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Effects of Deltamethrin on CAT, LPO and GSH in Tissues of Zebrafish *Danio rerio*

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ABSTRACT

Synthetic pyrethroids, such as deltamethrin, are toxic substances that lead to generation of reactive oxygen species, which harm living organisms. The present study deals with the level of imbalance evolved by sub-lethal concentrations of deltamethrin on the antioxidant enzyme catalase (CAT), lipid peroxidation (LPO) and the reduced glutathione (GSH) of *Danio rerio* brain and muscle during 16-days exposure period. For this study, matured adult fishes were exposed to different concentrations viz., 0.016, 0.025 and 0.043 μg L^{-1} (96-h LC_5, LC_{10} and LC_{20} of deltamethrin) for 16 days continuously. In the brain and muscle of fish an inhibition of catalase (CAT) activity was observed while for lipid peroxidation (LPO) a continuous enhancement was apparent for all exposure periods. GSH level in both the tissues were decreased for all exposure periods. The toxicity was time as well as concentration dependent. However, there is much to learn about the details of this phenomenon and further research is needed to fully elucidate the effect of pesticides and the environmental risks they pose in the aquatic organisms.

Key words: Deltamethrin, zebrafish, brain, muscle, oxidative stress

INTRODUCTION

Increasing number and amount of industrial, agricultural and domestic chemicals into the aquatic environment led to deleterious effects on the aquatic organisms. Synthetic pyrethroids have been introduced as viable substitutes for organochlorine, organophosphate and carbamate pesticides, recognized for their ecological hazards. They have become one of the most used pesticides due to their selective action, low toxicity to non-target organisms (Milam *et al*., 2000). In mammals and birds these substances are readily metabolized and excreted (Demoute, 1989). However, pyrethroids are extremely toxic to fish because the activity of the enzymatic system involved in their hydrolysis is reduced (Haya, 1989). One of the widely accepted synthetic compounds in this category is deltamethrin, a fourth-generation synthetic pyrethroid. It is extensively used in agriculture and forestry because of its high activity against a broad spectrum of insect pests (Mestres and Mestres, 1992).

Many toxic effects induced by deltamethrin in different fishes have been reported (Sayeed *et al*., 2003; Khalili *et al*., 2012). A contributing factor to the sensitivity of fish to deltamethrin exposure seems to be its high rate of gill absorption due to the lipophilicity. The main mode of its action is neurotoxicity. This compound has a great impact upon the central and peripheral nervous system and acts by modulating the opening and closing of channels that can

result in synaptic discharge, depolarization and ultimately death (Eells *et al*., 1993). Also, it has been reported that pyrethroids induce oxidative stress/alteration of antioxidant system and lipid peroxidation. Investigators showed that lipid peroxidation in cells increased with pyrethroids treatment and antioxidant enzyme activities and malondialdehyde were altered after exposure to pesticides in fish (Akhtar *et al*., 1994; Kale *et al*., 1999).

Oxidative stress is defined as a disruption of the prooxidant-antioxidant balance in favor of the former, leading to potential damage (Tuzmen *et al*., 2008). It is a result of an increase in Reactive Oxygen Species (ROS) and impairment of antioxidant defence systems or incapacity to repair oxidative damage. The main damage induced by ROS results in alterations of cellular macromolecules such as membrane lipids (lipid peroxidation), DNA (Marks *et al*., 1996), and/or proteins. The resulting damage may alter cell functions, eventually leading to cell death. Enzymatic (CAT) and non-enzymatic antioxidants such as reduced glutathione (GSH) and its precursor normally counteract damaging effects of ROS by either repairing the oxidative damage or directly scavenging oxygen radicals. Catalase is a common enzyme found in nearly all the organisms which are exposed to oxygen where it functions to catalyze the decomposition of H_2O_2 to O_2 and H_2O. Glutathione (GSH) is an antioxidant which helps to protect the cells from the ROS such as free radicals and peroxides. The glutathione defense enzyme systems in living cell detoxifies and eliminates the xenobiotics leading to the formation of products easily soluble in water and their rapid elimination from the organism. A previous study in our laboratory demonstrated that the effects of deltamethrin, characterized as a concentration-dependent loss of reproductive capacity of zebrafish (Sharma and Ansari, 2010).

