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Anti-implantation activity of *Terminalia bellirica* bark extracts in female albino rats

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ABSTRACT

Ancient literature mentions the use of number of plants, plant preparations and their phytochemical constituents for fertility regulation. Some local contraceptive agents have also been described in Ayurvedic, Sidda and Unani texts. Documented experiments or clinical data are, however, lacking. Therefore, the present investigation was undertaken to discover the antiimplantation activity of benzene and ethanol extracts of graded doses of the barks of *Terminalia bellirica*. A strong antiimplantation (74.20% inhibition) activity was observed at the dose level of 25mg/100g body weight. Thus, the *T. bellirica* barks extract may be explored as a female contraceptive.

KEYWORDS

Antiimplantation;
Fertility;
Terminalia bellirica;
Contraceptive;
Rats.

INTRODUCTION

Plant derived constituents that influence endocrine activities in both humans and animals have received a great deal of attention due to their possible beneficial as well as adverse effects[1]. Some of these plants are known to possess antifertility effect through their action on hypothalamo-pituitary-gonadal axis or direct hormonal effects on reproductive organs resulting in inhibition of ovarian steroidogenesis[2,3]. Example include *Mommordica charantia, Crotalaria juncea, Hibiscus rosa sinensis, Citrus medica, Oxalis corniculata, Rivea hypocrateriformis, Acalypa indica, Striga* specis which are used traditionally and reported to control fertility as well as abortifacient during early pregnancy[4-15].

Terminalia bellirica is a large deciduous tree. It belongs to Combertaceae family and available in forests of India, Burma, and Ceylon except in the dry and arid region of Sind and Rajaputana. The bark has medicinal value in anemia and leucoderma. The fruit are useful in bronchitis, sore throat, biliousness, inflammations, strangary, asthama and in diseases of the eye, the nose, the heart and the bladder. The seed is acrid, intoxicating useful in thirst, vomiting, bronchitis, corneal ulcers, relieves "*Vata*" (Ayurveda)[16].

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The fruit is one of the three constituents of the important Indian Ayurvedic preparation "*Tripala*". Antifertility effects of *Terminalia* species have been reported on mammals[17-22]. The present investigation is the first ever study undertaken to find the unexplored antiimplantation activity of the barks of *T. bellirica*, using benzene and ethanol being non-polar and highly polar, so as to extracts the maximum phytoconstituents present in the barks.

MATERIALS AND METHODS

Plant collection and preparation of extraction

The barks of the *Terminalia bellirica* were collected from around and near P.G. centre of Gulbarga University, Gulbarga, Nandihalli Camp, Sandur, (Karnataka, INDIA). The plant was authenticated by Dr.Y.N.Seetharam, Professor, Department of Botany, Gulbarga University, Gulbarga, Herbaria are made and their voucher specimen retained in the department for future references.

The shade dried barks of the plants were coarsely powdered (400g) and were extracted with benzene and ethanol (95%) in a soxhlet extractor for 72 hour. The concentrated to dryness under reduced pressure and controlled temperature (50-60°C) to yield a reddish brown semi powdered (47g). The extracts were stored in refrigerator. For administration to test the animals the extract were macerated in Tween-80 (1%) and resuspended in distilled water for their complete dissolution.

Animals

Colony bred female virgin albino rats (Wistar strain) of 60-70 days old and weighing 150-180g selected from the inbred animal colony was used for the antiimplantation activity. Adult albino mice of either sex were used for acute toxicity studies. They were fed with pellet diet as prescribed by CFTRI, Mysore, INDIA and water *ad libitum*. The temperature was maintained at 28±2°C with lighting schedule of 12 hrs light and 12 hrs darkness. The Institutional Ethical Committee for Animal Cares and use approved all experimental procedures.

Acute toxicity studies

Acute toxicity was carried out as described by Turner[23]. Adult albino mice of either sex were divided into three groups containing six animals in each group. The mice were fasted for 18h with water *ad libitum*. The suspension prepared as above were administered orally at two different doses 10 and 25mg/kg body weight, respectively to different extracts of different group of mice separately. Control mice received 0.1ml Tween-80 (1%). The animals were observed for 72h for behavioral changes and mortality.

Antiimplantation activity

Proven fertile female Wistar strain rats, with normal estrous cycle[24] were selected for this study. Antifertility activity was determined in female albino rats as described by Khanna and Choudhury**[25]**. Rats found in the estrous phase of the cycle were caged with males of proven fertility in the ration of 2:1. Animals, which showed thick clumps of spermatozoa in the vaginal smear, were separated for the experiment and that day was designated as day 1 of pregnancy[24]. These animals were divided into 7 groups consisting of 6 animals in each group. The group I received vehicle only and served as control. Groups II and III received benzene extract at doses of 10 and 25mg/kg body weight respectively. Group IV and V received ethanol extract at doses of 10 and 25mg /kg body weight respectively. All the above treatments were given from day 1 to 7 of pregnancy and on day 10, laparotomy was performed under light ether anesthesia using aseptic condition and uteri were observed for number and size of implantation sites.

