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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 Hirshfeild [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

Treatment	Dose mg/kg bw	Duration of Stages of Estrous Cycle (Days)				
		Estrus	Metaestrs	Diestrus	Proestrus	Diestrus Index
Control	1% DMSO	7.2 ± 0.23^{a}	5.8 ± 0.23^{a}	10.6 ± 0.23^{a}	6.4 ± 0.23^{a}	35.33^{a}
Pet. Ether	200	7.8 ± 0.50^{a}	5.5 ± 0.50^{a}	11.1 ± 0.50^{a}	5.8 ± 0.50^{a}	37.00^{a}
	600	7.3 ± 0.73^{a}	6.3 ± 0.73^{a}	10.8 ± 0.73^{a}	5.6 ± 0.73^{a}	36.00^{a}
Ethyl acetate	200	3.6 ± 0.51^{c}	3.8 ± 0.5^{c}	16.9 ± 0.51^{c}	5.9 ± 0.51^{a}	56.3b^{c}
	600	2.2 ± 0.20^{d}	2.9 ± 0.20^{d}	20.3 ± 0.20^{d}	5.6 ± 0.20^{a}	67.66^{d}
Ethanol	200	6.8 ± 0.37^{a}	5.4 ± 0.37^{a}	12.4 ± 0.37^{a}	5.4 ± 0.37^{a}	41.33^{a}
	600	3.7 ± 0.73^{b}	4.3 ± 0.73^{b}	17.0 ± 0.73^{b}	5.0 ± 0.73^{a}	56.66^{b}

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 1: Effect of various extracts of *E. jambolana* seeds on duration of different phases of the estrous cycle in female albino rat.

Treatment	Dose (mg/kg bw)	Ovary (mg/100 gm bw)	Uterus (mg/100 gm bw)
Control	1% DMSO	101.6 ± 4.10^{a}	271.6 ± 13.89^{a}
Pet. Ether	200	104.4 ± 2.22^{a}	287 ± 11.52^{a}
	600	104.8 ± 2.26^{a}	292.8 ± 17.95^{a}
Ethyl acetate	200	90.4 ± 1.63^{c}	187.2 ± 9.05^{c}
	600	75.6 ± 3.17^{d}	173.5 ± 3.27^{d}
Ethanol	200	101 ± 1.00^{a}	259.6 ± 2.57^{a}
	600	92.8 ± 2.20^{b}	195.2 ± 9.25^{b}

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)	Protein (µg/mg tissue)	Cholesterol (µg/mg tissue)	Glycogen (µg/mg tissue)	Ascorbic acid (µg/mg tissue)
Control	1% DMSO	13.15 ± 0.14^{a}	32.43 ± 0.37^{a}	4.13 ± 0.07^{a}	0.72 ± 0.05^{a}
Pet. Ether	200	13.0 ± 0.06^{a}	31.43 ± 0.14^{a}	3.98 ± 0.04^{a}	0.83 ± 0.07^{a}
	600	9.99 ± 0.29^{a}	28.31 ± 0.55^{a}	3.88 ± 0.06^{a}	0.76 ± 0.03^{a}
Ethyl acetate	200	7.05 ± 0.07^{c}	44.36 ± 0.34^{c}	2.78 ± 0.09^{c}	0.98 ± 0.06^{c}
	600	6.22 ± 0.23^{d}	56.03 ± 0.99^{d}	2.01 ± 0.02^{d}	1.34 ± 0.03^{d}
Ethanol	200	12.98 ± 0.08^{a}	29.68 ± 0.66^{a}	3.85 ± 0.37^{a}	0.79 ± 0.07^{a}
	600	8.16 ± 0.18^{b}	36.79 ± 0.43^{b}	3.01 ± 0.05^{b}	0.92 ± 0.09^{b}

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 3: Effect of various extracts of *E. jambolana* seed on ovarian Protein, Cholesterol, Glycogen and Ascorbic acid contents in female albino rat.

