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Histological Alteration in Different Tissues of Indian major carp, *Labeo rohita* (Hamilton) exposed to Profenofos 50% EC and Carbosulfan 25% EC formulations

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ABSTRACT

The objective of the study was to determine the histopathological changes in different tissues of fish, *Labeo rohita* exposed to two different pesticides. Gill, kidney, liver and Brain of fish were examined after exposure to lethal and sublethal concentrations of organophosphorus pesticide profenofos (96 h LC50 i.e.100 and 10 µg L^{-1}) and carbamates pesticide carbosulfan(1.2 and 0.12 mg L^{-1}) for 24 h and 8 days. Lesions were observed in gill, kidney and liver of *L.rohita* treated with both pesticide concentrations. Gill filaments of Hyperplasia, fusion due to separation of epithelium. Necrosis, degenerative changes in haemopoietic tissue, swelling of renal tubules, hypertrophy were observed in kidney. Degenerations of cytoplasm in hepatocytes, atrophy, and necrosis, vacuoles formation, rupture of blood vessels, necrosis and disappearance of hepatocyte wall and hepatic cords were observed in liver tissue. Behavioral changes, sensorial system like vision and smell, detection, attack and capture of prey, impair feeding, escape, and reproductive behavior all these functions are connected with various parts of the brain. There is no lesions were seen in control group.

Key words: Necrosis, Kidney, Formation of vacuoles, Blood vessels, Lesions, Cytoplasm, Disappearance.

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1. INTRODUCTION

Due to urban, industrial activities, freshwater sources are contaminated with different types of chemicals that affect the inhabiting biota. The complexity of the contaminants may induce a variety of biological responses (1). Aquatic ecosystem is the final sink for the many chemicals used in industry and agriculture has a global problem, the continuous release of these chemicals impair water quality and become unsuitable for aquatic organisms due to their persistence, bioaccumulation, toxicity and biomagnification in food chain and ecological balance (2, 3). Pesticide residues often reach the aquatic ecosystem and can be transferred to phytoplankton to fish and humans (4). The toxicity of any environmental contaminant is either acute or chronic (5). The damage of particular tissue depends on the toxic potentiality of a particular contaminant accumulated in the tissue (6). Disturbance of the homeostasis of an organism leads to compensatory, adaptive, and finally pathological processes, which are mostly energy-demanding (7, 8). Different biomonitoring programs have used the histological changes observed in fish tissues as biomarkers of aquatic ecosystems (9). Histopathological studies allowed the identification of several changes induced by environmental pollutants (10). Histopathological studies have proved to be a sensitive tool to detect direct effect of toxicants within in target organs of fish (11-13). Frequency and intensity of tissue damage depend on the concentrations of pesticides and the duration of the period fish are exposed to toxicants. In spite of that, numerous insecticides cause specific or non-specific histopathological damage (14, 15). Profenofos insecticide belongs to Organophosphorus classification, the IUPAC name was (RS)-(O-4-bromo-2-chlorophenyl O-ethyl S-propyl phosphorothioate) it was mainly used in different types of agricultural crops for controlling of pest and

pathogenic organisms. Carbosulfan belongs to carbamates pesticides, due to its broad spectrum insect control in several crops, which acts by inhibiting the activity of acetyl cholinesterase. The IUPAC name of the carbosulfan was 2, 3-dihydro-2, 2-dimethylbenzofuran-7-yl (dibutylaminothio) methylcarbamate (16). Hence, in the present study, an attempt has been made to observe possible histopathological changes in certain vital tissues like gill, liver, kidney and brain of the freshwater fish, *Labeo rohita* exposed to lethal (96 hr LC50) and sublethal concentration (1/10th of 96 hr LC50) of profenofos and carbosulfan EC formulation for 24 h and 8 days.

2. MATERIALS AND METHODS

2.1. Experimental animal

Freshwater fish*, Labeo rohita* of size 6±7 cm and 6.5±7.5 g weight were brought from a local fish farm Tenali, Guntur district of Andhra Pradesh, India and acclimated at 28 ± 2 ^{o}C in the laboratory for 15 days. During the acclimation period fish were fed twice a day with commercial fish pellets and rice bran. At the same time water was renewed with freshwater every two days. The same water supply was used during acclimation period and subsequent lethal and sublethal toxicity tests. Such acclimated fish were exposed to sublethal and lethal concentrations (1/10th 96 hr LC_{50} i.e.10 µg L^{-1}; 0.12 mg L^{-1} and 96 hr LC_{50} i.e. 100 µg L^{-1}; 1.2 mg L^{-1}) of profenofos and carbosulfan for 15 days. The water used in the experiments and water had following physic-chemical characteristics (17).

