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Histopathology as a Tool for Assessing the Extent of Toxicity of Quinalphos in Fish

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***Abstract*: Study was conducted to assess the histopathological damage induced by the organophosphate pesticide quinalphos on liver and brain in *Anabas testudineus* and *Oreochromis mossambicus*. In this study the fishes were exposed to a sub lethal concentration of pesticide for 30 days and examine the damage induced by the pesticide. Due to the effect of pesticide, changes occurs in the liver and brain of fishes. In liver, loss of normal architecture of parenchyma and hepatic cells were observed. Degeneration of neurons and dorsal and ventral olfactory area, blood streaks, vacuolation were observed in brain. Lesions in the liver leads to delayed function and also affect the central nervous system as a result of brain damage. This happens due to the neurotoxicity of pesticides. Which alters the physiology and normal health of fish. Increasing duration of exposure leads to, histopathological damage become more severe.**

***Keywords*: Histopathology, Quinalphos, Melanomacrophage centers.**

1. Introduction

Pesticides are substances meant for attracting, seducing, destroying, or mitigating any pest. Many pesticides can be grouped into chemical families. Prominent insecticide families include organochlorines, organophosphates, and carbamates. Pesticide leach out and contamination is the one of the factor which contribute to water pollution. Water pollution is the major crisis which affect both aquatic and terrestrial biota. Organophosphates (Ops) and organochlorides compounds widely used in Kerala to protect the crop plants from damage. OPs were first recognised in 1854, but their general toxicity was not established until the 1930s. Organophosphate or phosphoester is the general name for esters of phosphoric acid. Many Organophosphates are potent nerve agents, functioning by inhibiting the action of acetylcholinesterase (AChE) in nerve cells. Other effects of organophosphates are immunotoxicity, oxidative damage, inhibition of serine hydrolases or esterase etc. Quinalphos is an organo thio phosphate chemical pesticide used as an insecticide and acricide. It also produces ecotoxicity. Toxic effects mainly observed in amphibians, crustacean s, echinoderms, molluscs etc. Histopathology refers to the microscopic examination of tissue in order to study the manifestations of disease. Histopathology as a biomarker of pollutant stress. Histopathological biomarkers are useful indicators of the general health of the fish and are considered as a mirror that reflects exposure to a variety of anthropogenic pollutants. *Anabas testudienus* and *Oreochromis mossambicus* selected for the study as animal models. The present study analyzed histopathological damages caused by Quinalphos in the liver and brain of fishes. Liver is the detoxifying organ of animal body. Liver cells lost their caudal arrangement. Quinalphos is neurotoxic.it induces changes in the central nervous system of vertebrates. Quinalphos is similar to endosulfan, both are causes brain damage. This study reveals quinalphos induces abnormalities in vital organs of vertebrates especially liver and central nervous system.

2. Materials and Methods

Vertebrate models *Anabas testudienus* and *Oreochromis mossambicus* were used as experimental animals. After 60 days, fishes with normal health and similar weights selected for experiment purpose. The pesticide used in this experiment is quinalphos. LC 50 value calculated and was 0.022ppm for *Anabas testudineus* and 0.025 *for Oreochromis mossambicus*. The selected fishes were divided in to two groups and kept in separate aquarium tanks. Group 1 served as control and were kept in normal dechlorinated tap water and group 2 served as experimental group, exposed with sub lethal concentration of quinalphos (0.001ppm) for a period of 30 days.

After 30 days fishes catched from the tanks and anesthetized with tricaine methane sulphonate (MS-222) and placed on dissection board covered with a cotton towel. Dissected the fish liver and brain were preserved in 10% formaldehyde solution.

After the fixation of liver and brain follow the preparations of histological sections. Tissues were subjected to histopathological procedures using haematoxylin eosin as counter stain as per standard protocols. The first step of histopathological procedure is tissue fixation, most specimens are fixed in 10% formaldehyde to prevent tissue autolysis and putrefaction.it is the crucial step in tissue preparation. After the fixation, specimens are transferred to cassettes. Specimens are trimmed using a scalpel to enable them to fit in to an appropriately labelled tissue cassette. Next step is the tissue processing. Processing tissues in to thin microscopic sections is usually done using a paraffin block. Processing involves mainly 3 stages.

