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Harmful effects of pyrethroid ester insecticide on the male reproductive system mainly through affecting testicular function and inflammatory markers

Ha ABDEL MAKSOUD[1]; Mk MAHFOUZ[1]; Mi SOLIMAN[1]; Mohamed G ELHARRIF[2,*]; M ABBASS[1]; Ma EL-BADRY[1]

[1] Department of Biochemistry, Benha University, Benha, Egypt
[2] Department of Basic Medical Sciences, Shaqra University, Shaqraa, Saudi Arabia

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Abstract: Pyrethroid esters are widely used as insecticides worldwide. In this study, we aimed to evaluate the harmful effect of deltamethrin on the male reproductive system through the assessment of reproductive hormones, inflammatory markers, and testicular function. To achieve our aim, eighty male 7-9-week-old, Wistar rats were taken, weighed, and divided into four experimental groups. The first group was kept as a control group, and the other three groups were given deltamethrin orally at different concentrations (0.87, 8.7, and 17.4 mg/kg body weight) for nine weeks. The results indicated that deltamethrin administration associated with a significant decrease in reproductive hormones, especially FSH, LH, and significant elevation in the interleukin 2 (IL2), interleukin 6 (IL6), histamine, and cortisol levels. Also, the significance of inhibition of sperm motility and viability, decreased testis weights, sperm count, and fructose in semen were noted. These findings clarify the harmful effect of deltamethrin on the male reproductive system by producing a significant alteration in reproductive hormones, inflammatory markers as well as testicular function.

Introduction

Different types of pesticides have been commonly used for decades for agricultural purposes. Pyrethroid esters are synthetic organic compounds with plant origin, and they exert pesticidal activity through triggering the voltage-gated sodium channels (Nsibande and Forbes, 2016).

Pyrethroid ester insecticides are highly absorbed and more likely to cause toxicity; thus, this refers to a high lipophilic character of these compounds. Pyrethroid esters target the liver as the main site of metabolism, causing oxidative stress and generating reactive oxygen species (ROS) (Oliveiraa *et al*., 2018).

Long-term pyrethrin exposure produces harmful effects to many organs including the brain, the liver, and the kidney by free radical production and oxidative stress, leading to neurotoxic, hepatotoxic and nephrotoxic effects (Gunduz *et al*., 2015).

Occupational adverse effects of pyrethroids exposure appear when insufficient precautions are taken during pyrethroid preparation and application. Employees in this situation may develop cutaneous paraesthesia, ocular irritation, and upper respiratory tract inflammation (Meeker *et al*., 2008). Therefore, this study was designed to outline and assess the harmful effects of deltamethrin on the male reproductive system.

Materials and Methods

Eighty male 7-9-week-old Wistar rats of average weight (150-200 g) were obtained from Laboratory Animal Center at Benha University and were randomly distributed into four experimental groups after taking ethical approval from Benha University, following the NIH recommendations.

Rats were housed in separate metal cages and left one week for acclimatization. Then, four experimental groups were classified as follows: (1) control group (untreated), given corn oil as vehicle orally; (2) a group orally administered with deltamethrin at 0.87 mg/kg body weight (BW), equal to 0.01 of lethal dose 50% (LD50); (3) a group orally administered with deltamethrin at 8.7 mg/kg BW, equal to 0.1 of LD50; and (4) a group orally administered (by gastric tube) with deltamethrin at 17.4 mg/kg BW, equal to 0.5 of LD50. Each group comprised twenty male Wistar rats, which were treated for 9 weeks. By the end of the experiment, rats were weighed and sacrificed using light ether anesthesia and subjected to biochemical parameters.

Deltamethrin is a synthetic pyrethroid insecticide (C22H19Br2NO3) (98.1% purity) and was obtained from Kafr EL Zayat Co., Egypt.

*Address correspondence to: Mohamed G Elharrif, al_harrif@yahoo.com

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Data collection and estimated parameters

After the end of the experiment, blood samples were collected via direct heart puncture in centrifugation tubes without anticoagulant and kept at room temperature for 1 h to allow clotting. The samples were centrifuged at 3000 rpm for 10 min to separate clear serum.

