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Full Length Research Paper

Cyfluthrin-induced hepatotoxicity in rats

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The hepatotoxic effect of continous administration of cyfluthrin was investigated in rats. Rats (*Rattus norvegicus*) were grouped into A (0 ppm) control, B (100 ppm) and C (200 ppm) with the indicated amount of cyfluthrin administered orally for 15 weeks.The hepatotoxicity level was assessed by monitoring the changes in the organ to body; weight ratio, micronutrient level (iron, zinc, copper and selenium), the nutritional status (total carbohydrate, total glucose, total protein, total amino acids, total lipid and total cholesterol), the lipid peroxidation level (reduced glutathione and thiobarbiturate) and the antioxidant enzyme activities (glutathione peroxidase, glutathione reductase, catalase, and glucose-6-phosphate dehydrogenase). A dose-dependent decrease in the organ-to-body ratio was observed. The micronutrient level in the test groups increase significantly. The total carbohydrate, total glucose, total amino acids and total protein show a significant decrease in the test groups. There is no significant difference observed in the tissue cholesterol at both dosages under investigation. Lipid peroxidation was increased in the test groups as indicated by a significant increase in the thiobarbiturate level and a significant decrease in the reduced glutathione level. All the antioxidant enzymes studied increased significantly. Cyfluthrin is potentially hepatotoxic under continuous administration in rats.

Key words: Cyfluthrin, hepatotoxicity, nutritional status and antioxidant enzymes.

INTRODUCTION

Cyfluthrin is a synthetic type-2 pyrethroid insecticide. The original compound was isolated from the flower of chrysanthemum (Bloomquist, 1993). It has been effective in the control of a wide range of insects including cutworms, cockroaches, ants, termites, grain-beetle, weevil, fleas, flies and mosquitoes (Thompson, 1992). Due to its versatility it has become a useful active ingredient in the manufacture of many insecticides such as Baygon, Baythroid and Responsar (Meister, 1995). The mechanism of action of cyfluthrin has been traced to its interaction with sodium-ion-gated channels, leading to membrane depolarization and loss of electrical excitability in the central and the peripheral nervous system (Bloomquist, 1996). Cyfluthrin has been reported to exhibit selective toxicity to insects, with sparingly low toxicity in mammals due to rapid liver metabolism and urinary excretion of both the metabolized and intact compound. However, laboratory animal exposed to relatively high doses of cyfluthrin had shown the same toxic effect observed in insects; including convulsion, salivation, ataxia, weakness and apathy (Schimmel, 1983). Also, report of dermal toxicity has been reported for skin contact (Moran, 1989). Due to the central role played by the liver in the detoxification of cyfluthrin, there is a tendency for its accumulation and subsequent toxicity to the liver, disrupting the normal hepatic function.

Watanabe et al. (1982) reported liver necrosis and increased chromatin in the nuclei of hepatic cells after the administration of cyfluthrin. The absolute weight of the kidney was reported to decrease after the administration

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Table 1. Dry organ weight to body weight ratio (g/g) of rats administered with cyfluthrin.

Control	Cyfluthrin (100 ppm)	Cyfluthrin (200 ppm)
0.071±0.002	0.037*±0.001	0.051*±0.008

Values are mean±SD.
*Significant decrease ($p<0.05$) when compared with control.

of cyfluthrin (Suberg and Loser, 1983). In this study, the hepatotoxic effect of continous administration of sub-lethal dosages of cyfluthrin was monitored on the organ-body ratios, micronutrient levels, nutritional status, and the antioxidant enzyme activities in the liver of rats.

MATERIALS AND METHODS

Animals

45 rats (*Rattus norvegicus*) between 80-95 g were obtained from the Animal House of the Department of Biochemistry, Igbinedion University Okada. They were grouped into three of 15 rats each (control 20% lecithin in water, 100 ppm and 200 ppm of cyfluthrin dissolved in 20% lecithin in water). 1 ml of the solution was administered daily by gavages for 15 weeks. At the end the treatment the animals were sacrificed under light anesthesia and the organs perfused in physiologic saline solution.

Biochemical assays

Glutathione peroxidase assay is based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by glutathione peroxidase (GPX) coupled to the recycling of GSSG back to GSH utilizing NADPH (Beutler et al., 1977). The decrease in NADPH absorbance measures at 340 nm is indicative of glutathione peroxidase activity. The glutathione reductase activity is a measure of increase in the absorbance at 412 nm of 5,5'-dithiobis (2-nitrobenzoic acid) when it is reduced by the enzyme (Smith et al,. 1998). The *in vitro* catalase activity was determined using the modification of Ashiru and Singha (1971) method. Superoxide dismutase activity was estimated by Misra and Fridovish method (1972). Glucose-6-phosphate dehydrogenase activity was determined using the modification of Gupta and Baquer method (1998).