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They are dehydration, clearing, embedding. In dehydration, remove the formalin and water from the tissue. Xylene is an organic solvent and used for clearing process to remove alcohol and allow infiltration with paraffin wax. After clearing specimens are subjected to embedding. It is the final step of processing. Embedding provide tissue becomes surrounded by a large block of molten paraffin wax. It called as 'block '. Block is a support matrix that allows very thin sectioning. Next step is the sectioning. In sectioning, tissue specimens is to be cut into sections that can be placed in a slide. Microtome (Leica RM2125 RTS) is used for sectioning. Most tissues are cut at around 5 µm. After sectioning, followed the final stage is staining. Histochemical stains typically haematoxylin and eosin are used for staining. Staining provide contrast to tissue sections, making tissue structures more visible and easier to evaluate.

3. Result

Anabas testudineus and *Oreochromis mossambicus* were used as experimental models, exposed to quinalphos at a sub lethal concentration 0.001ppm for 30 days. The histopathological analysis showed considerable degree of alteration in the histoanatomy of liver and brain. These alterations were profound and the degree of changes in histoanatomy showed significant variation after exposure.

The LC 50 values of *Anabas testudineus* found to 0.002ppm and *Oreochromis mossambicus* is 0.025ppm were exposed to a sub lethal concentration (0.001ppm) of pesticide quinalphos for 30 days. Quinalphos exposures have induced discrete pathological changes in the liver tissue of the fish Anabas *testudineus* and *Oreochromis mossambicus*.

These changes include degenerated hepatoparenchyma (HP Plate 3 Fig. A1 Anabas), congestion of sinusoids (SC Plate 4 Fig. B3 Tilapia), formation of macrovacuolation (MV Plate 4 Fig. B2 Tilapia & Plate 3 Fig. A2 Anabas), and disappearance of hepatocytes cell wall, disposition and degeneration of hepatic cells (HD Plate 3 Fig. A2 Anabas, Plate 4 Fig. B1, B2, B3 Tilapia). One of the most observable changes are melanomacrophage centres (MMC Plate3 Fig. A1 & A3 Anabas). It also known as macrophage aggregates. They are distinctive groupings of pigment – containing cells within the heterothermous vertebrates. Formation of fibrosis (F Plate 3 Fig. A3 Anabas) is a characteristic feature in Anabas. Feathery degeneration (FD Plate 4 Fig. B1 & B3 Tilapia) occurs in Oreochromis. Other damages are picnotic nucleus (PN Plate 4 Fig. B1 & B2 Tilapia). Fish liver histology could therefore serve as a model for studying the interactions between environmental factors and hepatic structure and functions. It's revealed that the extent of quinalphos concentration causes cytoplasmic vacuolation by means of macro vacuolation, cellular degeneration, damage of nuclei, and congestion in the blood sinusoids.

The organ most associated with the detoxification and biotransformation process is liver, and due to its function, position and blood supply. Histopathological alterations in brain include degenerated dorsal olfactory area (DDOA Plate 1 Fig. A3 Anabas & Plate 2 B1 Tilapia), degenerated septal area (DSA Plate1 Fig. A3 Anabas & Plate 2 Fig. B2 Tilapia), complete degeneration of neurons (DN Plate 1 Fig. A1, A2 Anabas & Plate 2 Fig. B3 Tilapia), degenerated ventral area (DVA Plate1 Fig. A3 Anabas & Plate 2 Fig. B1 Tilapia), vacuolation, Blood Streaks (BS Plate 2 Fig. B2 Tilapia), congestion of medulla oblongata, blood cells damaged (BCD Plate 2 Fig. B2, B3 Tilapia & Plate 1 Fig. A1 Anabas). As Brain is the controlling and coordinating all body functions and behavioural aspects, any damage even insignificant will lead to alterations in the overall body functions.

