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ANTI-REPRODUCTIVE EFFECTS OF TOTAL GLYCOSIDES OF *Tripterygium wilfordii* IN MALE HOUSE RAT, *Rattus rattus*

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ABSTRACT

Present study was conducted to evaluate anti-reproductive effects of total glycosides of *Tripterygium wilfordii* (GTW) (Thunder God Vine), in male House rat, *Rattus rattus*, a predominant rodent pest species found in commensal situations in Punjab, India. Mature male rats were fed on bait (cracked wheat and powdered sugar, 98:2) containing 0.1, 0.2 and 0.3% GTW for 10 days in no-choice feeding tests. Results revealed 67.2-85.2% acceptability of treated bait over untreated bait. Autopsy of rats after 30 days of treatment withdrawal revealed significant ($P \leq 0.05$) reduction in weights of testes and epididymides. The sperm motility, live sperm count and sperm concentration in the cauda epididymal fluid of treated rats was also reduced significantly compared to that in untreated rats. The major abnormality in sperm morphology was sperm head tail separation. Results suggest the use of GTW for reducing reproduction of *R. rattus* in integration with chemical control.

Key words: Anti-reproductive effects, glycosides, *Rattus rattus, Tripterygium wilfordii*

INTRODUCTION

The economic damages and health problems caused by rodents (Singla *et al.,* 2008, 2013b; Meerburg, 2009; Singla and Babbar, 2010; Mulungu *et al.,* 2014) emphasize the need to develop new techniques for their management. After natural reduction or control with rodenticides and other methods, rodent population gets rebuild up to the original level by enhanced reproduction (Shilova and Tchabovsky, 2009). Fertility control using plant products is a non-lethal and environmentally safe strategy (Dubey *et al.,* 2008; Chambers *et al.,* 1999) that could be integrated with chemical control to maintain the population of rodents at lower level. The discovery of antifertility action of extracts from *Tripterygium wilfordii* (a twining wine of family *Celastraceae* common to southern China) 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). *T. wilfordii* extract contains a number of active compounds such as triptolide, tripdiolide, triptolidenol, tripchlorolide, 16-hydroxytriplide, etc, together called as glycosides. The antifertility effects of total glycosides and their individual compounds were studied in laboratory rats and mice keeping in view the development of human contraceptive (Matlin *et al.,* 1993, Qian *et al.,* 1995). Our previous studies evaluated the potential of triptolide, one of the most active components of *T. wilfordii* on wild rodent pest species keeping in view their integrated management (Dhar and Singla, 2013; Singla *et al.,* 2013a; Singla, 2014; Singla and Challana, 2014). Present studies were aimed to evaluate the anti-reproductive effects of total glycosides of *T. wilfordii* in male house rat, *Rattus rattus*, a predominant rodent pest species in commensal situations in India.

MATERIALS AND METHODS

Plant product used

The product containing total glycosides of *T. wilfordii* (GTW) used in present studies was extracted and supplied by Dr. Shi Da Zhao, College of Agronomy and Biotechnology, China Agricultural University, Beijing, China.

Animals

Mature male *R. rattus* were live trapped from poultry farms at Ludhiana (India) and acclimatized to laboratory conditions by keeping them individually in cages with food and water provided *ad libitum*. Food was prepared by mixing cracked wheat, powdered sugar and groundnut oil (WSO bait) in 96: 2: 2. Animals were used and maintained as per the guidelines of Institutional Animal Ethics Committee (vmc/12/3901-35 dated 6 August, 2012. During experimentation, healthy rats (n = 24) were weighed and divided into four groups with 6 animals in each group. Their body weight ranged from 170.8 to 176.7 g. There was no significant difference in mean body weight of rats among the four groups ($P>0.05$). Prior to treatment, all the rats were fed on plain WSO bait to determine their mean daily food consumption [(g 100 g^{-1} body weight (b.wt.)]. Food consumption was recorded after every 24 h.

Treatments

During treatment, rats of groups I, II and III were fed on bait (cracked wheat and powdered sugar, 98: 2) containing 0.1, 0.2 and 0.3% of GTW, respectively, in no-choice feeding test for 10 days. GTW was mixed after being dissolved in vehicle (1% sodium carboxymethyl cellulose solution). Rats of group IV, maintained as untreated control, were fed on plain WSO bait. During treatment, consumption of bait by all the rats was recorded after every 24 h to determine the mean daily bait consumption (g 100 g^{-1} b.wt.). From the total amount of the treated bait consumed in 10 days by rats of three groups, the total dose of the active ingredient (mg 100 g^{-1} b.wt.) ingested by rats was determined. After the termination of treatment, all the rats were again fed on plain WSO bait. Post-treatment consumption of food by all the rats was recorded after every 24 h to record the effect of treatment on appetite of rats. During treatment period and post treatment periods, rats were observed regularly for mortality and other toxic symptoms, if any.

