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Effect of ethanolic seed extract of *Caesalpinia bonducella* on female reproductive system of albino rat: a focus on antifertility efficacy

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ABSTRACT

Objective: To evaluate the effect of oral administration of the ethanolic seed extract of *Caesalpinia bonducella* on the reproductive system in Wistar female albino rat. **Methods:** The study was divided into four groups. The group I received distilled water and served as vehicle treated control. Groups II, III and IV were orally administered 100, 200 and 300 mg/kg body weight dose of seed extract respectively, for 10 consecutive days and subsequently euthanized twenty four hours after the last dose. **Results:** The treatment prolonged the length of estrous cycle with significant increase in the duration of diestrus stage. The analysis of the principal hormones *viz.* LH, FSH, estradiol and progesterone showed significantly decreased levels in dose-dependant manner. Ovarian and uterine weight was significantly reduced as compared to that of the control group. Histoarchitectural observations revealed follicular atresia and degeneration of corpora lutea in ovary. Oviduct showed degeneration of mucosal folds and epithelium cells. Uterus showed evidence of degeneration of endometrial epithelium and endometrial glands. Lamina propria and muscularis layer of vagina were found slightly disorganized. **Conclusions:** It can be concluded that significant decrease in the hormone levels, reproductive organs weight and alterations in histoarchitecture of reproductive organs might be due to antiestrogenic nature of seed extract.

1. Introduction

In the present era, overpopulation is becoming a global problem causing much pressure on economic, social and natural resources. Control of fertility with hormonal preparations containing estrogen and progesterone has been proved to be effective. The safety of long term use of these contraceptives, however, is controversial and they are not easily accessible and acceptable to all people.

Traditional medicines are practiced worldwide for fertility regulation since ancient times. A large number of plant species have been screened for their antifertility efficacy. The recent review of Kumar *et al*[1] reported 577 plant species belonging to 122 families, have been used

traditionally in fertility regulation in females. However, the search for an orally active, safe and effective plant preparation or its compound is yet needed for fertility regulation due to incomplete inhibition of fertility or side effects.

Caesalpinia bonducella F., (Caesalpinaceae) commonly known as Nata Karanja, a prickly shrub found throughout the hotter parts of India, Myanmar and Sri Lanka. Seeds consist of a thick, brittle shell with a yellowish white bitter fatty kernel[2]. It is reported to have multiple therapeutic properties like adaptogenic[3], antifilarial[4], antioxidative[5], anti-diabetic[6], anti-inflammatory, antipyretic and analgesic[7], muscle contractile activity[8], immunomodulatory[9], antimicrobial[10], and anti-estrogenic[11]. It has a high reputation among the folk as an agent that facilitates delivery and is widely used in the Ivory Coast[12]. Its leaves powder is used to treat gynecological disorders like menorrhagia and leucorrhoea[13].

The seeds of *Caesalpinia bonducella* are found to contain various chemical constituents such as furanoditerpenes, phytosterinin, β -sitosterol, flavonoids, bonducellin,

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aspartic acid, arginine, citrulline and β -carotene^[14].

The seeds of this plant are traditionally used in the fertility regulation in female in India^[15–17]. The preliminary studies on the root bark of the plant showed anti-implantation effect in rats^[18]. In view of these evidences, the effect of the ethanolic seed extract of *Caesalpinia bonducella* on female reproductive system was investigated in the present study.

2. Materials and methods

2.1. Plant material

The seeds of *Caesalpinia bonducella* F., (Caesalpinaceae) were procured from the local market of Dharwad, Karnataka, India. Further taxonomic identification was conducted by Dr. G.S. Mulgund and a voucher specimen number Bot/H/484 was deposited at Department of Studies and Research in Botany, Karnatak University, Dharwad, Karnataka, India.

2.2. Preparation of the extract

The seed extract was prepared by following the method of Shukla *et al*^[5]. Briefly, the air-dried seeds of *Caesalpinia bonducella* (50 g) were powdered and then extracted with 500 mL of 95% ethanol by using a Soxhlet apparatus. The crude extract obtained was filtered through Whatmann paper and the filtrate was evaporated to dryness on rotary flash evaporator below 50 °C. The yield of the extract was 4 % (w/w). *Caesalpinia bonducella* seed extracts (henceforth referred to as CBSE) obtained were preserved in sterile glass container at 4 °C until further use.

