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Antifertility effect of bait containing *Carica papaya* L. seed powder in male lesser bandicoot rat, *Bandicota bengalensis* (Gray and Hardwicke)

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In rodent control exercise, after successful control with rodenticides, the surviving population often rebuild up themselves rapidly due to reduced competition and high rate of reproduction. In this context, here, we investigated the potential of papaya (*Carica papaya* L.) seed powder as a natural reproduction inhibitor for male lesser bandicoot rat, *Bandicota bengalensis* (Gray and Hardwicke) in reducing the post control population rebuild up rate. Exposure of male *B. bengalensis* to cereal based bait containing 2, 3 and 5% papaya seed powder for 15 and 30 days durations in bi-choice laboratory feeding tests resulted in the ingestion of total 7.14-18.15 and 13.74-41.25 g/kg body wt. of the active ingredient, respectively. Breeding performance of treated rats was 33.33 and 16.67% after treatment with 5% papaya seed powder for 15 and 30 days, respectively. Autopsy of rats immediately after treatment withdrawal revealed a significant decrease in weights of testis, cauda epididymis, seminal vesicles and prostate gland. Sperm motility, sperm viability and sperm count were found reduced and sperm abnormality increased significantly in the cauda epididymal fluid of treated rats as compared to untreated rats. The total proteins, 17β-HSD, 3β-HSD and testosterone levels were also found decreased in treated rats as compared to untreated rats. All the changes observed in treated rats showed partial or no recovery after 30 days of treatment withdrawal. The study suggests the potential of papaya seed powder in regulating fertility of male *B. bengalensis* which may be exploited as a component of integrated rodent pest management.

Keywords: Antireproductive effect, Biological control, Indian mole rat, Integrated pest management (IPM), Reversibility, Rodents

The importance of medicinal plants as a source of antifertility drugs has been emphasized by many researchers[1-3]. Papaya (*Carica papaya* Linnaeus) (Cericaceae) seed extracts have shown significant scope in the quest for safer, cheaper and alternative method of regulating fertility in male rats[4], rabbits[5] and monkeys[6]. The *C. papaya* seeds contain active ingredients such as caricacin, an enzyme carpasemine, a plant growth inhibitor, and oleanolic glycoside which cause sterility in male rats[7]. Attempts to identify the chemical composition of chloroform[8,9], ethyl acetate[10], ethanol[11] and methanol[12] extracts of different parts of papaya plant have revealed the presence of compounds with antifertility property in rats.

Rodents are serious vertebrate pests causing huge losses to agricultural crops at pre and post harvest stages. Rodent damage up to 6.2% in wheat crop and 2.9% in rice crop has been observed in Punjab state (India)[13]. Among all the rodent species found throughout Southeast Asia, the lesser bandicoot rat or Indian mole rat, *Bandicota bengalensis* (Gray and Hardwicke) is the most destructive one causing heavy losses to field crops[14,15]. Conventional methods of controlling rodents all over the world include trapping, physical killing and use of repellents, antifeedants and rodenticides[16-18]. However, after a successful control with rodenticides, the remaining population of rodents rapidly rebuild up their population due to reduced competition and high rate of reproduction.

The antifertility effects of different extracts of *C. papaya* seeds were mostly evaluated in laboratory rats[9,19] in the process of development of human contraceptives. The present study attempts to evaluate the potential of papaya seed powder fed in bait as antifertility agent against a wild rodent pest species, *B. bengalensis* for managing rodent population.

Materials and Methods

Collection and maintenance of animals

Male *Bandicota bengalensis* were live trapped from crop fields and fish market, Ludhiana, Punjab (India). The rats were kept individually in cages for acclimatization in laboratory with food and water provided *ad libitum*. Food consisted of a mixture of

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cracked wheat, powdered sugar and groundnut oil (WSO bait) in ratio 96:2:2. Approval was obtained from Institutional Animal Ethics Committee of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, India for use of animals vide memo no. IAEC/2017/849-80 dated 07.08.2017 under Protocol no. GADVASU/2017/IAEC/41/15. Proper hygienic conditions were maintained. Aluminium trays were kept under each laboratory cage for collection and disposal of animal faeces and urine. After acclimatization, mature and healthy rats were weighed and divided into two sets of four groups each with 12 rats in each group.

Preparation of treatment bait with papaya seed powder

The ripe fruits of *C. papaya* var. Honey dew available in the local market were purchased to collect seeds. Seeds were washed, shade dried, ground to fine powder using domestic mixer grinder and stored in refrigerator. Treatment baits were prepared by mixing three different concentrations (2, 3 and 5%) of papaya seed powder in known quantity of WSO bait on w/w basis.

Feeding trials

During treatment, rats of three groups in first set were fed on treatment bait containing 2, 3 and 5% papaya seed powder, respectively in bi-choice with plain WSO bait for 15 days and the fourth group was kept as control (untreated) and was provided plain WSO bait. Similarly, rats of four groups of second set were exposed to similar concentrations of treated and untreated baits for 30 days. The positions of the bowls containing plain and treatment bait were changed daily to avoid any positional bias. Before and after the treatment, rats of all the groups were fed on plain WSO bait and water was available *ad libitum*. Bait consumption (g/100 g body wt.) was recorded after every 24 h to determine mean daily bait consumption. Based on the consumption of untreated and treatment baits during treatment period, per cent acceptance of treatment bait was determined as per the formula described by Dhar & Singla[20]. Total consumption of active ingredient (g/kg body wt.) in 15 and 30 days by treated groups of rats was also determined.

