The Pyrethroid Cypermethrin-Induced Biochemical and Histological Alterations in Rat Liver

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Cypermethrin, a synthetic pyrethroid, has broad-spectrum use in agriculture, domestic and veterinary applications due to its high bioefficacy, enhanced stability and considerably low mammalian toxicity. The objective of this study was to investigate the cypermetrin-induced alterations in the liver tissue of Wistar male rats, based on the histopatological, enzymological analyses and apoptotic changes. The animals of the experimental groups were orally fed with laboratory chow combined 60, 150, and 300 mg/kg Kral 250 EC during 28 consecutive days. At the end of the treatment, no significant change was found in relative liver weights, liver total proteins and cholinesterase enzyme activities of cypermethrin treated rats, when compared with control animals. Histopathological changes such as vacuolar degeneration, enlargement of the sinusoids, degeneration in hepatic cords and hepatocytes, vacuole formations in hepatocytes, pleomorphism in nucleus, and congestion were observed in liver tissues of only 150 and 300 mg/kg cypermethrin treated rats. Mononuclear cell infiltration and an increase in the Kupffer cells in liver parenchymatous tissue were also determined. In all cypermethrin treated groups, the apoptotic index in livers of rats was significantly increased compared to control group ($p < 0.001$). These results suggest that cypermethrin might cause hazardous effects in different levels to non-target organisms.

Key words —— cypermethrin, histopathology, apoptosis, cholinesterase activity, liver, rat

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INTRODUCTION

The undesired effects of pesticides have been recognized as a serious public health concern during the past decades. Synthetic pyrethroids such as cypermethrin, permethrin, and deltamethrin are increasingly used for indoor pest control because of their high insecticidal activity and considerably lower mammalian toxicity compared with other pesticides.[1)] In 1977, cypermethrin was allowed for turnover as a very active synthetic pyrethrin insecticide, effective in the control of many pest species in agriculture, animal breeding and the household.[2,3)] After household treatments, it persists in air and on walls and furniture for about three months.[4)] In spite of the low toxicity of pyretroids, persistence of these compounds in mammalian tissues may be dangerous.[5)] Permanence of cypermethrin and its fatty acid conjugates in adipose tissue, brain and liver was reported in rats.[6,7)] Several studies have demonstrated that cypermethrin has hepatotoxic potential in rodents and it also acts as a neurotoxin in mammals and insects and suppresses immune system.[1,4,8–12)]

The data in the literature about cypermethrin were obtained in different experimental conditions such as different doses, animals and different treatment schedule. This study is a repeated dose 28-day oral toxicity study in rodent (OECD 407)[13)] and it has been designed to assess histopathological, biochemical, and organ weight endpoints and changes in hepatocyte apoptosis. The aim of the current study was to analyze the subacute hepatotoxic effect of the orally administered cypermetrin in Wistar albino male rats, based on histopathological and biochemical findings. The TUNEL assay was used to detect apoptotic cells in liver.

MATERIALS AND METHODS

Chemicals —— Commercial formulation of cypermethrin (250 g of cypermethrin/l), Kral 250 EC (Safa Agriculture, Türkiye) was used. It was in the form of emulsion and adequate dilutions were done in water in order to reach test concentrations (60, 150, and 300 mg/kg). The test concentration of cypermethrin was calculated from the percentage of the active ingredient of commercial formulation of cypermethrin. Solutions were freshly made immediately before usage. All the other reagents used were of analytical reagent grade and obtained from Sigma (St Louis, U.S.A.).

Animals and Experimental Design —— The protocol was approved by the Animal Ethical Committee of Ege University, Faculty of Medicine. The study was conducted on 80 adult male (8-week old) Wistar albino rats weighting 120–160 g obtained from Breeding Center of Experimental Animals in Ege University, Faculty of Medicine. After 10 days of acclimation, the rats were assigned randomly to either the exposure groups (60, 150, and 300 mg/kg) or the control group, each containing 20 male rats and housed individually in labelled cages (19 × 19 × 12 cm) with solid plastic sides and stainless-steel grid tops and floors. They were maintained in controlled laboratory conditions of 12 hr dark/ light cycle, 21 ± 1°C temperature and 45–75% humidity. Animals of the control group were orally fed daily with a normal diet in standard laboratory chow (10 g/ rat/day), while the animals of the treated groups were fed with laboratory chow (10 g/rat/day) combined cypermethrin during 28 consecutive days as described in OECD guideline 407.[13] Tap water was also available *ad libitum*. All animals were weighed weekly throughout the study.

