Reproductive inhibition with gossypol in the lesser bandicoot rat, *Bandicota bengalensis*

Singla, N., Meenu, M.
Department of Zoology, Punjab Agricultural University, Ludhiana-141004, Punjab, India,
neenasingla1@gmail.com

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Abstract

Different concentrations of pure gossypol (PG), cottonseed extract containing 8.3% gossypol (CSE) and crude cotton seed oil containing 0.01% gossypol (CCSO) were fed to *Bandicota bengalensis* of both sexes to determine their effects on reproduction. Single oral administration of either 40 or 80mg PG/kg body weight (bw), or 100 or 200 mg CSE/kg bw did not affect reproductive parameters. Feeding of bait containing 0.05% PG for 4 days; 0.01 or 0.02% PG for 16 days and 0.2 or 0.5% CSE for 7 or 15 days each caused dose dependent decreases in weights of reproductive organs and accessory sex glands and motility, viability and density of sperm in cauda epididymal fluid. There was a dose dependent increase in sperm abnormalities. Gossypol-specific sperm abnormalities were also observed. No breeding was observed in untreated cyclic female rats paired with male rats treated with 0.05 or 0.02% PG for 4 or 16 days respectively, or 0.5% CSE for 15 days. A significant ($p \leq 0.05$) increase in oestrous cycle length was observed in female rats fed bait containing 0.01% PG for 18 days or 0.5% CSE for 15 days.

No effect on the oestrous cycle was observed in female rats fed bait containing 5 or 10% CCSO for 13 days. Feeding of bait containing 10% CCSO for 15 or 30 days to male rats also had no significant effect on reproductive parameters. Treatment of farmer's sugarcane fields with 2% zinc phosphide followed by treatment with 10% CCSO for 15 or 30 days had no significant effect on rodent activity. Our results reveal that reproduction of *B. bengalensis* can be inhibited with multiple doses of gossypol but the effects are dose and time dependent.

Keywords: cotton seed extract, crude cotton seed oil, gossypol, reproductive inhibition

Introduction

Gossypol produces species specific and dose dependent antifertility effects in mammals. Yu and Chan (1998) emphasized the need for continuing studies on gossypol. The lesser bandicoot rat, *B. bengalensis* is the major rodent pest of South Asia inflicting heavy losses on agricultural crops. The success of this species lies in its highly adaptable nature coupled with high reproductive capacity. Reproductive inhibition is a non-lethal alternative that has the potential to provide long lasting control. The present study was conducted to evaluate the potential of gossypol in inhibiting reproduction of *B. bengalensis.*

Materials and methods

Mature and healthy *B. bengalensis* of both sexes were trapped from crop fields and acclimatized to laboratory conditions. Gossypol (PG) was obtained from MP Biomedicals, USA. Cotton seed extracted compound (CSE) was obtained by etheral extraction of *G. hirsutum* seeds for 24 hr. Crude cotton seed oil (CCSO) was procured from an oil refinery in Ludhiana. Gossypol content in CSE and CCSO was estimated according to the method described by AOCS (Singh, 1991). Male rats were given single oral doses of 40 or 80mg PG /kg bw (n=4/group), or 100 or 200 mg CSE/kg bw (n=3/group), or fed multiple doses in bait (cracked wheat: powdered sugar: powdered milk: groundnut oil, 85:5:5:5) of 0.05% PG for 4 days (n=3/group), 0.01 or 0.02% PG for 16 days (n=3 /group), 0.2% CSE for 7 or 15 days (n=3 per group), 0.5% CSE for 7 or 15 days (n=3/group) or 10% CCSO for 15 or 30 days (n=7/group). Female rats were fed 0.01% pure gossypol for 18 days (n=3/group), 0.2% CSE for 7 or 15 days (n=3/group), 0.5% CSE for 7 or 15 days (n=3/group) and 5 or 10% CCSO for 13 days (n=5/group).

For each treatment group there were equivalent numbers of untreated controls. Fifteen days after the end of treatment, all treated and untreated male rats were bred with untreated cyclic female rats for 15 days after which all the male rats were autopsied to record the effect of treatment on weights of reproductive organs and accessory sex glands, sperm motility, viability, density and morphology in cauda epididymal fluid and histomorphology of testes. Female rats were observed for pregnancy and delivery of pups. Vaginal fluid of treated and untreated female rats was observed during pre- and post-treatment periods

twice each day to determine effects on the oestrous cycle. Sugarcane fields at village Iraq, district Ludhiana, Punjab (India) were treated with 2% zinc phosphide followed by treatment with 10% CCSO for 15 or 30 days to compare rodent activity, based on pre- and post-treatment period census bait consumption, with that in untreated fields and fields treated with only 2% zinc phosphide.

Results

Single oral administration of either 40 or 80mg PG/kg bw, or 100 or 200 mg CSE/kg bw to male *B. bengalensis* did not affect the weights of reproductive organs and accessory sex glands and sperm parameters in cauda epididymal fluid. Feeding of bait containing 0.05% PG for 4 days; 0.01 and 0.02% PG for 16 days and 0.2 and 0.5% CSE for 7 and 15 days each caused a dose dependent decrease in the weight of the testis, epididymis and seminal vesicles and motility, viability and density of sperm in cauda epididymal fluid, and a dose dependent increase in sperm abnormalities.

In rats treated with PG, gossypol-specific sperm abnormalities (missing axial fibers and sheaths in the flagellar region of spermatozoa) were observed. No breeding was observed in untreated cyclic female rats bred with male rats treated with 0.05 or 0.02% PG for 4 and 16 days respectively or with 0.5% CSE for 15 days. There was a significant ($P \leq 0.05$) increase in duration of the oestrous cycle from 4.51±0.02 to 4.58±0.03 (t=2.74, df=4) and from 4.52±0.02 to 4.64±0.07 days (t=2.33, df=4) in female rats fed on bait containing 0.01% PG for 18 days and 0.5% CSE for 15 days, respectively.

Feeding of bait containing 5 and 10% CCSO for 13 days to female rats did not have any effect on the duration of the oestrous cycle. Feeding of bait containing 10% CCSO for 15 or 30 days to male rats also had no significant effect on the weights of reproductive organs, sperm parameters, histomorphology of testis and reproductive success of rats. No significant difference in rodent activity was observed among sugarcane fields treated with only 2% zinc phosphide and those treated with 2% zinc phosohide followed by treatment with 10% CCSO for 15 and 30 days.

Discussion

The absence of effects on reproduction following single oral doses of PG and CSE may be due to the fast elimination of gossypol from the body (Othman and Abou-Donia, 1988). In our studies, a greater increase in oestrous cycle length was observed in females treated with CSE than in those treated with PG. This greater effect has been attributed to other factors present in seed extracted samples (Eagle and Bialek, 1952). A dose dependent decrease in reproductive organ weights and sperm quality and quantity in rats after gossypol treatment was also observed by Taylor et al. (1991).

The reduction in sperm concentration in gossypol-treated small rodents has been attributed mainly to the damage caused to the germinal epithelium leading to low production of sperm (Hoffer, 1983). Spermatocyte apoptosis during the meiosis of spermatogenesis is the main reason for the antifertility (Chang et al., 2010). Gossypol specific abnormalities in sperm have been reported previously (Nadakavkaren et al., 1979). Longer oestrous cycle lengths in gossypol-treated rats have also been reported (Lin et al., 1985). The present results reveal that reproduction of *B. bengalensis* can be inhibited with multiple doses of gossypol but the effects are dose and time dependent.

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