Evaluation of breeding potential

Immediately after termination of treatment in both the sets of rats, half of the treated and untreated rats in each group (n = 6 each) were weighed and kept for breeding with mature and cyclic female rats in male: female ratio of 1: 1. In each pair, body weight of male rats was more than that of the female rats. Large laboratory pens were used for breeding. Food (consisting of cracked wheat, powdered sugar, groundnut oil and milk powder in ratio 91:2:2:5 along with pre-soaked gram seeds) and water were provided *ad libitum*. Body weight of female rats of each grouped set was recorded at 10 days interval during breeding. Female rats were observed regularly for pregnancy and delivery of pups. The number and sex of pups delivered were also recorded.

Effect on organ weights and sperm parameters

Immediately after termination of treatment in both the sets of rats, rest half of the treated and untreated male rats in each group (n = 6 each) were weighed and autopsied to record antifertility effects of papaya seed powder. After 30 days of treatment withdrawal, male rats of each grouped set used for breeding experiments were also autopsied to record reversibility in antifertility effects. Immediately after autopsy, one of the cauda epididymis was taken out, incised longitudinally with a sharp blade and pressed in a watch glass to take out the cauda epididymal fluid in 0.5 mL of pre incubated 0.9 % saline solution. Various sperm parameters i.e. sperm motility (%), sperm viability (%), sperm count (millions/mL) and sperm abnormality (%) were recorded in the cauda epididymal fluid as per the method of Salisbury *et al.*[21] described by Singla *et al.*[22]. Reproductive organs (testes and cauda epididymes) and accessory sex glands (seminal vesicles and prostate gland) of all the rats were taken out, cleared of fat and weighed (g/100 g body wt.).

Effect on biochemical parameters

Total proteins (mg/g) were estimated as per the method of Lowry *et al.*[23] and specific activities of enzymes 17β-Hydroxy steroid dehydrogenase (17β-HSD) and 3β-HSD were estimated biochemically in testicular tissue homogenate of treated and untreated rats autopsied immediately and after 30 days of treatment withdrawal as per the methods of Agular *et al.*[24]. Enzyme activity (units/min/mg total proteins) was determined by formula given below:

$$\frac{\text{Absorbance (difference of first and last value)} \times \text{assay volume (2 mL)}}{\text{Volume of tissue extract (0.5 mL)} \times 1.5} \times 100$$

Effect on hormone level

Level of testosterone (ng/dL) of treated and untreated rats autopsied immediately and after 30 days of termination of treatment was estimated in

plasma using ELISA kit as per the manufacturer's protocol. Whole blood (0.9 mL) of all the male rats was collected immediately after dissection through cardiac puncture in a clean tube containing 0.1 mL of 3.2% tri-sodium citrate solution as an anticoagulant. Blood was centrifuged at 3000 rpm for 15 min. The supernatant plasma was collected in a separate tube and stored at –20°C until analysis.

Statistical analysis

Values were determined as Mean ± SE. Data for all the parameters was collected using factorial experiments in completely randomized design. Analysis was done using general linear model in SAS version 9.3. All pair wise treatment comparisons were made using Tukey's HSD test at 5% level of significance.

Results

Acceptance of treatment bait and active ingredient ingested

There was no significant difference in body weight among treated and untreated groups of rats used for experimentation. Feeding of WSO bait containing 2, 3 and 5 % papaya seed powder by treated groups of rats and plain WSO bait by untreated group of rats for 15 and 30 days durations in bi-choice revealed significantly lower mean daily consumption (g/100 g body wt.) of treatment baits at all the three test concentrations as compared to untreated bait. There was no significant difference in per cent acceptance of treatment bait among the three treated groups of rats of both sets. The acceptance of treatment bait varied from 36.44 to 44.21% and 35.18-37.07% in groups of rats treated for 15 and 30 days durations, respectively. The total active ingredient ingested after 15 and 30 days of treatment with different concentrations of papaya seed powder was 7.14-18.15 and 13.74-41.25 g/kg body wt., respectively. The difference in active ingredient ingested among groups of rats treated with 2, 3 and 5% papaya seed powder was found to be statistically significant during both 15 and 30 days treatments (Table 1).

Effect on reproductive success of male rats

Treatment for 15 days

Data presented in Table 2 revealed that out of the six untreated females paired with untreated male rats of first set, four (66.67%) delivered pups with a litter size of 5 to 8 (Mean: 6.25±0.53). Out of the six female rats paired with males treated with 2 and 3% papaya seed powder only two delivered pups in each case indicating 33.33% breeding success. The number of pups delivered was 4-6 (Mean: 5.00±0.40) per

Table 1 — Acceptance of bait treated with different concentrations of papaya seed powder by male *Bandicota bengalensis* during treatments for 15 and 30 days durations

Treatment duration	Conc.	Body weight (g) (n =12 each)	Mean daily bait consumption (g/100 g body wt.)		Per cent acceptance of treated bait	Active ingredient ingested (g/kg bwt)
			Untreated bait	Treated bait		
15 days	0%	275.00±20.50	5.65±1.23	-	-	-
	2%	341.83±20.90	4.15±0.14	2.38±0.20*	36.44±1.60^{a}	7.14±0.05^{a}
	3%	314.50±19.06	3.47±0.13	2.79±0.20*	44.21±1.76^{a}	12.25±0.20^{b}
	5%	277.33±7.60	3.19±0.11	2.42±0.11*	43.13±1.66^{a}	18.15±0.10^{c}
30 days	0%	281.00±10.20	5.66±0.19	-	-	-
	2%	328.16±20.20	3.87±0.27	2.29±0.18*	37.07±1.28^{a}	13.74±0.10^{a}
	3%	329.00±7.76	4.37±0.23	2.37±0.12*	35.18±1.12^{a}	21.33±0.80^{b}
	5%	321.50±12.45	5.05±0.13	2.75±0.27*	35.25±2.25^{a}	41.25±0.70^{c}

