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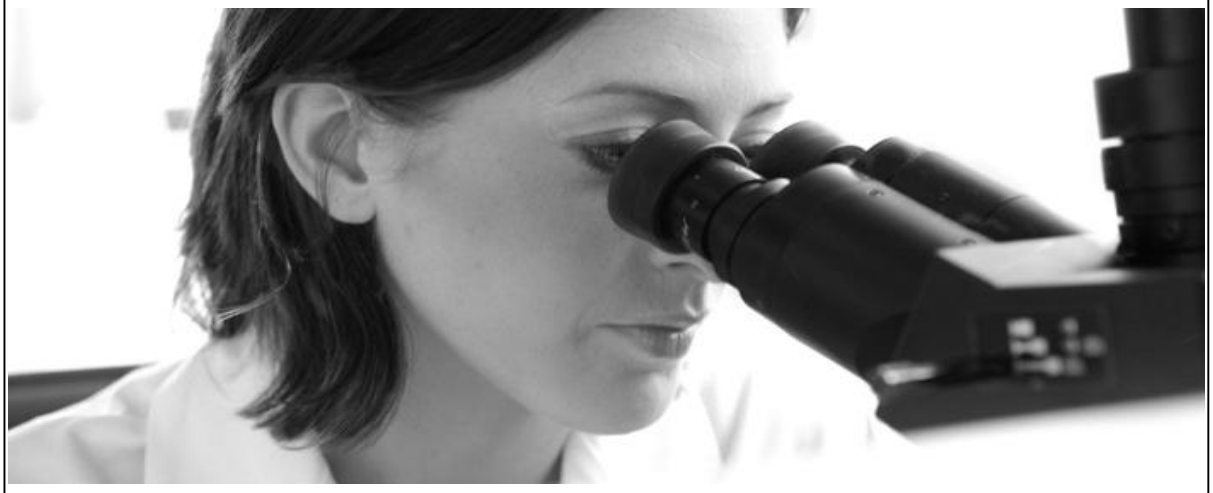

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RESEARCH ARTICLE

HISTOPATHOLOGICAL ALTERATIONS ON GILL, LIVER, KIDNEY AND SPLEEN OF CICHLID FISH, *ETROPLUS MACULATUS* EXPOSED TO CHLORPYRIFOS

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ABSTRACT

Acute toxicity of chlorpyrifos at 3, 4, 5, 6, 7, 8 and 9 µg/ L concentrations in *Etroplus maculatus* was investigated on different tissues for 96 h and the weights of organs exposed to the toxicant showed a significant decrease above 6 µg/ L concentrations when compared to control groups. Histopathological alterations on gill, liver, kidney and spleen were examined and compared with the control groups. Marked histological changes observed in gill were absence of secondary lamellae, necrosis, epithelial lifting, and aneurism. Hepatocytes showed necrosis, vacuolization, anucleated cells and eosinophilic materials in the cytoplasm. Leisons in kidney were necrosis as well as dialation of renal tubules and pyknotic nucleus whereas necrosis, anucleated cells, large numbers of melanomacrophage centers and vacuolization were prominent in spleen tissue. These findings represent the negative impact of chlorpyrifos on cichlid fish, *Etroplus maculatus*.

INTRODUCTION

Pesticides are the most toxic substances that are released intentionally into the environment to destroy certain forms of plant or animal life that are considered to be pests. Over 95% of pesticides that are released into the environment are known to reach non-target species. Run-off from lands is known to carry pesticides into the aquatic environment potentially affecting aquatic life, particularly fish. Exposure of fish to pesticide depends on its bioavailability, bioconcentration, biomagnification, and persistence in the environment. Bioavailability refers to the amount of pesticide in the environment available to fish where some pesticides rapidly breakdown after application while others bind tightly to soil particles suspended in the water column or to stream bottoms, thereby reducing their access. Some fish possess more bioconcentration in the body by concentrating pesticides in the tissues and organs, especially fats at levels 10 million times greater than in the water. However, biomagnification is another factor in which the accumulation of pesticides at each successive level of the food chain increases (Waynon and Finley, 1980).

