Toxicol. Int.Vol. 16, No. 2, 2009 pp 91-95

Protein metabolic profiles and histopathological studies in heart tissue of rats during cypermethrin toxicosis

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Abstract

Cypermethrin is the most widely used Type II pyrethroid pesticide because of its high effectiveness against target species and its low mammalian toxicity reported so far. It is a fast-acting neurotoxin and is known to cause free radical-mediated tissue damage. On exposure to oral sublethal doses (41 mg/kg bw) of cypermethrin as single dose, double dose and multiple dose with 48 h interval the various profiles of protein metabolism were studied in different groups of rat heart tissue. Total proteins showed decrement; whereas free amino acids, the activities of protease, aspartate aminotransferase, alanine aminotransferase and glutamate dehydrogenase and ammonia significantly increased in cypermethrin exposed rats. Urea content increase at all doses of exposure was not statistically significant. Thus variation in the protein metabolic profiles of the rat exposed to cypermethrin indicates its toxic effect on the cellular metabolism thereby leading to impaired protein synthetic machinery is clearly evidenced by histopathological studies.

Key words: Cypermethrin, pathophysiology and rat heart tissue

Introduction

Pesticides have made valuable contributions to human health by increasing food and fiber production and by reducing the occurrence of vector-borne diseases (Blindauer *et al.,* 1999). 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 clonic seizures (Verschoyle and Aldridge, 1980). These compounds have gained popularity over organochlorine and organophosphate pesticides due to their high effectiveness against target species (Elliot *et al.,* 1978), their relatively low mammalian toxicity (Parker *et al.,* 1984) and rapid biodegradability (Leahey, 1985). Cypermethrin 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, 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).

Present study critically examines the magnitude and relationships of the metabolites and enzymes involved in the metabolism of proteins and histopathological changes in heart tissue of rats treated with sublethal doses of cypermethrin, since the farmers, pesticide applicators, industrial workers and other pesticide users will be exposed to the pesticides repeatedly.

Materials and Methods

Test chemical

Technical grade cypermethrin (92% purity; *cis:trans* ratio 40:60) was obtained from Tagros Chemicals India Limited, Chennai.

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Experimental animals

About 40 adult, healthy Wister strain albino rats (70±5 days, 175±10 g) were obtained from the Indian Institute of Science (Bangalore, India) breeding colony and raised on a commercial pellet diet (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*. The animals were housed at constant temperature (28±2°C) and relative humidity (60±10%) with a 12 h light: 12 h dark cycle.

Experimental design

The study design included four groups consisting ten rats each. Toxicity evaluation was conducted by static bioassay method (Finney, 1971) and the LD_{50} for 48 h value of cypermethrin to rats was found to be 205 mg/kg bw. 1/5 LD_{50} value (41 mg/kg bw) was selected as sublethal dose and administered as single, double and multiple dose with one day of interval. The first group of animals was considered vehicle controls-corn oil. To the second group of animals single dose of cypermethrin (i.e., on 1st day) was administered orally (41 mg/kg bw). Double doses (82 mg/kg bw) were given with 48 h interval to the third group of animals on 1st and 3rd day. To the fourth group of animals multiple doses (164 mg/kg bw) were given with 48 h interval i.e., on 1st, 3rd, 5th and 7th day. After 48 h both control and experimental animals were sacrificed and heart tissues isolated and stored at -80°C for biochemical analysis and for histopathological studies a portion of tissue fixed in formalin for further processing.

Estimation of organic constituents

One percent homogenate of the heart tissue was 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 used as standard.

Analysis of nitrogenous end products

Five percent 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 percent 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°C 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 for 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 °C and stopped with 5.0 ml of glacial acetic acid. The color was extracted by shaking with 5.0 ml of toluene. After keeping the tubes overnight at 4°C, the color extract was measured. All spectrophotometric measurements were determined using Hitachi U-2800 model spectrophotometer.

Histopathology

Small pieces of heart tissue were fixed in 10% neutral buffered formalin. Sections of 6 μ thickness were cut and stained with hematoxylin and eosin (H&E) for observation under light microscope.

