**Carica Papaya modulates the organ histology, biochemicals, estrous cycle and fertility of Bandicota bengalensis rats**

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**ABSTRACT**

The present study is the first record of the effect of cereal bait formulated with papaya seed powder (PSP) on reproductive functions of female *Bandicota bengalensis*. Treatment baits prepared using WSO (wheat, sugar, oil mix) bait and PSP in ratios 97:3, 95:5 and 90:10 were fed to three groups of rats for 30 days in no-choice with one group as untreated control. The average weight of rats used was 235 g. Rats were evaluated immediately and after 30 days of treatment withdrawal. Acceptance of treatment baits by different groups was 79.06–89.45%, leading to the ingestion of 68.36–186.23 g/kg bwt of PSP. Results revealed significant (P < .05) anti-fertility effects of 10% PSP based bait in the reduction in the relative weight of the uterus; the number of primordial and pre-antral ovarian follicles, breeding success, litter size, duration of the estrous stage, and prolongation of proestrous and diestrous stages further indicated by significantly (P < .05) reduced levels of estradiol, 17β-HSD, ACP and ALP. The present study revealed anti-reproductive effects of 10% PSP-based cereal bait, which were recovered after 30 days of treatment withdrawal, thus suggesting further studies using higher concentrations of PSP for long-term irreversible effects.

1. **Introduction**

The lesser bandicoot rat, *Bandicota bengalensis* found throughout Southeast Asia (Singla and Babbar 2010), causes severe damage to agriculture (Singla and Babbar 2010) besides being implicated in the transmission of various zoonotic diseases (Singla et al. 2008, 2016). A growing concern, regarding environmental hazards caused due to excessive use of rodenticides (Smith and Shore 2015), has focused interest on using environment-friendly management approaches (Nattrass 2015).

The use of biologically active plant components in controlling fertility (Dhar and Singla 2014; Singla 2015) is a promising approach that can limit rodenticides (Mukhtar et al. 2018). Different parts of the papaya (*Carica papaya*) plant, such as leaves, seeds, fruit, latex and bark, have been investigated for developing effective contraceptive extracts (Chinoy et al. 1997; Udoh et al. 2005; Nwaehujor et al. 2014; Ekhtor and Shelu 2015; Julaeha et al. 2015; Odirichukwu 2015; Punitha et al. 2015; Odirichukwu et al. 2016; Kumari et al. 2017; Satriyasa et al. 2018). These plant parts contain enzymes, lycopenes, carotenoids, alkaloids, monoterpenoids, flavonoids, minerals, vitamins, etc. (Sindhu et al. 2019) that cause infertility (Adebiyi et al. 2003, 2004; Estella et al. 2020; Hakameri CS and Usman 2020). Ingestion of papaya seeds during pregnancy has been unsafe (Adebiyi et al. 2002). They affect the reproductive ability of female rats by disrupting the estrous cycle, increasing atresia in ovarian follicles, affecting androgens and reducing litter size (Dosumu et al. 2008; Memudu and Oluwole 2021).

Most of the previous studies have reported anti-fertility effects of different extracts of papaya seeds when administered orally to laboratory rats and mice, keeping in view the development of human contraceptives (Raji et al. 2005; Chinoy et al. 2006; Naik et al. 2015; Novitasari et al. 2018). No study has yet reported the effect of papaya seeds on the reproduction of wild female rodent species. Also, to use the anti-reproductive property of papaya seeds to manage the wild rodent population, there is a need to develop a bait formulation that can be easily applied under field conditions.

The present study was conducted to investigate the effect of cereal bait formulation based on papaya seed powder (PSP) as an ingredient on various reproductive functions such as estrous cycle, fertility, biochemicals and histology of reproductive organs in female *B. bengalensis* to provide a technology for integrated rodent pest management programme.

