Effects of Tebuconazole (A fungicide) on Reproduction of Male Rat

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Tebuconazole, a triazole fungicide, widely used in agriculture by farmers was evaluated to determine its effects on testes of rats. In the present study, Tebuconazole dissolved in olive oil was administered orally to albino male rats at dose level of 250 mg /kg b.wt/day for 15, 30 and 45 days. Reproductive toxicity of tebuconazole was evaluated on the basis of weight analysis of testes and accessory sex organs, fertility, sperm dynamics, hormonal analysis and histopathological studies. There was a decrease in the weight of testes, epididymis, ventral prostate and seminal vesicle. The result showed highly significant decline in sperm density and motility. Post fertility test showed 70%, 90%, and 100% negative results. A decrease in serum testosterone, FSH and LH levels were observed in all the treated groups. The histopathological observations also support the occurrence of toxicity being caused due to exposure of tebuconazole. The observations are thus indicative of the reproductive toxicity caused by tebuconazole at different dose durations in the testes of rats.

Keywords: Tebuconazole, testes, testosterone, LH, FSH, fertility

1. INTRODUCTION

The Pesticides are extensively being used to boost agriculture production. Apart from affecting target species, these toxic chemicals also influence physiology of numerous non-target species including man directly or through the food chain 1. Environmental pollution from pesticides is an important issue that attracts widespread public concern in developing countries 2. Unfortunately, the risk of acute exposure to these compounds is a constant threat in developing nations and these chemicals are responsible for numerous cases of poisoning annually in non-target wildlife and acute mammalian neurotoxicity and reprotoxicity 3. These are mostly non-selective, widespread applied, possess toxic properties 4, and in some cases are very refractory.
They include a great variety of substances like insecticides, acaricides, herbicides, fungicides and algaecides, different both in composition and properties with the purpose to kill, destroy or repel undesirable living organisms. Triazole fungicides are used agriculturally to control rust and mildew on fruit, vegetables, cereals and seeds, residential and commercial turf, and in pharmaceutical applications for the treatment of local and systemic fungal infections. Triazoles inhibit the biosynthesis of ergosterol, an essential component of fungal cell membranes, via. inhibition of cytochrome P450 dependent enzyme lanosterol 14α-demethylase. Cyp51 is evolutionarily conserved between plants, fungi and animals and in animals is critical for cholesterol synthesis and therefore steroid biosynthesis. Besides Cyp51, triazoles also modulate the gene expression and enzyme activity of multiple cytochrome P450 (CYP) and other metabolic enzymes in mammalian liver and other tissues.

Tebuconazole (TEB) ((RS)-1-p-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl) pentan-3-ol) is a systemic triazole fungicide used on crops such as barley, wheat, peanuts, and orchard fruits. Its mechanism of fungicidal activity is inhibition of a-lanosterol demethylase, which decreases ergosterol biosynthesis. Tebuconazole is a potent xenobiotic to which exposure can cause metabolic alterations and death of different organisms. The tebuconazole fungicide had been classified by the US EPA as Group C-Possible Human Carcinogen. TEB is persistent in soils and presents moderate mobility. Studies with rats show that perinatal exposure to tebuconazole produced immunological neurobehavioral and neuropathological deficits. However, there are no sufficient information was available concerning tebuconazole’s effects on the reproductive systems. Therefore, the present investigation was undertaken to elucidate the effect of tebuconazole on the reproductive system of adult albino rats.

2. MATERIALS AND METHODS

Animal model
Adult male albino rats (Rattus norvegicus) of an average body weight 180–200 g were used for experimentation. The animals were kept in clean polypropylene cages covered with chrome-plate grills and maintained in a room with controlled room temperature (20±5°C) with 12:12 h light/dark cycle. The animals were fed with food pellets procured from Ashirwad Industries, Chandigarh, as well as germinated/sprouted gram and wheat seeds as an alternative feed. Tap water was supplied ad libitum.

Chemical and dose
Technical grade Tebuconazole [(RS)-1-p-Chlorophenyl-4,4-Dimethyl-3-(1H-1,2,4-Triazol-1-ylmethyl) Pentan-3-ol], obtained from Gupta chemical Pvt. Ltd., Jaipur, was used for experimentation. The insecticide was dissolved in olive oil and administered to animals through oral intubations at daily doses of 250 mg kg⁻¹ b.wt. per day for 15, 30 and 45 days.

Experimental procedure
Animals were intended into four groups containing six animals each. Group I animals served as control and were administered olive only. The animals of group II, III, and IV were treated with tebuconazole of 250 mg kg⁻¹ bwt per day for 15, 30 and 45 days, respectively. At the end of the experimentation, the rats were weighed, and sacrificed under light ether anesthesia. The male reproductive organs were removed, washed with distilled water, dried, weighed, and processed for biochemical and histopathological studies.

Fertility test
The mating exposure test of the animals was performed. Rats were cohabited with normal proestrous females in the ratio of 1:3. The vaginal plug and presence of sperms in the vaginal smear were checked for positive mating. Females were separated and resultant pregnancies were noted when dams gave birth.