Evaluation of results

Average of six individual estimations were taken after pooling them and the mean values of control and experimental albino rats were subjected

to statistical treatment using one-way analysis of variance (ANOVA) and Duncan test for multiple comparison. The values were considered significant at 5% level.

Results and Discussion

The results of protein metabolic profiles of the control and experimental rats under cypermethrin are given in Table 1. The experimental rats exposed to cypermethrin showed statistically significant ($p < 0.01$) decrease of total protein content; whereas FAA, the activities of protease, ALT, AST and GDH and ammonia significantly ($p < 0.01$) increased. Urea content increase was not statistically significant.

In single dose cypermethrin administered rats, the heart did not show any marked pathological changes. In double dose administration congestion was observed (Fig. A). Under multiple dose administration congestion and slight infiltration

Table 1: Biochemical and enzymological changes in heart tissue of control and cypermethrin treated rats.

Muscle	Control	Single dose	Double dose	Multiple dose
Total proteins (mg/g we weight of the tissue)	$99.5613^{d} \pm 2.1676$	$91.6128^{c} \pm 1.0012$ (-7.98)	$83.5407^{b} \pm 1.5377$ (-16.09)	$76.8960^{a} \pm 1.3080$ (-22.76)
Free amino acids (μ moles of tyrosine/g wet weight of the tissue)	$59.3192^{a} \pm 0.6132$	$63.5278^{b} \pm 0.3403$ (7.09)	$67.7917^{c} \pm 0.7631$ (14.28)	$73.3057^{d} \pm 0.4398$ (23.58)
Protease (μ moles of tyrosine/mg protein/h)	$0.3872^{a} \pm 0.0554$	$0.4188^{ab} \pm 0.0358$ (8.16)	$0.4505^{bc} \pm 0.0257$ (16.35)	$0.4978^{c} \pm 0.0398$ (28.56)
AST (μ moles of pyruvate/ mg protein/h	$0.5853^{a} \pm 0.0095$	$0.6212^{b} \pm 0.0082$ (6.13)	$0.7017^{c} \pm 0.0099$ (19.89)	$0.7448d \pm 0.0104$ (27.25)
ALT (μ moles of pyruvate/ mg protein/h)	$0.6695^{a} \pm 0.0147$	$0.7217^{b} \pm 0.0150$ (7.80)	$0.7707^{c} \pm 0.0150$ (15.12)	$0.8195^{d} \pm 0.0184$ (22.40)
GDH (μ moles of formazone/ mg protein/h)	$0.1118^{a} \pm 0.0066$	$0.1237^{b} \pm 0.0037$ (10.64)	$0.1310^{c} \pm 0.0058$ (17.17)	$0.1468^{d} \pm 0.0037$ (31.31)
Ammonia (μ moles of ammonia/g wet weight of the tissue)	$5.3092^{a} \pm 0.3012$	$5.7388^{b} \pm 0.1273$ (8.09)	$6.1907^{c} \pm 0.1917$ (16.60)	$6.4242^{d} \pm 0.1268$ (21.00)
Urea (μ moles of urea/g wet weight of the tissue)	$0.1437^{a} \pm 0.0318$	$0.1540^{a} \pm 0.0337$ (7.17)	$0.1643^{a} \pm 0.0503$ (14.33)	$0.1848^{a} \pm 0.0551$ (28.60)

Values are means $\pm$ SD (n=6). Values with different superscripts are significantly different ($p < 0.05$) and same alphabet are not significantly different as determined by Duncan's Multiple range test. Values in parenthesis indicate per cent change over control.

One - Way Anova

Source of Variation	DF	Total proteins MS	FAA MS	Protease MS	AST MS	ALT MS	GDH MS	Ammonia MS	Urea MS
Between Groups	3	579.727**	214.654**	0.013**	0.032**	0.025**	0.001**	1.467**	0.002NS
Within Groups	20	6.196	0.317	0.002	0.000	0.000	0.000	0.040	0.002
Total	**23**								

