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ANTIFERTILITY EFFECTS OF SINGLE ORAL DOSES OF TRIPTOLIDE IN MALE HOUSE RAT (*Rattus rattus* L.)

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ABSTRACT

The aim of present study was to evaluate the effect of single oral doses of triptolide on fertility of male house rat, *Rattus rattus*. Single oral administration of triptolide to male *R. rattus* resulted in 16.67 and 33.33% mortality in rats treated with 100 and 150 mg kg^{-1} b.wt. of triptolide, respectively, within 1-3 days of treatment. The LD_{50} value of triptolide against male *R. rattus* was found to be 182.81 mg kg^{-1} b.wt. Autopsy of rats surviving till day 45 after treatment revealed no significant effect on weights of reproductive organs. The sperm motility and viability in cauda epididymal fluid were reduced significantly in all the treated groups. Sperm concentration was significantly reduced only in rats treated with 100 mg kg^{-1} b.wt. of triptolide. Treatments significantly affected sperm morphology. The sperms with head tail separation ranged from 15.8 to 24.0% in all treated groups. The present study suggests the potential of triptolide in regulating fertility of male *R. rattus*.

Key words: Antifertility, *Rattus rattus,* single dose, triptolide

INTRODUCTION

The house rat (*Rattus rattus* L.) is one of the most common rodent pest worldwide which causes severe economic losses directly by damaging food grains and other products and indirectly by transmitting several diseases to humans and animals (Singla *et al.,* 2008, 2013b; Meerburg, 2009). The economic damages and health problems caused by rodents emphasize the need to develop new techniques for management. After natural reduction or control with rodenticides, rodents rapidly re-buildup their population by enhancing their reproduction (Shilova and Tchabovsky, 2009). Repeated use of rodenticides may lead to non-target toxicity hazards, bait shyness and resistance (Mineau *et al.,* 2004; Brakes and Smith, 2005). For rodents, fertility control has been identified as the most effective and long term strategy to manage pest populations with high reproductive rate. Moreover, the use of plant products as antifertility agents (Singla and Mittal 2012; Singla and Garg 2013) will have an added advantage of being environmentally safe (Dubey *et al.,* 2008).

The antifertility effects of different parts of plant *Tripterygium wilfordii* Hook F. came into light from the studies on rats and mice and retrospective studies on men taking *T. wilfordii* preparations for some other medical reason (Chen *et al*., 2010). Triptolide, one of the major compounds found in this plant, reportedly causes infertility in male and female rats and mice (Sinha Hikim *et al*., 2000; Huynh *et al.,* 2000; Ni *et al.,* 2008; Liu *et al.,* 2010). Our earlier studies have reported antifertility effects of different concentrations of triptolide fed in bait for different durations of time to wild rat species (Dhar and Singla, 2013, 2014; Singla *et al.,* 2013a; Dhar *et al.,*

2014). No study has so far reported the antifertility effects of single dose of triptolide in rats. Present studies were hence undertaken to evaluate antifertility effects of single oral doses of triptolide in male *R. rattus* for its further use in population regulation of this species.

MATERIALS AND METHODS

Male *R. rattus* were live trapped with multi-catch rat traps from poultry farms in and around Ludhiana city (Punjab) during the year 2009-10. In the laboratory, rats were kept individually in cages (36 cm x 23c m x 23 cm) for 10-15 days for acclimatization before the commencement of experiment. Food and water were provided *ad libitum*. Food consisted of a loose mixture of cracked wheat, powdered sugar and groundnut oil (WSO bait) in ratio of 96:2:2. Proper hygienic conditions were maintained. Approval of Institutional Animal Ethics Committee was obtained for the use of animals.

Triptolide used in present study was supplied by M/s Pidilite Industries Pvt. Ltd., New Delhi, India. Mature and healthy rats (n = 24) were divided into 4 groups of 6 rats each. Rats of groups II, III and IV were orally administered with 50, 100 and 150 mg kg^{-1} b.wt. of triptolide, respectively, through gastric gavage in a single dose after being dissolved in vehicle (1% sodium carboxymethyl cellulose solution). Rats of group I, kept as untreated control, were administered vehicle only. Before and after treatment, rats of all the groups were fed on WSO bait and water provided *ad libitum.* Pre- and post-treatment bait consumption was recorded after every 24 hr. Rats were observed for mortality and other clinical symptoms up to 45 days of treatment. LD_{50} value of triptolide was calculated by probit analysis using Polo Software.