[Values are Mean±SE. *****Significance difference between treated and untreated bait consumptions. Values with different superscripts (a-c) in a column differ significantly at $P \leq 0.05$]

Table 2 — Effect of treatment with different concentration of papaya seed powder for 15 and 30 days durations on breeding success of male *Bandicota bengalensis*

Treatment duration	Conc. (n = 6 each)	No. of females delivered pups	Percent breeding success	No. of pups delivered (range)	No. of male pups	No. of female pups
15 days	0%	4	66.67%	6.25±0.53^{a}(5-8)	2.65±0.25^{a}	3.60±0.20^{a}
	2%	2	33.33%	5.00±0.40^{a}(4-6)	1.50±0.20^{a}	3.50±0.20^{a}
	3%	2	33.33%	5.00±0.40^{a}(4-6)	1.50±0.20^{a}	3.50±0.20^{a}
	5%	2	33.33%	3.50±0.20^{a}(3-4)	1.50±0.20^{a}	2.00±0.20^{b}
30 days	0%	3	50%	6.00±0.81^{a}(4-8)	2.00±0.40^{a}	4.00±0.41^{a}
	2%	2	33.33%	4.00±0.33^{a}(3-5)	1.33±0.15^{a}	2.66±0.19
	3%	2	33.33%	4.00±0.33^{a}(3-5)	1.33±0.15^{a}	2.66±0.19^{a}
	5%	1	16.67%	2.00±0.40^{a}(1-3)	1.50±0.20^{a}	0.50±0.20^{b}

[Values are Mean±SE. Values with different superscripts (a-b) in a column differ significantly at $P \leq 0.05$]

female. Likewise female rats paired with males treated with 5% papaya seed powder also registered 33.33% breeding success with a litter size of 3-4 (Mean: 3.50±0.20) pups per female (Table 2). There was no significant difference in total number of pups and male pups delivered by females paired with treated and untreated males. However, the number of female pups was significantly reduced when females were paired with males treated with 5% papaya seed powder for 15 days. There was an increase in body weight of all the pregnant female rats as observed after 10 days interval (Fig. 1A).

Treatment for 30 days

Out of the six female rats paired with males treated with 2 and 3% papaya seed powder for 30 days duration, two delivered pups in each case indicating 33.33% breeding success with a litter size of 3-5 (Mean: 4.00±0.33) pups per female, whereas when untreated females were paired with untreated males, the breeding success was 50% with a litter size of 4-8 (Mean: 6.00±0.81) per female. . In case of rats treated with 5% papaya seed powder for 30 days, breeding success was the least (16.67%) with a litter size of 1-3 (Mean: 2.00±0.40) (Table 2). There was no significant difference in total number of pups and male pups delivered by females paired with treated and untreated males. However, the number of female pups was significantly reduced when females were paired with males treated with 5% papaya seed powder for 30 days. There was an increase in body weight of all the pregnant female rats as observed after 10 days interval (Fig. 1B).

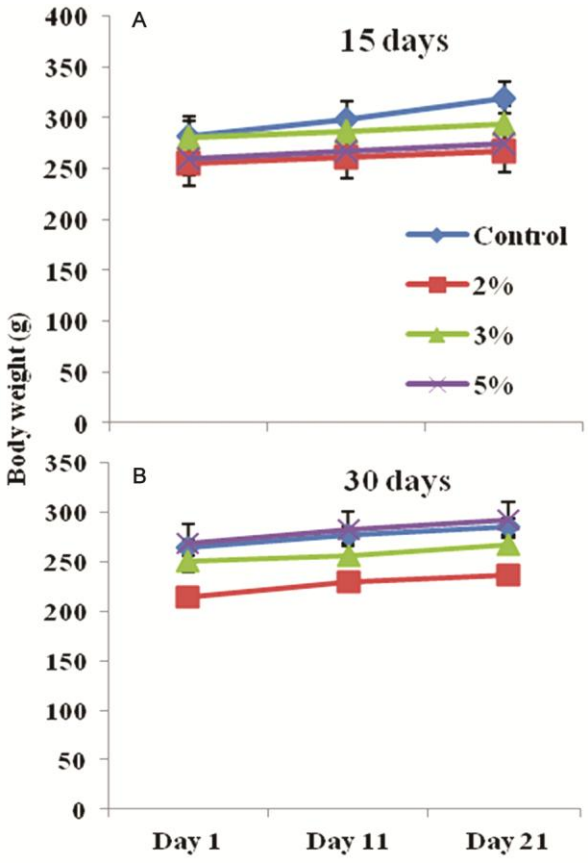

Fig. 1 —Increase in body weight of female rats during breeding with male rats treated for (A) 15; and (B) 30 days duration

Effect on sperm parameters

A significant effect of treatment with papaya seed powder was observed on sperm motility (%), sperm viability (%), sperm count (millions/ml) and sperm abnormality (%) in the cauda epididymal fluid of rats treated for both 15 and 30 days.