2. **Materials and methods**

2.1. **Experimental design**

2.1.1. **Study duration and location**

The present study was carried out in the Department of Zoology, Punjab Agricultural University (PAU), Ludhiana, Punjab, India, from September 2018 to August 2019.
2.1.2. Plant material and treatment bait preparation

The papaya fruits (C. papaya var. Honey dew) available in the local market were identified by the Department of Fruit Science, College of Horticulture and Forestry, PAU, Ludhiana. Fruits weighing 1.5–2.0 kg were elongated and oval with dark yellow skin and ridges on the surface (Gunnannavar et al. 2017). Their seeds were collected, washed gently under tap water, shade dried and pulverized into a fine powder using a domestic grinder (Kaur et al. 2021). The required quantity of treatment baits was prepared using WSO (wheat, sugar, oil mix) bait and PSP in ratios 97:3, 95:5 and 90:10, respectively.

2.1.3. Animal collection and maintenance

Animals were collected and maintained in the laboratory, as per the protocol described by Sidhu et al. (2020). Mature male (with scrotal testes) and female (with perforated vaginal opening) B. bengalensis with body weight of 200–300 g were live-trapped from crop fields and fish market, Ludhiana in Punjab province of India and kept in individual metallic cages (36 × 23 × 23 cm) for acclimatization under laboratory conditions for 10–15 days. Food and water were provided ad libitum. Food consisted of WSO bait (a loose mixture of cracked wheat, powdered sugar and refined groundnut oil in 96: 2: 2). The metallic trays were kept under each cage for daily collection and disposal of urine and faecal pellets. To prevent chances of any zoonotic disease transmission from wild rats, a full-sleeved apron, face mask and hand gloves were used while handling the animals.

2.1.4. Cyclicity of female rats

Before experimentation, the cyclicity of female rats was determined by observing their vaginal fluid for the presence, absence or proportional numbers of different types of cells such as leucocytes, epithelial cells and cornified cells (Goldman et al. 2007). Vagina was flushed with 0.2 ml of 0.9% saline solution and gently aspirated using a Pasteur pipette. A drop of vaginal fluid was then placed on a glass slide for immediate examination under a light microscope (Magnus, CH20i, Olympus (India) Pvt. Ltd., Noida, India), as per the method described in Sandhu and Singla (2019). Vaginal fluid was examined every 24 h and continued till the completion of one cycle. Three consecutive cycles were studied for each rat to determine cyclicity (Sahu and Maiti 1978). Non-cyclic female rats were not used for the experiment. All the rats were housed singly in laboratory cages for experimentation. The average body weight of all the cyclic female rats used was 235 g.

2.1.5. Animal grouping and treatment

Cyclic female rats were weighed and divided into four groups each (three treated and one control). Each group consisted of six replicated rats. During treatment, rats of treated groups I, II and III of all the four sets were fed on treatment baits containing different concentrations of PSP (3, 5 and 10%) for 30 days in a no-choice feeding test. Rats of group IV, kept as untreated control, were fed on WSO bait only. The treatment dosage was based on our earlier study (Kaur et al. 2021) on using a maximum 5% PSP in bait against male rats under bi-choice conditions where the anti-fertility effect was not much significant. Soin studied against female rats, the concentration of PSP was increased to 10% in bait in a no-choice condition. Before and after the treatment, rats in all the groups were fed on WSO bait. A 40 g formulated bait was fed to rats daily, which was replenished every 24 h. Bait consumption (g) by rats in all the sets and groups was recorded daily to calculate the mean daily consumption (g/100 g body weight (bwt)) of bait and the total amount of PSP ingested (g/kg bwt) in 30 days, as per the method described in Sidhu et al. (2020). Percent acceptance of treatment bait over untreated bait consumed during the pre-treatment period was determined as per the formula described in Singla and Parshad (2002) and given below:

Percent acceptance = \[ \frac{\text{Amount of papaya seed ingested (g/kg bwt)}}{\text{Amount of PSP mixed in 100g bait}} \times \frac{100}{Y} \times 10 \]

where Y = Total amount (g/100 g bwt) of treated bait containing a particular concentration of PSP consumed in 30 days by rats.

2.1.6. Animal ethics clearance

Approval from the Institutional Animal Ethics Committee (IAEC) for using animals (B. bengalensis of both sexes) was obtained vide memo no. IAEC/2018/1153-1188 under Protocol no. GADVASU/2018/IAEC/46/16. The procedures used in this study adhere to the Committee for the Purpose of Control and Supervision of Experiments on Animals, GADVASU (Guru Angad Dev Veterinary and Animal Sciences, University), Ludhiana, India.

