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# Effect of Triptolide on Vital Organs, Blood Biochemical Parameters and Histomorphology of Testis in Male *Bandicota Bengalensis*

KEYWORDS Triptolide, *Bandicota bengalensis*, testis, histomorphology.

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**ABSTRACT** *Present study reports the effect of triptolide in male Bandicota bengalensis. One group of rats was kept as vehicle control and the other three groups were fed on bait containing 0.15, 0.2 and 0.25% triptolide. Autopsy of rats immediately after 15 days of treatment period revealed no significant effect on weights of vital organs. A significant (P ≤ 0.05) increase in plasma levels of total proteins, alkaline and acid phosphatases and alanine and aspartate aminotransferases in response to stress induced by triptolide was observed in treated groups of rats. A significant (P ≤ 0.05) effect of treatment was also observed on histomorphology of testis in the form of decrease in seminiferous tubule diameter and number of various spermatogenic cells. Giant cells containing several round nuclei within a single cytoplasmic boundary were also observed in treated rats. Present studies suggest the antifertility potential of triptolide against male B. bengalensis.*

**Introduction**

Rodents constitute the largest and most successful group of mammals worldwide. Among different rodent species, lesser bandicoot rat, *Bandicota bengalensis* Gray and Hardwicke has been reported as the most detrimental pest. It is the predominant species inhabiting irrigated crop fields and causing heavy losses in Punjab, India (Singla and Parshad 2010, Singla and Babbar 2010, 2012). The species also acts as reservoir of a number of parasites of zoonotic importance (Singla et al 2008, 2013). The economic losses and health problems associated with this pest species emphasize the need to develop techniques for its management. The use of rodenticides is the main approach being followed to tackle rodent problems all over the world. However, the excessive use of rodenticides have resulted in non target toxicity hazards (Brakes and Smith, 2005) in addition to development of resistance among animals (Pelz and Klemann 2004). Moreover, due to their high rate of reproduction, rodents rapidly rebuildup their population after a successful control operation with rodenticides (Shilova and Tchabovsky 2009). So the challenge is to develop strategies which can reduce the reproductive output of rodents and be effective in long term.

Triptolide, a diterpenoid triepoxide obtained from Tripterygium wilfordii Hook, has several forms of pharmacological activities including anti-inflammatory, anti-fertility and anticancer activities (Zhang et al 2010). However, the clinical use of triptolide is known to present several practical disadvantages mainly due to its lower water solubility such as toxicity towards the heart, liver and kidney; reproductive dysfunction and hematopoietic dysfunction (Yang et al 2012, Zhang et al 2011). Earlier studies on antifertility effects of triptolide were conducted in laboratory rats and mice keeping in view the development of an oral human contraceptive (Lue et al 1998, Sinha Hikim et al 2000, Huynh et al 2000, Ni et al 2008, Liu et al 2010). Deng et al (2011) studied antifertility potential of oral doses of extracts of T. hypoglaucum containing triptolide against Mongolian gerbils keeping in view their management. In India, for the first time, Singla et al (2013) reported antifertility potential of triptolide fed in cereal based bait against house rat, Rattus rattus.

Our previous studies (Dhar and Singla 2013) have reported complete inhibition of reproduction in female *B. bengalensis* paired with male rats treated with 0.2 and 0.25% triptolide in bait immediately after treatment withdrawal. Autopsy of rats immediately after termination of treatment have revealed significant effect of triptolide treatment on weights reproductive organs, sperm motility, viability, density and sperm morphology. Present studies report the effect of triptolide treatment on weights of vital organs, blood biochemical parameters and histomorphology of testes in male *B. bengalensis*.

**Material and Methods**

The present study was carried out in the Animal House Laboratory, Department of Zoology, Punjab Agricultural University, Ludhiana, India.

**Collection and maintenance of animals**

Mature and healthy male lesser bandicoot rat, *B. bengalensis* were live-trapped with multi-catch rat traps from crop fields in and around Ludhiana. In the laboratory, rats were acclimatized individually in cages for 10-15 days before the experiment with food and water provided ad libitum. Food consisted of a loose mixture of cracked wheat, powdered sugar and groundnut oil (WSO bait) at a ratio of 96:2:2. Proper hygienic conditions were maintained. Approval of the Institutional Animal Ethics Committee was obtained for the use of animals.