Treatment for 15 days

In the rats treated for 15 days and autopsied immediately after termination of treatment, sperm motility, sperm viability and sperm count in cauda epididymal fluid were found to decrease significantly from that of untreated group, however, there was no significant difference between groups treated with 2 and 3% concentrations of papaya seed powder. There was a significant increase in sperm abnormalities in rats treated with 3 and 5% concentrations in comparison to the untreated rats and rats treated with 2% concentration. There was no significant difference in sperm abnormalities between untreated rats and those treated with 2% concentration (Fig. 2A).

In the rats treated for 15 days and autopsied after 30 days of treatment withdrawal, there was no significant difference in sperm motility in the cauda epididymal fluid among treated and untreated groups of rats indicating no recovery in antifertility effect. However, sperm viability and sperm count were found to differ significantly among all the treated and untreated groups. There was a significant increase in sperm abnormalities in rats of groups treated with 3 and 5% concentrations from that observed in untreated rats and rats treated with 2% concentration which are at par amongst them (Fig. 2A). Different sperm abnormalities observed were head shape, coiling of middle piece, coiling of tail and head tail separation.

Treatment for 30 days

In the rats treated for 30 days and autopsied immediately after treatment withdrawal, sperm motility and sperm viability were found to differ significantly among all the treated and untreated groups of rats. A significant difference in sperm count was observed

between untreated and treated groups of rats but the difference in sperm count between rats treated with 2 and 3% concentrations was not found to differ significantly. There was an increase in sperm abnormalities in rats of groups treated with 3 and 5% concentrations from that observed in untreated rats and rats treated with 2% concentration which was at par amongst each other. There was no significant difference in sperm abnormalities between untreated rats and those treated with 2% concentration (Fig. 2B).

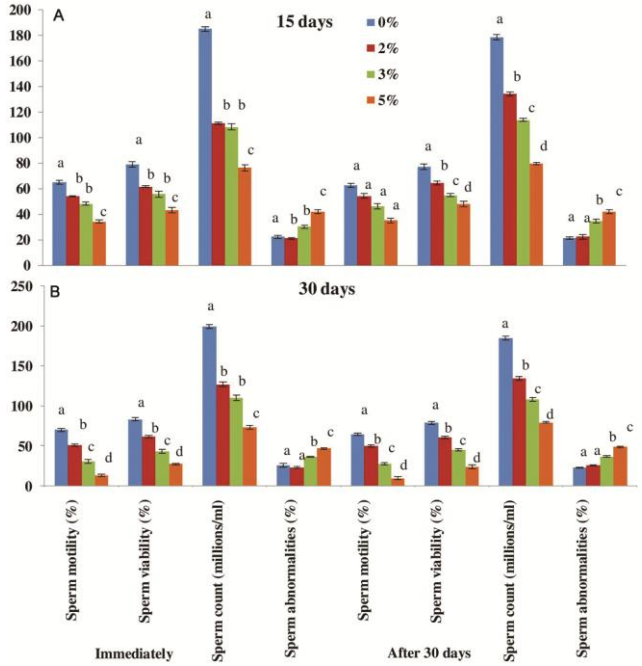

Fig. 2 —Comparison of sperm motility, sperm viability, sperm count and sperm abnormality in cauda epididymal fluid of rats autopsied immediately and after 30 days of termination of treatment with different concentrations of papaya seed powder for (A) 15 days; and (B) 30 days duration. Bars with different superscripts (a-d) indicate significant difference at $P \leq 0.05$

In rats treated for 30 days and autopsied after 30 days of treatment withdrawal, there was a significant difference in sperm motility, sperm viability and sperm count among all the treated and untreated groups of rats indicating no recovery in antifertility effects. There was a significant increase in sperm abnormalities in rats of groups treated with 3 and 5% concentrations from that observed in untreated rats and rats treated with 2% concentration. There was no significant difference in sperm abnormalities between untreated rats and those treated with 2% concentration (Fig. 2B). Different sperm abnormalities observed were head shape, coiling of middle piece, coiling of tail and head tail separation.

Overall, comparison of effect of treatment with different concentrations of papaya seed powder for 15 days on sperm parameters revealed no significant difference in sperm motility, sperm viability, sperm count and sperm abnormality between rats dissected immediately and after 30 days of treatment withdrawal indicating no recovery in antifertility effects of papaya seed powder (Fig. 3 A-D).

Overall, treatment with different concentrations of papaya seed powder for 30 days on sperm parameters in the cauda epididymal fluid also revealed no significant difference in sperm motility, sperm viability, sperm count and sperm abnormality between rats treated with different concentrations of papaya seed powder and dissected immediately and after 30 days of treatment withdrawal indicating no recovery in antifertility effects of papaya seed powder (Fig. 4 A-D).

Effect on weights of reproductive organs and accessory sex glands

A significant effect of treatment with 2, 3 and 5% papaya seed powder for 15 and 30 days durations was observed on weights of testis, cauda epididymis, seminal vesicles and prostate gland.