2.1.7. Animal sacrifice and tissue and blood collection

Animals in different sets and groups were evaluated per the protocol given in Table 1. As per CPCSEA guidelines (The Committee for the Purpose of Control and Supervision of Experiments on Animals), rats were sacrificed by cervical dislocation after sedation with pentobarbitone sodium. Rats of all the four groups in the first set were sacrificed immediately after treatment withdrawal and that of the second set after 30 days of treatment withdrawal. Rats were dissected to take out both the ovaries and uterus. These were cleared of fatty tissues, soaked on filter papers and weighed (g/100 g bwt) using an electronic balance to determine their relative weights, as described in Dhar and Singla (2014). The whole ovary (left) and piece of the uterus of three female rats in each set and group were collected for histological study. Blood (up to 1 ml) of all the female rats was collected by a cardiac puncture. A clean tube containing 0.1 ml anticoagulant (3.2% tri-sodium citrate solution) was used to collect 0.9 ml of blood. Blood was centrifuged at 3000 rotations per minute (rpm) for 15 min. The supernatant plasma obtained after centrifugation was stored at −20°C in a separate tube until analysis (Sidhu et al. 2020).
2.2. Histological analysis

The ovary and uterus of rats were processed and evaluated for histological alterations as per the method described in Dhar and Singla (2014). The whole ovary and piece of the uterus were fixed in Bouins’ fluid for 48 h, processed and embedded in paraffin. The paraffin sections of 6 μm thickness were obtained on glass slides with the help of a rotary microtome (Medimeas Instruments, Ambala Cantt, Haryana, India) and stained with haematoxylin and eosin (H&E) for histomorphological studies. Serial sections of the ovary were taken, and the number of different types of follicles was counted. In each section, only those follicles were counted in which the oocyte’s nucleus was visible, as described by Tilly (2003). In the section of the uterus, the number of endometrial glands was counted, and epithelial cell height (μm) and myometrium thickness (μm) were measured using Magvision Image Analysis Software under a light microscope.

2.3. Biochemical analysis

2.3.1. Total soluble proteins

Total soluble proteins (TSP) were estimated using the method given by Lowry et al. (1951). A spectrophotometer was used to record absorbance at 520 nm against a reagent blank. Protein concentration (mg/g) in the tissue sample was determined by preparing a standard curve of bovine serum albumin.

2.3.2. 17β-HSD and 3β-HSD activities

Activities of 17β-Hydroxysteroid dehydrogenase (17β-HSD) and 3β-Hydroxysteroid dehydrogenase (3β-HSD) were estimated in plasma with some modifications in the method of Agular et al. (1992). Specific activity was calculated in units/min/mg of total proteins.

Table 1. Experimental protocol for animal evaluation.

| Set 1 (all four groups) | Set 2 (all four groups) | Set 3 (all four groups) | Set 4 (all four groups) |
|------------------------|------------------------|------------------------|------------------------|
| 1. Group I (the low-dose group, fed on 3% PSP in bait) | Sacrificed immediately after treatment withdrawal for effect on organ weights, organ histology and biochemicals | Sacrificed after 30 days of treatment withdrawal to determine reversibility in effect on organ weights, organ histology and biochemicals | Fertility assessment immediately after treatment withdrawal |
| 2. Group II (the intermediate dose group, fed on 5% PSP in bait) | | | Evaluated for estrous cyclicity immediately after treatment withdrawal for up to 30 days, followed by fertility assessment to determine reversibility in effects |
| 3. Group III (the high-dose group, fed on 10% PSP in bait) | | | |
| 4. Group IV (untreated control, fed on bait without PSP) | | | |

Table 2. Mean daily consumption and acceptance of bait containing different concentrations of papaya seed powder and active ingredient ingested during 30 days of treatment in no-choice by female B. bengalensis.