Treatment	Dose (mg/kg bw)	Protein (µg/mg tissue)	Cholesterol (µg/mg tissue)	Glycogen (µg/mg tissue)	Ascorbic acid (µg/mg tissue)
Control	1% DMSO	10.15 ± 0.14^{a}	14.90 ± 0.11^{a}	2.11 ± 0.15^{a}	0.69 ± 0.06^{a}
Pet. Ether	200	10.0 ± 0.06^{a}	14.61 ± 0.24^{a}	2.17 ± 0.17^{a}	0.67 ± 0.02^{a}
	600	9.39 ± 0.29^{a}	14.96 ± 0.66^{a}	2.10 ± 0.28^{a}	0.70 ± 0.02^{a}
Ethyl acetate	200	7.45 ± 0.07^{c}	21.48 ± 0.47^{c}	1.71 ± 0.32^{c}	0.89 ± 0.07 ^{c}
	600	6.92 ± 0.23^{d}	23.30 ± 0.47^{d}	1.02 ± 0.19^{d}	1.02 ± 0.03^{d}
Ethanol	200	9.98 ± 0.08^{a}	15.88 ± 0.55^{a}	2.34 ± 0.13^{a}	0.71 ± 0.04^{a}
	600	8.16 ± 0.18^{b}	16.14 ± 0.34^{b}	2.05 ± 0.20^{b}	0.74 ± 0.09^{b}

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 4: Effect of various extracts of *E. jambolana* seed on uterine Protein, Cholesterol, Glycogen and Ascorbic acid contents in female rat.

Treatment	Dose (mg/kg bw)	Primary	Small pre-antral	Large preantral	Small antral	Graafian follicle	Total no. of follicles	Corpora lutea	Atretic follicle
Control	1% DMSO	81.10 ± 0.19^{a}	62.60 ±1.21^{a}	28.60 ± 0.58^{a}	11.7 ± 0.69^{a}	9.34 ± 0.36^{a}	198.91^{a}	8.34 ± 0.54^{a}	28.21 ± 0.93^{a}
Pet. Ether	200	85.40 ± 0.07^{a}	60.20 ± 0.91^{a}	28.0 ± 0.91^{a}	10.95 ± 0.67^{a}	9.37 ± 0.51^{a}	191.52^{a}	7.93 ± 0.43^{a}	31.60 ±1.44^{a}
	600	84.2 ± 0.53^{a}	59.4 ± 0.63^{a}	29.05 ± 0.39^{a}	10.01 ± 0.93^{a}	8.03 ± 0.32^{a}	190.69^{a}	7.60 ± 0.24^{a}	29.0 ±1.39^{a}
Ethyl acetate	200	72.32 ± 0.62^{a}	53.0 ± 0.30^{a}	26.40 ± 0.83^{a}	7.86 ± 0.51^{c}	4.56 ± 0.40^{c}	160.38^{c}	2.13 ± 0.59^{c}	57.28 ±1.10^{c}
	600	60.41 ±1.79^{c}	47.20 ±1.43^{c}	19.20 ± 0.40^{c}	6.42 ± 0.29^{d}	3.41 ± 0.80^{d}	128.8^{d}	1.00 ± 0.14^{d}	69.20 ± 0.62^{d}
Ethanol	200	80.79 ± 0.28^{a}	63.0 ± 0.46^{a}	29.90 ± 0.96^{a}	9.40 ± 0.53^{a}	8.06 ± 0.04^{a}	191.5^{a}	7.20 ± 0.42^{a}	33.20 ± 0.81^{a}
	600	76.80 ± 0.39^{b}	55.20 ±1.16^{b}	23.30 ± 0.59^{b}	7.0 ± 0.80^{b}	7.93 ± 0.50^{b}	170.23^{b}	6.23 ± 0.71^{b}	43.40 ± 0.53^{b}

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 5: Effect of various extracts of *E. jambolana* seed on ovarian follicular kinetics in female rat.

