



Biochemical Role of Pyrethroids on Male Fertility and Seminal Profile of Rat

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ABSTRACT

A total number of 80 Wister male rats, 9 -wk. old were taken, weighed and divided into four experimental groups to investigate the effect of Deltamethrin (DLM) oral administration (0.87, 8.7 and 17.4 mg/kg B.W) for a period of 9 weeks on reproductive organs and fertility indices of Wister male rats. The results indicated that DLM caused a significant reduction in Testis weights, sperm count, motility and viability and fructose in semen. Also induced decreases in serum FSH, LH and Estradiol levels however increase in sperm abnormalities. These results indicated that DLM is a toxic pyrethroid pesticide that produced significant reproductive toxicity in treated male rats as revealed by the severely affected parameters

(Key word: DLM, Reproductive toxicity, Sperm quality)

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1. INTRODUCTION:

During the past decades, different types of pesticides had widely been used in agriculture for high yield productions. Applications of pesticides have secured almost one-third of crop production in the whole world. Pesticides have led to the improvement of food production to secure the demands of a never increasing human population (Nsibande and Forbes, 2016).

Pyrethroids are synthetic organic compounds synthesized from *chrysanthemum* flowers that are used extensively as household and commercial insecticides (Hasibur *et al.*, 2014). Although Pyrethroids acute toxicity to mammals was known to be minimal, its potential endocrine effects have become a matter of public concern. Effects on reproductive and endocrine systems have been examined by *in vivo* experiments as well as by

occupational and environmental epidemiology. In some previous studies, association was found between urinary metabolite concentration and semen quality (Meeker *et al.*, 2008)

Deltamethrin (DLM) is a broad-spectrum synthetic dibromo-pyrethroid pesticide that is widely used for agricultural and veterinary purposes (Varol *et al.*, 2016) Voltage-gated sodium channels are the primary targets of these chemicals for toxicity to insects (Rune Zeng *et al.*, 2017). Several experimental studies have examined the effects of DLM various endpoints of male developmental and reproductive toxicity. Adverse effects on testis weight and sperm count, motility and quality, decreased serum testosterone levels, and/or changes in reproductive behavior, have been inconsistently reported in a number of

studies following oral administration of DLM to adult rabbits, rats and mice (Sharma *et al.*, 2014). Therefore, the objective of this study aimed to providing the evidence of reproductive toxicity of Deltamethrin.

2. MATERIALS AND METHODS:

This study was carried out at Institute of Medical Entomology. Eighty Wister male rats, 9 -wks-old were taken, weighed and randomly distributed into four experimental groups. Rats were housed in separate metal cages, then the four experimental groups were arranged as following, the first as a control group (un-treated) given corn oil as vehicle orally, the second group orally administrated with 0.87 mg/kg body weight (1/100th LD50) / 9 weeks, the third group orally administrated with 8.7 mg/kg body weight (1/10th LD50) / 9 weeks, the fourth group orally administrated with 17.4 mg/kg body weight (1/5th LD50) / 9 weeks. All the groups were treated for 9 weeks and at the end of experiment, animals were weighed and sacrificed using light ether anesthesia, to studying their effects on testis weights, sperm count, sperm motility, sperm viability, sperm abnormalities and fructose in semen, as well as serum FSH, LH and Estradiol levels.

The weights of the animals in all groups were determined (Scout Pro SPU601, Ohaus) once every week and this was used to calculate the amount of pesticide administered as shown in Eq.

$$X \text{ mg of pesticide} = \text{Group dose} \times \text{Kg body weight of animals}$$

X mg of the pesticide was orally administrated, at 48 hour intervals and fed to the rats. Deltamethrin is a synthetic pyrethroid insecticide (C₂₂H₁₉Br₂NO₃) (98.1% purity) were obtained from Kafr EL Zayat co.

Data collection and estimated parameters:

After Experiment period, blood samples were collected via direct heart puncture in centrifugation tubes from without anticoagulant and kept at room temperature for one hour to clot. The samples were centrifuged at 5000 rpm for 15 minutes to separate clear serum. After that, serum FSH (Marshall, 1975), LH (Knobil, 1980), Testosterone (Tateiki *et al.*, 1977) and E2 (Lichtenberg *et al.*, 1992) were determined using available commercial Kits.

After blood collection, rats were sacrificed by decapitation. within 5 to 10 sec to avoid stress and pain. The testes were excised quickly 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 (Nahas *et al.*, 1989), and fructose in semen (Foreman *et al.*, 1973) were determined

Statistical analysis: Using computer software SPSS version 22.0, simple 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 hour within each treatment and Duncan's multiple range tests was used to differentiate between significant means (Snedecor, 1989). The recorded data of rates was analyzed using two-sided Fisher's exact test, and $P < .05$ was considered as statistically significant.

3. RESULTS:

Significant differences in Sperm count were observed in all DLM treatment groups (0.87, 8.7 and 17.4 mg/kg B.W) at the end of the experiment when compared with normal control group, with group (IV) having the lowest mean value (Table 1). Also Sperm motility showed significant differences between the control and DLM treatment groups (Table 1), in a dose dependent manner.

