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Histomorphological and biochemical changes induced by triptolide treatment in male lesser bandicoot rat, *Bandicota bengalensis*

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ABSTRACT

Mature and healthy male lesser bandicoot rats, *Bandicota bengalensis* ($n = 40$) were fed on bait (mixture of cracked wheat and powdered sugar in 98:2) containing different concentrations of triptolide (0, 0.15, 0.20 and 0.25% w/w) for 15 days in two-choice trials. Results revealed no significant effect of triptolide treatment on weights of vital organs after 30 and 60 days of treatment withdrawal. A significant ($P \leq 0.05$) increase in plasma levels of TP, ALP, ACP, ALT and AST in response to stress induced in groups of rats treated with 0.20 and 0.25% triptolide was observed after 30 days of treatment withdrawal. No significant effect of treatment was observed on histomorphology of liver. A significant ($P \leq 0.05$) effect of triptolide treatment was, however, observed on testicular function in the form of reduced diameter of seminiferous tubules and number of various spermatogenic cells indicating effect on spermatogenesis and spermiogenesis. The cell stages affected did not recover fully within 60 days period following treatment withdrawal. The present study suggests the potential of triptolide in the reproductive management of *B. bengalensis* by way of affecting testicular function.

1. Introduction

Rodents are considered as the most destructive mammalian pests due to the damage they cause to standing crops and stored food products [1,2]. The lesser bandicoot rat, *Bandicota bengalensis* Gray and Hardwicke has been reported as one of the major pest in crop fields throughout Southeast Asia [3–6]. The species is also known to spread many diseases to man and livestock [7–12].

Use of rodenticides is the most common method to control rodents. However, excessive use of rodenticides may cause environmental hazards including non-target toxicity. For rodents, fertility control has been recognized as a more appropriate, environmentally benign, humane and long term strategy [13,14]. Triptolide, a diterpene triepoxide, is one of the major biologically active components purified from the Chinese herbal plant, *Tripterygium wilfordii* Hook F. having antifertility effects [15]. Most of the antifertility effects of triptolide have been evaluated against laboratory rats and mice [16–19] keeping in view the development of a human contraceptive. Studies were made on the antifertility potential of oral doses of extracts of *Tripterygium hypoglaucum* containing triptolide against Mongolian gerbils keeping in view their management [20]. However, for field scale use of an antifertility agent, it is required to be fed in bait form. In India, Singla et al. [21], for the first time reported the antifertility effect of triptolide fed in bait against house rat, *Rattus rattus*. Our previous studies [22] 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 and after 30 days of treatment withdrawal. Significant effect of triptolide treatment was observed on weights of reproductive organs, sperm motility, sperm viability, sperm density and sperm morphology of male *B. bengalensis*. The present study reports the reversibility/irreversibility in effects of triptolide on the histomorphology of testis and liver, body weight, weights of vital organs and biochemical parameters.

2. Materials and methods

The present study was carried out in the Animal House Laboratory, Department of Zoology, Punjab Agricultural University, Ludhiana, India. Approval of the Institutional Animal Ethics Committee was obtained for the use of animals.

2.1. Collection and maintenance of animals

Male *B. bengalensis* were live-trapped from crop fields in and around Ludhiana. In the laboratory, rats were acclimatized individually in cages ($36 \times 23 \times 23$ cm) with food and water provided *ad libitum* for 10–15 days before the commencement of the experiment. Food consisted of a loose mixture of cracked wheat, powdered sugar and groundnut oil (WSO bait) at a ratio of 96:2:2. The rats

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were kept in a room with 12 h light/12 h dark photoperiod and temperature of 24°–26 °C.

2.2. Treatment

Triptolide (molecular weight 360.41) was kindly supplied by Pidilite Industries, New Delhi, India. Mature and healthy rats ($n = 40$) were divided into 4 groups of 10 rats each. The average body weight of rats in different groups ranged from 241.73 to 270.27 g. There was no significant difference in average body weight of rats among the four groups. Rats of group I, fed on WSO bait were kept as untreated control. Rats of groups II–IV were fed on bait containing 0.15, 0.20 and 0.25% w/w triptolide, respectively for 15 days in two-choice trials 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 after being dissolved in vehicle (1% sodium carboxymethyl cellulose solution). In addition, 2 mL of the vehicle was also mixed in WSO bait fed to untreated rats during the treatment period. Water was provided *ad libitum*.

2.3. Effect on weight of vital organs

Rats were sacrificed after 30 and 60 days of treatment withdrawal ($n = 5$ each) and 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 body weight (bw)).