2.3. Animal model

Colony bred virgin Wistar female albino rats (*Rattus norvegicus*), approximately twelve weeks old and weighing between 190 and 200 g exhibiting regular estrous cycles were housed in polypropylene cages of size 35 cm long × 23 cm wide × 15 cm high under standard animal house conditions and controlled environmental conditions (24 ± 2) °C for 12 h light and 12 h darkness. They were fed pelleted standard rat feed (Sai Durga Feeds & Foods, Bangalore, India) and allowed free access to water.

2.4. Treatment protocol

Animals were equally distributed into four treatment groups containing eight animals in each. The group I animals were given distilled water alone for 10 days and served as control group. Group II, III & IV animals were treated with CBSE at the dose levels 100, 200, 300 mg/kg bodyweight /day for 10 consecutive days, respectively. The selected tested doses and duration were based upon the work of Chakrabarti

et al^[19] and Salunke *et al*^[20]. The required seed extract was dissolved in distilled water and administered orally with a straight ball-tipped needle.

2.5. Estrous cycle

The estrous cycle was studied by preparation of vaginal smear of the animals. The stages of estrous cycle and its duration were determined as described by Makonnen *et al*^[21].

2.6. Behavioral profile, body and organs weight

The animals were observed daily for behavioral activities. Body weight was recorded every day during the study period. 24 h after the last dose, the rats were weighed and sacrificed under Sodium pentobarbital anesthesia. The ovaries, oviducts, uteri and vagina were dissected out, freed from adherent tissues and blood and weighed to the nearest milligram (absolute weight).

2.7. Reproductive hormone levels

Blood samples were collected by cardiac puncture technique under Sodium pentobarbital anesthesia (40 mg/kg) in dry glass centrifuge tubes. The blood was then allowed to stand for 10 min at room temperature to clot and centrifuged at 3000 r/min for 10 min. The supernatant (serum) was then tipped off into separate vial and subsequently subjected for the assessment of FSH, LH, estradiol and progesterone levels by Fully Automated Bidirectionally Interfaced Chemiluminescent Immuno Assay.

2.8. Histological observations

The tissues were first examined for gross pathology and then fixed in 10% neutral buffered formalin solution. After proper fixation, the tissues were dehydrated in graded series of alcohol, cleared in benzene and embedded in paraffin wax. The paraffin blocks were sectioned at 5 μ m thickness by LEICA RM 2255 microtome according to the procedures of Kittel *et al*^[22], Morawietz *et al*^[23] and Fehlert *et al*^[24]. The tissue sections were subjected to rehydration by exposing them to decreasing concentrations of alcohol, 100–10% and then stained with haematoxylin. The sections were dehydrated by using increasing concentrations of alcohol 10–100% and then stained with eosin (Weesner, 1960)^[25]. The stained slides were photographed under Axio Imager M 2 for histological studies.

2.9. Ethical aspects

The study was approved by the Institutional Animal Ethical Committee, Department of Zoology, Karnatak University, Dharwad, India. CPCSEA (Committee for the

Table 1Effect of *Caesalpinia bonducella* seed extract on body and reproductive organs weight.

Parameters	Group I Control	Group II 100 mg	Group III 200 mg	Group IV 300 mg
Body weight (g)	201.6 \pm 1.0 ^a	200.3 \pm 1.3 ^a	202.3 \pm 1.0 ^a	201.6 \pm 0.9 ^a
Ovary (mg)	28.6 \pm 1.0 ^a	26.3 \pm 0.6 ^a	25.9 \pm 1.1 ^a	25.0 \pm 0.6 ^b
Oviduct (mg)	9.2 \pm 0.2 ^a	9.8 \pm 0.3 ^a	9.2 \pm 0.4 ^a	9.3 \pm 0.3 ^a
Uterus (mg)	399.7 \pm 11.5 ^a	363.2 \pm 19.1 ^a	333.0 \pm 3.4 ^b	331.3 \pm 25.5 ^b
Vagina (mg)	116.3 \pm 4.5 ^a	114.8 \pm 3.6 ^a	118.0 \pm 1.4 ^a	117.9 \pm 2.2 ^a

$n=8$. Data represents mean \pm SE; dissimilar letters indicate significant difference ($P \leq 0.05$) between the groups.

Table 2Effect of *Caesalpinia bonducella* seed extract on duration of estrous cycle and its phases.

