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Research Article

ANTIFERTILITY ACTIVITY OF *BRIDELIA CRENULATA ROXB*, AN ETHNOPLANT USED BY TRIBALS OF ORISSA TO PREVENT PREGNANCY

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ABSTRACT

Bridelia crenulata Roxb. (Euphorbiaceae family) is an ethnoplant, which is known to be used by the inhabitants of Mayurbhanj district in Orissa to prevent pregnancy. The present study was aimed at evaluating the antiovulatory activity of stem bark of Bridelia crenulata, to explore its therapeutic value and thereby scientifically validate the traditional claim. Petroleum ether extract of stem bark of Bridelia crenulata was tested for in vivo antiovulatory activity in female Swiss albino mice. Three different dose levels of the test extract were administered for 30 days (6 consecutive estrous cycles) and different phases of estrous cycle of mice were determined daily by microscopically observing vaginal smears of the treatment and control group. Antiovulatory activity was exhibited by petroleum ether extract [PEE] wherein a reduction of the total number of estrus phases (which is the ovulation phase) and a prolongation of diestrus phases (which is the non-ovulatory phase) were observed in the treatment group. Petroleum ether extract [PEE] showed a dose dependant, statistically significant, antiovulatory activity as compared to the control, at dose levels of 100 and 150 mg/Kg body weight of the mice. Also, the antiovulatory effect caused by PEE in mice was reversible after the dose was discontinued. Thus, it did not produce a permanent infertility in mice.

KEYWORDS: *Bridelia Crenulata Roxb., Euphorbiaceae, Ethnoplant, Antifertility.*

INTRODUCTION

Increasing population and its hazards is definitely one of the major global problems which are affecting global development and progress. Population control using contraception and family-planning is the only solution to prevent these hazards. Continuous studies are conducted on the safety and effectiveness of the modern contraceptives, but still there is a quest for alternative means. One such area to be explored is ethnomedicine. Ethnomedicinal plants are those plants which have high medicinal value and which have been used for several years, but are not studied scientifically.

Bridelia crenulata Roxb. (Euphorbiaceae family) [1, 2] is one such ethnoplant, which is known to be used by the inhabitants of Mayurbhanj district in Orissa to prevent pregnancy. The liquor of stem bark is given after menstruation [3]. This ethnoplant is commonly known as Kosi-gacha in Orriya and Adamaruthu, Maarivengai, Mulvengai and Oothiravengai in Tamil. 15 days = 3 estrous cycles [4]. It is a medium sized deciduous tree. These trees are found in India in some parts of Karnataka, Orissa and Tamil Nadu. *In vitro* antimicrobial activities have been reported for the stem bark of *Bridelia crenulata* [5, 6]. But though the plant has an ethnomedicinal use in preventing pregnancy, there are no studies reported on its antifertility activity. In view of the above, it seemed necessary to investigate the antiovulatory activity of stem bark of *Bridelia crenulata*, to explore its therapeutic value and thereby scientifically validate the traditional claim.

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DOI:

MATERIALS AND METHODS

Authentification of the research plant:

The stem bark of *Bridelia crenulata* (family- Euphorbiaceae) was authentified at *Survey of medicinal plants unit- Siddha* Govt. Siddha Medical College campus, Palayamkottai, Tirunelveli-627 002, Tamil Nadu [SMPU Spec.No.8324 February 2004].

Preparation of plant material and extracts:

The stem bark was separated from the tree, air-dried and powdered by hammer mill to obtain a coarse powder. Powdered crude drug was extracted with petroleum ether (60-80°C), using *Soxhlet* extraction method. The solvent was evaporated from the extract under reduced pressure using rotatory vacuum evaporator to give a yellow color extract. The percent yield (w/w) of the crude extract was determined with respect to the bark powder used for extraction.

Qualitative phytochemical screening:

The petroleum ether extract of stem bark of *Bridelia crenulata* was subjected to qualitative phytochemical screening to qualitatively detect presence or absence of various phytoconstituents using standard methods [7, 8].

Experimental Animals:

The antiovulatory activity was conducted as per test protocol [CUSCP/IAEC/02/2005-06], approved by Institutional Animal Ethics Committee [IAEC], C.U. Shah College of Pharmacy. Healthy female Swiss albino mice weighing between 20-35 grams and which showed 3 normal and regular estrous cycles were selected for the study. The 3 normal and regular estrous cycles of the female Swiss albino mice were

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observed, by microscopic examination of vaginal smears observed at the same time daily for 15 days [15 days = 3 estrous cycles].

