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RESEARCH ARTICLE

Effect of triptolide on reproductive output of male *Bandicota bengalensis*

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Abstract

Present study was aimed to evaluate the effect of triptolide on reproductive output of wild male rodent pest species, *Bandicota bengalensis* for its further use in integration with chemical control for management of this pest species under field conditions. One group of rats (n =15) was kept as vehicle control and other three groups (n = 15 each) were fed on bait containing different concentrations of triptolide. Feeding of bait containing 0.15, 0.2 and 0.25% triptolide for a period of 15 days in choice with plain bait resulted in per day ingestion of 19.99 ± 2.41, 19.28 ± 2.01 and 11.42 ± 1.30 mg/kg bw of triptolide, respectively. No pregnancy was observed in female rats paired with male rats treated with 0.2 and 0.25% triptolide immediately after treatment withdrawal. No pregnancy was observed in female rats paired with male rats treated with 0.25% triptolide even after 30 days of treatment withdrawal. Autopsy of rats immediately and after 30 and 60 days of termination of treatment revealed significant effect of triptolide treatment on weights reproductive organs, sperm motility, viability, density and sperm morphology. No effect of treatment was observed on plasma levels of testosterone. The present study suggests triptolide to be a strong candidate for reducing the reproductive output of male *B. bengalensis.*

Introduction

Among different rodent pest species, lesser bandicoot rat, *Bandicota bengalensis* Gray and Hardwicke has been reported to be the most detrimental inhabiting irrigated crop fields (Singla & Parshad, 2010) and causing heavy losses to field crops in Southeast Asia (Parshad, 1999; Rana *et al*., 2006; Singla & Babbar, 2010 and Borah & Bora, 2012). Improved irrigation and changes in cropping pattern during the last 4-5 decades in Punjab (India) has boosted the population of this species replacing the earlier species like *Nesokia indica*, *Tatera indica* and *Millardia meltada* whose population has declined (Parshad, 1999). The success of this species lies in its highly adaptable nature coupled with high reproductive capacity (Rao & Kishore, 2009). This species also acts as a reservoir for transmitting diseases to man and animals (Singla *et al*., 2008, 2012 and Meerburg, 2009).

The economic magnitude and health problems associated with rodent pests emphasize the need to develop new techniques for their management. After natural reduction or control with rodenticides, rodents rapidly rebuildup their population (Shilova & Tchabovsky, 2009) by enhancing their reproduction. Repeated use of rodenticides may lead to several problems including bait shyness, resistance and other non-target toxicity hazards (Mineau *et al*., 2004 and Brakes & Smith, 2005). Although poisons may be useful in the initial reduction of a high density population and, thereby, in reducing the immediate damage caused by them, fertility control could be used to maintain the population at lower level. Because of their low mammalian toxicity, cost effectiveness and biodegradable nature, plant products possess the potential in pest management (Dubey *et al*., 2008)**.**

Triptolide, a diterpene triepoxide, is one of the major compounds isolated from *Tripterygium wilfordii* and reported to have antifertility effects in male and female rats (Dai *et al*., 1994; Lue *et al.,* 1998; Sinha Hikim *et al.,* 2000; Huynh *et al.,* 2000; Ni *et al.,* 2008 and Liu *et al*., 2010). Earlier studies on antifertility effects of triptolide were conducted in laboratory rats and mice keeping in view the development of an oral human contraceptive. 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. However, for field scale use of an antifertility agent, it is required to be fed in bait form. 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*. No study has so far been conducted to evaluate the potential of triptolide fed in bait form in population regulation of *B. bengalensis*. Present study was hence undertaken to evaluate the effects of triptolide on reproductive output of male *B. bengalensis* for its further use in integration with chemical control for management of this pest species under field conditions.

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 (36 × 23 × 23 cm) with food and water provided *ad libitum* for 10–15 days before the commencement of experiment. 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 (IAEC) 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 = 60) were divided into 4 groups of 15 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 groups II–IV were fed on bait containing 0.15, 0.20 and 0.25% triptolide, respectively for 15 days in bi-choice with WSO bait. Rats of group I, fed on WSO bait were kept as untreated control. 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*.