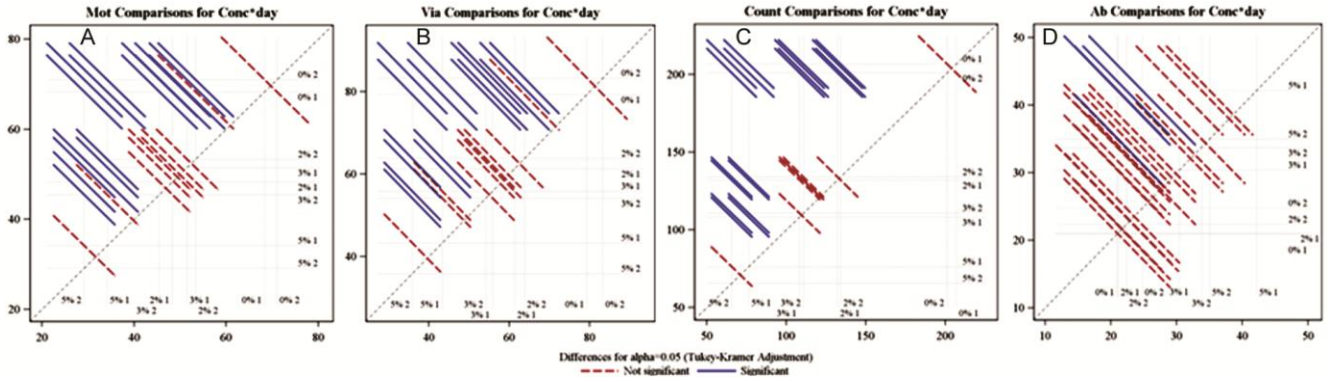

Fig. 3 — Comparison of (A) sperm motility; (B) sperm viability; (C) sperm count; and (D) and sperm abnormality in cauda epididymal fluid of rats treated with different concentrations of papaya seed powder for 15 days and autopsied immediately (1) and after 30 (2) days of termination of treatment

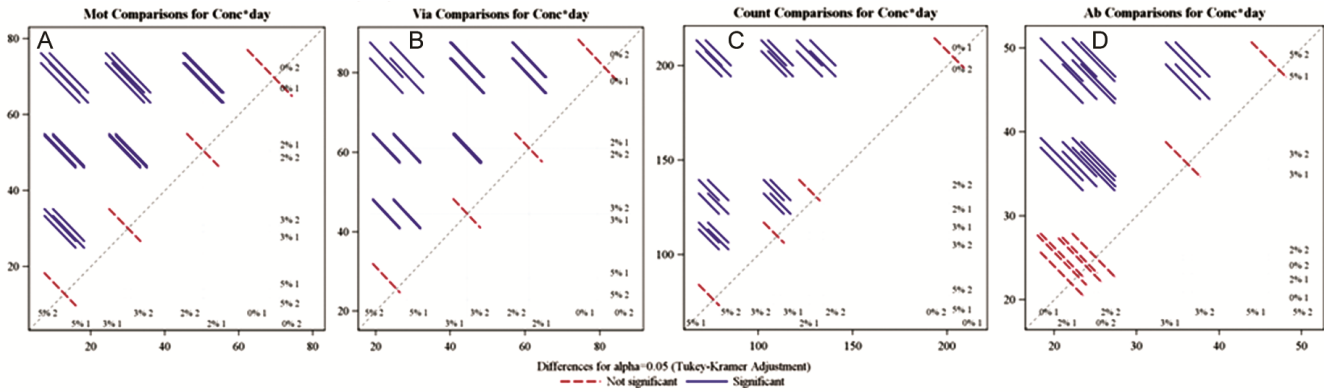

Fig. 4 — Comparison of (A) sperm motility; (B) sperm viability; (C) sperm count; and (D) sperm abnormality in cauda epididymal fluid of rats treated with different concentrations of papaya seed powder for 30 days and autopsied immediately (1) and after 30 (2) days of termination of treatment

Treatment for 15 days

Results revealed that the rats treated for 15 days and autopsied immediately after treatment withdrawal recorded a significant decrease in weight of testis in all the three treated groups with respect to each other as well as with untreated group. The weight of epididymis was significantly lower in rats of groups treated with 3 and 5% papaya seed powder as compared to untreated rats and rats treated with 2% concentration. There was no significant difference in weight of seminal vesicles among treated and untreated groups of rats. The weight of prostate gland in rats treated with 5% concentration was significantly lower from that of untreated rats and rats treated with 2 and 3% concentrations (Fig. 5A).

In rats treated for 15 days and autopsied after 30 days of treatment withdrawal, a significant decrease in weights of testis and seminal vesicles was observed in all the three treated groups with respect to each other as well as with respect to that of rats of untreated group. The weight of epididymis was significantly lower in rats of groups treated with 5% papaya seed powder as compared to untreated rats and rats treated with 2 and 3% concentrations. The weight of prostate glands in treated rats was significantly lower from that of untreated rats but there was no significant difference among the three treated groups of rats (Fig. 5A).

Treatment for 30 days

In the rats treated for 30 days and dissected after 30 days of termination of treatment, a significant decrease in weight of testis was observed between untreated group of rats and those treated with 3 and 5% concentrations of papaya seed powder. However, there was no significant difference between rats

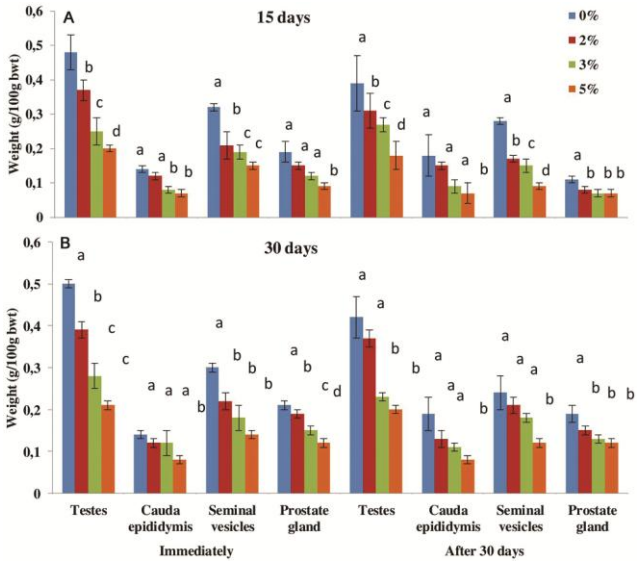

Fig. 5 —Comparison of weights of testis, cauda epididymis, seminal vesicles and prostate gland of rats autopsied immediately and after 30 days of termination of treatment with different concentrations of papaya seed powder for (A) 15; and (B) 30 days duration. Bars with different superscripts (a-d) indicate significant difference at $P \leq 0.05$

