ANTIFERTILITY ACTIVITY OF ROOT BARK OF *CAESALPINIA BONDUC* LINN. ROXB.IN FEMALE ALBINO RATS

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Summary

Birth control is a regimen of one or more actions, devices, sexual practices, or medications followed in order to deliberately prevent or reduce the likelihood of pregnancy or childbirth. Chemical agents of both natural and chemical origins are tried which can provide permanent sterility in female and males In present study 70% ethanolic extract root bark of *Caesalpinia bonduc* Linn. Roxb.(Caesalpiniaceae) have been screened for antifertility activity in female albino wistar rats. The preliminary phytochemical screening of 70% ethanolic extract of root bark of *C. bonduc* was showed to be carbohydrates i.e. non reducing polysaccharides starch, proteins, steroids, saponin glycosides, tannins and phenolic compounds. Implantation is calculated in terms of litters in control groups, when diethyl stilbestrol administered no implants were observed, where as administration of test drug 100% of inhibition of implant was observed. Oral administration of 70% ethanolic extract of root bark of *C. bonduc* at 300 mg/kg b.w. caused a significant increase in uterine wt. in rats ($P< 0.01$). It was found that, there is decrease in the ovarian weight significantly at 300 mg/kg b.w. ($P<0.01$). It may be concluded that the test drug are endowed with potential antifertility activity.

KEY WORDS : *Caesalpinia bonduc,* antifertility activity.

Introduction

World population is currently growing by approximately 74 million people per year. If current fertility rates continued, in 2050 the total world population would be 11 billion, with 169 million people added each year [1]. Use of traditional medicines especially ayurvedic based medicines for birth control among rural people is well practiced and is need to be scientifically established [2]. Birth control can be achieved temporarily by administration of various synthetic and semi synthetic estrogens or progesterone or in combination [3].

Caesalpinia bonduc L., commonly known as Nata Karanja, a prickly shrub found throughout the hotter parts of India, Myanmar and Sri Lanka, has grey, hard, globular shaped seeds with a smooth shining surface. Seeds consist of a thick, brittle shell with a yellowish white bitter fatty kernel [4]. Plant is reported to have multiple therapeutic properties like, antidiuretic, anthelmintic and antibacterial[5], anti-anaphylactic and antiviral [6], antiasthmatic [7], antiamebic and anti-estrogenic[8]. Blood sugarlowering activity of C. bonduc has been primarily evaluated with significant 1 result in rabbit [9] and rat models [10,11]. However, no reports are available on the antifertility activity of *C. bonduc* root bark, therefore present investigation was undertaken to examine the antifertility activity of 70% ethanolic extract of root bark of *C. bonduc* through various *in vivo* models.

Materials and Methods

Plant collection and authentication

Plant of *C. bonduc* were collected from the local areas of Kannur (Bijapur), Karnataka, India and were submitted and the Herbarium (Specimen Voucher Nos. Kascb1 and RMRC-474) were preserved and authenticated by Dr. P. G. Diwakar, Joint Director, Botanical Survey of India, Pune, Maharashtra, India and Dr. Harsha Hegade research scientist ICMR, Belgaum, Karnataka, India respectively.

Preparation of Extracts

The root and root bark were separated and dried and size reduced to a coarse powder. The root bark was extracted with 70 % ethanol. The ethanol was recovered on rotary flash evaporator. The root bark extract was further concentrated and dried over anhydrous sodium sulphate under vacuum to get brownish black sticky residue (21%, ERb).

1. Acute oral toxicity Test: (Acute Toxic Class Model)

The acute oral toxicity test was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD). [12]

All the animal experiments were approved by the institutional animal ethical committee of KLES's College of Pharmacy, Belgaum.

2. Screening for anti-implantation activity [13]:

Anti-implantation testing will be performed on adult Wistar female albino rats (150-200 g). The animals were maintained acrylic cages at room temperature with standard pellet diet and water. The rats in experiment were fertile first ascertain by breeding these virgin females with males of proven fertility. The female rats were used for the testes only after they had borne one or two litters, proving their fertility estrogenic activity of the extracts assess in immature female rats.

The vaginal smears of such female rats of known fertility were examined daily and the rats in proestrous phase of the estrous cycle will be left over night with known fertile males. The female rats will be examined following morning for evidence of copulation. Those rats which show thick clumps of spermatozoa in their vaginal smears were separated from the experiment and the day the spermatozoa will be found labeled as day one of pregnancy. Mated rats were randomly distributed into various groups of six animals in each. The test drug was fed orally to these

pregnant rats at a dose 50, 100 and 300 mg/kg by an intragastric catheter. The control group were received only vehicle in a similar manner. Treatment was given for ten days and the animals were laporotomised under mild ether anesthesia on day 16. The two horns of the uterus were examined for the number of implants and prominent corpora lutea. The animals were allowed to complete the gestation period (usually 21-23 days) and the number of litters delivered, if any counted.

