Antifertility Efficacy of the Plant *Balanites Roxburghii* (Balanitaceae) in Female Rats

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Petroleum ether, chloroform, ethanol, and distilled water extracts of the fruits of the plant *Balanites Roxburghii* (Balanitaceae) were tested for antifertility activity in female albino rats at a dose of 300 and 600 mg/kg body weight orally. Among these, the ethanol extract was found to be most effective in causing significant abortifacient activity. The antifertility activity was found to be dose dependent and reversible on withdrawal of the treatment. Phytochemical screening of the ethanol extract showed positive tests for the presence of alkaloids, glycosides, saponins, flavones, and phenolic compounds. The histological studies of the uterus and ovary were carried out to confirm the estrogenic activity. Acute toxicity studies of the crude extracts in mice revealed the non-toxic nature of the crude extracts.

The quest for the oral contraceptive agent that can control human fertility is as old as recorded history. Although a wide variety of synthetic contraceptive agents[1,2] are available, these cannot be used continuously due to their severe side effects[3,4]. Hence people are now looking back to age old tradition of using herbal medicines, which have minimum side effects. India in general and Western Ghat region in particular has enormous wealth of medicinal plants. Presently, a major research programme on systematic investigation of medicinal plants for their phytochemical, biological, and pharmacological properties, including antifertility properties, was undertaken in our laboratory[5,6]. As part of this research programme, we present in this paper antifertility efficacy of fruits of the plant *Balanites Roxburghii*.

Balanites Roxburghii is a small evergreen thorny tree found in drier parts of India. The bark, unripe fruits, and leaves of this plant are reported to have anthelmintic, analgesic, purgative, and antidysentric properties[7,8]. The antifeedant active saponins have been isolated from bark[9]. Aqueous suspension of dried fruits of this plant is being used as abortifacient by local herbal healers. This fact encouraged us to take up the fruits of *Balanites Roxburghii* for detailed investigation.

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MATERIALS AND METHODS

The plant *Balanites Roxburghii* (FDD. 822) was identified and compared with the herbarium maintained at the Department of Biotechnology, Kuvempu University. The fruits of *Balanites Roxburghii* were collected from uncultivated fields in and around Chitradurga (Karnataka) during fruiting season, i.e., June-July. The fruits were shade-dried, powdered, and subjected to soxhlet extraction (450 g) successively with petroleum ether (60-80°, 2 l), chloroform (2 l), ethanol (95%, 2 l), and finally it was macerated with distilled water (2 l) and kept for 7 d to get the crude extracts. The extracts were concentrated to dryness in a flash evaporator (Buchi type) under reduced pressure and controlled temperature (50-60°). The petroleum ether extract yielded yellow coloured mass, chloroform and ethanol extracts gave dark coloured residues, while water extract resulted in brown coloured amorphous solid.

Wistar rats of either sex (150-200 g; National College of Pharmacy, Shimoga) were used for evaluating antifertility activity. They were housed in polypropylene shoebox-type cages with stainless steel grill top and bedded with rice husk. The animals were provided with pelleted diet (Goldmohur, Lipton India, Mumbai) and water *ad libitum*.

They were allowed a one-week acclimatization period before the experimental session. All the experimental protocols were met with the approval of Institutional Animals Ethics Committee (Reg. No. 144/1999/CPCSEA/5-7-99).

Phytochemical screening:

The presence of various plant constituents in the extracts was determined by preliminary phytochemical screening as described by Kokate[10]. The observations made are presented in the Table 1.

Dose fixation:

Dose fixation was carried out by staircase method on Swiss mice (80-100 g). All the extracts were homogenised in Tween-80 (1%) and dissolved in distilled water, and they were administered to rats orally by means of intragastric catheter. It was observed that none of the extracts were found to be lethal even at the dose of 3000 mg/kg body weight. Hence one-tenth of 3000 mg/kg, i.e., 300 mg/kg body weight of the crude extracts was fixed as the dosage. As the extracts were found to be non-toxic, double of this dose, i.e., 600 mg/kg body weight was also chosen to ascertain the response of the animals and also to study the dose dependency.

Antiimplantation activity:

Colony-bred virgin female Wistar rats (150-200 g) were used. The vaginal smears from each rat were monitored daily. Only the rats with normal estrous cycle were selected for the experiment[11]. Antifertility activity was determined as described by Khanna and Chowdhary[12].

The female rats were caged with male rats of proven fertility in the ratio of 2:1 in the evening of proestrous and examined the following day for the evidence of copulation[13]. Rats exhibiting the copulation plug or thick clumps of spermatozoa in their vaginal smears were separated and that day was designated as day 1 of pregnancy, and those rats were divided into nine groups containing six rats in each group. The group I received vehicle only (Tween-80, 1%) and served as control. Groups II, IV, VI, and VIII received petroleum ether, chloroform, ethanol, and distilled water extracts at a dose of 300 mg/kg body weight, while groups III, V, VII and IX received the same extracts but at a dose of 600 mg/kg body weight. The above treatments were given from day 1 to 7 of pregnancy and on day 10, laparotomy was performed under light ether anaesthesia using sterile conditions. The uteri were examined to determine the number of implantation sites. The abdominal wound was sutured in layers, and the animals were allowed to recover and deliver after full term. Each pup was weighed and examined for gross defects. The litters were allowed to grow to check post-natal growth and monitor any congenital abnormalities. The results are tabulated in Table 2.

