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Time Dependent Variations of Antioxidant Enzyme Activities in Different Organs of Fresh Water Fish *Cyprinus Carpio* exposed to Synthetic Pyrethroids, Cypermethrin

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ABSTRACT

The indiscriminate usage of various types of pesticides in the modern world has led to serious environmental hazards, threatening the survival of mankind. The present study was undertaken to evaluate the influence of cypermethrin, a widely used synthetic pyrethroids pesticide, on antioxidant enzymatic defense system and lipid peroxidation in different organs of fresh water fish, *Cyprinus carpio*. Fishes were exposed to sub lethal concentration of cypermethrin for three different durations, 7, 14 and 21 days and were sacrificed 24 hrs after the exposure. The enzyme estimations were performed in gills, liver and brain by standard spectrophotometric methods and the values were compared with the controls. The activity of catalase was increased in gills and brain and decreased in the liver. The activity of superoxide dismutase was increased in gills and liver in all the durations and decreased in the brain. Glutathione peroxidase was decreased in all the three organs. In all the three organs, glutathione reductase and glutathione-s-transferase activity was significantly reduced. A significant increase in lipid peroxidation was observed in the liver, gills and brain of the fish following exposure to cypermethrin. In all the three organs studied the influence of cypermethrin was found to be exposure of time dependent.

Keywords: Cypermethrin, *Cyprinus carpio*, Antioxidant enzymes, Lipid peroxidation

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INTRODUCTION

The use of pesticides has increased with the growing awareness about their utility in agriculture production. The current trend of using excessive amount of insecticides and chemical fertilizers for increasing the productivity of crops and altogether overlooked the potential for secondary deleterious effects on non target flora and fauna. Synthetic pyrethroids have been introduced over the past two decades for agricultural and domestic use as replacements for more toxic pesticides, such as chlorinated hydrocarbons, organophosphates and carbamates[1].

Several studies have reported that pyrethroids are highly toxic to a number of non target organisms[2,3] and these pyrethroids are readily absorbed by the gills of fish even at very low concentration[4,5]. Thereby it progressively increases the sensitivity of fish to the toxic effect of these insecticides[6]. Among the synthetic pyrethroids, cypermethrin is one of the top ranked pesticides in annual usage. The widespread use of the cypermethrin in agricultural and public health applications, it is considered as the most effective pyrethroids[7] and extensively used in our regional agricultural fields.

In India, cypermethrin is registered for use on a wide array of crops including cotton, cabage, okra, brinjal, sugarcane, wheat and sunflower. Almost 70% of all sprays used on cotton in Andhra Pradesh in India are pyrethroids, which consists of mostly the cypermethrin[8]. It is found in many household ant and cockroach killers and ant chalk. These pesticides ultimately reach the aquatic systems through different pathways, affecting various aquatic organisms and reaches to human through food chain.

Pesticides may induce oxidative stress, leading to generation of free radicals and cause lipid peroxidation which shows the molecular mechanisms involved in pesticide-induced toxicity[9,10,11]. Antioxidant system plays a crucial role in maintaining cell homeostasis[12]. Oxidative stress may produce DNA damage, enzymatic inactivation, and peroxidation of cell constituents, especially lipid peroxidation when antioxidant defenses are impaired or overcome. Antioxidant defenses includes antioxidant enzymes and free-radical scavengers whose function is to remove reactive oxygen species, thus protecting organisms from oxidative stress[13].

Fish can serve as bio-indicators of environmental pollution and can play significant roles in assessing potential risk associated with contamination in aquatic environment and it may also serve as good monitoring tools for understanding oxidative stress. The response to oxidative stress has also an ecological significance, particularly, in aquatic environments. Hence, the present study was carried out to evaluate the toxicity of cypermethrin in antioxidant system of

fresh water fish Cyprinus Carpio, the common carp.