3. Screening for estrogenic activity [14]:

Estrogenic activity of the test drug was assessed in female rats. The selected rats were bilaterally ovariectomized under mild ether anesthesia through lateral incisions in the skin just below the last rib. The ovariectomized rats were divided into five groups of control, standard and test 70% ethanolic extract of root bark of *C. bonduc*.(50,100 and 300) respectively. The different groups of animals received through oral route: vehicle, diethylstilbestrol (1.5 mg/kg) or 70% ethanolic extract of root bark of *C. bonduc* (50, 100 and 300 mg/kg). The test drug was administered once daily for a period of 5 days. Twenty-four hours after the last dose, the animals were sacrificed and theirs uteri dissected, pressed and weighed. The weights of the uteri with fluid (wet weight) and without fluid (dry weight) were recorded. Mean values of each group was calculated and expressed as percent reduction of uterine weight compared to controls treated with diethylstilbestrol alone. Significance of the difference in the weight of the uteri of the animals treated with the test drug when compared with those of the animals in control groups was determined.

4. Hematological studies:

The blood samples were collected from each rat. The collected blood samples were analyzed for various hematological parameters like hemoglobin content, total red blood corpuscles, total white blood corpuscles and differential counts.

5. Ovarian weight in unilateral ovariectomized rats [14]**:**

The ovarian weight increases in control animal 7-14 days after removal of the ovary. Decreases in the ovarian weight in the treated animals compared to the control were indicating an inhibition of ovulation through suppression of follicular stimulating hormone. Ovarian histology may show corpus luteum, etc.

6. Fallopian tube studies:

The animal were sacrificed and to isolate the fallopian tube. The histo-pathalological changes of fallopian tube were studied.

Results

Acute toxicity study:

Studies were conducted all as the OECD guidelines. Results of LD_{50} is 300 mg/kg It was observed that the drug did not shown any toxicity at 3000 mg/kg

Antiimplantation activity:

The anti-implantation activity is expressed as percentage of animals showing absence of implantation in uteri when laparotomized on day 10 of pregnancy. The test drug given orally to the rats at a dose of 50 mg/kg showed 5.667 ± 0.55 no. of implantation sites, the reduction in implantation was 00%, 100 mg/kg showed 4.500 ± 1.52 no. of implantation sites, the reduction in implantation was 33.33%, and anti implantation 300 mg/kg exhibited a very potent anti-implantation activity since no implants 0.833 ± 0.03, in all the treated animals, were observed, indicating 100% anti-implantation activity. Anti-implantation activity was shown in table no-1.

Table no. 1: Anti-implantation activity of ESE.

Group	Treatment		No of rats pregnant/ treated	No. of litters	No of implantation mean (± SEM)	% Pre-implantation loss
I	Control		5,6,9,4,6,5.	5.833 ± 0.703	00	
II	Test drug	50 mg/ kg	06/06	5,6,8,4,5,6.	5.667 ± 0.55	00
		100 mg/ kg	04/06	0,5,6,0,7,9.	4.500 ± 1.52	33.33
		300 mg/ kg	00/06	0,0,0,5,0,0.	0.833 ± 0.03**	100
III	Diethyl stilbestol		0,0,0,0,0,0.	0.0 ± 0.0**	100	

Estrogenic activity:-

In this test the wt. of uterine (both dry and wet wt) was increases as compared to control. 50 mg/kg test drug doesn't showed any increase in wt. and it was non significant (105.4 ± 0.3385 dry wt. and 99.40 ± 0.7599 wet wt.), 100 mg/kg test drug showed less significant (150.4 ± 0.5673 dry wt and 140.9 ± 1.022 wet wt.) as compare to standard and 200 mg/kg test drug showed highly significant increase in wt. of uterine (198.5 ± 3.23 dry wt and 189.0 ± 4.88 wet wt.) shown in fig. 1 and 2 and vaginal opening showed up to 80%.