Phases of estrous cycle days	Group I Control	Group II 100 mg	Group III 200 mg	Group IV 300 mg
Total duration	4.57 \pm 0.17 ^a	5.28 \pm 0.15 ^b	6.00 \pm 0.18 ^c	6.50 \pm 0.18 ^c
Proestrous	1.00 \pm 0.00 ^a			
Estrous	1.00 \pm 0.00 ^a	1.00 \pm 0.00 ^a	1.14 \pm 0.16 ^a	1.00 \pm 0.00 ^a
Metestrous	0.57 \pm 0.17 ^a	0.57 \pm 0.17 ^a	1.00 \pm 0.00 ^a	1.00 \pm 0.00 ^a
Diestrous	2.00 \pm 0.00 ^a	2.75 \pm 0.15 ^b	3.00 \pm 0.00 ^b	3.50 \pm 0.18 ^c

$n=8$. Data represents mean \pm SE; dissimilar letters indicate significant difference ($P \leq 0.05$) between the groups.

Table 3Effect of *Caesalpinia bonducella* seed extract on reproductive hormone levels.

Hormones	Group I Control	Group II 100 mg	Group III 200 mg	Group IV 300 mg
FSH (mIU/mL)	1.44 \pm 0.00 ^a	0.86 \pm 0.00 ^b	0.50 \pm 0.00 ^c	0.28 \pm 0.00 ^d
LH(mIU/mL)	0.21 \pm 0.00 ^a	0.13 \pm 0.00 ^b	0.08 \pm 0.00 ^c	0.05 \pm 0.00 ^d
Estradiol(pg/mL)	160.41 \pm 0.14 ^a	144.40 \pm 0.00 ^b	139.56 \pm 0.00 ^c	128.53 \pm 0.09 ^d
Progesterone (pg/mL)	12.68 \pm 0.00 ^a	10.03 \pm 0.00 ^b	7.69 \pm 0.00 ^c	3.37 \pm 0.00 ^d

$n=8$. Data represents mean \pm SE; dissimilar letters indicate significant difference ($P \leq 0.05$) between the groups.

Purpose of Control and Supervision on Experiments on Animals) (Animal House Registration No. 639/02/a/ CPCSEA) guidelines were followed for maintenance and use of the experimental animals.

2.10. Statistical analysis

One-way ANOVA followed by Tukey's HSD post-hoc multiple comparisons tests was employed to analyze data. P values less than 0.05 were considered significant.

3. Results

3.1. Behavioral profile, body and organs weight

There was no treatment related changes in the behavioral profile at all the tested dose levels. All animals both in control and extract treated groups appeared healthy, alert and were responding to pain and touch. Vocalization, restlessness and irritability in animals were also not observed. The animals responded to loud noise, indicating the CNS excitation. No significant difference was observed in the body weight of the extract treated and control group (Table 1). A significant reduction ($P \leq 0.05$) in ovarian weight at 300 mg/kg; uterine weight at 200, 300 mg/kg dose level was

noticed when compared to their respective control groups (Table 1). The weight of oviduct and vagina showed no significant difference in the extract groups when compared with control group (Table 1).

3.2. Estrous cycle

Treatment with CBSE prolonged the length of estrous cycle significantly with a significant increase ($P \leq 0.05$) in duration of diestrous phase in a dose-dependent manner. On the other hand, no significant changes were noticed in length of proestrous, estrous and metestrous phases (Table 2).

3.3. Reproductive hormone levels

There was a significant decrease ($P \leq 0.05$) in the levels of FSH, LH, estradiol and progesterone hormone in dose-dependent manner in seed extract groups when compared to the control group (Table 3).

3.4. Histological observations

Histoarchitecture of the reproductive organs of female rats of vehicle treated control and following treatment with *Caesalpinia bonducella* seed extract at the doses

