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# DELTAMETHRIN INDUCED REPRODUCTIVE IMPAIRMENT IN ADULT MALE RATS EXPOSED DURING PREPUBERTAL STAGE

**DAVEEDU T AND SAINATH SB***

Department of Biotechnology, Vikrama Simhapuri University, Nellore-524320, India

***Corresponding Author: Dr. S. B. Sainath: E Mail: daveeduthathapudi@gmail.com**

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**ABSTRACT**

Deltamethrin (DTM) is a type II pyrethroids, it can adversely affect the male reproductive system by disturbing the steroidogenesis and spermatogenesis. However the consequence of prepubertal DTM intoxication and their impact on adult stage are not known. Current study aimed to investigate the significances of male reproductive parameters following DTM exposure from prepubertal stage to adult stage. Wherein prepubertal rats were treated with 3mg or 6mg/kg/day was given from PND 23 to PND 90 by oral gavage. Exposure of prepubertal rats to the DTM resulted in the significant weight loss in the animal and reduced sperm motility, sperm count, sperm viability and suppressed serum testosterone levels. Further DTM intoxicated groups also showed the reduced steroidogenic enzymes (3β HSD and 17β HSD levels) inn testis. Sperm morphological abnormalities and sperm DNA damage were observed along with the histopathological changes in the rat testis of DTM exposed groups. In conclusion, Deltamethrin treatment in the prepubertal rats induced the apparent reprotoxic effects at their adulthood it was confirmed by the observed adverse effects on reproductive organs.

**Keywords: Prepubertal rats, Deltamethrin, Reproductive toxicity, DNA damage**

## INTRODUCTION

Synthetic pyrethroids are widely used in agricultural sectors due to their effectiveness in controlling the range of insects. These pyrethroids are products of natural substances of pyrethrins attained from the plant flowers of pyrethrins species **[1]**. The others desired properties of pyrethroids including their bio-efficacy at low concentration, low toxicity to the mammalians when compare with other pesticides like organophosphates, in addition these pyrethroids are metabolized rapidly and their bioaccumulation in the tissues are also less **[2]**. Since pyrethroids have the attractive attributions their usage is enormously increased globally and their presence can be seen in many environmental compartments such as water, soil and also in agricultural fruits and vegetables. Recently the greater attention of many researchers are paid towards the pyrethroids and pyrithroidal agents mediated toxic effects to the environment, humans and wild life **[3]**. Pyrethroids have the additional advantage of broad spectrum activities and reduced resistance development in the insects. Due to the contamination of environment general public are exposed to these pyrithroidal agents, through different routes such as intake of food with pyrithroidal residues, inhalation the air contaminated with pyrethroids **[4]**.

DTM is a synthetic pyrethroids that belongs to the type II category of pyrethroids, widely used in agricultural, public health and veterinary sectors to control the pests, further used in poultry form to minimize the ectoparasites. One of the most common route through which DTM enters in to the human body are pyrethroids contaminated food and water intake, they have the tendency to readily absorb from the oral route **[5]**. Generally after entering in to the body these pyrethroids are readily metabolized and releases reactive oxygen species (ROS) that consequences in the oxidative stress in the animals exposed to the DTM, the mammalian sperm membrane contains the huge amount of the poly unsaturated fatty acids and low levels of defensive antioxidants, thus these sperm membrane is susceptible to undergo lipid peroxidation, although ROS production is normal and also beneficial for sperm capacitation, sperm and oocyte reaction and acrosome reaction, excess production of ROS is detrimental to the sperm and it finally leads to the male infertility **[6, 7]**.

There are several reports claim that reproductive toxicity of DTM the reprotoxic

effects of DTM includes reduction of sperm counts, quality and motility. DTM has the ability to decrease the testicular weight and testosterone production and changes the sexual behavior in the DTM intoxicated rats, rabbits and mice **[8]**, further DTM also induced the testicular degenerative changes **[7]**. Gilmora in 2006 reported in a book OEHHA the neurodevelopmental toxicity of DTM where its slightly delayed the preputial separation due to the in utero exposure **[9]**. In two generation toxic studies conducted in the rats reduced the weights ot both testes and brain weight ration in F1 male rats at 320 ppm dose and at the same dose it also decreased the gross weight and increased the offspring death however it was not affected the sexual performance and fertility.

