Original Article

Effects of Cypermethrin on Sexual Behaviour and Plasma Concentrations of Pituitary-Gonadal Hormones

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Abstract

Background: Pyrethroids are commonly used as insecticides for both household and agricultural applications, and have recently been linked to endocrine disruption. Cypermethrin is a type Π pyrethroid which is used widely throughout the world. The present study was aimed to investigate the effects of cypermethrin on the sexual behaviour and plasma level of pituitary-gonadal hormones of adult male mice.

Materials and Methods: Research methodology comprised injecting mice daily with cypermethrin (10, 15, 20 mg/kg i.p.) or DMSO (0.2 ml) for five weeks. Receptive female mice were used to test male sexual behaviors (sniffing, following, mounting, and coupling). Plasma concentrations of testosterone, luteinizing hormone (LH) and follicle stimulation hormone (FSH) were measured after five weeks treatment using the ELISA method.

Results: The results of the present study showed that cypermethrin-treated groups exhibited reduced sexual behavior when compared with the control group. Assay results demonstrate significantly reduced serum testosterone levels ($p<0.05$) versus the control group, whereas FSH and LH values increased significantly.

Conclusion: This study demonstrates that cypermethrin reduces plasma testosterone concentrations and thus is able to disrupt sexual behaviours.

Keywords: Cypermethrin, Pituatory-gonadal Axis, Sexual Behaviour, Endocrine Disruptors

Introduction

In compliance with the US Environmental Protection Agency (EPA) explanation of an endocrine-disrupting compound; an exogenous agent that hampers synthesis, secretion, transport, metabolism, binding action, or removal of natural blood-borne hormones that are existent in the body and are responsible for homeostasis reproduction and developmental process, is a matter of endocrine disruption (1). Pyrethroids are synthetic chemical versions of natural compounds isolated from chrysanthemum flowers. Pyrethroids are insecticides generally applied to fields for crop protection, in addition to their use as household and pet insecticide shampoos, and are sprayed by many cities in both tropical and temperate regions for mosquito control to prevent the spread of West Nile virus. To increase their lipophilicity and photostability the natural pyrethrin structure has been altered. This alteration produces a cogent pesticide resulting in an enhanced presence of pesticide in the environment. The major target of novel synthetic pyrethroids is sodium channels (2). The categorization of pyrethroids is based on their structure, clinical exhibitions in mammalian poisoning, as well as their actions on insect nerve preparations and insecticidal functions (3). Type I pyrethroids have a simple ester bond at the central linkage without the α cyano group. Type II pyrethroids (including cypermethrin) have α cyano group at the α carbon of this ester linkage (4). The α cyano group greatly increases the neurotoxicity of type II pyrethroids and they are mainly believed to be more efficient and toxic than type I pyrethroids. Among pyrethroids, cypermethrin is a fourth generation synthetic and a type Π pyrethroid in common and widespread use today. Its chemical structure is shown in figure 1 (5). The pyrethroids are more stable than natural pyrethrins, and systemic toxicity arises ensuing ingestion. Direct penetration of pyrethroids takes place across the skin to the peripheral sensory nerves (1, 6). Pyrethrins and pyrethroids make longer than activation of the voltage-dependent sodium channels by binding to it in the open position, causing a prolonged depolarization (3).

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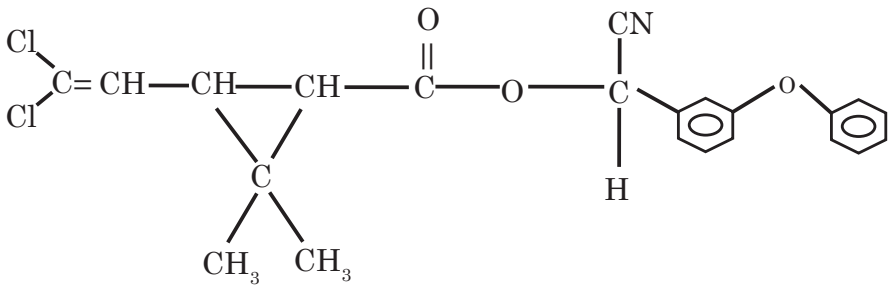


