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Full Length Research Paper

Studies on the hepatic antioxidant defense system in λ cyhalothrin-induced oxidative stress in fresh water tilapia (*OREOCHROMIS MOSSAMBICUS*)

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The present study was undertaken to determine the effects of λ cyhalothrin-induced oxidative stress in fresh water tilapia (*OREOCHROMIS MOSSAMBICUS*) with respect to changes in the levels of lipid peroxidation [LPO], reduced glutathione [GSH], glutathione-dependent antioxidant enzymes (glutathione peroxidase [GPX] and glutathione-S-transferase [GST]) and antiperoxidative enzymes (catalase [CAT] and superoxide dismutase [SOD]). Significant (P<0.05) elevation in the level of lipid peroxidation was observed in λ cyhalothrin-intoxicated fishes as compared to controls. A concomitant (P<0.05) decline in hepatic antioxidant status was also observed. The results of the present investigation have indicated that the tissue antioxidant defense system is operating at a lower rate despite increased λ cyhalothrin-induced oxidative stress.

Key words: Antioxidant status, λ cyhalothrin, lipid peroxidation, *Oreochromis mossambicus,* oxidative stress, synthetic pyrethroid.

INTRODUCTION

Pesticides not only alter the physico-chemical properties of water but also adversely affect the aquatic organisms (De Vlaming et al*.,* 2000; Parma et al., 2007). In aquatic organisms, the pollutants percolate up to the cellular level through the cell membrane and interact with the cellular macromolecules to inhibit the essential cellular meta-bolism (Siroka and Drastichova, 2004). Stress has been defined as the effect of any environmental alteration that extends homeostatic or stabilizing processes beyond their normal limits (Esch and Hazen, 1978). Oxidative stress usually characterizes chemically induced toxicity (Livingston et al., 1990; Cossu et al., 2000). Certain environmental contaminants can alter the reproductive physiology, growth and development of vertebrates by disrupting the normal functioning of the endocrine systems due to environmental stress (Colborn, 2002; Kumar et al., 2007). λ cyhalothrin (chloro trifluoropropenyl or dimethyl cyclopropane carboxylate) (Figure 1) insecticides are synthetic pyrethroid and acaricides used to control a wide range of pests, (Royal Society of Chemistry, 1991).

λ cyhalothrin is categorized as restricted use pesticide in Extension Toxicology Network for its toxicity to fish (Maund et al., 1998). Pyrethroids, synthetic derivatives of pyrethrins, have enjoyed increasing use as wide-spectrum insecticides (Casida and Quistad, 1998) reported that pyrethroids accounted for 23% of the worldwide insecticide market in 1995. Their use in the united states has increased substantially since 1998, due to Environment Protection Agency's (EPA's) concern about possible adverse effects of organophosphates on neurodevelopment in children. The wide spread use of pyrethroids in forestry, agriculture, and the home has resulted in their frequent detection in humans (Berkowitz et al., 2003; Heudorf et al., 2004; Whyatt et al., 2002). The environmental fate and effects of synthetic pyrethroid pesticides have been summarized by Hill (1989). Fish tend to lack the enzymatic machinery for the metabolism of this pyrethroid which is the obvious reason for the deleterious effect of this pesticide on fish (Demounte, 1989).

Exposure to λ cyhalothrin has been reported to induce many metabolic and morphologic aberrations in the liver

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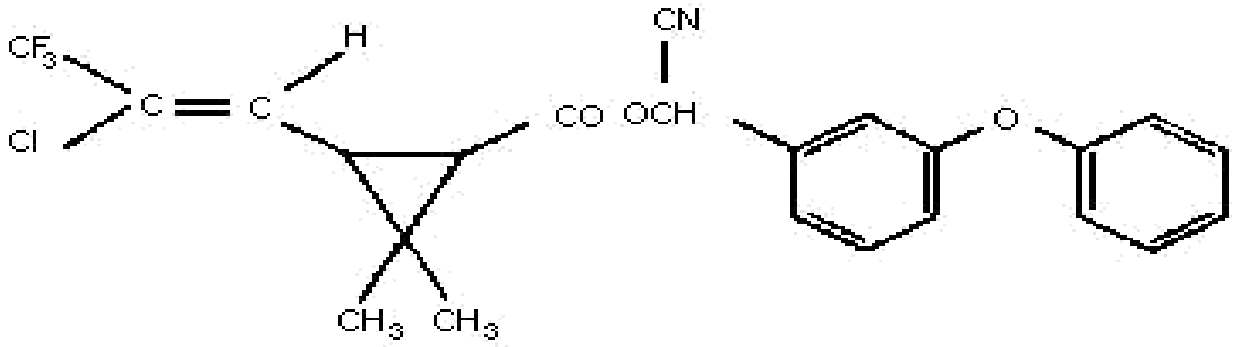