The clear serum used immediately for measuring the activity of the following biochemical parameters; serum FSH (Marshall, 1975), LH (Knobil, 1980), testosterone (Tateiki *et al*., 1977), IL2 Rat IL-2 ELISA (Ray Biotech, Inc Company, Cath#: ELR-IL-2) according to the manufacturer's instruction, IL6 (DRG(R) Interleukin-6 (rat) (EIA-4845) according to the manufacturer's instruction, histamine (Herman *et al*., 1994) and cortisol (Mullner *et al*., 1991).

The testes were excised and weighed. The relative weights were calculated. After that, sperm count (Ekaluo *et al*., 2008), sperm viability (Björndahl *et al*., 2003), sperm motility (Adeeko and Dada 1998), sperm abnormality (El Nahas *et al*., 1989), and fructose in semen (Foreman *et al*., 1973) were determined.

Statistical analysis

Using computer software SPSS version 22.0, one-way ANOVA was used to study the effect of treatment on each parameter of cooled and frozen semen at each hour and the effect of time (h) within each treatment, and Duncan's multiple range tests were used to differentiate between significant means (Snedecor, 1989). The recorded data of rates were analyzed using two-sided Fisher's exact test, and $p < 0.05$ was considered statistically significant.

Results

FSH levels revealed significant declines in both low and high doses of deltamethrin groups in a dose-dependent manner as compared to the control group (Tab. 1). Moreover, LH levels also show significant differences in low and high doses of deltamethrin groups in a dose-dependent manner as compared to the control group (Tab. 1). Furthermore, a non-significant declining trend in serum testosterone levels in low and high doses of deltamethrin groups as compared to the control group (Tab. 1).

IL2 levels showed a significant increase in different doses of deltamethrin groups (0.87, 8.7, and 17.4 mg/kg BW) as compared to the control group (Tab. 2). Also, rats of both low and high doses of deltamethrin (0.87, 8.7, and 17.4 mg/kg BW) revealed a significant elevation in IL6 levels in a dose-dependent manner as compared to control group (Tab. 1). Significant elevation noted in serum cortisol level in the low- and high-dose deltamethrin groups (0.87, 8.7, and 17.4 mg/kg BW) in a dose-dependent manner as compared to the control group (Tab. 2). Additionally, serum histamine levels revealed significant increases in low and high doses of deltamethrin groups (0.87, 8.7, and 17.4 mg/kg BW) in a dose-dependent manner as compared to the control group (Tab. 2).

Significant differences in sperm count were observed in all deltamethrin-administered groups (0.87, 8.7, and 17.4 mg/kg BW) at the end of the experiment when compared with the normal control group. Also, sperm motility showed significant differences between control and deltamethrin administered groups (Tab. 3), in a dose-dependent manner. Moreover, sperm viability showed significant reductions between control and deltamethrin groups (Tab. 3), in a dose-dependent manner. Also, significant reductions in testis weight were observed in deltamethrin administered groups (Tab. 3) at the end of the experiment when compared with normal control group, with intermediate reductions in (0.87 and 8.7 mg/kg BW) groups and lowest mean value in (17.4 mg/kg BW) group (Tab. 3). Furthermore, the deltamethrin administered group with the high dose (17.4 mg/kg BW) revealed a significant decline of fructose level in semen, as compared to the control group, while there were non-significant differences in the other groups (0.87 and 8.7 mg/kg BW) (Tab. 3). On the other hand, the administration of deltamethrin revealed significant increases in sperm abnormality in all treated groups, as compared with the control group (Tab. 3).

TABLE 1

Effect of deltamethrin administration on reproductive hormones

Parameters	Control	Deltamethrin, mg/kg BW	Deltamethrin, mg/kg BW	Deltamethrin, mg/kg BW	Sig.
		0.87	8.7	17.4	
FSH (mIU/mL)	0.88 ± 0.03 [a]	0.63 ± 0.07 [b]	0.38 ± 0.04 [c]	0.22 ± 0.04 [d]	0.001
LH (mIU/mL)	1.95 ± 0.07 [a]	1.65 ± 0.09 [b]	1.41 ± 0.09 [c]	1.12 ± 0.05 [d]	0.001
Testosterone (mIU/mL)	2.54 ± 0. 23 [a]	2.40 ± 0. 31 [a]	2.13 ± 0.34 [a]	1.67 ± 0.17 [a]	0.167

Data are presented as mean ± SE.