Estimation of reduced glutathione level was determined by the method described by Moron et al. (1979). Thiobarbiturate level was determined by the modification of Ohokawa et al. (1979) method.

Determination of nutritional status

Total lipid in the tissue was determined using the soxhlet extractor method according to AOAC (1980). Total tissue nitrogen content was determined by the Kjedahl's method (AOAC, 1980). The total carbohydrate was determined using the phenol/sulphuric acid method (Dubois et al., 1956). The cholesterol level was estimated using cholesterol oxidase method (Allain et al., 1974). Free glucose was determined by glucose oxidase method (Randox Kit). Total amino acid level was determined by the ninhydrin method (Magne and Larher, 1992).

Absorption spectrophotometer (Perkin-Elmer Analyst 100) was used in the determination of the micronutrient concentration after a wet digestion of the sample (perchlorate/HNO_3) (3:2, v:v) using a modification of Turnland and Acord (1986) method.

Statistical analysis

The values are recorded as mean ± standard deviation. The statistical significance of difference in the mean and standard deviation ($p<0.05$) was analyzed by the student T-test for comparism of each of the test groups and the control.

RESULTS AND DISCUSSION

Table 1 shows the organ to body weight ratio. There is a significant decrease ($p< 0.05$) in the body-weight ratio at both dosages in a dose-depend manner. This agrees with the result of Oikawa and Iyatomi (1983) who reported decrease in the absolute weights of the liver, heart, and lungs in male rats at high cyfluthrin dosage, and females at this dose had depressed liver, kidney, adrenal, and ovarian weights. Shaw et al. (1983) reported the highest concentration of cyfluthrin in the liver when 0.5 mg/kg of cyfluthrin was administered daily for five days to a dairy cow. The accumulation of cyfluthrin in the liver is likely the primary factor which leads to the reduction in the absolute weight or the organ to body ratio. A reduction in the weight can be traced to two related mechanisms: a reduction in the rate of cell division which affects the genetic composition or through the necrosis of the tissue. Since cyfluthrin has no direct interaction with the DNA (Suberg.and Loser, 1983b), the reduction in tissue weight is most likely caused by tissue necrosis, possibly occurring at a basal rate. Gross pathological examination of hens administered high dose of cyfluthrin revealed spotty, brittle livers and pale, slightly mottled kidneys (Thyssen et al., 1981).

Necrosis caused by cyfluthrin is possibly due to free radical generation, causing peroxidation of lipids as confirmed by significant increase in the thiobarbiturate levels in the test groups, and the significant reduction in the reduced glutathione level decreased significantly in the test groups at both dosages (Table 5). Oikawa and Iyatomi (1983) reported that rats treated with cyfluthrin at 300 or 1000 mg/kg feed had significantly increased blood urea nitrogen levels, the blood glucose levels were depressed in males at 300 or 1000 mg/kg feed, and in females at 1000 mg/kg feed. Males at 1000 mg/kg feed had significantly increased liver aspartate amino transaminase activity. These parameters reveal that the metabolism in the liver is affected by cyfluthrin administration. Table 2 shows the nutritional status of the liver indicating significant reduction in the total carbohydrate, total glucose and total lipid with the total protein decreesing in a dose-dependent manner. There is also a significant increase in the total amino acid. There is no signifi-

Table 2. Nutritional Status of tissue (mg/g of tissue) of rats administered with cyfluthrin.

Nutrient	Control	Cyfluthrin (100 ppm)	Cyfluthrin (200 ppm)
Total Carbohydrate	61.22 ± 1.42	45.20*±3.22	38.36*± 3.56
Total glucose	68.32±	20.33* ± 1.68	24.84*±0.15
Total Protein	95.56±6.82	41.11*±6.18	24.87* ± 2.43
Total Amino acid	06.18 ± 2.11	21.12* ± 2.14	11.01*±1.21
Total Lipid	68.56 ± 1.11	22.51*± 5.11	20.15* ± 6.10
Total Cholesterol	15.73±2.72	14.17 ± 1.54	16.30 ± 4.23

Values are mean±SD * significant decrease ($p<0.05$) when compared with control.
* Significant increase ($p<0.05$) when compared with control.

Table 3. Tissue Micronutrient Level in (µg/g) tissue of rats administered with cyfluthrin.