Figure 1. Chemical structure of λ cyhalothrin.

tissue of the experimental animals (Anandan et al., 1998; Kucera et al., 2006). It induces hepatic necrosis by a multiple step mechanism. Peroxidation of endogenous lipid is a major factor in the cytotoxic action of λ cyhalothrin (Padma et al., 2006). Oxygen free radicals are reportedly involved in toxicity of numerous chemicals and also in pathogenesis of many diseases (Kehrer, 1993; Ray and Banerjee, 1998). Tilapias (*Oreochromis mossambicus*) is one of the most salinity-tolerant species, reproducing both in fresh water and in seawater (Stickney, 1986; Suresh and Lin, 1992). Tilapia are omnivorous warm water fish of major commercial importance. In the present study, we have investigated the deleterious effects of λ cyhalothrin-induced oxidative stress on hepatic defense system in fresh water tilapia (*O. mossambicus*) with respect to changes in the levels of lipid peroxidation and antioxidant status in liver tissue.

MATERIALS AND METHODS

Chemicals

Epinephrine, tetraethoxy propane and λ cyhalothrin were obtained from M/s. Sigma Chemical Company, St. Louis. MO, USA. All the other chemicals used were of analytical grade.

Animals

Tilapia (*O. mossambicus*) of length ranging between 9 to 13 cm and weight 2 to 7 g collected from Pallathuruthy pond, Cochin, India were selected for the study. The fishes were kept in fibre plastic tanks and maintained at normal room temperature (30 ± 2°C, 12 h light/ dark cycle).

Experimental protocol

After acclimatization, the fishes were divided into four groups of 10 fishes each. Group I served as control. Group II were normal fishes exposed to acetone alone (vehicle control). Groups III and IV fishes were exposed to λ cyhalothrin [0.3 µg (dissolved in acetone)/L] and [1.1 µg (dissolved in acetone)/L] respectively, for the induction of oxidative stress. The tanks were covered with nylon nets.

The toxicant solution was renewed every 24 h and the experiment was continued for a period of 15 days. At the end of the experiment, the fishes were killed and liver tissue excised was used for biochemical analyses. The liver tissue was excised immediately and washed with chilled isotonic saline. The liver tissue homogenates prepared in ice cold 0.1 M Tris-HCl buffer, pH 7.2 were used for the determination of lipid peroxides (LPO), reduced glutathione (GSH), glutathione-dependent antioxidant enzymes (GPX and GST) and antiperoxidative enzymes (CAT and SOD).

Biochemical assays

Tissue lipid peroxidation level was determined as TBA-reactive substances by the method described by Ohkawa et al. (1979). GSH was determined by the method of Ellman (1959). Glutathione peroxidase [EC 1.11.1.9] (GPx) activity was measured by the method of Paglia and Valentine (1967). Glutathione-S-[EC 2.5.1.18] (GST) activity was determined by the method of Habig et al. (1974). Catalase [EC 1.11.1.6] (CAT) activity was assayed according to the method of Takahara et al. (1960). Superoxide dismutase [EC 1.15.1.1] (SOD) activity was determined according to the method of Misra and Fridovich (1974).

Statistical analysis

All data were analyzed using ANOVA with the aid of SPSS 10.0 for Windows. Data obtained were expressed as mean $\pm$ SD. Multiple comparisons of the means were separated using the Duncan multiple range test at 5% probability.