Forty five days after treatment, male rats of all the groups were weighed, anaesthetized and sacrificed for observing the antifertility effects of triptolide. Their reproductive organs including testis, epididymis, seminal vesicles and prostate gland were dissected out, cleared off fat tissue and weighed. One of the cauda epididymis of each rat was incised and pressed to take out the cauda epididymal fluid. The fluid was liquefied in 0.5 mL of 0.9% saline solution pre-incubated at 37°C. The effect of triptolide on sperm motility, sperm viability, sperm concentration and sperm morphology (in terms of % abnormality) in the cauda epididymal fluid was determined as per the methods of Salisbury *et al.* (1978) and Singla and Garg (2013).

The data was expressed as mean ± SD. Significance of differences was determined using one 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*). Probability value $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

In the present study no significant difference in mean daily food consumption of WSO bait before and after the treatment was observed (Table 1) indicating no adverse single dose effect of triptolide treatment on food consumption or appetite of rats. No mortality was observed in control group and in the rats administered 50 mg kg^{-1} b.wt. of triptolide. However, the rats administered 100 and 150 mg kg^{-1} b.wt. of triptolide exhibited 16.67 and 33.33% mortality, respectively, within 1-3 days of treatment (Table 1). No other clinical symptom was observed in rats of any group. The LD_{50} value of triptolide in male *R. rattus* was 182.81 mg kg^{-1} b.wt. Xue *et al.* (2011) reported that inactivation of hepatic microsomal enzymes P450s by triptolide treatment abolishes the ability of animal to metabolize triptolide in liver, subsequently resulting in increase in half-life and toxicity of triptolide *in vivo*.

Table 1: Effect of single oral dose of triptolide in male *R. rattus*

Group (n = 6 each)	Dose (mg kg^{-1} b.wt.)	Mean daily food consumption (g 100 g^{-1} b.wt.)		Mortality (%)
		Before treatment	After treatment	
I	0	7.36 ± 1.16	6.79 ± 1.04	nil
II	50	5.60 ± 0.49	6.79 ± 0.85	nil
III	100	8.84 ± 0.93	8.60 ± 1.83	16.67
IV	150	7.55 ± 0.53	5.91 ± 2.10	33.33

The house rats, surviving till 45 days after treatment, were anaesthetized to record antifertility effects. There was no significant difference in the weights of reproductive organs such as testis and epididymis and accessory sex glands such as seminal vesicles and prostate gland among groups of treated and untreated rats (Fig. 1) indicating no significant effect of single oral doses of triptolide. Singla *et al.* (2013a) did not find any significant effect of 0.1% triptolide treatment for 14 days on reproductive organ weights of male rats. However, Dhar and Singla (2013) reported a significant decrease in the weights of reproductive organs of male *Bandicota bengalensis* treated with 0.25% triptolide for 15 days.

In present study a significant difference was observed in sperm motility and sperm viability between treated and untreated rats at all the tested doses of triptolide. The values decreased in treated groups. However, the difference in values of these parameters between rats treated with 50 and 150 mg kg^{-1} b.wt. of triptolide and similarly between rats treated with 100 and 150 mg kg^{-1} b.wt. of triptolide was non-significant (Table2). Sperm concentration also decreased in treated rat groups but the difference in sperm concentration between untreated rats and the rats of groups II and IV was non-significant. It decreased significantly ($P \leq 0.05$) only in the rats treated with 100 mg kg^{-1} b.wt. of triptolide. This may be due to individual variations observed in response of *R. rattus* towards triptolide treatment. Similar individual variations in response of *R. rattus* have also been reported in other studies (Faleh *et al.*, 2012; Singla *et al.*, 2013a).