Chlorpyrifos (O, O-Diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate) belongs to a group of broad-spectrum organophosphorus insecticide widely used in agriculture for control of insect pest on crops such as citrus, alfalfa, wine grapes, nut orchards, pineapple, tomato, maize, tobacco etc. Due to repeated application of chlorpyrifos to control pests, large quantities are being exposed to aquatic ecosystem. The main pathways by which they enter into the water bodies include careless handling, accidental spillage or discharge of untreated effluents into natural water ways that have harmful effects on fish population and also contribute to long term effects in the environment (Carter and Graves, 1973). Fish provides 16% of high quality protein, is a vital and primary source of food to human. Therefore, health status of fish provides an ecological impact of any toxicant in the aquatic ecosystem. Health of fish can be determined by several ways, among which histopathological observations are considered as a valuable tool for toxicological studies and monitoring water pollutions.

Histopathological techniques are rapid, sensitive, reliable, comparatively inexpensive tool for studying the stress response of toxicants and it is a proven document to assess the architecture of tissues or organs. Therefore, the present study was aimed to evaluate the acute toxicity effects of chlorpyrifos on histopathological alterations in different tissues of freshwater cichlid fish, *Etroplus maculatus* for 96h.

MATERIALS AND METHODS

Etroplus maculates

Etroplus maculatus, freshwater cichlid fish weighing 3.5 ± 0.5 g and length 6 ± 0.3 cm collected from local fish farm near Parappanangadi, Malappuram district, Kerala, India, were

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adjusted to the laboratory conditions for 15 days before experiment. Fish were fed with standard fish pellets during acclimatization and at the time of experiment, and are maintained in large cement tank containing dechlorinated water and well aeration. The physiochemical features of the tap water were analysed by maintaining water temperature at 28 ± 2°C, dissolved oxygen at 8.5 and pH at 7.6 according to the method as described in APHA (1998).

Grouping of test animals

Fishes were randomly selected and transferred into 40 L cement tanks and nine groups were maintained each group with ten animals. It consisted of two control groups – positive (propylene glycol as vehicle solvent) and negative control, and 7 treatment groups with different concentrations, i.e 3, 4, 5, 6, 7, 8 and 9 µg/ L of chlorpyrifos for 96 h as exposure period. The median lethal concentration (LC_{50}-96 h) of chlorpyrifos was estimated as 6.61 µg/ L concentration (Raibeemol and Chitra, 2015).

Collection of tissues

Tissues such as gill, liver, kidney and spleen were isolated immediately after the end of mortality observed from every group. Weight of the organs was recorded and tissues were stored in 10% buffered formalin for 24 to 48 h for histopathological examination.

Histopathological analysis

Tissues were dehydrated in ascending grades of alcohol and were cleared in xylene until they became translucent. Tissues were then transferred to molten paraffin wax for 1hour to remove xylene completely and impregnated with wax. Then the blocks were cut and sections of thickness 4 to 6 microns were prepared using rotary microtome. The sections were stained with haematoxylin and eosin and mounted in DPX (Roberts and Smail, 2001). The slides were examined and photomicrographs were taken using Cannon shot camera fitted to the Carl Zeiss Axioscope 2 Plus Trinocular Research Microscope.

Statistical analyses

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test using statistical package SPSS 19.0.

RESULTS

Organ weights

Chlorpyrifos exposure above 6 µg/ L concentrations showed a significant ($p<0.05$) reduction in the weights of tissues as gill, liver, kidney and spleen when compared to control groups (Table 1). Test groups at 3 and 4 µg/ L concentrations did not showed any lethality whereas at 5 µg/ L concentration showed mortality of one animal at the end of 96 h, therefore the mean values observed was for 9 animals. Chlorpyrifos at 6 µg/ L killed four animals among the ten exposed at the end of 96 h, accordingly the mean values were presented for 6 animals in the given table. Toxicant at 7µg/ L showed mortality of six animals after 65 h where 'n' values represented in this group is for four animals and concentrations of 8 µg/ L and 9µg/ L showed 100% mortality at 26 and 24 h, respectively, therefore, the organ weights are not presented in the Table. Differences in the organ weights were considered to be significant at $p<0.05$ against control groups using ANOVA and data are presented as mean ± SD in each group.

Histological analysis

Histopathological observations revealed alterations in various tissues as gill, liver, kidney and spleen of fish exposed to chlorpyrifos at concentrations above 6 µg/ L. In the present study gill, liver, kidney and spleen tissues of both control groups showed normal architecture (Figure 1 and 2).