In vivo studies conducted in rats and mice shown that pyrethroids might impairs the testosterone production via inhibition of key enzymes involved in the testicular steroidogenesis. However most of these studies were evaluated in adults and reproductive toxic studies pertaining to DTM in prepubertal stage are dearth. Generally pre-and peri pubertal stages are critical phases in the postnatal development phases, since it is the first life developmental stage wherein organisms will directly expose to toxicants without intervention of maternal metabolism. Further it is the period where immune, endocrine and morphological development occurs. Moreover the most vital functions of testis such as steroidogenesis and spermatogenesis have not been well developed, which makes the animal most sensitive to the endocrine disruption posed by the toxic chemicals **[10]**. Since the effect of DTM exposure on the later male reproductive functions remain unknown, we aimed to study the DTM effect on such a sensitive stage. Main objective of present study was to assess the DTM detrimental effects on adult rats following their prepubertal exposure to the DTM.

## MATERIALS AND METHODS

### Chemicals

Chemicals like Deltamethrin and other were purcahed from sigma chemicals Co. (St. Louis, MO, USA). Kits and other reagent used in this study were obtained from Himedia Chemicals Co. (Mumbai India) and local suppliers.

### Animals

Prepubertal male albino Wistar rats (30±5g) were used in this study, they were purchased from the NIRFBR-Hyderabad. Rats were maintained under well controlled laboratory settings i.e. 12 hour light/dark cycle and temperature: 22-25ºC and relative humidity adjusted to: 50 ± 5°C throughout the

experimental study. Rats were fed with laboratory pellets (Obtained from NIN, Hyderabad, and Telangana, INDIA) and given tap water ad libitum. All the experimental studies conducted were in accordance with the current guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals, INDIA (CPCSEA 2003). Regn. 1837/PO/RcBiBt/S/15/CPCSEA.

**Experimental design**

After maintaining the acclimation period, experimentation of the prepubertal rats were started. Animals (n=40) were allocated equally into four groups (One control and three treated groups), each group contains the 10 rats. Groups I animals were vehicle control (Peanut oil), group III treated with high concentration of DTM was dissolved in peanut oil 6 mg/kg, group IV animals were treated with low concentration DTM (3mg/kg).

**Necropsy**

Individual weight of the animal were recorded at the beginning of the experiment and cessation of the experiment (Before necropsy), rats were kept on overnight fast, weigh up and sacrificed on the 90th day of study period. Collected the whole blood from cardiac organ by puncturing the heart prior to necropsy. Serum was carefully separated and kept in -20C for the further study. Organs like testis, kidney, liver, prostate gland, epididymis, brain, seminal vesicles, and vasdeference were rapidly harvested removed adherent tissues and weighed organs to the nearest values. Testes was used to estimate the enzymatic analysis.

**Spermatozoa count**

Sperm was obtained from the epididymis tissue by chopping the epididymis in few ml of Hams F-12 medium, filtered over the nylon comprising mesh and kept incubated for a period of 5 min at room temperature. The obtained sperm was counted used to count the sperm using the neubauer chamber as described in previous studies **[11]**. 5µl of epididymis sperm aliquot was mixed with the 95 µl of Hams F-12 medium. 10 µl of above solution was kept on hemocytometer and it was kept over a humidified chamber for 5 min. Intact spermatozoa calculated with the help of light microscope at 200X.

**Sperm motility assay**

Motility of the sperm was analysed with the aid of microscope, within the 5 min following the isolation from the section of cauda epididymis at 32°C **[11]**. Fluids of the cauda epididymis was collected and they were briefly diluted with the 2ml of Ham's F-12 medium. 10 µl of aliquot was kept over the Neubauer chamber and number of motile

and non-motile sperms were noted. Nonmoving sperms were counted initially followed by the motile sperms. Percentage of motility in the sperms were expressed as motility sperms in the total number of sperms counted.