Acute Toxicity studies:

The acute oral toxicity of petroleum ether extract of stem bark of *Bridelia crenulata* was carried out as per OECD 423 – guidelines.

Evaluation of Antiovulatory activity:

The female Swiss albino mice were divided into 4 groups, each group containing 6 mice; three treatment groups and a control group. The three treatment groups, Group I, II & III was administered petroleum ether extract of *Bridelia crenulata,* by using olive oil as the vehicle. Group I, II & III was administered the extract at dose levels of 50mg/kg, 100mg/kg and 150mg/kg body weight of mice. Control group (Group IV) was administered olive oil.

All the treatment and control groups received the respective dose for 30 days [6 estrous cycles] orally. The dosing was started in the diestrus phase or the proestrus phase of the estrous cycle that is before the estrus phase. Dosing the female animals before estrus phase would target on development of the ova/egg within the ovaries and prevent release of the ova from the ovaries [antiovulation effect]. Female mice were not allowed to mate for 6 estrous cycles till the dosing was complete. Along with daily dosing for 30 days the stages of estrous cycle were checked daily by observing vaginal smears of all the groups. The focus was to observe a reduction of the total number of estrus phases in the six estrous cycles and a prolongation of diestrus phases [9].

Reversibility of the antiovulatory effect : After 30 days when the dosing was discontinued, mating of mice was still not allowed and vaginal smears were further observed for 15 more days [3 more estrous cycles] to see if the antiovulatory effect caused by the test extract is reversible after the dose is discontinued.

Statistical analysis:

Values are expressed as mean ± SEM [n=6 animals/group]. The numerical results were evaluated by application of One-way ANOVA with post Bonferroni multiple comparisons test for statistical significance.

RESULTS AND DISCUSSION

Qualitative phytochemical screening of petroleum ether extract of stem bark of *Bridelia crenulata* revealed the presence of flavonoids, steroids and triterpenoids.

Acute toxicity studies were conducted according to OECD 423 guidelines. The petroleum ether extract of stem bark of *Bridelia crenulata* was found to be non-toxic up to a dose of 2000 mg/kg body weight of mice.

Antiovulatory activity:

Healthy female Swiss albino mice which showed 3 normal and regular estrous cycles were selected for *in vivo* antiovulatory activity. The 3 normal and regular estrous cycles of these mice were observed, by microscopic examination of vaginal smears daily for the different phases of estrous cycle for 15 days [15 days = 3 estrous cycles]. The vaginal smears were checked for the presence of three cell types (a) leukocytes, (b) epithelial cells, & (c) cornified cells. According to the presence or absence of these cells and the relative proportion of each cell type, the phases of the estrous cycle of each animal were recorded. The estrous cycle of mice is normally of four to five days duration. It consists of four phases, Day 1: proestrus phase, Day 2: estrus phase, Day 3: metaestrus phase, Day 4: diestrus phase I [diestrus phase is of 1 or 2 days] & Day 5: diestrus phase II [10].

The microscopic images of the vaginal smears showing different phases of estrous cycle of mice were captured by the image analyser during the antiovulatory study. These images are given in figure1-5.

