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Histopathological alterations in the gill, liver and brain of *cyprinus carpio* on exposure to quinalphos

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Abstract: Study was conducted to assess the histopathological damage of Gill, Liver and Brain in common carp, *Cyprinus carpio* after sublethal exposure to Quinalphos. Exposed to sublethal concentration (One tenth (1/10th, 0.75 µl/L) of commercial grade quinalphos (25% Emulsified Concentration) for 1, 7, 14, 21 and 30 days and a parallel control was run simultaneously. Gill, Liver and Brain of exposed individuals exhibited some remarkable changes in their histology in comparison to control. Prominent changes include shrinkage of the glomerulus, and dilation of tubular lumen. Vacuolization, desquamation, hydropic swelling and hyaline degeneration of tubular epithelium is also observed. Cyst formation and hemorrhage also appear in certain specimens. Duration of exposure appears to have a profound effect on Gill, Liver and Brain as with increasing duration of exposure histopathological damages become more severe.

Keywords: Histopathology, *Cyprinus Carpio,* Quinalphos, Gill, Liver, Brain

1. Introduction

Pesticides have been widely used all over the world to control insects, pests and disease vectors. They ultimately find their way into aquatic habitats such as rivers, lakes and ponds, and have been found to be highly toxic not only to fish but also to the organisms, which constitute the food chain [1-3]. Moreover, Agriculture, as the largest consumer of freshwater and as a major cause of reduction of surface and groundwater resources through erosion and chemical runoff directly correlates with the loss of water quality [4-13]. Pesticides in general, are used very extensively in agriculture, forestry, public health and in veterinary practices. Hence, it is necessary to study the immediate and chronic effects of pesticides on fish, which form a part of human diet. These compounds have a tendency to accumulate in small quantities in lower fish food organisms and ultimately biomagnify in the fish species. A broad-spectrum organophosphate used heavily throughout the world for agriculture and domestic purposes [14]. The frequent occurrence of organophosphate pesticides has been regarded as a serious global public health problem and a major environmental issue. To a lesser extent, they can also absorb the toxins directly from the water [15, 16]. Therefore, it would be pertinent to study the effect of such organophosphate pesticides on long-term exposure by chronic studies to ascertain the residual toxicity.

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory [17, 18] and field studies [19-21]. One of the great advantages of using histopathological biomarkers in environmental monitoring

is that this category of biomarkers allows examining specific target organs, including gills and liver that are responsible for vital functions, such as respiration, excretion and accumulation and biotransformation of xenobiotics in the fish [22], and serve as warning signs of damage to animal health [23].

Quinalphos is one of the organophosphate insecticides extensively used in agriculture in our area. The Environment Centre of National Toxicology declared that, there are a dozen highly dangerous chemicals including quinalphos. Quinalphos is a hard insecticide, which has become a matter of concern because of its potentiality and hazardous effect. The present study was performed to evaluate the sub-lethal effects of quinalphos on histopathological alterations in the vital organs like brain, gill and liver of fresh water teleost (*Cyprinus Carpio)* as a laboratory animal model. The *Cyprinus Carpio* was selected for the bioassay experiments since it is one of the most economically important freshwater fish that is extensively cultured in India, China and other countries.

2. Materials and Methods

Fish were collected from ponds of local government hatchery with the help of fishermen. Fishes were caught by fishing net and carefully packaged into aerated polythene bags filled with tube well water. Fishes were brought to laboratory and immediately given 0.05% potassium permanganate treatment for two minutes for disinfecting them. After disinfectant treatment they were transferred into plastic pools of 500 L capacity for two weeks acclimatization to laboratory conditions. Fishes were starved for first 24 hr and then fed ad lib rice bran mixed with mustard oilcake in the ratio of 2:1, during acclimatization. Water of the pool was changed daily and dead fishes were removed immediately whenever located.

The experiment was conducted under natural photoperiod and temperature. Water quality was measured as per APHA [24]. The temperature of the experimental water was 23 ± 1.5º C, pH was 7.2 ± 0.4, Dissolved oxygen was 7.2 ± 0.6mgl^{-1}, and free carbon dioxide was 6.2 ± 0.4 mgl^{-1} and total hardness as calcium carbonate was 112 ± 3.2 mgl^{-1}.

