Antifertility Activity of *Eugenia Jambolana* Seed Extract in Female Albino Rat

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**Abstract**

The present study was conducted to investigate the effect of *Eugenia jambolana* seeds on estrous cycle and ovulation. Petroleum ether, ethyl acetate and ethanol extracts of seeds of *E. jambolana* administered orally at the dose level of 200 mg and 600 mg/kg body weight to adult female rat for 30 days. Out of the three extracts, ethyl acetate extracts at both the dose level seems to be more effective resulted in irregular estrous cycle with prolonged diestrus and reduced estrus and metaestrus during the experimental period. The data revealed that the ethyl acetate extract caused a significant decline in the wet weight of ovary and uterus, as well as protein and glycogen level, however, cholesterol and total ascorbic acid level increased significantly. The extract also significantly reduced the number of developing follicles, graffian follicles and corpora lutea and increase the number of atretic follicles. Micrometric measurement of the uterus showed the decrease in the diameter of the uterus and thickness of the myometrium and endometrium significantly in the ethyl acetate extract treated rats. These observations showed the anti-ovulatory activity of *Eugenia jambolana* seed in female albino rats.

**Keywords:** *Eugenia jambolana*; Estrous cycle; Follicular kinetics; Anti-ovulatory; Ovary

**Introduction**

A large number of medicinal plants have been screened to explore the possibility for selecting a potential antifertility agent [1-5]. But so far no single plant is available which can safely be used to prevent pregnancy. Although few plants have shown promising result in preventing pregnancy but they have failed in the course of their investigations. Hence, the search needs to be continued to find out the potent antifertility plant. *Eugenia jambolana* Lam (Myrtaceae) is used as Ayurvedic plant for various medicinal purposes [1,2,5]. The flowers of this plant have been reported to possess contraceptive activity in male albino rats [6]. Earlier studies of our laboratory have also shown the anti-implantation and antiestrogenic effect in female rats [7]. Hence the present study has been undertaken to evaluate the effects of various extracts of *E. jambolana* seeds on estrous cycle and female reproductive organs in rats.

**Materials and Methods**

**Collection and authentication of plant**

The fully matured seeds of *E. jambolana* were obtained from fields in and around madikeri districts of Karnataka, India, during June-August 2010 from a single tree. The seed identified and authenticated by Dr. Sudarshan, Professor of the Department of Botany, University of Mysore, Manasagangotri, Mysore and the plant bearing herbarium number of 1634, where voucher specimens were deposited.

**Extraction of plant material**

The seeds were shade dried, powdered and subjected to soxhlet extraction successively and separately with petroleum ether (40°C-60°C), ethyl acetate (76°C-77°C) and ethanol (70°C-80°C). The decoction so obtained was evaporated under reduced pressure and controlled temperature (50°C-60°C). The dried mass was considered as the extract and preserved at 6°C in dark and diluted as required.

**Animals**

Adult, healthy, virgin female albino rats of wistar strain (60-70-days-old weighing 160-190 g body weight) with normal estrus cycle were selected. All the animals were bred in a standard animal house. The animals were housed in polypropylene cages and maintained under controlled conditions of light (12 h) and temperature 24 ± 3°C they were fed on pellets and water ad libitum. The animals were allowed to acclimatize to the laboratory environment for 1 hr before being subject to the experiments. The experimental protocol was approved by the Institutional Animal Ethics Committee.

**Experimental design**

The animals were divided into seven groups consisting of six animals in each group.

- **Group I:** Control, received 0.2 ml DMSO (1%).
- **Group II and III:** Received 200 mg and 600 mg/kg body weight of Pet. ether extract in 0.2 ml DMSO respectively.
- **Group IV and V:** Received 200 mg and 600 mg/kg body weight of ethyl acetate extract in 0.2 ml DMSO respectively.
- **Group VI and VII:** Received 200 mg and 600 mg/kg body weight of ethanol extract in 0.2 ml DMSO respectively.

All the above treatments were given orally by using intragastric catheter for 30 days to cover 6 regular estrous cycles. The treatment was started from estrous phase only, as the ovarian activities change markedly from one phase to another phase of estrous cycle. The treatment was given orally everyday between 10.00 and 11.00 h. The stages of estrous cycle were recorded daily by observing vaginal smears according to Vogel [8]. The control and treated animals were sacrificed on day 31st by cervical dislocation 24 hrs after the last treatment.

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Organ weight

Ovaries and uteri were dissected out, freed from surrounding tissues, blotted on filter paper and weighed quickly to the nearest milligrams on an electronic balance.