2.2. Physico-chemical analysis of water

Turbidity–8 Silica units, Electrical conductivity at 28^{o}C - 816 micro ohms/cm,Alkalinity-1, Phenolphthalein-Nil,Methylorange-472, Total hardness (as $CaCO_3$) - 232, Non carbonate hardness (as $CaCO_3$)-Nil, Calcium hardness (As N) -Nil, Sulphate (as SO_4) -Trace, Chloride (as Cl)- 40, Fluoride (as Fl)- 1.8, Iron (as Fe) -Nil , Dissolved oxygen-8-10 ppm, Temperature- 28±2^{o}C. All the precautions laid by committee on toxicity tests to aquatic organisms were followed (17).

2.3. Pesticides

Commercial grade formulations of two pesticides: organophosphorus pesticide profenofos, available in 50% EC [emulsible concentrate active ingrédient, carbamates carbosulfan 25% EC formulations] were used as the test pesticides. Solutions of the both pesticides were made by diluting with acetone to obtain required concentrations.

2.3.1. Sublethal toxicity tests

Based on the preliminary lethal toxicity tests (1/10th 96 h LC50 i.e.10 µg L^{-1} for profenofos and 0.12mg L^{-1}; 96 h LC50 i.e.100 µg L^{-1}; 1.2 mg L^{-1} for carbosulfan were selected as sublethal and lethal concentrations. After the acclimation, fish were exposed to sublethal concentrations in groups of 10 fish in 15 L of the test water in glass chambers for 24 h and 8 days. The experiments were conducted in triplicates for each concentration of pesticides. Control group were maintained in two glass chambers for each pesticide. Concentrations of the pesticides were re-established to maintain the original levels while the test solutions were renewed each day to improve the water quality (17, 18).

2.4. Histopathology

After 24 h and 8 days of pesticide exposure, gill, liver, brain and kidney tissues were isolated from control and pesticide treated fish. Physiological saline solution (0.75% NaCl) was used to rinse and clean the tissue. They were fixed in Bouins aqueous solution for 48 h processed through graded sequence of alcohols cleared in xylene and embedded in paraffin wax. Gill tissue was processed by double embedding technique. Sections were cut of 6 µ (microns) thickness; stained with Ehrlich haematoxilin and Eosin (H&E) (dissolved in 70% alcohol) 19 and were mounted in Canada balsam. Histopathological lesions were examined and photographed with the help of Olympus computer attached light microscope under 400X lens (19).

3. RESULTS AND DISCUSSION

Histological lesions in different tissues of freshwater fish, *L.rohita* exposed to lethal and sublethal concentrations of profenofos and carbosulfan were summarized. Hyperplasia, fusion of gill filaments due to separation of epithelium, necrosis of gill epithelium, degeneration of pillar cells, development of vacuoles in the epithelium are the pathological changes observed in profenofos and carbosulfan treated fish (Figure 1).