The data available in regard to the oxidative stress and antioxidant systems in fish exposed to deltamethrin are limited. A study on freshwater fish *Channa punctatus* Bloch showed that, after 48 h of exposure to deltamethrin, oxidative stress was induced in all the investigated tissues (Sayeed *et al*., 2003). Fish, among the group of non-target aquatic organisms, represent the largest and most diverse group of vertebrates. Hence, this study was aimed to look in to the toxic effects of sub-lethal concentrations of deltamethrin on the activity of catalase, reduced glutathione and lipid peroxidation in brain and muscle of *Danio rerio*. Zebrafish was selected for the present study because they are model organisms for toxicological research and also recommended by the Organization for Economic Co-operation and Development (OECD, 1992).

MATERIALS AND METHODS

Fish maintenance and treatments: For the present study, matured adult fishes were procured from our stock aquarium and exposed to different concentrations viz., 0.016, 0.025 and 0.043 μg L^{-1} (96 h LC_5, LC_{10} and LC_{20} of deltamethrin) as calculated earlier (Sharma *et al*., 2012) for 16 days continuously. The concentrations of deltamethrin (Decis®) used in our experiments, was selected because no mortality was observed but the biochemical investigated parameters, such as oxidative stress biomarker and antioxidant defense system of exposed fishes were affected. Six aquaria, with two replicates for control and deltamethrin treatment, were used. Fifty fishes for each concentration of the pesticide were used. In these aquaria water was replaced daily with fresh treatment of pesticide so as to maintain the constant concentration of the toxicant. In order to obtain the desired concentration, deltamethrin was appropriately diluted and mixed with aquarium water just before use in the experiment. Fish from the control group were maintained in pesticide-free water.

Preparation of tissue homogenates: After the expiry of the exposure periods (4, 8, 12 and 16 days), required number of exposed fishes were taken out from experimental and control groups and their tissues (brain and muscle) were dissected. Homogenates (prepared as 1 g tissue per 10 volumes of buffer) of the tissues were prepared in ice-cold buffer (0.1 M Tris-EDTA buffer, pH 7.4) using a homogenizer. The resulting homogenate was centrifuged at 8,000 g for 30 min in a Remi centrifuge at 4°C. The supernatant was decanted and used for analyses.

Biochemical analysis: The levels of LPO were measured *via* the thio-barbituric acid reacting substances (TBARS) colour reaction for malondialdehyde (MDA) according to the method of Placer *et al.* (1966) and results were expressed as μmol of MDA formed/mg protein. Catalase (CAT) (EC 1.11.1.6) activity was assayed by monitoring the disappearance of hydrogen peroxide (H_2O_2) at 570 nm, according to the method of Sinha (1972) and values were expressed as μmol/min/mg protein. Reduced glutathione (GSH) level was estimated according to the method of Paglia *et al.* (1975) determined by its reaction with 5,5'-dithio-bis (2-nitro-benzoic acid) (DTNB) to yield a yellow chromophore which was measured spectrophotometrically at 412 nm and results were expressed as GSH mg/mg protein.

Total protein determination: In tissues the protein contents were assayed using the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Statistical analysis: Statistical analysis was done by Analysis of Variance (ANOVA). Two-way ANOVA was used for multiple group comparisons of the data using StatPlus® version 2009 computer software purchased from Analystsoft Vancouver, Canada. All data are expressed as Mean (n = 6)±Standard Deviation (SD) and differences were considered significant at $p<0.05$.

RESULTS

In the present investigation there was a significant ($p<0.05$) alterations in the catalase activity, GSH level and LPO in brain and muscle of zebrafish exposed to deltamethrin at different concentrations and exposure periods.