MATERIALS AND METHODS

Maintenance of fishes

Healthy specimens of adult Cyprinus carpio of both sexes, with uniform weight of 95 ± 5 g were procured from Government fish pond, Puducherry. While collection, care was taken to avoid stress and injury to fishes, then they were carefully transported to the laboratory in oxygen pack. The active and healthy Cyprinus carpio were selected for acclimatization during which they were kept in glass aquaria for 10 days. During acclimatization, the fish were fed with commercial food pellets. The water was changed regularly; the remaining food and faecal matters were removed periodically. The water quality is also monitored periodically. Physico-chemical characteristics of the experimental medium such as temperature, pH, salinity, dissolved oxygen and total hardness were analyzed following standard procedure (APHA, 1998). The healthy fishes were subsequently used for the present study. The fishes were examined carefully for any pathological symptoms and placed in dilute water containing 0.1 mg/l of potassium permanganate solution to avoid the possibility of any dermal infection.

Experimental design

Healthy and same sized Cyprinus carpio were chosen and sorted into 4 groups of 15 fishes each.

Group I: Control fishes

Group II: Fishes exposed to 1/10 of Lc_{50} value of cypermethrin (0.6mg/L), for 7 days (Expt. I)

Group III: Fishes exposed to 1/10 of Lc_{50} value of cypermethrin (0.6mg/L), for 14 days (Expt. II)

Group IV: Fishes exposed 1/10 of Lc_{50} value of cypermethrin (0.6mg/L), for 21 days (Expt. III)

The dose was selected based on 96 hrs LC_{50} value.

Test solution was renewed daily, which facilitated the removal of nitrogenous waste excreted by the test fishes and for the removal of unconsumed food. The fish were fed during the experiment at least twice (morning and evening) a day. Feeding was stopped 24 h prior to sacrifice. The stock and test solution was prepared by dissolving the pesticide in acetone. Fish kept in a pesticide free medium served as control. The same volume of acetone used in the dissolution of pesticide was maintained in the control.

24 hours after the respective experimental period the fishes were sacrificed and the key organs such as gills, brain and liver were surgically removed. Tissues were thoroughly washed in normal cold saline (4–6◦C), blotted dry, weighed, and homogenized in 50mMTris-HCl buffer (pH7.5) using Potter- Elvehjam homogenizer fitted with a Teflon-coated pestle. The

homogenates were centrifuged at 4°C for 20 min at 10,000 g in a refrigerated centrifuge. The corresponding supernatants were either used fresh or kept frozen at -20◦C until further use for biochemical analysis.

Biochemical analysis

Total protein was estimated by the method of Lowry et al[14]. Superoxide dismutase activity (EC 1.15.1.1) was assayed following the method of Marklund and Marklund[15]. Catalase (EC 1.11.1.6) was assayed according to the method of Sinha[16]. Glutathione peroxidase (EC 1.11.1.9) activity was assayed according to the method of Rotruck et al[17]. Glutathione reductase (EC 1.6.4.2) activity was determined by the method of Stall and Vegal[18]. Glutathione-s-transferase activity (EC 2.5.1.1.8) was estimated by the method of Habig et al[19]. Lipid peroxidation was measured by the method of Devasagayam and Tarachand[20].

Statistical analysis

All the data were analyzed using Student's t- test and the data were expressed as mean± SEM. The p value of <0.05 was considered as significant against control.

RESULTS AND DISCUSSION

Catalase

(Table 1): In seven days exposed group in the brain slight but significant ($p< 0.05$) increase was observed in the specific activity of catalase and no change was evident in liver and gills. The specific activity of catalase was increased in gills and brain in 14 days ($p<0.05$) and 21 days ($p<0.01$) exposed group. Interestingly in the liver the specific activity of catalase was decreased in 14 days ($p<0.01$) and 21 days ($p<0.001$) exposed group.

Table 1 Effect of cypermethrin on catalase activity in different organs of fresh waterfish *Cyprinus carpio*

Organs	Control	(E1)	(E2)	(E3)
Gills	61.23 ± 2.26	65.54 ± 2.75	73.34 ± 3.039 *	74.68 ± 2.22 * *
Brain	52.64 ± 2.38	56.63 ± 1.15 *	59.34 ± 1.15 *	64.85 ± 2.14 * *
Liver	79.67 ± 2.49	76.65 ± 3.89	68.39 ± 1.36 * *	60.37 ± 1.86 * * *

Superoxide dismutase

(Table 2): The activity of SOD decreased significantly ($p< 0.05$) in the brain of seven days exposed fishes. No significant change was evident in liver and gills. However, in 14 days and 21 days exposed group significant changes were observed in all the three organs studied. The specific activity of superoxide dismutase was increased in gills and liver in 14 days ($p<0.05$) and 21 days ($p<0.01$) exposed group. Interestingly in the brain the specific activity of superoxide dismutase was decreased in 14 days ($p<0.01$) and 21 days ($p<0.01$) exposed group.

