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Assessement of reproductive toxicity induced by deltamethrin in male albino rats

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ABSTRACT

Introduction: Pyrethroids are important insecticides because of their high activity and low mammalian toxicity. Some studies have demonstrated that these insecticides, especially compounds with an α-cyano group, produce toxic effects on various biological systems. Deltamethrin (DTM) is a Type II synthetic pyrethroid insecticide containing -cyano group and is used worldwide in agriculture, home pest control, protection of foodstuff, and disease vector control. In this study, the toxic effects of DTM were evaluated in adult male albino rats.

Material & methods: Treated groups were given DTM orally at various dose levels (7.5, 17.5 and 27.5mg/kg b. wt./day for 45 days) with a positive control group-receiving vehicle (olive oil) only and their fertility rate and male sex organs were evaluated.

Results: Signs of toxicity and reduction in the weight of testes and accessory sex organs were observed in a dose-dependent manner. DTM brought about marked reduction in epididymal and testicular sperm counts and fertility in insecticide-exposed males. A significant decrease in the sialic acid content of testes, epididymis, ventral prostate, seminal vesicle and testicular glycogen was noticed. However, the cholesterol and protein content of testes and accessory sex organs was raised significantly in all treated groups. A significant reduction in seminal vesicle fructose and serum testosterone was also observed after DTM administration. Histopathological examination revealed severe degenerative changes in seminiferous tubules while these effects were milder at the lowest dose level.

Conclusion: These results provide first hand information that DTM adversely affects the reproductive system of male rats.

Key-words: Deltamethrin, Rats, Testis, Spermatozoa, Sperm count, Sperm motility, Pathology

INTRODUCTION

Over 80,000 new synthetic chemicals have been developed and dispersed globally over the past 50 years (1); however, pesticides have given rise to serious problems due to poisonings and toxic exposures worldwide. As much as 25-33% of the global burden of disease is attributed to environment risks associated with the use of chemicals (2). Adverse health consequences of toxic-related hazardous incidents involving mass casualties, environmental and occupational exposures and other toxic exposures are of the highest priority as public health concerns (3).

The pyrethroids are synthetic analogs of the original pyrethrins and represent a diverse group of over 1,000 powerful insecticides (4,5). Pyrethroid insecticides are used preferably over organochlorines, organophosphates

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and carbamates due to their high effectiveness against a wide range of insects, low toxicity to non-target organism (mammals) and biodegradability (6). However, the uncontrolled, indiscriminate and widespread usage of these chemicals in agriculture, public health and public protection make them potent environmental contaminants causing several harmful effects to the ecosystem, wildlife and finally the public (7,8).

The toxicity of pyrethroid insecticides to mammalian animals has received much attention in recent years (9). There have been cases of accidental and occupational exposures after dermal contact which leads to acute pyrethroid poisonings (10-12).

Toxicity of Deltamethrin, a synthetic pyrethroid insecticide, was studied in different animals and it was found that this insecticide has neurotoxic (13,14), genotoxic (15,16) and immunotoxic effects (17,18). Also, some adverse reproductive (19,20) effects have been reported.

The aim of this study was, therefore, to clarify whether deltamethrin has a detectable adverse effect on male reproductive system of rats, which could support the idea of the generalized harmfulness of this chemical on male reproduction.

MATERIALS AND METHODS

For the present investigation, albino rats were used as biological models to represent the mammalian species. An oral route of compound administration was adopted to mimic the most likely route of human exposure. Toxic implications of these insecticides with reference to body and sex organs weight analysis; sperm dynamics, biochemical profile, testosterone level, histopathological changes and mating trials have been assessed in male rats.

Test material: Deltamethrin [(S)-α-cyano-3-phenoxybenzyl-(1R)-cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate] (Technical grade 90.7%) from Gupta Chemicals Pvt. Ltd. Jaipur, India was dissolved in olive oil (0.5ml) for oral administration.

Animals: Proven fertile healthy adult Swiss albino male rats (Rattus norvegicus) of Wistar strain, weighing 200-250g were used. The animals were maintained in separated cages at room temperature (26 ±1^{0}C) and 12h light: 12h dark cycles. The rats were given standard pellet diet (Ashirwad Indusrtries Ltd., Chandigarh, India) and water was provided ad libitum throughout the study.