**Sperm viability and abnormalities**

The epididymis sperm viability was analysed by staining in 1% solution of trypan blue solution **[12]** and they were calculated using light microscope at the magnifications of 200x. Single drop of epidymal sperm was diversified with the single drop of 0.5% eosin solution on a microscopic slide and they were covered with coverslip. After incubation of 60sec they were calculated using microscope. Stained spermatozoa were considered as dead and unstained spermatozoa was considered as live spermatozoa. Viability of sperm was expressed as percentage of unstained sperms of all sperms totaled. The morphological anomalies in the sperm were counted by means of light microscope in accordance with the previous methodology of Hemavathi and Rahiman *et al*., **[13]**.

**Hypo osmotic swelling test**

Integrity of sperm membrane was evaluated by treating them to the hypo osmotic medium, the percentage of sperms with the tail coiling was estimated by the method of jayanendran *et al*., **[14]**. 1 ml of hypo osmotic solution was prepared and kept incubated at 37°C for 10 min. To the prepared solution 100µl of spermatozoa was added and kept a side for incubation at 37°C for 30 min. Single drop of this solution was taken on glass slide and coved with coverslip. And it was observed for the coiled sperm tail at a magnification of 200x under the light microscope, sperms about 100 in number were studied from every animal.

**Estimation of testicular steroidogenic enzyme assay**

Fraction of testis was thoroughly homogenized in 1:9 ratio in 0.2 M Tris- HCL (Ice cold buffer solution) containing the pH of 6.8 containing the 0.1% of acetyl trimethyl ammonium bromide with the help of homogenizer for the evaluation of hydroxyl steroid dehydrogenases. Fraction of microsomes were collected and used as enzyme source. Testicular enzymes like 3β-hydroxysteriod dehydrogenase another enzyme 17β-hydroxysteriod dehydrogenase level were evaluated according to the Bergmeyer *et al*., **[15]**. The enzymatic conditions were set at zero order kinetics, later the 1Ostandardization regarding the linearity with deference to the time of enzymatic concentration and incubation. The protein content/ enzyme source was used to determine testicular enzymes in accordance

with Lowry *et al* **[16]** using bovine serum albumin protein as a standard protein.

**Measurement of testosterone**

Serum testosterone levels were estimated by the enzyme linked immunosorbent assay (ELISA) using kit method. Assay was performed by strictly following the protocol given by the kit manufacturers. The sensitivity of the assay was analysed as 0.02ng and internal difference was 6.5%. All the samples were executed for the testosterone measurement at the same time to minimize the internal variation. The testosterone was denoted in ng/ml.

**Enzymatic antioxidants**

Small sample of the aliquots were studied for each enzymatic activities. The catalase activity was analysed at 240 nm using spectrophotometer based on its capacity to decompose the molecule hydrogen peroxide. Similarly superoxide dismutase levels were determined spectrophotometrically at 480 nm. Wherein its capacity to inhibit the epinephrine auto-oxidation at alkaline medium.

**Glutathione assay**

Quantification of glutathione (GSH) in both epididymis and testis was done as described by the Beutler *et al* **[17]**, by using a Ellmans reagent (5,5′-dithio-bis-[2-nitrobenzoic acid]: DTNB). Extracts were obtained from the tissue and they were added to the 12% trichloro acetic acid (1:4 v/v) and the contents were centrifuged. To the formed supernatant 0.25 mM concentration of DTNB in 0.1 M NaH2PO4 with pH 8.0 was added and the resultant thiolate anion was estimated spectrophotometrically at 412 nm.

**Estimation of Lipid peroxidation levels**

Lipid peroxidation levels in the testis and epididymis was estimated in terms of malandialdehyde (MDA) a degraded product of lipid peroxidation and they were estimated by the using the reagent thibarbituric acid (TBA). The levels of LPX were performed according to the Ohkawa *et al* **[18]**. 0.5 ml of the homogenate was freshly prepared in phosphate buffer (pH 7.2) to this 1.0 ml of TCA and TBA 1.0 ml was added. The contents were mixed thoroughly followed by 20 min boiling in water bath, cooled and centrifuged at 1000x g for 10 min. The absorbance of the supernatant content was then estimated spectrophotometrically at 535nm. Formation of LPx was denoted as μmoles of MDA produced/g wet wt. of tissue.