The abdominal incision was closed with sutures and the rats were allowed to recover and deliver full term pregnancy[24]. Those rats showing implantation sites but not delivered were again laparotomised on day 23, and the uteri were examined for implantation sites. Each fetus was weighed and examined for a genital distance and gross defects. The litters were allowed to grow to check postnatal growth and congenital anomalies.

Statistical analysis

The statistical analysis was done to determine the significant difference of results between treated and control groups using as described by Snedchor and Cochran[26], All the values were statistically analyzed by using Student's-'*t*' test. The values were judged al-

most significant if $p<0.05$, significant if $p<0.01$ and highly significant if $p<0.001$.

RESULTS

Acute toxicity studies

No mortality and changes in the behavior were observed in all treated and control groups of the mice up to the dose of 250mg/kg body weight. Hence, one tenth of the doses were used for antiimplantation activity.

Antiimplantation activity

The antiimplantation activity is expressed as percentage of animals showing absence of implantation in uteri where laparotomized on day 10 of pregnancy. The mean number of implants in control rats is 10.50±1.22. Whereas, 10 and 25mg/100g body weight of benzene extract has inhibited the implantation slightly, as a results 9.18±1.07 and 8.33±1.21 implants with respective inhibition of 12.58% and 20.67% are seen. However, the ethanol extract at the dose level of 10 and 25mg/100g body weight have shown significant reduction with 6.28±1.62 ($p<0.05$) and 2.71±1.19 ($p<0.01$) implants with respective inhibition of 30.60% and 74.20%. Among the two extracts of *T. bellirica* barks evaluated for antiimplantation activity the ethanol extract is more effective. Though 6/6 rats in all the groups have shown implantation sites, it is considerably less in high dose of ethanol extract administration.

No toxic effects were observed in the animals and their pups by gross visual examination when the pregnancy is continued. Soon after the parturition all the experimentation animals exhibited normal estrous cycle and breeding they underwent normal pregnancy and delivered normal litters, including that the action of the extract was reversible.

DISCUSSION

In the present study oral administration of benzene and ethanol extract of *Termilalia bellirica* barks at the doses 10 and 25mg/100g body weight orally from day 1 to 7 of pregnancy, produced a dose dependent adverse effect on fertility index (Quantal pregnancy) and number of implantations in uterine horns of the female rats by virtue of an increase in the percentage of the preimplantation embryonic loss. These results are in agreement with the earlier findings of Sharangouda et al.[3].

TABLE 1 : Effect of benzene and ethanol extracts of *Terminalia bellirica* barks on implantation in rat when administered orally day 1 to 7 of pregnancy

Group	Treatment	Dose	No. of rats having no implantation sites on day 10	Mean no. of implants	%inhibition of implantation
I	Control	0.1ml Tween-80	Nil	10.50±1.22	Nil
II	Benzene	10mg	Nil	9.18±1.07	12.58
III		25mg	Nil	8.33±1.21	20.67
IV	Ethanol	10mg	Nil	6.278±1.06*	30.60
V		25mg	Nil	2.71±1.19**	74.20

Duration: 07 days
Values are mean ± S.E.
Six animals were maintained each group
***p<0.05, **p<0.05 and ***p<0.001 when compared to control**

The loss of implantation caused by both the extract may be due to antizygotic, blastocytotoxic or antiimplantation activity[27]. Pregnancy interceptive effect of the extracts of *T. bellirica* barks can be interrupted as due to the estrogenic nature of the plant. Regular development of all the events leading to nidation, at least in rats and mice is chiefly under the direct command of estrogen-progesterone interplay at the cellular level[28] and a slight disturbance in this hormonal balance may result in an unfavorable endometrial environment.

Pre-implantation losses can also arise due to disruption of events that are prerequisite for fertilization or impairment in the production of cytokines, growth factors and various types of adhesion molecules, either by the developing blastocyst or by uterine epithelium around the site of implantation[29-30].

Plant products exhibiting estrogenic activity and producing antifertility effects are known in literature, viz., the ingestion of 10 and 20mg/100g body weight of ethanol extract of *Oxalis corniculata* whole plant from 1-7 of pregnancy and not cause pregnancy failure but reduced the number of viable fetuses and increased the number of resorptions in female pregnant rats[3]. Flavonoides isolated from *Striga lutea* and *Striga orobranchoides* possessed estrogenic and antifertility property[12,13]. Phenolphthalein isolated from *Momordica charantia* seeds showed potent

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antiimplantation and pregnancy interruption in albino rats[4]. Withdrawal of the treatment to adult rats has resulted in resuming the normal estrous cycle within 6-8 days.

In conclusion, among the two extracts of *Terminalia bellirica* barks evaluated fro antiimplantation activity the ethanol extract at the dose level of 25mg/100g body weight is more effective and potential.

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