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Effect of acute exposure to Chlorpyrifos on hepatic antioxidant system in the freshwater fish, *Pseudetroplus maculatus* (Bloch, 1795)

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Abstract

The present study was undertaken to evaluate the effect of chlorpyrifos on hepatic antioxidant system in the freshwater fish, *Pseudetroplus maculatus*. Fish was exposed at sublethal concentration (one-tenth of LC_{50}-96 h; 0.661µg/L) of chlorpyrifos for 24, 48, 72 and 96h maintaining the control group. The weight of liver decreased significantly after 96 h of chlorpyrifos exposure. The activities of antioxidant enzymes superoxide dismutase and catalase decreased significantly ($P<0.05$) while the levels of hydrogen peroxide generation and lipid peroxidation was increased significantly ($P<0.05$) in the treatment groups. Aspartate and alanine aminotransferase activities were found to be increased after 72 and 96 h of chlorpyrifos treatment and this could be due to the toxicant-induced stress. The present findings illustrates that acute exposure to chlorpyrifos altered antioxidant defense system in liver tissue thereby inducing oxidative stress.

Keywords: Chlorpyrifos, Antioxidant enzymes, Alanine aminotransferase, Aspartate aminotransferase, Liver, *Pseudetroplus maculatus*

Introduction

There is an increasing concern on the negative impact of various pollutants like pesticides in the aquatic environment, which seriously affect the normal physiology of aquatic animals. Worldwide pesticide production increased at a rate of about 11% per year, from 0.2 million tons in 1950s to more than 5 million tons by 2000 (FAO, 2017). Extensive use and release of pesticides in the aquatic ecosystem affect non-target animals such as insects, birds, amphibians, fishes and mammals (Kohler and Triebskorn, 2013; WHO, 2017). Fish is considered as a good indicator of environmental pollutants because their biochemical effects are similar to mammals. Apart from this fish is the major source of food to human worldwide and also have an important role in food chain.

Organophosphate pesticides are highly toxic to fish even at recommended levels due to their persistence in the environment and ability of bioaccumulation in various organs (Fulton and Key, 2001). The toxicity of these organophosphorus pesticides mainly includes inhibition of acetylcholinesterase (AChE) by their active metabolites or oxygen analogs generated during the metabolism (Jokanovic, 2009). Chlorpyrifos (O,O-diethyl-O-3,5,6-trichlor-2-pyridyl pho-

sphorothioate; CPF) is a broad spectrum organophosphate insecticide widely used to control foliar insects in agricultural crops and subterranean termites (Rusyniak and Nanagas, 2004). It is the second highest selling organophosphate insecticide in India and is more toxic to fish than organochlorine compounds. Chlorpyrifos may be absorbed to fish from the surface water through the gill, skin and digestive system and is distributed to various tissues through the blood and mainly get accumulates in fatty tissues due to its lipophilic property (Hunter *et al.,* 1999).

Fish have been used as an experimental model for the evaluation of the fitness of aquatic ecosystems that are exposed to environmental toxicants (Xing *et al.,* 2012). Several studies suggest that the toxicity in fish exposed to pesticides have a direct link on the imbalance of the antioxidant defense system. Increased production of reactive oxygen species (ROS) as a result of pesticide exposure lead to oxidative damages to vital tissues. Fish are endowed with antioxidant defense mechanism to neutralize the impact of ROS generated resulting from the metabolism of various toxicants. Antioxidant enzymes are crucial in the effort to counteract oxidative stress caused by toxicants once when the supply of other antioxidant enzymes is depleted (Martinez- Alvarez *et al.,* 2005).

Fish liver is the critical organ as it is the major centre for detoxification and hence a target organ for many environmental toxicants. Liver tissue includes various antioxidant defense enzymes as superoxide dismutase (SOD), catalase (CAT), glutathione reductase and peroxidase etc. to protect them from oxidative stress (Sulfath *et al*., 2013). Antioxidants contributes to the maintenance of relatively low level of the reactive and harmful free radicals such as hydroxide radical, superoxide radical and hydrogen peroxide in the presence of Cu^{2+} and/or Fe^{3+}. Such markers measured at the molecular or cellular level in fish have been proposed as sensitive 'early warning' tool in environmental quality assessments (Suvetha *et al.,* 2010).