Bait consumption and toxicity

Two cages each of size 36 × 23 × 23 cm were tied together to provide the option of two baits to each rat of treated groups. Bait was kept in bowls and the position of bowls was changed daily to avoid preference to any particular side. Water was provided *ad libitum*. Bait consumption was recorded after every 24 hrs and every time, bait was replaced to the original 30 g. Before weighing, the bait of all the treated and untreated rats was cleared of fecal pellets and dried. Before and after the treatment, all the rats were fed on WSO bait. Mean daily consumption of bait (g/100 g body weight (bw)) during pre-treatment, treatment and post-treatment periods (average of 15 days each) by different groups of rats was calculated. Based on the amount of treatment bait consumed, total and mean daily dose (mg/kg bw) of triptolide ingested by each group of rats was calculated. Percent acceptance of treated bait over WSO bait was determined as per the formula described below:

$$\text{Percent acceptance} = \frac{\text{Treated bait consumed during treatment period}}{\text{WSO bait consumed + treated bait consumed during treatment period}} \times 100$$

Effect on breeding performance

Immediately after the termination of treatment, five male rats of each group were paired with healthy, untreated and cyclic female rats in ratio 1:1. Before pairing, the vaginal fluid of all the female rats was examined twice a day for two weeks to determine their cyclic nature. Female rats in the proestrous stage of the estrous cycle were used for pairing. Pairing was carried out in breeding pens. During pairing, WSO bait and water were provided *ad libitum*. In addition, green fodder and soaked black grams were also provided to rats. Vaginal fluid of all the female rats was

examined daily in the morning hours for the presence of sperms. The day on which sperm was observed in the vaginal smear was considered as day 1 of pregnancy and the female rats were separated from their male partners. After 15 days of pairing, all the male rats were separated from their female partners. Female rats were observed for pregnancy and delivery of pups. After 30 days of termination of treatment, another set of five male rats of each group were paired with healthy, untreated and cyclic female rats as per the procedure described above to record the breeding performance of rats after 30 days of treatment withdrawal.

Anti-reproductive effects immediately after termination of treatment

Immediately after the termination of treatment, five male rats of each group were weighed, anaesthetized and dissected to record anti-reproductive effects of triptolide.

Effect on weights of reproductive organs

Reproductive organs such as testis, epididymis, seminal vesicles and prostate gland were dissected out, cleared of fat tissue and weighed (g/100g bw).

Effect on sperm parameters

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 37^0C. The effect of triptolide on sperm motility (%), sperm viability (%), sperm density (millions/ml) and sperm morphology (% abnormality) in the cauda epididymal fluid was determined as per the methods described by Salisbury *et al.* (1978) and Singla & Garg (2013).

To estimate the sperm motility, a drop of cauda epididymal fluid was taken on a clean glass slide and observed under microscope. Percent sperm motility was scored on the basis of the number of progressively moving sperms amongst the total number of sperms visible in a microscopic field at 100X.

Sperm viability (%) was determined by taking one drop of cauda epididymal fluid on clean glass slide and mixing it with one drop of 0.1% eosin solution in 0.9% saline maintained at 37^0C. After two minutes, a coverslip was placed on it. Live spermatozoa with transparent heads and dead spermatozoa with pink heads were counted per 100 sperms per animal at 400X magnification.

Sperm density was determined by using Neubauer haemocytometer, commonly used for counting RBC's. The cauda epididymal fluid was diluted in the ratio of 1:200 in a pipette with 0.2% eosin solution in 0.9% saline. The haemocytometer chamber was charged with diluted cauda epididymal fluid and observed under microscope. The spermatozoa lying with in the square and those touching two sides of a square in five secondary squares (four at corners and one in center) having sixteen tertiary squares in each were counted. The mean of total number of spermatozoa in five secondary squares was multiplied by 10^7 to obtain the number of spermatozoa in millions/ml.

Smears of cauda epididymal fluid of each rat were drawn on clean glass slides, air dried, fixed in methanol and stained with Giemsa stain. Staining was carried out for 40 – 45 minutes. The slides were then washed under running tap water to remove excess of the stain. Air dried slides were mounted in DPX. To determine percent abnormality in sperm morphology, the numbers of normal and abnormal sperms were counted per 100 sperm per slide of an animal at 400X.

Anti-reproductive effects after 30 and 60 days of termination of treatment

After 30 and 60 days of termination of treatment, again sets of five rats in each group were dissected to record anti-reproductive effects as per the procedure described above to see reversibility/irreversibility in effects of triptolide after 30 and 60 days of treatment withdrawal.

