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Effect of a Synthetic Pyrethyroid, Cypermethrin, on Aminotransferases and Glutamate Dehydrogenase Activities in Gill, Liver and Muscles of a Freshwater Fish, *Cyprinus carpio*

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Abstract.- Freshwater fish, *Cyprinus carpio* were exposed to acute dose of cypermethrin at 7.5 µg/L for 1, 3, 5, 7 and 9 days and sub-lethal concentrations (1.5 µg/L) for 1,7, 14, 21 and 28 days to determine the change in enzyme activity in three functionally different tissues *viz.*, gill, liver and muscle. Cypermethrin induced alterations that are both time dependent and tissue-specific, with glutamate dehydrogenase (GDH), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) enzymes showing a significant elevation in all tissues after both lethal and sub-lethal exposure. The activities of ASAT, ALAT and GDH increased in all the tissues as exposure time increased.

Keywords: Cypermethrin, *Cyprinus carpio*, ALAT, ASAT, GDH, aminotransferases.

INTRODUCTION

The indiscriminate use of various pesticides to reduce the impact of pests on agricultural crops has increased over the years, especially in developing countries (Santhakumar and Balaji, 2000). These pesticides, even when applied in restricted areas may be washed by rains and floods into large water bodies such as ponds and rivers, consequently altering the physico-chemical properties of these water bodies (Bhalchandra and Lomte, 2001). These changes are proving to be highly toxic, not only to fish, but also to other organisms, which form the food of fish (Shakoori *et al.*, 1976; Madhab and Ajit, 2002; Shoaib *et al*., 2013). In recent years, synthetic pyrethroids have been developed for use in agriculture and for public health purposes. The current commercial products were evolved from natural pyrethrins, which possess high insecticidal potency, low mammalian toxicity and very short persistence. These are highly toxic to fish and some aquatic invertebrates, however (Coats *et al.*, 1989). Of these pyrethroids, cypermethrin is increasingly being used as the active ingredient in many dips that are used to prevent and treat ticks, lice and scab on sheep, and as a treatment against infestation by the parasitic sea louse in aquaculture. Contamination of river courses may occur as a result of the direct use of pyrethroid-based dips, and also from the processing of sheep skin and knitwear manufacture.

Das and Mukherjee (2003) and David *et al*. (2004) have investigated the effects of cypermethrin on fish, but there is little information on the comprehensive effects of cypermethrin during the exposure and post-exposure periods and there have been some previous research on the sub-lethal effects of cypermethrin on lipids, free fatty acids, metabolites and enzymes of protein and carbohydrate metabolism of fish during the exposure and recovery phases (Begum, 2005a,b).

The activity of aspartate and alanine amino transferases (ASAT and ALAT), which serve as strategic links between protein and carbohydrate metabolisms, is known to alter under several physiological and pathological conditions (Shivaknmar, 2005). Glutamate dehydrogenase (GDH), a mitochondrial enzyme, catalyses the oxidative deamination of glutamate, providing α-ketoglutarate to the Krebs cycle (Reddy and Venugopal, 1990). This enzyme has a number of physiologically significant metabolic functions. It is closely associated with the detoxification mechanisms of tissues and, in extra-hepatic tissues, can be utilized for the channelling of ammonia released during proteolysis for detoxification into

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urea in the liver. Hence, the activities of ASAT, ALAT and GDH are considered as sensitive indicators of stress (Gould *et al*., 1976).

The present study, therefore, aims to investigate the effect of the synthetic pyrethroid, cypermethrin, on ASAT, ALAT and GDH activity in the economically important freshwater fish, *Cyprinus 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 and then acclimatised for 30 days in large fiber tanks (22 **x** 12 **x** 5 feet) and fed with commercial dry feed pellets.

In the laboratory, the fish were held in 100 L glass aquaria (120 cm **x** 45 cm **x** 80 cm) containing dechlorinated tap water for acclimatization (20 days) at 22±1°C. The physico-chemical characteristics of the tap water followed those described in APHA (2005). Water was renewed every day and a 12-12 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 phases, but feeding was stopped 2 days before exposure to the test medium for the acute toxicity test.

Procedure adopted

Technical grade cypermethrin (95%) was obtained from Merck. After the normal process of acclimatization, a group of twenty five fish each was transferred to the aquaria. Acute dose at 7.5 µg/L and a chornic dose at 1.5 µg/L of cypermethrin were added in the water in aquaria for 1, 3, 5, 7 and 9 days and for 1,7, 14, 21 and 28 days, respectively (Finney, 1971). Both control and exposed fish were sacrificed at the end of each day. Gill, liver and muscle tissues were isolated and immediately transferred to a deep-freezer prior to analysis. Total protein content was estimated using the method of Lowry *et al*. (1951), amino acids according to Moore and Stein (1954), and ammonia with the Nessler reagent as described by Bergmeyer (1965).

ASAT and ALAT were assayed by the colorimetric method of Reitman and Frankel (1957) and expressed as µM pyruvate/mg protein/h and µM oxaloacetate/mg protein/h. GDH was assayed using the method of Lee and Hardy (1965), and expressed as µM formozan/mg protein/h. The experiments were repeated seven times to get concurrent values.