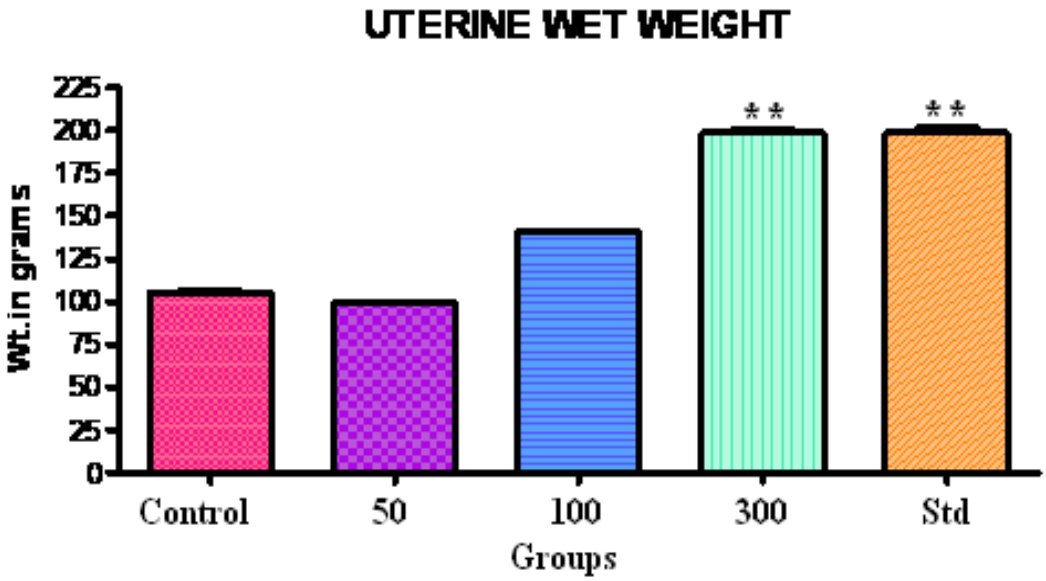

Fig. 1: Estrogenic activity: uterine wet weight of ERb.

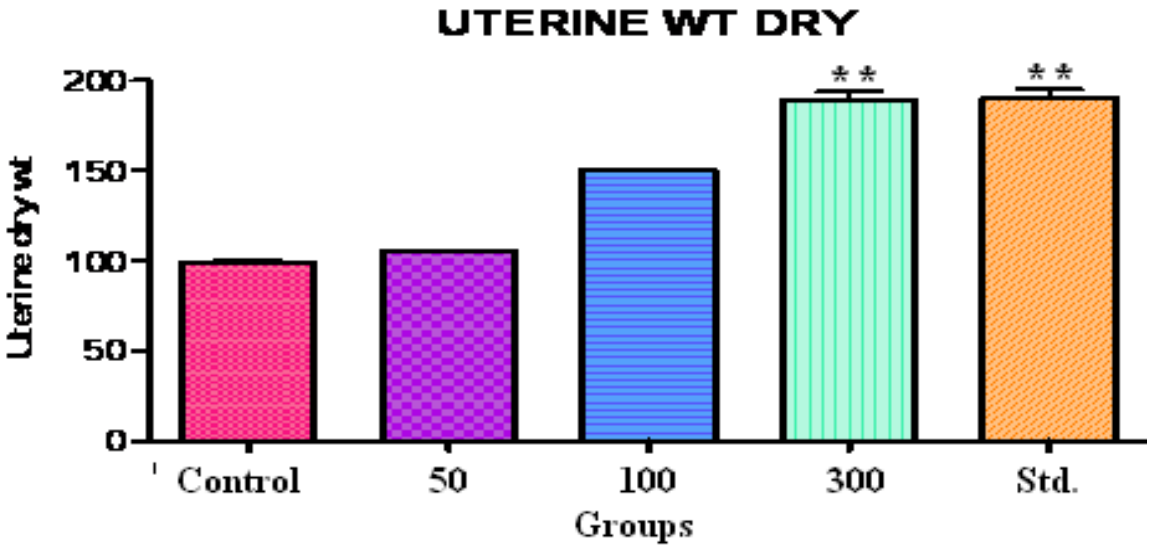

Fig. 2: Estrogenic activity: uterine dry weight of ERb.

Haematological Parameters: There was a non significant change in haematological parameters seen in all three doses. Haemoglobin, Total erythrocyte count, Total leucocyte counts, Nutrophils, Lymphocytes, Monocytes, Eosinophils values found to be within normal range when compared to control.

Ovarian weight in unilateral ovariectomized rats:-

Ovarian wt was decrease in 100 mg/kg and 200 mg/kg test drug (52.05 ± 0.7364 and 46.09 ± 1.04) respectively as compare to control group and this result showed that 200mg/kg was more significant shown in table no. 2 and graphically presented in fig-3.

Table no.4 : Ovarian weight in unilateral ovariectomized rats of ERb.

