

Pharmacological Evaluation of Anti-Fertility Activity of the List of Extract of *Embelia ribes* Burm F in Female Albino Mice

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Abstract

Anti-fertility activity of methanolic extract of *Embelia ribes* Burm F in female albino mice. *Embelia ribes* Burm F extracts, when administered orally, altered the estrous cycle pattern in female mice, prolong the length of estrous cycle with significant increase in the duration of diestrus stage and reduced significantly the number of litters in albino mice. Treatment of mice with extract of Group 1 (Embelin Std), Group 2 (Estradiol Std), Group 3 (Mother Ext 55), Group 4 (55 Green), Group 5 (55 Blue), Group 6 (55c visible), Group 7 (55 Yellow), Group 8 (55 Pink), Group 10 (Mother Ext 91), Group 10 (91 Toulene), Group 11 (91 CHCl₃), for day for 21 days caused a prolonged estrous cycle with significant increase in the duration of diestrus phase and elongation of estrus stage in treatment with higher dose (as per the table for the regulated days).

Results: The analysis of the principal hormones involved in estrous cycle regulation showed that the fruits extract altered gonadotrophin release (LH, FSH and prolactin) and estradiol secretion. The result indicated the anti-fertility effect of *Embelia ribes* Burm F fruits extract in female albino mice.

Keywords: *Embelia ribes* Burm. F; Anti-fertility activity; Estrous cycle; Integrative medicine

Introduction

The promissory note for the oral contraceptive agent that can control fertility is a thrust from the old history to future journey. Although a wide variety of synthetic contraceptive agent are available, that cannot be used continuously due to their Obesity side effect. Presently systematic investigation *Embelia ribes* Burm F Fresh fruits were collected from I.I.H.R. Hessarghatta fields, Bangalore with accession No.1001 [1]. Which is an altitude of 800 meters (2800 Sqft) latitude: 13.1323 North,

longitude: 77.49332 East. Wind at east 12 km/hr, temperature (110-250°C) Humidity at 45% which is attunciated in FRLHT Collection No. 55181), dated January 03, 2012, to study an identified the species by a Dr. Ravi kumar. K. Asst. Director, RMR Division, at Institute of Ayurveda and Integrative Medicine (IAIM), for their biologically the Fruit is a drupe, 5 mm across, hairless, green with beaked apex on ripening the fruit is dark black with distinct scaly, the phytochemical. revealed the presence of alkaloids, quinones, coumarins, volatile oil, Flavanoids, Glycosides, Lipids, Resin, Tannins. Respectively, and pharmacological antifertility activity approved by Dr. Chidambaranath I.A.E.C. chairman reference no. I.A.E.C./syed asadulla/rgu/Ph.D/KMCP/42/2018, The estrous cycle of mice are changes occurring are proestrus, estrus, metestrus and diestrus, in cell types observed in the vaginal smear lavage from the mice is observed in the microscope, and the determination of the estrous cycle phase of vaginal secretion was collected from female mice, The proportion the three types of cells was used for the determination of the estrous cycle phases, Round and nucleated ones are epithelial cells, irregular ones without nucleus are the cornified [2]. Cells, and the little round ones are the leukocytes, A proestrus smear consists of a predominance of nucleated epithelial cells, an estrous smear primarily consists of anucleated cornified cells, a metestrus smear consists of the same proportion among leukocytes, cornified, and nucleated epithelial cells, and a diestrus smear primarily consists of a predominance of leukocytes [3].

Methodology

Collection of plant material and extraction

Embelia ribes BURM F Fresh fruits were collected and shade dried. The dried fruits were coarse powdered and the powder was packed in to soxhlet column and extracted with methanol was concentrated under reduced pressure (bath temp 500°C)

The dried extract was stored in airtight container in refrigerator [4-6].

Phytochemical studies of the extract

Phytochemical studies of the methanolic fruits extract were carried out by qualitative and TLC methods produced [7].

Animals

Laboratory bred virgin female swiss albino mice aged 85-100 days weighing between 22-25 g, showing regular estrous cycle were used and were allowed free access to water and libtium etc. throughout the study [8-10].

Test material administration

The fruits extract was administered orally in two different doses as per the table in two experimental groups of albino mice. The dose for each group was calculated considering the human dose based on ethno-medical uses of the plant for birth control. The hepatotoxicity of the extract was tested by carrying out liver function tests after regular intervals. The Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) level in the mice administered with dose of as per the table showed no significant change ($P>0.05$). Initially, the other low and high dose kg body weight/days (which were the double of the first dose) were selected. However, this dose was observed to cause a significant elevation in SGOT and SGPT levels of the mice after 10 days of extract administration. Hence, a lesser dose as per the table was selected, as this dose caused no significant change in transaminase activity during the study period [11].