| Treatment (n = 24 each) | Mean daily bait consumption (g/100 g bwt) | Percent acceptance of treatment bait over untreated bait | Papaya seed powder ingested in 30 days (g/kg bwt) |
|------------------------|-----------------------------------------|--------------------------------------------------------|-----------------------------------------------|
| Pre-treatment (n = 24) | Treatment (n = 24) | Post-treatment (n = 12) | | |
| Control | 8.63 ± 0.98 | 7.65 ± 0.79 | 8.15 ± 1.67 | – | – |
| 3% | 8.95 ± 1.32 | 7.62 ± 1.60 | 6.60 ± 4.46 | 89.45 ± 11.21 | 68.36 ± 21.02 |
| 5% | 9.20 ± 2.64 | 7.59 ± 1.80 | 7.53 ± 3.82 | 86.82 ± 12.30 | 114.40 ± 37.82 |
| 10% | 8.21 ± 2.50 | 6.18 ± 0.90 | 6.20 ± 2.70 | 79.06 ± 32.04 | 186.23 ± 40.56 |

Values are Mean ± SD.
2.3.3. Alkaline and acid phosphatases
Alkaline phosphatase (ALP) and acid phosphatase (ACP) activity in plasma were estimated per the method given by Bessey et al. (1946). The rate of formation of p-Nitrophenol was measured as absorbance at 410 nm as it is directly proportional to the activity of ALP and ACP in the sample. The \( \mu \) moles read off from the standard curve corresponding to the measured optical density were multiplied by 20 to obtain units of ALP and multiplied by 4.68 to obtain units of ACP. The activity was measured in \( \mu \) moles of p-Nitrophenol lib/min/mg protein.

2.3.4. Estradiol
The level of estradiol (ES) in plasma (ng/ml) was estimated using an ELISA kit, as per the manufacturer’s (Xema Medica Co. Ltd., Russia) protocol.

2.4. Fertility assessment
The fertility assessment of rats was done per the procedure described in Kaur et al. (2021). Immediately after treatment withdrawal, six female rats in each group of the third set were paired with untreated mature male \textit{B. bengalensis} rats in 1:1 in large laboratory pens. Six female rats in each group of the fourth set were paired with mature male rats in a ratio of 1:1 after 30 days of treatment withdrawal. During pairing, food and water were provided \textit{ad libitum}. Food consisted of a loose mixture of cracked wheat, powdered sugar, refined groundnut oil and milk powder in the ratio of 91:2:2:5. Pre-soaked black gram seeds (50 g) were also provided in a separate bowl to eliminate the chances of any nutritional deficiency. Male rats were kept with female rats for up to 15 days of pairing to ensure successful mating, after which the males were separated. Female rats were observed for pregnancy and delivery of pups. The number and sex of pups delivered were also recorded. Percent breeding success was determined as per the formula given below:

\[
\text{Percent breeding success} = \frac{\text{Number of females pregnant}}{\text{Total number of females paired}} \times 100
\]

The proportion of male and female pups delivered was determined as per the formula given below:

\[
\text{Proportion of pups of particular sex delivered} = \frac{\text{Number of pups of particular sex delivered}}{\text{Total number of pups delivered}}
\]

2.5. Effect on estrous cyclicity
The duration of the estrous cycle and its stages was determined as per the method described in Dhar and Singla (2014). Immediately after treatment withdrawal, the vaginal fluid of...
ingestion of 68.36, 114.40–186.23 g/kg bwt of PSP, respectively, in 30 days’ treatment. Percent acceptance of treatment bait over untreated bait consumed before treatment by rats of all the treated groups varied from 79.06 to 89.45% (Table 2). There was no significant (P > .05) difference in mean daily consumption of treated and untreated baits among different groups of rats.

3.2. Effect on reproductive organ weights

In rats sacrificed immediately and after 30 days of treatment withdrawal, the relative weight (g/100 g bwt) of ovaries didn’t differ significantly (P > .05) among groups of treated and control rats at all the three concentrations of PSP (Figure 1(A)). However, the relative weight of the uterus was to be reduced significantly (P = .003) in rats of the group treated with 10% PSP and sacrificed immediately after treatment withdrawals compared to rats of the control group and those treated with 3% and 5% PSP. In rats treated with different concentrations of PSP and sacrificed after 30 days of treatment withdrawal, no significant (P > .05) effect was observed on the relative weights of ovaries and uterus (Figure 1(B)).