Biochemical Assays —— After 28 days of the experiment, ten animals of each group were killed by cervical dislocation and the livers them were dissected out, weighed and stored at –70°C until analysis. The liver tissues (1 g) homogenized with Ultra Turrax homogeniser in 5 ml of 50 mM phosphate buffer (pH: 7.4). The particle free supernatant was obtained by centrifugation at 5000 g for 20 min at 4°C and used as enzyme source. Liver cholinesterase (ChE) activities were determined by the spectrophometric method of Ellman *et al*.[14] The assay mixture contained 0.259 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB) in 67 mM phosphate buffer, pH: 7.4, 0.298 mM buthirylthiocholine iyodide and 20 μl of 250-fold dilution of the enzyme source in a total volume of 3.02 ml. Reaction was followed at 410 nm for 10 min intervals at 37°C against blank containing buthirylthiocholine iyodide and phosphate buffer. The extinction coefficient of the product of the chemical reaction, 5-thio-2-nitrobenzoate is 13.61 mM^{-1} cm^{-1}. The protein content was estimated, described previously by Lowry *et al*.[15] On the other hand, the liver weights were recorded and relative liver weights of each animal were calculated.

Histopathological Examination —— For light microscopic examination, other 10 animals of each group were anaesthetized (0.10 mg/kg Ketalar® + 0.02 mg/kg Rompun®, i.p.) and perfused transcardially with 100 ml heparinised saline followed by 300 ml of 4% para-formaldehyde in 0.1 mol/l phosphate buffer (pH: 7.4). Livers were removed, post-fixed for 24 hr in the same fixative, and processed for paraffin embedding. After routine processing, paraffin sections of each tissue were cut into 5–6 μm thickness and stained with haematoxylin and eosin (H&E).

Analysis of Apoptosis in Tissue Sections —— Apoptosis in the liver was defined and quantitated as previously described by Promega DeadEnd™ Colorimetric Apoptosis Detection System (TUNEL) (Promega Corp., Madison, U.S.A., Cat No: G7130).[16] For TUNEL, cells were fixed in 4% paraformaldehyde solution for 25 min at room temperature, rinsed in phosphate buffered saline (PBS) and permeabilized by immersing the slides in 20 μg/ml Proteinase K solution. Cells were incubated with terminal deoxynucleotidyl transferase (rTdT) reaction mixture containing biotinylated nucleotides and rTdT at 37°C for 60 min, rinsed with sodium chloride-sodium citrate buffer (SSC) and PBS. Streptavidin horseradish peroxidase (HRP) was added to each slide and incubates for 30 min at room temperature. Slides were then stained with diamino benzidyne system (DAB). In this study, TUNEL (+) immunoreactivity was assessed by light microscopy (Olympus BX-51 light microscope, Olympus C-5050 digital camera) at a magnification of ×100. TUNEL (+) cells were counted with the use of Image-Pro Express (Media-Cybernetics, 2002, U.S.A.) image software in rat liver. The number of apoptotic hepatocytes (at any morphological phase) was divided by the total number of hepatocytes and multiplied by 100 to achieve and apoptotic index. For each group, a minimum of 2000 hepatocytes was counted.

Statistics —— The results of biochemical analysis, liver and relative liver weights were presented as the mean ± standard error of mean (S.E.M.). Comparisons were made between control and treatment groups using one-way analysis of variance (ANOVA) followed by Dunnett's test. Values of $p < 0.05$ were regarded as statistically significant.

RESULTS

Body and Liver Weights

Mean changes in body weight, liver and relative liver weights are summarized in Table 1. At the end of the experiment, no statistically significant differences were occurred in any treatment group com-

pared with control group for absolute change in weight gain ($p > 0.05$). When compared with the control animals, while a significant decrease was determined in the liver weight of the rats treated with 150 and 300 mg/kg cypermethrin ($p < 0.05$), no significant change was found in any treatment group for relative liver weights.