Experimental Design: The experimental protocol met the National Guidelines on the proper care and use of animals in laboratory research. The animals were divided into four groups of 10 animals each. Animals of group I were given olive oil and served as controls. Groups II, III and IV received deltamethrin at the tested dose levels of 7.5, 17.5 and 27.5mg/kg b. wt. /day in 0.5ml olive oil for 45 days.

The male rats were cohabited with proestrous females in the ratio of 1:3. The vaginal plug and presence of sperm in the vaginal smear was checked for positive mating. Females were separated and resultant pregnancies were noticed when dams gave birth. Fertility was calculated in control as well as treated groups.

The animals were weighed before and after the treatment and autopsied under light ether anesthesia. The testes, epididymides, seminal vesicle, ventral prostate and vas deferens were dissected out. The adherent fatty tissues and blood vessels were removed and weighed. To ensure normalization of data for statistical analysis, organs weight was expressed per 100g of body weight. Sperm motility in cauda epididymides and density of testicular and cauda epididymides suspended sperm were calculated (40). Blood was collected by cardiac puncture from the heart and serum was separated by centrifugation and stored at –20 C.

Histopathological studies: The testes of the treated and control rats were fixed in Bouin's fixative, embedded in paraffin wax, sectioned at 5 m thickness

and stained in hematoxylin-eosin. Sections were examined under light microscope and general histopathological changes were examined.

Biochemical studies: Freshly removed testes and accessory sex organs were weighed to required milligram for biochemical analysis of cholesterol (41), glycogen (42) sialic acid (43), protein (44) and fructose (45). Serum testosterone concentration was measured by radio immunoassay method (46).

Statistical analysis: The data were analyzed statistically by using Students't' test (47) with SPSS software version 13 and the significance of differences were set at P<0.01 and P< 0.001.

RESULTS

Oral administration of deltamethrin at all the dose levels (7.5, 17.5 and 27.5mg/kg b. wt./day for 45 days) produced signs of reproductive toxicity in male rats in a dose-dependent manner i.e. all the effects were mild at low doses and became severe at higher dose levels.

Body and reproductive organs weight: Significant difference in the body weight was noticed at the end of the treatment period among treated groups when compared with the control group. Similarly, significant reduction (P<0.01 and P< 0.001) in the weight of testes and accessory sex organs was observed after deltamethrin treatment at 7.5, 17.5 and 27.5mg/kg b. wt./day dose levels for 45 days. (Table 1)

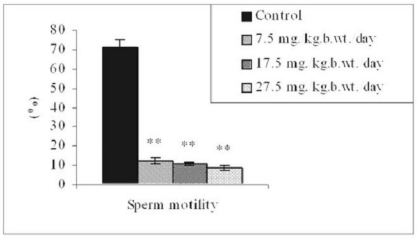
Table 1: **Body and sex organ weight (Deltamethrin 7.5, 17.5 and 27.5 mg/kg b.wt./day for 45 days)**

Treatment	Body weight		Testes	Epididymides	Seminal Vesicle	Ventral Prostate
	Initial	Final				
	G		mg/100g body wt.			
Group I Control (vehicle only)	175.82 ±3.9	194.00 ±8.42	1209.34 ±50.91	512.01 ±8.23	414.63 ±26.01	337.82 ±24.33
Group II 7.5 mg/kg.b.wt./day	174.93 ±6.88	200.03ns ±6.29	863.471** ±34.29	330.51** ±34.22	252.61* ±32.37	217.86* ±29.33
Group III 17.5 mg/kg.b.wt./day	173.29 ±3.68	158.42* ±6.02	837.10** ±44.37	324.01** ±12.83	211.19* ±29.22	209.71* ±27.94
Group IV 27.5 mg/kg.b.wt./day	180.71** ±3.93	145.00** ±2.56	811.00** ±15.69	302.21** ±12.23	207.11** ±11.28	175.74** ±13.68

(Mean ±SE of 10 animals) (Group II; III; IV, compared with Group I)

*ns = P<0.05 * = P<0.01 ** = P<0.001*

Fig 1: **Effects of different dose levels of deltamethrin for 45 days on cauda epididymal sperm motility**



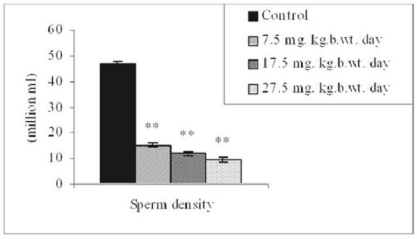
(Mean ±SE of 10 animals) ** = P<0.001

Sperm dynamics and Fertility: The sperm motility in cauda epididymides at various dose levels was severely impaired (P<0.001) in comparison to controls (Fig.1)

Also, marked reduction (P<0.001) in epididymal and testicular sperm density was noticed after deltamethrin administration in all treated groups (Fig. 2, 3).

