Early exposure to deltamethrin impaired steroidogenesis in male rats at their adult stage

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Abstract:

Deltamethrin (DTM) is a type II synthetic pyrethroids and endocrine disruptor that can impair steroidogenesis and cause serious adverse effects on male reproduction. However, the impact of prepubertal DTM exposure and its effect on adult reproduction is yet unknown. The present study investigated the DTM exposure effect from the prepubertal stage to the adult stage on several reproductive endpoints in male rats. Prepubertal rats were exposed to 3mg or 6 mg/kg/day from the postnatal day (PND) 23 to the adult stage (90 PND). Significant reductions in the activity levels of 3\(\beta\)- and 17\(\beta\)-hydroxysteriod dehydrogenases were observed in the DTM-treated rats over the control group. Both low and high doses of DTM were able to disrupt the steroidogenesis process, as evidenced by the decreased hormonal production (Testosterone). Furthermore, DTM-exposed rats showed decreased sperm count, motility, and viability. Moreover, DTM exposed group also showed severe sperm DNA damage along with severe testicular histopathological abnormalities in the treated groups. Further molecular docking studies results specified that DTM is interacting with 3\(\beta\)-HSD a key enzyme involved in steroidogenesis and that might lead to impaired steroidogenesis. These findings showed that exposure to prepubescent DTM negatively affected the male reproductive system in adults' life indicating that a comprehensive risk assessment of DTM exposure during the prepubescent period is necessary.

Key words:

Prepubertal rats, Deltamethrin, Reproductive toxicity, 3\(\beta\)-HSD, Molecular docking
Introduction

Pesticides are being used worldwide more frequently in both agriculture and non-agriculture purpose to control the pests population. The most widely used pesticides in India are insecticides, which account for more than 65% of all pesticide usage. The pesticide sector in India is the biggest sector when compared with all other countries in Asia (Panda et al. 2003). Since pesticides are widely employed for both agricultural and non-agricultural uses, that results in the negative impact on human health and the environment. Most of the insecticides and herbicides that are sprayed on target with the intention controlling their population are also reaching into the environment such air, water, and soil. Insecticides that are both synthetic and natural (Derived from plants, such as pyrethrins) are causing serious threat to human health. Pyrethroids are the synthetic versions of pyrethrins flowers. The general toxicity nature of pyrethroids is high for insects but low for mammals and birds. Pyrethroids are divided into two types those are type-I and type- II based on their mechanism of action, and the majority of pyrethroids act by delaying the closing of sodium channels in the neuronal membrane, which is the main cause of their toxicity (Breckenridge et al. 2009), further pyrethroids can also targets the voltage-sensitive calcium channels and sodium channels, and thus causing the excess release of neurotransmitters that finally leads to the toxicity (Costa et al. 2015; Wolansky et al. 2013). Inhibition of sodium potassium ATPase in the neuronal membranes is another aspect of pyrethroids toxicity (Tan et al. 2010; Soderlund et al. 2012) Epidemiological studies shown that pyrethroids exposure during pregnancy and childhood is linked to poor neuronal development (Burns et al. 2013). Several toxicity issues were reported from the DTM exposure those are oxidative stress mediated DNA damage and cell death (Wang et al. 2016) thyroid and liver cancers (Osimitz et al. 2009; Price et al. 2007; Finch et al. 2006). Deltamethrin is a synthetic pyrethroids type-II, most commonly used as insecticide all over the world. It is one of the strongest insecticides now available and is frequently used to manage a variety of ectoparasites, such as lice, flies, and ticks, to protect fish, fruits, and vegetables from pests and parasites in the terrestrial and
aquatic animal sectors (Chandra et al. 2013). Deltamethrin-contaminated food and drink consumption were harmful to the humans (Barlow et al. 2001). Initially, deltamethrin was thought to be the least hazardous to mammals, but investigations have shown that it has a variety of toxic effects on both mammals and non-mammalian organs (Bradberry et al. 2005).