Effect on plasma levels of testosterone

At necropsy of rats immediately after termination of treatment and after 30 and 60 days of termination of treatment, blood samples from all the treated and untreated rats were collected by cardiac puncture in Ethylenediamine tetra-acetic acid (EDTA) rinsed tubes. Blood was centrifuged at 3000g for 10 min and plasma collected was stored at −20°C until analysis. Plasma level of testosterone was measured by enzyme immunoassay, using 96-well ELISA plate.

Statistical analysis

All values were expressed as mean ± SEM and analyzed using analysis of variance. The statistical differences were considered significant at $P \leq 0.05$. Values in percentages were analyzed after arc sign transformation.

Results and Discussion

Bait consumption and toxicity

Data on bait consumption (Table 1) revealed significantly ($P \leq 0.05$) low consumption of treatment bait from that of WSO bait offered in choice during treatment period by all the three treated groups. Mean daily consumption (g/100g bw) of treated bait, however, did not differ significant among the three treated groups. There was no significant difference in mean daily consumption of WSO bait during pre-treatment and post-treatment periods among all the four groups of rats. However, during post-treatment period, the mean daily consumption of WSO bait by rats of groups II and III, treated with 0.15 and 0.2% triptolide, respectively was significantly low from that consumed during pre-treatment period (Table 1). This may be due to toxicity of triptolide caused during treatment period in rats of these two groups. The percent acceptance of treatment bait over WSO bait offered in bi-choice during treatment period was found to vary from 20.69 to 25.91% in the three treated groups.

Effect on breeding performance

None of the untreated cyclic female rats (n =5) paired with male rats fed on bait containing 0.2 and 0.25% triptolide for 15 days in bi-choice, delivered pups when paired immediately after the termination of treatment. However, two females out of the five (40%) paired with male rats fed on bait containing 0.15% triptolide delivered 5 and 2 pups, respectively. Four out of the five (80%) female rats paired with untreated male rats delivered on an average 5.25±0.41 number of pups (Table 2). None of the untreated cyclic female rats (n =5) paired with male rats fed on bait containing 0.25% triptolide delivered pups even when paired even after 30 days of termination of treatment. One female out of five (20%) paired with male rats treated with 0.2% triptolide, however, delivered 5 pups when paired after 30 days of termination of treatment. Percent pregnancy was increased to 60% in female rats paired with male rats treated with 0.15% triptolide after 30 days of termination of treatment (Table 2). Results thus revealed partial improvement in breeding success of male rats treated with 0.15 and 0.25 triptolide after 30 days of treatment withdrawal. In male rats treated with 0.25% triptolide, however, there was no breeding even after 30 days of treatment withdrawal.

Miao *et al*. (2001) carried out studies with multiglycosides of *T. wilfordii* on farmland rats and mice at the dosage of 30 and 50mg/kg/day and reported a decrease in birth rate by 32.6%. The pregnancy rates measured by housing each male with two untreated females were 100, 67 and 0% in control, low dose (50µg/kg bw/day) and high dose (100µg/kg bw/day) treated rats, respectively (Lue *et al*., 1998). Huynh *et al*. (2000) by housing each male with two untreated females measured 100 and 0% pregnancy rates in control rats and rats fed daily with 100µg/kg bw of triptolide for 82 days.

Effect on weights of reproductive organs

Autopsy of male rats (5 of each group) immediately after termination of treatment revealed significant effect of triptolide treatment on reduction in weights (g/100gbw) of testis, epididymis, seminal vesicles and prostate gland (Table 3). A significant ($P \leq 0.05$) difference in weight of testis was found among all the groups of treated and untreated rats. However, the weights of epididymis, seminal vesicles and prostate gland were found to be significantly ($P \leq 0.05$) low only in rats of group IV treated with 0.25% triptolide from that of rats of untreated group I and treated groups II and III. The highest effect of treatment was observed in rats treated with 0.25% triptolide.

In rats autopsied after 30 days of termination of treatment, the weights of above organs were again found reduced significantly in treated groups of rats from that of untreated group. The weight of testis in group of rats treated with 0.25% triptolide was found to differ significantly from that of untreated group of rats and rats of treated groups II and III. There was no significant difference in weight of testis between the rats of groups II and III (Table 4). After 30 days of treatment withdrawal, the weight of epididymis of rats treated with 0.15 and 0.25% triptolide was found to differ significantly from that untreated group. The weight of seminal vesicles was not found to differ significantly among the treated and untreated groups of rats after 30 days of treatment withdrawal. The weight of prostate gland in rats treated with 0.25% triptolide was found to differ significantly from that of untreated rats but the similar differences from other two groups of treated rats were non-significant.

