Cypermethrin on protein metabolic profiles in rat kidney
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ABSTRACT
Cypermethrin is most widely used 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. An attempt has been made in estimating its toxicity in rat kidney at molecular levels. Following 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 kidney. 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 rats. These effects on the protein metabolism of rats exposed to cypermethrin, which caused impairment of protein synthetic machinery and indicated its toxic effects on cellular functioning.

Key words : Cypermethrin, Protein metabolism, Rat kidney.

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 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).

Cypermethrin has been classified by the US Environmental Protection Agency (US EPA, 1989) as a possible carcinogen. The pesticide has been shown to induce chromosomal aberrations and micronucleus formation in mouse bone marrow as well as in spleen (Amer and Aboul-ela, 1985; Amer et al., 1993). It also increases the frequency of sister chromatid exchange in bone marrow cells of mice (Giri et al., 2003). DNA damage was observed in lymphocytes of workers occupationally exposed to pesticides such as cypermethrin (Undeger and Basaran, 2002).

The present study critically examines the magnitude and relationships of the metabolites and enzymes involved in the metabolism of proteins in rat kidney 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.

Experimental animals:
About 40 adult, healthy, wistar 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 ºC) and relative humidity (60±10%), with a 12 h light: 12 h dark cycle.

**Experimental design:**

The study design comprised four groups consisting of ten rats each. Toxicity evaluation was conducted by static bioassay method (Finney, 1971), and the LD₅₀ for 48 h value of cypermethrin to rats was found to be 205 mg/kg bw. 1/5 LD₅₀ value (41 mg/kg bw) was selected as sublethal dose and administered as single, double and multiple dose with one day interval in between. The first group of animals was treated as vehicle controls, and administered 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 kidney tissues isolated and stored in -80 ºC for biochemical analysis.

**Estimation of organic constituents:**

One per cent homogenate of the kidney 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 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 contained 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 rats were subjected to statistical analysis using Duncan test for multiple comparison. The values were considered significant at 5% level.

**RESULTS AND DISCUSSION**

**Effect on protein metabolic profiles:**

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

Since proteins are involved in the architecture and physiology of the cell, they appear 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 (Table 1) can be correlated with 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.

The increase in protease activity (Table 1) observed at different doses 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). Moreover, histopathological damage and hydromineral imbalance during pesticide stress has been reported to account for the elevated protease activity (Moorthy et al., 1984).

Enhanced protease activity and decreased protein level resulted in a marked elevation of FAA content in the kidney tissues at different doses of cypermethrin-exposed rats (Table 1). Presumably, the degradation of proteins led to FAA accumulation. This higher level of FAA can also be attributed to the decreased utilization amino acids, and is also suggestive of its involvement in the maintenance of osmotic and acid base balance (Moorthy et al., 1984).

The elevation of AST and ALT activities observed in this study (Table 1) offers an excellent corroboration 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. It has also been suggested that stress conditions in general induce 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 succinate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase and cytochrome-c-oxidase, a situation also demonstrated by Ghosh (1989) in Labeo rohita under cypermethrin toxicity.

The elevation observed in the GDH activity (Table 1) 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, the elevation in transaminase reactions and GDH helps in supplying keto acids to the TCA cycle in order to compensate the energy crisis in kidney tissues during cypermethrin toxicity.

In the present study, ammonia content increased in kidney tissues of rats exposed to sublethal doses of cypermethrin (Table 1). Elevated activities of proteases, transaminase reactions, and increased deamination (GDH) support the augmented ammonia levels during cypermethrin toxicosis. The enhanced ammonia levels in kidney tissues of cypermethrin treated rats may lead to ammonotoxaemia, and shows deleterious effects on the animal metabolism. Though ammonia is essential for the synthesis of important compounds such as purines, pyrimidines, and non-essential amino acids, and also functions as a key factor in acid-base regulation, it is toxic in non-physiological concentrations, and excess ammonia therefore has to be disposed off (Murray et al., 2007). Ammonia, a toxic nitrogenous end product, is released exogenously into the digestive tract, and endogenously into the tissues through catabolism of amino acids,