3.3. Effect on histomorphology

3.3.1. In the uterus

In rats treated with different concentrations of PSP for 30 days and sacrificed immediately after treatment withdrawal, an effect was found on histomorphology of the uterus (Figure 2). Loss of cellular integrity was there. The number of endometrial glands, luminal epithelium cell height and myometrium thickness were found to be reduced, but the difference between the treated and control groups of rats was not found to differ significantly (P > .05) (Figure 3(A)). In rats sacrificed after 30 days of treatment withdrawal also, no significant (P > .05) difference was found in many endometrial glands, luminal epithelium cell height and myometrium thickness among the control and treated groups (Figure 3(B)).

3.3.2. In the ovary

In rats treated with different concentrations of PSP for 30 days and sacrificed immediately after treatment withdrawal, an effect was found on histomorphology of the ovary (Figure 4). Follicular cells’ proliferation was grossly affected. In rats sacrificed immediately after treatment withdrawal, the number of primordial (P = .003) and pre-antral follicles (P = .004) was found to decrease significantly in rats treated with 10% concentration compared to rats of the control group and those treated with 3% and 5% concentrations of PSP. However, no significant (P > .05) difference was found in the number of primary, secondary and antral follicles among treated and control groups of rats (Table 3). In rats sacrificed after 30 days of treatment withdrawal, no significant (P > .05) difference was found in many primordial, primary, secondary, preantral and antral follicles among rats of treated and control groups, indicating reversibility in effects (Table 3).

2.6. Statistical analysis

Values were determined as Mean ± SD (standard deviation). General linear model in statistical analysis software (SAS) version 9.3 was used for the statistical analysis of data obtained by using a factorial, completely randomized design. Tukey’s HSD (honestly significant difference) test at a 5% level of significance was used to obtain all pair-wise treatment comparisons.

3. Results

3.1. Bait consumption and acceptance of treatment bait

Mean daily consumption (g/100 g bwt) of treatment bait by rats of groups I, II and III treated with 3, 5 and 10% PSP was 7.62, 7.59 and 6.18 g/100 g bwt, respectively, leading to mean total consumption of treated and untreated bait by rats.
3.4. Effect on biochemicals

The plasma levels of 17β-HSD, ES, ALP and ACP were significantly ($P < .001–0.007$) low in rats of the group treated with 10% PSP and sacrificed immediately after treatment withdrawal from the control group and groups treated with 3% and 5% PSP. No significant ($P > .05$) difference was observed in plasma levels of TSP and 3β-HSD among rats in the treated and control groups (Table 4).

In rats sacrificed after 30 days of treatment withdrawal, no significant ($P > .05$) difference was observed in plasma levels of 3β-HSD, 17β-HSD, ALP and ACP among rats of treated and control groups, indicating reversibility in effects; however, the level of ES was still found reduced in rats treated with 10% PSP, indicating no reversibility in the effect caused (Table 4). No significant effect of treatment was observed on the plasma level of TSP immediately and after 30 days of treatment withdrawal.

Table 3. Effect of treatment with different concentrations of papaya seed powder for 30 days on the number of different types of follicles in the ovary of *B. bengalensis*.

| Treatment (n = 3 each) | Primordial | Primary | Secondary | Pre-antral | Antral |
|-----------------------|------------|---------|-----------|------------|--------|
| Control               | 26.67 ± 3.05$^a$ | 13.67 ± 2.08$^a$ | 8.33 ± 2.08$^a$ | 6.00 ± 1.00$^a$ | 6.00 ± 1.00$^a$ |
| Immediately after treatment withdrawal | 21.67 ± 2.87$^a$ | 15.67 ± 1.52$^a$ | 8.33 ± 2.08$^a$ | 6.33 ± 1.52$^a$ | 4.33 ± 2.08$^a$ |
| 3%                    | 26.00 ± 2.00$^a$ | 16.33 ± 1.52$^a$ | 10.33 ± 0.57$^a$ | 8.00 ± 1.00$^a$ | 6.67 ± 1.16$^a$ |
| 10%                   | 15.00 ± 0.99$^b$ | 11.33 ± 2.30$^a$ | 4.33 ± 2.51$^a$ | 2.67 ± 0.57$^b$ | 3.00 ± 1.00$^a$ |
| After 30 days of treatment withdrawal | 24.00 ± 4.57$^a$ | 14.33 ± 2.51$^a$ | 8.00 ± 1.00$^a$ | 8.67 ± 0.57$^a$ | 11.67 ± 1.52$^a$ |
| 3%                    | 26.33 ± 2.51$^a$ | 13.33 ± 4.94$^a$ | 8.67 ± 1.52$^a$ | 6.33 ± 0.57$^a$ | 8.33 ± 0.57$^a$ |
| 10%                   | 25.33 ± 1.14$^a$ | 13.67 ± 5.56$^a$ | 11.67 ± 1.52$^a$ | 6.00 ± 1.00$^a$ | 7.00 ± 1.73$^a$ |