The effects of the deltamethrin exposure on LPO in brain of zebrafish are presented in (Fig. 1). In the brain, a significant ($p<0.05$) increase of LPO is apparent at all pesticidal concentration on

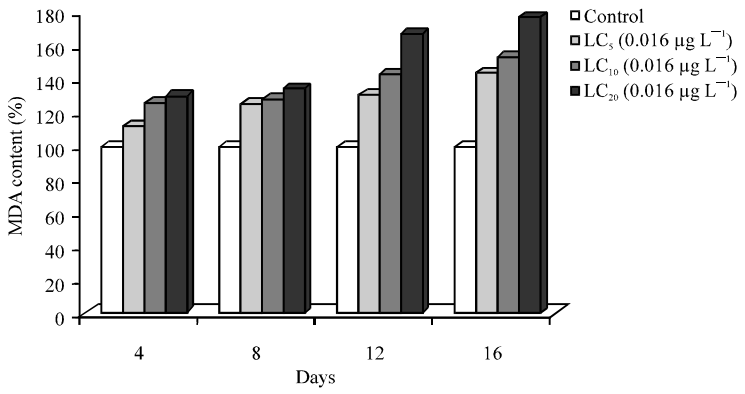

Fig. 1: Effect of deltamethrin on LPO in brain of zebrafish

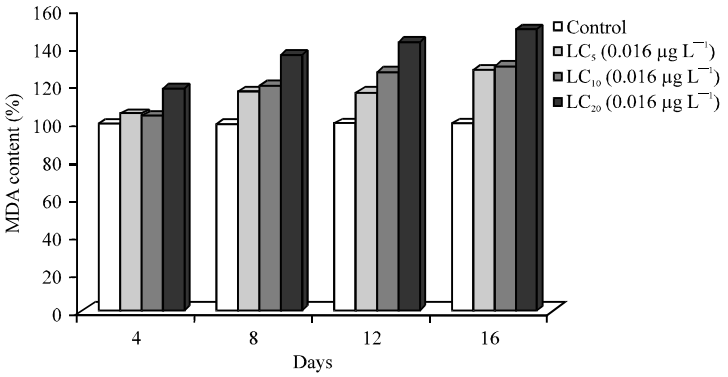

Fig. 2: Effect of deltamethrin on LPO in muscle of zebrafish

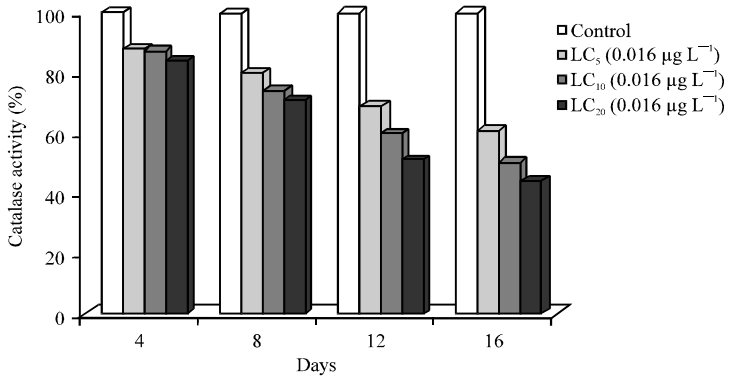

Fig. 3: Effect of deltamethrin on CAT activity in brain of zebrafish

the 16-days post-exposure. Significant elevation of LPO in the muscle was observed by the 4-days post-exposure, with the maximum (by 150% of control) recorded at the end of the studied period (Fig. 2).

Figure 3 shows the effects of deltamethrin exposure on the activities of CAT in fish brain. The CAT activities decreased throughout the post-exposure periods. The highly significant ($p<0.001$) change was recorded on the 8-days, when CAT activity was decreased by 71% after exposure to LC_{20} of deltamethrin. The inhibition rate persisted up to the end of the experiment. In the muscle, CAT activity was decreased, with maximum thresholds on the 16-days (by 54%, compared with controls) at LC_{20} of pesticide treatment. A time and concentration dependent decrease was also noticed in CAT activity (Fig. 4).

In the brain of fish a significant ($p<0.05$) decrease of GSH levels up to the 16-days was observed (Fig. 5). In the muscle, a sudden decrease by approximately 69% of GSH was recorded on the 8-days post-exposure while by the end of the experimental tenure it decreased to approximately 43% of control (Fig. 6) at highest pesticidal treatment.