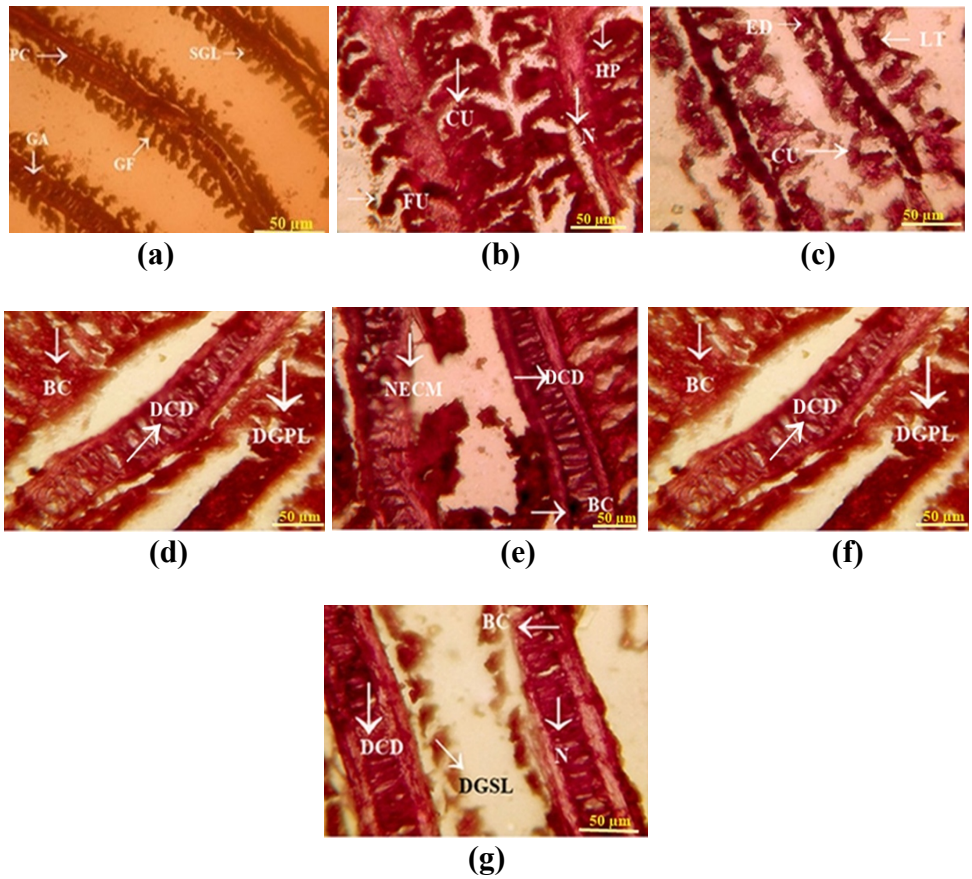

Figure 1. a) Normal Gill lamella of *L. rohita,* Bouin, Scale bars= 50µm, HEx 400. PGL, Primary gill lamella;GA, Gill arch;SGL, Secondary gill lamella; GF, Gill filaments;PC, Pillar cells; b) *L. rohita* exposed to sublethal concentration of profenofos for 24 hr; Bouin, Scale bars= 50µm, HEx 400; CU, Secondary lamellar culing; HP, Hyperplasia; Necrosis; c) *L. rohita* exposed to lethal concentration of profenofos for 24 hr; Bouin, Scale bars= 50µm, HEx 400; CU, Secondary lamellar culing; HP, Hyperplasia; Necrosis; d) *L. rohita* exposed to sublethal concentration of carbosulfan for 24 hr; Bouin, Scale bars= 50µm, HEx 400;BC, Blood Congestion;CD, Degenerated Chondrocyte;DGPL, Degenerated primary lamella; e) *L. rohita* exposed to lethal concentration of carbosulfan for 24 hr; Bouin, Scale bars= 50µm, HEx 400; NECM, Necrotic extracellular gill filaments CD, Degenerated Chondrocyte;BC, Blood Congestion; f) *L. rohita* exposed to profenofos sublethal concentration for 8 days; Bouin, Scale bars= 50µm, HEx 400; DGPL; Degenerated primary lamella;DSGL; Degenerated Secondary lamella;LT, Lamellar telangiectesis; g) *L. rohita* exposed to carbosulfan sublethal concentration for 8 days; Bouin, Scale bars= 50µm, HEx 400;BC, Blood Congestion;DCD, Degenerated Chondrocyte;DGSL, Degenerated Secondary lamella;N, Necrosis.

Profenofos and carbosulfan toxicity caused marked pathological changes in the kidney of exposed fish which include severe necrosis, degenerative changes in haemopoietic tissue, cloudy swelling in renal tubules, cellular hypertrophy and granular cytoplasm were evident. Distal convoluted tubules decreased in size, the interstitial renal tissue was less affected. Renal interstitial tissue showed vacuoles formation and cellular contours were not clearly distinguished. Highly degenerative changes in haemopaitic tissue, which include cellular hypertrophy and granular cytoplasm, were evident (Figure 2).