Statistical analysis of data

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

RESULTS AND DISCUSSION

The results of the present study revealed that cypermethrin induced alterations that are both time dependent and tissue-specific, with GDH, ASAT and ALAT enzymes showing a significant elevation in all tissues after both lethal and sub-lethal exposure (Tables I-II). In particular, a progressive increase was observed in the activities of ALAT, ASAT and GDH in all the organs of the fish exposed to cypermethrin. This suggests the active trans-deamination of amino acids for the incorporation of ketoacids into the Krebs cycle in order to release the energy required for the synthesis of new proteins (Sivaramakrishna and Radhakrishnaiah, 1998). The elevation of these enzymes as a whole indicates the utilization of amino acids, while the elevation in transaminases suggests the existence of a heavy drain on metabolites through cypermethrin stress, since stress conditions in general induce an elevation in the transamination pathway (Awasthi *et al*., 1984). Involvement of alternate pathways, such as 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* exposed to cypermethrin. Alterations in the activities of the aminotransferases 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 (Raju and Ramna, 1985) and ASAT, a key enzyme for nitrogen metabolism and energy mobilization in invertebrates, is often

Table I.- Effect of acute doses of cypermethrin administered at 7.5 µg/L for 9 days on the activities (Mean±SD) of glutamate dehydrodegase (GDH, µM formozan/mg protein/h), aspartate aminotransferase (ASAT, µM oxaloacetate/mg protein/h) and alanine aminotransferase (ALAT, µM pyruvate/mg protein/h) of gill, muscles and liver of a freshwater fish, *Cyprinus carpio.*

Tissue	Enzymes	Control (n=7)	Acute dose of cypermethrin (7.5 µg/L)				
			1 day (n=7)	3 days (n=7)	5 days (n=7)	7 days (n=7)	9 days (n=7)
Gill	GDH activity	0.13 ± 0.03^{F}	0.15 ± 0.04^{E}	0.16 ± 0.01^{D}	0.18 ± 0.01^{C}	0.20 ± 0.01^{B}	0.23 ± 0.06^{A}
	ASAT activity	1.46 ± 0.00^{F}	1.68 ± 0.01^{E}	1.82 ± 0.02^{D}	1.93 ± 0.03^{C}	2.18 ± 0.04^{B}	2.43 ± 0.05^{A}
	ALAT activity	1.66 ± 0.01^{F}	1.89 ± 0.02^{E}	2.11 ± 0.03^{D}	2.48 ± 0.02^{C}	2.88 ± 0.01^{B}	3.12 ± 0.02^{A}
Muscle	GDH activity	0.18 ± 0.01^{F}	0.19 ± 0.00^{E}	0.21 ± 0.01^{D}	0.24 ± 0.00^{C}	0.26 ± 0.01^{B}	0.28 ± 0.00^{A}
	ASAT activity	2.20 ± 0.08^{F}	2.55 ± 0.01^{E}	2.94 ± 0.01^{D}	3.221 ± 0.02^{C}	3.54 ± 0.01^{B}	3.62 ± 0.02^{A}
	ALAT activity	4.57 ± 0.02^{F}	4.98 ± 0.04^{E}	5.89 ± 0.05^{D}	6.32 ± 0.06^{C}	6.78 ± 0.05^{B}	6.93 ± 0.06^{A}
Liver	GDH activity	0.42 ± 0.01^{E}	0.45 ± 0.02^{D}	0.57 ± 0.0^{C}	$0,63\pm0.03^{B}$	0.67 ± 0.02^{A}	0.40 ± 0.01^{F}
	ASAT activity	2.20 ± 0.02^{F}	2.55 ± 0.03^{E}	2.94 ± 0.03^{D}	3.22 ± 0.04^{C}	3.54 ± 0.05^{B}	3.62 ± 0.06^{A}
	ALAT activity	6.13 ± 0.05^{F}	6.78 ± 0.07^{E}	7.72 ± 0.08^{D}	8.89 ± 0.07^{C}	9.99 ± 0.06^{B}	10.23 ± 0.08^{A}

Table II.- Effect of chronic doses of cypermethrin administered at 1.5 µg/L for 28 days on the activities (Mean±SD) of glutamate dehydrodegase (GDH, µM formozan/mg protein/h), aspartate aminotransferase (ASAT, µM oxaloacetate/mg protein/h) and alanine aminotransferase (ALAT, µM pyruvate/mg protein/h) of gill, muscles and liver of a freshwater fish, *Cyprinus carpio.*