DISCUSSION

Aquatic organisms have developed several cellular defense paths, which under normal metabolic conditions regulate the level of ROS and protect against the deleterious effects of free radicals. This defense system includes both antioxidant enzymes, such as SOD, CAT, GPX, GR and

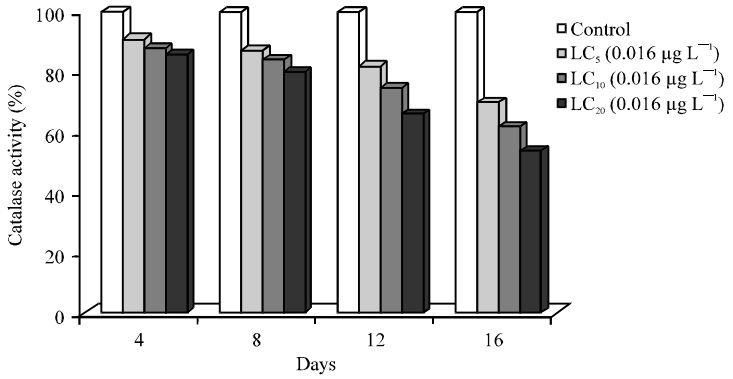

Fig. 4: Effect of deltamethrin on CAT activity in muscle of zebrafish

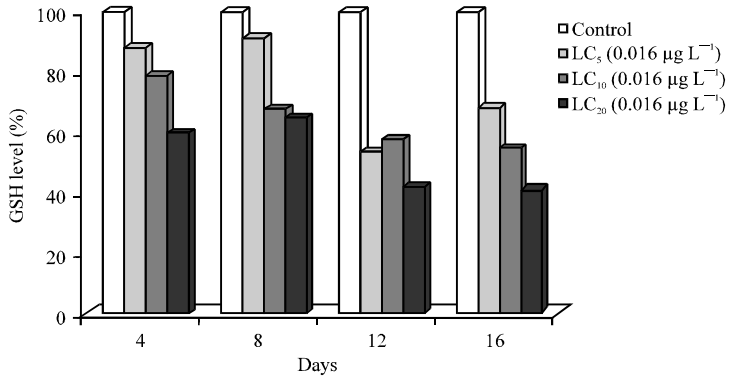

Fig. 5: Effect of deltamethrin on GSH level in brain of zebrafish

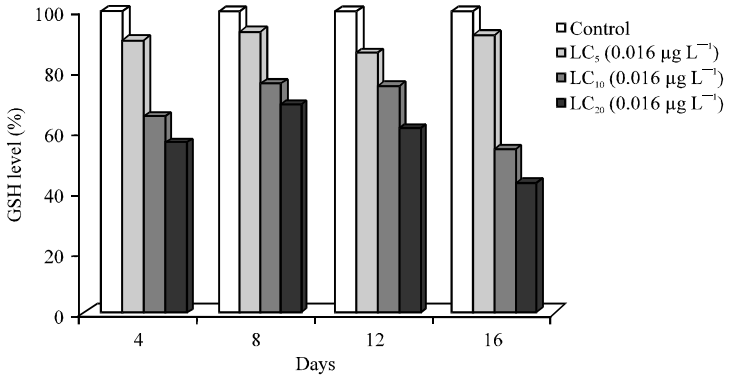

Fig. 6: Effect of deltamethrin on GSH level in muscle of zebrafish

GST and low-molecular-weight free-radical scavengers, such as the tripeptide glutathione. Most of the research on oxidative stress in fish has focused on toxicological effects of different xenobiotics on antioxidant systems and LPO (Bainy *et al.*, 1996; Fatima *et al.*, 2000). The consequences of deltamethrin, particularly its effects on oxidative stress and antioxidant system, were investigated in fishes (Sayeed *et al.*, 2003; Atif *et al.*, 2005; Yildirim *et al.*, 2006). The relevant induction of LPO observed in present study in tissues of pesticide exposed fish, suggested an oxidative stress

condition due to over accumulation of ROS. A similar response was found in fish exposed sub-chronically to industrial pollutants (Sayeed *et al.*, 2003; Lima *et al.*, 2006; Gultekin *et al.*, 2000).