2.4. Effect on biochemical parameters

At necropsy of rats after 30 and 60 days of treatment withdrawal ($n = 5$ each), blood samples from all the treated and untreated rats were collected by cardiac puncture in ethylenediamine tetraacetic acid rinsed tubes. From control group I, blood samples were collected of 5 rats only. Blood was centrifuged at 3000g for 10 min and plasma collected was stored at –20 °C until analysis. Plasma level (g/dL) of total protein (TP) was determined according to the method of Lowry et al. [23]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (IU/L) were assayed by the method described by Bergmeyer [24]. Alkaline phosphatase (ALP) and acid phosphatase (ACP) activities (IU/L) were assayed by the method of Bessey et al. [25]. The levels of these marker enzymes were measured because of being considered as functional indicators of liver and testis. ACP is considered to be a functional indicator of spermatogenesis.

2.5. Effect on histomorphology of testis

At autopsy of rats after 30 and 60 days of treatment withdrawal, a piece of testicular tissue was dissected out, cut into two pieces and fixed for 48 h in 10% formal saline. After fixation, the tissue was washed in distilled water, dehydrated in ascending series of ethanol to water, cleared in xylene and embedded in paraffin wax having a melting point of 58–60 °C [26]. The sections were cut at 5 µm on a microtome and after usual dewaxing, sections were hydrated in descending series of ethanol to water, stained with haematoxylin, counter-stained with eosin, dehydrated in ascending series of ethanol to water, cleared with xylene and mounted in DPX [26].

Haematoxylin and eosin (HE) stained sections of testis of all the rats were studied under light microscope for the presence/absence of Sertoli cells and germinal cells and development of cellular associations (the 'stages' and 'phases' of spermatogenesis, which progress through precisely timed and highly organized cycles essential for continuous sperm production) in all the 8 stages of seminiferous epithelial cycle (SEC) in seminiferous tubules as classified with HE method by Bilaspuri and Guraya [27]. Twenty five seminiferous tubules complete in a cross section were selected for analyses. The diameter (mm) of each seminiferous tubule across both major and minor axes was measured at 400× magnification. The average of both minor and major axis was used to calculate the average diameter of a seminiferous tubule (STD). Measurement was made using ocular micrometer calibrated with stage micrometer.

The effect of triptolide treatment on spermatogenesis was accessed quantitatively by counting the number of nuclei of spermatogonia (SG), preleptotene (PL), leptotene (L), zygotene (Z), pachytene (P), diplotene (D), round spermatids (RS), elongating spermatids (EL), elongated spermatids (ED), spermatozoa (SZ) and Sertoli cells (SC) in round cross sections of 25 seminiferous tubules per animal. 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 [28] as described by Singla et al. [21].

2.6. Effect on histomorphology of liver

At autopsy of rats, a 5 mm piece of liver of untreated and treated rats was taken and processed for histological study as per the method described above. HE stained sections of liver were observed for any apparent focal necrosis with inflammatory cell infiltration in hepatocytes.

2.7. Statistical analysis

All values were expressed as mean ± SEM. Significance of differences was determined using two way analysis of variance and Tukey's test. The statistical analyses were performed using Graph Pad Instat Version 3.0 for Windows (Graph Pad Software, San Diego, CA, USA and http://www.graphpad.com). Critical differences were considered significant at $P \leq 0.05$.

3. Results

Feeding of bait containing 0.15, 0.20 and 0.25% w/w triptolide for a period of 15 days in choice with plain bait resulted in total ingestion of 44.77 ± 5.40, 45.37 ± 5.25 and 57.90 ± 6.00 mg/100 g bw of triptolide, respectively.

3.1. Effect on weight of vital organs

No significant difference was observed on the weights of vital organs such as liver, spleen, kidneys, adrenal glands and heart between untreated rats and rats treated with different concentrations of triptolide after 30 as well as 60 days of treatment withdrawal (Table 1) indicating no adverse effect of triptolide treatment on vital organs of the male rats. Also no significant difference was found in weights of different organs of rats of all the treated groups autopsied after 30 and 60 days of treatment withdrawal.

3.2. Effect on biochemical parameters

A dose dependent increase in the level of various biochemical parameters after 30 and 60 days of treatment withdrawal was observed. After 30 days of treatment withdrawal, the plasma levels of TP, ALP, ACP, ALT and AST were found to differ significantly ($P \leq 0.05$) between treated and untreated groups of rats (Table 2) except in rats of the group treated with 0.15% triptolide whose plasma levels of ALP and ALT were not found to differ significantly from that of the untreated group. After 60 days of treatment withdrawal (Table 2), no significant difference was found in the plasma levels of ALP among the treated and untreated groups of rats and in plasma levels of ALT and AST between untreated group of rats