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Effect of cypermethrin toxicity on enzyme activities in the freshwater fish *Cyprinus carpio*

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Cyprinus carpio **a freshwater fish, was exposed to lethal concentration (7.5 µg/L) for one, three, five, seven and nine days and, sublethal concentration (1.5 µg/L) for 1, 7, 14, 21 and 28 days of cypermethrin, respectively to observe the enzyme activity in functionally three different tissues; that is, muscle, gill and liver. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and glutamate dehydrogenase (GDH) were increased in all the tissues with an increase in exposure time of cypermethrin. Though, under sublethal concentration of cypermethrin for 14, 21 and 28 days, a decreasing trend was observed in all the three tissues. The increased levels of amino transferase might be attributed to tissue damage under toxic stress in *C. carpio*. It has been concluded that the usefulness of the enzymes as biomarkers of cypermethrin toxicity appeared to be concentration and tissue dependent and can be effectively used to assess the impact of the agrochemical on the fish.**

Key words: Cypermethrin, *Cyprinus carpio*, enzymes activity, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamate dehydrogenase (GDH).

INTRODUCTION

In agriculture, indiscriminate use of different pesticides to prevent the crop from pest peril has increased in the developing countries (Santhakumar and Balaji, 2000). The pesticides, even when applied to restricted areas are washed and carried away by rains and floods to large water bodies like ponds and rivers and change the water chemistry (Bhalchandra et al., 2001). It may be highly toxic, not only to fishes but also to other organisms, including man (Madhab et al., 2002).

In recent years, synthetic pyrethroids have been developed for major uses in agriculture and public health purposes. The current commercial products were evolved from the natural pyrethrins, which possess high insecticidal potency, low mammalian toxicity and very short persistence. These are highly toxic to fish and some aquatic invertebrates (Coats et al., 1989). Cypermethrin is being increasingly used as the active ingredient in many dips that are used to prevent and treat ticks, lice, and scrab on sheep and as a treatment against infestation by the parasitic sea louse in aquaculture. The sources of contamination of river courses occur as a result of the direct use of pyrethroid-based dips and also from the processing of sheep skin, wood industry and knitwear manufacture. The environmental concentrations of cypermethrin are often below those that are lethal to many freshwater teleost (McLeese et al., 1980; Stephenson, 1982; Ansari and Kumar, 1988; Philip et al., 1995). Studies have been done on the effects of a synthetic pyrethroid and cypermethrin on fish (Polat et al., 2002; Das and Mukherjee, 2003; David et al., 2004), but

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Abbreviations: AST, Aspartate aminotransferase; **ALT**, alanine aminotransferase; **GDH**, glutamate dehydrogenase.

there is little information on the comprehensive effects of cypermethrin during exposure and post exposure period. Earlier reports are available on the sublethal effects of cypermethrin on lipids, free fatty acids, metabolites and enzymes of protein and carbohydrate metabolism of fish during exposure and recovery phase (Begum 2005a, b).

Shivaknmar (2005) reported that the activity of aspartate and alanine amino transferases (AST and ALT), may serve as strategic links between protein and carbohydrate metabolisms, which is known to alter under several physiological and pathological conditions. Reddy and Venugopal (1990) stated that glutamate dehydrogenase (GDH), a mitochondrial enzyme, catalysis the oxidative deamination of glutamate, provides α-ketoglutarate to the Krebs cycle. This enzyme has several metabolic functions with great physiological significance. It is closely associated with the detoxification mechanisms of tissues. GDH in extra-hepatic tissues could be utilized for the channeling of ammonia released during proteolysis for its detoxification into urea in the liver. Hence, the activities of AST, ALT and GDH are considered as sensitive indicators of stress (Gould et al., 1976).

Enhancement in GDH activity in the tissues provided ketoglutarate and reduced nucleotides, which may fulfill the energy requirements during toxicity manifestations (Chandravathy and Reddy, 1994). The regulatory roles of GDH enzyme as observed in mammalian models in checking the deamination process reported earlier (Philip et al., 1988; Ramana et al., 1990; Reddy and Venugopal, 1990; Reddy and Yellama, 1991; David, 1995; Deva, 2000 and Shobha Rani et al., 2001; Prashanth and Neelagund, 2008).

