Cypermethrin toxicosis on protein metabolic profiles in *Rana hexadactyla*

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SUMMARY

Cypermethrin is a synthetic pyrethroid insecticide with low mammalian toxicity but high insecticidal activity. Frogs, *Rana hexadactyla* were exposed to sublethal concentration (*i.e.*, 1/10 LC_{50} 1.63mg/l) of cypermethrin for 7 days and 30 days to analyze various protein metabolic profiles in different tissues. Total proteins showed decrement, whereas free amino acids and the activity of protease, aspartate aminotransferase, alanine aminotransferase and glutamate dehydrogenase as well as ammonia and urea significantly increased in cypermethrin –exposed frogs. It was also observed that alterations steadily increased with the days of exposure and exhibited tissue specificity. These effects on the protein metabolism of frogs exposed to cypermethrin, which cause impairment on protein synthetic machinery, indicate its toxic effects on cellular functioning.

Key words : Cypermethrin, Protein metabolic profiles and Frog.

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Pesticides are unique contaminants in that they are intentionally released into the environment to elicit toxicity in certain 'pest' species. Unfortunately, lack of selectively often leads to problems in humans and other non-target species.

An increase in global food demand has resulted in a significant increase in the use of pesticides in agriculture. This has caused great concern among health and environmental scientists, since some of these chemicals induce mutations (somatic as well as germ-line) in experimental systems (Meng *et al.*, 2000). In humans, exposure to pesticides has been associated with cancer (Dich *et al.*, 1997).

Synthetic pyrethroid pesticides account for over 30% of the global pesticide use (Eisler, 1992). Two distinct classes of pyrethroids have been identified based on different behavioral, neuropsychological and biochemical profiles. Type I pyrethroids mainly cause hyper-excitation and fine tremors, while Type II pyrethroids possess a cyano-group and produce a more complex syndrome, including chronic seizures (Verschoyle and Aldridge, 1980). These compounds have gained popularity over organochlorine and organophosphate pesticides due to their high efficacy against target species (Elliot *et al.*, 1978), their relatively low mammalian toxicity (Parker *et al.*, 1984), and rapid biodegradability (Leahey, 1985). Cypermethrin [alpha – cyano-3-phenoxybenzyl ester of 2, 2-dimethyl-3-(2, 2-dichlorovinyl) cyclopropane carboxylic acid], is a composite synthetic pyrethroid, a broad spectrum, biodegradable insecticide, and a fast – acting neurotoxin with good contact and stomach action. It is used to control many pests, including moths, and pests of cotton, fruit and vegetable crops. Consistent with its lipophilic nature, cypermethrin has been found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries and brain (Hall *et al.*, 1980).

The present study critically examines the magnitude and relationships of the metabolites and enzymes involved in the metabolism of proteins in functionally different tissues of frogs treated with sublethal concentration of cypermethrin.

MATERIALS AND METHODS

Experimental animals:

About 30 adult, healthy frogs, *Rana hexadactyla* were collected from their natural habitat, in and around Tirupati with a mean weight of 50±5gm. They were housed in glass tanks partially filled with wet soil and covered with wire mesh. They were acclimated to laboratory conditions for one week prior to the experiment and water temperature 27± 2 ºC, pH=7±0.1 and light period of 12 h. They are fed with earthworms and cockroaches *ad libitum* to prevent starvation. The animals were starved for 24 h before they were exposed to pesticide. Each frog was examined for signs of abnormality or parasitic infection, if found they were rejected. Technical grade cypermethrin (92% purity; *cis:trans* ratio 40:60) was obtained from Tagros Chemicals India Limited, Chennai. Cypermethrin was

dissolved in acetone to study subacute and subchronic effects.

Experimental design:

The study design comprised three groups consisting ten frogs each. 1/10 of the LC_{50} value (0.163 mg/l) of cypermethrin was selected as sublethal concentration and exposed for 7 days and 30 days with one day of interval and control frogs were exposed with acetone. After the stipulated time, both control and experimental animals were sacrificed and different tissues isolated and stored in -80 ºC for biochemical analysis.

Estimation of organic constituents

One per cent homogenate of the tissues were prepared in 0.25 M ice cold sucrose solution using a motor-driven Teflon-coated pestle control homogenizer for the estimation of total proteins (TP) with Folin phenol reagent (Lowry *et al.,* 1951), using bovine albumen serum as standard. This homogenate was precipitated with 10% trichloro acetic acid, and the protein free supernatant was processed for free amino acids (FAA) estimation by the addition of ninhydrin reagent (Moore and Stein 1954). tyrosine was as standard.

Analysis of nitrogenous end products

Five per cent homogenate of the tissues were prepared in distilled water for ammonia, and in 15% perchloric acid for urea. Levels of ammonia were known using ammonium chloride as standard, and urea by diacetyl monoxime method (Natelson, 1971).