Lipid peroxidation was enhanced in tissues (liver, gill, muscle and brain) of *Oreochromis mossambicus* treated with (RPR-V) an organophosphate insecticide (Rao, 2006). Agrahari *et al.* (2006) also observed an increase in the total lipid content in liver, gill, muscle and brain of *Channa punctatus* exposed with monocrotophos. Lipid peroxidation was increased in the brain of freshwater fish, *Oreochromis niloticus* treated with diazinon (Uner *et al.*, 2006). Recently, Parthasarathy and Joseph (2011) found that λ-cyhalothrin caused a significant increase in the LPO in the liver as compared to control.

Our results have shown that CAT activities and GSH level were highly decreased in brain of exposed fish, particularly at the 16-day after deltamethrin exposure. Similar effects were reported in *Channa punctata* Bloch (Atif *et al.*, 2005) but the increases noticed for the liver antioxidants enzymes and GSH. This observation suggested that, CAT is responsible for the catabolism of hydrogen peroxide generated in the tissues of deltamethrin exposed fishes. It is suggest that the decrease in the activities of CAT, noticed in the 16-days of deltamethrin exposure, were sufficient to prevent the rise of LPO in brain and muscle.

The concentration dependent decrease in the CAT activity observed in this investigation is in the agreement with the report of other workers (Gultekin *et al.*, 2000; Pascual *et al.*, 2003). The explanation of decreased CAT activity could be provoke by the flux of superoxide radicals (O^{2-}) induced by the pollutants (Ahmad *et al.*, 2000). According to Mekail and Sharafaddin (2009) the activity of CAT was decreased in brain, liver and kidney of weanling rat treated with diazinon, carbaryl and λ-cyhalothrin. Our results show decrease in CAT activity in brain and muscle of zebrafish exposed to deltamethrin with respect to control which may be due to oxyradical production. A time-dependent decrease in the CAT activity was also observed by Rosety *et al.* (2005) in sea bream gills exposed to malathion. Wielgomas and Krechniak (2007) have been reported the alteration in CAT activity in wistar rat liver after exposure to alpha-cypermethrin and chlorpyriphos. Recently, Naveed *et al.* (2011) reported the decrease in CAT activity in tissues (brain, liver and kidney) of *Channa punctatus* exposed to triazophos. Tripathi and Bandooni (2011), reported that the specific activity of CAT declined significantly ($p<0.05$) in liver of *Clarias batrachus* after treatment with alphamethrin (0.018 ppm) for 14 days. It might be due to binding of deltamethrin to CAT or by inhibiting CAT synthesis as reported by Sayeed *et al.* (2003).

The increase in GSH has been described as one of the protective mechanisms that fish adopt in the exposure to aquatic pollutants (Ansari *et al.*, 2009). Banerjee *et al.* (1999) studied the effect of some pesticides on GSH and observed the decreased level of GSH by pesticide exposure. Rao (2006) studied the depletion of GSH in the gill, liver, brain and muscle of *Oreochromis mossambicus* after pesticide treatment. Farombi *et al.* (2008) observed the reduction in GSH in tissues (kidney, gill and heart) of African catfish, *Clarias gariepinus* treated with butachlor. The insecticide raid decreased the GSH levels in the liver and this has implications for the ability of the animal to withstand oxidative stress (Achudume *et al.*, 2010). Also, Jin *et al.* (2011) studied the effects of cypermethrin on the hepatic oxidative stress and DNA damage in zebrafish.

CONCLUSION

In conclusion, the available experimental data provide support to the concept that oxidative stress is a highly important mechanism in pesticide-induced toxicity. Our study revealed that

deltamethrin induced a distinct oxidative stress in the brain and muscle of zebrafish. The difference in the level of CAT, LPO and GSH detected in brain and muscle may be correlated with the specific metabolic pathways of these two tissues. These biochemical adaptive mechanisms may help to keep the oxidative stress at a tolerable level during pesticide exposure. This notwithstanding, there is much yet to learn about the details of this phenomenon and further research is needed to more fully elucidate the effects that pesticides have and the environmental risks they pose in the aquatic organisms.

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