Asian Pacific Journal of Reproduction

Journal homepage: www.apjr.net

Document heading doi: 10.1016/S2305-0500(13)60124-4

Effect of ethanolic seed extract of *Caesalpinia bonducella* on fertility in pregnant female albino rats

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ARTICLE INFO

Article history:
Received 3 May 2013
Received in revised form 12 May 2013
Accepted 12 May 2013
Available online 20 June 2013

Keywords:
Fertility
Implantation
Caesalpinia bonducella
Corpora lutea
Albino rats

ABSTRACT

Objective: In the present study, an attempt has been made to investigate the effect of ethanolic seed extract of *Caesalpinia bonducella* (C. *bonducella*) on fertility in female Wistar albino rats. **Methods:** Pregnant rats were randomized into two groups containing eight animals in each group. Control group rats were orally administered with 1 mL/100 g b.wt of distilled water while rats in second group received 300 mg/kg of the seed extract from gestation day 1-7, once daily. The animals were sacrificed twenty four hours after the last dose on gestation day 12. **Results:** A significant reduction in implantation index and a contrasting significant increase in resorption index, pre-implantation and post-implantation loss was recorded in the seed extract treated rats. Progesterone level declined significantly in rats treated with seed extract. Ovary revealed degenerated corpora lutea in treated rats. The embryos were noticed with craniofacial deformities in extract treated group. **Conclusions:** The results obtained in this study suggest that the seeds of *C. bonducella* possess antifertility activity probably due to its antiprogesterogenic hormonal properties that can modulate the reproductive function of the experimental rats.

1. Introduction

The use of many plants and herbs for fertility regulation especially among woman has been prevalent in India for many centuries. Natural plant substances possessing mild inherent estrogenic or anti-estrogenic properties offer themselves as effective non-conventional source of contraception with less deleterious side effects. Many plants and herbs have been reported to possess anti-ovulatory, interceptive, abortifacient and emmenagogue activity in laboratory animals [1].

Caesalpinia bonducella F., (Caesalpiniaceae) (C. *bonducella)* 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 and anti-diabetic [3,4] antifilarial [5], anthelmintic [6], anti-inflammatory, antipyretic and analgesic [7], antimalarial[8], immunomodulatory[9], antibacterial, antifungal, antispasmodic[10], antiproliferative[11], antipsoriatic[12], anticonvulsant[13], antiulcer[14], anti-venom[15] and antiurolithiatic[16]. The seeds of C.*bonducella* are found to contain various chemical constituents such as furanoditerpenes, phytosterinin, β-sitosterol, flavonoids, bonducellin, aspartic acid, arginine, citrulline and β -carotene[17].

The seeds of this plant are traditionally used in the fertility regulation in female in India [18-20]. The leaves of *Caesalpinia bonduc*, as well as those of other species are used as an emmenagogue and to facilitate delivery in pregnant women [21, 22]. It has a high reputation among the folk as an agent that facilitates delivery and is widely used in the Ivory Coast [23]. The preliminary studies on the root bark of the plant showed anti-implantation effect in rats [24]. Recent work from our laboratory illustrated antifertility efficacy of its seeds in female rats by decreasing hormone (gonadotropins and steroids) levels, alterations in histoarchitecture of reproductive organs [25]. In view of all these evidences, the present work was intended to check the antifertility activity of ethanolic seed extract C. *bonducella* in pregnant rats.

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2. Materials and methods

2.1. Plant material

The seeds of C. *bonducella* F. (Caesalpiniaceae) were procured from the local market of Dharwad district, 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* [26]. Briefly, the air-dried seeds of C. *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 Whatman paper and the filtrate was evaporated to dryness on rotary flash evaporator below 50 ℃. The yield of the extract was 4 % (w/w). C. *bonducella* seed extracts (henceforth referred to as CBSE) obtained were preserved in sterile glass container at 4 ℃ until further use.