Assay of enzymes:

Five per cent homogenate of the tissues were prepared in 0.25 M ice-cold sucrose solution for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and glutamate dehydrogenase (GDH); in ice cold distilled for protease, and these were centrifuged at 2500 rpm for 10 min in a refrigerated centrifuge at 4 ºC to remove cell debris, and clear cell-free extracts were used as enzyme source. Protease activity was measured (Moore and Stein, 1954) with the reaction mixture containing 100 µm of phosphate buffer (pH 7.0) and 12 mg of denatured protein. AST (E.C.2.6.1.1) and ALT (E.C.2.6.1.2) activities were assayed following the method of (Reitman and Frankel, 1957). The incubation mixture for AST contain 100 µm of phosphate buffer (pH 7.4), 2 µm of ketoglutarate, and 50 µm of L-aspartic acid (pH 7.4). For ALT, incubation steps followed were the same as described for AST, except that the substrate used was D-alanine (50 µm). The standard graph was prepared with sodium pyruvate. GDH activity was measured by the method of Lee and Lardy (1965). Incubation mixture contained 100 µm of phosphate buffer (pH 7.4), 40 µm of sodium glutamate, 0.1 µm of NAD, 4 µm of 2,4-iodophenyl-3-(nitrophenyl)-5-phenyltetrazolium chloride (INT), and the enzyme source. This was incubated for 30 min at 37 ºC, and stopped with 5.0 ml of glacial acetic acid. The colour was extracted by shaking with 5.0 ml of toluene. After keeping the tubes overnight at 4 ºC, the colour extract was measured. All spectrophotometric measurements were determined using Hitachi U-2800 model spectrophotometer.

Evaluation of results:

An average of six individual estimations were taken after pooling them, and the mean values of control and experimental frogs were subjected to statistical analysis using 't' test for comparison. The values were considered significant at $p<0.05$ or $p < 0.01$ level.

RESULTS AND DISCUSSION

The results of protein metabolic profiles of the control and experimental frogs under cypermethrin are mentioned in Table 1 and 2. The experimental frogs exposed to cypermethrin showed statistically significant ($p<0.05$/ $p<0.01$) decrease of total protein content, whereas FAA, the activities of protease, ALT, AST and GDH as well as ammonia and urea significantly increased in different tissues. Alteration in protein metabolic profiles was in the form of a sublethal concentration- and time-dependent manner in treated frogs.

Catabolism of proteins and amino acids make a major contribution to the total energy production in rats. The depletion of total protein content observed in this investigation (Table 1) can be correlated with this fact. These results are in agreement with the earlier report of Nagarjuna *et al.* (2008), who demonstrated a similar situation in rats exposed to cypermethrin. The increase in protease activity (Table 2) observed at different periods of exposure of cypermethrin was clearly reflected in the breakdown of proteins. Under proteolysis, enhanced breakdown dominates over synthesis, while in the case of anabolic process; increased synthesis dominates the protein breakdown (Murray *et al.,* 2007).

Enhanced protease activity and decreased protein level resulted in a marked elevation of FAA content in the tissues at different periods of exposure of cypermethrin- exposed frogs (Table 2). Presumably, the degradation of proteins led to FAA accumulation.

The elevation of AST and ALT activities observed in this study (Table 2) offers an excellent corroboration

Table 1: Biochemical changes in different tissues of frog, *Rana hexadactyla* exposed to cypermethrin

Tissue	Control	7 days	30 days
Total proteins [a]	91.8144±1.3863	82.0368±0.8063**	65.8944±1.3949**
Brain		(-10.65)	(-28.23)
Liver	188.9424 ±0.7889	161.4960±5.0573**	107.6260±1.0159**
		(-14.53)	(-43.04)
Kidney	85.3344±0.7969	73.9008±1.4567**	59.0256±0.6021**
		(-13.40)	(-30.83)
Muscle	110.6352±0.5539	98.4096±3.2718**	81.9936±3.2036**
		(-11.05)	(-25.89)
Free amino acids [b]	13.6800±0.5373	15.0480±0.3342**	19.4688±0.4690**
Brain		(10.00)	(42.32)
Liver	12.4416±0.4052	13.9680±0.5203**	20.5776±0.3683**
		(12.27)	(65.39)
Kidney	9.3744±0.4971	10.8432±0.3221**	14.8464±0.3723**
		(15.67)	(58.37)
Muscle	9.8208±0.3398	11.2464±0.4141**	13.1184±0.3111**
		(14.52)	(33.58)
Ammonia [c]	2.1486±1.7208	2.4490±1.7688*	2.9096±2.0185**
Brain		(13.98)	(35.42)
Liver	3.1152±0.0074	3.6489±0.0676**	4.4831±0.0860**
		(17.13)	(43.91)
Kidney	3.5184±0.0057	4.0758±0.0656**	4.9278±0.0048**
		(15.84)	(40.06)
Muscle	2.5558±0.0037	2.9274±0.0562*	3.3702±0.0103*
		(14.54)	(31.86)
Urea [d]	0.3614±0.0254	0.3963±0.0189*	0.4476±0.0389**
Brain		(9.66)	(23.86)
Liver	0.4609±0.0163	0.5554±0.0987*	0.6776±0.0896**
		(20.49)	(46.99)
Kidney	0.4158±0.0233	0.4876±0.0479*	0.5739±0.0901**
		(17.28)	(38.02)
Muscle	0.1725±0.0103	0.1930±0.0149*	0.2166±0.0458**
		(11.90)	(25.59)

