Exposure to chlorpyrifos altered histoarchitecture of hepatocytes in the freshwater fish, *Pseudetroplus maculatus* (Bloch, 1795)

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Abstract

Chlorpyrifos (O,O-Diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate) is an organophosphate pesticide widely used in agriculture and aquaculture. Chlorpyrifos at sublethal concentration (0.661 µg/ L) was exposed to the cichlid fish, *Pseudetroplus maculatus* for 24, 48, 72 and 96 h. At the end of every treatment period, liver tissue was collected and examined for structural modifications. The hepatic alterations observed due to chlorpyrifos treatment were necrosis, vacuolization, segmentation of hepatic cells, anucleated cells, pyknotic nuclei of the cell, degenerative cytoplasm, melano macrophage aggregation and few spindle shaped nucleus. These hepatic alterations were more prominent in fish when exposed to chlorpyrifos for longer durations. In the present investigation, *Pseudetroplus maculatus* is used as biological indicator to assess aquatic pollution, which clearly demonstrates that chlorpyrifos altered the histoarchitecture of hepatocytes in fish. Therefore, chlorpyrifos could be considered as extremely toxic chemical when exposed to aquatic organisms even at sublethal concentration.

Keyword: *Pseudetroplus maculatus*, Pesticide, Chlorpyrifos, Histopathology, Hepatocytes, Acute toxicity

1. Introduction

Pesticides are the toxic substances released by human intentionally into the environment to kill pest, which is an effective weapon to protect agricultural products. They are the major cause of concern for aquatic environment due to its direct release into water bodies, nature of persistence, bioaccumulation and bioconcentration in the organisms (Joseph and Raj, 2010) [9]. So it is difficult to remove the toxicants once released into the aquatic ecosystem. Aquatic resources and fisheries are exceptionally valuable natural assets to human population because apart from proteinaceous fish food products, commercial and recreational values are added to it. When pesticides released into the aquatic ecosystem are known to cause broad range of responses to aquatic organisms, particularly fish and the effects of compounds depends on the nature of toxic compound, exposure time, dose rate, persistence in the environment and the species exposed (Fisher, 1991) [8]. Fishes are very sensitive to a wide variety of toxicants due to its accumulation in different tissues thereby producing many physiological and biochemical changes (Nagarathnamma and Ramamurthi, 1982) [13].

Organophosphates are more frequently used pesticide which are the derivative of phosphoric acid and have the highest level of vertebrate toxicity. It is well known that the residues of organophosphates remain unaltered for extended period of time in organic soils and surrounding drainage systems (Harris and Miles, 1975) [7]. Chlorpyrifos (O,O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate; CPF) is a broad spectrum organophosphate insecticide widely used to control foliar insects in agricultural crops (Rusyniak and Nanagas, 2004) [17] and subterranean termites (Venkateswara Rao et al., 2005) [20]. It is the second highest selling organophosphate insecticides which are moderately persistent in soil with the half-life ranging between 60 and 120 days (Howard, 1991) [8]. Chlorpyrifos that are found on the surface water may be absorbed through the gill, skin and digestive system of fish and distributed in various tissues through the blood where it is known to accumulate mainly in fatty tissues due to its lipophilic property (Mahdi et al., 2013) [10]. Although organophosphates are widely used in terrestrial environment, there is substantial evidence proving the movement of chemicals to aquatic environment.

Histopathological assessment in fish has been recognized as a highly valuable tool to identify the toxicological impact of pollutants since they provide early warning signs of disease and the health status of aquatic environment (Meyers and Hendrick, 1985) [12]. It allows examining specific target organs, including gill, kidney and liver that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer et al., 2001) [8].
In the light of above information and ideas, the present investigation is aimed to study the effect of sub-lethal concentration of chlorpyrifos on liver histopathology of the freshwater teleost fish, *Pseudetroplus maculatus*.

2. Materials and methods

2.1 Experimental organism

*Pseudetroplus 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 adjusted to the laboratory conditions for 15 days before experiment. Fish were fed with standard fish pellets during 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) [1].

2.2 Chemical

Chlorpyrifos (O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate) of technical grade (97%) was obtained commercially from Hikal Chemical Industries, Gujarat, India

2.3 Experimental design

Sublethal concentration of chlorpyrifos i.e., 0.661μg/L (one-tenth of LC₉₀-96 h) was chosen for the present study and the animal was exposed to toxicant upto 96 h at 24 h interval along with the control group. Ten fishes were maintained in each group, and after the end of every treatment period fishes were sacrificed from both control and treated groups for histopathological study.