**Evaluating the sperm DNA damage**

DNA damage was tested using the comet assay approach with slighy modification **[19]**. The slides were treated with comet imagery dye (Ethidium bromide) for comet

viewing. The creation of comets was observed. Olympus, Japan) and the pictures were taken with a microscope with a computer linked to it. The pictures were taken. We used the image analysis program CASP Lab. To prevent sperm samples from being exposed to light, all the slides were kept in darkness and experiments were conducted. Every experiment were carried out three times (100 cells were examined for A total of three slides per species and one comet formation each slide were ready (prepared) separately for each treatment

**Histology**

A small section of testis was embedded in the paraffin and after fixing in the Boulin's solution for 1 day followed by the completed dehydration of tissue using the ascending series of the ethanol and washed in xylene. The tissue sectioned specimen 5µM of testis was stained in eosin and hematoxylin and studied them under the microscope (Hovers microscope: Model no. HV-12 TR).

**Statistical analysis**

The experimental data was expressed in mean± S.D and obtained results were analysed statistically using one – way analysis of variance using the post-hoc Tucky test (Graph pad prism), values were considered significant difference at $P<0.01$.

**RESULTS**

**Clinical and general toxicity**

All the animals used in the present study were carefully observed for the clinical signs and symptoms it includes urination, salivation, lethargic behavior and vocalization. But no signs of clinical toxicity was observed in both control and also in treated rats.

However, significant weight loss ($P<0.05$) was observed in the DTM treated groups over the control group. Most importantly loss of weight in the testis and epididymis and other accessory organs like cauda, carpus and caput were observed in DTM treated groups ($P< 0.01$) as compared to the control group. Further significant decline ($P< 0.01$) in the weight of vas deferens, seminal vesicles and prostate gland were also seen in the DTM treated rats when compare with the control rats **(Table 1)**. The macroscopic testis images shown in **Figure 1**, indicates the apparent testis size reduction in the animals treated with DTM ($P<0.01$) when compare with the control group.

**Sperm, testicular steroidogenic enzymes and serum testosterone levels**

Daily sperm production (DSP) in the control testis was observed as an average of 12.96 million/g testis. Wherein animals treated with DTM showed significant decrease in the DSP as compared with the

(P< 0.01) control group. Apparent reduction in the sperm count was observed in the epididymis of DTM treated rats (average 7.86 mn/g testis), and other key sperm parameters like sperm motility, sperm viability were significantly declined (P<0.001), whereas sperm coiling in rats treated with DTM were significantly increased due to the DTM effect, they when compared with untreated control rats **Table 2**.

All animals in control group clearly shown the normal morphological shape in the sperm structure, however significant morphological alterations were observed in rats exposed to DTM **(Figure 2)**.

Testicular steroidogenic enzymes such as 3β-HSD and 17β-HSD levels were found to be significantly reduced (P<0.001) in both low dose and high dose DTM exposed groups, when compared with control group **(Table 2)**. Significant reduction in the levels of serum testosterone was also observed in the DTM treated rats (P< 0.001) at low dose and high dose over control group.

**Testicular oxidative stress parameters**

Lipid peroxidation (LPx) levels were significantly increased (P<0.001) in the testis of the DTM exposed (Low and high dose) groups as compared with its control **(Table 2)**. Correspondingly the antioxidants like CAT, SOD, GSH levels were markedly declined (P<0.01) in the DTM exposed rats as compared to control group, shown in **Table 3**.

**Evaluating the sperm DNA damage**

DNA damage studies with comet assay shown that, DTM exposure at both high and low concentrations negatively impacted the sperm DNA integrity when compared with control groups **(Figure 4)**.

**Histology**

Testicular histopathological studies were performed for both control and treated rats. Testicular transverse section of control group showed intact basement membrane containing different stages of germ cells and intestinal lumen was surrounded by the sperms. Whereas DTM exposed rats demonstrated the disrupted testicular architecture, damaged epithelium and lumen without sperms were observed **(Figure 3)**. No structural deformities were observed in the vehicle treated rats testis.