**Treatment**

Triptolide (molecular formula $C_{20}H_{24}O_6$, molecular weight 360.41) was kindly supplied by Pidilite Industries, New Delhi, India. Rats (n = 20) were divided into 4 groups of 5 rats each. The average body weight of rats in different groups ranged from 248 to 311.2 g. There was no significant difference in average body weight of rats among the four groups. Rats of groups II–IV were fed on bait containing 0.15, 0.2 and 0.25% triptolide, respectively for 15 days in bi-choice with WSO bait. Treatment bait was prepared by mixing the desired concentration of triptolide in a mixture of cracked wheat and powdered sugar (98:2). Triptolide was mixed in bait after being dissolved in vehicle (1% sodium carboxymethyl cellulose solution). Rats of group I, fed on WSO bait containing vehicle were kept as untreated control. Water was provided ad libitum throughout the treatment.

**Effect on weights of vital organs**

At autopsy of rats immediately after termination of treat-

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ment, their vital organs such as liver, spleen, kidneys, adrenal glands and heart were dissected out, cleared of fat and blotted dry to determine their weights (g/100 g bw).

**Effect on blood biochemical parameters**

At autopsy of all the treated and untreated rats immediately after termination of treatment, blood samples were collected by cardiac puncture in Ethylene diamine tetra-acetic acid (EDTA) rinsed tubes. Blood was centrifuged at 3000g for 10 min and plasma collected was stored at −20°C until analysis. In the plasma, level of total proteins (g/dl) was determined according to the method of Lowry et al (1951). Alkaline phosphatase (ALP) and Acid phosphatase (ACP) activities (IU/L) were assayed according to the method of Bessey et al (1946). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (IU/L) were assayed according to the method of Reitman and Frankel as described by Bergmeyer (1974).

**Effect on histomorphology of testes**

At autopsy of rats immediately after termination of treatment, a piece of testicular tissue was fixed for 48 hours in the 10% formalin. After washing in running water and dehydration in alcohol series, tissue was embedded in paraffin and 5 μm thick sections were cut and stained with haematoxylin and eosin (HE) as per the method of Humason (1979). Stained sections of testis of each rat were studied under light microscope for the presence/absence of Sertoli cells and germinal cells and development of complete/incomplete cellular associations in all the 8 stages of Seminiferous Epithelial Cycle (SEC) in seminiferous tubules as classified with HE method by Bilaspuri and Guraya (1980). The diameter of the seminiferous tubule itself (STD) and the numbers of nuclei of germinal cells such as spermatogonia (SG), leptotene (L), zygotene (Z), pachytene (P), diplotene (D), round spermatids (RS), elongating spermatids (EL), elongated spermatids (ED), spermatozoa (SZ) and Sertoli cells (SC) were counted in cross sections of 25 seminiferous tubules per rat at 400X magnification. The crude spermatogenic cell counts of round and almost round nuclei were corrected for differences in their nuclear diameter to obtain true count of cells by Abercrombie's formula (Abercrombie 1946) as described by Singla et al (2013).

**Statistical analysis**

All values were expressed as mean ± SEM and analyzed using analysis of variance. The statistical differences were considered significant at $P \leq 0.05$.

**Results and Discussion**

**Effect on weights of vital organs and blood biochemical parameters**

Results of present studies revealed no significant difference in the weights of vital organs such as liver, spleen, kidneys, adrenal glands and heart among untreated group of rats and rats of groups treated with different concentrations of triptolide, immediately after termination of treatment (Table 1). This indicates no toxicity of triptolide treatment on vital organs of the male *B. bengalensis.*

Immediately after termination of treatment, the plasma level of total proteins, ALP, ACP, ALT and AST were found increased significantly ($P \leq 0.05$) in all the treated groups of rats from that of untreated group (Table 1). However, the level of ALP between the rats of untreated group (group I) and those treated with 0.15% triptolide (group II) was not found to differ significantly. Xu et al (2013) reported some toxicity of triptolide to liver, kidney and spleen at intravenous dose levels of 0.1 and 0.3 mg/kg bw for 14 days. Mei et al (2005) have reported that triptolide treatment can increase the levels of ALT and AST if taken for a long time.

