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Preliminary study on the anti - implantation activity of *Leptadenia reticulata* in female rats

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ABSTRACT: The effect of ethanolic extract of the whole plants of *leptadenia reticulata* has been studied in albino rats to explore it's the anti-implantation and hormonal activities. A strong anti-implantation (inhibition 100%) and uterotropic activity was observed at the dose level of 300 mg /kg. No antiestrogenic activity could be detected.
Keyword: Leptadenia reticulate; Antifertility; Anti-implantation; Estrogenic; Uterotropic

1. INTRODUCTION

Efforts are being made to develop antifertility products from plants. Ethno medical literature contains thousands references to the use of plants for a variety of reproduction related purposes (1). One such plant is *leptadenia reticulate* belonging to family Asclepiadaceae, well known for its tonic, restorative and stimulant property in the Indian system of medicine. This plant is distributed in the southern parts of India. The main constituents reported are stigma sterol, beta-sitosterol, flavonoids, pregnane glycosides and proteins (2). Aerial parts of *leptadenia reticulata* is reported to contain tocopherol and possess several pharmacological activities such as galactogogue,antimicrobial and anti-inflammatory activity. Seeds of L.reticulata are reported to contain hyperoside,aflavonoid glycoside. L.reticulata is claimed to have hypotensive effect in dogs. Antioxidant principles derived from plants are reported to have antitumor activity (3). Hence it was decided to illustrate the ethno botanical use the plant and the study was planned find out the unexplored anti fertility and hormonal activity of the ethanolic extract of the leaves *leptadenia reticulata.*

2. EXPERIMENTAL

2.1. Plant material

The whole plants were collected in the summer season from srimushnam village in Tamil Nadu, India. The drug was identified by the department of Botany. Annamalai University, Chidambaram, India and voucher specimen was deposited in institutional herbarium (voucher no 2299)

2.2. Preparation of extract

Powdered whole plants of *Leptadenia reticulata* (500g) was extracted (soxhlet) with 70% ethyl alcohol in 1; 10 w/v ratio for 72hrs.they yield was 20g. Qualitative chemical analysis revealed that the extract contains alkaloids, carbohydrates steroids, glycosides, flavonoids and reducing sugars. (4-5)

2.3. Animals

Colony-bred Female albino rats (wistar strain) were maintained in standard environmental condition of temperature, relative humidity, light/dark cycle and fed a commercial diet and water ad libitum. All the experimental were performed according to the CPCSEA norms after obtaining the approval of the institutional animal's ethics committee (IAEC).

Acute toxicity studies

Acute toxicity study was carried out on whole plant extract of *Leptadenia reticulate. The extract was found to be safe up* 3000mg/kg of body weight. The oral acute toxicity study was performed using the up and down procedure (OECD guidelines)

2.4. Anti-implantation activity

Proven fertile females wistar rats, weighing between 200 and 250 g were selected and left overnight with males of proven fertility in the ratio of 3:1. Day 1 of the pregnancy was confirmed by the presence of spermatozoa in the vaginal smear. Twelve pregnant rats were selected for anti- implantation activity. Six rats received vehicle while the other six received LRA (300mg/kg) orally suspended in tween80 from day 1 to 7 of the pregnancy. On the 10th day of pregnancy, laparotomy was performed under ether

anesthesia and the uterine horns were inspected for number of implants.Anti-implantation activity was determined as described by Khanna and Chaudhary (1968).

2.5. Hormonal profile

2.5.1. Estrogenic activity

Colony- bred immature female rats (25-30 days old) were divided into four groups (six in each group) and treated as follows;

- Group I-control (1%tween 80 suspension);
- Group II-LRA suspension (500mg/kg,p.o.);
- Group III-Ethynyl estradiol EED)(0.1ug/rat,i.m);
- Group-EED 0.1ug/rat,i.m) +LRA(250mg/kg,p.o.)

Treatment was continued for 7days and the animal were killed by cervical dislocation 24h after the last treatment .the uteri were dissected out, blotted dry and weighed.(8-9)

2.5.2. Ponderal changes in genital organs.

Female rats were bilaterally ovariactomized. Sixteen days after the ovariactomy, the rats were divided into four groups of six each and were treated in a similar way for estrogenic activity .treatment was continued for 5days .The animals were killed 24h after last treatment. The uterus, cervix and vagina of each animal was dissected out, blotted dry and weighed.