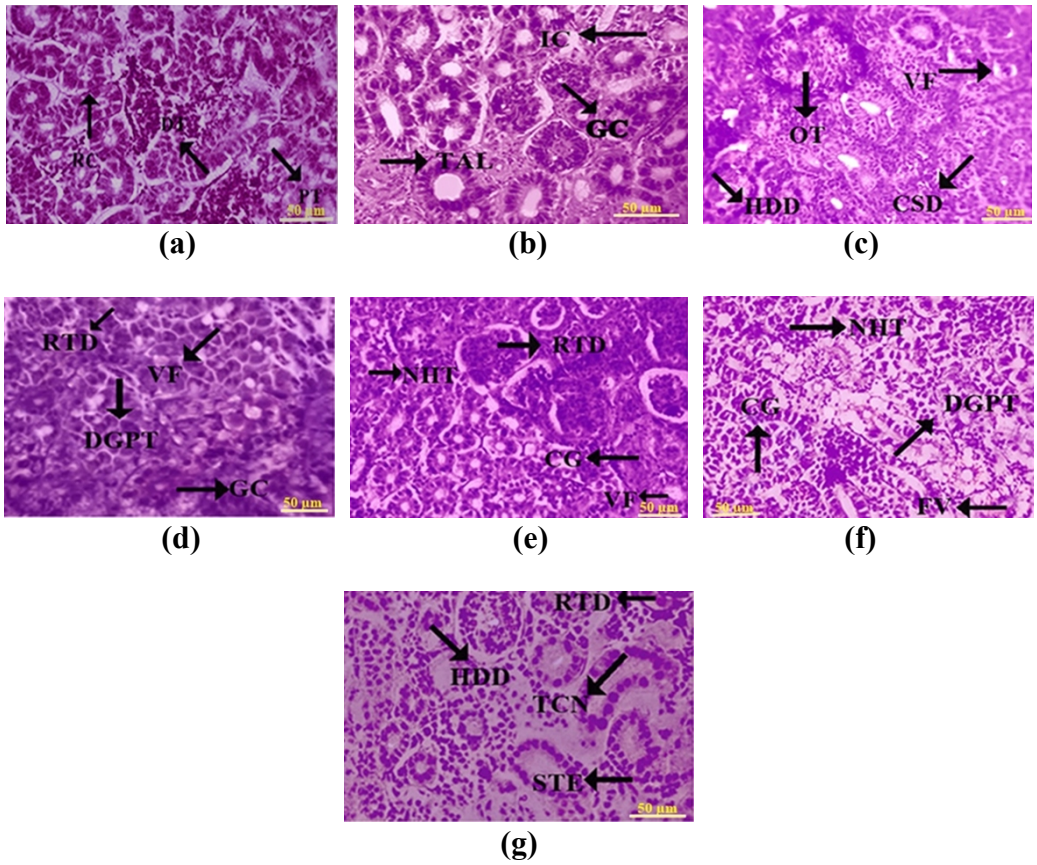

Figure 2. a) Control: Normal structure of Kidney in L. rohita; Bouin, Scale bars= 50µm, HEx 400;RC, PT, Proximal Tubule; DT, Distal convoluted tubule; b) L. rohita exposed to sublethal concentration of profenofos for 24 hr ; Bouin, Scale bars= 50µm, HEx 400;IC, Interstitial cells; GC, Glomerular congestion; TAL, Tubular Architectural loss; c) L. rohita exposed to lethal concentration of profenofos for 24 hr; Bouin, Scale bars= 50µm,OT, Occlusion of Tubular lumen;VF, Vacuole Formation;HDD, Hyaline Droplets Degeneration;CSD, Cloudy Swelling Degeneration; d) L. rohita exposed to sublethal concentration of carbosulfan for 24 hr; Bouin, Scale bars= 50µm, RTD, Renal tubular degeneration; VF, Vacuole Formation; DGPT, Degenerated primary tubule; GC, Glomerular congestion; e) L. rohita exposed to lethal concentration of carbosulfan for 24 hr; Bouin, Scale bars= 50µm, NHT, Necrotic haemopoietic tissue;RTD, Renal tubular degeneration, GC, Glomerular congestion; f) L. rohita exposed to sublethal concentration of profenofos for 8 days; Bouin, Scale bars= 50µm, NHT, Necrotic haemopoietic tissue;GC, Glomerular congestion, DGPT, Degenerated primary tubule; FV, Formation of Vacuole; g) L. rohita exposed to sublethal concentration of carbosulfan for 8 days; Bouin, Scale bars= 50µm, RTD, Renal tubular degeneration, HDD, Hyaline Droplets Degeneration;TCN, Tubular cell necrosis; STE, Seperation of tubular epithelium.