treated with 3 and 5% concentrations and between untreated rats and rats treated with 2% concentration. The weight of cauda epididymis was significantly low in rats of group treated with 5% papaya seed powder (0.08 g/100 g body wt.) as compared to untreated rats (0.11 g/100 g body wt.) and rats treated with 2% (0.13 g/100g bwt) and 3% (0.08 g/100 g body wt.) concentrations. There was no significant difference in weight of seminal vesicles among treated and untreated groups of rats. The weight of prostate gland among treated groups of rats was significantly at par but lower than in untreated rats (Fig. 5B).

Overall, comparison of effect of treatment with different concentrations of papaya seed powder for 15

days on weights of different reproductive organs and accessory sex glands revealed a significant increase in weight of cauda epididymis and a significant decrease in weight of prostate gland at 2% concentration after 30 days of treatment withdrawal as compared to that observed immediately after treatment withdrawal. There was no significant difference in weights of organs at other concentrations immediately and after 30 days of treatment withdrawal indicating no or partial recovery in antifertility effects of papaya seed powder (Fig. 6 A-D).

Comparison of effect of treatment with different concentrations of papaya seed powder for 30 days on weights of different reproductive organs and accessory sex glands revealed a significant decrease in weight of testis at all the concentrations after 30 days of treatment withdrawal as compared to that observed immediately after treatment withdrawal. There was no significant difference in weights of other organs immediately and after 30 days of treatment withdrawal indicating no or partial recovery in antifertility effects of papaya seed powder (Fig. 7 A-D).

Effect on biochemical parameters and level of testosterone

Treatment for 15 days

Results revealed that the rats treated for 15 days and autopsied immediately after treatment withdrawal recorded a dose dependent decrease in level of total proteins, 17β-HSD and 13β-HSD in testicular tissue with a significant difference in level between rats treated with 3 and 5% concentrations from untreated rats and those treated with 2% concentration. The level of testosterone in blood plasma decreased in a dose dependent manner with a significant difference among all the treated and untreated groups of rats (Fig. 8A).

In rats autopsied after 30 days of treatment withdrawal also, the level of total proteins, 17β-HSD and 13β-HSD in testicular tissue and plasma level of testosterone were reduced in a dose dependent manner. A significant difference was found in their level among all the treated and untreated groups of rats (Fig. 8A).

Treatment for 30 days

In rats treated for 30 days and autopsied immediately after treatment withdrawal, there was a

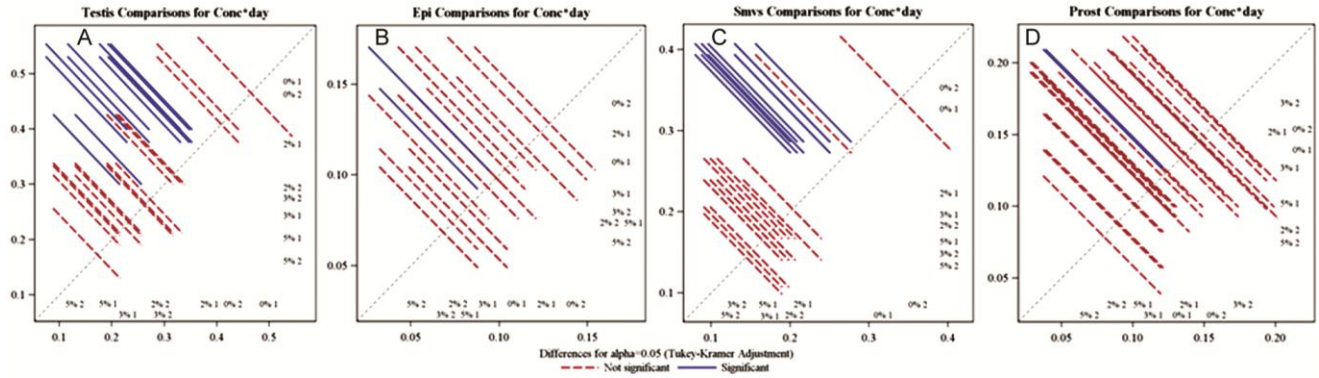

Fig. 6 — Comparison of (A) testis; (B) cauda epididymis; (C) seminal vesicles; and (D) prostate gland of rats treated with different concentrations of papaya seed powder for 15 days and autopsied immediately (1) and after 30 (2) days of termination of treatment