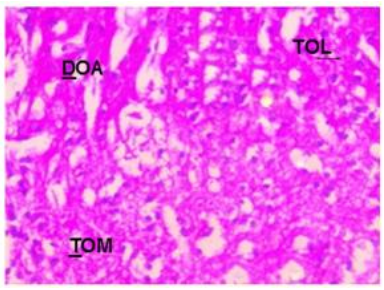


Fig. A. Control

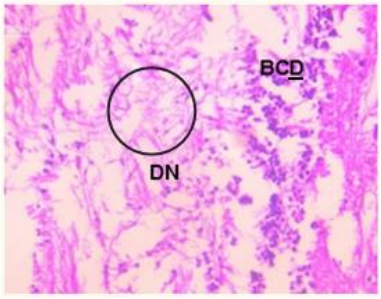


Fig. A1. Treated

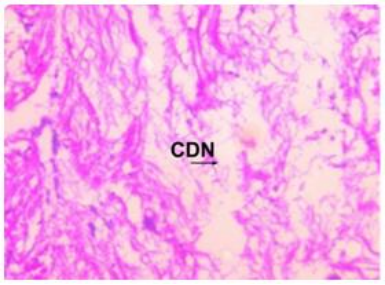


Fig. A2. Treated

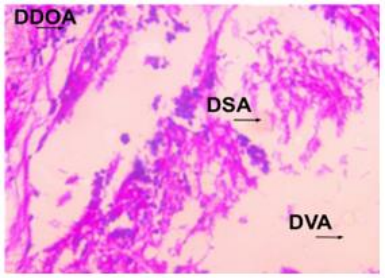


Fig. A3. Treated

Fig. 1. Histopathology of anabas brain- showing damage – Plate 1

Plate 1 Fig. A Brain (control):

DOA – Dorsal Olfactory area, TOL –Tractus olfactorius

lateralis, TOM - Tractus olfactorius medialis.

Plate 1 Fig. A1 Brain (treated):

DN – Degenerated neurons, BCD – Blood cells damage.

Plate 1 Fig. A2 Brain (treated):

CDN - Complete degeneration of neurons.

Plate1 Fig. A3 Brain (treated):

DDOA – Degenerated dorsal olfactory area, DSA – Degenerated septal area, DVOA – Degenerated ventral olfactory area.

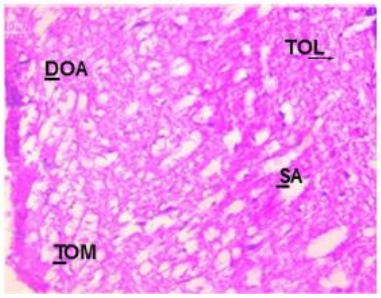


Fig. B. Control

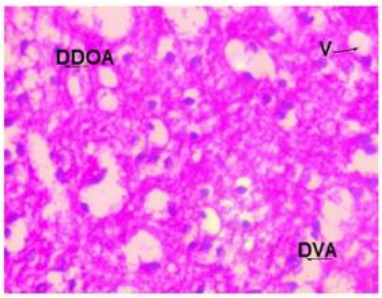
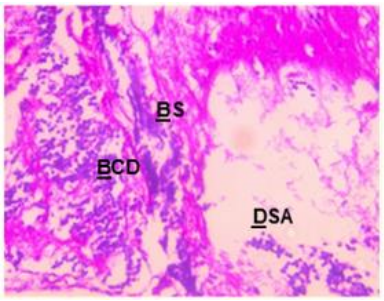


Fig. B1. Treated



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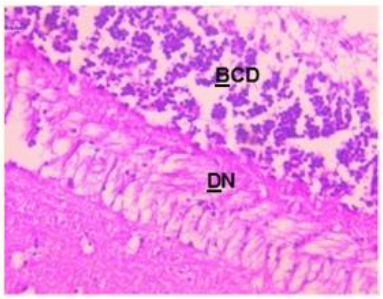


Fig. B3. Treated

Fig. 2. Histopatholog of tilapia brain-showing damage-Plate 2

Plate 2 Fig. B Brain (control):

DOA – Degenerated olfactory area, TOL – Tractus olfactorius lateralis, TOM – Tractus olfactorius medialis, SA – Septal area.