RESULTS AND DISCUSSION

Lipid peroxidation *in vivo* has been identified as one of the basic deteriorative reaction in cellular mechanisms of the λ cyhalothrin induced oxidative stress in fresh water fishes. In the present investigation on exposure to λ cyhalothrin induced a significant ($P<0.05$) increase in the level of lipid peroxidation in the liver tissue of group IV, 1.1 µg/L fishes as compared to group I control (Table 1). This indicates that high vulnerability to peroxidative damage in λ cyhalothrin induced toxicity, is probably due to a decline in the level of free radicals for scavengers. Antioxidants are necessary for preventing the formation of free radicals and they inhibit some of the deleterious

Table 1. Levels of lipid peroxidation (LPO), reduced glutathione (GSH), and the activities of glutathione peroxidase (GP_X), glutathione-S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD), in the liver tissue of normal and experimental groups of fishes.

Parameters	Control	Acetone	Pesticide+Acetone(0.3 µg/L)	Pesticide+Acetone (1.1 µg/L)
	Group I	Group II	Group III	Group IV
LPO	0.45+0.025^{a}	0.41 + 0.02^{b}	0.48+0.03^{a}	0.895+0.045^{c}
GSH	3.72+0.17^{a}	3.99+0.15^{b}	3.59+0.14^{a}	1.63+0.09^{c}
GP_X	4.81+0.36^{a}	4.94+0.32^{a}	4.36+0.38^{b}	2.17+0.11^{c}
GST	631.5+59.0^{a}	597.5+51.0^{a}	536.5+47.5^{b}	323.5+27.5^{c}
CAT	11.4+0.07^{a}	11.35+0.06^{a}	10.35+0.08^{b}	6.15+0.04^{c}
SOD	5.75+0.40^{a}	5.11+0.37^{b}	4.71+0.35^{c}	3.21+0.16^{d}

Results are mean+SD for 10 fishes. Values expressed: LPO, nmol malondialdehyde released/mg protein; GSH, nmolg^{-1} wet tissue; GP_X, nmol GSH oxidized min^{-1} mg^{-1} protein; GST, µmol 1-chloro- 2,4-dinitrobenzene conjugate formed min^{-1} mg^{-1} protein; CAT, nmol H_2O_2 decomposed min^{-1} mg^{-1} protein; SOD, one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation Values that have a different superscript letter (a, b, c, d) differ significantly with each other ($P<0.05$; Duncan's multiple range test). Group II, acetone treated 1.1 µg/L (vehicle control) for 15 days: Group III, λ cyhalothrin dissolved in acetone (0.3 µg/L) for 15 days: Group IV, λ cyhalothrin dissolved in acetone (1.1 µg/L) for 15 days.

actions of reactive oxygen species that damage lipids, DNA and proteins (Haidara et al., 2006). There were no significant alterations observed in the level of lipid peroxidaion in groups II and III fishes, as compared to group I fishes. Reports by Anandan et al. (2004) showed that lipid peroxidation is a complex sequence of reactions that leads to the disruption of membrane functions.

Our results also confirmed the same pattern and showed that λ cyhalothrin exposed fishes might be less resistant and more susceptible to lipid peroxidation. Three different mechanisms are able to induce lipid peroxidation: autoxidation (by free radical reaction), photo-oxidation and enzyme action. Autoxidation is a radical-chain process involving 3 sequences, initiation, propagation and termination. The general process of lipid peroxidation consists of three stages: initiation, propagation and termination. Initiation occurs when oxygen is partly reduced by Fe^{2+} to species able to abstract a hydrogen atom from a methylene carbon. Resulting alkyl radical reacts rapidly with oxygen to form a peroxy radical ($LOO^{\cdot}$), which itself can liberate LOOH via hydrogen abstraction from a neighbouring allyl bond. In this reaction, new alkyl radicals are produced which propagate lipid peroxidation.

$nFe^{2+} + O_2 \rightarrow nFe^{3+}$ + reduced O_2 (I)

$I + LH \rightarrow IH + L$ initiation

$L + O \rightarrow LOO^{\cdot}$

$LOO^{\cdot} + LH \rightarrow LOOH + L$ Propagation

Fe^{2+} can substantially enhance lipid peroxidation by decomposing LOOH to highly reactive lipid alkoxy radicals (LO) that behave as organic initiators and branch lipid peroxidation.

$Fe^{2+} + LOOH \rightarrow Fe^{3+} + OH^{\cdot} + LO$

$LO + LH \rightarrow LOH + L$ chain branching

Excess Fe^{2+} can also complete, as electron donors, for $LOO^{\cdot}$ and LO inhibiting both the propagation and chain branching reactions and causing the Fe^{2+} dependent termination of lipid peroxidation.