Pharmacological screening

Study of estrous cycle: Animals were divided into three groups consisting of five animals in each group. One group served as control and received vehicle orally for 21 days. The other groups received Group 1 (Embelin Std), Group 2 (Estradiol Std), Group 3 (Mother Ext 55), Group 4 (55 Green), Group 5 (55 Blue), Group 6 (55c visible), Group 7 (55 Yellow), Group 8 (55 Pink), Group 10 (Mother Ext 91), Group 10 (91 Toulene), Group 11 (91 CHCL₃), per day. The estrous cycle was studied by stained preparation of vaginal smear of the animals. The stage of estrous cycle and its duration were determined, After 21 days of treatment, extract was withdrawn from the mice and estrous cycle was studied for another 21 days, 1000 µg for mother extracts and coloumn samples 40 µg/kg and 80 µg/kg body weight/days is the dose [12].

Study of reproductive outcome in mice: Three groups of mature female mice (five mice/group) were selected as mentioned above. Two groups received Fruits extract for 8 days control group received vehicle for the same period. All the

experimental mice were then allowed to mate with mature fertile mate mice and the treatment was continued for 21 days. The number of litter was determined after the completion of one gestation period in all-experimental groups. The litters were allowed to grow and the growth of litters produced from the extract-administered group was compared with those of control group [13].

The reversibility of antifertility effect of the extract was also studied in the treated groups according to the method listed. For this study, the extract was administered continuously for 21 days and then the extract was withdrawn. After 21 days of extract withdrawal, animals were allowed to mate with male mice. The number of litter was determined after the completion of one gestation period [14].

Study of reproductive hormones: Blood sample were collected from the caudal vein of the animal in all the stages of estrous cycle. Serum FSH, LH, prolactin, 17 β estradiol and 17 OH progesterone concentrations were measured by ELISA micro well kits.

Determiation of oral LD50: LD50 of the fruits extract was determined as described and the extract was administered orally into six groups of mice (10 mice/group) in six different doses. i.e., 3, 4, 5, 7 and 8 g/kg body weight/day. Based on rat mortality, the oral LD50 value for 24 h was calculated.

Statistical analysis

The data were statistically analyzed and expressed as mean S.E.M. Statistical analysis of the variance between control and experimental values was done student's t-test.

Results

Phytochemical studies

Qualitative TLC studies of the extract revealed the presence of alkaloids, quinones, coumarins volatile oil, flavanoids, glycosides, lipids, resin, tannins etc.

Effect of the extract on the estrous cycle and the reproductive hormones

The result from the cytological, hormonal and reproductive screening (**Tables 1 and 2**) in the present study revealed that the methanolic extract of *Embelia ribes* Burm F Fruits could be responsible for the antifertility effect. Treatment of mice with extract of Group 1 (Embelin Std), Group 2 (Estradiol Std), Group 3 (Mother Ext 55), Group 4 (55 Green), Group 5 (55 Blue), Group 6 (55 c visible), Group 7 (55 Yellow), Group 8 (55 Pink), Group 10 (Mother Ext 91), Group 10 (91 Toulene), Group 11 (91 CHCL₃), for 21 days caused a prolonged estrous cycle with significant increase in the duration of diestrus phase (**Table 1**) and elongation of estrus stage in treatment with higher dose (80 µg/kg body weight/day) (**Tables 1 and 2**).

Table 1: Effect of list of extract of *Embelia ribes* Burm F Fruits on the estrous cycle of mice for 21 days and number of litters produced in different groups of mice.