2.3. Animal model

Colony bred virgin Wistar female albino rats (Rattus norvegicus), approximately twelve weeks old and weighing between 180 and 200 g exhibiting regular estrous cycle 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)℃ 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

Normal cycling proestrous or estrous female Wistar rats were caged over-night with males (2:1 ratio) of proven fertility. Next morning, insemination was confirmed by the presence of the vaginal plug and spermatozoa in the vaginal smear. This day of mating was designated as first day of gestation (GD1). These mated females were isolated, weighed and divided into two groups (A, B) of eight animals each. The animals of Group A received distilled water (1 mL/100 g b.wt) and served as control. Animals of group B received ethanolic seed extract at 300 mg/kg b.wt./day (suspended in distilled water) dose, from the first to the seventh day of gestation (GD1 to GD7).

2.5. Maternal parameters

The animals were observed daily for behavioral activities. Body weight was recorded every day during the study period. Feed and water intake of all the rats was recorded throughout the experimental period.

2.6. Implantation parameters

Twenty-four hours after the last dose, on gestation day twelfth, the rat were sacrificed under sodium pentobarbital anesthesia (40 mg/kg). The ovaries were excised, weighed and examined for the number of fresh corpora lutea. Both the uterine horns were examined for the number of implantation sites, live or dead/resorbed fetuses and weighed on Sartorius electronic balance. Embryos with bright reddish aspect and clear margins were considered to be normal and those with dull blue colour, no clear margin, smaller in size and with some surrounding exudate were considered to be resorbing. The following parameters were computed:

Implantation index = Total number of implantation sites/number of Corpora lutea × 100

Resorption index= Total number of resorption sites/Total number of implantation sites × 100

Pre-implantation loss = Number of Corpora lutea − Number of implantations/Number of Corpora lutea × 100

Post-implantation loss = Number of implantations − Number of life fetuses/Number of implantations × 100

2.7. Hormone estimation

Blood samples were collected by cardiac puncture technique in dry glass centrifuge tubes. The blood was then allowed to stand for 10 min at room temperature to clot and centrifuged at 3 000 r/min for 10 min. The supernatant (serum) was then tipped off into separate vial and subsequently subjected for the assessment of progesterone level by Fully Automated Bidirectionally Interfaced Chemi Luminescent Immuno Assay.

2.8. Histological examination

The ovaries were fixed in 10% neutral buffered formalin solution overnight. After proper fixation, the tissues were dehydrated in graded series of alcohol, cleared in benzene and embedded in paraffin wax. The tissue sections were cut at 5 μm thicknesses by Leica RM 2255 microtome and stained with hematoxylin and eosin. The stained slides were photographed under Axio Imager M.2 microscope for histological examination.

2.9. Gross morphology of embryo

The embryos were removed from the uterine horns immediately and were fixed in Bouin's fixative for gross observations under stereoscopic microscope.

2.10. Statistical analysis

Independent-samples t test was employed to analyze data. P values less than 0.05 were considered significant.

2.11. Ethical aspects

The study was approved by the Institutional Animal Ethical Committee, Department of Zoology, Karnatak

University, Dharwad, India. CPCSEA (Committee for the 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.

3. Results

3.1. Maternal parameters

Clinical signs of toxicity such as respiratory distress, salivation, diarrhoea, changes in the appearance of hair tuft as well as maternal mortality were not observed at any time during the treatment period in the extract-treated animals. All animals both in control and extract treated groups appeared healthy, alert and were responding to pain and touch. No significant difference was noticed in final body weights, feed and water intake in both the groups (Table 1).

3.2. Implantation parameters

The weights of ovary and uterus were reduced significantly ($P<0.05$) in extract group when compared to their respective control. The number of the corpora lutea and implantation sites decreased significantly ($P<0.05$) in extracts treated rats. Further, a significant ($P<0.05$) reduction in implantation index as well as the contrasting increase in resorption index, pre-implantation and post-implantation loss of 47.63%, 34.27%, 52.31%, and 43.65% against their respective control, respectively, was recorded in the seed extract treated rats.

3.3. Hormone estimation

Progesterone level was reduced significantly ($P<0.05$) in treated rats when compared with the control group (Table 1).

3.4. Histological examination

The ovary of the control group rats revealed compactly arranged corpora lutea whereas the ovary in seed extract treated group showed degenerated and shrunken corpora lutea.

