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Effect of 28-day exposure to fenpropathrin on the activities of serum alanine transaminase and liver antioxidant enzymes in mice

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Abstract

Fenpropathrin (FEN) was administered intraperitoneally in doses of 2.38 mg/kg, 5.9 5mg/kg, or 11.9 mg/kg b.w. to mice for 28 consecutive days. FEN did not significantly affect the activity of alanine transaminase (ALT) in the sera. Superoxide dismutase and glutathione peroxidase activities were significantly elevated in the liver after a 28-d exposure to moderate or highest doses of the pesticide. These results demonstrate that the 28-day exposure to FEN leads to an up-regulation of expression of antioxidant enzymes in response to an oxidative stress in mouse liver without causing a significant increase in ALT activity.

Keywords: mouse, fenpropathrin, antioxidant enzymes.

Introduction

Fenpropathrin (FEN) is a synthetic pyrethroid (PYR). PYRs are used as insecticides (25) and acaricides (5). They are used in agriculture and horticulture to increase crops (25). Humans and animals are constantly exposed to traces of PYRs *via* water and food of plant origin (4). In Poland, current annual use of PYRs is estimated to be over 80 000 kg of active ingredient (16). However, among the 13 PYRs registered in Poland, FEN is absent.

PYRs are used in veterinary medicine to protect animals against ectoparasites (1). They are also in use for indoor pest control (*i.e.* flies, mosquitoes, termites, ants) (18), malaria (27), and tick-borne diseases prevention (5). The present global climate change, with a temperature increase by 2 to 3ºC, has raised the human population living in endemic zones of malaria by 3% to 5% (25). It compels both locals and tourists to use insecticides more frequently and sleep under bed nets soaked with PYRs for protection against mosquito bites. There is a growing number of reports of PYR abuse in veterinary medicine (1). All these data suggest that many non target organisms are at risk of sub-acute or even chronic exposure to PYRs.

PYRs are neurotoxins. The main mechanism of PYR toxicity in insects is the inhibition of voltage-dependent sodium channels in neuron cell membranes (22, 25). It produces paralysis and death of target organisms (8). In the literature, descriptions of PYR's acute toxicity predominate. In 1980, Vershoyle and Aldridge (26) described acute toxicity of 36 PYRs following their intravenous administration. They established the first taxonomy of PYR intoxication in mammals. Based on the clinical signs of intoxication, they divided the chemicals into the ones producing aggressive sparring, sensitivity to external stimuli, tremor progressing to prostration (T syndrome), and those inducing choreoatetosis, salivation, pawing, burrowing behaviour, increased startle response, and terminal clonic seizures (CS syndrome). This classification was confirmed in studies of the intracerebral toxicity of 29 PYRs in mice, when type I PYRS (parallel to producing the T syndrome) and type II (analogous to producing the CS syndrome) were distinguished (15). The main difference in the results of these studies was that FEN, classified as producing T syndrome in the first study, was classified as producing both syndromes of intoxication (tremor and salivation) in the second

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study. FEN is an α-cyano pyrethroid, albeit not a typical one. The majority of pyrethroids having the structure of esters of α–cyano-3-phenoxybenzyl alcohols produce the CS syndrome in mammals, similar to deltamethrin (the most potent PYR insecticide).

For over half a century it was believed that PYRs acted only *via* fast deregulation of the nervous system, without any significant cytotoxic effect. However, there is evidence that the exposure to pyrethroids may produce oxidative stress (6, 7) and damage to internal organs (7).

Intoxication with various xenobiotics is a form of environmental stress, which may lead to imbalance of the oxidative/antioxidative (redox) system in living organisms. The oxidative/antioxidative system under conditions of homeostasis is the organism's defence mechanism against the harmful effects of reactive oxygen species (ROS). ROS are mainly free radicals with unpaired electrons. They are constantly formed as side products of biological reactions. Cells protect themselves against excessive ROS with enzymatic (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)) and non-enzymatic (glutathione, vitamin C, melatonin) antioxidant systems. The ROS can damage DNA, proteins and lipids; change cell's metabolism; affect gene expression and posttranslational modifications of proteins, which accelerate ageing and the development of atherosclerosis, hypertension, type II diabetes, and cancer (14).

The objective of the study was to determine the effect of the 28-d exposure to FEN on the activity of alanine transaminase (ALT) in the sera and activities of antioxidant enzymes in mouse liver.

Material and Methods

Non-gravid female albino Swiss mice weighing from 18 to 24 g, approximately 6 weeks of age, purchased from a licensed breeder, were used in the study. All animals were given a 7-d acclimatisation period and maintained on a 12 h light/dark cycle. Food and tap water were provided *ad libitum.* Temperature was maintained at 21 ± 2ºC.

Fenpropathrin (RS)-α-cyjano-3-phenoxybenzyl 2, 2, 3, 3-tetramethylcyclopropane carboxylate, 99% purity, was purchased from the Institute of Industrial Organic Chemistry (Annopol, Warsaw, Poland). As PYRs poorly dissolve in water, Tween 60 (poloxyethylene sorbitan monostearate) purchased from the Laboratorium Reagenzien, Germany, was used to prepare solutions in saline (0.1 mL per 10 mL solution).