Tissue	Enzymes	Control (n=7)	Chronic dose of cypermethrin (1.5 µg/L)				
			1 day (n=7)	7 days (n=7)	14 days (n=7)	21 days (n=7)	28 days (n=7)
Gill	GDH activity	0.13 ± 0.03^{F}	0.26 ± 0.01^{C}	0.27 ± 0.01^{B}	0.29 ± 0.01^{A}	0.25 ± 0.01^{D}	0.23 ± 0.00^{E}
	ASAT activity	1.46 ± 0.00^{F}	2.72 ± 0.03^{B}	2.88 ± 0.04^{A}	2.01 ± 0.04^{C}	1.73 ± 0.03^{D}	1.57 ± 0.02^{E}
	ALAT activity	1.66 ± 0.01^{F}	3.24 ± 0.04^{B}	3.43 ± 0.03^{A}	3.21 ± 0.02^{B}	2.75 ± 0.01^{D}	2.55 ± 0.02^{E}
Muscle	GDH activity	0.18 ± 0.01^{C}	0.21 ± 0.01^{B}	0.23 ± 0.01^{A}	0.24 ± 0.00^{A}	0.24 ± 0.01^{A}	0.24 ± 0.01^{A}
	ASAT activity	4.57 ± 0.02^{A}	3.88 ± 0.05^{C}	3.92 ± 0.06^{B}	3.39 ± 0.04^{D}	3.02 ± 0.03^{E}	2.72 ± 0.02^{F}
	ALAT activity	6.13 ± 0.06^{B}	6.11 ± 0.04^{B}	6.45 ± 0.05^{A}	5.99 ± 0.05^{C}	5.58 ± 0.04^{D}	5.34 ± 0.06^{E}
Liver	GDH activity	0.42 ± 0.01^{D}	0.45 ± 0.00^{C}	0.55 ± 0.01^{B}	0.57 ± 0.02^{A}	0.40 ± 0.01^{E}	0.34 ± 0.00^{F}
	ASAT activity	2.20 ± 0.02^{F}	9.89 ± 0.07^{A}	9.24 ± 0.08^{B}	8.69 ± 0.05^{C}	7.96 ± 0.04^{D}	6.88 ± 0.03^{E}
	ALAT activity	6.13 ± 0.05^{F}	6.78 ± 0.06^{E}	7.72 ± 0.05^{D}	8.89 ± 0.06^{C}	9.99 ± 0.07^{B}	10.23 ± 0.07^{A}

used as a biochemical indicator of stress (Shobha *et al*., 2001; Reddy and Venugopal, 1990). Overall, therefore, the increases in ASAT and ALAT levels indicate that the *Cyprinus carpio* were 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 (Shobha *et al*., 2001).

In the present study the significant elevation in the activities of these enzymes in the organs of fish exposed to the acute dose of cypermethrin indicates the greater association of oligomers of these enzymes in response to toxic stress. This shows that oxidative deamination is contributing higher ammonia production. The high levels of ammonia produced are not eliminated, however, but are salvaged through GDH activity which is utilized for amino acid synthesis through transaminases (Prashanth, 2003). The GDH elevation in all tissues (Table I) also suggests the possibility of a need for α-ketoglutarate in the Krebs cycle for the liberation of energy. The GDH activity (Table I) in the present study showed a progressive enhancement in all tissues (gill, muscle and liver), throughout the exposure, suggesting a developing need for α-ketoglutarate. The regulatory role of this enzyme in

checking the deamination process has been reported previously from mammalian models (Reddy and Yellama, 1991; Shobha *et al*., 2001). GDH catalyses 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 acids. GDH activity was enhanced in muscle and kidney tissues after 28 days of cypermethrin toxicity, which indicates increased deamination of glutamate and formation of ammonia. This increased ammonia content observed in muscle and kidney tissues supports the above statement.

Overall, it can be observed that exposure to sub-lethal concentrations of cypermethrin resulted in less change in the protein metabolism. It can also be noticed that the effects were more pronounced in the gill and liver tissues than in the muscle tissue. Despite the toxic effects of exposure to chronic concentrations of cypermethrin, the fish attempted to withstand the toxic effects of the pesticide by modulating their physiological and metabolic response towards proper utilization of enzymes and proteins for synthetic processes.

The increased activities of ASAT and ALAT (Tables I-II) shown in this study indicate that there may be an active transamination of amino acids, possibly to provide keto acid in the Krebs cycle. The steady rise in the activities of GDH, ASAT and ALAT in the organs of fish exposed to sub-lethal concentrations of cypermethrin (Tables I-II) may be due to the synthesis of these enzymes under chronic cypermethrin stress. The increase in these enzyme activities could be helpful to the fish in that the structural reorganization of proteins and incorporation of keto acids into the Krebs cycle serves to favour gluconeogenesis or energy production. The increase in transaminases can also be linked to the formation of urea (Ramna and Ramamurthi, 1983). The steady increase in the activities of ASAT ALAT and GDH, therefore, leads to metabolic compensation and allows the animal to adapt to the imposed toxic stress. Furthermore, the elevation in GDH activity in the chronic concentration of cypermethrin (Table II) could lead to increased production of glutamate in order to eliminate ammonia.

CONCLUSIONS

It can be concluded from the current study that protein metabolism in the muscle and liver tissues is disrupted on sub-lethal exposure to cypermethrin for 28 days. These kinds of studies will help to determine what remedial measures need to be undertaken in water bodies polluted with cypermethrin, so as to minimise adverse effects on fish and on fish consumers.

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