In the present study, *Psuedetroplus maculatus* is used as an experimental model in order to assess the toxicity effect of chlorpyrifos. It is an important indigenous cichlid fish of south India especially Kerala, commonly found in freshwater and brackish water habitats. The present study was accordingly designed to evaluate the acute toxic sublethal effects of chlorpyrifos on the antioxidant defense system in hepatocytes of the cichlid fish, *Pseudetroplus maculatus.*

Materials and methods

Experimental organism

Pseudetroplus maculatus, 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 (40 L capacity) containing dechlorinated water and well aeration. The physiochemical features of the tap water were analyzed 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).

Chemicals

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. All other chemicals were purchased from local commercial sources.

Experimental design

Sublethal concentration of chlorpyrifos i.e., 0.661µg/L (one-tenth of LC_{50}-96 h) was chosen for the present study and the animal

was exposed to toxicant for 24 to 96 hours along with the control group.
Group I: Control group maintained for 96 h.
Group II: Chlorpyrifos-treated group maintained for 24, 48, 72 and 96 h.

At the end of every treatment period, fishes were caught very gently using a small dip net, one at a time with least disturbance and were decapitated. Liver tissue were dissected, weighed and 1% tissue homogenate was prepared for the biochemical analyses. A 1% (w/ v) homogenate of liver tissue was prepared in ice-cold normal saline with the help of a motor-driven glass Teflon homogenizer on crushed ice for a minute. The homogenate was centrifuged at 800 *g* for 15 min at 4ºC to obtain the supernatant, which was then used for the biochemical analyses. Protein was estimated by the method of Lowry *et al*. (1951) with bovine serum albumin as the standard. Activities of superoxide dismutase (Marklund and Marklund, 1974), catalase (Claiborne, 1985), glutathione reductase (Carlberg and Mannervik, 1985), levels of hydrogen peroxide generation (Pick and Keisari, 1981) and lipid peroxidation (Ohkawa *et al*., 1979); activities of alanine and aspartate aminotransferases (Reitman and Frankel, 1957) were measured in the supernatant of crude homogenate.

Statistical analysis

Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test using statistical package SPSS 19.0. Differences were considered to be significant at $p<0.05$ against the control group. Data are presented as mean ± SD for ten animals per group. All biochemical estimations were carried out in duplicate.

Results

In the present study, chlorpyrifos exposure significantly ($P<0.05$) decreased the weight of liver after 96 h when compared to the corresponding control group (Fig.1). Sublethal exposure of chlorpyrifos caused significant decrease ($P<0.05$) in the activities of antioxidant enzymes such as superoxide dismutase and catalase in a time-dependent manner (Figs. 2 and 3). However, the levels of hydrogen peroxide generation and lipid peroxidation increased significantly ($P<0.05$) when compared to corresponding control group (Figs. 4 and 5). Chlorpyrifos treatment significantly increased ($P<0.05$) the activities of alanine and aspartate aminotransferse in hepatocytes after 72 h exposure (Figs. 6 and 7).

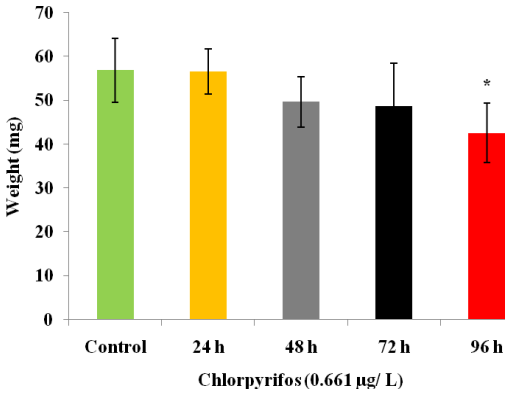

Figure 1: Effect of chlorpyrifos on the weight of liver in the fish, *Pseudetroplus maculatus*.

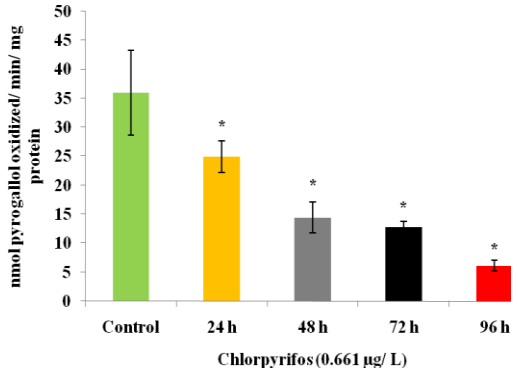

Figure 2: Effect of chlorpyrifos on the activity of superoxide dismutase in the liver of fish, *Pseudetroplus maculatus*.