Biochemical Changes

Table 2 shows the effect of cypermethrin on liver total protein values and ChE activities of experimental rats. There was no significant change in liver total protein values among control and treatment groups. Liver ChE activities were increased in all treatment groups compared with control values but these inductions were not statistically significant (p values for 60, 150, and 300 mg/kg doses are 0.448, 0.307, and 0.059, respectively).

Histopathological Changes

The liver of control animal is showed in Fig. 1A. The administration of cypermethrin for 28 days resulted in dose-dependent degenerative changes of variable degrees in many areas of the liver. Histopathological effects of 150 and 300 mg/kg cypermethrin on the liver of treated animals are presented in Fig. 1B–1D. An enlargement of the sinusoids, degeneration in hepatic cords and hepatocytes, vacuole formations in hepatocytes were determined in the liver of the rats in 150 and 300 mg/kg cypermethrin treatment (Fig. 1B). Mononuclear cell infiltration and an increase in the number of Kupffer cells (hyperplasia) were designated in the liver parenchymatous tissue of rats (Fig. 1C). In addition, congestion was noted in the liver of all treated rats (Fig. 1D). Vacuolar degeneration as a result of an increase in the number of lipid vacuoles in cytoplasm and therefore a cytoplasmic damage were observed in the liver tissues of 150 and 300 mg/kg cypermethrin treatment.

Apoptotic Index in Rat Liver

When compared with control animals, there were significant increases on apoptotic index in liver tissues of cypermethrin treated rats ($p < 0.001$). Table 3 and Fig. 2A–2D is represented apoptotic nuclei of rat hepatocytes. In comparison with the control group, 91, 253, and 595% dose-dependent increases of apoptotic nuclei in liver tissues were observed in rats treated with 60, 150, and 300 mg/kg cypermethrin, respectively.

Table 1. Mean Changes in Body Weight, Liver and Relative Liver Weights of Rats in Control and Cypermethrin Treated Groups

Parameters	Control ($n = 10$)	60 mg/kg ($n = 10$)	150 mg/kg ($n = 10$)	300 mg/kg ($n = 10$)
Initial Weight (g)	157.1 ± 24.2	120.0 ± 28.7	121.0 ± 15.9	126.3 ± 14.3
Final Weight (g)	180.0 ± 25.1	173.1 ± 21.4	154.8 ± 13.7	155.9 ± 11.3
Weight Change (g)	22.9	53.1	33.8	29.6
Liver Weight (g)	6.56 ± 0.65	6.59 ± 0.66	4.75 ± 0.19[a)]	5.30 ± 0.92[a)]
Relative Liver Weight	0.030 ± 0.002	0.038 ± 0.003	0.031 ± 0.003	0.034 ± 0.002

Values are given as mean ± S.E.M. *a*) Statistically significant difference from control by Dunnett test ($p < 0.05$). n is total number of animal in each group.

Table 2. Liver Total Protein Values and ChE Enzyme Activities of Rats in Control and Treatment Groups

Groups	n	Total Protein (mg/ml)	ChE (μmol/min/mg protein)
Control	10	16.87 ± 2.93	0.0045 ± 0.0009
60 mg/kg	10	22.35 ± 4.70	0.0066 ± 0.0013
150 mg/kg	10	26.00 ± 1.28	0.0073 ± 0.0017
300 mg/kg	10	23.48 ± 5.29	0.0099 ± 0.0030

Values are given as mean ± S.E.M. n is total number of animal in each group.