Glutamate dehydrogenase (GDH), a mitochondrial enzyme, catalysis the oxidative deamination of glutamate, provides α-ketoglutarate to the Krebs cycle (Reddy and Venugopal, 1990). Therefore, the present study was aimed at investigating the effect of the synthetic pyrethroid, cypermethrin on aspartate aminotransferase, alanine amino transferase and glutamate dehydrogenase activity in the economically important freshwater fish, *C. carpio*.

MATERIALS AND METHODS

Collection and maintenance of fish

Healthy and active *C. carpio* fingerlings were procured from the Fisheries Department. Fish were brought to the laboratory in large aerated crates, acclimated for 30 days in large fiber tanks (7×4 × 2 m) and fed with commercial dry feed pellets.

In the laboratory, the fish were held in 100 L glass aquaria (120 × 45 × 80 cm) containing dechlorinated tap water for acclimatization for a period of 20 days at 22±1°C. The physio-chemical characteristics of the tap water were followed as described in APHA (1998). Water was renewed every day and a 12 to 12 h photoperiod was maintained during the acclimatization and test periods. The fish were fed regularly with commercial fish food pellets during the acclimatization and test tenures, but feeding was stopped two days before the exposure to test medium for acute toxicity test.

Preparation of stock and acute toxicity test

Technical grade cypermethrin (95%) was obtained from Merck. After the normal process of acclimatization, a group of 10 fish each was transferred to the aquaria. Stock solution of lethal cypermethrin (7.5 µg/L) and sub-lethal concentration (1/5th of LC_{50} that is, 1.5 µg/L for 96 h) added into the water in the aquaria. Predetermined exposure of lethal concentration of fish was given for one, three, five, seven and nine days and sub-lethal concentration 1, 7, 14, 21 and 28 days according to Finney (1971). Each treatment was replicated three times. Control and challenged fishes were sacrificed at the end of each day in the laboratory. At the end of the experiment, the fish were killed with a blow on the head and gill, liver and muscle tissue were excised and immediately transferred to the deep freezer prior to analysis. Total protein content was estimated by the method of Lowry et al. (1951), amino acids by Moore and Stein (1954), and ammonia with the Nessler reagent as described by Bergmeyer (1965); Prashanth and Neelagund (2008).

Aspartate aminotransferase and alanine aminotransferase were assayed by the colorimetric method of Reitman and Frankell (1957). Alanine aminotransferase activity was expressed as µM pyruvate formed/mg protein/h and the AST activity as µM oxaloacetate formed/mg protein/h. Glutamate dehydrogenase was assayed by the method of Lee and Hardy (1965). GDH activity was expressed as µM formozan formed/mg protein/h. The experiments were repeated for seven times to get concurrent values.

Statistical analysis of data

The data obtained was analyzed statistically by following Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSIONS

The present results show that cypermethrin-induced alterations are time dependent, tissue-specific and they point to disrupt activity. GDH, AST and ALT enzymes has shown significant elevation in all tissues after lethal and sub lethal exposure (Tables 1 to 3). A progressive increase was noticed in the activities of ALT, AST and GDH in all the organs of the fish exposed to cypermethrin. It might be due to the active trans-deamination of amino acids for the incorporation of ketoacids into the tricarboxylic acid (TCA) cycle to release necessary energy required for the synthesis of new proteins (Sreedevi et al., 1992; Sivaramakrishna and Radhakrishnaiah, 1998). The elevation in these enzymes was probably due to the utilization of amino acids during this cycle. The elevation in transaminases suggests the existence of the heavy drain on metabolites during cypermethrin stress. Awasthi et al. (1984) proposed that stress conditions in general induce elevation in the transamination pathway. The present results are in line with the findings of above mentioned studes. Involvement of alternate pathways like aminotransferase reactions are also possible due to inhibition of oxidative enzymes like isocitrate dehydrogenase and cytochrome C -oxidase, a situation demonstrated by Ghosh (1989) in *Labeo rohita*

Table 1. GDH activity (µM glutamine/mg protein/h) in the organs of fish, *Cyprinus carpio* on exposure to the lethal and sub lethal concentrations of cypermethrin.