After 60 days of termination of treatment also the weights of testis in rats of all the three treated groups were found reduced significantly from that of untreated group. A significant difference in weight of epididymis of rats of groups II and IV was found from that of untreated group (Table 5). There was no significant difference observed in weight of seminal vesicles among the treated and untreated groups of rats. After 60 days of treatment withdrawal, a significant reduction in weight of prostate gland was found only in rats of treated group IV.

The present results thus reveal irreversibility in effects of triptolide on weights of testis, epididymis and prostate gland. The testicular weights (1.09 ± 0.1g) of male rats treated with triptolide over a prolonged period (100µg/Kg bw/day for 82 days) were 26% less than those of the vehicle control (1.48 ± 0.05g) (Huynh *et al*., 2000).

Lue *et al*. (1998) did not observed any significant differences in mean weights of testis, epididymis, ventral prostate and seminal vesicles among untreated rats and rats administered 50 and 100µg/kg bw/day of triptolide for 35 and 70 days. Singla *et al*. (2013) also did not observe any significant effect of triptolide treatment on weights of reproductive organs of groups of rats treated with 0.025, 0.05 and 0.1% of triptolide in bait for 7 and 14 days durations and autopsied after 30 and 60 days of termination of treatment.

Effect on sperm parameters

A significant ($P \leq 0.05$) decrease in percent sperm motility, sperm viability and sperm density and increase in sperm abnormality was found in all the treated groups of rats compared to untreated group (Table 3). There was a dose dependent effect of triptolide treatment on sperm parameters in the cauda epididymal fluid. The highest effect of treatment was observed in rats treated with 0.25% triptolide. Immediately after withdrawal of treatment, the percent sperm motility in rats of groups treated with 0.2% (17.20 ± 3.9) and 0.25% (3.50±0.94) triptolide was found reduced significantly ($P \leq 0.05$) from that of rats of untreated group (69.70%). A significant difference was observed in sperm viability among the rats of all the treated and untreated groups. Sperm density was also found to be differ significantly among the rats of treated and untreated groups, however, the similar difference between rats of groups treated with 0.2 and 0.25% triptolide was not significant statistically. The sperm motility, sperm viability and sperm density in the cauda epididymal fluid of treated groups of rats were found to be decreased significantly from that of untreated group of rats even after 30 (Table 4) and 60 days (Table 5) of treatment withdrawal indicating irreversibility in antifertility effects of triptolide.

The sperm motility, which averaged 58.20% in the control rats, was reduced to almost zero in male rats treated orally with 100µg/kg bw/day of triptolide for 70 days (Lue *et al*., 1998). In rats treated with 100µg/kg bw/day of triptolide for 82 days also, the sperm motility was reduced to nil by the end of treatment compared with control rats (57.70 ± 0.4%) (Huynh *et al*., 2000). Lue *et al.* (1998) observed a decrease in cauda epididymal sperm content by 68% in male rats treated orally with 100µg/kg bw/day of triptolide for 70 days. In adult male rats fed daily with 100µg/kgbw of triptolide for 82 days, cauda epididymal sperm content was found decreased by 84% by the end of treatment (Huynh *et al*., 2000). The sperm motility and viability were found reduced to 5.0 and 18.0%, respectively in *R. rattus* treated with 0.1% triptolide for 14 days duration in bi-choice test compared to 53.7 and 56.9%, respectively in rats of untreated group after 30 days of termination of treatment (Singla *et al*., 2013). 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 & Storey, 1989 & Bernacchi *et al*., 1993). The poor sperm motility is independent of mitochondrial function as the ATP levels of triptolide treated rats and controls were not found statistically different (Huynh *et al*., 2000).