$Fe^{2+} + LOO^{\cdot}/LO \rightarrow Fe^{3+} + LOOH/LOH$ termination

The results of the present study demonstrated that λ cyhalothrin might have stimulated lipid peroxidation by influencing a variety of these reactions. λ cyhalothrin might have enhanced the initiation process not only by producing $OH^{\cdot}$ but also by activating the Fe^{2+} autoxidation. This action of λ cyhalothrin may alter other molecules of biological relevance in cellular and sub-cellular membranes. λ cyhalothrin might have activated the Fenton-like reaction that causes the formation of the alkoxy radicals initiator of lipid peroxidation. It elevates the amount of Fe^{2+} oxidized probably by acting with a site specific mechanism similar to that described for other $OH^{\cdot}$ producers stimulating the Fenton reaction. The alteration of redox recycling of iron, affects the Fe^{2+}/Fe^{3+} ratio in the reaction mixture.

Both of these phenomena may account for the active-tions exerted by λ cyhalothrin on the peroxidation of cell membranes. As there is some evidence that λ cyhalothrin alters the Ca^{2+} binding sites on membrane acidic phospholipids, in particular of the phosphatidylserine and phosphatidylinositol classes. λ cyhalothrin may activate peroxidation by enhancing cellular as it does for other Ca^{2+} dependent processes. Glutathione is one of the abundant tripeptide nonenzymatic biological antioxidants present in the liver (Anderson, 1998). It acts as a sub-strate for H_2O_2 removing enzyme glutathione peroxidase and for dehydroascorbate reductase (Ahmed and Khater, 2001).

It also plays a critical role in cellular function, which includes the maintenance of membrane protein, the

removal of free oxygen radicals such as peroxyl radical, superoxide radical, alkoxy radical, translocation of amino acids across cell membranes, the detoxification of foreign compounds and biotransformation of drugs (Comporti et al., 1991; Muriel et al., 1992). In the present study a significant (P<00.05) decline in the level of GSH in group IV fishes was observed compared to group I normal control fishes (Table 1). The tissue antioxidant status might be operating at a diminished level in λ cyhalothrin induced fishes.

Reduction noticed in the level of GSH in λ cyhalothrin induced fishes was either due to increased degradation or decreased synthesis of glutathione. GSH protects the hepatocellular membranes from the oxidative damaging action of lipid peroxides.

Significant (P<0.05) decline was noticed in the activities of GSH-dependent antioxidant enzymes, GP_X and GST, in the liver tissue of group IV fishes compared to normal controls (Table 1), reflecting an increased oxidative stress in λ cyhalothrin induced fishes. GP_X offers protection to the cellular and subcellular membranes from the peroxidative damage by eliminating hydrogen peroxide and lipid peroxide. GST binds to many different lipophilic drugs; so it would be expected to bind λ cyhalothrin and act as an enzyme for GSH conjugation reactions. Inhibition of these enzymes may lead to the accumulation of these oxidants and makes liver cell membranes more susceptible to oxidative damage. GSH and GSH-dependent enzyme systems may be directly related to the pathogenic mechanisms of λ cyhalothrin induced fishes.

Activities of antiperoxidative enzymes (SOD and CAT) were also significantly (P<0.05) decreased in the liver tissue of λ cyhalothrin induced fishes as compared to controls (Table 1). Reduction in the activities of the antiperoxidative enzymes in λ cyhalothrin induced fishes, might be due to the increased generation of reactive oxygen radicals such as superoxide and hydrogen peroxide, which in turn leads to the inactivation of these enzyme activities. λ cyhalothrin treated with *O. mossambicus* in the present study showed a significant (P<0.05) elevation in the level of lipid peroxidation along with a marked decline in the activities of superoxide dismutase and catalase, thus indicating the increased λ cyhalothrin induced oxidative stress condition. In conclusion, the effect of oxidative stress can be evidenced by cellular accumulation of lipid peroxides. The present study indicated that λ cyhalothrin, induced significant alterations in liver toxicity of fish and in the activities of antioxidant enzymes. The overall hepatotoxic effect of λ cyhalothrin is probably related to a generation of free radicals, which alters the antioxidant status and membrane stability.

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