Profenofos and carbosulfan has induced discrete pathological changes in the liver tissues. These changes include degenerations of cytoplasm in hepatocytes, atrophy, and necrosis, formation of vacuoles, rupture in blood vessels, necrosis and disappearance of hepatocyte wall and disposition of hepatic cords, formation of vacuoles, rupture in blood vessels, blood congestion, necrosis and disappearance of hepatocyte wall and disposition of hepatic cords, decrease in the size of nucleus as evident (Figure 3).

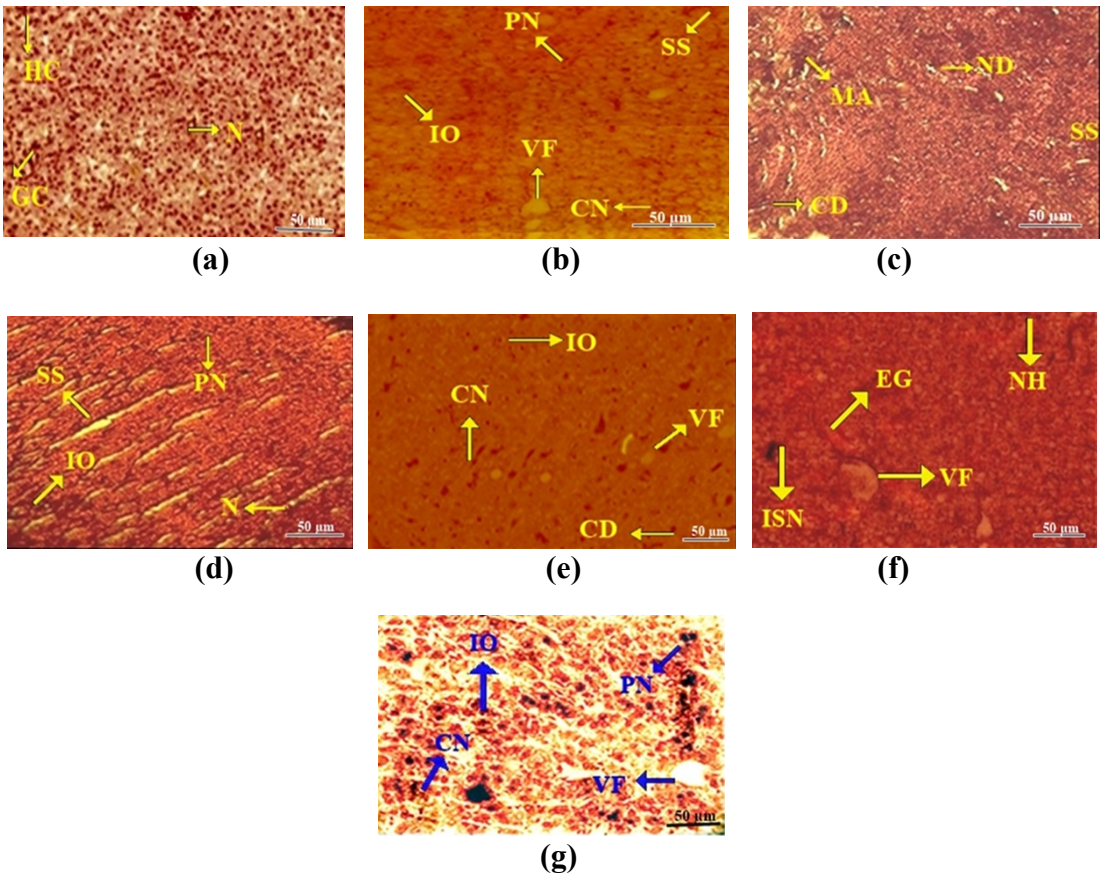

Figure 3. a) Normal structure of Liver in L. rohita; Bouin, Scale bars= 50µm, HEx 400; N, Nucleus; HC, Hepatic cell;GC, Granular Cytoplasm; b) L. rohita exposed to sublethal concentration of profenofos for 24 hr ; Bouin, Scale bars= 50µm, HEx 400; EG, Eosinophilic granules, NH, Nuclear Hypertrophy,VF, Vacuole formation;ISN, Irregular shaped nucleus; c) L. rohita exposed to lethal concentration of profenofos for 24 hr ; Bouin, Scale bars= 50µm, HEx 400;MA, Melanomacrophases Aggregate;ND, nuclear degeneration; CD, Cytoplasmic Degeneration; d) L. rohita exposed to sublethal concentration of carbosulfan for 24 hr ; Bouin, Scale bars= 50µm, HEx 400;PN, Pyknotic nuclei;SS, Increased sinusoidal space; IO, Intracellular oedema;N, Necrosis; e) L. rohita exposed to lethal concentration of carbosulfan for 24 hr ; Bouin, Scale bars= 50µm, HEx 400;IO, Intracellular oedema;CD, Cytoplasmic Degeneration;VF, Vacuole formation;CN, Cell necrosis; f) L. rohita exposed to sublethal concentration of profenofos for 8 days ; Bouin, Scale bars= 50µm, HEx 400, PN, Pyknotic nuclei;SS, Increased sinusoidal space;IO, Intracellular oedema;CN, Cell necrosis;VF, Vacuole formation; g) L. rohita exposed to sublethal concentration of carbosulfan for 8 days; Bouin, Scale bars= 50µm, HEx 400, PN, pynotic nuclei; IO, Intracellular oedema, CN, Cell necrosis; VF, Vacuole formation.