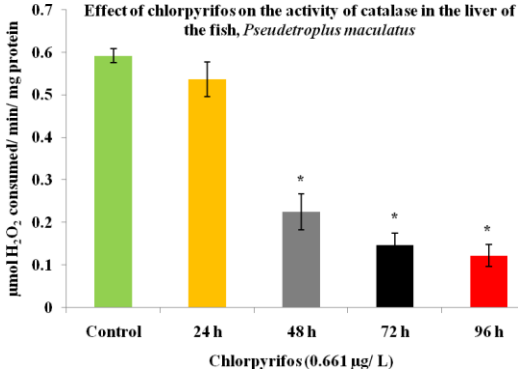

Figure 3: Effect of chlorpyrifos on the activity of catalase in the liver of fish, *Pseudetroplus maculatus*.

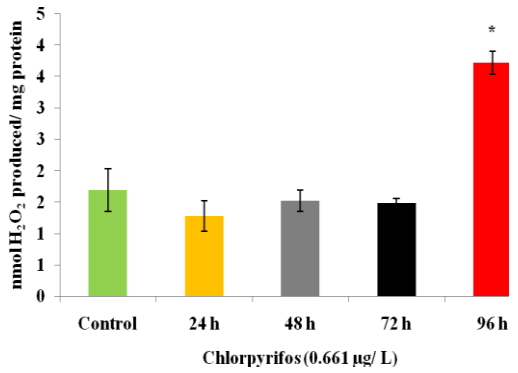

Figure 4: Effect of chlorpyrifos on the level of hydrogen peroxide in the liver of the fish, *Pseudetroplus maculatus*.

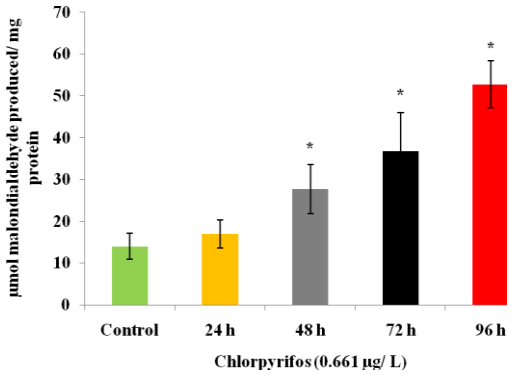

Figure 5: Effect of chlorpyrifos on the level of lipid peroxidation in the liver of the fish, *Pseudetroplus maculatus*.

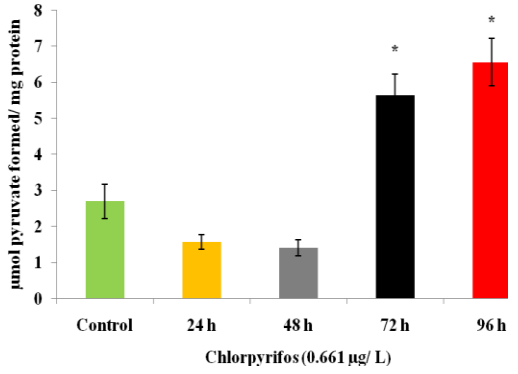

Figure 6: Effect of chlorpyrifos on the activity of alanine aminotransferase in the liver of the fish, *Pseudetroplus maculatus*.

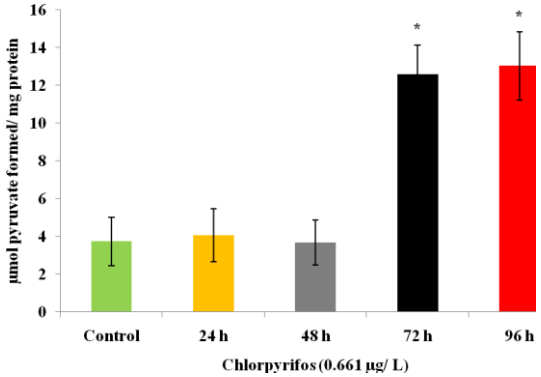

Figure 7: Effect of chlorpyrifos on the activity of aspartate aminotransferase in the liver of the fish, *Pseudetroplus maculatus*.