Biochemical analysis

The ovary and uterus from one side of each animal were processed for biochemical analysis such as Cholesterol by Peters and Vanslyke [9], Ascorbic acid by Roe and Krether [10], Protein by Lowry et al. [11] and Glycogen by Carrol et al. [12].

Ovarian follicular kinetics

Ovary from another side of each animal were fixed in Bouin's fluid, embedded in paraffin wax, sectioned at 5 µm thickness of the ovary were prepared for the study of follicular kinetics. To quantitatively evaluate ovarian follicles, the methods described by Hirshfeld [13] Sanjay and Joshi [14] were used in the present study. Ovarian follicles were classified as primary, small preantral, large preantral, small antral and Graafian follicle according to the morphological classification scheme used by Lundey et al. [15].

Histometry

The diameter of the uterus, thickness of the myometrium, endometrium and height of epithelial cells were measured by using stage and ocular micrometer.

Statistical Analysis

One way analysis of variance (ANOVA) followed by Duncan's multiple test were used to find out significant difference among mean values of each parameter of different experimental groups by fixing minimum significance level at P<0.05. Values with same superscript letters are not significantly (P<0.05) different whereas those with different superscript letters are significantly (P<0.05) different when compared to control.

Results

Estrous cycle

The present study revealed that the ethyl acetate extract at both dose levels showed an antifertility effect. It is observed that the administration of pet.ether at both the dose levels and low dose level of ethanol has shown no significant difference in the length of any of the phase of the estrous cycle. Ethyl acetate extract of both the doses has decreased significantly (P<0.01) the estrus and metaestrus phases and also caused prolongation of diestrus phase. High dose of ethanol extract treatment has arrested the normal estrus cycle at diestrus phase at later stages of the cycle and decreased the total number of estrus and metaestrus phases significantly (P<0.01) (Table 1).

Organs weight

There was a significant (P<0.01) decrease in the wet weight of ovary and uterus at low dose level of ethyl acetate extract with high dose of ethyl acetate and ethanol extract administration the reduction in the weight is highly significant (P<0.001) when compared to control (Table 2).

Biochemical change

Administration of ethyl acetate extract at both dose levels has caused highly significant decrease (P<0.001) of protein and glycogen and an increase in the cholesterol and ascorbic acid level in the ovary and uterus of rats (Table 3, 4).

Follicular kinetics

The animals treated with ethyl acetate at low dose level caused a statistically less significant (P<0.05) reduction in the number of small antral and graafian follicles with concomitant significant increase in the number of atretic follicles of the same stage. At high dose caused a highly significant decrease (P<0.001) in the number of healthy small preantral, large preantral, small antral, graafian follicles and active

### Table 1: Effect of various extracts of *E. jambolana* on weight of Ovary and Uterus in female albino rat.

| Treatment | Dose (mg/kg bw) | Ovary (mg/100 gm bw) | Uterus (mg/100 gm bw) |
|------------|----------------|----------------------|----------------------|
| Control    | 1% DMSO        | 101.6 ± 1.10         | 271.6 ± 13.89        |
| Pet. Ether | 200            | 104.4 ± 2.22         | 287 ± 11.52          |
| Ethyl acetate | 200  | 104.8 ± 2.26         | 292.8 ± 17.95        |
| Ethanol    | 200            | 90.4 ± 1.63          | 187.2 ± 9.05         |
| Ethanol    | 600            | 75.6 ± 3.17          | 173.5 ± 3.27         |

All values are expressed as Mean ± Standard error. The data was analyzed by one way ANOVA. Analysis of Variance. Values with same superscript letters are not significantly (P>0.05) different whereas those with different superscript letters are significantly (P<0.05) different as judged by Duncan’s Multiple Test.

### Table 2: Effect of various extracts of *E. jambolana* seeds on weight of Ovary and Uterus in female albino rat.

| Treatment | Dose (mg/kg bw) | Ovary (mg/100 gm bw) | Uterus (mg/100 gm bw) |
|------------|----------------|----------------------|----------------------|
| Control    | 1% DMSO        | 92.8 ± 2.20          | 195.2 ± 9.25         |
| Pet. Ether | 200            | 92.8 ± 2.20          | 195.2 ± 9.25         |
| Ethyl acetate | 200  | 101 ± 1.00          | 259.6 ± 2.57         |
| Ethanol    | 600            | 75.6 ± 3.17          | 173.5 ± 3.27         |

All values are expressed as Mean ± Standard error. The data was analyzed by one way ANOVA. Analysis of Variance. Values with same superscript letters are not significantly (P>0.05) different whereas those with different superscript letters are significantly (P<0.05) different as judged by Duncan’s Multiple Test.
and fresh corpora lutea with a concomitant significant increase in the number of atretic follicles of the same stage. The result also showed a significant reduction in the total number of follicles in the ethyl acetate extract treated ovary (Table 5).