Organ	Control	Exposure period in days									
		Lethal					Sublethal				
		1	3	5	7	9	1	7	14	21	28
Gill	0.130^{J}	0.146^{I}	0.159^{H}	0.182^{G}	0.198^{F}	0.233^{E}	0.257^{C}	0.274^{B}	0.288^{A}	0.252^{D}	0.231^{E}
SD±	0.003	0.004	0.006	0.005	0.007	0.006	0.009	0.010	0.008	0.009	0.007
Muscle	0.176^{H}	0.186^{G}	0.209^{F}	0.242^{C}	0.256^{B}	0.276^{A}	0.211^{F}	0.233^{B}	0.244^{C}	0.238^{D}	0.229^{E}
SD±	0.001	0.002	0.001	0.003	0.003	0.004	0.002	0.003	0.005	0.002	0.001
Liver	0.421^{F}	0.449^{E}	0.557^{C}	0.631^{B}	0.671^{A}	0.404^{F}	0.445^{E}	0.553^{D}	0.559^{C}	0.401^{G}	0.336^{H}
SD±	0.003	0.004	0.005	0.003	0.003	0.002	0.004	0.003	0.006	0.007	0.005

Values are means ± SD (n=6) for a tissue. Values in a column followed by the same letter are not significantly different ($P < 0.05$) from each other according to Duncun's multiple range (DMR) test.

exposed to cypermethrin. The changes in the activities of the amino transferases would often be reflected in nitrogen metabolism and interdependent biochemical reactions. The increased levels of amino transferase might be attributed to tissue damage under toxic stress in *C. carpio*. Similar findings were reported by Raju and Ramna, (1985). AST, a key enzyme of nitrogen metabolism and energy mobilization in invertebrates, is often used as a biochemical indicator of stress (Shobha Rani et al., 2001; Reddy and Venugopal, 1990). We are of the view that the increase in AST and ALT levels indicate that *C. carpio* was under toxic stress. The amino acids appear to be mobilized to get transamination to 2-keto acids, for use in the production of energy rich compounds (David, 1995; Rajamannar and Manohar, 1998; Deva, 2000).

Enhancement of GDH activity in the tissues provided ketoglutarate and reduced nucleotides, which may fulfill the energy requirements during toxicity manifestations (Chandravathy and Reddy 1994). GDH is also known to play a crucial role in ammonia metabolism and is known to be affected by a variety of effectors (Shakoori et al., 1976; David, 1995). After several metabolic functions with great physiological significance and known to be closely associated with the detoxification mechanisms of tissues, GDH in extrahepatic tissue could be utilized for its ultimate detoxification to urea in the liver. In the present study the significant elevation in the activities of these enzymes in the organs of fish exposed to the lethal concentration of cypermethrin was probably due to greater association of oilgomers of these enzymes in response to toxic stress. This shows that oxidative deamination contributes to higher ammonia production. The high levels of ammonia produced is not eliminated but is salvaged through GDH activity, which is utilized for amino acid synthesis through transaminases (David, 1995; Deva, 2000 and Prashanth 2003; Begum, 2004). The GDH elevation in all tissues (Table 1) also suggests the possibility of a need for α-ketoglutarate to the TCA cycle for the liberation of energy. In the present study, the GDH activity showed a progressive enhancement in all tissues (gill, muscle and liver), throughout the exposure, suggesting a need for α-ketoglutarate.

The regulatory roles of this enzyme as observed in mammalian models in checking the deamination process were reported earlier (Moorthy et al., 1984; Philip et al., 1988; Ramana et al., 1990; Reddy and Venugopal, 1990; Reddy and Yellama, 1991; David, 1995; Deva, 2000; Shobha et al., 2001; Prashanth and Neelagund, 2008). GDH catalyzes the reversible reaction of oxidative deamination of glutamate to α-ketoglutarate and ammonia (Begum and Vijayaraghavan, 1998) and plays an important role in the catabolism and biosynthesis of amino acid. GDH activity was enhanced in muscle and liver tissues for 28 days of cypermethrin toxicity, which indicates increased deamination of glutamate and formation of ammonia. It has been observed that the sublethal exposure of cypermethrin produced less change in the protein metabolism. It has also been noticed that the liver, gill and muscle were affected and the disturbances were found to be more in those tissues than that of muscle tissue.

Increased activities of AST (Table 2) and ALT (Table 3) in the study indicate that there may be an active transamination of amino acids, possibly to provide keto acid in the TCA cycle. The steady rise in the activities of GDH, AST and ALT in the organs of *C. carpio* exposed to sublethal concentrations of cypermethrin (Tables 1 to 3) may be due to the synthesis of these enzymes under sub acute cypermethrin stress. The increase in these enzyme activities could be helpful to the fish for structural reorganization of proteins and incorporation of keto acids into the TCA cycle to favor gluconeogenesis or energy production. The increase in transaminases can also link to the formation of urea (Ramna and Ramamurthi, 1983). The gradual increase in the activities of AST, ALT and GDH lead to metabolic compensation and allow the

Table 2. The aspartate aminotransferase (AST) activity (µM oxaloacetate /mg protein/h) in the organs of fish, *Cyprinus carpio* on exposure to the lethal and sub lethal concentrations of cypermethrin.