Group	Treatment	Dose	Ovarian weight (± SEM)
I	Control	2ml/kg	62.32 ± 0.545
II	Test drug of ERb	50 mg/ kg	60.38 ± 0.239
III		100 mg/ kg	50.05 ±0.723**
IV		300 mg/ kg	42.29 ± 1.45**
V	Diethyl stilbestol	1.5 ml/kg	38.35± 0.217**

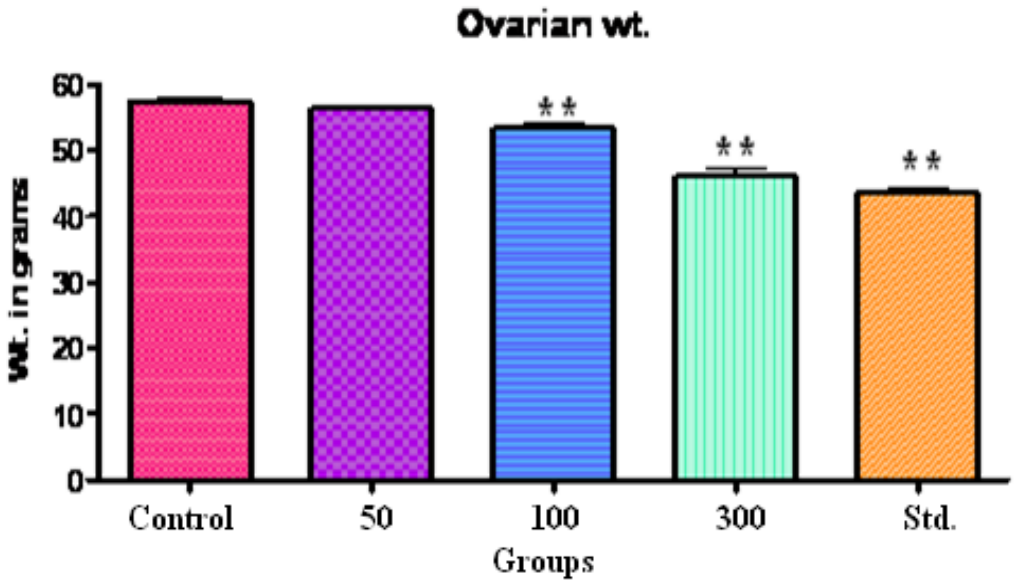

Fig.3: Ovarian weight in unilateral ovariectomized rats of ERb.

Discussion

To assess the antifertility activity of ERb in female albino rats by using parameters like Antiimplantation activity, Estrogenic activity, Haematological Parameters, Ovarian weight in unilateral ovariectomized rats were carried out and the antifertility effect has observed. Rats typically have rapid cycle times of 4 to 5 days. Although they ovulate spontaneously, they do not develop a fully functioning corpus luteum unless they receive coital stimulation. Fertile mating leads to pregnancy in this way, but infertile mating leads to a state of pseudopregnancy which lasts about 10 days. Mice and hamsters have similar behavior. The events of the cycle are strongly influenced by lighting periodicity. A set of follicles start to develop near the end of proestrus and grow at a nearly constant rate until the beginning of the subsequent estrus when the growth rates accelerate eightfold. They then ovulate about 109 hours after starting growth. Oestrogen peaks at about 11am on the day of proestrus. Between then and midnight there is a surge in progesterone, LH and FSH, and ovulation occurs at about 4am on the next, estrus day. The following day, metestrus, is called early diestrus or diestrus I by some authors. During this day the corpora lutea grow to a maximal volume, achieved within 24 hours of ovulation. They remain at that size for 3 days, halve in size before the metestrus of the next cycle and then shrink abruptly before estrus of the cycle after that. Thus the ovaries of cycling rats contain three different sets of corpora lutea at different phases of development [15]. The results of qualitative chemical investigation of *Caesalpini bonduc* root bark indicated the presence of the compounds like- carbohydrates i.e. non reducing polysaccharides (starch), proteins, sterols, saponin glycosides, tanines and phenolic compounds. Gross behavioural studies were carried out by method Choudhury NSK et.al. In this study the animals were kept under supervision up to 14 days, there is no mortality was observed in all treated rats.

Anti-implantation activity was carried out by method Choudhury NSK et. al. Implantation is calculated in terms of litters in control groups, when diethyl stilbestrol administered no implants were observed, where as administration of test drug 100% of inhibition of implant was observed [13]. It is well known that for implantation exact equilibrium of estrogen and progesterone is essential, any disturbance in level of these hormones causes infertility. The test drug contains sterols of estrogen property [16]. Standard drug used i.e. diethyl stilbestrol inhibites implant as Estrogen binds to specific nuclear receptors in target cells and produce effects by regulating protein synthesis.

Conclusion

In conclusion, the administration of test drug *Caesalpinia bonduc* exhibite potent anti-implantation activity, anti-estrogenic activity, abortion and anti-estrogenic activity, anti-ovulatery activity. Thus the scientific validation for the traditional claims for antifertility use of plants may be justified. Further studies are recommended for isolation of single active constituent and understanding the exact mechanism(s) of the test drug. Hence, *Caesalpinia bonduc* Linn. Roxb. Root bark (ERb) can be used for induction in sterility i.e. it can be used as anti-fertility agent.

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