Original Paper

Cyfluthrin-induced Nephrotoxicity in Rats

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ABSTRACT

This study was designed to evaluate the nephrotoxic effect of cyfluthrin in rats. Cyfluthrin at 0ppm (control), 100ppm and 200ppm (test-groups) was administered orally for 15 weeks.The 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) were estimated. The results were statistically compared ($p<0.05$) with the control. A significant decrease in the organ-to-body ratio was observed in the test groups. The concentration of the micronutrient in the test groups increased significantly. The total carbohydrate, total glucose, total amino acids, total lipids and total cholesterol showed a significant decrease in the test groups, but a significant increase was observed in the tissue protein level of the test groups. 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 in the test groups when compared with the control. Cyfluthrin is nephrotoxic under continuous administration in rats.

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Introduction

Cyfluthrin is a synthetic type-2 pyrethroid insecticide. The original compound was isolated from the flower of chrysanthemum.[1] It has been effective in the control of a wide range of insects including cutworms, cockroaches, ants, termites, grain-beetles, weevil, fleas, flies and mosquitoes.[2] Due to its versatility it has become a useful active ingredient in the manufacture of many insecticides.[3] The insecticidal action of cyfluthrin has been traced to its ability to block sodium-ion-gated channels, leading to membrane depolarization and loss of electrical excitability in the central and the peripheral nervous system.[4] Typical signs of intoxication include hyper-excitability, convulsions, body tremor, ataxia, incordination, choreoathetosis, and salivation.

Early reports have shown that cyfluthrin exhibits low toxicity to mammals due to rapid metabolism and excretion; however laboratory animals exposed to very high dosages of the compound had exhibited the same toxic effect observed in insects.[5] Also, reports of dermal toxicity from skin contact have been published.[6] Cyfluthrin triggers inflammatory reaction in the kidneys of female rats, and an irreversible damage to the sciatic nerves.[7]

In this study, we assessed the nephrotoxic effect of cyfluthrin by monitoring the effect of continuous administration of sub-lethal dose of cyfluthrin on the organ weight changes, micronutrient levels, nutritional status, and the antioxidant enzyme activities in the kidneys of rats.

Materials and Methods

Chemicals and Assay Kits:

1. 3-(2,2-dichloro-vinyl)-2,2-dimethyl-cyclopropane-carboxylic acid cyano-(4-fluoro-3-phenoxy-phenyl)-methyl ester (BETA-cyfluthrin) - a product of Bayer agricultural products (M.O., USA)
2. Sodium sulphate, copper sulphate, cholesterol, lecithin and thiobarbiturate standard - products of Sigma Chemicals Co. (St. Louis, M.O., USA)
3. Concentrated sulphuric acid, ethanol, acetic acid diethyl ether - products of British Drug House (Poole, England)
4. Glutathione peroxidase kit, catalase kit, glutathione reductase kit, and superoxide dismutase kit - products of Sigma-Aldrich

   All other fine chemicals were of analytical grade.

Animals: 45 rats (*Rattus norvegicus*) between 80-95g 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, 100ppm and 200ppm of cyfluthrin dissolved in 20% lecithin in water). 1ml of the solution was administered daily by gavages for 15 weeks. At the end of the treatment, the animals were sacrificed under light anesthesia and the organs perfused in physiologic saline solution.

Assay for Glutathione Peroxidase Activity[8]: Glutathione peroxidase assay was 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. The decrease in NADPH absorbance measured at 340nm is indicative of glutathione peroxidase activity.

Assay for Glutathione Reductase Activity[9]: The enzyme activity is a measure of increase in the absorbance at 412nm of 5, 5'-dithiobis (2-nitrobenzoic acid) when it is reduced by glutathione reductase.

The in vitro catalase activity was determined using a modification of Ashiru and Singha method.[10] Superoxide dismutase activity was estimated by Misra and Fridovish method.[11] Glucose-6-phosphate dehydrogenase activity was determined using a modification of Gupta and Baquer method.[12]

Determination of Lipid Peroxidation Level: Estimation of reduced glutathione level was determined by the method described by Moron et al.[13] Thiobarbiturate level was determined by a modification of Ohokawa et al method.[14]

Determination of Nutritional Status: Total lipid in the tissue was determined using the soxhlet extractor method according to the AOAC method.[15] Total Nitrogen content was determined by the Kjedahl's method AOAC.[15] The total carbohydrate was determined using the phenol/sulphuric acid method.[16] The cholesterol level was estimated using cholesterol oxidase method.[17] Free glucose was determined by glucose oxidase method (Randox Kit). Total amino acid level was determined by the Ninhydrin method.[18]

Micro-nutrient Analysis: 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) modification of Turnland and Acord method.[19]

Statistical Analysis: The values were 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 comparison of each of the test groups and the control.