Values are Mean ± SD. Values with different superscripts (a–b) in a column separately for those sacrificed immediately and after 30 days of treatment withdrawal differ significantly at $P < .05$ for the same control.

Figure 4. H&E stained sections of the ovary of *B. bengalensis* treated with different concentrations of papaya seed powder and sacrificed immediately after the withdrawal at 200× magnification showing the effect on follicular development, (A) control rat, (B) 3% treated rat, (C) 5% treated rat, and (D) 10% treated rat (PrF-Primordial follicle, SF-Secondary normal follicle, AnF-Antral follicle, AtF-Atretic follicle).
Table 4. Effect of treatment with different concentrations of papaya seed powder for 30 days on different biochemical parameters in the plasma of female B. bengalensis.

| Treatment (n = 6 each) | TSP (mg/g) | 17β-HSD (units/min/mg protein) | 3β-HSD (units/min/mg protein) | ES (μ moles of p-Nitrophenol lib/min/mg protein) | ALP (μ moles of p-Nitrophenol lib/min/mg protein) | ACP (μ moles of p-Nitrophenol lib/min/mg protein) |
|-----------------------|------------|--------------------------------|-------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Control               | 24.77 ± 3.72a | 1.84 ± 0.42a                         | 3.04 ± 1.05a                     | 1.14 ± 0.171a                                     | 9.10 ± 1.69a                                     | 42.42 ± 10.34a                                     |
| Immediately after treatment withdrawal |            |                                  |                                |                                               |                                               |                                               |
| 3%                   | 22.70 ± 1.54a | 1.20 ± 0.44a                         | 1.70 ± 0.244a                   | 0.91 ± 0.171a                                     | 7.27 ± 1.27a                                     | 29.45 ± 6.30a                                     |
| 5%                   | 22.02 ± 8.13a | 1.37 ± 0.416a                        | 2.20 ± 0.49a                    | 0.96 ± 0.44a                                     | 11.49 ± 2.81a                                    | 28.26 ± 16.67a                                    |
| 10%                  | 20.51 ± 7.01a | 0.43 ± 0.22b                          | 1.80 ± 0.51a                    | 0.53 ± 0.34b                                     | 8.71 ± 2.23b                                     | 19.86 ± 12.32b                                    |
| After 30 days of treatment withdrawal |            |                                  |                                |                                               |                                               |                                               |
| 3%                   | 26.12 ± 2.94a | 1.64 ± 0.37a                          | 2.38 ± 0.56a                    | 1.16 ± 0.27a                                     | 34.78 ± 4.85a                                    | 34.78 ± 4.85a                                     |
| 5%                   | 25.54 ± 1.15a | 1.31 ± 0.42a                          | 2.55 ± 0.67a                    | 1.12 ± 0.07a                                     | 28.26 ± 16.17a                                   | 28.26 ± 16.17a                                    |
| 10%                  | 24.63 ± 5.14a | 1.73 ± 0.78a                          | 3.22 ± 0.91a                    | 1.05 ± 0.122b                                    | 36.88 ± 10.26a                                   | 36.88 ± 10.26a                                    |

Values are Mean ± SD.
Values with different superscripts (a–b) in a column separately for those sacrificed immediately and after 30 days of treatment withdrawal differ significantly at P < .05 regarding the same control.