Mean values with different superscript letters in the same row are significantly different at ($p < 0.05$).

TABLE 2

Effect of deltamethrin administration on inflammatory markers

Parameters	Control	Deltamethrin, mg/kg BW 0.87	Deltamethrin, mg/kg BW 8.7	Deltamethrin, mg/kg BW 17.4	Sig.
IL2	0.31 ± 0.03 [b]	0.38 ± 0.04 [b]	0.49 ± 0.07 [b]	1.02 ± 0.10 [a]	0.03
IL6	5.02 ± 0.41 [c]	5.42 ± 0.33 [bc]	7.87 ± 0.71 [b]	11.41 ± 1.39 [a]	0.001
Histamine	1.78 ± 0.16 [c]	3.40 ± 0.67 [c]	9.96 ± 0.84 [b]	17.99 ± 2.27 [a]	0.001
Cortisol	5.38 ± 0.48 [c]	13.38 ± 1.02 [c]	23.30 ± 3.41 [b]	41.36 ± 4.60 [a]	0.001

Data are presented as mean ± SE.
Mean values with different superscript letters in the same row are significantly different at ($p < 0.05$).

TABLE 3

Effect of deltamethrin administration on epididymal sperm parameters and testis weight

Parameters	Control	Deltamethrin, mg/kg BW 0.87	Deltamethrin, mg/kg BW 8.7	Deltamethrin, mg/kg BW 17.4	Sig.
Sperm Count ($x10^6$ spermatozoa/mL)	41.25 ± 2.25[a]	32.75 ± 1.65[b]	31.5 ± 1.75[b]	22.75 ± 1.49[c]	0.001
Sperm Motility (%)	86.75 ± 1.37[a]	72.00 ± 1.77[b]	51.00 ± 1.58[c]	34.75 ± 1.79[d]	0.001
Sperm Viability (%)	90.00 ± 1.77[a]	76.00 ± 1.35[b]	54.00 ± 1.68[c]	37.25 ± 0.85[d]	0.001
Abnormalities (%)	3.50 ± 0.64[c]	5.50 ± 0.66[b]	8.50 ± 1.44[ab]	15.25 ± 1.54[a]	0.001
Fructose in semen (μg/mL)	74.81 ± 3.07 [ab]	76.35 ± 2.66 [a]	64.00 ± 5.48 [b]	50.77 ± 2.73 [c]	0.001
Testis weight (g)	1.37 ± .048[a]	1.19 ± 0.11[ab]	1.18 ± .079[ab]	0.99 ± 0.05 [b]	0.03

Data are presented as mean ± SE.
Mean values with different superscript letters in the same row are significantly different at ($p < 0.05$).

Discussion

The current research aimed to identify and evaluate the effect of long-term exposure to deltamethrin on the male reproductive system through the determination of its effect on testicular function, reproductive hormones, and inflammatory markers. Our obtained results showed that the testosterone concentrations tend to be decreased but not significantly, while a highly significant decrease in FSH and LH levels.

The effect of pyrethroids on pituitary gonadotropin hormones and testicular hormones is dependent on time of exposure and testicular tissue (Sharma *et al*., 2018). The decline in hormone levels was related to either direct effect of pyrethroids on androgen biosynthesis pathway in the testes or its effect on the hypothalamus/anterior pituitary gland, which might have indirectly affected the testis and sexual function (Rajawat *et al*., 2014).