DTM can disrupt the testicular steroidogenesis by inhibiting key enzymes involved in testosterone production. However, the majority of these studies were conducted at the adult stage. Studies on DTM mediated reproductive toxicities were obscure. Pre- and peripubertal stages are often considered as key stages in postnatal development, where the organisms are directly exposed to toxins without the help of maternal metabolism. Additionally, during this time morphological, endocrine, and immunological development take place. Most importantly, testicular activities, such as steroidogenesis and spermatogenesis, have not yet fully evolved in the prepubertal stage and the animal is more vulnerable to the endocrine disruption caused by toxic chemicals. Because of the immature testicular activities, such as steroidogenesis and spermatogenesis, and animals tend to be more vulnerable to the endocrine disruption caused by hazardous substances (Filler et al. 1993), these prepubertal stage is very sensitive.

Although several researchers studied the DTM mediated reproductive toxicity effects in adult male rats, studies related to DTM effect in rats exposed at their juvenile stage are obscure. Hence the present was undertaken and to the best of our knowledge, this is the first investigation into how DTM impairs the steroidogenesis in adult rats exposed during their prepubertal stage. The purpose of this research work is to assess the potential reproductive toxic effects of DTM on steroidogenesis.

**Materials and methods:**

**Chemicals**

Pure deltamethrin powder (>98%) was purchased from TCI Chemicals in India. Remaining all chemicals were procured from Himedia laboratory (Mumbai, India) with the analytical-grade substances for the current investigation.

**Animals**

Prepubertal male rats (25±5g) were procured from NIRFBR- Hyderabad, Rats were fed with rat pellets made up of 10% wheat bran, 44% soy bean powder, 22% net protein, 4.7% lipids, 3.3% fibre, fish meal, molasses, salts (sodium chloride, calcium carbonate, and calcium phosphate), and methionine. Rats have unlimited access to water. Current research work was performed in accordance with the present guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals, INDIA (CPCSEA 2003) and present study was approved by the Institutional Animal Ethical Committee 1837/PO/RcBiBt/S/15/CPCSEA. The National norms and standards on animal welfare and institutional guidelines were strictly followed in performing the experiments on rats.

**Experimental design**

Prepubertal rats used in the current study were acclimatized prior to the commencement of the experiment. All rats (n=40) were divided equally into four groups, each group containing 10 rats (one control and one vehicle control remaining two were treatment groups). Animals in Groups I were given tap water and group-II peanut oil as the vehicle control, Group III received low dosage of DTM (3 mg/kg) and group IV received treatment with high concentration of DTM (6 mg/kg) DTM and treatment were continued from PND 23 to 90 days. At the end of the experimental studies rats were maintained for overnight fast, weighed, and sacrificed on the 90th day of the study period. Before performing necropsy, a heart puncture was used to collect the entire blood from the cardiac organ. For the purpose of hormonal investigation, serum was carefully isolated and stored at -20°C. Testes adherent tissues were removed, and the organs were weighed to the nearest values.

**Blood Sampling and Hormonal Assay**
Serum testosterone levels were quantified using ELISA kit method, purchased from regional ELISA kit suppliers, testosterone levels were measured in serum from the treatment and control groups. Estimating testosterone levels was done strictly in accordance with the suppliers' protocol. Testosterone concentrations were expressed as ng/ml, and test range sensitivities ranged from 0 to 16 ng/ml, the volume of the testosterone was denoted as ng/ml.

**Semen morphology analysis**

Sperm morphology was evaluated using computerised methods. Samples were fixed with Hancock's solution after semen collection, and smears were prepared. Giemsa's dye was applied to stain the slides, which were then examined under a 400x magnification (Utilizing the light microscope Olympus). Each rat's 500 spermatozoa were analysed and classified as normal or abnormal based on the exact sperm morphological standards. The head and tail deformities were separated from the morphological abnormalities. As part of the evaluation of the sperm tail, mid-piece sperm abnormalities were taken into consideration. The proportions of sperms with normal and unusual shapes were estimated (Adamkovicova et al. 2016)

**Testicular enzyme steroidogenic marker estimation**

Small piece of the testis was finely homogenized in in 0.2 M Tris- HCL containing the 0.1% of acetyl trimethyl ammonium bromide (pH: 6.8) and 0.1% of acetyl trimethyl ammonium bromide was also added to quantify the hydroxyl steroid dehydrogenases. Microsomal fraction was taken and used as an enzyme source. Testicular enzymes such as 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase were estimated as suggested by the Bergmeyer (1974). Initial conditions for enzymatic quantification were set to zero and next to the 1st order kinetics with respect to the linearity with the differences in the enzymatic concentrations and incubation. The amount of the protein concentration/ enzymatic source was employed to estimate the testicular enzymes level as described by the Lowry et al (1951) and bovine serum albumin protein was used as a standard.
protein. Spectrophotometric analysis was used to quantify the sample absorbance at 340 nm (Bergmeyer et al. 1974)