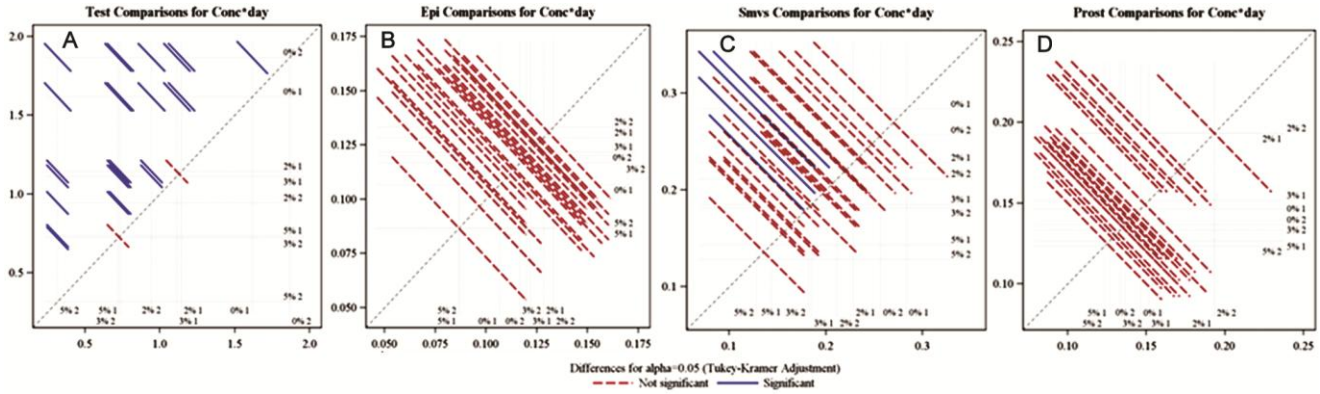

Fig. 7 — Comparison of (A) testis; (B) cauda epididymis; (C) seminal vesicles; and (D) prostate gland of rats treated with different concentrations of papaya seed powder for 30 days and autopsied immediately (1) and after 30 (2) days of termination of treatment

dose dependent decrease in level of total proteins, 17β-HSD and 13β-HSD in testicular tissue and the level of testosterone in blood plasma. A significant difference was found among all the treated and untreated groups of rats (Fig. 8B).

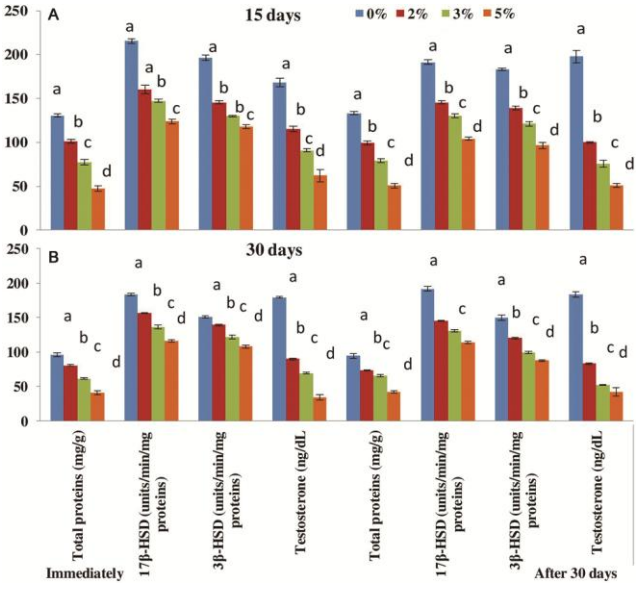

Fig. 8 —Comparison of level of total proteins, 17β-HSD, 3β-HSD and testosterone in rats autopsied immediately and after 30 days of termination of treatment with different concentrations of papaya seed powder for (A) 15; and (B) 30 days duration. Bars with different superscripts (A-D) indicate significant difference at $P \leq 0.05$

In rats autopsied after 30 days of treatment withdrawal also, the level of total proteins, 17β-HSD and 13β-HSD in testicular tissue and plasma level of testosterone were found reduced in a dose dependent manner. A significant difference was found in their level among all the treated and untreated groups of rats (Fig. 8B).

Overall, comparison of effect of treatment with different concentrations of papaya seed powder for 15 days on biochemical parameters and testosterone level revealed significant difference in total proteins at 5% concentration, 17β-HSD at 2% concentration and 3β-HSD at both 2 and 5% concentration between rats dissected immediately and after 30 days of treatment withdrawal. All these parameters were found further reduced in rats after 30 days of treatment withdrawal. Level of testosterone at 2% concentration was also found reduced further in rats after 30 days of treatment withdrawal thus indicating no recovery in antifertility effects of papaya seed powder (Fig. 9A).

Overall, the treatment with different concentrations of papaya for 30 days on biochemical parameters in testicular tissue revealed no significant difference in total proteins, 17β-HSD and 3β-HSD between rats dissected immediately and after 30 days of treatment withdrawal. Level of testosterone at 2% concentration