A similar reduction in serum levels of testosterone, follicle-stimulating hormone, and luteinizing hormone was obtained in alpha-cypermethrin treated rats (Alaa-Eldin *et al*., 2016). Hence, the reduced testosterone might be responsible for the decreased sperm counts and motility and also morphological abnormality of the testis. The possible mechanism for the reduction of testosterone, FSH, and LH level advocates extra testicular targets of pyrethroids. Pyrethroids may also be affecting the hypothalamus-pituitary axis. LH stimulates Leydig cells to produce testosterone; hence, the decrease in LH may also be a contributing factor for the low level of testosterone. The potential hormonal activity of deltamethrin has multiple effects on the endocrine system.

The obtained data showed significant variation in the mean levels of serum IL2, IL6, cortisol, and histamine in deltamethrin groups in a dose-dependent manner. The presented results were in agreement with the data reported by Arora *et al.* (2016), who stated that deltamethrin was able to

induce an inflammatory response directly by up-regulating the levels of cytokines and indirectly by increasing ROS production and altered enzymatic antioxidant defense in the liver, causing oxidative damage and triggering inflammation via a cytokine-mediated immune response. This involves the release of anti-inflammatory and pro-inflammatory cytokines (Mani *et al.*, 2017) and activates the transcription of multiple inflammatory genes (Heneka and O'Banion, 2007). Furthermore, Reza khalatbary *et al.* (2016) considered that inflammation is the main mechanism for the deltamethrin toxicity due to the up-regulation of cyclooxygenase 2, which plays a key role in promoting inflammation (Maalej *et al.*, 2017).

Also, Ramzy *et al.* (2014) revealed that the elevation of serum cortisol levels in acute and chronic exposure to pesticides are due to primary response to stressors caused by pesticides and a probable increase in cortisol biosynthesis.

The obtained data showed significant decreases in the mean value of sperm motility, sperm viability, sperm count, testis weight, fructose in semen, and a significant increase in sperm abnormalities levels in deltamethrin groups, in comparison with the normal control group. The decrease in testes weight may be due to the direct cytotoxic action of deltamethrin on testicular tissue. Cremonese *et al.*, (2017) suggested that accumulation of the insecticides in the testicular tissue may have adversely affected the Sertoli cell population, leading to compromised spermatogenesis and reduction in sperm head counts.

Our results were in agreement with results reported by Desai *et al.* (2016), who revealed that the decreased testicular weight after exposure to pyrethroid derivatives due to declined testosterone levels, a decreased number of germ cells, inhibition of spermatogenesis, and reduced steroidogenic enzyme activities. Also, Ben Slima *et al.* (2017) reported that the reduction in sperm count, motility, viability, and morphology may be due to an adverse effect of deltamethrin on spermatogenesis by acting at a molecular level.

Reduction in sperm count may be due to the degeneration of Leydig cells, decreased testosterone production, or even necrosis of seminiferous tubules. Reduction in sperm motility may be due to decreased mitochondrial enzyme activity of the spermatozoa, altered fructose synthesis and secretion by the accessory glands, or corruption of microtubule structure of the spermatozoa (Issam *et al.*, 2009). It was suggested that the chronic occupational exposure to modern pesticides, may adversely affect semen quality, potentially leading to poorer morphology and chronically alter sex hormone levels acting at the pituitary level through prolactin and LH suppression, inhibiting compensatory responses to testicular dysfunction (Cremonese *et al.*, 2017). The depletion of fructose content hampers the glycolytic metabolism of spermatozoa resulting in abnormal sperm functions, which ultimately cause complete male sterility. It is well known that the function of seminal vesicles is under androgen control, and a direct association exists between serum testosterone, seminal fructose, and spermatozoa motility/fertility (Gonzales, 2001). Additionally, the sugar composition of seminal plasma correlates positively with fertility, mainly due to its importance to spermatozoa energy production. Fructose and glucose are essential for adenosine triphosphate production and motility of spermatozoa (Ben Slima *et al.*, 2017).

Conclusion

By the end of the study and depending on our data, we concluded that, long term exposure of deltamethrin at different concentration followed by alteration of reproductive hormones, significance elevation of inflammatory biomarkers as well as inhibition of testicular tissue functions. So, more efforts to limit exposure which may be a significant contributory factor to the development of male infertility.

Conflicts of Interest

The authors declare that they have no conflicts of interest to report regarding the present study.

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