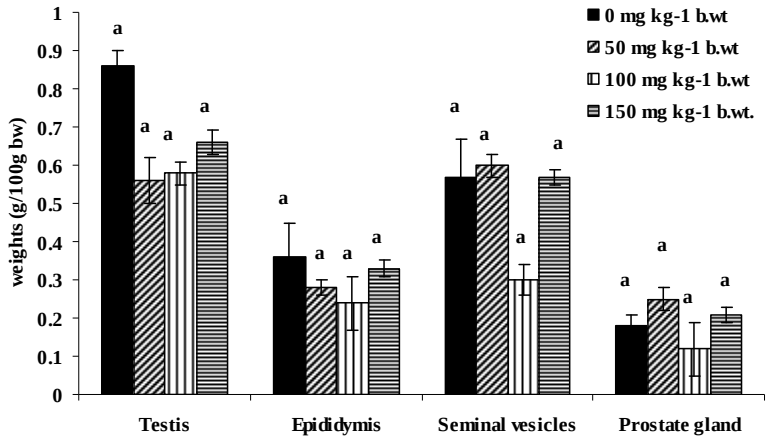

Fig. 1: Effect of single oral dose of triptolide on the weights of reproductive organs of male *R. rattus*

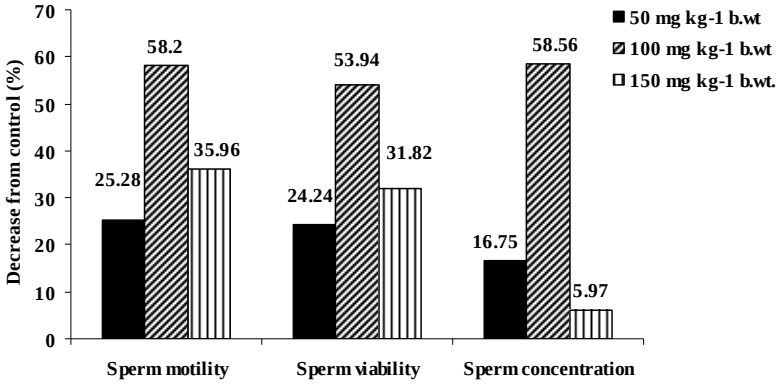

Fig. 2: Decrease (%) in some sperm parameters in male rats treated with single oral dose of triptolide from that of untreated rats

Decrease in sperm motility, viability and concentration in treated rats from that of untreated rats are presented in Fig. 2. Reports reveal that the sperm motility, which averaged 58.2 and 57.7% in untreated rats, was reduced to almost zero in male rats treated orally with 100 µg kg^{-1} b.wt. of triptolide for 70 days (Lue *et al.*, 1998)

Table 2: Effect of single oral dose of triptolide on sperm parameters in cauda epididymal fluid of male *R. rattus*

Group	Dose (mg kg^{-1} b.wt)	Sperm motility (%)	Sperm viability (%)	Sperm concentration (millions mL^{-1})	Sperm abnormality (%)	
					Head tail separation	Other abnormalities
I (n = 6)	0	74.17 ± 1.83^{a}	82.50 ± 2.28^{a}	466.75 ± 79.33^{a}	0.00 ± 0.00^{a}	3.17 ± 1.09^{a}
II (n = 6)	50	55.00 ± 2.04^{b}	62.50 ± 2.57^{b}	388.58 ± 64.67^{a}	15.83 ± 1.83^{b}	9.17 ± 1.40^{b}
III (n = 5)	100	31.00 ± 4.10^{c}	38.00 ± 4.38^{c}	193.40 ± 46.27^{b}	24.00 ± 5.37^{b}	22.00 ± 3.03^{c}
IV (n = 4)	150	47.50 ± 4.14^{bc}	56.25 ± 3.25^{bc}	438.87 ± 104.31^{a}	16.25 ± 2.07^{b}	7.50 ± 1.25^{b}

n = Sample size, Mean values in a column not sharing a common superscript differ significantly ($P \leq 0.05$)