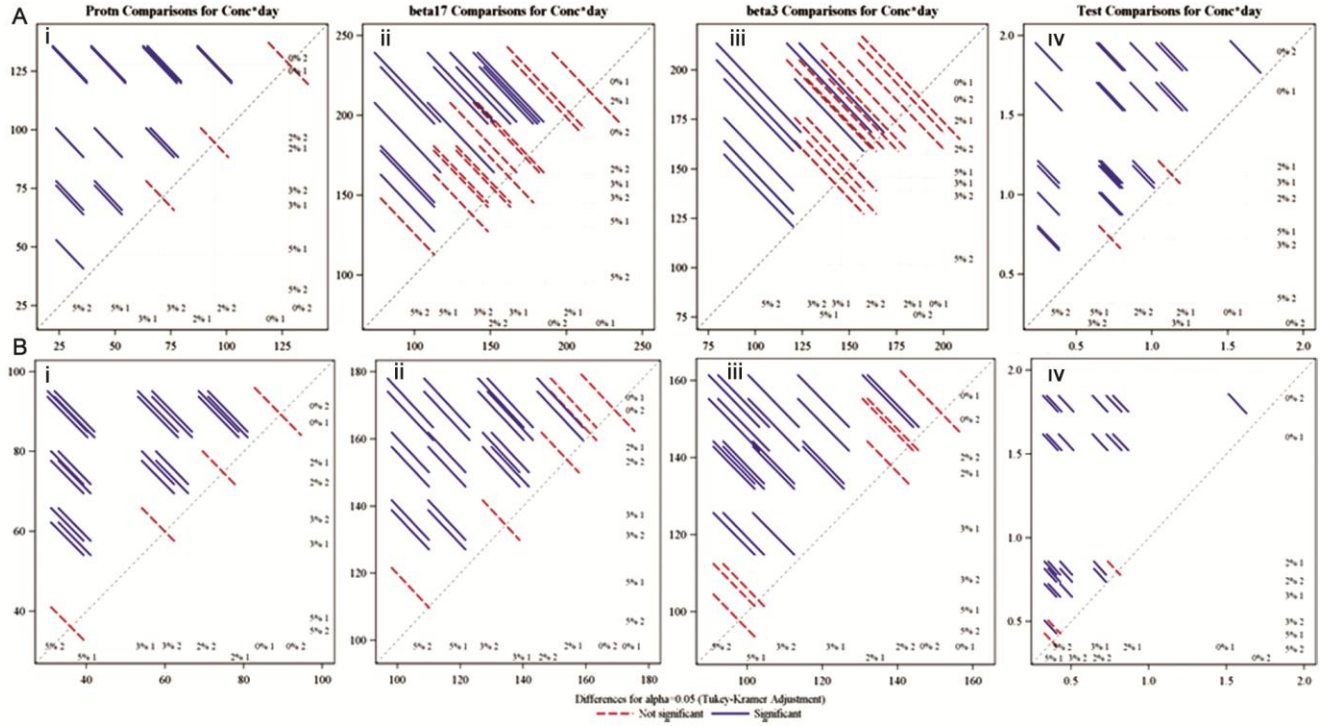

Fig. 9 — Comparison of level of (i) total proteins; (ii) 17β-HSD; (iii) 3β-HSD; and (iv) testosterone of rats treated with different concentrations of papaya seed powder for (A) 15; and (B) 30 days and autopsied immediately (1) and after 30 (2) days of termination of treatment

was found reduced in rats after 30 days of treatment withdrawal thus indicating no recovery in antifertility effects of papaya seed powder (Fig. 9B).

Discussion

The seeds of *C. papaya* have been very much perceived to have the compound that has potential to control fertility. Fertility status of a male individual can be identified by semen quality which depends upon the sperm motility, sperm viability and sperm count[22]. The decrease in these parameters proves impaired level of male androgens that are involved in regulating the testicular functions[25]. Significant reduction in plasma testosterone in treated groups reported in present study indicate the antispermatogenic effect of *C. papaya* seed powder through decreased steroidogenesis. 3β-HSD and 17β-HSD, the key enzymes of androgenesis convert androstenedione to testosterone through hydrogenation[26]. In present study, the activity levels of these two enzymes were also found decreased significantly in testes of treated rats suggesting that papaya seed powder interferes with steroid hormone biosynthesis, which ultimately results in impaired spermatogenesis and infertility.

Similar to present studies, suppression of epididymal sperm motility, sperm viability and sperm concentration with increase in sperm abnormality in laboratory rats and mice was observed after treatment with papaya seed extracts[8,9]. Unripe papaya seed extract deteriorated quantity and quality of the sperm with suppression of spermatogenesis in rats[27]. Oral administration of chloroform extract of papaya seed in the langur monkey revealed decrease in sperm concentration leading to azoospermia, total inhibition of sperm viability and increase in sperm abnormality after 60 days of treatment[28]. As observed in present study, Chinoy *et al.*[29] also observed reversible alterations in the sperm motility and morphology thereby impairing fertility in male rodents after treatment with crude aqueous extract of papaya seeds. Oral administration of papaya seed extract also induced reversible infertility in male rabbits[5].

Present studies are in conformity with the reports of, Lohiya & Goyal[8] who observed complete loss of fertility after 40 to 60 days of treatment with crude chloroform extract (5 mg/kg body wt./day for 20, 40 and 60 days) of *C. papaya* seeds in male albino rats that is similar to present investigation with male *B. bengalensis*. Lohiya *et al.*[5] concluded that the methanol extract of *C. papaya* seeds was safe to use as male antifertility agent and the isolated compounds from the seeds of *C. papaya* were equally effective in terms of contraceptive efficacy, reversibility, and with no adverse side effects.

Most of the previous studies have reported reversible antifertility effects of papaya seed after treatment withdrawal[5,19], but in present study, the effects caused by papaya seed powder were not reversed or partially reversed even after 30 days of treatment withdrawal.

Conclusion

Present study thus reveals a significant effect of different concentrations of papaya (*Carica papaya*) seed powder fed for 15 and 30 days on weight of reproductive organs, sperm parameters and level of androgens of male *Bandicota bengalensis* which were not reversed or partially reversed after 30 days of treatment withdrawal. The study establishes highest antifertility effects of 5% papaya seed powder fed for 30 days against male *B. bengalensis*. However, further studies under field condition are required for including this fertility regulating strategy in integrated rodent pest management programs.

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Conflict of interest

Authors declare no competing interests.

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