There were 4 groups of 8 randomly selected animals. They were injected intraperitoneally (i.p.) once daily for 28 consecutive days with:

- group I (control) - saline (10 mL/kg b.w.)
- group II - FEN at a dose of 2.38 mg/kg/b.w.
- group III - FEN at a dose of 5.95 mg/kg/b.w.
- group IV - FEN at a dose of 11.9 mg/kg/b.w.

All doses of FEN were dissolved in saline with Tween 60 (0.1 mL of Tween per 9.9 mL of saline). Each mouse weighing 20 g received 0.2 mL of an appropriate solution per injection.

On day 29, the animals were decapitated and their blood samples were collected. The samples were centrifuged for 4 min at 3400 rpm. ALT activity was measured in serum using Cobas Integra 800 apparatus (Roche, France). SOD and GPx activities were measured in the liver. The livers were homogenised with a mechanic blender MPW- 120 in a 0.1 M buffer of Tris-HCl, 7.4 pH (0.5 g of tissue per 5 mL of buffer). The homogenates were centrifuged for 15 min twice at 5000 rpm. SOD activity was determined in the supernatant with the use of RANSOD kit (Randox Laboratories, U.K.) with a spectrophotometric method (3). The activity of GPx was measured with the use of RANSEL kit (Randox Laboratories, U.K.), also with a spectrophotometric method (20).

The results were shown as means ± SEM, and evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test. The P value <0.05 was considered statistically significant.

Results

FEN administered to mice did not significantly affect the activity of ALT in experimental animals (Table 1). The highest activity of ALT was recorded in mice receiving the highest dose of FEN. SOD activity was significantly elevated after the 28-d exposure to the insecticide (Fig. 1). The highest activity of SOD was observed in group IV receiving the highest dose of FEN (2720 ± 94 U/g of tissue). It was significantly higher than that in the control group (1420 ± 85 U/g of tissue). SOD activity was also significantly higher in comparison to the control group (2641 ± 106 U/g of tissue) in group III receiving the moderate dose of FEN. In the group receiving the lowest dose of FEN (group II), SOD activity (1279 ± 154 U/g of tissue) did not significantly differ from that in the control group. The activity of GPx was also increased after the 28-d exposure to the pesticide (Fig. 2). The activity was significantly elevated in groups III (90 ± 7.7 U/g of tissue) and IV (106 ± 5.4 U/g of tissue) when compared to the controls (40 ± 15.4 U/g of tissue). In group II, receiving the lowest dose of FEN, the activity of GPx (49 ± 6 U/g of tissue) did not significantly differ from that in the control group.

Table 1. Activity of alanine transaminase (ALT) in the sera of mice exposed to fenpropathrin (FEN) for 28 days, evaluated on day 29. The results are shown as means ± SEM ANOVA, n = 8

Day	Group	ALT (U/l)
29	Control	54 ± 4
	Fen 2.38 mg/kg	62.5 ± 4
	Fen 5.95 mg/kg	70.5 ± 9
	Fen 11.9 mg/kg	73.7 ± 8

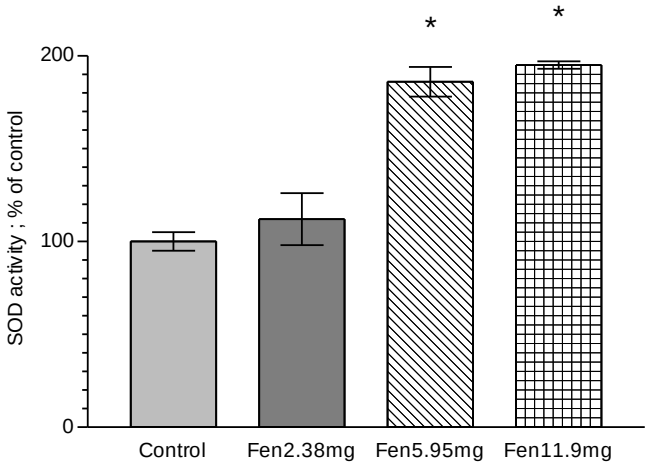


Fig. 1. Effect of the 28-d exposure to fenpropathrin (Fen) on superoxide dismutase (SOD) activity in the liver, ANOVA, Dunnett's test, n = 8, * P < 0.05

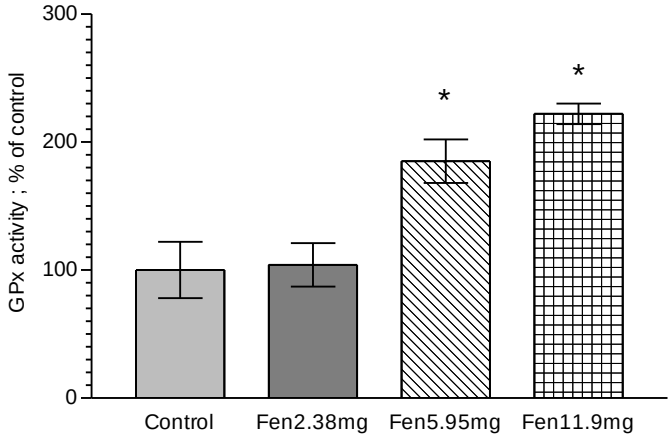


Fig. 2. Effect of the 28-d exposure to fenpropathrin (Fen) on glutathione peroxidase (GPx) activity in the liver, ANOVA, Dunnett's test, n = 8, *P < 0.05 *vs* Control

