertrophy vacillation in primary gill lamellae, pycnotic nuclei and increase in

volume of pillar cells. Tilak et al (27) reported that the effect of butachlor technical and machete 50% EC has induced marked pathological changes in fish gills. The changes included secondary filaments lost their original shape and cutting of secondary gill filaments, pillar cell nucleus showed necrosis anddeveloped vacuoles in the secondary gill epithelium. The degeneration in gill is due to intimate contact of gills withtoxicant may lead to defection of normalrespiratory area that is damage of gilltissue which in turn may reduce thediffusion capacity of the gill. Hyperplasia refers to an increase in the number of normal cells that constitute a given tissue. Gill alterations such as hyperplasia of the epithelial cells can be considered adaptive, since they increase the distance between the external environment and blood, serving as a barrier to the entrance of contaminants. Hyperplasia observed may be the fish's response is to ward off or block something that irritates tissues. Hyperplasia, however, may play a role in the early stages of neoplasia.

4. CONCLUSION

Our study indicates the effects of LCT and Marshal produces histological changes in various tissues like gills, liver and kidney tissues of L. *rohita*. As a conclusion, the findings of the current histological observations indicate a correlation between exposure to both the pesticides and histopathological disorders observed in several tissues.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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