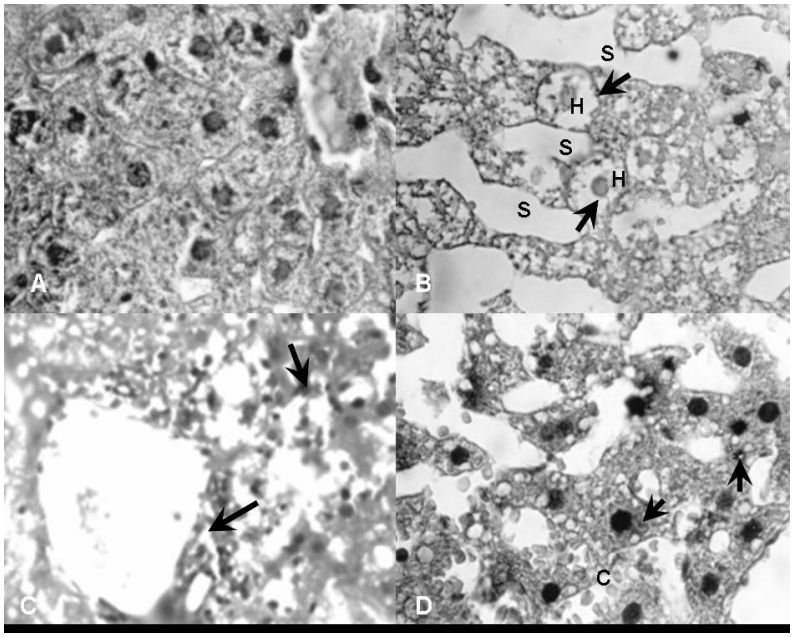

Fig. 1. The Administration of Cypermethrin for 28 days (150 and 300 mg/kg) Resulted in Damage of Architecture of Liver Cells along with Disarrangement of Hepatic Cords

A: The liver of control animal. B–D: An enlargement of sinusoids (S), vacuole formation in hepatic paranchyma (arrow), degenerative changes in hepatocyte (H) could only be observed in 150 and 300 mg/kg dosed animals. 1D also exhibit erythrocytes in liver paranchyma (C). All such changes some time also appears when animals are killed using faulty way of killing. H&E Stain; Original magnification, × 40 (A, B, and D), × 10 (C).

Table 3. Apoptotic Index of Rats in Control and Treatment Groups

Groups	n	Apoptosis	Increase (%)
Control	10	4.85 ± 0.26	—
60 mg/kg	10	$9.28 \pm 0.35^{a)}$	91.3
150 mg/kg	10	$17.14 \pm 0.50^{a)}$	253.4
300 mg/kg	10	$33.71 \pm 1.22^{a)}$	595.0

Values are given as mean ± S.E.M. *a*) Statistically significant difference from control by LSD by Dunnett test ($p < 0.001$). n is total number of animal in each group.

DISCUSSION

The prolonged and indiscriminate use of cypermethrin is reported to cause both acute and chronic toxicity in non-target species including humans.[17,18] Most toxic chemicals are metabolized in liver and these processes may cause liver injuries. In our study, the evaluation of liver tissue using biochemical and histopathological assays indicated that subacute doses of cypermethrin induce only dose-dependent histopathological changes in the tissue; however, in biochemical examination, no significant change in total protein and ChE enzyme levels was determined.

Long-term feeding studies with laboratory animals have shown adverse effects of cypermethrin: it caused reduced growth rate and increased liver weight in rats.[19] However, our study is a repeated dose 28-day oral toxicity study in rat and it is a short-term feeding study. Therefore, in this study, while there was no significant change in relative liver weight of rats of all cypermethrin treated groups, there was a significant decrease in the liver weight of the rats treated with only 150 and 300 mg/kg per day cypermethrin. Body weight gain of rats in experimental groups did not change throughout the experimental period when compared to control. It was reported that some synthetic pyrethroids such as permethrin, deltamethrin had no effect on body weight gain of rats.[20,21] These results support with ours.

There is limited research about the effects of cypermethrin on ChE enzyme activity in liver. In our study, cypermethrin had no significant effect in total protein and ChE enzyme values of liver. Similarly, in another study, no statistically significant changes were detected in total protein levels during 7 days of i.p. doses of 300 mg/kg cypermethrin treatment.[22] However, in chronic studies it was observed that cypermethrin caused an increase in protein con-