**Histometry**

The diameter of the uterus, thickness of endometrium and myometrium and epithelial cell height has decreased highly significantly (P<0.001) with both the dose levels of ethyl acetate extract treated rats (Table 6).

**Discussion**

In the present investigation, pet.ether, ethyl acetate and ethanol extracts of *E. jambolana* seeds were tested for estrous cycle and ovulation. Out of these the ethyl acetate extract has shown most promising antiovulatory activity in female albino rats. In this study, treatment of ethyl acetate extract of *E. jambolana* at both doses for 30 days showed a significant decrease in the estrus and metaestrus phases with concomitant increase in diestrus phase, resulting in the reduction
of total number of cycles. The prolongation of diestrus phase indicates that maturation of the follicle in the preovulatory phase was delayed, leading to non-maturation of graafian follicle. Non-availability of matured graafian follicle was indicated by reduction in the metaestrus phase. As a result the extracts provoked inhibition of the ovulation with consequent reduction of the cyclicity. Similar results have been obtained with Azadirachta indica [16], Jatropha curcus [17] and Mimoso pudica [18]. This may be due to the fact that the decreased estrogen availability at regular intervals which is due to administration of the crude extract of seeds of Eugenia jambolana. Ovulation takes place under the combined and balanced influence of ovarian and extra ovarian hormones. Imbalance in these hormones leads to irregularity in the ovarian function and duration of estrous cycle [19,20].

There is a decrease in wet weight of ovary in ethyl acetate treated groups shows the antiiovulatory effect via suppression of FSH. Similar results have also obtained with administration of Artobotrys odoratissimus [21,22].

In the present study, the decrease in the relative wet weight of the uterus indicates the weak estrogenic and strong antiestrogenic effects of the ethyl acetate extract. An active antiestrogen has been reported to decrease the wet weight of uterus [23,24]. These finding clearly corroborate the potent antiestrogenic nature of the extract which was tested by using immature ovariectomized rats [7].

Protein is considered to be the building material and is involved in the alteration of almost every physiological function. In the present study the low protein content of ovary and uterus indicates the retarded growth. Glycogen is involved in providing energy to various processes like, ovulation, transportation and survival of eggs and implantation. All these changes are hormone dependent [25]. The decreased glycogen content in ethyl extract of Eugenia jambolana seed treated rats may be due to lowered steroidogenesis, which attributed to non-availability of gonadotrophins.

Ascorbic acid plays an important role in ovarian steroidogenesis [26]. Therefore, in the present data the accumulation of ascorbic acid in the ovaries and uterus gives additional support to the inhibition of steroidogenic activities. The significant elevation in cholesterol content of ovary and uterus indicates the non-availability of pituitary gonadotrophins which are necessary for conversion of cholesterol to estrogen/progesterone [27,28]. In the present investigation, the significant elevation in cholesterol content of ovary and uterus in ethyl acetate extract treated rats suggests the non-utilization of cholesterol towards biosynthesis of hormone in ovaries.

There is an increase in the number of atretic follicles and concomitant decrease in the number of primary, preantral, antral, graafian follicles in ethyl acetate extract treated rats may be due to the non-availability of a required amount of extra ovarian regulators (FSH and LH). The formation of corpus luteum is a direct continuation of preovulatory follicle development. The decrease in the number of corpora lutea indicates that the ethyl acetate extract inhibited the conversion of the preovulatory follicles into corpus luteum arresting ovulation. Similar results have been obtained in Momordica charantia [29], Malva viscus [22].

The ethyl acetate extract caused atrophic effect on the uterine tissue as revealed by the significant reduction in the epithelial cell height and the thickness of the endometrium and myometrium. The result observed in the present study on the histology of the uterus is in agreement with the studies made by Pal [30] on Sesbania sesban seeds.

Conclusion

In conclusion, the ethyl acetate extract of Eugenia jambolana seed exerted a strong antiovulatory effect. Administration of ethyl acetate extract of Eugenia jambolana may block ovulation by altering estrous cycle with a prolonged diestrus, decreases the uterine and ovary weight and may cause antiovulation effect. The antiestrogenic activity of the ethyl acetate extract of Eugenia jambolana L might be the reason for antiovulatory activity.

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