| Table 1 : Biochemical and enzymological changes in kidney tissue of control and cypermethrin treated albino rats |
|-------------------------------|----------------|---------------|--------------|----------------|
| Kidney                        | Control        | Single dose   | Double dose  | Multiple dose  |
| Total proteins (mg/g wet weight of the tissue) | 121.4353 ± 6.877 | 112.8672 ± 1.6971 | 104.8752 ± 1.1637 | 95.7457 ± 0.6567 |
| Free amino acids (µ moles of tyrosine/g wet weight of the tissue) | 50.3888 ± 0.6702 | 55.0695 ± 0.5332 | 58.3610 ± 0.6700 | 63.4167 ± 0.5136 |
| Protease (µ moles of tyrosine /mg protein/h) | 0.3083 ± 0.0496 | 0.3398 ± 0.0354 | 0.3715 ± 0.0467 | 0.4110 ± 0.0248 |
| AST (µ moles of pyruvate /mg protein/h) | 0.2342 ± 0.0052 | 0.2512 ± 0.0134 | 0.2700 ± 0.0129 | 0.2835 ± 0.0078 |
| ALT (µ moles of pyruvate /mg protein/h) | 0.4928 ± 0.0148 | 0.5200 ± 0.0060 | 0.5732 ± 0.0111 | 0.6237 ± 0.0104 |
| GDH (µ moles of formazone /mg protein/h) | 0.2097 ± 0.0144 | 0.2253 ± 0.0052 | 0.2397 ± 0.0072 | 0.2627 ± 0.0088 |
| Ammonia (µ moles of ammonia /g wet weight of the tissue) | 4.3170 ± 0.1373 | 4.6847 ± 0.1099 | 5.0603 ± 0.0933 | 5.7255 ± 0.0733 |
| Urea (µ moles of urea/g wet weight of the tissue) | 0.7289 ± 0.0720 | 0.7640 ± 0.0553 | 0.8153 ± 0.0606 | 0.9240 ± 0.0871 |

Values are means ± S.D. (n=6). Values with different superscripts are significantly different (p<0.05), and those without, are not significantly different, as determined by Duncan’s Multiple range test. Values in parentheses indicate per cent change over control.

pyrimidines and purines (Lowenstein and Goodman, 1978). Ammonia cannot be stored for longer periods of time in the body as it leads to endogenous ammonotoxicity. The reduction in ammonia content suggests that the ammonia might have been converted into non-toxic compounds, glutamine and urea in cypermethrin-exposed Cyprinus carpio (David et al., 2004).

The presence of urea in kidney tissue might be due to the vascular mobilization and translocation from liver. (Table 1). The elevation in urea levels is in consonance with increased proteolytic activity, enhanced transamination, and elevated ammonia levels during cypermethrin toxicosis. Thus, an increase in urea enunciates the role being played by liver tissues of exposed rats in the elevation of ammonia toxicity, besides their pivotal role in replenishing the protein nitrogen to synthesize useful precursors for the maintenance of homeostasis and dynamic equilibrium. Increased levels of urea under cypermethrin stress reveal that the rats might have adapted to the biosynthesis of urea as a major pathway of detoxification of ammonia. Probably this pathway may be beneficial to animals in detoxification and physiological compensation, or adjustment to various exogenous and endogenous toxicants.

The status of protein metabolic profiles changes in kidney tissues in the present study corroborates the findings of Begum et al. (2007) and Nagarjuna et al. (2008). At repeated dose levels of Cypermethrin administered orally, a damaging effect on the cell metabolism occurs, thereby leading to impaired protein synthetic machinery. In conclusion, it can be stated that long term exposure to sublethal doses of pyrethroid pesticides can result in cell metabolism toxicosis.

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