Discussion

In the present study, it was shown that the 28-d exposure of mice to subtoxic doses of FEN did not significantly affect the activity of ALT in the sera and significantly increased the activities of selected antioxidant enzymes (SOD and GPX) in the liver.

The results of our experiment are in agreement with the results obtained by Popa-Wagner (21), according to whom an up-regulation of genes coding antioxidant enzymes occurs in response to xenobiotics, which produce an oxidative stress. An exposure of a non–target organism to repeated subtoxic doses of PYRs is an extreme oxidative challenge. The

antioxidant system protects the exposed cells and tissues from damage. Probably, thanks to the reaction of the antioxidant system, a 28-d exposure of mice to FEN in our experiment did not lead to clinical symptoms of liver failure nor significant changes in laboratory parameters of liver insufficiency (for instance, an increase in ALT activity). In the case of exposure to higher doses of the xenobiotic, the antioxidant system might have become overwhelmed and the depletion of antioxidant parameters might have occurred. An imbalance between the ROS and antioxidants might have led to the development of clinical symptoms of poisoning or even the development of serious diseases (10, 11, 14).

However, sub-acute exposure to xenobiotics results in a decrease in antioxidant enzyme activity. This refers not only to the enzymatic, but also to the non enzymatic elements of the antioxidant defence system. Krzepiłko (12) studied the effect of exposure of yeast cell extracts to bifenthrin, deltamethrin, and cypermethrin on their oxidative/antioxidative system. The author demonstrated that the investigated PYR insecticides reduced concentration of thiol groups in the yeast cell extracts. These results confirmed that the xenobiotics produced oxidative stress. Moreover, the concentration of thiol groups in yeast samples was considered a very good marker of oxidative stress (23). Even an application of antioxidants such as ascorbic acid or tocopherol did not protect yeast cells from the toxicity of the used PYRs (13). However, Musik *et al.* (17) have shown that organic selenium compounds supplementation improved the oxidant/antioxidant balance in rats. The GPx are enzymes containing selenium. The activity of GPx may increase until an adequate supply of selenium in the diet maximises the enzyme activity. SODs are metalloproteins with different catalytic metal ions: copper, zinc, manganese, iron, or nickel. The lack of all these elements in the animal feed is unlikely. The animals used in our study received a regular animal feed containing all major and trace elements at the average requirement level for laboratory animals.

Pyrethroids are metabolised mainly by the liver esterases and cytochrome P450 (2, 9). Dar *et al.* (6) showed that repeated oral administration of bifenthrin (a non-cyano type I PYR producing the T syndrome) at a dose of 5.8 mg/kg daily for 20 or 30 d led to an increase in lipid peroxidation, and a decrease in SOD activity. Dubey *et al.* (7) reported a significant hepatic oxidative stress and hepatic damage in rats exposed to deltamethrin, which is also an α–cyano PYR (type II–producing the CS syndrome), administered with fluoride. Unfortunately, the experimental data about FEN are scarce. They usually refer to acute neurotoxicity, confirming significant neurotoxic effects of poisoning with FEN (19). Because mammals maintain constant, and higher than in insects, internal body temperature, have faster metabolism, and lower sensitivity of sodium channels PYRs are considered to be relatively safe for them (25). Our findings, which show that a 28-d exposure of mice to FEN did not increase the activity of ALT, support this theory.

In the past, the majority of studies on pesticide toxicity used to focus on the risk assessment of an occupational exposure of humans (24). At present, the theory predominates that in humans a non-occupational exposure to subtoxic doses of PYRs comes from drinking water (4), indoor air and dust (18), textiles (5), and food. Therefore, there is a need for further studies on effects of sub-acute and chronic exposure of non-target organisms to PYRs. Our study did not reveal any significant effect of FEN in the course of a 28-d exposure of the experimental animals on ALT activity in their sera, but did confirm that a significant increase in the activities of selected antioxidant enzymes in the liver occurs in response to subtoxic doses of the insecticide.

In conclusion, the current results show that the 28-d exposure of mice to FEN leads to an up-regulation of antioxidant enzyme expression in response to an oxidative stress in mouse liver without causing a significant increase in ALT activity in their sera. With such a toxicity profile, FEN could be under consideration for a wider use as a pesticide.

Conflict of Interests Statement: The authors of the article declare that there is no conflict of interest.

Animal Rights Statement: All experimental procedures were conducted with respect for the laws and regulations of the European Union and Poland. The experiment was approved by the Local Ethics Committee for Animal Experiments in Lublin.

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