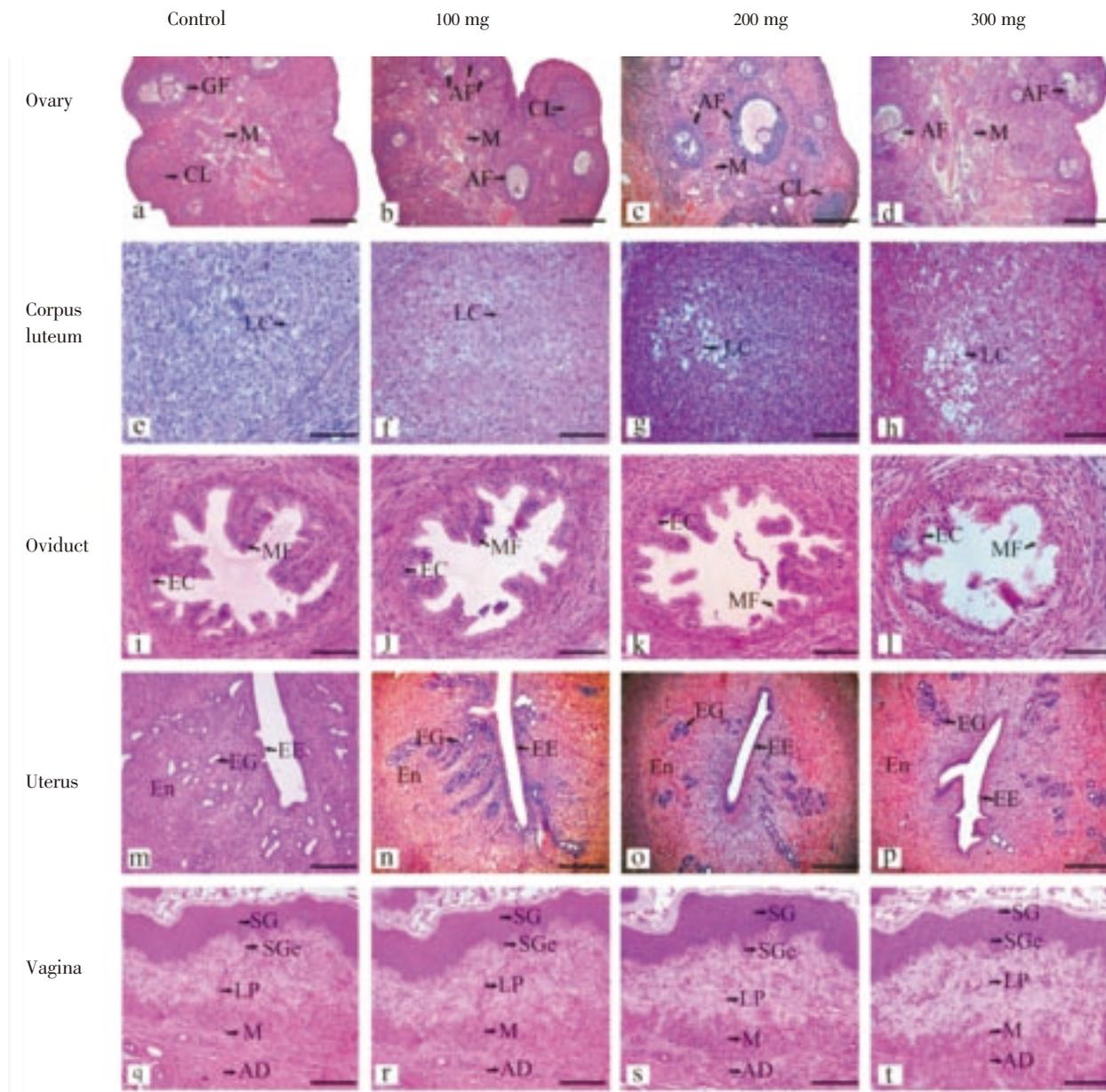


Figure 1. Histoarchitecture of the reproductive organs of female rats of vehicle treated control and following treatment with *Caesalpinia bonducella* seed extract at the doses 100, 200, 300 mg/kg bodyweight for 10 days.

AF–Atretic follicle, CL–Corpus luteum, M–Medulla, LC–Lutein cells, MF–Mucosal fold, EC–Epithelial cells, EG–Endometrial glands, EE–Endometrial epithelial, En–Endometrium, SG–Stratum granulosum, SGe–Stratum germinativum LP–Lamina propria, M–Muscularis, AD–Adventia.

100, 200, 300 mg/kg for 10 days are presented in Figure 1. The histological analysis of control ovary revealed healthy Graafian follicle, corpus luteum and compactly arranged medulla region (Figure 1a). The treatment with CBSE caused degeneration of developing follicles leading to follicular atresia (Figure 1b–d) and degeneration of lutein cells of corpus luteum (Figure 1f–h). These symptoms were much more prominent at 200–300 mg/kg dose levels. The ampulla region of oviduct of the control group showed normal histological features, illustrating a well defined internal mucosa and muscular layer (Figure 1i). The treated groups showed degeneration of mucosal folds and its associated

columnar epithelium cells and muscular layer (Figure 1j–l). These degenerative and atrophic changes were more pronounced in those that received 300 mg/kg of CBSE (Figure 1l). The histology of uterus revealed that treatment with CBSE caused degeneration of endometrial epithelium. The uterine glands were reduced in number and had shrunken morphology with non–secretory function (Figure 1n–p). The vascularity was poor and stroma was compact as compared to vehicle control (Figure 1m). Further, lamina propria and muscular layer of vagina showed mild degeneration at dose level 300 mg/kg (Figure 1t) when compared to the control group (Figure 1q). On the other hand, stratum granulosum,

stratum germinativum, lamina propria, muscular layer and adventitia were found to be unaffected in the rest of treatment group (Figure 1r–s).