2.4 Histopathological analysis

After the end of every treatment, fishes from both control and treated groups were sacrificed and liver was removed. Tissue was then fixed in buffered formalin, dehydrated in ascending alcohol series and cleared in xylene. Tissue was embedded in molten paraffin wax and sections of 5-6 μm thickness were made with a rotary microtome. Preparations were stained with eosin-hematoxylin and mounted in DPx and the stained sections were observed under trinocular research microscope and photographed.

3. Results and discussion

Histological analysis is the most effective and sensitive tool to determine cellular changes that may occur in vital organs due to toxicant exposure. Suitable organs for histological examination include skin, gills, liver, spleen, and kidney (Dutta, 1996) [10]. In the present study a variety of histopathological changes were observed in the liver of *Pseudetroplus maculatus* after chlorpyrifos treatment. The severity and frequency of organ lesions were more pronounced according to the increase in the duration of the treatment. The structure of normal liver of the fish consists of a continuous mass of large hexagonal cells. The hepatocytes are large in size with homogenous granular cytoplasm, centrally located distinct spherical nuclei where each cord separated by the thick wall of the peripheral cells (Figure 1). Liver is the organ associated with the detoxification and biotransformation process and it may be due to its function, position and blood supply (Van der Oost et al., 2003) [19]. It is the most affected organ when exposed to any contaminants in the water and the major deformalities includes necrosis, vacuolization, segmentation of hepatic cells, anucleated cells, pyknotic nuclei of the cell, degenerative cytoplasm (Rodrigues and Fanta, 1998) [16].

Chlorpyrifos when exposed at sublethal concentration for 24 h showed only slight modification in the histoarchitecture of hepatocytes (Figure 2). When the exposure period is extended to 48 h showed hepatocytes with degenerated cytoplasm (Figure 3). This finding is similar to the hepatocytes when exposed to chlordecone in the fish *Pseudetroplus maculatus* (Asifa et al., 2014) [2] and chlorpyrifos-treated zebrafish (*Danio rerio*) (Manjunatha and Philip, 2015) [11]. After 72 h of chlorpyrifos exposure, several lesions were observed in the hepatocytes such as segmentation of hepatic cells, anucleated cells, pyknotic nuclei of the cell, degenerative cytoplasm, aggregation of melanomacrophages and few spindle nucleus (Figure 4). However, toxicant-related severe pathological lesions were noted after 96 h of chlorpyrifos exposure with complete degeneration of cytoplasm and presence of few spindle nucleus (Figure 5).

The function of the melanomacrophages in the liver of fishes remains uncertain, but some studies have suggested that it is related to destruction, detoxification or recycling of endogenous and exogenous compounds (Haaparanta et al., 1996) [6]. An increase in the density of the melanomacrophage aggregates is generally related to important hepatic lesions, such as degenerative and necrotic processes (Pacheco and Santos, 2002) [14]. The liver of the exposed fish had slightly vacuolated hepatocytes showing evidence of fatty degeneration, necrosis of some portions of the liver tissue and this could be probably resulted from the excessive effort and stress acquired by the fish to get rid-off the toxicant from its body during the process of detoxification in the liver.
inability of fish to regenerate new liver cells may also have led to necrosis and segmentation of hepatic cells (Olufayo and Alade, 2012)\textsuperscript{[14]}. Pycnotic nuclei were also observed in liver of treated fish, it is an irreversible condensation of chromatin in the nucleus of cell and the observed changes indicate that the hepatocytes became hypofunctional after chlorpyrifos exposure (Tiwari et al., 2017)\textsuperscript{[18]}.

![Fig 1: Photomicrograph showing normal architecture of liver tissue in *Pseudetroplus maculatus*](image1)

Fig 1: Photomicrograph showing normal architecture of liver tissue in *Pseudetroplus maculatus*

![Fig 2: Photomicrograph showing slight modification in histoarchitecture of liver tissue after exposure to chlorpyrifos for 24 h in *Pseudetroplus maculatus*](image2)

Fig 2: Photomicrograph showing slight modification in histoarchitecture of liver tissue after exposure to chlorpyrifos for 24 h in *Pseudetroplus maculatus*

![Fig 3: Photomicrograph showing chlorpyrifos-treated hepatocytes with degenerated cytoplasm (→) after 48 h in *Pseudetroplus maculatus*](image3)

Fig 3: Photomicrograph showing chlorpyrifos-treated hepatocytes with degenerated cytoplasm (→) after 48 h in *Pseudetroplus maculatus*
4. Conclusions
The current study concluded that the organophosphorus insecticide chlorpyrifos is potent to cause hepatic toxicity through damage in cellular morphology in *Pseudetroplus maculatus*. As food source, fish interferes food chain including human’s life quality. But an agricultural effort by reducing the use of pesticides and implementing natural remedies for pest encroachment can be one of the solutions for pesticide pollution.

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6. References


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