3.5. Gross morphology of embryo

The embryo in the control group showed well developed head region, nostril region, branchial arches, heart, forelimb and hind limb bud and tail region whereas the treatment with seed extract has caused deformities in almost all the region. Head and nostril region were comparatively condensed in size compared to control embryo. Branchial arches, heart, fore limb bud and hind limb bud region and tail region seemed poorly developed (Figure 1).

4. Discussion

Maternal parameters (body weight changes, food and water intake) and clinical signs of toxicity (respiratory distress, salivation, diarrhoea, changes in the appearance of hair as well as maternal mortality) can be used to evaluate the integrity of maternal homeostasis [27]. In the present study, the increase in the final weight of all the experimental animals suggests that growth was not impaired by the extract. This is an indication towards selective effect of seed extract on maternal homeostasis.

It is well established fact that that seed extract of *C. bonducella* is antiestrogenic in nature [28]. So the decreased uterine weight in the present study may be taken as an indicative of the antiestrogenic nature of *C. bonducella* since antiestrogenic substances decrease the wet weight of the uterus [29]. The uterine weight in pregnant rats serves as an index of uterine decidualization [30]. Thus a significant decrease in uterine weight indicates suppression of uterine decidualization in the present study. A successful implantation depends on embryo quality, uterine receptivity and synchronization of embryonic development and endometrial maturity. So it can be assumed that, component of the seed extract might have acted directly on the uterus and made endometrial environment hostile for implantation leading to decrease in the number of implantation sites. Our recent work [25] also support the present investigation where treatment with graded doses of ethanolic seed extract of *C. bonducella* in non-pregnant rats revealed degeneration of uterine endometrial epithelium, reduced uterine glands and

Table 1
Effect of ethanolic extract of *C. bonducella* seed on maternal parameters, ovarian and uterine weight, implantation parameters and progesterone level.

Parameters	Control	300 mg/kg b. wt.	t and P value
Maternal final weight (g)	213.50 ± 2.65	207.50 ± 1.79	t 14= 1.871, P = 0.082
Feed intake (g/d per rat)	10.42 ± 0.30	9.87 ± 0.13	t 14=1.652, P = 0.121
Water intake (mL/d per rat)	17.72 ± 0.23	17.17 ± 0.13	t 14=2.016, P = 0.063
Weight of ovary(mg)	30.12 ± 0.24	24.13 ± 1.36*	t 14=4.320, P = 0.001
Weight of uterus (g)	2.81 ± 0.07	1.37 ± 0.19*	t 14=6.771, P = 0.000
No. of Corpora lutea	11.87 ± 0.12	9.6 ± 0.67*	t 14=3.255, P =0.006
No. of implantation	11.62 ± 0.18	5.12 ± 0.93*	t 14=6.828, P =0.000
Implantation index (%)	97.90 ± 3.88	47.63 ± 9.05*	t 14=5.486, P =0.000
Resorption index (%)	0.00 ± 0.00	34.27 ± 11.10*	t 14= 3.087, P =0.008
Pre-implantation loss (%)	2.07 ± 1.35	52.31 ± 9.05*	t 14= 5.486, P =0.000
Post-implantation loss (%)	0.00 ± 0.00	43.65 ± 11.90*	t 14=3.667, P =0.003
Progesterone (ng/mL)	67.21 ± 0.25	52.42 ± 0.72*	t 14=19.198, P =0.000

n =8, Data represents mean ± SE; symbol * indicate significant difference ($P < 0.05$) as compared to the control.