3.5. Effect on fertility

In the present study, the breeding success of female rats of the control group was only 66.67%. Breeding success in female rats of the group treated with 10% PSP and evaluated immediately after treatment withdrawal was to be reduced to 50%, which was increased to 66.67% in rats evaluated after 30 days of treatment withdrawal, indicating partial reversibility in effects. No breeding of rats was observed in the group of rats treated with 3% PSP and evaluated immediately and after 30 days. Breeding success in rats treated with 5% PSP was 83.33% and 100% immediately and after 30 days of treatment withdrawal indicating no much effect (Table 5). The average number of pups (4.00–5.00) delivered by female rats treated with 5% and 10% PSP and evaluated immediately and after 30 days of treatment withdrawal was, however, found to be significantly (P < .05) less than those delivered by control rats (7.00). However, no significant difference in the proportion of male and female pups delivered was observed among treated and control rats (Table 5).

3.6. Effect on the estrous cycle

During the first 15 days after treatment withdrawal (Table 6), the duration of one estrous cycle (117.12 h) was found to be significantly (P < .0001) prolonged and that of the estrous stage (10.67 h) significantly (P < .0001) reduced in rats treated with 10% concentration of PSP compared to rats of the control group and those treated with 3% and 5% PSP. The duration of the diestrous stage in the first 15 days was significantly (P < .0001) prolonged in rats treated with all the three concentrations of PSP, with a significantly higher effect on rats treated with 10% PSP. The duration of the proestrous stage was significantly (P < .001) prolonged in rats treated with 3% and 10% PSP compared to control rats and rats treated with 5% PSP, while the duration of the metestrous stage was reduced significantly (P < .001) in rats of all the three treated groups compared to the control group.

Between 16 and 30 days of treatment withdrawal (Table 6), no significant (P > .05) difference was found in the duration of one estrous cycle and metestrous stage among rats of treated and control groups, indicating partial reversibility in effects. The duration of proestrous (11.89 h) and diestrous (71.89 h) stages was still found to increase significantly (P < .001) and that of the estrous stage (9.67 h) decreased significantly (P < .0001) in rats treated with 10% PSP, indicating no reversibility.

4. Discussion

The overall control of the female reproductive system is a complex mechanism involving numerous neuroendocrine factors and hormones that affect the successful production of mature gametes. The present study investigated the effect of cereal bait formulation based on PSP as an ingredient on various reproductive functions, such as histology of reproductive organs, biochemicals, reproductive hormones, estrous cycle and fertility of B. bengalensis, to provide a technology.

Table 5. Effect of treatment with different concentrations of papaya seed powder for 30 days on breeding success of female B. bengalensis.

| Treatment (n = 6 each) | Number of pregnant females | Breeding success (%) | Number of pups delivered | Proportion of male pups | Proportion of female pups |
|-----------------------|-----------------------------|---------------------|--------------------------|-------------------------|---------------------------|
| Immediately after treatment withdrawal |                              |                     |                          |                         |                           |
| Control               | 66.67                       | 7.00 ± 1.70a                     | 0.55 ± 0.08a               | 0.45 ± 0.08a           |                           |
| 3%                   | 0                           | –                   | –                         | –                       | –                         |
| 5%                   | 83.33                       | 5.00 ± 1.50b                     | 0.53 ± 0.16a               | 0.47 ± 0.16a           |                           |
| 10%                  | 50.00                       | 4.00 ± 0.99b                     | 0.33 ± 0.07a               | 0.67 ± 0.07a           |                           |
| After 30 days of treatment withdrawal |                              |                     |                          |                         |                           |
| Control               | 66.67                       | 7.00 ± 2.20b                     | 0.59 ± 0.14a               | 0.41 ± 0.14a           |                           |
| 3%                   | 0                           | –                   | –                         | –                       | –                         |
| 5%                   | 100.00                      | 5.00 ± 2.20b                     | 0.50 ± 0.147a              | 0.50 ± 0.147a          |                           |
| 10%                  | 66.67                       | 5.00 ± 1.21b                     | 0.41 ± 0.134a              | 0.59 ± 0.134a          |                           |

Values are Mean ± SD.
Values with different superscripts (a–b) in a column for the number and proportion of pups separately for those evaluated immediately and after 30 days of treatment withdrawal differ significantly at P < .05.
for integrated rodent pest management programme. The study revealed dose-dependent adverse effects of PSP on the histomorphology of the ovary and uterus, biochemicals, reproductive hormones, estrous cycle and fertility of *B. bengalensis* thus justifying its reported use as a female anti-fertility agent (Chinoy et al. 1995, 2006; Dosumu et al. 2008; Julaeha et al. 2015; Memudu and Oluwole 2021).