Table 2 Effect of cypermethrin on superoxide dismutase activity in different organs of fresh water fish *Cyprinus carpio*

Organs	Control	(E1)	(E2)	(E3)
Gills	45.53 ± 2.37	50.67 ± 1.49	57.64 ± 2.36 *	56.32 ± 1.132 * *
Brain	59.65 ± 1.49	52.62 ± 1.62 *	51.27 ± 1.24 * *	48.67 ± 1.97 * *
Liver	50.38 ± 1.31	55.63 ± 1.74	61.75 ± 1.4 *	63.74 ± 2.18 * *

Glutathione peroxidase

(Table 3): In seven days cypermethrin exposed group no appreciable changes were observed in the activity of GPx in all the three organs. In the gills of 14 days exposed group no significant change was observed while in the brain and liver activity is significantly ($p<0.05$) decreased. In 21 days exposed group the enzyme activity was significantly decreased in gills ($p<0.05$), brain ($p<0.05$) and liver ($p<0.01$).

Table 3 Effect of cypermethrin on glutathione peroxidase activity in different organs of fresh water fish *Cyprinus carpio*

Organs	Control	(E1)	(E2)	(E3)
Gills	80.75 ± 3.25	78.67 ± 2.97	76.61 ± 1.75	69.38 ± 1.57 *
Brain	77.94 ± 1.84	72.33 ± 1.82	69.64 ± 1.12 *	65.28 ± 2.12 *
Liver	84.65 ± 2.19	85.24 ± 2.27	76.36 ± 1.59 *	70.69 ± 1.66 * *

Glutathione reductase

(Table 4): No significant changes were observed in the activity of this enzyme in the gills and brain of seven days exposed fishes. However, the activity is markedly reduced ($p<0.05$) in the brain of seven days exposed group. In all the three organs, glutathione reductase activity is significantly reduced ($p<0.05$) in 14 days exposed fishes and further declined ($p<0.01$) in all the three organs of fishes exposed with cypermethrin for 21 days.

Table 4 Effect of cypermethrin on glutathione reductase activity in different organs of fresh water fish *Cyprinus carpio*

Organs	Control	(E1)	(E2)	(E3)
Gills	54.69 ± 2.57	52.39 ± 2.75	47.34 ± 1.59 *	38.58 ± 1.18 * * *
Brain	59.37 ± 2.46	52.41 ± 1.12 *	52.76 ± 1.48 *	46.37 ± 1.73 * *
Liver	53.62 ± 1.89	51.69 ± 1.67	47.33 ± 1.29 *	41.55 ± 1.61 * *

Gluthione-s-transferase

(Table 5): There was no significant alteration in this enzyme activity in fishes exposed to cypermethrin for seven days. In fishes exposed for 14 days, glutathione-s-transferase activity was significantly decreased in gills ($p<0.01$), brain ($p<0.01$) and liver ($p<0.05$). In 21 days exposed fishes in all the three organs glutathione-s-transferase activity was drastically decreased ($p<0.001$)

Table 5 Effect of cypermethrin on glutathione transferase activity in different organs of fresh water fish *Cyprinus carpio*

Organs	Control	(E1)	(E2)	(E3)
Gills	44.36 ± 1.75	46.52 ± 2.46	35.82 ± 1.43 **	32.74 ± 0.947 * *
Brain	39.47 ± 1.68	42.68 ± 1.32	31.49 ± 0.894 * *	26.12 ± 1.34 * * *
Liver	41.35 ± 1.23	42.67 ± 1.27	35.57 ± 1.33 *	32.42 ± 0.843 * *

Lipid peroxidation

(Table 6): No significant changes were observed in seven days exposed fishes. Lipid peroxidation is increased significantly in all the three organs in 14 days exposed group and increased further in 21 days exposed fishes.