Anti-reproductive effects

After 30 days of termination of treatment, the treated and untreated rats were weighed and sacrificed to record anti-reproductive effects. Their reproductive organs and accessory sex glands such as testes, caput and cauda epididymides and seminal vesicles were dissected out and weighed. Sperm motility, live sperm count, sperm concentration, and sperm abnormality were determined in cauda epididymal fluid (Singla and Garg, 2013).

Statistical analyses

All the values were calculated as mean ± SE. Analysis of variance and calculation of critical difference was carried out to rank the results of various anti-reproductive parameters at 5% level of significance.

RESULTS AND DISCUSSION

Effect on food consumption and active ingredient ingested

Feeding of bait containing 0.1, 0.2 and 0.3% GTW in no-choice for 10 days to male *R. rattus* resulted in the total ingestion of 50.2, 84.6 and 119.1 mg 100 g^{-1} b.wt. of active ingredient by rats of

Table 1: Acceptance of bait containing GTW by male *R. rattus* in no-choice for 10 days

Group (n = 6)	Dose in bait (%)	Body weight (g)	Mean daily consumption of treated food (g 100 g^{-1} b.wt.)	Acceptance over untreated food (%)	Total active ingredient administered (mg 100 g^{-1} b.wt.)	Mortality (%)
I	0.1	170.8±8.0	4.9±0.3 [a]	85.2	50.2±3.3	Nil
II	0.2	172.5±4.9	4.2±0.4 [a]	78.9	84.6±9.8	16.7
III	0.3	176.7±10.5	3.9±0.2 [b]	67.2	119.1±4.8	16.7
IV	0	173.3±7.9	6.0±0.5[a]	-	-	Nil

All values are mean ±SE; Values with different superscripts (a, b) in a column differ significantly at P<0.05

groups I, II and III, respectively (Table 1). At 0.2 and 0.3% concentrations each, 16.7% mortality of rats was also observed. No such mortality was observed in rats treated with 0.1% GTW. No significant (P >0.05) difference in food consumption (g 100 g^{-1} b.wt.) of rats between pre- and post-treatment periods was observed indicating no effect of treatment on appetite of rats (Fig. 1). Also no significant difference was observed between the consumption of treated bait and plain WSO bait before treatment by rats, except at 0.3% concentration indicating good acceptability (85.2, 78.9 and 67.2% at 0.1, 0.2 and 0.3% concentration, respectively) of treated bait over untreated bait. A dose dependent decrease in the consumption of treated bait among the three treated groups of rats was observed (Table 1). The mean daily consumption of bait containing 0.3% GTW was significantly low which may be due to toxicity caused by GTW leading to bait shyness.

Effect on weights of reproductive organs and accessory sex glands

Feeding of bait containing 0.1, 0.2 and 0.3% GTW in no-choice for 10 days resulted in significant reduction (P ≤0.05) in weights (g 100 g^{-1} b.wt) of testes, caput and cauda epididymides from that of untreated rats (Table 2). The difference in weights of these organs among the three treated groups was, however, found to be non-significant, P>0.05 (Table 2). The differences in weight of seminal vesicles between treated and untreated rats and among treated groups was also found to be non-significant (P>0.05). Dhar and Singla (2013) have reported a significant decrease in weights of reproductive organs of male lesser bandicoot rat, *Bandicota bengalensis* treated with 0.25% triptolide (a triterpene epoxide obtained from *T. wilfordii*) for 15 days. Singla (2014) observed no significant effect on weights of reproductive organs of male *R. rattus* administered single oral dose of 100 and 150 mg kg^{-1} b.wt. of triptolide.

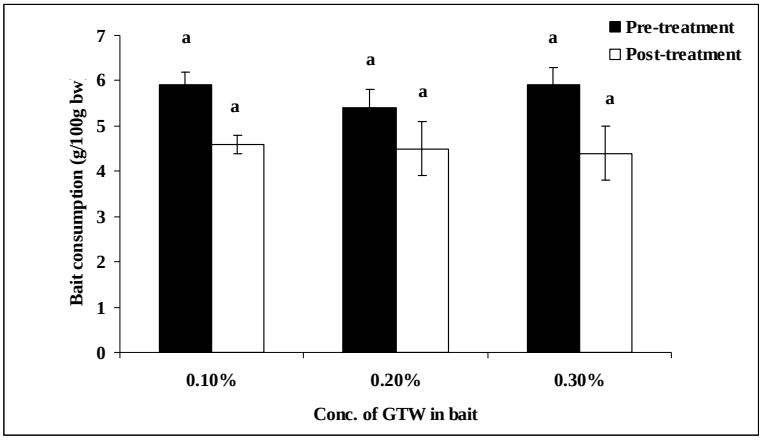

Fig. 1: Effect of feeding bait containing GTW in no-choice for 10 days on post-treatment bait consumption by male *R. rattus.* Superscript 'a' above bars indicates no significant difference at P<0.05 between pre- and post-treatment bait consumption