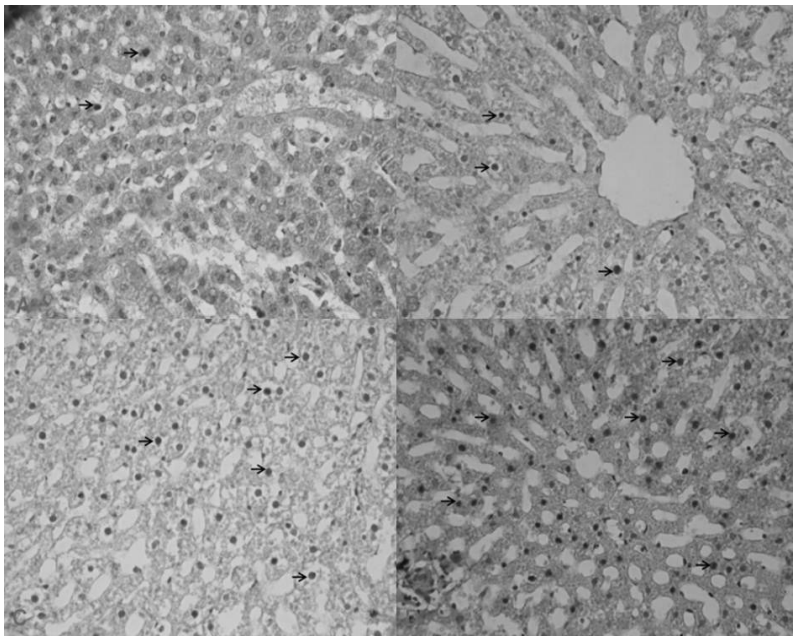

Fig. 2. A: The Liver Tissue of Rats in Control Group, B–D: The Liver Tissue of Rats Treated with 60, 150, and 300 mg/kg Cypermethrin, Respectively

The arrows points apoptotic nuclei of rat hepatocytes. Slides were stained with DAB. Original magnification, × 10.

tent.[23)]

Our histological results showed that histopathological changes such as mononuclear cell infiltrations, an enlargement of the sinusoids, degeneration in hepatic cords and hepatocytes in liver of rats exposed to 150 and 300 mg/kg cypermethrin. Similar histolopathological changes such as mononuclear cell infiltration and parenchymatous degenerations of hepatocyte in liver were observed in Wistar rats exposed 250 mg/kg orally alpha-cypermethrin.[3)] However, it was reported that slight histopathological changes in liver was observed in dermally cypermethrin applied rats.[24–26)] Daily oral cyhalothrin administration in mice at levels up to 2000 mg/kg diet for 28 days showed dose-related histopathological changes in liver of rats exposed to dosages of 100 mg/kg and above.[27)] Histopathological liver damages caused by cypermethrin were determined in other studies as well.[22,28,29)]

In vivo and *in vitro* studies of cypermethrin have shown that it causes necrosis, inflammation and cytoplasmic hypertrophy in hepatocytes.[30,31)] Previous studies demonstrated cypermethrin-induced hepatotoxicity in rats.[22,24,32)] We have also observed that subacute hepatotoxicity of cypermethrin on the rats. There were significant increases on apoptotic index in liver tissues of cypermethrin treated rats. In comparison with the control group, dose-dependent increases of apoptotic nuclei in liver tissues were observed in rats treated with 60, 150, and 300 mg/kg cypermethrin, respectively. Programmed cell death or apoptosis is important during embryonic development, maintaining tissue homeostasis, and for removing damaged or infected cells. However, apoptosis has been shown to be triggered by several factors, including exposure to pesticide.[33–36)] Our results showed that cypermethrin produced cell injury and apoptosis in rat hepatocytes. Also cypermethrin-induced apoptosis in the telencephalon of tadpoles has been reported by Izaguirre *et al.* (2000).[37)] Permethrin, another synthetic pyrethroid, caused concentration-dependent increase in both apoptotic and necrotic cell death in thymocytes.[38)] Though these findings indicate a specific role for apoptosis in cypermethrin induced toxicity, it is still premature to draw definite conclusions on the role of apoptosis in environmental pollutant-induced toxicity.

The overall results of this study showed that oral exposure to cypermethrin introduces significant histopathological alteration in liver of rats. Based on our results and literature data, we suggest that cypermethrin usage might cause hazardous effects in various levels to non-target organisms, including

human being.

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