Sperm dynamics
The sperm motility in cauda epididymis and sperm density of testicular and cauda epididymis were determined.

Biochemical parameters
The total protein, sialic acid, glycogen and cholesterol levels were determined by Zlatkis, Zak, and Boyle 1953.

Hormonal analysis
Testosterone, leutinizing hormone (LH), and follicle stimulating hormone (FSH) were estimated through chemiluminescence in fully automatic Advia Cemtaus Immuno Assay System.

Testicular histology
Testes of rats exposed to tebuconazole and control were fixed in Bouin’s fixative for at least 48 h, processed by paraffin wax impregnation method, cut using a rotary microtome at 5 mm thickness, and stained with hematoxylin and eosin (HE) for light microscopic examination.

Statistical analysis
The data were analyzed statistically using Student’s “t” test and the significance of differences was set at p<0.01 and p<0.001.
**Sperm Dynamics, Motility and Fertility**

The sperm density in testes and cauda epididymis decreased highly significantly (P ≤ 0.001) after tebuconazole administration in the all dose duration (Table 1.2) and a severe impairment of sperm motility in cauda epididymis was also observed in the experimental rats. Control rats showed 100% positive fertility in the mating exposure test while the rats exposed to 250 mg/kg b. wt/day dose level showed 70, 90 and 100% negative fertility in the duration of 15, 30 an 45 days respectively (Table 1.2).

**Biochemical changes in testes after administration of tebuconazole**

The glycan and sialic acid contents in testes decreased significantly (p ≤0.01 and p ≤0.001) while testicular protein and cholesterol increased significantly (p ≤0.01 and p ≤0.001) in tebuconazole treated rats (Table 1.3). These changes were in dose duration dependent manner.

**Serum concentration of total testosterone, FSH and LH**

Rats administered with tebuconazole exhibited significant (p ≤0.01 and p ≤0.001) reduction in the level of serum testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in the 30 and 45 days at the dose of 250 mg/kg b wt/ day in comparison to control rats (Table 1.4). However, tebuconazole showed a non significant decrease in the level of serum testosterone, FSH and LH at the duration of 15 days.

**Testicular histopathology**

Histoarchitecture of control rat testes exhibits normal morphology of seminiferous tubule with all successive stages of spermatogenesis, lumen filled with spermatozoa and sertoli cells are present. Testes of rats treated with tebuconazole (250 mg/kg b w/day for 15 days) showed degenerated germinal epithelium and lumen with less sperms. Further, loosened tunica propria, few spermatocytes, and few numbers of Leydig and Sertoli cells with increased intertubular space and lumen with cellular debris were found in testes of rats treated with tebuconazole (250 mg/ kg b.w/day for 30 days). Spermatogetic elements changed into debris in the lumen causing complete arrest of spermatogenesis at high dose level of acephate. Ruptured germinal epithelium with disrupted interstitial cells with damaged Leydig cells was also seen in 45 days duration (Figure 1a-d).

### 4. DISCUSSION

Tebuconazole, is one of the triazole fungicide that produced neuropathological, neurobehavioral and immunological defects in different species, however disturbances in reproductive functions are less known 20. The present study revealed that administration of tebuconazole at the dose level of 250 mg/kg/b.wt./day for 15, 30 and 45 days to male rats resulted in reproductive toxicity. Administration of tebuconazole showed a significant decrease in weight of testes and accessory sex organs. The weight of testes is largely dependent on the mass of differentiated spermatogenic cells and the reduction in the weight of testes may be due to reduced tubule size, decreased number of germ cells, elongated spermatids, spermatogenic arrest and inhibition of steroid synthesis by Leydig cells 21 22 23. The reduction in weight of accessory sex organs may be due to low availability of androgens or antiandrogenic activity of tebuconazole 24. Generally, maintenance of weights of accessory reproductive glands depends on testosterone level 25. Reduction in sperm motility and density suggested an undersupply of testosterone to the testes and epididymis, thereby possibly causing impaired epididymal function, as it is known that the structure and function of the testes and epididymis is dependent on androgens 26 27. Reduced sperm count in testes and epididymis may also be due to inhibition of meiotic division of spermatocytes or may be due to the suppressive effect of the tebuconazole on spermatogenesis 28 29. The decrease in fertility potentials reported after the treatment of male rats has been attributed to impairment in sperm motility and viability 30.

**Table 1.1: Testes and accessory sex organ weight analysis (Tebuconazole 250 mg/kg, b.wt./day)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Testes</th>
<th>Epididymis</th>
<th>Seminal Vesicle</th>
<th>Ventral Prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong> (Vehicle only)</td>
<td>1212.42 ±53.61</td>
<td>510.26 ±6.76</td>
<td>412.62 ±12.21</td>
<td>344.27 ±17.24</td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td>998.46 ±39.41</td>
<td>461.34 ±15.41</td>
<td>358.21 ±16.41</td>
<td>310.12 ±27.84</td>
</tr>
<tr>
<td><strong>Group III</strong></td>
<td>886.32 ±63.21</td>
<td>412.34 ±15.41</td>
<td>349.26 ±18.26</td>
<td>281.36 ±19.21</td>
</tr>
<tr>
<td><strong>Group IV</strong></td>
<td>836.24 ±43.36</td>
<td>397.41 ±17.26</td>
<td>332.61 ±19.34</td>
<td>242.81 ±27.23</td>
</tr>
</tbody>
</table>