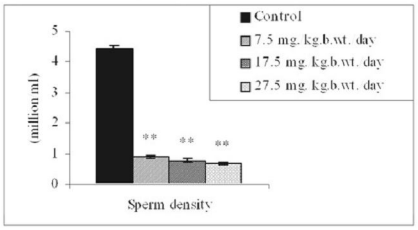
Mating exposure tests revealed that control rats showed 100% (+) ve fertility whereas a sharp decline in fertility (60%, 90% and 100%-ve) in deltamethrin treated rats was observed (Fig. 4)

Fig 2: **Effects of different dose levels of deltamethrin for 45 days on cauda epididymal sperm density**



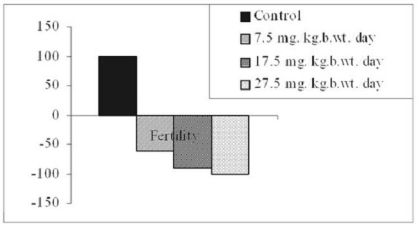
(Mean ±SE of 10 animals) ** = P<0.001

Fig 3: **Effects of different dose levels of deltamethrin for 45 days on testicular sperm density**



(Mean ±SE of 10 animals) ** = P<0.001

Fig 4: **Effects of different dose levels of deltamethrin for 45 days on fertility**



(Mean ±SE of 10 animals)

Biochemical Findings: Protein content of testes and accessory sex organs was increased significantly (P<0.01 and P< 0.001) at all dose levels; however, marked reduction (P<0.01 and P< 0.001) in sialic acid content of testes, epididymides, ventral prostate, seminal vesicle and vas deferens was noticed.(Table 2)

Table 2: **Tissue protein and sialic acid concentrations (Deltamethrin 7.5, 17.5 and 27.5 mg/kg b.wt./day for 45 days)**

Treatment	Protein (mg/g)					Sialic acid (mg/g)				
	Testes	Cauda Epididymides	Seminal Vesicle	Ventral Prostate	Vas Deferens	Testes	Cauda Epididymides	Seminal Vesicle	Ventral Prostate	Vas Deferens
Group I Control (vehicle only)	249.97 ±11.92	217.75 ±3.14	232.19 ±3.14	238.19 ±4.80	263.81 ±5.92	4.90 ±0.06	4.50 ±0.04	5.09 ±0.02	5.16 ±0.04	5.86 ±0.18
Group II 7.5 mg/kg. b.wt./day	302.08* ±6.18	257.78* ±8.47	276.21* ±8.89	280.80ns ±15.26	294.38* ±8.11	3.00* ±0.46	3.08* ±0.34	3.98* ±0.28	3.91** ±0.39	3.83* ±0.42
Group III 17.5 mg/kg. b.wt./day	333.21** ±12.07	265.43** ±4.34	289.98** ±8.34	289.33** ±8.25	310.11* ±8.73	2.51** ±0.47	2.73** ±0.36	3.76** ±0.26	3.46* ±0.31	2.77** ±0.53
Group IV 27.5 mg/kg. b.wt./day	339.29** ±3.69	271.22** ±7.13	299.15** ±9.34	293.21** ±6.38	315.51* ±8.98	2.13* ±0.83	2.63** ±0.39	3.54** ±0.22	3.11* ±0.47	2.36** ±0.51

A significant decrease (P< 0.001) in seminal vesiclefructose level was observed in rats treated with deltamethrin at various calculated doses. Testicular glycogen content was decreased significantly (P<0.01 and P< 0.001) in all treated groups i.e. Group II, III and IV. Significant elevation (P< 0.001) was noticed in testicular cholesterol in deltamethrin treated animals at all dose levels. (Table 3)