**Figure 1: The macroscopic testis showing altered testicular size in response to DTM exposure**

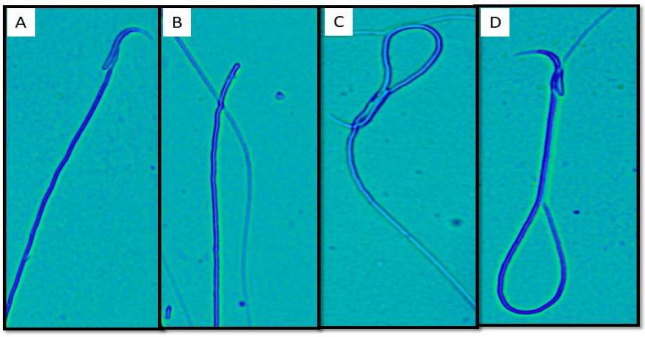


**Figure 2: Morphological abnormalities in the sperm structure in rats exposed to DTM**

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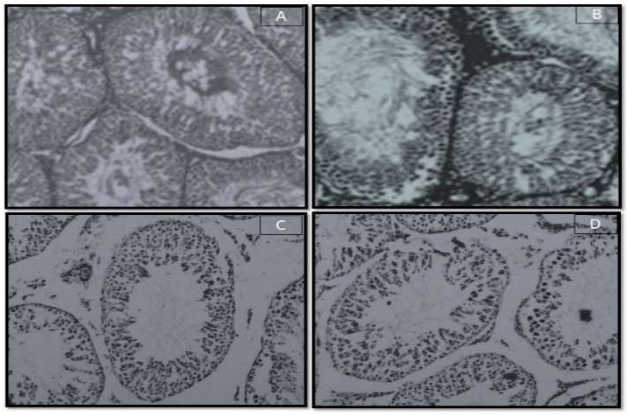


**Figure 3: Histological studies indicating the disrupted testicular architecture in DTM exposed rats**

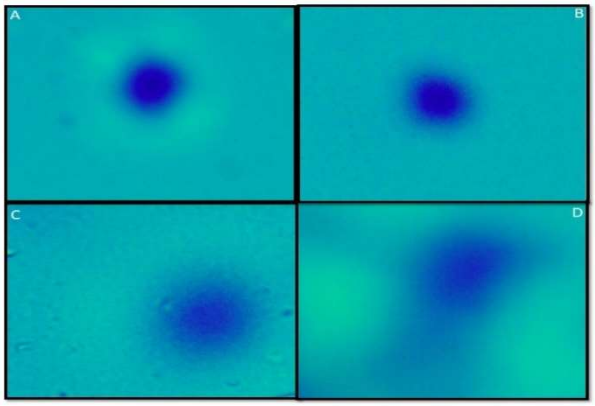


**Figure 4: DNA damage studies indicating fragmented sperm DNA in DTM treated rats**

**Table 1: Body and organ weight of control and DTM exposed rats**

| | Control | Vehicle | Low dose (DTM) | High dose (DTM) |
|---|---|---|---|---|
| **Body Weight** | **184.26$^{a}$ ± 9.56** | **181.25$^{a}$ ± 9.12** | **160.43$^{a}$ ± 10.22** | **152.42$^{a}$ ± 5.70** |
| **Testes** | **1.19$^{a}$ ± 0.16** | **1.18$^{a}$ ± 0.079** | **0.81$^{b}$ ± 0.101** | **0.68$^{c}$ ± 0.039** |
| **Epididymis** | **1.009$^{a}$ ± 0.059** | **1.0081$^{a}$ ± 0.121** | **0.756$^{b}$ ± 0.029** | **0.708$^{c}$ ± 0.039** |
| **Vas deference** | **0.166 $^{a}$ ± 0.0211** | **0.169$^{a}$ ± 0.0159** | **0.98$^{b}$ ± 0.0311** | **0.76$^{a}$ ± 0.0255** |
| **Prostate gland** | **0.151 $^{a}$ ± 0.018** | **0.152$^{a}$ ± 0.025** | **0.90$^{b}$ ± 0.024** | **0.83$^{a}$ ± 0.032** |
| **Seminal vesicle** | **0.450$^{a}$ ± 0.061** | **0.441$^{a}$ ± 0.091** | **0.362$^{b}$ ± 0.069** | **0.314$^{b}$ ± 0.030** |
| **Brain** | **0.646a ± 0.040** | **0.649a ± 0.052** | **0.633a ± 0.050** | **0.629a ± 0.041** |
| **Liver** | **3.107a ± 0.262** | **3.10a ± 0.39** | **3.12a ± 0.49** | **2.99a ± 0.36** |
| **Kidney** | **0.673a ± 0.022** | **0.681a ± 0.051** | **0.663a ± 0.031** | **651a ± 0.07** |