2.5.3 Estrous cycle study

Female rat's shows normal estrous cycle were selected and divided into two groups of six rats. The first group served as control and received only vehicle of formulation orally for 30 days. The second group received LRA suspension (300 mg/kg) orally every day for the same period. The vaginal smears were observed every morning in both the groups of animals to check any variation in the estrous cycle.(12)

3. RESULT

No mortality and changes in the behavior were observed in all the treated and control groups of mice up to a dose of 300mg/kg body weight. Hence, one-tenth of this dose, i.e. up to 300mg/kg body weight, was used for anti-implantation testing.

The ethanolic extract of *leptadenia reticulata* roots given orally to rats at does 300mg/kg exhibited a very potent anti-implantation activity since no implants, in the entire treated animal, were observed indicating a 100% antiimpantation activity (Table1)

When given to immature rats.LRA induced a significant increase in the weight of the uterus when compared with the control. LRA also potentiated the estrogenic activity of EED.LRA was able to induce a significant increase in the weight of genital organs of ovariactomized rats and potentiate the effect of EED

As far as the estrous cycle is concerned, LRA prolonged the during of estrous phase when compared to the control (72 to 96 vs. 48 to 72 h, respectively).

Table 1: Anti-implantation activity of LRA in female rats when fed orally from days 1to 7 of pregnancy.

Treatment (day 1- day 7)	No. of pregnancies/ no. of treated	No. of implantation (mean ± S.E)	Average litters delivered
Vehicle (0.5ml/rat p.o)	6/6	8.5±8.2	8.5
LRA (300mg/kg p.o.)	6/6	0	0

Table 2: Effect of LRA on uterine weight of immature female rats in the presence and absence of estrogens

Treatment(dose, mg/kg body weight)	Uterine weight mg/100g	Vaginal cornification
Vehicle (0.5ml/rat p.o.)	33.64 ± 0.98	Vagina not open (0 to +)
LRA (300mg/kg p.o.)	78.62 ± 6.37*	Open (+++)
EED (0.1µg/rat i.m.)+ LRA (300mg/kg p.o.)	129.97 ± 9.13*	Open (+ to ++)
EED (0.1µg/rat i.m.)	120.60 ± 6.02*	Open (+++)

***p < 0.001 when compared with control +, nucleated epithelial cells; + + nucleated and cornified cells, + ++, cornified cell.**

***p < 0.001, when compared with control**

Table 3; Effect of LRA on the weight of uterus, cervix, and vagina of ovariactomized rats

Treatment (dose, mg/kg body weight)	weight in mg/100g (mean ± SEM)		
	Uterus	Cervix	Vagina
Control (tween-80, 1%)	69.081±0.069	29.42±0128	36.42±0108
Ethanolic extract (300 mg/kg p.o)	107.236±0.423[a]	37.27±0.157[a]	42.78±0.102[a]
Ethynyl estradiol (0. 1 µg/kg i.m)	158.366±0.322[a]	47.29±0.149[a]	50.48±0.062[a]
Ethynyl estradiol (0.1 µg/kg i.m) and ethanolic extract (300 mg/kg p. o)	261.4188±0.3040[b]	77.34±0.26[b]	80.27±0212[b]

a = p<0.001 vs. control group.
b = P<0.001 vs. EED group.

4. DISCUSSION

In the present study, the whole plants of LRA were tested for its anti-implantation and estrogenic properties. The loss of implantation activity caused by ethanol may be due to antizygotic, blastocytotoxic or anti-implantation activity as described by Hafez (1970). From these overall results, even if preliminary and done only with one dose, we can conclude that LRA possesses a significant estrogenic activity shown by its uterotropic effects in immature females rats and by its ability to increase the weight to genital organs in ovariactomized rats. Both activities were confirmed by the potentiating of the effect of ethynyilestradiol, LRA also possesses a very strong anti-implantation activity (100) which may be due to its estrogenic activity. In fact, it is well know that estrogenic substances inhibit pregnancy by suppressing the level of both follicular stimulating hormone (FSH) and luteinizing (LH) which in turn prevent the implantation. Further studies should be necessary to confirm he potent estrogenic effect of this extract and to isolate the active principles responsible for the activity.

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