Alterations in brain tissue were shown in (Figure 4), vision and smell, detection, attack and capture of prey, impair feeding, escape, and reproductive behavior all these functions are connected with various parts of the brain.

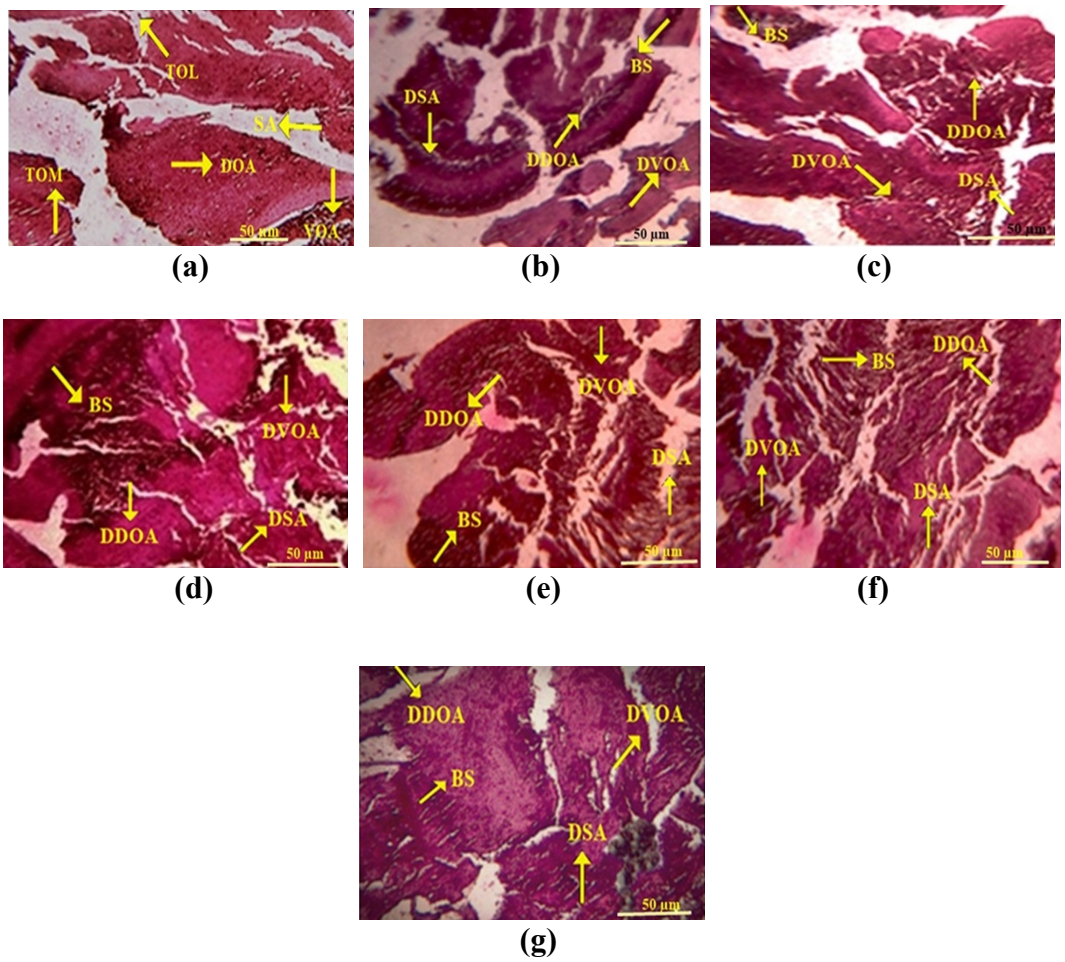

Figure 4. a) Normal structure of Brain in L. rohita; Bouin, Scale bars= 50µm, HEx 400, SA, Septal area; DOA, Dorsal olfactory area;VOA, Ventral olfactory area. TOM, Tractus olfactorius medialis, TOL, Tractus olfactorius lateralis; b) L. rohita exposed to sublethal concentration of profenofos for 24 hr ; Bouin, Scale bars= 50µm, HEx 400; DSA, Degenerated septal area; BS, Blood streeks;DDOA, Degenerated dorsal olfactory area; DVOA, Degenerated ventral olfactory area; c) L. rohita exposed to lethal concentration of profenofos for 24 hr ; Bouin, Scale bars= 50µm, HEx 400;BS, Blood streeks; DSA, Degenerated septal area; BS, DDOA, Degenerated dorsal olfactory area; DVOA, Degenerated ventral olfactory area; d) L. rohita exposed to sublethal concentration of carbosulfan for 24 hr ; Bouin, Scale bars= 50µm, HEx 400; Blood streeks; DSA, Degenerated septal area; BS, DDOA, Degenerated dorsal olfactory area;DVOA, Degenerated ventral olfactory area; e) L. rohita exposed to lethal concentration of carbosulfan for 24 hr ; Bouin, Scale bars= 50µm, HEx 400; Blood streeks; DSA, Degenerated septal area; BS, DDOA, Degenerated dorsal olfactory area;DVOA, Degenerated ventral olfactory area; f) L. rohita exposed to sublethal concentration of profenofos for 8 days ; Bouin, Scale bars= 50µm, HEx 400; Blood streeks; DSA, Degenerated septal area; BS, DDOA, Degenerated dorsal olfactory area;DVOA, Degenerated ventral olfactory area; g) L. rohita exposed to sublethal concentration of carbosulfan for 8 days ; Bouin, Scale bars= 50µm, HEx 400; Blood streeks; DSA, Degenerated septal area; BS, DDOA, Degenerated dorsal olfactory area; DVOA, Degenerated ventral olfactory area.