Organ	Control	Exposure period in days									
		Lethal					Sublethal				
		1	3	5	7	9	1	7	14	21	28
Gill	1.462^{I}	1.68^{F}	1.825^{F}	1.973^{E}	2.177^{D}	2.431^{C}	2.720^{B}	2.882^{A}	2.015^{H}	1.732^{G}	1.574^{H}
SD±	0.006	0.010	0.007	0.006	0.053	0.0557	0.002	0.004	0.050	0.007	0.009
Muscle	2.205^{J}	2.555^{I}	2.942^{G}	3.221^{F}	3.543^{D}	3.620^{C}	3.885^{B}	3.917^{A}	3.392^{E}	3.002^{G}	2.723^{H}
SD±	0.008	0.012	0.015	0.020	0.018	0.022	0.027	0.021	0.028	0.019	0.017
Liver	2.467^{H}	2.884^{G}	3.221^{F}	3.478^{E}	3.886^{C}	4.123^{B}	4.234^{A}	4.112^{B}	3.946^{C}	3.657^{D}	3.23^{F}
SD±	0.030	0.040	0.055	0.047	0.063	0.074	0.079	0.081	0.066	0.007	0.008

Values are means ± SD (n=6) for a tissue. Values for a tissue in a column followed by the same letter are not significantly different ($P < 0.05$) from each other according to Duncun's multiple range (DMR) test.

Table 3. The alanine aminotransferase (ALT) activity (µM pyruvate formed /mg protein/h) in the organs of fish, *Cyprinus carpio* on exposure to the lethal and sub lethal concentrations of cypermethrin.

Organ	Control	Exposure period in days									
		Lethal					Sublethal				
		1	3	5	7	9	1	7	14	21	28
Gill	1.662^{I}	1.889^{H}	2.112^{G}	2.477^{B}	2.876D	3.112^{C}	3.237^{B}	3.431^{A}	3.214^{B}	2.750E	2.554^{F}
SD±	0.009	0.011	0.014	0.019	0.018	0.023	0.026	0.029	0.022	0.027	0.018
Muscle	4.575^{J}	4.984^{I}	5.886^{F}	6.324^{D}	6.778^{B}	6.931^{A}	6.114^{D}	6.448^{C}	5.994^{E}	5.576^{G}	5.341^{H}
SD±	0.022	0.029	0.034	0.039	0.041	0.044	0.033	0.051	0.052	0.047	0.061
Liver	6.131^{K}	6.778^{J}	7.723^{H}	8.886^{E}	9.997^{B}	10.234^{A}	9.886^{C}	9.236^{D}	8.687^{F}	7.965^{G}	6.876^{I}
SD±	0.056	0.060	0.550	0.623	0.675	0.723	0.765	0.663	0.559	0.721	0.645

Values are means ± SD (n=6) for a tissue. Values for a tissue in a column followed by the same letter are not significantly different ($P < 0.05$) from each other according to Duncun's multiple range (DMR) test.

animal to adapt to the imposed toxic stress. The increase in GDH activity at the sub lethal concentration (Table 1) could lead to increased production of glutamate in order to eliminate ammonia. To have an insight into the role of these enzymes in the altered metabolism of cypermethrin intoxicated fish, the activities of both AST and ALT were investigated in the present experiment. Elevated levels of AST and ALT indicate the enhanced transamination of amino acids, which may provide keto acids to serve as precursors in the synthesis of essential organic elements. It is likely that toxic stress imposed by cypermethrin might be one of the factors for the observed activities of AST and ALT in the present study.

Conclusion

It can be concluded from the current study that the sublethal exposure of cypermethrin produced less change in the protein metabolism. It has also been observed that the liver, gill and muscle were affected and the stress was found to be more in liver and gill than that of muscle tissue. With respect to the toxic effects on exposure to sublethal concentration of cypermethrin, the fish tries to withstand the toxic effects imposed by the pesticide by modulating their physiological and metabolic response towards proper utilization of enzymes and proteins for synthetic processes but after 28 days exposure recovery was not possible. These kinds of studies will help to determine remedial measures to be taken at appropriate times in a polluted organism, particularly fish and thus prevent ill effects in fish consumers.

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