During present studies also the major effect of triptolide treatment on sperm morphology was in the form of sperm head tail separation (Fig. 1). The sperm head tail separation was found to be highest in rats of group treated with 0.25% triptolide (54.06±3.14%). A significant difference in sperm abnormality was observed between rats of untreated and treated groups (Table 3) autopsied immediately after the termination of treatment. The abnormality in sperm morphology was found increased significantly in all the treated groups of rats from that of untreated group even after 30 (Table 4) and 60 (Table 5) days of termination of treatment. The sperm head tail separation in rats of group treated with 0.25% triptolide was found to be 55.87±1.87 and 51.27±2.04%, respectively after 30 and 60 days of treatment withdrawal. Other abnormalities in sperms morphology included acrosomeless heads, knob shaped heads, straight heads, triangular heads, banana shaped heads, heads coiled over mid-piece, bent or coiled tails etc. (Fig. 2).

Singla *et al*. (2013) observed 49.7 and 30.8% sperm head-tail separation in rats treated with 0.1% triptolide for 14 days in bi-choice after 30 and 60 days of termination of treatment, respectively. 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. Structural abnormalities in epididymal spermatozoa including disrupted connecting pieces, cracked midpieces and more than 80% of the spermatozoa decapitated in rats treated with 0.05mg/kg bw/day of tripchlorolide (also obtained from *T. wilfordii*) for 7 weeks were observed by Ye *et al.* (1994). Virtually all the cauda epididymal sperms in adult Sprague-Dawley rats fed daily with 100µg/kg bw 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 the plasma membrane of the entire middle and principal pieces, disorganization of the mitochondrial sheath and aggregation of many sperm tails (Huynh *et al*., 2000). Deng *et al.* (2011) observed a significant increase in number of mis-shapen sperms in the cauda epidudymal fluid of rats administrated orally with 160 mg/kg bw/day of crude ethanol extracts of *T. hypoglaucum* root containing triptolide

for 30 days. Testis and epididymal weights, the sperm motility and density were also reduced significantly. They also observed a gradual recovery in effects of triptolide after 30-days of cessation of treatment.

Effect on plasma levels of male sex hormone

During present studies, though the plasma level of testosterone was found increased in rats of groups treated with triptolide, but the difference in level from that of untreated group of rats was not found to differ significantly (Table 6). This suggests that triptolide has no adverse effect on the normal endocrine function of the testis. Lue *et al.* (1998) also did not observed significant difference in plasma levels of testosterone between control and triptolide treated rats.

Table. 1- Acceptance of triptolide mixed in bait by male *B. bengalensis* in bi-choice feeding test

Groups	Conc. in bait (%)	Mean daily bait consumption (g/100 g bw)				Acceptance of treated bait over WSO bait (%)
		Before treatment (n=15)	During treatment (n=15)		After treatment (n=5)	
			WSO bait	Treated bait		
I	0	8.09±0.34	7.06±0.36	-	6.75±0.33	-
II	0.15	10.24±0.31	6.33±0.32[a]	1.99±0.24[b]	5.48±0.18*	23.46±2.60
III	0.2	12.56±0.45	7.26±0.28 [a]	1.93±0.20[b]	5.96±0.33*	20.69±1.89
IV	0.25	7.43±0.37	3.44±0.25 [a]	1.21±0.14 [b]	6.58±0.23	25.91±2.40

-Values are Mean ± SEM, n=Number of rats, *significantly low consumption from pre-treatment
[a,b]Values with different superscripts in a row for during treatment consumption differ significantly at $P \leq 0.05$
[b]Values with similar superscripts in a column for treated bait consumption do not differ significantly

Table. 2-Effect of triptolide treatment on breeding success of male *B. bengalensis* immediately and 30 days after termination of treatment.

Group (n=5 each)	Conc. in bait (%)	Immediately after termination of treatment		30 days after termination of treatment	
		No. of females delivered pups (Percent pregnancy)	No. of pups delivered (Mean±SEM)	No. of females delivered pups (Percent pregnancy)	No. of pups delivered Mean±SEM)
I	0	4 (80%)	5.25±0.41 (6,6,5,4 pups)	5 (100%)	5.40±0.37 (6,6,6,5,4 pups)
II	0.15	2 (40%)	3.5±1.06 (5,2 pups)	3 (60%)	3.33±0.72 (5,3,2 pups)
III	0.2	0 (0%)	nil	1 (20%)	5.00±0.00 (5 pups)
IV	0.25	0 (0%)	nil	0 (0%)	Nil

Table. 3- Effect of triptolide treatment on weights of reproductive organs and cauda epididymal sperm parameters immediately after termination of treatment