Discussion

The present findings showed that acute exposure of chlorpyrifos induced oxidative stress in the hepatocytes of fish, *Pseudetroplus maculatus*. Liver performs various functions associated with the metabolism of xenobiotics and as other tissues, it depend on antioxidant enzymes for the protection against the free radicals produced during the biotransformation of toxicants (Londis and Yu, 1995). In the present study the weight of the liver decreased significantly after chlorpyrifos treatment and this may be due to the systemic toxicity of the exposed compound.

During intoxication of chlorpyrifos there was significant decrease in the activities of antioxidant enzymes as superoxide dismutase and catalase. Oxidative stress caused by pesticides in aquatic organisms may lead to the generation of reactive oxygen species (ROS) and alterations in antioxidants enzymes (Livingstone, 2001). The production of ROS has been shown to attack nearby molecules resulting in damage of the molecular structure and function or dysfunction of many organs and systems. The excess ROS production and their damaging effects can be minimized by the cellular antioxidant defense systems (Sureda *et al*., 2004). The antioxidant enzymes such as SOD and catalase play a major role in eliminating the ROS produced during bioactivation of xenobiotics and the induction of SOD-catalase system may be the first line of defensive mechanism against the ROS production (Nwani *et al*., 2010). Catalase and SOD have a remarkable importance for aquatic organisms because these enzymes protect the tissues from free radicals that cause oxidative stress (Saglam *et al.*, 2014). Thus the variation in the activity of antioxidant enzymes may be used as indicators of pollutant mediated oxidative stress (Sayeed *et al*., 2003).

In general, superoxide dismutase prevents the accumulation of superoxide radical and it is rapidly eliminated. The reduction in the activity of superoxide dismutase indicates the potential effect of chlorpyrifos and failure of SOD to eliminate superoxide radicals. Catalase is the key enzyme that converts hydrogen peroxide to water and molecular oxygen, which is crucial for the fish to detoxify the hydrogen peroxide generated into water molecule (Attila *et al*., 2001). The decrease in the activity of catalase after chlorpyrifos exposure indicates the oxidative stress caused by the toxicant (Kono and Fridovich, 1982). Thus accumulation of superoxide radical is associated with the induction of oxidative stress in liver tissues. Chlorpyrifos inhibited the activity of catalase that lead to failure in scavenging hydrogen peroxide from the hepatocytes, which was proved by the significant increase in the level of hydrogen peroxide at the end of 96 h. Similar observation was reported in hepatocytes of fish, *Pseudetroplus maculatus* exposed to nonylphenol (Asifa and Chitra, 2016).

The present results clearly illustrates that acute exposure to chlorpyrifos generated reactive oxygen species as hydrogen peroxide and superoxide anion, which in turn are responsible for cell and tissue damage. Chlorpyrifos exposure resulted in the significant increase in the level of lipid peroxidation in hepatocytes of fish. Lipid peroxidation may be considered as the toxicant induced oxidative damage to membrane lipids (Xing *et al*., 2012). It has been reported that organophosphorus pesticides may increase lipid peroxidation by direct interaction with the cell membrane (Hazarika *et al*., 2003). Lipid peroxidation byproduct, such as a malondialdehyde (MAD), is widely used as indicator of increased concentration of cellular reactive oxygen species and a sign of tissue injuries (Christia and Costa, 1984). Similar to pesticides, even the exposure of nanoparticles as fullerene C_{60} has been shown to increase the level of lipid

peroxidation in the fish *Pseudetroplus maculatus* (Sumi and Chitra, 2016).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are liver-specific enzymes and they are more sensitive measure of hepatotoxicity and histopathologic changes which can be measured for acute toxicity testing (Balint *et al*., 1977). The present observation showed that the activities of alanine and aspartate aminotransferases were significantly increased at the end of 72 and 96 h. The elevated level of aminotransferases indicate the rapid synthesis and deamination of amino acids, enabling carbohydrate and protein metabolism during fluctuation of energy demands as a result of chlorpyrifos-induced stress. It also reveals one of the adaptive measures of the fish to increase energy production under toxicant stress (Gabriel *et al.*, 2012). Fish exposed to ethoxyquin has been shown to increase the activities of aminotransferases and altered normal metabolism of the fish (Neethumohan *et al*., 2017).

Conclusion

The results of the present investigation indicate that acute sublethal exposure of chlorpyrifos imbalance the hepatic antioxidant defense system in the fish, *Pseudetroplus maculatus*. Acute toxicity of chlorpyrifos at sublethal concentration is therefore, proved as a threat to the survival of fish in natural environment.

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