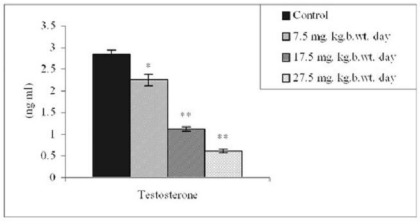
Table 3: **Tissue glycogen, cholestrol, and fructose concentrations (Deltamethrin 7.5, 17.5 and 27.5 mg/kg b.wt./day for 45 days)**

Treatment	Glycogen (mg/g)	Cholesterol (mg/g)	Fructose (mg/g)
	Testes	Testes	Seminal Vesicle
Group I Control (vehicle only)	2.50 ±0.09	6.00 ±0.41	4.95 ±0.27
Group II 7.5 mg/kg.b.wt./day	0.52* ±0.39	9.28** ±0.55	2.57** ±0.23
Group III 17.5 mg/kg.b.wt./day	0.29** ±0.25	10.11** ±0.73	2.28** ±0.28
Group IV 27.5 mg/kg.b.wt./day	0.18** ±0.02	12.37** ±0.36	2.10** ±0.18

(Mean ±SE of 10 animals) (Group II; III; IV, Compared with Group I)
ns = P<0.05
* = P<0.01
** = P<0.001

Radio immunoassay: The reduction in serum testosterone level was much more pronounced (P<0.01 and P< 0.001) at higher doses of DTM exposure. (Fig. 5)

Fig 5: **Effects of different dose levels of deltamethrin for 45 days on testosterone**



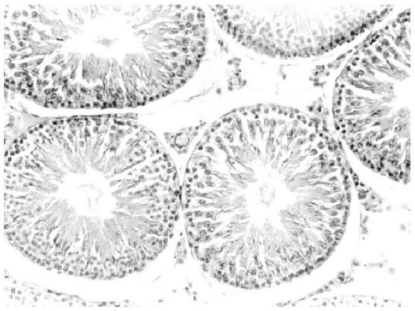
(Mean ± of 10 animals) * = P<0.01 ** = P<0.001

Histopathological Studies: Histological observation of the testes showed normal spermatogenesis with spermatogenic cells at different stages of development in control rats (Fig. 6).

Fig 6: **Control**
Microphotograph of control rat testis showing normal morphological architecture with different stages of spermatogenesis. Lumen filled with spermatozoa. (H&E 100X)

The testes of rat at lower dose level (7.5mg/kg b. wt./day) showed mild changes in the seminiferous tubules (Fig. 7).

Fig 7: **Deltamethrin 7.5mg/kg b.wt./day for 45 days**
Seminiferous tubules photograph exhibiting loosened tunica propria. Lumen contains fewer spermatozoons. (H&E 100X)



Histological observation of the testes with increasing dose level (i.e. 17.5 and 27.5mg/kg b. wt./day) of deltamethrin treated rats for 45 days revealed inhibition of spermatogenesis, reflected by completely damaged and shrunken seminiferous tubules and absence of sperm in the lumen. Interstitial cells were also severely affected. (Fig. 8, 9)

Fig 8: **Deltamethrin 17.5mg/kg b.wt./day for 45 days**
Microphotographs of seminiferous tubules depicting decreased size and complete spermatogenic arrest. Lumen contains cellular debris. Interstitial cells are disorganized. (H&E 100X)

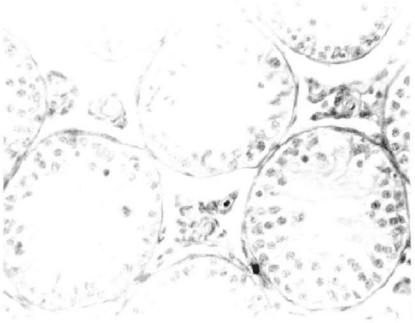
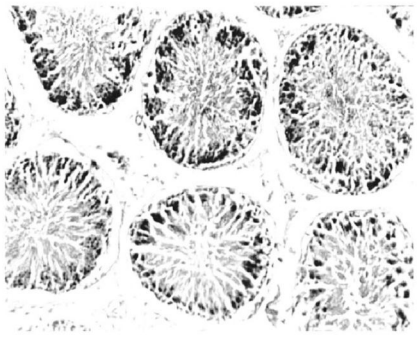


Fig 9: **Deltamethrin 2.5mg/kg b.wt./day for 45 days**
Microphotograph showing damaged and shrunken seminiferous tubules with highly loosened tunica propria. Increased interstitial space with disrupted interstitial cells and blood vessels could be seen. (H&E 100X)



DISCUSSION

Despite unlimited access to food, there was a significant decrease in the body weight gain in male rats exposed to deltamethrin which was a clear indication of general toxicity. It could be concluded that the magnitude of toxicity, which made the animals rather lethargic, might have affected their fertility indirectly rather than having any specific effect on reproductive function; however, Chapin et al., (1993) showed that the reproductive system of male rats was relatively resistant to body weight reductions of down to even 70% of the body weight (21).