Organ wt./sperm parameters	Treatment (n=5 each) (Mean±SEM)			
	0%	0.15%	0.20%	0.25%
Testis (g/100g bw)	0.34±0.04[a]	0.19±0.02[b]	0.19±0.01[b]	0.09±0.01[c]
Epididymis (g/100g bw)	0.08±0.007[a]	0.07±0.004[a]	0.06±0.004[a]	0.03±0.001[b]
Seminal vesicles (g/100g bw)	0.57±0.05[a]	0.54±0.07[a]	0.53±0.03[a]	0.24±0.003[b]
Prostate gland (g/100g bw)	0.32±0.03[a]	0.39±0.06[a]	0.28±0.04[a]	0.08±0.01[b]
Sperm motility (%)	69.70±1.94[a]	19.50±2.17[b]	17.20±3.93[bc]	3.50±0.94[c]
Sperm viability (%)	77.75±1.60[a]	36.70±0.97[b]	27.40±0.38[c]	18.78±1.37[d]
Sperm density (millions/ml)	365.00±13.93[a]	243.00±12.62[b]	177.00±13.01[c]	133.33±9.81[c]
Sperm head tail separation (%)	11.22±1.52[a]	20.88±2.27[b]	26.63±1.27[b]	54.06±3.14[c]
Other sperm abnormalities (%)	8.59±2.18[a]	21.07±1.96[b]	29.54±1.72[c]	34.00±1.33[c]

[a-c]Values with different superscripts in a row differ significantly at $P \leq 0.05$

Table. 4- Effect of triptolide treatment on weights of reproductive organs and cauda epididymal sperm parameters after 30 days of termination of treatment

Organ wt./sperm parameters	Treatment (n=5 each) (Mean±SEM)			
	0%	0.15%	0.20%	0.25%
Testis (g/100g bw)	0.25±0.01[a]	0.17±0.02[b]	0.16±0.01[b]	0.09±0.02[c]
Epididymis (g/100g bw)	0.07±0.002[a]	0.05±0.008[b]	0.06±0.005[ab]	0.05±0.002[b]
Seminal vesicles (g/100g bw)	0.51±0.01[a]	0.38±0.11[a]	0.33±0.04[a]	0.33±0.01[a]
Prostate gland (g/100g bw)	0.34±0.01[a]	0.15±0.04[ab]	0.18±0.04[ab]	0.08±0.02[b]

Sperm motility (%)	66.60±0.81[a]	21.70±10.06[b]	8.88±2.53[b]	2.75±0.54[b]
Sperm viability (%)	72.65±1.22[a]	26.56±3.48[b]	22.95±2.00[b]	20.25±2.60[b]
Sperm density (millions/ml)	342.00±31.21[a]	226.00±25.55[b]	192.50±19.49[b]	158.75±8.17[b]
Sperm head tail separation (%)	9.40±1.92[a]	25.20±2.66[b]	40.23±0.42[c]	55.87±1.87[d]
Other sperm abnormalities (%)	4.71±0.79[a]	25.58±3.09[b]	29.92±1.25[b]	32.47±2.28[b]

[a,b]Values with different superscripts in a row differ significantly at $P \leq 0.05$

Table. 5- **E**ffect of triptolide treatment on weights of reproductive organs and cauda epididymal sperm parameters after 60 days of termination of treatment

Organ wt./sperm parameters	Treatment (n=5 each) (Mean±SEM)			
	0%	0.15%	0.20%	0.25%
Testis (g/100g bw)	0.24±0.01[a]	0.21±0.03 [b]	0.18±0.04 [b]	0.14±0.03 [b]
Epididymis (g/100g bw)	0.07±0.003[a]	0.06±0.006 [b]	0.06±0.01[ab]	0.05±0.01[b]
Seminal vesicles (g/100g bw)	0.50±0.01[a]	0.45±0.10[a]	0.41±0.06[a]	0.21±0.04[a]
Prostate gland (g/100g bw)	0.33±0.01[a]	0.24±0.05[a]	0.28±0.02[a]	0.09± 0. 01[b]
Sperm motility (%)	71.50±0.96[a]	22.70±8.32[b]	9.33±0.95[b]	3.13±1.34[b]
Sperm viability (%)	71.76±3011[a]	27.01±2.48[b]	23.73±0.97[b]	20.65±2.82[b]
Sperm density (millions/ml)	356.00±17.71[a]	240.00±18.60[b]	226.67±28.80[bc]	171.25±10.51[c]
Sperm head tail separation (%)	9.28±2.18[a]	22.51±1.82[b]	38.84±0.77[c]	51.27±2.04[d]
Other sperm abnormalities (%)	4.28±0.93[a]	22.60±1.11[b]	29.98±0.99[c]	36.73±0.87[d]