Sl. No	Standard/extracts/coloumn	Duration of different	Phages	Cycle	(days)
	Fractions	Proestrus (days)	Estrus (days)	Diestrus (days)	Number of litters
1	Group 1 control	0.97 ± 0.07	0.99 ± 0.19	1.98 ± 0.21	7.9 ± 0.12
2	Group 2 embelin std	0.47 ± 0.23	1.35 ± 0.48	2.38 ± 0.25	5.9 ± 0.38
3	Group 3 estradiol std	0.47 ± 0.23	1.35 ± 0.48	2.38 ± 0.25	5.9 ± 0.38
4	Group 4 Mother ext 55	0.49 ± 0.32	0.49 ± 0.32	3.50 ± 0.46	2.5 ± 0.23
5	Group 5 55 green	0.56 ± 0.13	1.25 ± 0.23	3.72 ± 0.45	3.8 ± 0.34
6	Group 6 55 blue	0.86 ± 0.56	1.23 ± 0.23	1.85 ± 0.21	7.4 ± 0.42
7	Group 7 55 c visible	2.6 ± 0.43	2.3 ± 0.92	8.0 ± 0.63*	5.0 ± 0.86
8	Group 8 55 yellow	2.8 ± 0.02	1.7 ± 0.23	8.6 ± 0.23*	5.7 ± 0.21
9	Group 9 55 pink	2.6 ± 0.43	2.3 ± 0.92	8.0 ± 0.63*	5.0 ± 0.86
10	Group 10 Mother ext 91	3.1 ± 0.11	2.0 ± 0.16	9.5 ± 0.13*	5.9 ± 0.64
11	Group 11 91 toulene	3.0 ± 0.62	2.5 ± 0.68	6.2 ± 0.56	4.0 ± 0.82
12	Group 12 91 CHCL ₃	2.8 ± 0.02	1.7 ± 0.23	8.6 ± 0.23*	5.7 ± 0.21

N= 6 data are Mean ± SEM

Note: Group 4 and group 10 compared with group 1, 2 and 3: *= (P ≤ highly significant 0.001)

Table 2: Hormone levels in various groups of animals during study.

Sl. No	Standard/extracts/Coloumn fractions	Duration of different	phages	cycle	(days)
	Table 2	Proestrus (days)	Estrus (days)	Diestrus (days)	Number of litters
1	Group 1 control	0.97 ± 0.07	0.99 ± 0.19	1.98 ± 0.21	7.9 ± 0.12
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8	Group 8 55 yellow	2.8 ± 0.02	1.7 ± 0.23	8.6 ± 0.23	5.7 ± 0.21
9	Group 9 55 pink	2.6 ± 0.43	2.3 ± 0.92	8.0 ± 0.63	5.0 ± 0.86
10	Group 10 Mother ext 91	3.1 ± 0.11	2.0 ± 0.16	9.5 ± 0.13	5.9 ± 0.64

11	Group 11 91 toluene	3.0 ± 0.62	2.5 ± 0.68	6.2 ± 0.56	4.0 ± 0.82
12	Group 12 91 CHCL ₃	2.8 ± 0.02	1.7 ± 0.23	8.6 ± 0.23	5.7 ± 0.21

Discussion

The present antifertility effect significantly decreases the duration of proestrus and metestrus stage in experiment group was recorded than those of control group animals. These changes were found to revert back after withdrawal of the treatment except proestrus stage in groups with higher dose of treatment. The prolongation of diestrus phase may lower the chance of pregnancy in treatment of mice with fruits extract decreased the mean number of litters (**Table 1**), the number of litters appeared to decrease more with higher dose of treatment, which may suggest dose dependant antifertility effect. All the litters of treated mice grew up normally without showing any physical abnormality indicating that the *Embelia ribes* Burm F fruits were not teratogenic in albino mice. Absence of toxicity in the doses administered justifies the safe nature of the fruit extract. The LD50 of fruits extract was found to 40-80 µg as per table in mice (**Table 2**) [15,16].

Conclusion

The increase in the number of litters observed in both the post-treatment groups may suggest reversibility of the antifertility effect. In the present study the decrease in the LH in both the treated groups and FSH in higher dose of treatment and compared to the control, animals may indicate that the disturbance of estrous cycle and ovulation through suppression of FSH. In the present study an increase in prolactin level was observed which was more pronounced during proestrus stage with higher dose of extract. These observations are comparable with the studies, who reported that a combination of enhanced prolactin and suppressed LH secretion in adult mice is due to prolongation of estrus cycle. In our study, no detectable change was observed in the level of progesterone with treatment of fruit extract. Disturbance on the estradiol secretion with significant decrease during estrous stage of the cycle observed with the extract treatment may be due to impairment in the release of LH and FSH causing hormonal imbalance. These observations could also suggest the antifertility effect of *Embelia ribes* Burm F Fruits.

The present study demonstrated that a methanolic extract and column samples of *Embelia ribes* Burm F Fruits has the antifertility activity. Enhancement of prolactin and suppressed of LH secretion in adult mice which increase the prolongation of estrus cycle. The analysis of the principal hormones which involved in estrous cycle regulation shows the alteration in gonadotrophin release (LH, FSH and prolactin) and estradiol secretion indicated in table's.

Conflict of Interest Statement

We declare that we have future interest to reach up to microsomal study.

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