Moreover, sperm Viability showed significant reductions between the control and DLM treatment groups (Table 1), in a dose dependent manner. Also, significant reductions in Testis weight were observed in DLM treatment groups (Table 1) at the end of the experiment when compared with normal control group, With intermediate reductions in (0.87 and 8.7 mg/kg B.W) groups and lowest mean value in (17.4 mg/kg B.W) group (Table 1). Furthermore, the DLM treated group with high dose (17.4 mg/kg B.W) after 9 weeks revealed a significant decline in fructose level of seminal vesicle as compared to control group, while there were non-significant differences in group (0.87 and 8.7 mg/kg B.W) (Table 1). On other hand, administration of DLM revealed Significant increases in sperm abnormality in all treated groups, in compare with control group (Table 1).

Animals of both low dose and high doses DLM treated groups after 9 weeks revealed significant declines in FSH levels in a dose dependent manner as compared to control group (Table 1). Moreover, LH levels also show significant differences in low dose and high doses DLM groups in a dose dependent manner after 9 weeks as compared to control group (Table 1). Furthermore, Significant differences in Serum E2 were observed in DLM treatment groups (8.7 and 17.4 mg/kg B.W) at the end of the experiment when compared with normal control group. With no significant differences in (0.87 mg/kg B.W) group (Table 1). Meanwhile, Non-significant declining trend in serum Testosterone after 9 weeks treatment in low dose and high doses DLM treated groups as compared to control group (Table 1).

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Table 1: Effect of DLM treatment on epididymal sperm parameters and reproductive hormones.

Parameters	Control (-ve)	DLM , mg/kg B.W	DLM , mg/kg B.W	DLM , mg/kg B.W	Sig.
		0.87	8.7	17.4	
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
Abnormities (%)	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
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
E2 (mIU/ml)	22.80 ± 2.49 ^a	18.90 ± 1.99 ^a	13.52 ± 1.08 ^b	10.00 ± 0.85 ^b	0.001

Data are presented as (Mean ± S.E).

S.E = Standard error.

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

4. DISCUSSION:

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 DLM treated groups in comparison with normal control group. The decrease in testes weight may be due to the direct cytotoxic action of DLM on testicular tissue. Cleber *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 observed the adverse effect of low and high doses of DLM for 45 days on reproductive organs and fertility indices of male Albino mice. The author revealed that Decline in testicular weight might be due to declined serum testosterone concentration, decreased number of germ cells, inhibition of spermatogenesis and decline in steroidogenic enzyme activities. Also, (Ben Slima *et al.*, 2013) reported that, epididymal sperm count, motility and viability, morphology were significantly altered after DLM treatment. The reduction in sperm count may be due to an adverse effect of DLM on spermatogenesis. Moreover, Ekaluo *et al.*, (2013) revealed that DLM is capable of disrupting spermatogenesis/spermiogenesis, even though it is not usually considered a reproductive toxin. In addition, Madhubabu and Allethrin, (2017) reported that pyrethroids toxicity affects reproduction by acting at a molecular level to affect spermatogenesis, androgen production, sperm production, and function.

Reduction in sperm count may be due to degeneration of Leydig cells, reduced testosterone production, or even necrosis of

seminiferous tubules. Reduction in sperm motility may be due to decreased mitochondrial enzyme activity of the spermatozoan, altered fructose synthesis and secretion by the accessory glands, or corruption of microtubule structure of the spermatozoan 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 (Cleber *et al.*, 2017). 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).

Our obtained results showed that testosterone concentrations tend to be decreased but not significantly increased due to individual variations while a highly significant decreases in FSH, LH and E2 levels. moreover Sharma *et al.*, (2018), demonstrated that, the effect of pyrethroids on pituitary gonadotropin hormones and testicular hormones is dependent on time of exposure and testicular tissue being their primary target. Similar reduction in serum levels of testosterone, luteinizing hormone and follicle

stimulating hormone was obtained in alpha-cypermethrin treated rats (Alaa-Eldin *et al.*, 2016). The decline in hormone levels was attributed to either direct effect of toxicant on androgen biosynthesis pathway in testis or its effect on hypothalamus/anterior pituitary gland which might have indirectly affected the testis and sexual function (Rajawat *et al.*, 2014). Hence, the reduced testosterone might be responsible for the decreased sperm counts and motility and also morphological abnormality of testis in treated mice. Joshi *et al.*, (2011) stated that the possible mechanism in the reduction of testosterone, FSH, and LH level advocates extra testicular targets of Pyrethroids. Pyrethroids may also be affecting hypothalamus-pituitary axis. LH stimulates Leydig cells to produce testosterone; hence, decrease in LH may also be a contributing factor for low level of testosterone. The potential hormonal activity of DLM has multiple effects on the endocrine system. The decreased levels of estradiol upon DLM treatment are consistent with those reported by (Marettovaa *et al.*, 2017). Calculating serum testosterone and estradiol appears to be a more reliable tool than measuring serum testosterone only in assessment of male fertility (Gomaa *et al.*, 2018).

5. CONCLUSION:

Based on the present data, oral administration of DLM at doses of 0.87, 8.7 and 17.4 mg/kg b.wt for 9 weeks to male rats causes reproductive toxicity. This toxicity is manifested by significant alter in sperm parameters and significant reduction in reproductive hormones. So, more attention should be given to sources and environmental impact of DLM, with more efforts to limit exposure to which may be a significant contributory factor to the development of male infertility.

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