Fig 1: Molecular structure of cypermethrin

This effect on voltage-sensitive sodium channels is responsible for the insecticidal activity, as well as the toxicity of the pyrethroids to nontarget species (6, 7). Type II pyrethroids are more potent, and lead to considerable after-potentials and eventual nerve conduction block. Furthermore, pyrethroids block voltage-sensitive chloride channels which may increase CNS toxicity (8). The pyrethroids and pyrethrins are lipophilic and are rapidly distributed to the central nervous system. Pyrethroids, including cypermethrin, have been designed to be effective longer than pyrethrins. Since cypermethrin is one of the most consumed insecticides in Iran, in the present study, we investigated the effects of cypermethrin on sexual behaviours, plasma level of pituitary-gonadal hormones, and its capability to disrupt endocrine system homeostasis.

Materials and Methods

Chemicals and reagents

Heparinized haematocrit tubes (Haematokrit Kapillaren, Hirschmann. Laborgerate, Eberstadt, Germany) were purchased from BRAND GMBH + CO KG. DMSO and diethyl ether was purchased from Merck (Darmstadt, Germany). Serum testosterone, LH and FSH commercial enzyme-linked immunosorbent assay (ELISA) kits were acquired from Demeditec Diagnostics Ltd., Germany. Commercial cypermethrin was purchased from local dealers.

Animals and maintenance

Normal adult male NMRI mice weighing 20-30 g were obtained from Razi Vaccine and Serum Research Institute, Karaj, Iran. Mice were housed in groups of four in standard plexiglas cages with sterilized pine shavings as bedding material at a temperature of 22-26 °C with a humidity-controlled and an air-conditioned colony room. The animals were given tap water and a commercial standard pelleted diet ad libitum and maintained on 12 hours light: 12 hours dark controlled photoperiod conditions (lights on from 0800 a.m. to 0800 p.m.). All animal experiments were performed in accordance with the Institutional Guide for the Care and Use of Laboratory Animals.

The mice were randomly allocated to three experimental groups and a control (DMSO) group, each consisting of eight animals. Animals in the control group were injected with 0.2 ml of DMSO for 1 month of daily treatment. Three experimental groups were given three different doses of cypermethrin (10, 15, and 20 mg/kg per day) i.p. for five weeks. Adult male NMRI mice were administered a suspension solution of cypermethrin in 0.2 ml DMSO at the mentioned doses. In the first phase of the research mice sexual behaviours were assessed after the completion of the dosing period; and then, under light anesthesia with diethylether, blood samples were collected via heparinized haematocrit tubes which were implanted into the retro-orbital sinus plexus of the eyes to determine serum testosterone, follicle stimulation hormone (FSH) and luteinizing hormone (LH) concentrations.

Sexual behavior tests

Receptive female mice were used to test male sexual behaviors (sniffing, following, mounting, coupling) in such a way that males were placed in the female's acrylic cage (25 cm× 25 cm × 40 cm; l × w × h) containing wood chips with food and water provided ad lib. Before studies of sexual behaviours, control (DMSO) and cypermethrin treated males were separately placed in a cage with a sexually experienced male and a receptive female to have prior learning or experience. Early morning of the assessment day, sexually naive males were separated and kept apart until evening. Each naive male, currently sexually experienced, was given 60 minutes to accompany a receptive female, during which male behaviours were assessed and compared.

Table 1: Effects of varying doses of cypermethrin on behavioural components of mice treated with the insecticide in comparison with the control group (Mean± SE).