Table 2: Effect of feeding bait containing GTW in no-choice for 10 days on weights of reproductive organs of male *R. rattus*

Group	Dose in bait (%)	Body weight (g)	Testis	Epididymis weight (g 100 g^{-1} b.wt.)		Seminal vesicles
				Caput	Cauda	
I	0.1	146.2±11.7 (n = 6)	0.30±0.03[b]	0.07±0.01[b]	0.13±0.01[b]	0.77±0.15[a]
II	0.2	165.3± 6.7 (n = 5)	0.32±0.07[b]	0.08±0.01[b]	0.11±0.01[b]	0.64±0.06[a]
III	0.3	163.9±12.8 (n = 5)	0.31±0.06[b]	0.08±0.01[b]	0.11±0.01[b]	0.63±0.06[a]
IV	0	175.2± 9.7 (n = 6)	0.65±0.00[a]	0.12±0.02[a]	0.16±0.01[a]	0.68±0.08[a]

All values are mean ±SE; Values with different superscripts (a, b) in a column differ significantly at P<0.05

Table 3: Effect of feeding bait containing GTW in no-choice for 10 days on sperm parameters in cauda epididymal fluid of male *R. rattus*

Group	Dose in bait (%)	Sperm motility (%)	Live sperm count (%)	Sperm concentration (million/ml)	Sperms with head tail separation (%)
I	0.1	0.2 ±0.2[b]	1.7 ±0.8[b]	56.2 ±21.3[b]	80-90
II	0.2	10.0 ±6.3[b]	16.0 ±10.3[b]	117.6 ±85.4[b]	80-90
III	0.3	0.0 ±0.0[b]	3.0 ±3.0[b]	3.6 ±1.9[b]*	80-90
IV	0.0	75.0 ±2.2[a]	80.0 ±1.8[a]	461.7 ±44.4[a]	0-10

All values are mean ±SE; *Two rats were having azoospermia; Values with different superscripts (a, b) in a column differ significantly at P <0.05

Effect on sperm parameters

Results revealed significant ($P \leq 0.05$) difference in sperm motility (%), live sperm count (%) and sperm concentration (sperms x 10^6 mL^{-1}) between treated and untreated rats (Table 3). The difference in all the above parameters among the three treated groups was, however, non-significant. At 0.3% concentration, two rats were found to have azoospermia and rest with sperms having no motility. The sperm motility and live sperm count which averaged 75 and 80%, respectively, in untreated rats were reduced to 0-10 and 1.7-16%, respectively, in treated rats. The concentration of spermatozoa in cauda epididymal fluid of all the treated rats was reduced significantly ($P \leq 0.05$) from 461.7 million mL^{-1} in untreated group of rats to 3.6-117.6 million mL^{-1} in treated groups of rats. Within epididymis, there are a number of glutathione transferases that play an important role in sperm motility (Alvarez and Storey, 1989; Gandy *et al.*, 1996).

In all the treated groups sperm head tail separation was 80-90% as compared to upto 10% in untreated rats (Table 3). Dhar and Singla (2013) found sperm head tail separation to be 54.1% in *B. bengalensis* treated with 0.25% triptolide for 15 days, whereas Singla *et al.* (2013a) observed 49.7% head tail separation in *R. rattus* treated with 0.1% triptolide for 14 days.

The mechanism by which glycosides of *T. wilfordii* induce oligozoospermia is unknown. *In vitro* evidence indicate that these can inhibit Ca^{+2} influx in human sperm (Qian *et al.*, 1995). So, it is possible that glycoside induced azoospermia could be attributed, at least in part, to the inhibition of Ca^{+2} influx. Intracellular Ca^{+2} is known to play a major role in sperm motility (Suarez, 1996), however, the validity of this interpretation remains to be tested in rats. Sperm abnormality is significant prognostic factor for fertilization and pregnancy (Franken, 1998). Chromatin de-condensation of cauda epididymal sperm nuclei leading to sperm head tail separation is indicative of sperm malfunction (Aravindan *et al.*, 1997; Huynh *et al.*, 2000) and could contribute to the observed sterility. The study revealed anti-reproductive effects of total glycosides of *T. wilfordii* thus suggesting their use for control of *R. rattus* population in integration with chemical control.

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