**Table 2: Sperm parameters and serum testosterone of DTM exposed group**

| Parameter | Control | Vehicle | Low dose (DTM) | High dose (DTM) |
|---|---|---|---|---|
| **Daily sperm count (millions/g testis)** | **12.96$^{a}$ ± 2.94** | **11.87$^{a}$ ±1.83** | **8.99$^{b}$ ± 0.91** | **7.86$^{c}$ ± 0.92** |
| **Sperm count (millions/ml)** | **70.32$^{a}$ ± 6.68** | **71.38$^{a}$ ± 5.46** | **57.39c ± 5.49** | **49.39$^{d}$ ± 5.49** |
| **Sperm viability (%)** | **69.34$^{a}$ ± 3.35** | **66.54$^{a}$ ± 5.16** | **55.48$^{c}$ ± 5.56** | **42.47$^{b}$ ± 2.79** |
| **Sperm motility (%)** | **64.18$^{a}$ ± 5.20** | **64.25$^{a}$ ± 5.25** | **45.13$^{c}$ ± 6.10** | **41.59$^{b}$ ± 7.11** |
| **HOS Tail coiled sperm (%)** | **60.66$^{a}$ ± 6.82** | **61.06$^{a}$ ± 5.11** | **42.16$^{c}$ ± 4.70** | **39.47$^{b}$ ± 5.66** |
| **Testosterone (ng/ml)** | **7.47$^{a}$ ± 0.26** | **6.99$^{a}$ ± 0.16** | **3.89$^{c}$ ± 0.19** | **3.28$^{b}$ ± 0.43** |
| **3βHSD (nmol of NAD converted to NADH/mg protein/min)** | **13.29$^{a}$ ± 3.72** | **14.66$^{a}$ ± 2.77** | **11.44$^{c}$ ± 1.96** | **7.99$^{b}$ ± 2.56** |
| **17βHSD (nmol of NADPH converted to NADP/mg protein/min)** | **11.60$^{a}$ ± 1.12** | **11.99$^{a}$ ± 1.53** | **8.05$^{c}$ ± 0.36** | **4.30$^{b}$ ± 0.79** |

**Table 3: Effect of DTM on oxidative stress parameters in rat testis**

| Parameter | Control | Vehicle | Low dose (DTM) | High dose (DTM) |
|---|---|---|---|---|
| **SOD** | **17.6$^{a}$ ± 1.9** | **18.80$^{a}$ ±1.12** | **13.91$^{b}$ ± 0.98** | **8.80$^{c}$ ± 0.22** |
| **Catalase** | **11.11$^{a}$ ± 1.60** | **12.38$^{a}$ ± 1.40** | **9.99c ± 0.98** | **7.5.30$^{d}$ ± 1.09** |
| **GSH** | **14.30$^{a}$ ± 08.35** | **13.59$^{a}$ ± 0.11** | **9.11$^{c}$ ± 0.41** | **7.40$^{b}$ ± 1.11** |
| **LPx** | **8.99$^{a}$ ± 0.20** | **8.20$^{a}$ ± 0.15** | **12.11$^{c}$ ± 1.10** | **14.50$^{b}$ ± 1.02** |

## DISCUSSION

In toxicological studies estimating the gross body weight of of animal and individual organ weight is considered as benchmark. In our study we observed the significant weight loss in rats treated with DTM at both concentration (Low dose and high dose). The reduced body weight might be linked with the DTM induced anorectic. Hence animal exposed to the DTM might suffered with lack of appetite and thus decreased food intake directly affect the weight of animal, Or it could also due to the DTM indirect effect on CNS which generally regulates the food and water intake **[7]**. Further it might also because of the cytotoxic effect of DTM that eventually targeted the animal somatic cells. Quantifying the weight of reproductive organs is essential in estimating the male reproductive toxicity, most importantly weight and size of the testis, because it gives the clue about the spermatogenesis. In genaral testicular mass around 98% made up of germinal cells and spermatogenic tubules.