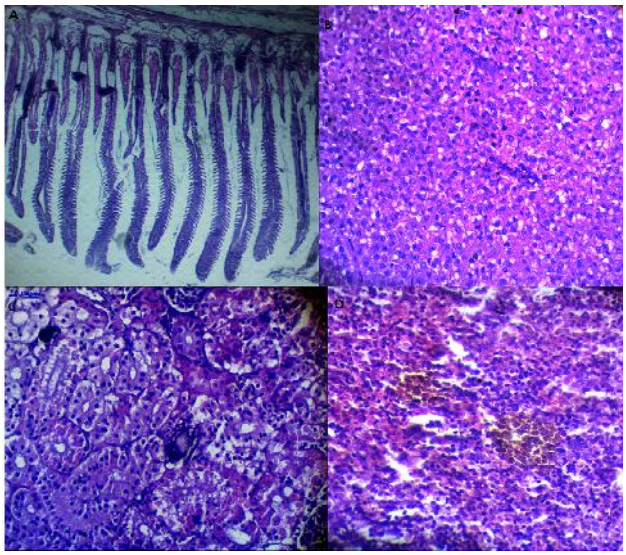

Figure 1 Photomicrograph of Negative control tissues A-Gill, B-Liver, C-Kidney, D- Spleen at X 400 magnification showing normal architecture

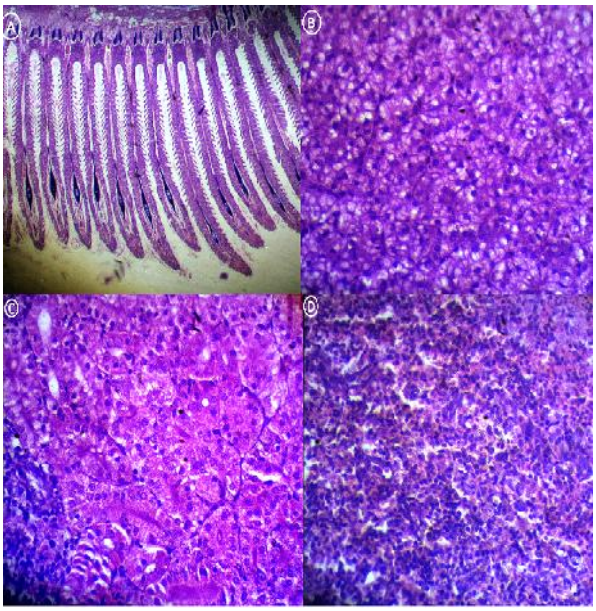

Figure 2 Photomicrograph of Positive control tissues (propylene glycol) A-Gill, B-Liver, C-Kidney, D- Spleen at X 400 magnification showing normal architecture

Table 1 Weights of gill, liver, kidney and spleen of *Etroplus maculatus* exposed to chlorpyrifos

Weights of tissues (g)	Negative Control	Positive Control (Vehicle)	Chlorpyrifos (µg/ L)				
			3 (96 h)	4 (96 h)	5 (96 h)	6 (96 h)	7 (65 h)
Gill	126.2 ± 6.5	125.1 ± 3.5	122.2 ± 7	122.3 ± 3.05	120.12 ± 5.5	104.2 ± 6.9*	109.2 ± 3.4*
Liver	56.85 ± 7.24	60.18 ± 6.16	56.2 ± 5.97	55.7 ± 2.95	47.43 ± 3.78	48.24 ± 3.01*	36.7 ± 6.76*
Kidney	6.58 ± 1.14	6.57 ± 0.54	6.5 ± 0.42	6.2 ± 0.31*	6.31 ± 1.12	6.29 ± 0.18*	6.07 ± 0.53*
Spleen	1.79 ± 0.59	1.87 ± 0.46	1.67 ± 0.06	1.85 ± 0.26	1.7 ± 0.41	1.65 ± 0.43*	1.51 ± 0.18*

Gill

Fish when treated above 6 µg/ L concentrations showed absence of secondary lamellae, necrosis, epithelial lifting, and aneurism (Figure 3).