4. Discussion

The present study shows no significant change in the final body weight after 10 days of oral administration with *Caesalpinia bonducella* seed extract at all the three dose levels tested. However, a significant reduction in genital organs weight was noticed. Since body weight gain was not altered significantly in the extract treated rats in comparison with the controls, the histomorphological changes observed in ovary and uterus of the female reproductive system following treatment may be attributed to the effect of the extract itself on this specific system. In addition, the absence of any sign and symptom of toxicity in the extract treated animals suggests that the extract was safe to the female rats at the doses employed in the present study.

The most important hormones involved in the regulation of the alterations detected in the feminine genital tract are estrogen and progesterone, which are regulated by pituitary–secreted gonadotropin hormones^[26]. The reduced FSH and LH level in serum of treated animals is an indication towards the possibility of effect of extract on the anterior pituitary or the hypothalamus since the secretion of FSH and LH is regulated by the gonadotropic releasing hormone secreted by the hypothalamus. Moreover, alkaloids have been shown to reduce concentrations of LH, estradiol and FSH^[27]. Therefore, the presence of alkaloids in the seed extract may account for the alterations in the levels of the gonadotropic hormone observed in the present study. It is well established fact that the pulsatile secretions of pituitary gonadotropins regulate steroidogenesis in the ovary. The reduction in levels of steroid hormone (estradiol and progesterone) in the seed extract treated animals may be attributed to the decreased levels of gonadotropins hormone. Moreover, the work of Yakubu *et al*^[28] where administration of the *Cnidioscolous aconitifolius* leaf extract has significantly reduced concentration of estradiol, progesterone, follicle stimulating and luteinizing hormones also support the present investigation.

The decrease in the ovarian and uterine weight may be taken as an indicative of the antiestrogenic nature of *Caesalpinia bonducella* since antiestrogenic substance decreases the wet weight of the uterus^[29]. In addition, Khedkar *et al*^[18] and Salunke *et al*^[20] have also reported that ethanolic extract of root bark and seed of *Caesalpinia bonducella* caused a significant decrease in the ovarian weight and possess anti–estrogenic activity.

The prolonged estrous cycle and its diestrous phase in the extract treated groups are due to unavailability of estrogen at a required level in treated animals and suggest the antifertility effect of *Caesalpinia bonducella* seeds since

prolongation of diestrous phase reduce the chances of the rats to get pregnant. The work of Gebrie *et al*^[30] corroborates the present finding where administration of methanolic root extracts of *Rumex steudelii* in female rats has prolonged estrous cycle and its diestrous phase.

The presence of degenerated follicles and corpora luteum in the ovary of treated animals in this experiment can be correlated with the low level of estradiol and extended diestrous phase of the estrous cycle. The results observed in the present study on the histology of the ovaries are also more or less similar with study of Sharanabasappa *et al*^[31] where treatment of female rats with petroleum ether, benzene, chloroform and alcohol extract of *Momordica charantia* seed extracts caused significant decrease in the number of developing follicles, Graafian follicles and corpora lutea and an increased number of atretic follicles in rats treated with these extracts.

Degeneration of mucosal folds, its associated columnar epithelium cells in ampulla region of oviduct may be attributed to non availability of gonadotropic or steroidal hormones or both. Similarly, Okoko and Yama^[32] have also reported marked mucosal reduction in oviduct of *Abrus precatorius* seed extract treated rats and supports the present study.

It is well established fact that the cell proliferation that occurs in the rodents uterus every 4–5 days depends on the direct action of estrogen^[33]. So the degeneration of endometrial epithelium, uterine glands, and compact stroma of uterus in the present study is due to estrogen deprivation in the blood circulation of seed extract treated animals.

Vagina showed mild degeneration of lamina propria and muscularis. Several studies in animal models have demonstrated that fluctuations in ovarian hormones cause measurable changes in overall vaginal wall thickness, nerve density and structural organization of the muscularis layer^[34]. In the present study it can be conferred the reduced level of estradiol and progesterone might have caused mild degeneration of lamina propria and muscularis at higher doses of CBSE.

The present study demonstrated estrogen antagonist effects of the ethanolic seed extract of *Caesalpinia bonducella* in albino rats mediated through direct effect of the extract on the reproductive organs possibly by suppressing follicular growth in the ovary and/ or by disruption of the hormonal balance in the hypothalamo–hypophysial ovarian and uterine axis.

Conflict of interest statement

We declare that we have no conflict of interest.

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