Inflammatory alterations of lamellar epithelium and hyperplasia were reported in the gill of freshwater fish, *Cyprinus carpio communis* (L) exposure to sublethal concentration of lead and cadmium (20). Edema with lifting of lamellar epithelium and hyperplasia of lamellar epithelium were observed in the gills of yellow perch (Perca flavescens) and gold fish (*Carassius auratus*) polluted water containing residues of oil sands (21). Similar findings were noted in the gills of White seabass (22). Lates calcarifer on exposure to acute and chronic cadmium. Swellings of inflammation in almost all the respiratory lamellae of gills of *Oreochromis niloticus* on exposure to copper (10). In the present study, gills were found to be the most effected tissue compared to liver and kidney. Because of the direct contact with the both toxicants, there may be a similarity with Hyperplacia, desquamation, and necrosis of epithelial lifiting oedema and collapsed secondary lamellae were observed in the freshwater fish, *Cirrhinus mrigala* exposed to dichlorvos (23). Toxicity of formalin cause of pathological damage in the gills, gill dysfunction, osmoregulatory and respiratory imbalance in ornamental fish amazon blue spotted corydora (*Corydoras melanistius*) (24). This is due to the gill epithelium is the primary contact surface, with the external environment, became a target of the environmental contaminants present in the polluted water. Blood congestion in between tubules and haemorrhage in the interstitium albino rats treated with dimethoate, carbendazim or carbofuran pesticides (25). Disorientation in glomerular structure, cloudy swelling, dilation in the