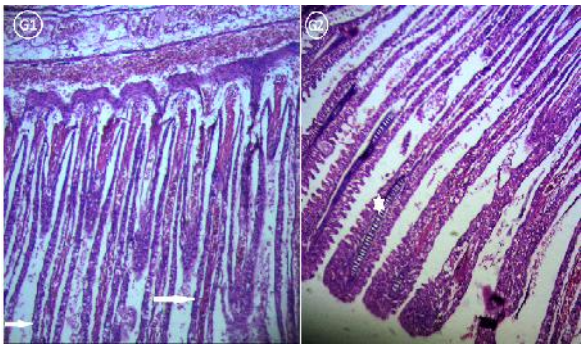

Figure 3 Photomicrograph of chlorpyrifos-treated gill (above 6 µg/ L concentrations) showing absence of secondary lamellae (arrow); and aneurism (asterisk) at X 100 magnification

Liver

Treated animals above 6 µg/ L concentrations demonstrate necrosis, vacuolization, anucleated cells and eosinophilic materials in the cytoplasm of hepatocytes (Figure 4).

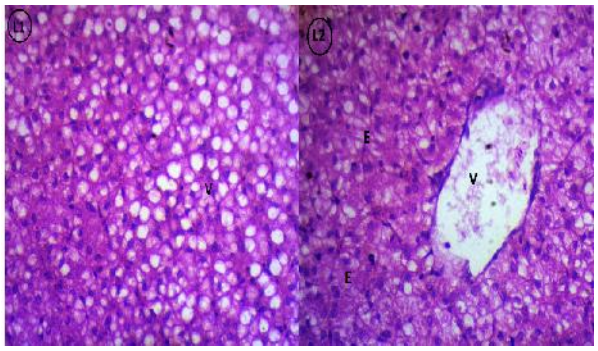

Figure 4 Photomicrograph of chlorpyrifos-treated hepatocytes (above 6 µg/ L concentrations) showing V- vacuolization, E- anucleated cells (x 100 magnification)

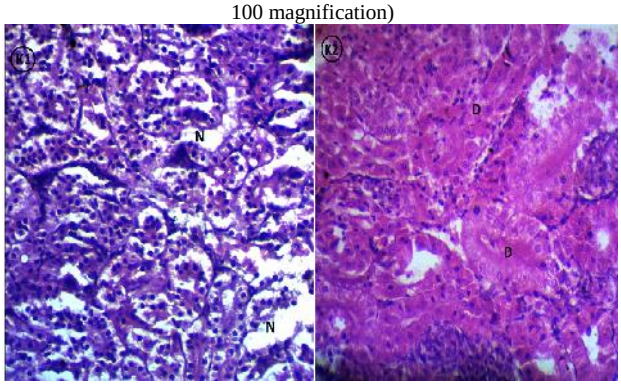

Figure 5 Photomicrograph of chlorpyrifos-treated kidney tissues (6 µg/ L concentration) showing N- necrosis in tubular epithelium; D- dialation od renal tubule and pyknotic nuclei (7 µg/ L concentration) at X 400 magnification

Kidney

Chlorpyrifos treatment showed necrosis as well as dialation of renal tubules and pyknotic nucleus in kidney of fish when compared to control groups (Figure 5).

Spleen

Exposure to chlorpyrifos above 6 µg/ L concentrations was noted with necrosis, anucleated cells, large numbers of melanomacrophage centers and vacuolization in spleen tissue (Figure 6).

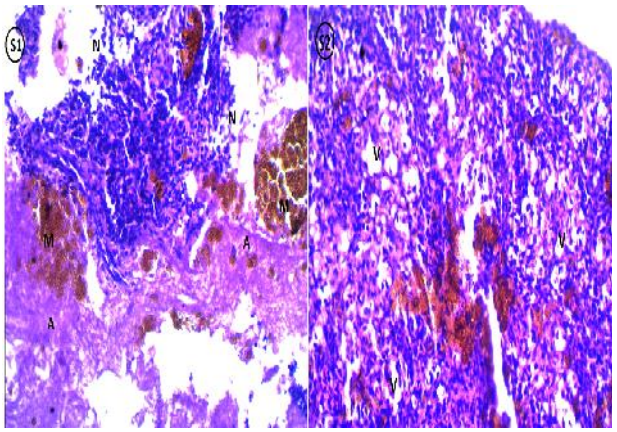

Figure 6 Photomicrograph of chlorpyrifos-treated spleen tissues (above 6 µg/ L concentrations) showing N- necrosis, A- anucleated cells, M- melanomacrophage centers, V- vacuolization at X 400 magnification

DISCUSSION

Histopathological studies are increasingly being used as an important indicator in environmental monitoring since they provide a definite biological end-point in examining toxicant-exposed specific target organs. Histological observations found in all tissues of *Etroplus maculatus* in the present study indicate that different concentrations of chlorpyrifos above 6 µg/ L showed different degrees of pathological changes.