and 82 days (Huynh *et al.,* 2000). Similarly, the cauda epididymal sperm content decreased by 68 and 84% in male rats treated orally with 100 µg kg^{-1} b.wt. of triptolide for 70 days (Lue *et al.,* 1998) and 82 days (Huynh *et al.,* 2000), respectively. The sperm motility and viability reduced to 5.0 and 18.4%, respectively, in *R. rattus* treated with 0.1% triptolide in bait for 14 days as compared to 53.7 and 56.9%, respectively, in untreated rats after 30 days of treatment withdrawal (Singla *et al.,* 2013a). The same were reduced to 3.50 and 18.78% in male *B. bengalensis* treated with 0.25% triptolide in bait for 15 days as compared to 69.70 and 77.75%, respectively, in untreated rats after 30 days of treatment withdrawal (Dhar and Singla, 2013). The sperm concentration was reduced to 133.3 millions mL^{-1} in *B. bengalensis* treated with 0.25% triptolide for 15 days as compared to 365.0 millions mL^{-1} in untreated rats (Dhar and Singla 2013). The same was reduced to 159.9 millions mL^{-1} in *R. rattus* treated with 0.1% triptolide for 14 days as compared to 416.2 millions mL^{-1} in untreated rat (Singla *et al*., 2013a). Within the epididymis, there are a number of glutathione transferases (Papp *et al.,* 1995; Gandy *et al.,* 1996) that play an important role in sperm motility (Alvarez and Storey, 1989; Bernacchi *et al.,* 1993). The poor sperm motility is independent of mitochondrial function as the ATP levels of triptolide treated and untreated rats were statistically at par (Huynh *et al.,* 2000).

In present study, sperms with head tail separation, acrosomeless heads, knob shaped heads, straight heads, triangular heads, banana shaped heads, heads coiled over mid-piece and coiled tail were considered abnormal. The head tail separation was found only in treated rats which ranged from 15.83 to 24.00% in all the 3 concentrations tested. Other abnormalities in sperm morphology were found in all the treated and untreated rats which were 3.17% in untreated rats and increased significantly ($P \leq 0.05$) from 7.5 to 22.0% in treated rats (Table 2). No significant difference was found in other abnormalities between rats treated with 50 and 150 mg kg^{-1} b.wt. of triptolide. Other abnormalities were observed to be significantly more in rats treated with 100 mg kg^{-1} b.wt. This may again be due to individual variations observed in *R. rattus*. Dhar and Singla (2013) found sperm head tail separation to be 54.06% in *B. bengalensis* treated with 0.25% triptolide for 15 days whereas Singla *et al*. (2013a) observed 49.70% in *R. rattus* treated with 0.1% triptolide for 14 days. Virtually all the cauda epididymal sperms in adult Sprague-Dawley rats fed daily with 100 µg kg^{-1} b.wt. of triptolide for 82 days exhibited severe structural abnormalities. The most striking changes observed were head tail separation, premature chromatin decondensation of sperm nuclei, a complete absence of plasma membrane of the entire middle and principal pieces, disorganization of the mitochondrial sheath and aggregation of many sperm tails (Huynh *et al.,* 2000). Structural abnormalities in epididymal spermatozoa including disrupted connecting pieces, cracked mid-pieces and more than 80% of the spermatozoa decapitated in rats treated with 0.05 mg kg^{-1} b.wt. of tripchlorolide (also obtained from *T. wilfordii*) for 7 weeks were observed by Ye *et al.* (1994). Triptolide treatment results in nuclear decondensation leading to head-tail separation in a severe case and nuclear decondensation without head-tail separation in mildly affected cases (Huynh *et al.,* 2000). Any chromatin decondensation of cauda epididymal sperm nuclei is indicative of sperm malfunction (Aravindan *et al.,* 1997) and could also contribute to the observed sterility.

Conclusion: The present study demonstrates mortality with single oral doses of 100 and 150 mg kg^{-1} b.wt. of triptolide in male *R. rattus* with LD_{50} value of 182.81 mg kg^{-1} b.wt. The major antifertility effects of triptolide include the effects on sperm motility, live sperm count and sperm morphology at all the doses tested.

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