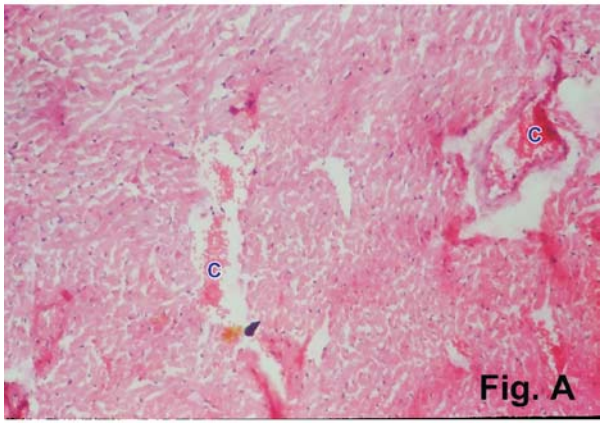


Fig. A: Double dose cypermethrin administered rat heart tissue showing congestion (C). H & E. 200x.

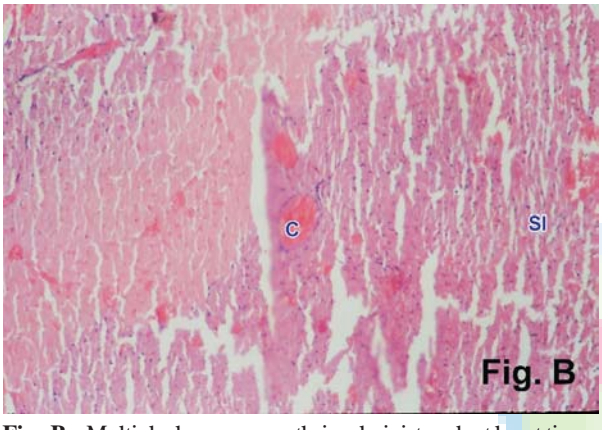


Fig. B: Multiple dose cypermethrin administered rat heart tissue showing congestion (C) and slight infiltration (SI). H&E. 200x.

were observed (Fig. B). Alteration in protein metabolic profiles and histopathological changes were dose and time-dependent manner in treated rats.

Proteins being involved in the architecture and physiology of the cell, they seem to occupy a key role in cell metabolism (Murray *et al.,* 2007). 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 can be correlated to this fact. These results are in agreement with the earlier report of David *et al.* (2004) who demonstrated a similar situation in *Cyprinus carpio* exposed to cypermethrin.

Increase in protease activity observed at different doses of cypermethrin was clearly reflected the breakdown of proteins. Enhanced protease activity and decreased protein level has resulted in a marked elevation of FAA content in the heart tissues at different doses of cypermethrin exposed rats. The elevation of AST and ALT activities observed in this investigation offers an excellent support to 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 was necessary to cope up with the energy crisis during pyrethroid stress.

The elevation observed in the GDH activity indicates its contribution to enhanced ammonia levels and glutamate oxidation during cypermethrin toxicity. Increased free amino acid 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 elevation in transaminases, GDH helps in supplying keto acids to the TCA cycle in order to compensate the energy crisis in heart tissues during cypermethrin toxicity. In the present study ammonia content was increased in heart tissue of rats exposed to sublethal doses of cypermethrin. Elevated activities of proteases, transaminase reactions and increased deamination reaction (GDH) support the augmented ammonia levels during cypermethrin toxicosis. The presence of urea in heart tissue might be due to the vascular mobilization and translocation from liver. The elevation in urea levels was in consonance with increased proteolytic activity, enhanced transamination and elevated ammonia levels during cypermethrin toxicosis.

In heart tissues, pathological changes include congestion and slight infiltration. Tos-Luty *et al.* (2003) observed focal parenchymatous degeneration of cardiomyocytes and the presence of single basophils in the heart of rats orally exposed to malathion. The present status of protein metabolic profiles changes correlated with histopathological changes of tissues, which corroborated with the findings of Begum (2007). Such changes would definitely results in malfunctioning of heart tissue. At repeated dose

level tested of cypermethrin administered orally, and exert a damaging effect on the cell metabolism thereby leading to impaired protein synthetic machinery. In conclusion, long term exposure to sublethal doses of pesticide results in cell metabolism toxicosis.

Acknowledgements

Dr. A. Nagarjuna thanks to Andhra Pradesh Council of Science and Technology, Hyderabad for the financial support (Young Scientist Fellowship).

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Received : 22.07.2008
Accepted: 10.11.2008