Values are means ± SD (n=6). Values in parentheses indicate per cent change over control.
NS: Non-significant, * and ** indicates significance of value at P<0.05 and P<0.01, respectively
[a] mg/g wet weight of the tissue
[b] µ moles of tyrosine/g wet weight of the tissue
[c] µ moles of ammonia/g wet weight of the tissue
[d] µ moles of urea/g wet weight of the tissue

of the above trend. This is a clear indication of shunting of amino acids into TCA cycle through oxidative deamination and active transamination. Such a phenomenon is necessary to cope up with the energy crisis during pyrethroid stress.

The elevation observed in the GDH activity (Table 2) indicates its contribution to enhanced ammonia levels and glutamate oxidation during cypermethrin toxicity. Increased FAA levels and their subsequent transamination results in greater production of glutamate, thus increasing the intracellular availability of substrate, glutamate, for consequent oxidative deamination reaction through GDH. Besides, the elevation in transaminases and GDH helps in supplying keto acids to the TCA cycle in order to compensate the energy crisis in tissues during cypermethrin toxicity.

In the present study, ammonia content increased in tissues of frogs exposed to sublethal concentrations of

Table 2: Enzymological changes in different tissues of frog, *Rana hexadactyla* exposed to cypermethrin

Tissue	Control	7 days	30 days
Proteins [c]			
Brain	0.0605±0.0032	0.0687±0.0168* (13.64)	0.0790±0.0173** (30.58)
Liver	0.1057±0.0053	0.1200±0.0065** (13.47)	0.1537±0.0069** (45.39)
Kidney	0.0907±0.0031	0.1085±0.0154** (19.56)	0.1265±0.0213** (39.39)
Muscle	0.0477±0.0037	0.0547±0.0219* (14.66)	0.0640±0.0203** (34.03)
AST [f]			
Brain	2.0201±0.0063	2.2988±0.0093** (13.80)	2.8454±0.0649** (40.85)
Liver	2.4033±0.0102	2.8526±0.0153** (18.69)	3.9170±0.0052** (62.98)
Kidney	2.9684±0.0068	3.4790±0.0696** (17.20)	4.4895±0.0546** (51.24)
Muscle	4.1044±0.0189	4.655±0.0219** (13.41)	5.6358±0.0097** (37.31)
ALT [f]			
Brain	0.6366±0.0110	0.7446±0.0603** (16.95)	0.8880±0.0701** (38.32)
Liver	2.0667±0.0087	2.5725±0.1326** (24.46)	3.2098±0.0084** (55.31)
Kidney	1.2118±0.0093	1.4708±0.1342** (21.37)	1.7648±0.1908** (45.63)
Muscle	2.3588±0.0110	2.7900±0.3617** (18.28)	3.2550±0.2725** (38.02)
GDH [g]			
Brain	0.2150±0.0109	0.2463±0.0106** (14.55)	0.2955±0.0074** (37.47)
Liver	0.2613±0.0076	0.3213±0.0174** (22.95)	0.4193±0.0232** (60.42)
Kidney	0.2150±0.0072	0.2561±0.0121** (19.14)	0.3320±0.0104** (54.45)
Muscle	0.2344±0.0023	0.2752±0.0105** (17.43)	0.3268±0.0116** (39.43)

Values are means ± SD (n=6). Values in parentheses indicate per cent change over control.
NS: Non-significant, * and ** indicates significance of value at P<0.05 and P<0.01, resepectively
[e] µ moles of tyrosine/mg protein/h
[f] µ moles of pyruvate/mg protein/h
[g] µ moles of formazone/mg protein/h

cypermethrin (Table 1). Elevated activities of proteases, transaminase reactions, and increased deamination reaction (GDH) support the augmented ammonia levels during cypermethrin toxicosis. The enhanced ammonia levels in tissues of cypermethrin treated frogs may lead to ammonotoxaemia and shows deleterious effects on the animal metabolism.

Increased urea levels in liver tissue might be due to activation of urea cycle (Table 1). The presence of urea in extra hepatic tissues might be due to the vascular mobilization and translocation from liver. The elevation in urea levels is in consonance with increased proteolytic activity, enhanced transamination, and elevated ammonia levels during cypermethrin toxicosis. The status of protein metabolic profiles change in tissues in the present study corroborates the findings of Begum *et al*. (2007) and Nagarjuna *et al*. (2008). In conclusion, it can be stated that long term exposure to sublethal concentration of

pyrethroid pesticides can result in cell metabolism poisoning.

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