For the histological study, 0.75µl/L of commercial grade quinalphos (25% Emulsified Concentration) was selected as sub lethal concentration. Common carp individuals of size, 17-22 cm, and weight, 50-65 gm was sorted and starved for 24 hr before starting the experiment. Six specimens were exposed to the sub lethal dose for 1, 7, 14, 21 and 30 days and a control was run simultaneously.

Fish were sacrificed at 1, 7, 14, 21 and 30 days of exposure. Fish were first immobilized in ice and then dissected out carefully; Gill, Liver and Brain were removed and fixed in bouins fluid for 24 hr and then processed and embedded in paraffin for block preparation. The sections were about 5-6 micron and stained with haematoxylin and eosin. The slides were examined under a light microscope and photographed for histopathological effects.

3. Results

3.1. Gill

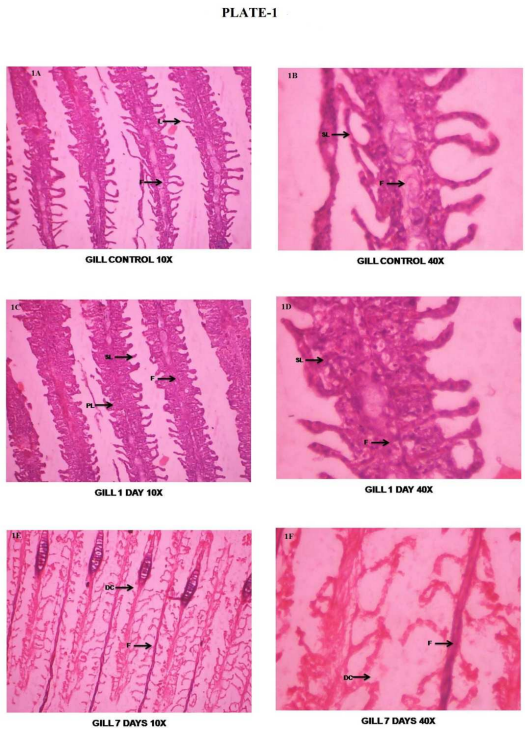

Plate 1, Figure 1. *A (10 X) & B (40 X) Control fish gill, C (10 X) & D (40 X) experimental fish gill 1st day, E (10 X) & F (40 X) experimental fish gill 7 days, showing Lamellae (L), Filament (F), Secondary Lamella (SL), Primary Lamella (PL) and Degenerative Changes (DC).*

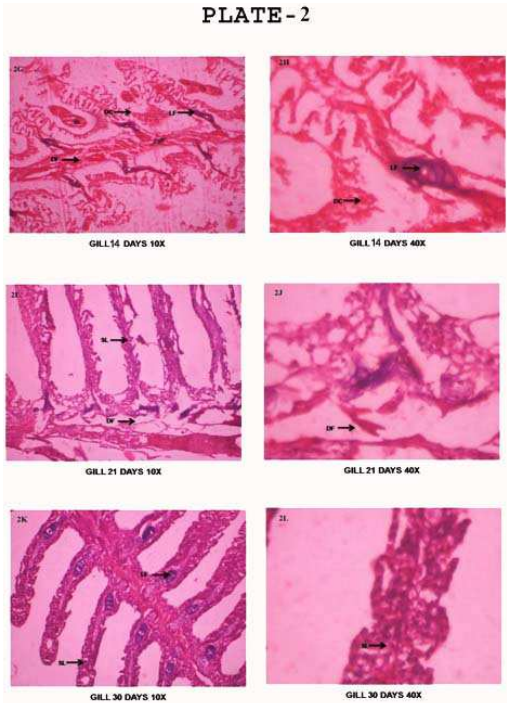

Plate 2, Figure 2. *G (10 X) & H (40 X) experimental fish gill 14 days, I (10 X) & J (40 X) experimental fish gill 21 days, K (10 X) & L (40 X) experimental fish gill 30 days, showing Damage of Filament (DF), Degenerative Changes (DC), Lamellar Fusion (LF) and Secondary Lamella (SL).*

The structure of the gill of control fish consists of primary lamellae, secondary lamellae with well marked inter lamellar spaces. The gill lamella consists of respiratory epithelial cells and pilaster cells situated in between the blood capillaries. An exposure of the fish for a period of 1 day to sub lethal concentration of quinalphos showed that there were no significant pathological changes in the gills of the fish (Plate-1, Fig.1c and1d). On exposure for a period of 7 days has shown some degenerative changes and lamellar fusion (Plate-1, Fig. 1e and 1f). On exposure for a period of 14 days a further damage was occurring in the gill structure. The primary and secondary gill lamellae showed heavy degenerative changes and lamellar fusion (Plate. 1, Fig.1c and 1d). Additional exposure for a period of 21days less degenerative changes were observed in the structure of the gills appeared with mild degree of degenerative changes and lamellar fusion (Plate. 2, Fig.2i and 2j). Exposure for a period of 30 days has shown very less degenerative changes (Plate. 2, Fig. 2k and 2l).