Micronutrient	Control	Cyfluthrin (100 ppm)	Cyfluthrin (200 ppm)
Iron	281.71±11.43	232.21*±17.21	215.38*±21.03
Zinc	119.21 ± 12.11	141.19* ± 11.25	131.79* ± 20.34
Copper	85.15±5.32	196.35*±20.15	152.72* ±11.98
Selenium	98.17±13.52	116.28* ± 23.54	124.12* ±21.96

Values: (Mean ± SD). * Significant increase ($p<0.05$) when compared with control

Table 4. Activity of anti-oxidant enzymes (U/L) of rats administered with cyfluthrin.

Anti-oxidant enzyme	Control	Cyfluthrin (100 ppm)	Cyfluthrin (200 ppm)
Glutathione Peroxidase	51.13±4.11	107.18*±10.22	142.63*±13.71
Glutathione Reductase	54.39±10.22	96.66*±6.18	88.96*±10.38
Catalase	109.23±19.38	288.13*±12.67	126.17*±13.15
Glucose-6-phosphate dehydrogenase	81.13±9.87	83.85 ± 2.56	108.86*±10.15

Values: (Mean± SD). * Significant increase ($P<0.05$) when compared with control

Table 5. Lipid peroxidation Level (mg/g of dry tissue) of rats administered with cyfluthrin.

Parameter	Control	Cyfluthrin (100 ppm)	Cyfluthrin (200 ppm)
Thiobarbiturate level	45.78±5.18	108.96*±10.85	163.53*±16.77
Reduced glutathione	75.18±11.56	25.80*±6.18	17.58*±8.12

Values: (Mean ± SD). * Significant increase ($P<0.05$), * significant decrease ($p<0.05$) when compared with control

ficant difference in the cholesterol level in the tests groups under investigation.

The carbohydrate are broken down for ATP formation which is required for the synthesis of protein needed for cyfluthrin detoxification, while some of the free glucose and amino acids are needed for the conjugation reaction. Since the metabolism has shifted to catabolism, there must be a halt on anabolism, especially the liver-derived lipids accounting for the reduction in the tissues levels of these metabolites.

The possible explanation for the reduction in the total protein is likely due to proteolysis of structural protein (likely playing an important role in the reduction of the organ-body weight ratio) using their amino acids for translation of detoxifying enzyme synthesis, conjugation of cyfluthrin metabolite or for energy metabolism with the amino group channeled into urea cycle. This accounts for the high blood level of urea and liver aspartate transaminase leading to the hypoglycemia reported by Oikawa and Iyatomi (1983). The micronutrients studied (Table 3) were those that play important roles in the detoxification of free radical; some are cofactors to antioxidant enzyme studied (Table 4). Their analysis is necessary to establish the role of free radicals generation

in the toxicity of cyfluthrin and its metabolites. The activity of the antioxidant enzymes, glutathione peroxidase increased significantly in both test groups when compared with the control. This may be due to increase in the concentration of selenium in the test groups. Glutathione peroxidase requires selenium for its optimum activity. Catalase is a hemoprotein that requires central iron for its catalysis. It plays important role in the detoxification of hydrogen peroxide. Catalase activity increased significantly in the test groups as also the iron levels. This means hydrogen peroxide generation plays important role in the toxicity of cyfluthrin. Copper and zinc play important roles in the three dimensional structures and catalysis of superoxide dismutase. There is a significant increase in the concentration of copper and zinc in the test groups. This is likely the reason for the increase in the glutathione peroxidase level, assisting catalase in the detoxification of the hydrogen peroxide and other peroxides generated from superoxide radical and superoxide dismutase. This clearly shows that under continous administration of cyfluthrin, superoxide radical is likely generated requiring the presence of Cu/Zn superoxide dismutase for detoxification. The oxidized glutathione produced during the detoxification of hydrogen peroxide must be reduced; this requires FAD-dependent glutathione reductase, whose activity increased significantly in both test groups, confirming the role of free radical in the toxicity of cyfluthrin. The reducing equivalent needed for the reduction of the oxidized glutathione is ultimately derived from NADPH, which is also generated from glucose-6-phosphate, catalyzed by glucose-6-phosphate dehydrogenase, whose activity is slightly higher than the control at 100 ppm, but significantly increases at 200 ppm concentration of cyfluthrin. This indicates that the extent of free radical generation is possibly dose-dependent. Thus, accumulation of cyfluthrin and its intermediates can cause oxidative damage in tissues under continous administration.

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