Paired testicular mass is a valuable index of reproductive toxicity in male animals (22). In the present study, weight of the testes decreased significantly with increasing dose levels of pesticide in rats revealing two principle impacts on the male reproductive system namely, the anti-spermatogenic and anti-androgenic effects. Decreased diameter of seminiferous tubules and reduced number of spermatogenic elements in the seminiferous tubules reflect the anti-spermatogenic toxicity (23). The anti-androgenic action of deltamethrin is possibly reflected by decrease in the number of sperm in the tubules of testes and decrease in the weight of prostate gland (24).

It is well established that androgens are the major regulators of growth, structure and functions of accessory sex organs. In the present study, significant reduction in the accessory sex organs weights were recorded, indicating that the circulating levels of androgens were not enough to maintain the weight of these organs. In accessory sex organs, it is not testosterone, but rather the 5 -reduced metabolites, dihydrotestosterone and 3 , 17 androstenediol are the primary regulating hormones controlling the structure and function (25). A decrease in such androgen metabolites might eventually result in decreased sex organ weight.

It is well known that a large part of the male reproductive system depends on testosterone. The process of spermatogenesis will eventually cease in the absence of this hormone. Reduction in testosterone dependent parameters by deltamethrin may cause the so called "androgen deprived effect" to target organs by affecting testosterone synthesis in the testes.

Sperm motility serves as an important measure of sperm functional capability and its decline is a marker of male reproductive toxicity and also an indication of the onset of infertility (26). Marked inhibition of sperm motility may be due to low levels of ATP content (27) but low fructose concentration in seminal vesicles can be another cause of low sperm motility (28).

The reduction of sperm density in cauda epididymis is of importance with regard to fertilization (29). In the present investigation deltamethrin caused a significant reduction in sperm density in cauda epididymides as well as testes. Reduction in sperm counts in testes may be due to altered gonadotrophins (30).

The 60%, 90% and 100% negative fertility rate may be attributed to lack of forward progression and reduction in density of spermatozoa and altered biochemical milieu of cauda epididymis due to insecticide intoxication. Glycogen is important source of energy for general body metabolism. Constant supply of carbohydrate (glucose) is essential for gonadal maturation and the proper functioning of the testes (31). Leydig cell function is also suppressed in the absence of carbohydrates and significant depletion in glycogen content after the administration of deltamethrin was noticed in the present study. This interference may affect the maturational process of spermatozoa and their motility and inhibition of glycogen synthesis might eventually decrease spermatogenic processes (32).

Cholesterol is an important precursor in the synthesis of steroid hormones (33) its requirement for normal activity of testes has been well established. Present investigation revealed hypercholesterolemia in insecticide exposed rats. Increased level of cholesterol is attributed to decreased androgen concentration, which resulted in impaired spermatogenesis (31). Also, increased testicular cholesterol content indicates that pituitary gonadotrophins may not be available for steroidogenesis (34).

Structural integrity of acrosomal membrane is dependent upon sialic acid; an alteration in its content may lead to changes in motility and fertilizing ability of sperm (35,36).

Measurement of fructose has been used in almost all laboratories of the world as a marker of the seminal vesicle function (28). WHO includes measurement of this sugar in the assessment of these glands funtion (37). The decrease in the fructose level of seminal vesicles of insecticide treated rats was another important observation. The depletion of fructose content hampers the glycolytic metabolism of spermatozoa. This results in abnormal sperm functions, which ultimately gave rise to complete male sterility (38,39).

In conclusion, our result clearly suggest that exposure to insecticide deltamethrin have adverse effects on accessory sex organs and biochemical parameters of male rats.

DECLARATION

We, the authors of this research paper hereby, declare that this research work carried out in Reproductive Toxicology Unit, Department of Zoology, University of Rajasthan, Jaipur (INDIA) had met the National Guidelines on the proper care and use of animals in laboratory research and the experimental protocol comply with the current laws of our country.

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