3.2. Liver

The structure of the normal liver of the fish consists of a continuous mass of large hexagonal cells. The hepatocytes are large in size with homogenous granular cytoplasm and either centrally located distinct nuclei. Each cord separated by the thick wall of the peripheral cells (Plate. 3, Fig. 3a and 3b). No significant changes were observed in the structure of the liver of the fish exposed for a period of 1 day to the quinolphos (Plate. 3, Fig. 3c and 3d). However, in the fish after 7 days of exposure has shown necrotic changes (Plate. 3, Fig. 3e and 3f). An exposure of 14 days witnessed hepatocytes with widespread vacuoles, clear necrotic changes and picnotic nuclei of cells (Plate. 4, Fig. 4g and 4h). Exposure for a period of 21 days resulted in degenerative hepatocytes, changes in central vein with congestion (Plate. 4, Fig. 4i and 4j). Only the degenerative changes were witnessed after 30 days of exposure.

PLATE-3

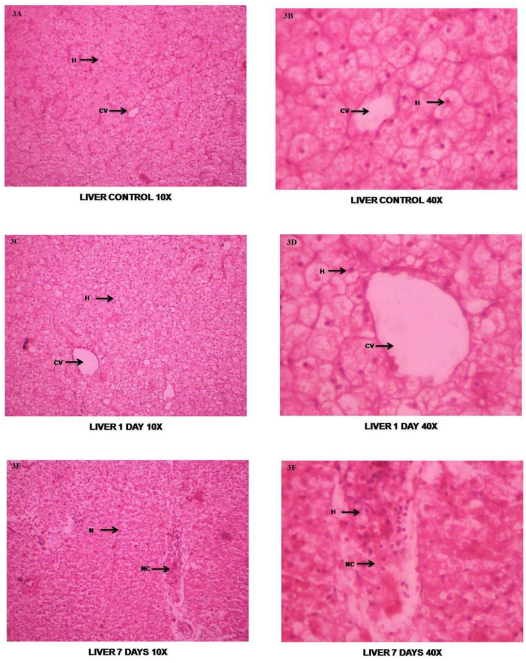

Plate 3, Figure 3. *A (10 X) & B (40 X) Control fish liver, C (10 X) & D (40 X) experimental fish liver 1st day, E (10 X) & F (40 X) experimental fish liver 7 days, showing Hepatocytes (H), Central Vein (CV) and Necrotic Changes (NC).*

PLATE-4

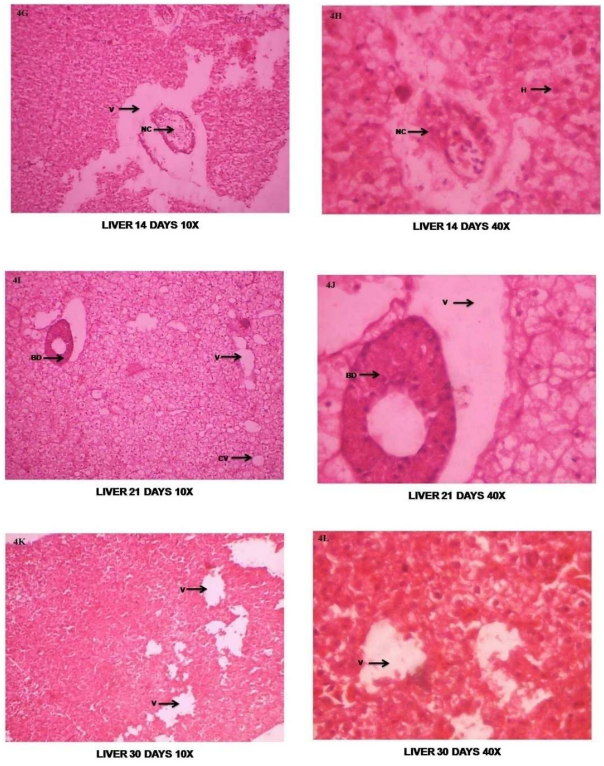

Plate 4, Figure 4. *G (10 X) & H (40 X) experimental fish liver 14 days, I (10 X) & J (40 X) experimental fish liver 21 days, K (10 X) & L (40 X) experimental fish liver 30 days, showing Hepatocytes (H), Vacuolization (V), Necrotic Changes (NC) and Bile Duct (BD)*