Table 6 Effect of cypermethrin on lipid peroxidation in different organs of fresh waterfish *Cyprinus carpio*

Organs	Control	(E1)	(E2)	(E3)
Gills	5.178 ± 0.219	5.249 ± 0.194	6.168 ± 0.178 * *	7.324 ± 0.219 * * *
Brain	6.492 ± 0.175	6.531 ± 0.169	7.461 ± 0.229 * *	9.147 ± 0.224 * * *
Liver	7.241 ± 0.317	6.948 ± 0.250	8.347 ± 0.247 *	8.968 ± 0.215 * *

Antioxidant systems can be considered as biomarkers of exposure to pollutants, and also as an indicator of toxicity. The induction levels of primary antioxidant defenses preventing cell damage can be regarded as an adaptive response to an altered environment; in contrast an inhibition can lead to cell damage and toxicity in a dose-dependent manner.

Fish are endowed with defensive mechanisms to counteract the impact of reactive oxygen species (ROS) resulting from the metabolism of various chemicals. These systems include various antioxidant defense enzymes such as superoxide dismutases which catalyze the dismutation of superoxide radical to hydrogen peroxide, catalase acting on hydrogen peroxide, glutathione S-transferase family possessing detoxifying activities towards lipid hydroperoxides generated by organic pollutants such as heavy metals[21, 22].

Under normal physiological status, the antioxidant defense systems including SOD, CAT and GST can be induced by a slight oxidative stress as a compensatory response, and thus the reactive oxygen species (ROS) can be removed to protect the organisms from oxidative damage. The liver in fish is an organ that performs various functions associated with the metabolism of xenobiotics[23]. Hepatocytes like other cells are dependent on antioxidant enzymes for the protection against reactive oxygen species produced during the bio transformation of xenobiotics[24]. SOD is responsible for the removal of hydrogen peroxide which is metabolized to oxygen and water[25]. Also SOD is the enzyme metabolizing superoxide radical and its levels are directly related to CAT activity.

The present study revealed that CAT activity increased in the gills and brain of Cyprinus carpio following cypermethrin exposure at sub lethal concentrations for 7, 14 and 21 days and in the liver, CAT activity was found to be decreased. The activity of SOD increased in the gills and liver, while in brain the activity of SOD decreased. The induction of SOD and CAT in the present study suggests that oxidative stress response still works well under the current conditions, and the increase of antioxidative enzymes may be a physiological adaptation for the elimination of ROS generation. Similar results have been observed in gilthead sea bream (Sparus aurata) and Carassius auratus exposed to polyaromatic hydrocarbons as phenanthrene[26, 27].

The activity of antioxidant may be increased or inhibited under chemical stress depending on the intensity and the duration of the stress applied as well as susceptibility of the exposed species. It is not a general rule that an increase in xenobiotic concentrations induces antioxidant activity[28]. In the presence of xenobiotic, an initial decreased response in the antioxidant system may be followed by an induction. Thus the existence of an inducible antioxidant system may reflect an adaptation of organism[29]. The response of antioxidant system to oxidative stress in various tissues shows differences from one species to another due to the differences in antioxidant potential of these tissues[30].

Superoxide dismutase is a primary oxygen radical scavenger of tissues converting superoxide anion into hydrogen peroxide and oxygen. Catalase is mainly located in the peroxisomes, and is responsible for the reduction of hydrogen peroxide produced from the metabolism of long chain fatty acids in peroxisomes[31]. Catalase activity was sensitive to pollutants in the liver of freshwater fish[32].

The decrease in the catalase activity of liver in the present study could also be due to flux of superoxide radicals inhibiting catalase activity[33]. The decline in the antioxidant enzyme activities with the increase in the duration of cypermethrin exposure may reflect an inability of the sub cellular structures to provide the required enzymes due to tissue damage under condition of oxidative stress. Pesticide-induced inhibition in catalase activity was reported by various studies in fish species. For example, Gupta et al[34] reported that methoxychlor inhibits mitochondrial respiration, causes reactive oxygen species production, and decreases the activity of antioxidant enzymes such as superoxide dismutase and catalase.