TABLE 1: PHYTOCHEMICAL SCREENING OF CRUDE EXTRACTS OF FRUITS OF *BALANITES ROXBURGHII*

Class of compounds	Petroleum ether extract	Chloroform extract	Ethanol extract	Distilled water extract
Alkaloids	-ve	-ve	-ve	+ve
Steroids	+ve	+ve	-ve	-ve
Saponin glycosides	+ve	+ve	+ve	-ve
Protiens	-ve	-ve	+ve	+ve
Flavonoids	-ve	-ve	+ve	-ve
Fixed oils and fats	+ve	+ve	-ve	-ve
Tannins and phenolics	-ve	-ve	+ve	-ve

+ve indicates presence of chemical constituents and; -ve indicates absence of chemical constituents

TABLE 2: ABORTIFACIENT ACTIVITY OF THE CRUDE EXTRACTS OF FRUITS OF *BALANITES ROXBURGHII*

Treatment	Dose mg/kg body weight	Group	No. of rats pregnant /treated	Mean No. of implants on day 10	Mean No. of litters delivered	Percentage reduction in pregnancies
Control	-	I	6/6	11.00	11.00	-
Pet. ether extract	300	II	6/6	11.28	8.33	26.15
	600	III	6/6	10.86	7.33	32.50
Chloroform extract	300	IV	6/6	11.63	9.50	18.31
	600	V	6/6	11.92	9.16	23.15
Ethanol extract	300	VI	6/6	12.37	6.66	46.16
	600	VII	6/6	12.94	5.16	60.12
Water extract	300	VIII	6/6	12.60	10.16	19.36
	600	IX	6/6	11.62	9.04	22.20

No. of rats used in each group=6

Petroleum ether, chloroform, and ethanol extract of the plant *Balanites Roxburghii* were found to possess abortifacient activity. The phytochemical investigations have proved the presence of steroids in petroleum ether and chloroform extracts. As our search is aimed at isolation of nonsteroidal antifertility agent, only the ethanolic extract was taken up for the detailed investigation of estrogenic and antiestrogenic activity in Wistar rats to ascertain the possible mechanism of its antifertility action.

Estrogenic/antiestrogenic activity:

Colony-bred Wistar female rats, aged 21-23 d, weighing between 50-55 g were bilaterally ovariectomised under light ether anaesthesia and under sterile conditions. They were divided into four groups consisting of six rats each. The group I served as control and received vehicle only (Tween-80, 1%, 5 ml/kg). The group II received ethinyl estradiol in olive oil (1 μg/rat/day) subcutaneously. The group III received ethanol extract at the dose of 600 mg/kg body weight. The group IV received, in addition to ethinyl estradiol (1 μg/rat/day), ethanol extract at the dose of 600 mg/kg body weight. All the above treatments were given for 7 d. Vagina and the vaginal smears were examined in all the animals in the treated groups for 7 d of treatment. On day 8, the rats were sacrificed by decapitation, the uteri were dissected out, and the exact weights of the uteri were recorded. The two horns of the uterus were separated; one of the horns was used for investigation of biochemical changes and the other was utilized for histological studies. The separated horn was fixed in Bouin's fluid for 25 h, dehydrated in alcohol, and then embedded in paraffin. The paraffin blocks were sectioned at 6 μm intervals and then stained with haematoxylin-eosin for histological studies. The diameter of the uterus, thickness of endometrium, and the height of endometrial epithelium were measured in 10 randomly selected sections using a calibrated ocular micrometer. The statistical analysis was carried out by using Student's *t*-test. The results were expressed as mean ± SEM. The findings are summarized in Tables 3 and 4.

Biochemical studies:

The biochemical changes in the uterus of the treated rats were carried out to know the effect of ethanol extract on the total protein content and total glycogen content of the uterus. Total protein content and glycogen content were estimated by the method as described by Lowry *et al.*[14] and Good *et al.*[15] respectively. The results are tabulated in Table 5.

Statistical analysis:

The statistical analysis was done so as to determine the significant difference of results between treated and control groups using Student's *t*-test as described by Kulkarni[16].