**Table 1. Effect of triptolide treatment on weights of vital organs and biochemical parameters in plasma of male *B. bengalensis* immediately after termination of treatment**

| Organ weights/ biochemical parameters | Treatment (n=5 each) (Mean±SEM) | | | |
|---|---|---|---|---|
| | 0% (I) | 0.15% (II) | 0.20% (III) | 0.25% (IV) |
| Liver (g/100g bw) | 3.63±0.31 | 4.72±0.17 | 4.62±0.14 | 3.52±0.07 |
| Spleen (g/100g bw) | 0.41±0.10 | 0.27±0.03 | 0.31±0.02 | 0.47±0.09 |
| Kidneys (g/100g bw) | 0.31±0.02 | 0.30±0.03 | 0.37±0.02 | 0.20±0.00 |
| Adrenal glands (g/100g bw) | 0.01±0.00 | 0.02±0.00 | 0.02±0.00 | 0.01±0.00 |
| Heart (g/100g bw) | 0.29±0.03 | 0.31±0.01 | 0.35±0.01 | 0.22±0.02 |
| Total proteins (g/dl) | $5.76\pm0.07^{a}$ | $6.39\pm0.02^{b}$ | $6.66\pm0.03^{bc}$ | $6.74\pm0.03^{c}$ |
| ALP (IU/L) | $54.51\pm7.97^{a}$ | $63.60\pm6.12^{ab}$ | $82.55\pm5.88^{bc}$ | $86.91\pm32.26^{c}$ |
| ACP (IU/L ) | $20.84\pm0.24^{a}$ | $27.53\pm0.40^{b}$ | $35.70\pm0.72^{c}$ | $47.66\pm0.20^{d}$ |
| ALT (IU/L) | $29.32\pm1.12^{a}$ | $37.78\pm3.35^{b}$ | $41.40\pm2.82^{b}$ | $47.22\pm1.11^{b}$ |
| AST (IU/L) | $15.07\pm0.60^{a}$ | $23.63\pm1.92^{b}$ | $32.03\pm0.99^{c}$ | $38.32\pm0.71^{d}$ |

**$^{a-d}$Values with different superscripts in a row differ significantly at $P \leq 0.05$**

The changes in levels of various blood biochemical parameters in triptolide treated rats indicate various aspects of metabolism in animals. Phosphates are involved in many different processes that require mobilization of phosphate ions or dephosphorylation as part of anabolic, catabolic or transfer processes (Kaur and Dhanju 2004). The phosphatases transfer phosphate group from nucleotides to further synthesize the chemical energy to overcome stress (Naveed et al 2004). A change in enzyme activity is generally related to intensity of cellular damage (Muthuviveganandavel et al 2008, Sangha et al 2013). Abnormalities in plasma proteins are not indica-

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tive of a specific disease but of a condition that alters the tissue responsible for the balance between protein synthesis and catabolism or mechanical loss (Dimopoullos 1970). The increased level of ALT and AST in plasma of rats indicates their enhanced metabolic activity to meet the stress induced by exposure of pesticide (Kaur and Dhanju 2004).

**Histomorphology of testis**

At light microscopy level, the degree of testicular damage ranged from normal seminiferous tubules in untreated group of rats (Plate Ia) to seminiferous tubules with seminiferous epithelium containing varying levels of degenerated germ cells along with the presence of intraepithelial vacuoles of varying sizes in groups of rats treated with triptolide (Plate Ib and c). In untreated rats (group I) all the stages of seminiferous epithelial cycle (SEC) (i.e. from stages 1a to 8) were fully matured in the cross-sections of testis stained with HE. In group of rats treated with 0.15% triptolide (group II), also all the stages of SEC were observed. In group of rats treated with 0.2% triptolide (group III), no spermatozoa were found in the lumen of seminiferous tubules. In group of rats treated with 0.25% triptolide (group IV) no definite stage of SEC was observed. The SEC consisted only of Sertoli cells and few spermatogonia. Other germ cells from leptotene to spermatozoa were found absent. The diameter of seminiferous tubules among treated and untreated groups of rats were found to differ significantly ($P \leq 0.05$) with dose-dependent decrease in diameter. True count of cells in the seminiferous tubules of rats treated with triptolide was found decreased significantly ($P \leq 0.05$) in groups of rats treated with triptolide from that of rats of untreated group (Table 2).