	Control	Sham	Cypermethrin (10 mg/kg)	Cypermethrin (15 mg/kg)	Cypermethrin (20 mg/kg)
Numbers of sniffing	14 ± 1.1	12.88 ± 0.97	9.23 ± 0.88*	8.38 ± 0.99**	8.38 ± 1.1**
Numbers of following	8.88 ± 0.83	6.75 ± 0.86	5.38 ± 1	4.25 ± 8.8**	4.25 ± 0.64*
Numbers of mounting	3.25 ± 0.5	3 ± 0.53	1.8 ± 0.47	1.25 ± 0.45*	1.25 ± 0.31*
Numbers of coupling	0.9 ± 0.1	0.8 ± 0.13	0.3 ± 0.15*	0.2 ± 0.13**	0.2 ± 0.13**

** p<0.05 and ** p<0.01, difference from the control group.*

Three separate repetitions of the experiment were run for each male. Sniffing, following, mounting and coupling were the assessed sexual behavior parameters. During sexual behavior tests, if the male mice showed no mounts during 30 minutes the mounting component was over. If not, they were permitted up to 60 minutes additional time for coupling and ejaculation (9-11). During all testing sessions behavioural parameters were recorded on videotape and analyzed after the completion of the experiments.

Hormone assay

Testosterone, LH and FSH hormones were assayed by solid phase enzyme-linked immunosorbent assay (ELISA) kits, based on the principle of competitive binding and according to the manufacturer's instructions.

The microtiter wells are coated with an antibody directed toward an unique antigenic site on the hormone molecule.

Endogenous hormone of a serum sample competes with a hormone horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is removed by washing.

The amount of bound peroxidase conjugate is in reverse proportion to the concentration of in the sample. After addition of the substrate solution, the intensity of color developed is also in reverse proportion to the concentration of hormone in the serum sample.

Statistical analysis

Since data displayed normality of distribution and homogeneity of variance, one-way ANOVA and Tukey post hoc test (SPSS software) were used for comparison between the effects of different doses of extract with the control. Microsoft excel software was used for drawing graphs.

Results

The results of this research suggested that cypermethrin significantly decreased sexual behaviour components including sniffing, following, mounting, and coupling in comparison with the control group (Table 1).

Measuring sexual hormones after injecting cypermethrin and assessing behavioural components showed that plasma testosterone concentrations were significantly reduced in the cypermethrin treated groups (Fig 1).

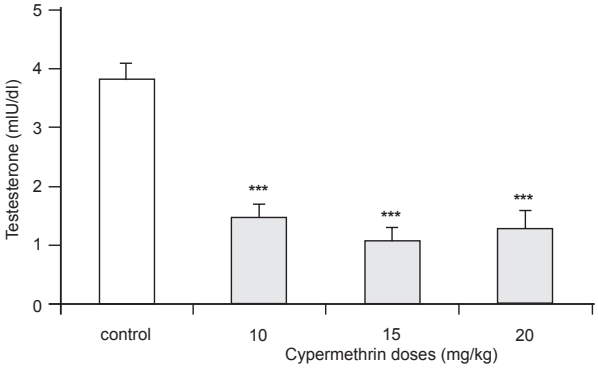
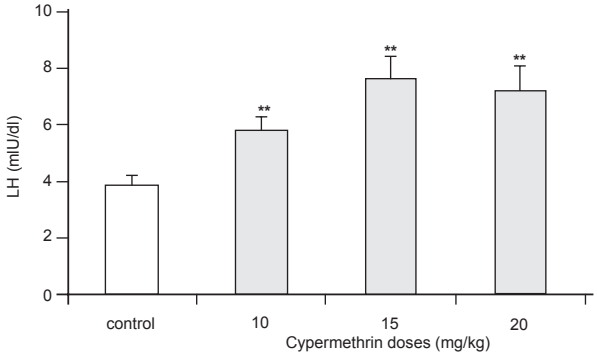


*Fig 1: Effect of different doses of cypermethrin on serum testosterone levels in comparison with the control group (Mean± SE). *** p< 0.001, difference from the control group.*



*Fige 2: Effect of different doses of cypermethrin on serum LH levels in comparison with the control group (Mean± SE).** p < 0.01, difference from the control group.*

The present results also show that cypermethrin-treated mice have significantly elevated se-

rum FSH and LH concentrations vs. the control group (Fig 2, 3).