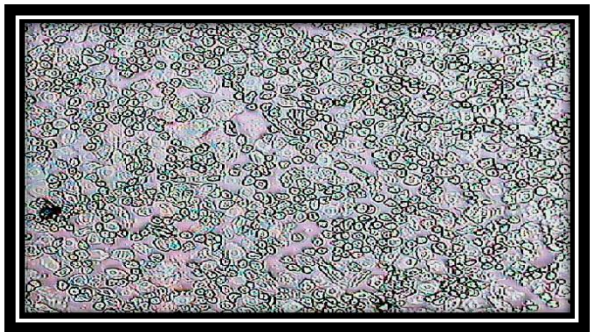

Fig. 1: Day 1 of estrous cycle - Proestrus phase

Fig. 2: Day 2 of estrous cycle - Estrus phase

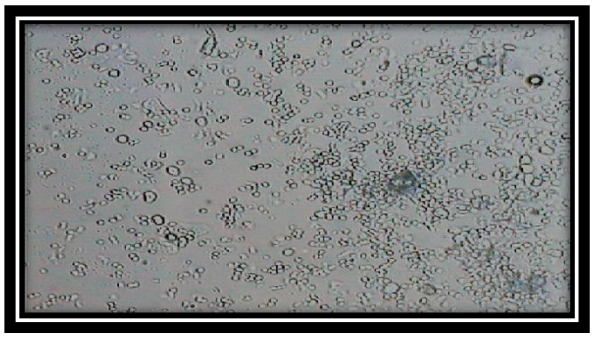

Fig. 3: Day 3 of estrous cycle - Metaestrus phase

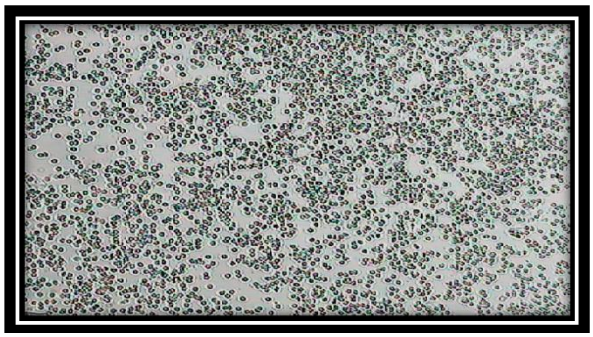

Fig. 4: Day 4 of estrous cycle -Diestrus phase I

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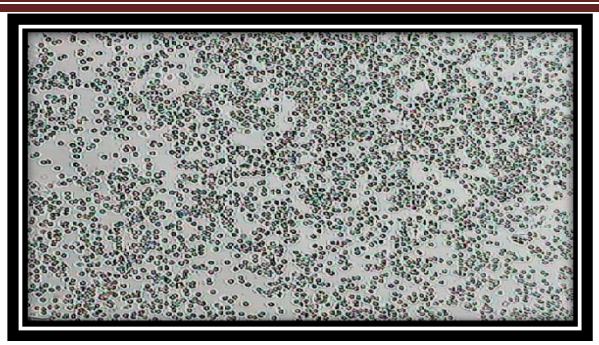

Fig. 5: Day 5 of estrous cycle - Diestrus phase II

The indication for antiovulatory effect in mice was reduction in estrus phases [ovulatory phase] and prolongation of diestrus phases of estrous cycle of mice [11]. In case of all PEE treated mice, the diestrus phases were prolonged while the estrus phases were reduced, indicating an antiovulatory effect [table no.1]. In case of the Group I, which received PEE in a dose of 50mg/kg body weight of the mice, the diestrus prolongation was observed for a shorter duration that is 5-6 days. This diestrus prolongation was statistically non-significant as compared to the control [table no.1] [figure no.6]. The diestrus arrest was reversed before the dosing was discontinued, showing a weak antiovulatory effect in mice. For an ideal contraceptive effect, the antiovulatory effect should continue till the test extract is administered. In case of Group II and III, strong antiovulatory action was observed [table no.1]. The prolongation of the diestrus phase in these two groups continued till the dose was administered, that is till the end of 6th estrous cycle. Thus, PEE at dose levels 100 and 150 mg/Kg body weight showed statistically significant, dose dependant antiovulatory activity as compared to the control [figure no. 6]. After 30 days, when the dosing was discontinued, the test animals were further not allowed to mate for 15 more days [3 more estrous cycles] and vaginal smears were observed to see if the antiovulatory effect caused by the test extract is reversible after the dose is discontinued. It was observed that for both 100mg/kg and 150mg/kg body weight dose, diestrus arrest was reversible within few days as soon as the dosing was discontinued. Thus, petroleum ether extract [PEE] showed a statistically significant, dose dependant antiovulatory activity as compared to the control. The antiovulatory effect was reversible, as soon as the dosing was discontinued. Thus, it did not produce a permanent infertility in mice.

Group VI i.e. Control group showed no prolongation of diestrus phases and exhibited a regular and normal estrous cycles, throughout the 30 days of dosing. Also, when the vaginal smears were further checked for 15 more days, without the dosing, the control group exhibited a normal and regular estrous cycle, indicating that olive oil that was used as vehicle for non-polar extracts have no antiovulatory effect on the mice.

Table No. 1: Antiovulatory effects of all the three doses of petroleum ether extract [PEE]

Data	Control [Olive oil]	Doses of PEE [Per kg body weight]		
		50mg	100mg	150mg
No. of estrous cycles studied while giving the test extract	6	6	6	6
Reduction of estrus phases in 6 cycles [mean ± SEM] after giving the test extract	6.00	5.66 ± 0.211 ^{ns}	3.6 ± 0.211***	2.16 ± 0. 307***
Prolongation of diestrus phases in 6 cycles [mean ± SEM] after giving the test extract	12.00	12.83 ± 0.401^{ns}	18.83 ± 0.601***	23.16 ± 0.307***

Values are expressed as mean ± SEM [n=6 animals/group]. The numerical results were evaluated by application of One-way ANOVA with post Bonferroni multiple comparisons test for statistical significance. ***p<0.001[statistically significant when compared to the control group]; ^{ns}p>0.05[non-significant as compared to control group].