Plate 2 Fig. B1 Brain (treated):

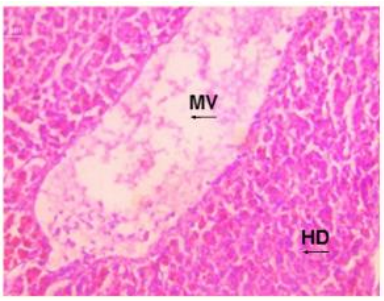
DDOA – Degenerated dorsal olfactory area, DVA – Degenerated ventral area, V – Vacuolation.

Plate 2 Fig. B2 Brain (treated):

BS – Blood streaks, DSA – Degenerated septal area, BCD – Blood cells damage.

Plate 2 Fig. B3 Brain (treated):

DN – Degenerated neurons, BCD – Blood cells damage.



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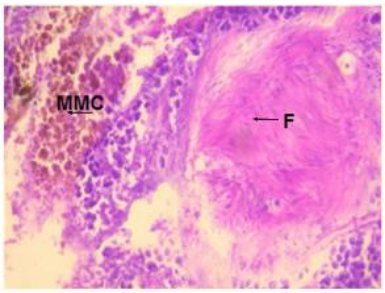

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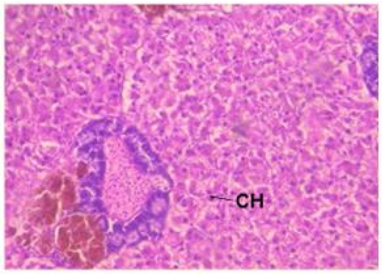

Fig. A. Control

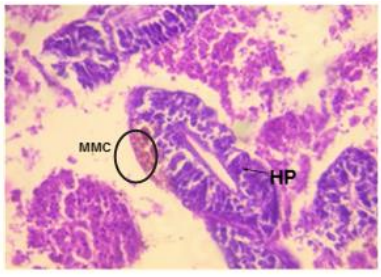

Fig. A1. Treated

Fig. 3. Histopathology anabas liver- showing damage-Plate 3

Plate 3 Fig. A Liver (control):

CH –Caudal arrangement of hepatic cells.

Plate 3 Fig. A1 Liver (treated):

MMC – Melan macrophage centre, HP – Hepatoparenchyma

Plate 3 Fig. A2 Liver (treated):

MV – Macrovacuolation, HD – Hepatic cells degenerated.

Plate 3 Fig. A3 Liver (treated):