Results (*see Tables 1–5*)

Table 1: Dry Organ Weight to Body Weight Ratio (g/g)

A (control)	B (100ppm)	C (200ppm)
0.087±0.004	0.032*±0.006	0.025*±0.003

Values: (mean SD), *Significant decrease ($p<0.05$) when compared with control

Table 2: Nutritional Status of the Kidney (mg/g of tissue)

Nutrient	A	B	C
Total Carbohydrate	45.31±1013	20.13*±1.32	08.36*±1.86
Total glucose	18.68±2.32	10.13*±2.51	04.84*±0.15
Total Protein	105.56±6.82	185.56*±6.18	248.87*±7.13
Total Amino acid	49.98±2.18	19.18*±2.56	11.01*±2.76
Total Lipid	84.56±1.87	32.11*±6.32	20.15*±4.56
Total Cholesterol	26.75±6.52	7.17*±0.91	05.30*±1.36

Values: (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 (mg/g) of Tissue

Micronutrient	A	B	C
Iron	201.11±20.85	245.15*±11.15	295.91*±15.79
Zinc	96.13±18.17	181.19*±9.25	201.49*±14.35
Copper	85.15±5.32	196.35*±20.15	241.83*±18.13
Selenium	87.18±10.35	189.97*±12.15	213.01*±10.85

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

Table 4: Specific Activity of Anti-Oxidant Enzymes Unit of Enzyme/mg of Protein

Anti-oxidant Enzyme	A	B	C
Glutathione Peroxidase	51.13±4.11	107.18*±10.22	142.63*±13.71
Glutathione Reductase	64.39±10.22	96.66*±6.18	138.96*±10.38
Catalase	109.23±19.38	288.13*±12.67	296.17*±13.15
Glucose-6-phosphate dehydrogenase	81.13±9.87	193.85*±18.56	208.86*±10.15

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

Table 5: Lipid Peroxidation Level (mg/g of dry tissue)

	A	B	C
Thiobarbiturate level	45.78±5.18	108.96?±10.85	163.53?±6.77
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

Discussion

Cyfluthrin is excreted mainly as metabolites in the urine with 40% of cyfluthrin-metabolite recovered in 48 hours.[20,21] This suggests that the kidneys play a vital role in the elimination of cyfluthrin and its metabolites. The accumulation in the nephrons must have accounted for the significant decrease ($p<0.05$) in the organ weight to body ratio (Table 1). The un-metabolized cyfluthrin or its metabolites may have a necrotic effect on the organ. Up to 9% of administered cyfluthrin remains unchanged (un-metabolized) in analyzed kidneys of cows.[22]

Lipid peroxidation level (Table 5) shows a significant increase in both study groups when compared with the control, as reflected by an increase in the thiobarbiturate level and a decrease in the level of reduced glutathione. Although, no previous studies have implicated kidney damage in recurrent exposure of a subject, its effects on the liver have been documented.[23] Since both liver and kidney play synergetic roles in xenobiotic metabolism, an adverse effect on the liver may in all likelihood affect the kidneys.

The specific activities of antioxidant enzymes were also examined (Table 4). Although they all show significant increase ($P<0.05$), catalase shows the highest value denoting that cyfluthrin nephrotoxicity is via a hydrogen peroxide pathway; this is supported by Villarini,[24] who reported an increase in the hepatic catalase activity in the administration of Type II pyrethroid. The concentrations of tissue micronutrient necessary for catalase **(**haemoprotein**), (**Fe**),** RNA polymerase (Zn^{2+})**,** superoxide dismutase (Cu^{2+}/Zn^{2+}**)** and glutathione peroxidase (Se) production were also estimated (Table 3). The significant increase ($P<0.05$) in the kidney concentration of the micronutrient further suggests that the accumulation of cyfluthrin and its metabolites in the kidney triggers induction of the synthesis of the enzymes responsible for free radical detoxification, an observation typical of type II pyrethroid.[24] Nutrition plays an important role in the detoxification of the free radical.[25] The nutritional component of the cell has a direct link with the energy level of the cell and the functional integrity. The metabolic products of the metabolism of cyfluthrin are majorly glucuronidated or conjugated to glycine.[26, 21]

The utilization of the nucleotide triphosphates as energetic in the expression of the genes involved in the metabolism of the cyfluthrin, and the use of glucose as the precursor of UDP-glucuronic acid all depend on the availability of glucose, which also depends on the carbohydrate store, thus accounting for the significant reduction ($p<0.05$) in the glycogen and free glucose in the test groups when compared with the control. The significant increase ($p<0.05$) observed for the protein is consistent with the increased protein synthesis associated with the detoxification of the compound, and possibly the expression of the enzymes responsible for detoxification of the free radical generated during the accumulation and metabolism of cyfluthrin. The significant increase ($p<0.05$) in the amino acid concentration is explained with respect to the utilization of the amino acids for the conjugation reactions of the cyfluthrin metabolites like glycine and possibly glutathione, which is a derivative of glycine, glutamate, and cysteine, or its conversion to glucose via gluconeogenesis, which is required for the conjugation reaction or the energy metabolism.

The continuous exposure of the kidney to cyfluthrin is toxic and may result in the loss of kidney functions and a large alteration in the body homeostasis.

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