In the present study, a significant decrease in the relative weight of the uterus of treated *B. bengalensis* was found. On the contrary, a significant increase in the weight of the uterus of rats treated with papaya seed extract has been reported by Novitasari et al. (2018). The hormonal and histological changes caused due to papaya seed treatment may be the reason for the reduction in uterine weights (Memudu and Oluwole 2021).

Follicles are the basic functional units of reproduction in the ovary as the development, maturation and ovulation of female gamete occur within the ovarian follicle. The present study revealed a significant decrease in the number of primordial and pre-antral follicles in the ovary of rats treated with 10% PSP, which can be correlated with a decrease in the plasma level of estradiol found in the present study. A similar decrease in the serum levels of estradiol in rats treated with papaya seed extract was also observed by Raji et al. (2005). Such changes in hormone levels affect the histological appearance of female reproductive organs (Aritonang et al. 2017). The decline in the level of estradiol results in unbalanced ovarian functions, which invariably affect the follicular development, uterine glands, endometrium and fertility index (Biswal 2014). In the present study, a non-significant decrease in luminal epithelial cell height, myometrium and number of endometrial glands was observed in the uterus of rats treated with different concentrations of PSP.

The pituitary-gonadal axis controls the pattern and frequency of the estrous cycling through the gonadotropin hormones i.e. follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Evaluation of the estrous cycle in rats is an index to estimate the functional status of the hypothalamic-pituitary-ovarian axis (Cora et al. 2015). Change in the estradiol level may also be the cause of irregularity in estrous cycle of treated female rats (Aritonang et al. 2017). In the present study, treated rats showed significantly prolonged proestrous and diestrous stages and reduced estrous stage. Similar irregularity in the estrous cycle in rats treated with oral doses of papaya seed extract was also observed earlier (Raji et al. 2005; Dosumu et al. 2008; Naik et al. 2015; Odirichukwu et al. 2016).

The estrous stage is the period of heat or sexual receptivity and a decrease in its duration suggests a reduction in receptivity and fertility (Nayaka et al. 2014). The rate of ovulation also decreases due to a prolonged diestrous phase (Naik et al. 2015).

In the present study, fertility was reduced to 50% in rats treated with 10% PSP. Keshri et al. (1993) reported only 30% pregnancy in rats administered hexane extract of papaya seeds administered at 1 g/kg bwt daily. The number of pups delivered by female *B. bengalensis* treated with 5 and 10% papaya seed powder in present study was significantly lower than that delivered by control rats. Similar to the present results, Raji et al. (2005) also reported a reduced number of pups in females rats orally treated with chloroform extract of papaya seeds. The decrease in estradiol level acts to down-regulate the brain’s hypothalamic mechanisms that reduce the release of LH and hence the ovulation. Reduced ovulation may further lead to a fewer implantation sites.

3β-HSD is directly involved in the conversion of pregnenolone into progesterone, which is responsible for the maintenance of pregnancy, while 17β-HSD is involved in the major pathway of the formation of androstenedione and testosterone, which are subsequently aromatized, leading to the formation of estradiol. Suppression in activities of 17β-HSD and 3β-HSD found in the present study, also observed by Uche-Nwachi et al. (2011), may be the cause of the reduced level of estradiol. A decreased level of ACP and ALP was also observed in the present study, which indicates cellular damage (Muthuviveganandavel et al. 2008).

### 5. Conclusion

The present study is the first record on the effect of cereal bait formulated with papaya seed powder as an ingredient on reproductive functions of female *B. bengalensis* to find a field applicable approach to controlling the rodent pest population. The findings revealed significant anti-reproductive effects of 10% papaya seed powder, which were found reversed after 30 days of treatment withdrawal. The study suggests a need to conduct further studies using higher concentrations of papaya seed powder or its extract to attain long-term irreversible effects.
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