3.3. Brain

The architecture of brain in the control fish showed clear neural cells with distinct nuclei (Plate. 5, Fig. 5a and 5b). On 1st day of exposure to sub lethal toxicity of quinalphos showed mild degenerative changes in neural cells when compared to control. After 7 days of exposure period the brain tissue has shown structural damage, necrotic changes in neural cells and intracellular edema (Plate. 5, Fig. 5e and 5f). On exposure of 14 days more degenerative changes, increased necrotic condition of neural cells and cytoplasmic vacuolization observed (Plate. 6, Fig. 6g and 6h). From day 21 to day 30 showed slight degenerative changes and vacuolization (Plate. 6, Fig. 6k and 6l) when compared to 14 day exposure period.

PLATE - 5

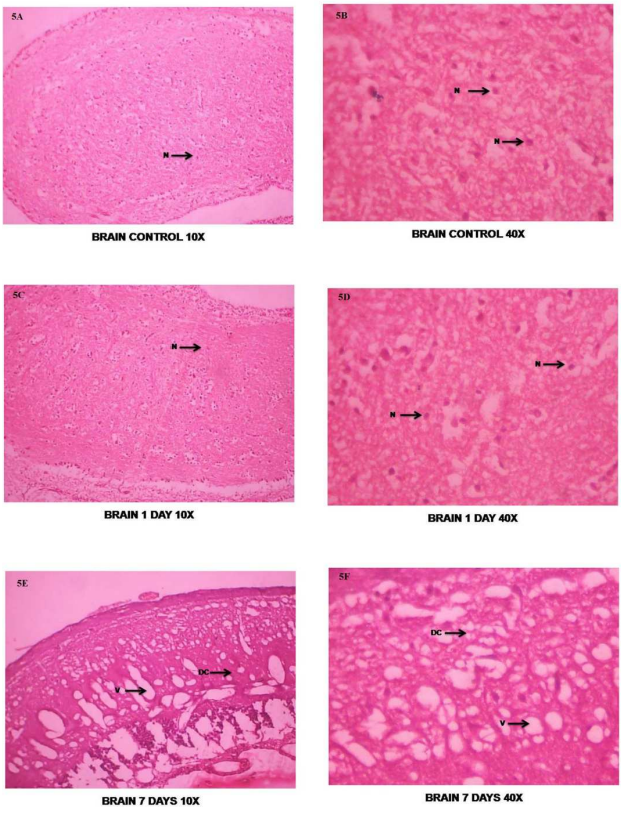

Plate 5, Figure 5. *A (10 X) & B (40 X) Control fish brain, C (10 X) & D (40 X) experimental fish brain 1st day, E (10 X) & F (40 X) experimental fish brain 7 days, showing Nucleus (N), Vacoulation (V) and Degenerative Changes (DC).*

PLATE - 6

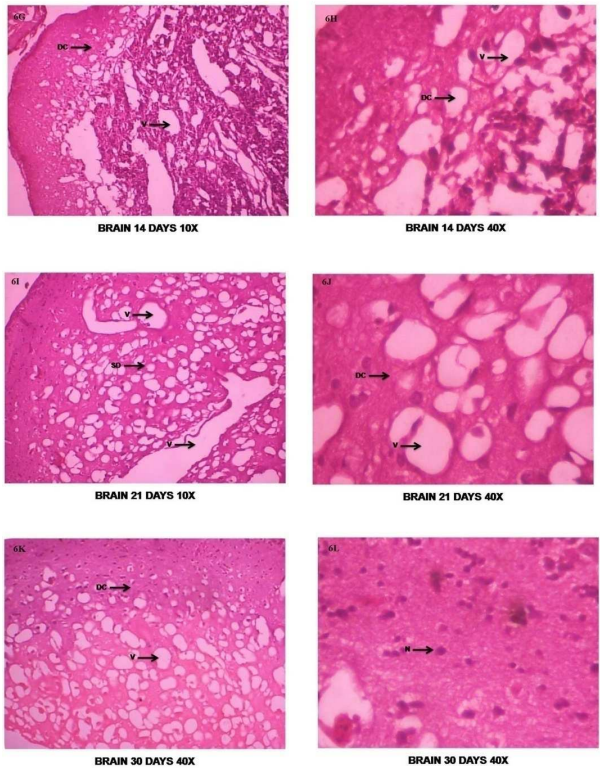

Plate 6, Figure 6. *G (10 X) & H (40 X) experimental fish brain 14 days, I (10 X) & J (40 X) experimental fish brain 21 days, K (10 X) & L (40 X) experimental fish brain 30 days, showing Nucleus (N), Vacoulation (V) and Degenerative Changes (DC).*