In our investigation significant reduction in the testicular weight was found

it could be due to the decline in the testosterone levels in the serum. These results support the earlier findings reported by the **[7]**, where they confirmed the exposure of mice to the DTM showed the decreased testicular weight at a dose of 3mg and 6mg. In the current study, decreased testicular weight could be attributed to the decline in the number of germ cells, and inhibition of spermatogenesis, further it also could be due to the decrease in the testicular enzyme activities.

Interestingly DTM exposure significantly deteriorated the sperm count, viability and motility at both high and low dose exposure, however DTM exposure rats demonstrated the significant elevation in the sperm coiling and sperm morphological abnormalities.

The testicular weight was reduced because of the damage in the histological architecture, germ cell atrophy and necrosis might be the one of the cause the testis weight loss. 3β and 17β HSD were the key steroidogenic enzymes in void in steroidogenic pathway they were essential for the production of testosterone from the molecules of pregnenolone and androstenedione. In the current study DTM negatively influenced the levels of both enzymes at both concentration, decreased steroidogenic enzymes levels that could be due to the fall in testosterone that might be impaired the steroidogenic pathway **[7]**. In male reproductive system generally androgens regulates the key function of reproductive organs growth and their structural integrity. In the current study weight of the accessory sex organs such as epididymis, seminal vesicles and prostate glands were significantly decreased as result of DTM exposure. The large extent of male reproductive system depends on the testosterone. Most importantly spermatogenesis, that occurs in the presence of testosterone, any absence in testosterone leads to the arrest of spermatogenesis. DTM deprives the androgens in the exposed male rats and thus decreased the activities of testosterone dependent male reproductive sperm parameters.

Similar results were also seen the pyrethroids exposed male animal where exposure of cypermethrin decreased the levels of male hormones such as LH, FSH and Testosterone **[20]**. Reduction in the testicular hormones as a result of DTM exposure might occurred due to their direct effect on the androgens in the testis, or indirectly by influencing the brain Hypothalamo-pitutory gonadal axis, that eventually decreased the production of those male reproductive hormones **[7]**.

Since the sperm membrane contains the large amount of poly unsaturated fatty acids that could easily undergo attack by the ROS and eventually results in the altered sperm capacitation and decreased sperm motility **[21]**. In DTM treated rat's abnormalities in the sperm parameters were apparently observed in sperm head damage, decreased motility and viability that points toward the compromised fertility in the rats. We also observed the significant decline in the levels of CAT, SOD and GSH with sharp rise in the LPx levels.

Additionally, a significant rise in the sperm DNA damage in DTM-intoxicated rats could have genotoxic effects at the level of gametes from males **[22]**. Our findings demonstrated that spermatotoxicity was caused by DTM exposure as seen by sperm DNA damage (Comet assay), decreased motility, decreased viability, decreased sperm membrane integrity, increased sperm head abnormalities, as well as decreased sperm number

Histopathological investigation of testis revealed that animals treated with DTM showed disrupted epithelium, and lumen devoid of sperm. Further tubular atrophy, cell necrosis, vacuolization with sloughing with degenerated spermatids and spermatocytes were observed in Sertoli cells. Further degeneration in the Sertoli and Leydig cell was also seen as an effect of DTM intoxicants in the treated rats. These results were in accordance with the reports suggested by the Desai *et al* **[7]**. Vacuolization in the Sertoli and germ cells were also observed, it might be due to the increased diameter / swelling of endoplasmic reticulum that indicates the altered cellular permeability caused by the pyrethroids **[23]**.

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**Conflicts of Interest**

The authors declare there is no conflict of interest.

**REFERENCES**

**[1]** Luty S, Latuszynska J, Obuchowska-Przebirowska D, Tokarska M, Haratym-Maj A. Subacute toxicity of orally applied alpha-cypermethrin in swiss mice. Ann Agric Environ Med 2000; 7: 33 41.

**[2]** Saillenfait, A.M., Ndiaye, D., Sabate, J.P., 2016. The estrogenic and androgenic potential of pyrethroids in vitro. Review. Toxicology in vitro: an international journal published in association with BIBRA 34, 321–332.

**[3]** Gan, J., Lee, S.J., Liu, W.P., Haver, D.L., Kabashima, J.N., 2005. Distribution and persistence of pyrethroids in runoff sediments. Journal of environmental quality 34, 836–841.