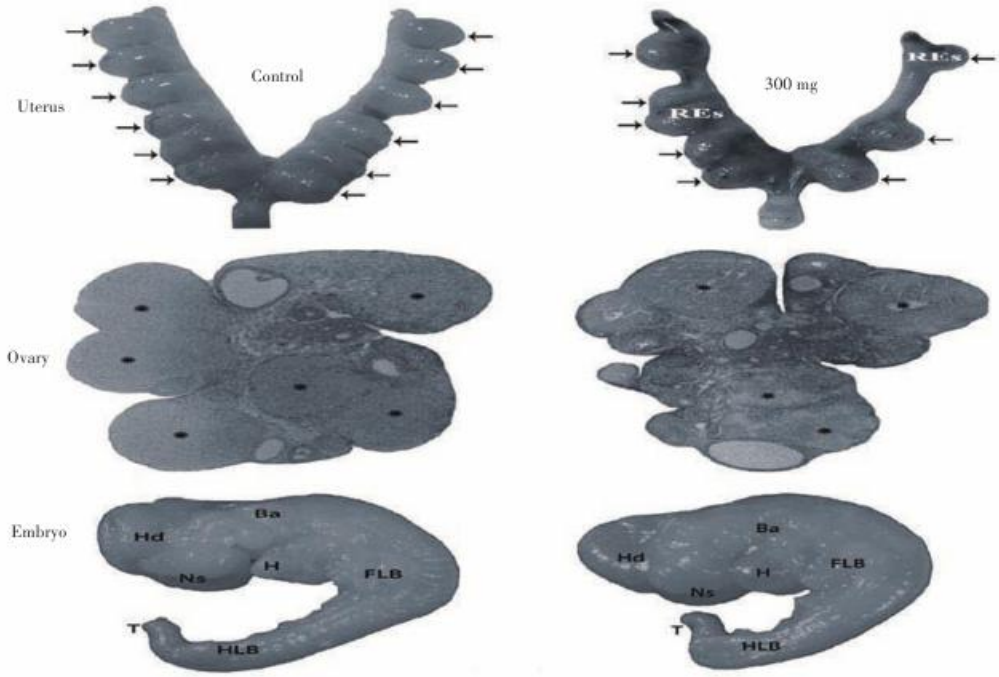

Figure 1. Uteri, histoarchitecture of ovary and gross morphology of embryos in pregnant rat on GD 12 in control group and *C. bonducella* seed extract (300 mg/kg b.wt) treated group.

Arrow marks(→)-Implantation sites, REs-Resorbing sites,(*)represent Corpora lutea, Hd-Head region, Ns-Nostril region, Ba-Branchial arches, H-Heart, FLB-Fore limb bud, HLB-Hind limb, T-Tail.

poor vascularity and stroma. The reduction in ovarian weight and number of corpora lutea by the extract suggests that the reproductive performance of the mother was hindered and further supports the antipregnancy potentials of the extract.

The implantation index and pre-implantation loss evaluates the number of blastocytes implanted in the uterus while the resorption index and post-implantation loss establishes correlation between the number of implanted blastocysts and those that have not developed [27, 31]. The reduction in the computed implantation index as well as the contrasting increases in the pre- and post-implantation losses in the present study is probably due to decrease in the blood levels of progesterone which in turn might have impaired endometrium function. This observation is further strengthened by degenerated corpora lutea in the ovary of seed extract treated rats. Furthermore, the increase in the resorption index by the extract in this study is an indication of failure in the development of the embryo. Such occurrence of foetal resorption suggests that interruption of pregnancy occurred after implantation of the foetus.

Moreover, previous reports indicated the presence of flavonoids, [32] alkaloids [33] and terpenoids [34] in medicinal plants are responsible for contraceptive or pregnancy interceptory effects. Therefore, it can be assumed that the presence of these phytochemical in the seed of C. bonducella might have caused pregnancy interceptory effect in the present study. Poorly developed embryo might be due to inhibition of mitotic division of embryo the as the animal were treated during post-coital period.

The present study thus suggests that ethanolic seed extract of *C. bonducella* possesses antifertility activity probably due to its antiprogesterogenic nature. However, it is very difficult to explain the exact mechanism of antigestational activity of the extract. At present, it can be postulated that its effect is probably due to multiple attributes. Further studies are however needed to establish its mechanism of action and to isolate specific components responsible for it.

Conflict of interest statement

We declare that we have no conflict of interest statement

Acknowledgments

The authors acknowledge financial assistance from DBT-KUD-IPLS Programmme, New Delhi for granting research fellowship to the first author and Department of Zoology, Karnatak University, Dharwad, to undertake the present work.

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