**Comet assay for assessing the damage to the sperm DNA**

Sperm DNA was analysed using the comet assay (Daveedu et al. 2023) sperm slides were prepared and treated with the ethidium bromide dye for viewing comet. All sperm sample slides were performed and kept in dark conditions, each experiment was performed three times with 100 cells. To avoid the damage caused by the sun light The formed comets observed and images were captured using the fluorescence microscope (Olympus, Japan) connected with the computer. Software CASP Lab program was employed to measure the DNA damage.

**Molecular docking 3β HSD (Gao et al. 2021)**

3β - HSD protein sequence was retrieved from Uniprotein database with ID: P22072, retrieved sequence was modelled using software modeller and human 3β HSD protein chosen as template (PDB: 1JTV). Modelled protein was docked with the DTM using Autodockvina as a molecular docking tool. Binding interaction between DTM and 3β HSD were visualized using Discovery Studio 2.1. 2.16.

**Statistical analysis**

Results are reported as mean ± S.D. Statistical analysis was performed using one-way analysis of variance (ANOVA) using post-hoc Tukey test. All statistical tests were performed by using Graphpad prism version5.0. The differences in the values between the groups were considered significant at $p < 0.05$.

**Histology**

Each testis from a rat in the control and experimental groups was fixed for 24 hours in Bouin's solution, dehydrated with an alcoholic series that got stronger, washed in xylol, and then embedded in paraffin wax. For histological analysis, hematoxylin and eosin Y were used to stain the sectioned
specimens. Hovers microscope (Model No. HV-12TR) was used to determine the testis' histological findings.

**Results**

The clinical signs and symptoms of all the animals included in the current investigation, including urine, salivation, sluggish behavior, and vocalization, were closely monitored but neither the control nor the treated rats showed any outward symptoms of clinical toxicity. However, the DTM treated groups lost weight significantly decreased when compared with its respective control group (Data not shown). The testis, epididymis, and other ancillary organs such the cauda, carpus, and caput weights in the DTM exposed groups were reduced than the control groups, similarly weight of the prostate gland, seminal vesicles, and vas deferens was also found to be decreased.

**Serum testosterone levels, sperm, and testicular steroidogenic enzymes**

All of the animals in the control group had sperm structures that were obviously in their usual morphological shape, while rats subjected to DTM showed significant morphological changes.

When compared to the control group, both low dosage and high dose DTM exposure groups showed significantly lower levels of testicular steroidogenic enzymes such 3β-HSD and 17β-HSD (P < 0.05).

When compared to the control group (Fig.1A and 1B), DTM exposed groups also demonstrated the significant reduction in the serum testosterone levels (P < 0.05) at low and high concentrations as shown in Fig 1C.

**Sperm morphology analysis**

Sperm morphological abnormalities were depicted in Table.1. Sperm morphological observations of DTM exposed groups were compared with its counter control group. Normal morphology of sperm were significantly decreased in DTM intoxicated groups with 3mg/kg exposure, mean while these values were further reduced (P< 0.05) in DTM at their high dose (6mg/kg). Sperm abnormalities in the head and tail were also studied, where the abnormalities in the sperm head were significantly increased (P< 0.05) in the DTM treated rats, similarly morphological abnormalities of sperm tail
were also analysed, the abnormal tails were significantly increased (P< 0.05) in DTM exposed groups than that of control groups.

**Assessing the damage to the sperm DNA**

DTM intoxication demonstrated the sperm DNA damage at both high and low concentrations and it showed the detrimental effect on the sperm DNA integrity when compared with its respective control groups. The sperm DNA damage studies were shown in the Fig.1.

Fig.1. Effect of pesticide DTM on sperm DNA damage were depicted here, A & B represents the control and vehicle control group where in no sperm DNA damage were observed in two groups. However C & D images showing sperm DNA fragments due to damages in the sperm DNA in DTM exposed groups both in low dose treatment: C, and high DNA damage in high DTM exposed groups respectively.