**[4]** Saillenfait AM, Ndiaye D, Sabaté JP. 2015. Pyrethroids: Exposure and health effects – an update. Int. J. Hyg. Environ. Health 218: 281–292.

**[5]** Barlow SM, Sullivan FM, Lines J. Risk assessment of the use of deltamethrin on bednets for the prevention of malaria. Food Chem Toxicol 2001; 39: 407–22.

**[6]** Kale M, Rathore N, John S, Bhatnagar D. Lipid peroxidative damage on pyrethroids exposure and alterations in antioxidant status in rat erythrocyte: a possible involvement of reactive oxygen species. Toxicol Lett 1999; 105: 197–205.

**[7]** Desai, Ketaki & Moid, Nilofer & Patel, Pragnesh & Highland, Hyacinth. (2016). Evaluation of Deltamethrin induced reproductive toxicity in male Swiss Albino mice. Asian Pacific Journal of Reproduction. 5. 10.1016/j.apjr.2015.12.004.

**[8]** Sharma, P., Huq, A.U., Singh, R., 2014. Cypermethrin-induced reproductive toxicity in the rat is prevented by resveratrol. Journal of human reproductive sciences 7, 99–106.

**[9]** Office of Environmental Health Hazard Assessment (OEHHA). California Environmental Protection Agency. Reproductive and Cancer Hazard Assessment Branch. March 2013. Evidence on the developmental and reproductive toxicity of deltamethrin

**[10]** Filler R. (1993) Methods for evaluation of rat epididymal sperm morphology. In: Methods in Toxicology: Male Reproductive Toxicology, Part A. Chapin, R. E., pp. 334–343 (ed. J.J. Heindel), Chapin, R. E. 1st, San Diego: Academic Press.

**[11]** M. A. Belsey, K. S. Moshissi, R. Eliasson, C. A. Paulsen, A. J. Callegos, M.R. N. Prasad, *Press Concern*, **1980**.

**[12]** Talbot P, Chacon P. A triple stain technique for evaluating normal acrosome reaction of human sperm. J Exp Zool 1981; 215. 210–208.

**[13]** Hemavathi E, Rahiman MA. Toxicological effects of ziram, thiram, and dithane M-45 assessed by sperm shape abnormalities in mice. J Toxicol Environ Health. 1993 Apr;38(4):393-8. doi: 10.1080/15287399309531727. PMID: 8478981.

**[14]** R.S. Jeyendran, H.H. Van Der Ven, L.J.D. Zaneveld, The hypo-osmotic swelling test:

**[15]** an update, Arch. Androl. 29 (2) (1992) 105–116.

**[16]** O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein Measurement with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.

**[17]** E. Beutler, in Red CellMetabolism: AManual of Biochemical Methods (Ed: E. Beutler), Grune and Straton Company, New York 1975, pp. 8

**[18]** H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95 (1979) 351–358.

**[19]** Naresh Dumala, Bhanuramya Mangalampalli, Srinivas Chinde, Srinivas Indu Kumari, Mohammad Mahoob, Mohammad Fazlur Rahman, Paramjit Grover, Genotoxicity study of nickel oxide nanoparticles in female Wistar rats after acute oral exposure, *Mutagenesis*, Volume 32, Issue 4, July 2017, Pages 417–427, https://doi.org/10.1093/mutage/gex007

**[20]** Gupta RS, Sharma R, Sharma A, Bhatnager AK, Dobhal MP, Joshi YC, *et al*. Effect of Alstonia scholaris bark extract on testicular function of Wistar rats. Asian J Androl 2002; 4(3): 175-178.

**[21]** Gheri G, Vichi D, Thyrion GD, Bonaccini L, Vannelli GB, Marini M, *et al*. Sialic acid in human testis and changes with ageing. Reprod Fertil Dev 2009; 21(5): 25-33.

**[22]** Zlatkis A, Zak B, Boyle AJ (1953) A new method for the direct determination of serum cholesterol. J Lab Clin Med 41:486–492

**[23]** Creasy DM, Foster PMD. Male reproductive system. In: Haschek WM, Rousseaux CG, editors. Handbook of toxicologic pathology. Amsterdam: Elsevier; 2002, p. 785-846.