4. Discussion

Tissue histology is considered as an indicator of exposure to pollutants, represents a useful tool to assess the degree of pollution, particularly for sublethal and chronic effects [25]. Histopathological changes in the present study were in agreement with the study recorded in the liver of fish (*O. niloticus* L.) treated with lorsban and vitamin E + lorsban for four consecutive weeks. Hepatosomatic index of the vitamin E treated group were insignificant throughout the experimental periods. Few studies recorded that, the hepatosomatic index was not affected by the dietary α-tocopherol levels. The common liver abnormalities observed in the present study were loss of parenchymal architecture, fatty degeneration, vacuolar degeneration, atrophy and necrosis of hepatic and pancreatic cells with leucocytic infiltration. The recorded results in the present study were similar to those observed by Tilak *et al.*, and Kunjamma *et al.,* [26, 27] recorded pyknotic nucleus, protein precipitation, pancreatic acini appeared with the loss normal structure and necrosis of the hepatic and pancreatic tissue in freshwater fish (*Catla catla*) and (*Oreochromis mossambicus*) treated with chlorpyrifos. The present results were more or less in agreement with other studies in which necrosis and lipidosis vacuolization, an increase of macrophage aggregates and eosinophilic

granular cells were recorded in fish treated with insecticides malathion and paraquat, respectively [28, 29]. Changes in the liver were time and concentration dependent.

Histological changes in the liver could be attributed to the fact that, the liver is the major site of detoxification [30], it is expected that the toxicant insecticide would reach there in abundance for detoxification and disposal [31]. Fatty vacuolar degenerations of hepatic tissues could reflect abnormal lipid metabolism (e.g., lipid peroxidation), since lipid accumulation was prominently observed in the present study, it might be an indication of earlier liver damage [32]. While, [33] recorded that, accumulation of the lipid droplets in the hepatocyte cytoplasm could be considered as a defense mechanism. In the present study, the appearance of pycnotic nuclei indicated that the cells became hypofunctional. Focal necrosis were also observed in the liver of the fishes *Heteropneustes fossilis* and *Brachydanio were* exposed to organophosphate insecticide malathion and dimethoate 500, respectively [34]. Focal necrosis is probably due to the involvement of liver cells in the metabolic transformation of the insecticide, causing functional and structural changes to the cells [35]. Morphological changes in the liver supported many various biochemical reactions as indicated by enzyme activities [36].

Brain is the controlling center of all functions and movements in the body organisms like fish serving as a relay station. In the present study hyperplasia, edema, necrosis and an increase in brain cells were some of the histological changes observed in the brain of the fish *Cyprinus carpio* exposed to sub lethal concentration of quinolphos toxicity. These changes could be related to possible inhibition or decreased cholinergic activity on exposure to quinalphos. Since, quinalphos is a potent neurotoxic agent which inhibits acetyl cholinesterase activity of brain [37]. Similarly, membrane bound K^{1+}, Mg^{1+}ATPase and Ca^{2+}ATPase activities have been decreased in dose and time depend manner in the brain regions (hen mesencephalon, cerebellum and medulla oblongata) of the fish *Oreochromis mocsombicus* [38], which supports the histological changes observed in the brain of *Cyprinus Carpio* in the present study.

Thus the histological changes that were taking place in the present study, as the initial period of exposure in the organs of the fish on exposure to quinolphos toxicity might be a part of defense mechanism. The further accumulation of quinolphos in the organs of the fish on prolonged exposure caused destruction in the organ structures. The slight structural reorganization of the gill, liver, and brain of the fish observed at day 30 of exposure to quinolphos toxicity gives support to some extent that the ability of the fish to resist the sublethal stress and in the repair of the damage caused to the organ by enhancing the protein synthetic potentials and other associated activities of the cell.

Conflict of Interest

The authors declare that we have no conflict of interest.

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