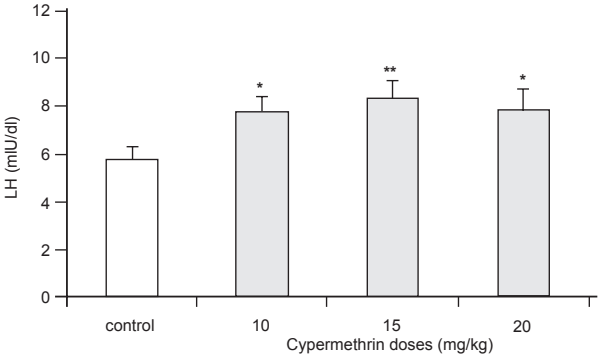


Fig 3: Effect of different doses of cypermethrin on serum FSH levels in comparison with the control group (Mean± SE) * p < 0.05 and ** p < 0.01, difference from the control group.

Discussion

The results of the present study demonstrate that i.p. dosing of adult male NMRI mice for 35 consecutive days with cypermethrin decreases sexual behaviors (sniffing, following, mounting and coupling). However continued treatment with cypermethrin lowers circulating testosterone levels significantly and increases the serum FSH and LH levels.

Previous studies show that cypermethrin is able to influence some reproductive and fertility parameters as exposure to this chemical can cause significant increase in the production of nonviable or abnormal sperm in mice (5, 12, 13). Ingestion of cypermethrin at high doses (18.93 or 39.66 mg per day) resulted in a significant increase in the weights of testes and seminal vesicles of male Sprague-Dawley rats. Also epididymal and testicular sperm counts as well as daily sperm production were significantly decreased in exposed males (5). It has been determined that pyrethroid type II insecticides are involved in interplay with the picrotoxin site of the GABA receptor complex, thus activating it (1, 7). From another standpoint, male sexual behaviour is encompassed by the many behaviours affected by the GABA neurotransmitter (14). This neurotransmitter is deemed to suppress male copulatory behaviour and the thorough erectile response in particular. Stimulation of GABAA receptors in the middle preoptic area (MPOA) has been shown to diminish the number of animals that demonstrated mounts and ejaculations (15). Fenvalerate, another different type II pyrethroid, was proclaimed to bear behavioural manifestations in rats that that resembled those provoked by anxiogenic-like drugs (3, 16), most probably by acting on GABAA receptor sites within the central nervous system (CNS) (17-20).

Studies by Elbetieha and his co-workers have documented that treatment of rats with cypermethrin at doses of 18.93 or 39.66 mg per day decreased FSH and LH levels as well as testosterone levels (5). However our study showed that treatment with lower doses of cypermethrin decreased serum testosterone levels while increasing serum LH and FSH levels.

The key role of testosterone in health (enhanced production of red blood cells, increased energy and protection against osteoporosis) as well as sexual functioning (increased libido) is noticeable, and the disruption of its production may impair male reproductive health (21-24). Previous studies have shown that decreased levels of testosterone or disorders involving testosterone synthesis caused decreases in sexual activity (23, 24). Some pyrethroid exposures in rats have been reported to cause significant decreases in testicular enzymes for testosterone biosynthesis, such as 17ß-hydroxysteroid dehydrogenase (17ß-HSD) and glucose-6-phosphate dehydrogenase, which might be due to interference with testicular testosterone synthesis (25-27). Insufficiency of the above-mentioned proteins significantly decreases testosterone biosynthesis (10, 26).

Elevated levels of FSH and LH can result from the direct effects of pyrethroid on the CNS or they can be in response to decreased testosterone by the negative feedback of the anterior pituitary (28-30).

The results of this study in conjunction with the conclusions of other studies suggest that the insecticide cypermethrin modifies normal sexual behavior and testosterone levels and might have exert certain toxic effects on humans. Therefore it should be used with extreme caution.

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