**Table 2. Variations in the true count of cells and diameter of the seminiferous tubules of male *B. bengalensis* after triptolide treatment**

| Treatment (n=5 each) | SG | L | Z | P | D | RS | EL | ED | SZ | SC | STD (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0% (I) | 5.43±0.30$^{a}$ | 1.16±0.49$^{a}$ | 6.95± 2.27$^{a}$ | 24.46±3.12$^{a}$ | 12.91±4.17 | 51.63±6.49$^{a}$ | 17.74±7.10$^{a}$ | 18.70±7.50$^{a}$ | 24.30±7.82$^{a}$ | 4.84± 0.25$^{a}$ | 0.19± 0.004$^{a}$ |
| 0.15% (II) | 7.07±0.17$^{b}$ | 0.60±0.18$^{a}$ | 1.28± 0.60$^{b}$ | 17.42±1.59$^{ab}$ | 2.56± 1.39$^{b}$ | 29.05±2.92$^{b}$ | 4.20± 1.97$^{b}$ | 1.00± 0.98$^{b}$ | 8.83±3.05$^{ab}$ | 14.11±1.12$^{b}$ | 0.17± 0.002$^{b}$ |
| 0.2% (III) | 7.87±0.38$^{b}$ | 0.32±0.15$^{a}$ | 1.28± 0.48$^{b}$ | 14.19±1.63$^{b}$ | 3.56± 1.30$^{b}$ | 29.17±3.35$^{b}$ | 3.68± 1.32$^{b}$ | 0.24± 0.23$^{b}$ | 0.00± 0.00$^{b}$ | 18.98±0.66$^{c}$ | 0.17± 0.004$^{b}$ |
| 0.25% (IV) | 9.31±0.28$^{c}$ | 0.00±0.00$^{b}$ | 0.00± 0.00$^{b}$ | 0.00± 0.00$^{c}$ | 0.00± 0.00$^{b}$ | 0.00± 0.00$^{c}$ | 0.00± 0.00$^{c}$ | 0.00± 0.00$^{b}$ | 0.00± 0.00$^{b}$ | 25.93±0.58$^{d}$ | 0.14± 0.004$^{c}$ |

**-Values are Mean±SEM**
**-Values with different superscripts in a column differ significantly at $P \leq 0.05$.**

This indicates some form of interference by triptolide during spermatogenesis and spermiogenesis.

Miao et al (2001) and Singla et al (2013) also reported damage in testicular tissue in the form of decreased amount of spermatogenic cells in triptolide-treated testis sections of rats, suggesting the effect of triptolide on growth and development of spermatogenic cells. Lue et al (1998) reported no significant effect of triptolide on histomorphology of testes of rats treated with 50 and 100μg/kg bw of triptolide for 35 and 70 days. Sinha Hikkim et al (2000) reported little or no effect of triptolide (100μg/kg bw for 70 days) on spermatogenesis and Leydig cell function. Longer treatment duration of triptolide treatment in adult male rats (100 μg/kg bw for 82 days), however, affected spermatogenesis with marked variability in the response of individual animals (Huynh et al 2000).

In the present study, giant cells containing several round nuclei within a single cytoplasmic boundary were observed in rats treated with all the three concentrations of triptolide (Plate Id). These cells appeared to have detached from the seminiferous epithelium and were seen towards tubular lumen. Similar cells were also reported by Huynh et al (2000) in rats treated with 100 μg/kg bw of triptolide for 82 days. They referred to these giant cells as symplasts. Evidence from the experimental models and humans suggests that the impairment of the complex post-meiotic chromatin remodelling process during spermiogenesis and defects in spermatogenesis involving spermatocytes could contribute to spermatid malformation and infertility (Huynh et al 2000). Present studies thus suggest the antifertility potential of triptolide against male *B. bengalensis*.

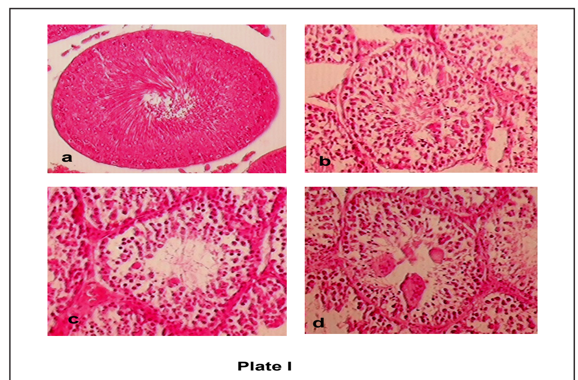


**Plate I: Haematoxylin-eosin stained sections of testis of *Bandicota bengalensis* at 400 X magnification showing antifertility effect of triptolide: (a) Normal seminiferous tubules in untreated rat; (b and c) Seminiferous tubules of treated rats showing varying levels of spermatogenic cell degeneration and vacuolization; (d) Seminiferous tubule of treated rat showing giant cells containing several round nuclei within a single cytoplasmic boundary.**

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