**Molecular docking 3β-HSD**

The molecular docking interactions between 3β-HSD and pyrethroids DTM were analysed in the present study. Where the modelled 3β-HSD protein interacted with the DTM with a binding affinity about -8.3 kcal/mol. There are four amino acid residues were participating in forming the binding
interaction with DTM were shown in the Fig. 3. The amino acids were methionine -180, isoleucine-190, valine -15, phenylalanine-14 and methionine -86. The significance of 3β-HSD and DTM interaction in impairing the steroidogenesis mechanism was shown in schematic diagram Fig.2.

![Diagram](image)

Fig.2. Testicular steroidogenesis (Testosterone production) in Leydig cell. Cholesterol is a precursor molecule and it get transported from intracellular site to the mitochondrial site using a channeling protein called steroidogenic acute regulatory (StAR) protein. After entering into the mitochondria cholesterol undergoes transformation as pregnenolone and further converted to progesterone by 3β-hydroxysteroid dehydrogenases 3β-HSD.

**Histology**

Studies on the testicles' histopathology were carried out on both untreated and treated rats. Intact basement membrane containing various stages of germ cells and sperms surrounding the intestinal lumen were visible in the testicular transverse section of the control group, in contrast the altered
testicular architecture seen in DTM-exposed animals, damaged epithelium and a lumen devoid of sperm were seen (Fig.3).

![Fig.3](image)

**Fig.3.** Testis transverse sections in control, vehicle control and experimental rats. From the above figure, A&B Photomicroscopical images of control rat and vehicle rats testis shows the compact arrangements in the seminiferous tubules with full spermatogenesis. C&D Photomicroscopic images of testis from DTM exposure at low and high concentrations show disorganized and ruptured epithelium with lumen empty of sperm.

The testis of the rats exposed to the vehicle showed no structural abnormalities.
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Fig. 4. A) Depicts the effect of DTM on testicular steroidogenic enzymes such as 3β-HSD. Fig.1B) represents the effect of DTM on 17β-HSD and Fig.1C) indicates DTM effect on testosterone levels. Values are expressed as mean ± SD. *P<0.05

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Fig.5. The 3β-HSD residues that are participating or interacting with the pesticide DTM are Isoleucine-190, Methionine-86, Phenylalanine-14 and Valine-15 respectively. Thus there are four hydrogen bonds were observed between 3β-HSD and DTM. The binding interactions were visualized using Discovery studio.

Table 1 Sperm morphological abnormalities

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vehicle control</th>
<th>DTM Low dose</th>
<th>DTM high dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal morphology</td>
<td>85.05 ±0.82 a</td>
<td>83.01 ±0.99 a</td>
<td>62.02 ±0.57 b</td>
<td>44.19 ±0.26 c</td>
</tr>
<tr>
<td>Abnormal head</td>
<td>1.94 ±0.59 a</td>
<td>2.07 ±0.34 a</td>
<td>2.99 ±0.29 b</td>
<td>3.64 ±0.31 c</td>
</tr>
<tr>
<td>Abnormal tail</td>
<td>0.96 ±0.19 a</td>
<td>1.04 ±0.23 a</td>
<td>3.99 ±0.36 b</td>
<td>4.7 ±0.14 c</td>
</tr>
</tbody>
</table>

Above results are shown in mean + SD (standard deviation) of 10 animals (single group)

Different superscripts in same row represents significant difference (p < 0.05).

Discussion
The gross body weight of the animal and individual organ weights were used as standards in toxicological research. In our investigation, we saw that rats given DTM at 3mg/kg and 6mg/kg dosage diminished the weight lost significantly (data not shown). Possible reasons for this weight loss caused by the DTM might be attributed to the anorectic effect of DTM, thus animals exposed to the DTM might have experienced loss of appetite, which has a direct impact on their body weight. Alternatively, it might also due to the indirect effects of the DTM on the central nervous system (CNS), which normally regulates how much amount of the food and water an animal consumes (Kumar et al. 2018; Sandhia et al. 2013; Rajwat et al. 2014; Desai et al. 2016).