RESULTS AND DISCUSSION

All the extracts were evaluated for post-coital antifertiltiy activity at the dose of 300 mg/kg and 600 mg/kg body weight. No toxic effects were observed either by gross visual examination or in the weight of rats. After discontinuation of the treatment, all the rats were mated. This resulted in pregnancy and delivery of normal litters,

TABLE 3: ESTROGENIC/ANTIESTROGENIC ACTIVITY OF THE ETHANOL EXTRACT OF FRUITS OF *BALANITES ROXBURGHII*

Group	Treatment group (dose/day)	Uterine weight in mg ± SEM	Vaginal cornification
I	Control	62.14±4.52	nucleated and cornified cells
II	Ethinyl estradiol(1 μg/rat)	302.72±13.38*	cornified cells
III	Ethanol extract(600 mg/kg)	114.81±9.17*	nucleated and cornified cells
IV	Ethinyl estradiol (1 μg/rat) + ethanol extract (600 mg/kg)	322.16±9.62*	cornified cells

*P <0.01 *vs.* control, Student's *t*-test, n=6

TABLE 4: HISTOLOGICAL CHANGES IN THE UTERUS

Group	Treatment (Dose/day)	Diameter of the uterus (μm)±SEM	Thickness of the endometrium (μm)±SEM	Height of the endometrial epithelium (μm)±SEM
I	Control	356.56±6.06	52.82±2.18	28.14±1.06
II	Ethinyl estradiol (1 μg/rat)	861.24±2.84	247.63±1.92*	48.72±9.12
III	Ethanol extract (600 mg/kg)	571.40±1.48	75.92±1.12	37.63±1.52*
IV	Ethinyl estradiol (1 μg/rat) + ethanol extract (600 mg/kg)	911.10±2.66*	268.32±4.38*	54.44±3.72*

*P <0.01 *vs.* control, Student's *t*-test, n=6

TABLE 5: BIOCHEMICAL CHANGES IN THE UTERUS OF TREATED RATS

Treatment (Dose)	Protein content µg/mg±SEM	Glycogen content µg/mg±SEM
Control	8.24±0.40	1.52±0.20
Ethinyl estradiol (1 µg/kg)	10.92±0.84	1.92±0.45
Ethanol extract (600 mg/kg)	8.78±0.30*	1.50±0.11
Ethinyl estradiol (1 µg/kg) + ethanol extract (600 mg/kg)	12.41±0.15	2.06±0.5

*p <0.01 *vs.* control, Student's *t*-test, n=6

indicating that the action of the extracts of fruits of *Balanites Roxburghii* is reversible.

There was no noticeable change in the colour and size of the implants when compared with rats of control group. These observations clearly indicate that none of the extracts possess antiimplantation activity. However, all the extracts exhibited abortifacient activity. Amongst these, ethanol extract showed 46.16% abortifacient activity at a dose of 300 mg/kg body weight and 60.12% at a dose of 600 mg/kg body weight. All the animals in the treated group showed open vagina, while the control group had closed vagina, which further support the abortifacient activity. No vaginal bleeding was observed in any of the animals. This observation revealed that all the implants have been resorbed by the uterus.

Oral administration of the ethanol extract caused significant increase in uterine weight. The uterotropic potency, as shown by the increase in weight of the uterus, was about 40% in the case of ethanol extract, when compared with that of ethinyl estradiol. This suggested mild estrogenic activity of the extract. This fact was well supported by other parameters such as increase in diameter of the uterus, thickness of endometrium, and height of endometrial epithelium.

It was also observed that the ethanol extract potentiated the action of ethinyl estradiol when administered together. This fact was well indicated by the significant increase in diameter of uterus, thickness of endometrium, and height of the endometrial epithelium. Examination of the vaginal smears of the treated rats showed predominantly cornified and nucleated epithelial cells.

The results of biochemical changes also support the observations made in histological studies that the ethanol extract of *Balanites Roxburghii* has mild estrogenic activity. Administration of ethanol extract along with ethinyl estradiol at the dose of 600 mg/kg body weight and 1 µg/rat/day, respectively for 7 d resulted in increase in protein as well as glycogen content in the uterus.

In the present study, the extracts of the fruits of the *Balanites Roxburghii* were tested at the dose of 300 mg/kg and 600 mg/kg body weight. All these extracts did not exhibit any antiimplantation activity. However, abortifacient activity was exhibited by these extracts.

It is a well known fact that for implantation and sustenance of pregnancy, exact equilibrium of secretion of estrogen and progesterone is necessary. Any imbalance in the levels of these hormones can cause antiimplantation or can induce abortion[17]. Compounds disturbing hormonal functions may evoke infertility[18]. In this study, the histological changes observed in the uterus of animals treated with various extracts support an unfavourable uterine milieu. Therefore, the abortifacient activity may be due to mild estrogenic activity of the extracts causing expulsion or resorption of the implants by the uterus. The abortifacient activity of the ethanol extract was found to be reversible on withdrawal of the treatment. The ethanol extract at a dose of 600 mg/kg body weight was found to possess 60.12% of abortifacient activity. The abortifacient activity may be due to mild estrogenic activity of the extract. Two known flavones – apigenin and luteolin – have been isolated and found to possess antifertility activity[19]. The phytochemical investigations of ethanol extract have indicated the presence of saponin glycosides and flavonoids. The antifertility activity of ethanol extract may be due to the presence of saponin glycosides[20] and flavonoids[21].

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