[a-c]Values with different superscripts in a row differ significantly at $P \leq 0.05$

Table. 6-Effect of triptolide treatment on plasma levels of male sex hormone after different intervals of treatment withdrawal

Treatment (n=5 each)	Testosterone (ng/ml) (Mean ±SEM)			
	0%	0.15%	0.20%	0.25%
Immediately after termination of treatment	0.66±0.24	2.98±0.74	2.94±1.52	5.07±1.33
30 days after termination of treatment	0.66±0.24	2.26±0.95	4.15±0.82	4.10±1.61
60 days after termination of treatment	0.66±0.24	1.22±0.44	3.68±1.66	3.28±1.74

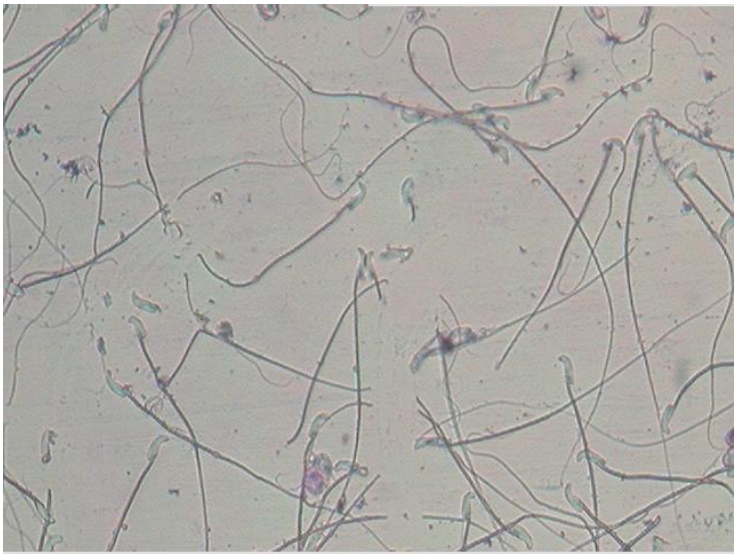

Figure 1. Giemsa stained cauda epididymal fluid smear of treated male rats showing sperm head tail separation.

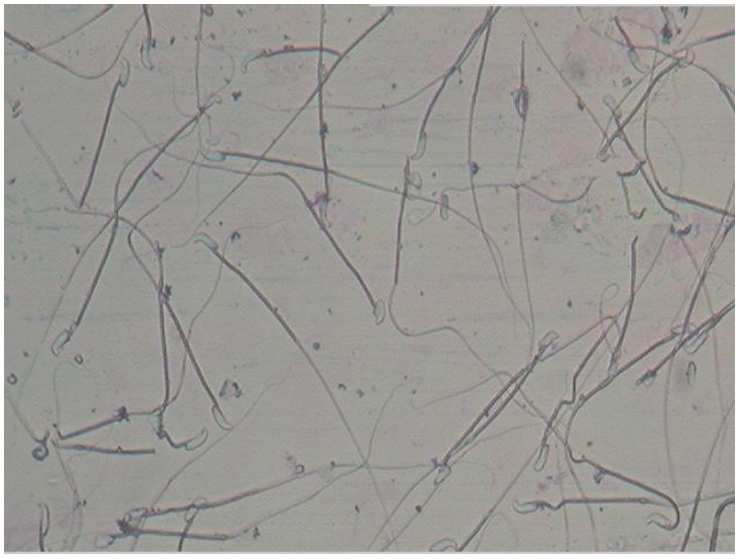

Figure 2. Giemsa stained cauda epididymal fluid smear of treated male rats showing other abnormalities in sperm head and tail regions.

Conclusion

The present study suggests triptolide to be a strong candidate for reducing reproductive output of male *B. bengalensis* via significantly affecting reproductive organs, cauda epididymal sperm motility, viability, density and morphology without causing much effect on the level of testosterone. Triptolide treatment in bait can be used as a follow up of chemical control for regulating post control population rebuildup of *B. bengalensis* under field conditions.

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