MMC – Melano macrophage centre, F – Fibrosis

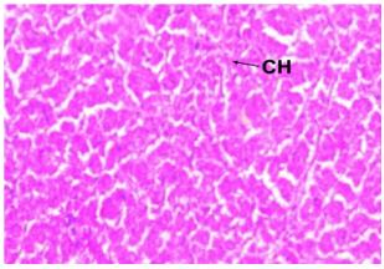

Fig. B. Control

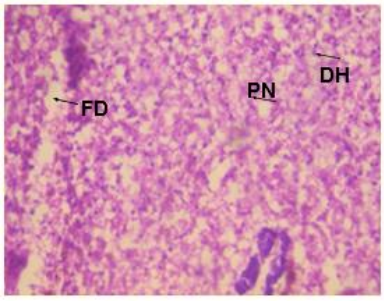

Fig. B1. Treated

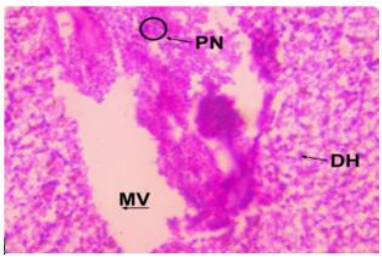

Fig. B2. Treated

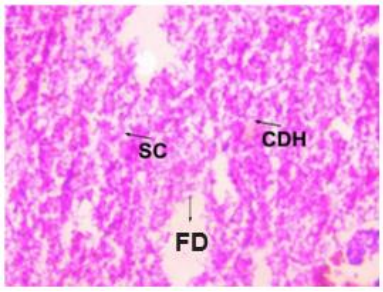

Fig. B3. Treated

Plate 4 Fig. B Liver (control):

CH – Caudal arrangement of hepatic cells

Plate 4 Fig. B1 Liver (treated):

FD- Feathery degeneration, PN- Picnotic nucleus, DH- Degenerated hepatic cells.

Plate 4 Fig. B2 Liver (treated):

PN – Picnotic nucleus, MV- Macrovacuolation, DH- Degenerated hepatic cells.

Plate 4 Fig. B3 Liver (treated):

SC – Sinusoid congestion, CDH – Complete degeneration of hepatic cells, FD – Feathery degeneration.

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 sub lethal and chronic effects. The common liver abnormalities observed in the present study were loss of parenchymal architecture, Macrovacuolatisation, presence of picnotic nuclei, melanomacrophage centers, fibrosis, and congestion in sinusoids, blood streaks among hepatic cell, feathery degeneration, and loss of caudal arrangement of hepatic cells. The similar observation were found in *Cyprinus carpio* due to the quinalphos (25% emulsified concentration) [14]. And also, they found that the necrotic changes, bile ducts in liver, fatty degeneration, atrophy and pancreatic cells with leucocyte infiltration. The recorded results in the present study were similar to those observed by Tilak et *al* and Kunjamma *et al* [19], [9] recorded picnotic 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 [14] granular cells were recorded in fish treated with insecticides malathion and paraquat, respectively. Brain is the controlling centre of all functions and movements in the body organisms like fish serving as a relay station. In the present study degenerated dorsal olfactory area, formation of blood streaks, degenerated septal area, degenerated ventral area, degeneration of neurons, were some of the histological changes observed in the brain of the fish *Anabas testudineus* and *Oreochromis mossambicus* exposed to sub lethal concentration of quinalphos 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.

The results of the present study are similar to the toxicity of Quinalphos 25% EC exposure induced pathological changes in the brain of exposed fish *channa punctatus.* In atrophy, necrosis, pycnosis, dissolution of the nissel bodies, swelling of the axon and demyelination or vacuolisation of the myelin sheath of nerve fibres also observed, since quinalphos is an organophosphate compound and organophosphates are neuropoisons, the quinalphos intoxication caused atrophy, chromatolysis i.e., dissolution of the nissel bodies and loss of stainable substances within the cytoplasm. Congestion in the medulla oblongata, which in turn causes abnormalities in the blood circulation [13].

The results from the present study suggest that the histopathological lesions observed in the test organisms due to exposure to quinalphos. Even sub lethal concentration of quinalphos induced great extent of damage to the brain, which may alters the physiological and behavioural functions of the fish.

5. Conclusion

The study reveals the extent of toxicity in *Anabas testudineus* and *Oreochromis mossambicus*. Pesticide use is one of many factors contributing to the decline of fish and other aquatic species. If pesticides are selected wisely, used in combination with other pest control measures, and applied safely, the pollution of our surface waters and contamination of aquatic life can be minimized. Quinalphos is organophosphate pesticide. It is a neuropoison. It has been reported that the effects of quinalphos is similar to endosulfan. So, the both pesticides are banned due to their harmful effects, but it is commonly and widely used without any regulations. This compound can adversely affect terrestrial and aquatic life. It is suggested that pollution is the social crisis and pesticide contamination is the main reason of pollution. It is better to avoid the pesticide in order to protect the environment and biodiversity there by maintaining equilibrium in the nature.

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