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

HA ABDEL MAKSOUD1; MR MAHFOUZ1; MI SOLIMAN1; MOHAMED G ELHARRIF2,*; M ABBASS1; MA EL-BADRY1

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) (Oliveira 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
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 b          | 2.13 ± 0.34 c          | 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 \( p < 0.05 \).
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

### Table 2

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

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

References

Adeeko AO, Dada OA (1998). Chloroquine reduces the fertility capacity of epididymal sperm in rats. African Journal of Medicine and Medical Sciences 27: 63-68.

Alaa-Eldin EA, El-Shafei DA, Abouhashem NS (2016). Individual and combined effect of chlorpyrifos and cypermethrin on reproductive system of adult male albino rats. Environmental Science and Pollution Research 24: 1532-1543.

Arora D, Siddiqui MH, Sharma PK, Singh SP, Tripathi A, Mandal P, Singh US, Singh PK, Shukla Y (2016). Evaluation and physiological correlation of plasma prostatec fingerprints for deltamethrin-induced hepatotoxicity in Wistar rats. Life Sciences 160: 72-83.

Ben Slima A, Chtourou Y, Barkallah M, Fetoui H, Boudawara T, Gdoura R (2017). Endocrine disrupting potential and reproductive dysfunction in male mice exposed to deltamethrin. Human & Experimental Toxicology, 36: 218-226.

Björndahl L, Barratt CL, Mortimer D, Jouannet P (2015). ‘How to count sperm properly’: checklist for acceptability of studies based on human semen analysis. Human Reproduction 31: 227-232.

Cremonese C, Piccolo C, Pasqualotto F, Clapauch R, Koifman RJ, Koifman S, Freire C (2017). Occupational exposure to pesticides, reproductive hormone levels and sperm quality in young Brazilian men. Reproductive Toxicology 30: 720-730.

Desai K, Moid N, Patel P, Highland H (2015). Evaluation of deltamethrin induced reproductive toxicity in male Swiss Albino mice. Asian Pacific Journal of Reproduction 5: 24-30.

Ekaluo UB, Udokpoh AE, Ikpeme EV, Peter EU (2008). Effect of chloroquine treatments on sperm count and weight of testes in male rats. Global Journal of Pure and Applied Sciences 14: 175-177.

El Nahas SM, de Hondt HA, Abdou HA (1989). Chromosome aberrations in spermatogonia and sperm abnormalities in Curacron-treated mice. Mutation Research/Genetic Toxicology 222: 409-414.

Foreman D, Gaylor L, Evans E, Trella C (1973). A modification of the Roe procedure for determination of fructose in tissues with increased specificity. Analytical Biochemistry 56: 584-590.

Gonzales GF (2001). Function of seminal vesicles and their role on male fertility. Asian Journal of Andrology 3: 251-258.

Gunduz S, Muthu H, Tural D, Yildiz O, Uysal M, Coskun HS, Bozcuk H (2015). Platelet to lymphocyte ratio as a new prognostic
for patients with metastatic renal cell cancer. *Asia-Pacific Journal of Clinical Oncology* **11**: 288-292.

Heneka MT, O’Banion MK (2007). Inflammatory processes in Alzheimer’s diseases. *Journal of Neuroimmunology* **184**: 69-91.

Herman K, Frank G, Ring J (1994). Contamination of heparin by histamine: measurement and characterization by high-performance liquid chromatography and radioimmunoassay. *Allergy* **49**: 569-572.

Issam C, Samir H, Monia Z, Hassen BC (2009). Toxic responses to deltamethrin (DM) low doses on gonads, sex hormones and lipoperoxidation in male rats following subcutaneous treatment. *The Journal of Toxicological Sciences* **34**: 663-670.

Reza khalatbary A, Ahmadvandb H, Ghabaeea DNZ, Malekshah AK, Navazesh A (2016). Virgin olive oil ameliorates deltamethrin induced nephrotoxicity in mice. A biochemical and immunohistochemical assessment. *Toxicology Reports* **3**: 584-590.

Knobil E (1980). The neuroendocrine control of menstrual cycle. *Proceedings of the 1979 Laurentian Hormone Conference* **36**: 52-88.

Maalej A, Mahmoudi A, Bouallagui Z, Fki I, Marrekchi R, Sayadi S (2017). Olive phenolic compounds attenuate deltamethrin-induced liver and kidney toxicity through regulating oxidative stress, inflammation and apoptosis. *Food and Chemical Toxicology* **106**: 455-465.

Mani VM, Gokulakrishnan A, Sadiq AM (2017). Molecular mechanism of neurodevelopmental toxicity risks of occupational exposure of pyrethroid pesticide with reference to Deltamethrin - a critical review. *BAOJ Pathology* **1**: 008.

Marshall JC (1975). Clinics in endocrinology and metabolism. Investigative procedures. *Clinics in Endocrinology and Metabolism* **4**: 545-567.

Meeker JD, Barr DB, Hauser R (2008). Human semen quality and sperm DNA damage in relation to urinary metabolites of pyrethroid insecticides. *Human Reproduction* **23**: 32-40.

Müllner S, Neubauer H, König W (1991). A radio immunoassay for determination of insulin in several animal species, insulin derivatives. *Journal of Immunological Methods* **140**: 211-198.

Nsibande SA, Forbes PBC (2016). Fluorescence detection of pesticides using quantum dot materials - a review. *Analytica Chimica Acta* **945**: 9-22.

Oliveira JM, Losanob NF, Condessab SS, de Freitas RMP, Cardoso SA, Freitas MB, de Oliveira LL (2018). Exposure to Deltamethrin induces oxidative stress and decreases of energy reserve in tissues of the Neotropical fruit-eating bat *Artibeus lituratus*. *Ecotoxicology and Environmental Safety* **148**: 684-692.

Rajawat NK, Soni I, Mathur P, Gupta D (2014). Cyfluthrin-induced toxicity on testes of Swiss albino mice. *International Journal of Current Microbiology and Applied Sciences* **3**: 334-343.

Ramzy EM, Aly AM, Ibrahim LA (2014). Biomarker studies of potential hazards of chlorpyrifos to Nile tilapia, *Oreochromis niloticus*. *International Journal of Environment* **3**: 94-105.

Sharma P, Singh R, Jan M (2014). Dose-dependent effect of Deltamethrin in testis, liver, and kidney of Wistar rats. *Toxicology International* **21**: 131-139.

Snedecor GW, Cochran WG (1989). Statistical methods. 8th Ed. Ames, IA, USA: Iowa State Univ. Press.

Tateishi K, Yamamoto H, Ogihara T, Hayashi C (1977). Enzyme immunoassay of serum testosterone. *Steroids* **30**: 25-32.