inter space of urinary tubular, necrosis in the hematopoietic tissue, appearance of vacuoles in the cytoplasm epithelial cells of renal tubules and narrowing of the tubular lumen due to diazinon toxicity in fish rainbow trout (*Oncorhynchus mykiss*) (26). Histopathological changes in kidney of fish rainbow trout exposed to methiocarb, kidney had tubular necrosis and renal tubules were filled with eosinophilic material (27). Reduced glomerular filtration rate, glomerular lesions, degeneration of cellular boundaries and clumping of glomeruli at some places in the kidney of rainbow trout (*Oncorhynchus mykiss*) exposed to fungicide captanin (28). The present observations are in agreement with the reports of observed renal damage, rupture in the glomeruli and reduced renal tubules and reduced lumen in Brown Trout (*Salmo trutta m. fario*) and Rosy Barb (*Puntius conchonius*) and mphioxus (*Branchiostoma belcheri)* (29). As mentioned above, histopathology of kidney highly corroborate with our present work on the effect of profenofos and carbosulfan on the kidney. In this present study histopathological alteration in the kidney were degenerative changes in epithelial cells of proximal tubules and haemopoietic tissues, severe necrosis in the proximal tubules leading to the formation of vacuoles, degenerative changes in epithelial cells of collecting tubules. In the current study it has been observed that some important histopathological changes were observed in the liver of the fish *L.rohita* exposed to both pesticides. Some of the alterations of the kidney were focal necrosis with inflammatory infiltration, vacuoles in the hepatocytes and hepatic sinusoids congestion in albino rats treated with dimthoate. Formation of vacuoles, cytoplasm in hepatocytes, atrophy, blood streaks among hepatocytes, intercellular empty space and disintegration of lattice fibres (30). Observed cytoplasm with nuclear degeneration, cellular degeneration and damaged hepatocytes in fish *Cyprinus carpio* exposed to chromium. Necrosis of hepatocytes with enlarged sinusoid in freshwater fish *Cirrhinus mrigala* exposed to dichlorvos (23, 31). reported that nuclear degeneration, hepatic cord disarray and precipitation of cytoplasm in Anabas testudineus teleost exposed to acute and chronic levels of cythion (32). reported moderate focal necrosis, granular glycogen, nuclei pyknosis, loss of the architecture structure, onion-like cells in fish *Cyprinus carpio* due to presence of microcystins (33). Observed the cytoplasmic degeneration in hepatocytes, formation of vacuoles, rupture in blood vessels and appearance of blood cells among hepatocytes, formation of vacuoles, picnotic nuclei in the liver of *Oreochromis niloticus* exposed to aluminium. All the observed changes in the present investigation indicate the irreparable damage to vital organs of the fish exposed to sublethal and lethal concentrations of both pesticides making it less fit for better survival (34). Reported that mononuclear infiltration, neuronal degeneration, discoloration, severe spongiosis in African catfish *Clarias gariepinus* exposed to cypermethrin. Similar findings were also observed by in spotted murrel *Channa punctatus* and rainbow trout exposed to endosulfan. Swelling of pyramidal cells with binucleated nuclei, necrosis of neuronal cells of cerebrum indicates loss of nissal substances, vacuolar changes were observed in fish on exposure to Chlorpyriphos and *Datura stramonium* was reported (35, 36). Eosinophilic granule cells (EGC) in the meninges and cerebral cortex, degeneration of neuronal bodies, necrosis and apoptosis and glyosis observed by in Cachama blanca (*Piaractus brachypomus)*, alterations in brain tissue indicate the degenerative neuronal processes (37). Tongo Isioma and Ezemonye Lawrence reported that degeneration of dark stained Purkinje neurons, oedema, necrosis and vacuolar changes in the cerebrum in African toad *Bufo regularis* exposed to endosulfan (38). Profenofos and carbosulfan causes several injuries and damages to the brain, in the present study was more in lethal than in sublethal concentrations. Both toxicants inhibit the normal functioning of the body metabolism and behavioral changes, sensorial system like vision and smell, detection, attack and capture of prey, impair feeding, escape, and reproductive behavior all these functions are connected with various parts of the brain, all of them are altered due to AChE inhibition caused by these agrochemicals treated fish then leads to impaired neuronal dysfunction of central nervous system. Thus, when fish are exposed to pesticides, they suffer irreparable architectural changes in various vital organs marking the fish less fit for better survival. These histopathological changes can alter various physiological activities of the fish such as release of various enzymes and the metabolic processes.

4. CONCLUSION

Chief histopathological changes in the Gill, liver, kidney and brain were necrosis of gill epithelium, degeneration of pillar cells, haemopoietic tissue, cloudy swelling in renal tubules, cellular hypertrophy, cell necrosis, formation of vacuoles, disposition of hepatic cords, sinusoidal space, disposition of hepatic cords decrease in size of nucleus, intracellular oedema, lipid infiltration, disappearance hepatocyte wall and pycnotic nucleus was evident. The pathological changes noticed in the all the tissues might affect the physiological activity of the fish such as metabolic activities and enzymatic systems. This reduces the functional ability of liver, kidney and brain, which indirectly effects all metabolic.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration among all

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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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