Mean ± SEM of 6 animals (Group II; III; IV compared with Group I)

*ns = Non significant, * = P ≤ 0.01 (Significant), ** = P ≤ 0.001 (Highly significant)

**Table 1.2: Sperm Dynamics and Fertility (Tebuconazole 250 mg/kg, b.wt./day)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sperm motility (%)</th>
<th>Sperm density (million/ml)</th>
<th>Fertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong> (Vehicle only)</td>
<td>70.46 ±4.02</td>
<td>4.35 ±0.37</td>
<td>46.62 ±0.86</td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td>34.32 ±5.38</td>
<td>1.87 ±0.69</td>
<td>36.14 ±0.91</td>
</tr>
<tr>
<td><strong>Group III</strong></td>
<td>18.96 ±6.34</td>
<td>0.98 ±0.54</td>
<td>21.24 ±0.87</td>
</tr>
<tr>
<td><strong>Group IV</strong></td>
<td>11.07 ±7.65</td>
<td>0.76 ±0.65</td>
<td>16.01 ±1.10</td>
</tr>
</tbody>
</table>

Mean ± SEM of 6 animals (Group II; III; IV compared with Group I)

*ns = Non significant, * = P ≤ 0.01 (Significant), ** = P ≤ 0.001 (Highly significant)
Tebuconazole treatment caused a decrease in cholesterol level. A decrease in glycogen content could also affect the metabolism, motility and fertilizing capacity of spermatozoa and testosterone in the testis are mainly regulated by the follicle stimulating hormone (FSH) and luteinizing hormone (LH). The reduction in the serum level of testosterone could probably be due to the decrease of serum levels of FSH, LH and prolactin which are essential for the gonadal development and steroidogenesis in rats. The decrease in the serum concentrations of FSH, LH and testosterone in the treated animals clearly indicates the action of extract on the secretion of pituitary gonadotropins and in turn in the testosterone biosynthesis in the testis and reproductive organs. Thus the present investigation suggests that fungicide tebuconazole is highly toxic to reproductive function and alter the fertility of animals.

Administration of tebuconazole also changes the biochemical parameters of the reproductive tract. Protein biosynthesis is a key factor for testicular development and spermatogenesis. Elevation in the total Protein content in testes may be due to hepatic detoxification activity which results in the inhibitory effect on the activity of enzyme involved in the androgen biotransformation. The structural integrity of the acrosomal membrane is dependent upon sialic acid; the reduced sialic acid content might alter the structural integrity of acrosomal membrane, ultimately affects the metabolism, motility and fertilizing capacity of spermatozoa. A significant increase in cholesterol level was observed after tebuconazole treatment. This increased testicular cholesterol concentration may reflect reduced conversion of cholesterol to testosterone which resulted in accumulation of cholesterol in the testes, and thus impaired spermatogenesis. Tebuconazole treated rats showed a significant decline in glycogen content. Reduced glycogen reflects decrease in the number of postmeiotic germ cells, which are thought to be the sites of glucose metabolism. A decrease in glycogen content could also be due to inhibited the glycogen synthesis which eventually decreases spermatogenesis.

Testosterone, an important androgen, plays a pivotal role in maturation, spermatogenesis and the maintenance of accessory sex organs. Tebuconazole treatment caused a significant decrease in the testosterone level of treated rats. The significant reduction of testosterone level in blood indicates the reduction of androgen level in treated animal. The production of the sperm cells (spermatozoa) and testosterone in the testis are mainly regulated by the follicle stimulating hormone (FSH) and luteinizing hormone (LH).

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Fig 1(a) Micro-photograph of control rat testes showing all the successive stages of spermatogenesis i.e. normal morphology of seminiferous tubules. Lumen is filled with sperm. Leydig cells are also present.

(b) Photograph of testes treated with Tebuconazole 250mg/kg b.wt. for 15 days showing vacuolated interstitial tissues with disrupted connective tissues. Tunica propria loosened at several sites. Inhibition of spermatogenesis seen as there is presence of cellular debris in the lumen.

(c) Photograph of testes treated with Tebuconazole 250mg/kg b.wt. for 30 days showing reduced size of seminiferous tubules with increased Intertubular spaces. Germinal epithelium loosened and even damaged.
at some sites. Spermatogenic elements changed into debris in the lumen, causing complete arrest of spermatogenesis.

5. CONCLUSION
The present study revealed that oral administration of tebuconazole induced reproductive toxicity in male albino rats. Toxic effects of tebuconazole were pronounced at high dose level. The conclusion can be drawn that tebuconazole administration affects spermatogenesis leading to poor semen quality and reduced male fertility but these effects were dose and duration dependent. These observations suggested the limited use of such toxic insecticides to improve the quality of life for human welfare.

6. REFERENCE

Conflict of Interest: None
Source of Funding: Nil