Oxidative stress biomarkers assessed in liver tissue of Channa punctatus exposed to deltamethrin showed a decrease in catalase activity[35]. Bagnyukova et al[36] also reported a gradual decrease in the brain catalase activity of Carassius auratus in response to aminotriazole induced oxidative stress. Catalase activity was reported to be significantly declined in Channa punctatus exposed

to deltamethrin[35]. In Heteropneutes fossilis, monocrotophos treatment resulted in decrease in the catalase activity[37]. Deltamethrin exposure also caused significant decreases in catalase activities in liver, kidney and gill tissues of Channa punctatus[35]. This decline in catalase activity could be due to the excess production of superoxide anion as indicated by[38].

The apparent increase in SOD activity in the gills and liver of the fish may be due to the production of superoxide anions which led to the induction of SOD, to convert the superoxide radical to H_2O_2. The increase in CAT activities in the gills and brain may be a response to the hydrogen peroxide produced by SOD activity since CAT is responsible for the detoxification of hydrogen peroxide to water. Increase in the activity of CAT and SOD is usually observed in the face of environmental pollutants since SOD-CAT system represents the first line of defense against oxidative stress[39].

CAT activity, found mainly in peroxisomes, is associated with elevated concentrations of H_2O_2. GPX catalyses the reduction of H_2O_2 derived from oxidative metabolism as well as peroxides from oxidation of lipids and is considered the most effective enzyme against lipid peroxidation[31]. Its activity is considered complementary to CAT activity, being especially suited for hydroperoxide detoxification at low substrate concentrations[40, 41]

Glutathione peroxidase (GPx) is the most important peroxidase that has been postulated to protect the erythrocytes from damage by H_2O_2. It catalyzes the glutathione dependent reduction of hydroperoxides and of hydrogen peroxide. Therefore, it is hypothesized that this enzyme may protect tissues against oxidative damage due to lipid peroxidation. Environmental pollutants may induce glutathione peroxidase activity.

The activity of glutathione peroxidase and glutathione reductase in all the organs studied was found to be decreased gradually during the exposure period with cypermethrin. This indicates the defect in the protective role of the enzyme against lipid peroxidation. This probably reflects an unfavorable adaptation to the oxidative conditions to which the fish have been exposed.

Xenobiotic metabolizing enzymes glutathione reductase (GSH), glutathione S-trans ferase (GST), which bio-transform different toxic agents to water soluble products and used as important biomarkers for environmental condition. Fish exposed to pollutants is thought to generate free radicals especially reactive oxygen species (ROS) with subsequent alteration in fish antioxidant defense. GSH is important in protecting against deleterious effects of the cell exposed to ROS by reacting with them to form glutathione disulphide. This antioxidant effect occurs spontaneously through GSH or may also be catalyzed by glutathione S-transferase[42].

Lipid peroxidation is one of the main processes induced by oxidative stress. Lipid peroxides are formed from the oxidative deterioration of poly unsaturated lipids in the membranes of cells and organelles. Lipid peroxidation bi-products, such as malondialdehyde are used as indicators of increased concentration of cellular reactive oxygen species and a sign of cellular injuries[43]. Diverse contamination can initiate lipid peroxidation, including organic compounds, pesticides and heavy metals.

A significant increase in lipid peroxidation as MDA formation was observed in the liver, gills and brain of the fish following exposure to cypermethrin at sub lethal concentrations. The data indicate that reactive oxygen species may be associated with the metabolism of cypermethrin leading to peroxidation of membrane lipids of the respective organs. Previous investigations have reported the induction of lipid peroxidation by other pesticides such as endosulfan[44] and cypermethrin[45] in fish. The observed lipid peroxidation resulting possibly from ROS generated by the compound may lead to cell apoptosis. ROS and oxidative stress have been shown to be triggers of apoptosis[46]. Exogenous ROS such as H_2O_2 at moderate levels induce apoptosis in many cell types[47]. Endogenously produced ROS have also been found to be important in the apoptotic cell death triggered by many other stimuli including environmental chemicals[48].

CONCLUSION

It is evident from the present study that cypermethrin influences antioxidant system differently in different organs and also it is exposure time dependant. The present results suggest that when reactive oxygen species are generated in excess or there is not enough oxygen radical scavenging activity, free radical chain reactions are stimulated and interactions with protein, lipids, and nucleic acids cause cellular damage and even systemic disease in stressed fish.

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