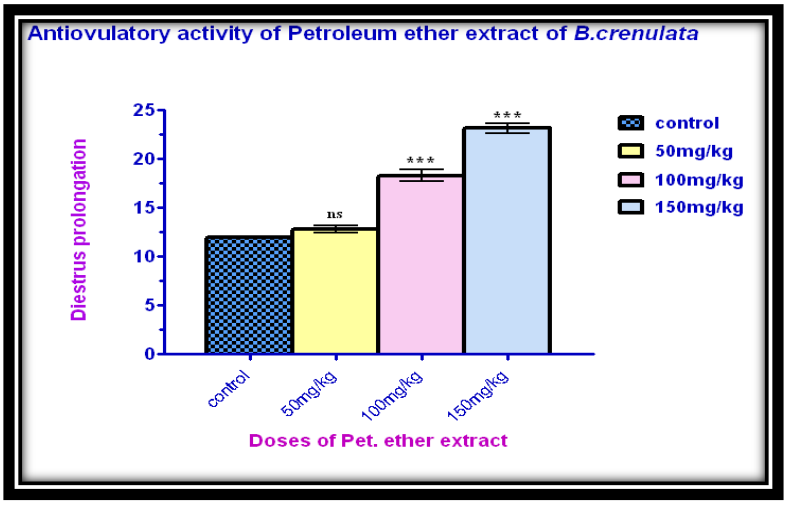

Fig. 6: Antiovulatory activity of all the 3 doses of PEE compared to control

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CONCLUSION

Petroleum ether extract [PEE] showed a dose dependant, statistically significant, antiovulatory activity. Also, the antiovulatory effect produced by PEE in mice was reversible, as soon as the dosing was discontinued. Thus from the present pharmacological investigation we can conclude that stem bark of *B.crenulata* can be considered as a potential source for developing an effective and safe herbal contraceptive formulation.

REFERENCES

1. Khory and Khatrak's, Materia medica of India and their therapeutics, 539.
2. Ramesh N, Viswanathan MB, Saraswathy A, Brindha P, Balakrishna K, Lakshmanaperumalsamy P. Phytochemical and Antimicrobial Studies of Bridelia crenulata. *Pharm Biol (Formerly Int J Pharmacog)* **2001**;39(6):460.
3. Mudgal V, Pal DC. Medicinal Plants used by tribals of Mayurbhanj (Orissa). *Bull. Bot. Surv. India*, **1980**;22:59-62.
4. Jain SK, Sinha BK and Gupta RC. Notable Plants in Ethnomedicines of India, Deep Publications, New Delhi, **1991**;50.
5. Ramesh N, Viswanathan MB, Saraswathy A, Brindha P, Balakrishna K, Lakshmanaperumalsamy P, Patra A. Antibacterial activity of luteoforol from Bridelia crenulata. *Fitoterapia* **2001**;72(4):409-411
6. Ramesh N, Viswanathan MB, Saraswathy A, Brindha P, Balakrishna K, Lakshmanaperumalsamy P. Phytochemical and Antimicrobial Studies of Bridelia crenulata. *Pharm Biol; Formerly Int J Pharmacog* **2001**;39(6):462-464.
7. Khandelwal KR. Practical Pharmacognosy- Techniques and Experiments, Nirali Prakashan, Pune, **2001**;149-154.
8. Kokate CK, Purohit AP, Gokhale SB. 'Text Book of Pharmacognosy' 14th edition, Nirali Prakashan, **2000**;81.
9. Bhaskaran S, Khanam S. Effect of alcoholic extract of Ananas, Bamboosa and Phoenix on oestrous cycle of female albino rats. *Ind J Nat Prod* **2001**:17(2):43.
10. Thompson E. Drug Bioscreening-Drug Evaluation Techniques in Pharmacology**,** VCH Publishers, Inc. New York, **1990**;299-319.
11. Bhaskaran S, Khanam S. Effect of alcoholic extract of Ananas, Bamboosa and Phoenix on oestrous cycle of female albino rats. *Ind J Nat Prod* **2001**:17(2):46.

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