Fish gills are composed of primary and secondary lamellae, which are formed by three different cell types such as pillar cells, respiratory cells and erythrocytes. It is well known that gills are the primary target of waterborne toxicants as it is constantly in contact with the water (Mallatt, 1985). Respiration, osmoregulation and excretion are the important functions of fish gills and they are sensitive to changes in the environment as it remained in close contact with the external environment (Poleksic and Mitrovic-Tutundzic, 1994). In general some of the alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, partial fusion of some secondary lamellae are examples of defense mechanisms, and these results increase the distance between the external environment and the blood and serve as a barrier to

the entrance of toxicants (Mallatt, 1985). Therefore, histological changes observed in fish gills are acknowledged as a fast and valid method to determine the damages caused by exposure to different pollutants (Arellano *et al*., 2001). Various alterations in gill tissue were recorded in the present study such as absence of secondary lamellae, necrosis, epithelial lifting, and aneurism. These modifications were considered as a first degree of gill lesions and lamellar aneurism thereby also associated due to the disturbance of blood flow in blood channels (Neskovic *et al*., 1996).

Liver is considered as the most important organ linked with detoxification and bioaccumulation process. In addition, due to its function, position and blood supply it is also one of the organs used as reliable biomarkers of toxic injury and representative of biological endpoints of contaminant exposure (Stentiford *et al*., 2003). In the present study chlorpyrifos above 6 µg/ L concentrations showed necrosis, vacuolization, anucleated cells and eosinophilic materials in the cytoplasm of hepatocytes. These changes may be attributed to the direct acute toxic effects of chlorpyrifos on liver tissue.

Renal lesions may be good indicators of environmental pollution because the fish kidney receives largest portion of post-branchial blood (Ortiz *et al*., 2003). Although kidney does not possess high levels of xenobiotic metabolizing enzymes as does the liver, many of the enzymatic reactions occurring in the liver have been shown to occur in the kidney. As it receives the bulk of the post branchial blood flow, kidney is considered as an important organ in the detoxification and elimination of aquatic contaminants in fish (Ortiz *et al*., 2003). Proliferation of renal tubules, necrosis of tubular epithelium, dialation of renal tubules and pyknotic nucleus were observed in kidney tissue treated with chlorpyrifos. Similar observations were observed in kidney of *Gambusia affinis* exposed to chlorpyrifos (Sabiha and Neelam, 2013).

In teleost fish the only lymph-node organ is spleen (Roberts, 2001). Histological investigations of the spleen have been mainly focused on the compartments such as lymphocytes and the macrophages that are important for the defense systems of the fishes (Montero *et al*., 1999). The main elements of the spleen parenchyma are white and red pulp. The white pulp is composed of lymphoid tissue, surrounding small arteries and diffusely intermeshing with the red pulp. The red pulp is composed of a reticular cell network and supporting blood-filled sinusoids that hold diverse cell populations, including macrophages and lymphocytes. Scattered through the parenchyma are numerous accumulations of the pigmented macrophages namely melanomacrophage centers. In the present study control tissues were observed with thin capsules enclosing homogenous red and while pulp with well organized melanomacrophage centers. After chlorpyrifos exposure melanomacrophage centers are poorly organized forming irregular cell clusters found between the white and the red pulp, usually concentrated in a large amount around the blood vessels as dark brown-black deposits. Necrosis, anucleated cells, large numbers of melanomacrophage centers and vacuolization in spleen tissue are associated with the toxicity of chlorpyrifos in the environment (Gogal *et al*., 1999).

CONCLUSION

The histopathological alterations observed in gill, liver, kidney and spleen of fishes are due to the acute toxic effect of chlorpyrifos in *Etroplus maculatus*.

Aknowledgment

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