In the present study we also identified the considerable loss in testicular weight (data not shown), which may be related to a drop in serum testosterone levels. These findings supports the earlier research done by the Desai et al., 2015 where exposure of mice to the different concentrations of DTM (3 mg/kg and 6 mg/kg) resulted in decreased testicular weight, decreased testicular weight might be due to the decreased spermatogenesis, decreased sperm count, or decreased testicular enzyme activity (Desai et al. 2016).

Steroidogenic enzymes such as 3β- HSD and 17β-HSD, two key enzymes participating in the steroidogenesis were decreased. These two enzymes were crucial for the synthesis of testosterone by converting the various precursor molecules required for the steroidogenesis, interestingly these two steroidogenic enzymes levels were significantly diminished (P<0.05) in the DTM exposed rats, that might had the direct affect in decreasing the testosterone production. Thus the current investigation found that DTM had a detrimental impact on both enzyme ie 3β-HSD and 17β- HSD levels at both concentrations, that finally leading to the drop in testosterone and inducing the impairment of the steroidogenic pathway (Sengupta et al. 2004). Our results are in agreement with the earlier studies conducted by group of researchers demonstrated that impairment in the steroidogenesis as a result of pesticidal exposure, that eventually results in the impaired physiological mechanisms in the male reproductive system (Fattahi et al. 2009; Issam et al. 2009) Germ cell atrophy and necrosis may be
one of the causes of the testicular weight loss. The testicular weight was decreased due to damage to the histological architecture. Androgens govern the primary function of reproductive organ growth and structural integrity in the male reproductive system. Weight of the seminal vesicles, epididymis, and other auxiliary sex organs was measured in the current study. The testosterone has a major role in the male reproductive system. Most significantly, in spermatogenesis, which takes place when testosterone is available, and spermatogenesis is shot down when testosterone levels are decreased.

As a result of DTM depriving exposed male rats of androgens, the activities of testosterone-dependent male reproductive sperm parameters were reduced (Andersson et al. 2004; Tilbrook et al. 2000).

DTM exposure may have directly affected the androgens in the testis, or it may have had an indirect effect by affecting the hypothalamo-pituitary gonadal axis in the brain, which ultimately reduced the production of those male reproductive hormones (Gajraj et al. 2009; Sharma et al. 2013). Additionally, a marked increase in the DNA damage to sperm in DTM-intoxicated rats may have genotoxic consequences on male gametes (Daveedu et al. 2023). Our results showed that DTM exposure produced spermatotoxicity as evidenced by sperm DNA damage (Comet assay), decreased sperm membrane integrity, increased sperm head abnormalities, and decreased sperm quantity were found in the DTM exposed rats. Surprisingly molecular docking studies revealed that pesticide DTM made interactions with the 3β-HSD and thus interfered with the natural converting mechanism of 3β-HSD protein which are essential for testosterone biosynthesis. Testis from animals treated with DTM had damaged epithelium and lumens devoid of sperm, according to histopathological analysis. Sertoli cells showed further tubular shrinkage, cell necrosis, vacuolization with sloughing, and degraded spermatids and spermatocytes. DTM intoxicants were also observed to cause more Sertoli and Leydig cell degeneration in the treated rats (Rashid et al. 2012). These findings were consistent with the reports recommended by Creasy et al. (2002). Vacuolization was also seen in the Sertoli and germ cells, and this could be related to the enlarged endoplasmic reticulum, which would imply that the
pyrethroids have altered the cellular permeability. The potential toxic studies observed in the current study such as altered hormonal balance and impaired steroidogenesis are supporting the studies of Slima et al., 2017.

**Conclusion**

The current investigation proved that animals exposed to the toxicants at their early stage of life i.e., prepubertal stage can be detrimental to their reproductive system. In our study exposure to pesticide DTM manifested different harmful effects like hormonal disturbance and testicular and sperm morphological abnormalities. From our study it could be hypnotized that the reduced testosterone production in the DTM exposed rats might be due to their ability of the DTM disturb the functions of 3 β-HSD. However, more research work is warranted to support this notion.

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**Authors contribution statement**

The authors contributions are as follows, Daveedu Thathapudi designed, executed and prepared the manuscript for the research work, Paturi Raja Vijaya Manohar, Raja Jayarao Yendluriand SB Sainath assisted in interpreting the results and analysis.

**Conflict of interest**

Conflict of interest declared none.
References


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