Treatment	Dose (mg/kg bw)	Diameter of uterus (µm)	Thickness of myometrium (µm)	Thickness of endometrium (µm)	Epithelial cell height (µm)
Control	1% DMSO	2162.10 ± 4.17^{a}	559.09 ± 3.13^{a}	129.90 ± 2.17^{a}	22.06 ± 0.43^{a}
Pet. Ether	200	2132.59 ± 6.14^{a}	567.91±1.59^{a}	118.16 ±1.84^{a}	24.89 ± 0.77^{a}
	600	2175.00 ± 7.68^{a}	554.70 ± 5.28^{a}	121.19 ± 1.74^{a}	23.16 ± 0.83^{a}
Ethyl acetate	200	2011.70 ± 5.79^{c}	492.13 ± 5.79^{c}	101.31 ± 2.18^{c}	19.51 ± 0.64^{c}
	600	1807.03 ± 3.78^{d}	430.34 ± 7.20^{d}	96.27 ± 1.43^{d}	16.92 ± 1.31^{d}
Ethanol	200	2159.38 ± 3.77^{a}	561.63 ± 4.55^{a}	122.51 ± 1.32^{a}	23.99 ± 1.68^{a}
	600	2098.00 ±7.15^{b}	505.61 ± 5.95^{b}	018.12 ± 1.59^{b}	20.19 ± 0.70^{b}

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 6: Effect of various extracts of *E. jambolana* seed on histometric parameters of the uterus in female rat.

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 conmitant 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 *Azardirachta indica* [16], *Jatropha curcus* [17] and *Mimosa 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 *E. 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 antiovulatory 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 *E. 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 *E. jambolana* seed exerted a strong antiovulatory effect. Administration of ethyl acetate extract of *E. jambolana* may block ovulation by altering estrous cycle with a prolonged diestrous, decreases the uterine and ovary weight and may cause antiovulation effect. The antiestrogenic activity of the ethyl acetate extract of *E. jambolana* L might be the reason for antiovulatory activity.

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References

1. https://trove.nla.gov.au/work/10930451
2. Nadkarni AK, Nadkarni KM (1954). Indian Materia Medica. In: (3rd eds) Popular Book Prakashan, Bombay.
3. Chopra AN, Nayar S, Chopra IC (1965) Glossary of Indian Medicinal Plants, CSIR, New Delhi.
4. Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN, Mahrotra BN (1969). Screening of Indian Plants for Biological Activity – Part II. J Exp Biol 7: 250-262.
5. Kamboj VP (1988) A review of Indian Medicinal Plants with interceptive activity. Indian J Med Res 87: 336-355.
6. Rajasekaran M, Bapana JS, Lakshmanan S, Ramachandran Nair AG, Veliath AJ, et al.(1998) Antifertility effect in male rats of oleanolic acid, a triterpine from *Eugenia jambolana* flowers. J Ethnopharmacology 24: 115-121.
7. Sarita M, Bhagya M (2012) Antiimplantation and Antiestrogenic activity of *Eugenia Jambolana* seed. J Pharmacy Research 5: 2607-2609.
8. Vogel HG (1997) Ovarian hormones, In: Pharmacological Assay. Springer-Verlag Berlin Hidelberg (2nd eds) New York, USA.pp 637-647.
9. Peter JP, Vanslyke DD (1964) Quantitative Clinical Chemistry, Williams and Wilkins (2nd eds), Baltimore.
10. Roe JH, Kuether CA (1943) The determination of ascorbic acid in whole blood and urine through the 2-4 dinitrophenyl hydrazine derivative of dehydroascorbic acid. J Biol Chem 147: 399-407.
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 193: 265-275.
12. Carol NV, Langelly RW, Row RH (1956) Glycogen determination in liver and muscle by use of anthrone reagent. J Biol Chem 29: 583-593.
13. Hirshfield AN (1991) Development of follicles in the mammalian ovary. Int Rev Cytol 124: 43-101.
14. Sanjay VS, Joshi BN (1997) Melatonin and exposure to constant light/darkness affects ovarian follicular kinetics and estrous cycle in Indian desert Gerbil Meriones hurrianae (Jerdon).Gen Comp Endocrionol 180: 352-357.
15. Lundy T, Smith PO, Connell A, Hudson NL, Mc Natty KP (1999) Population of grannulosa cells in small follicles of the sheep ovary.J Reprod Fertil 155: 251-262.
16. Gbolotorun SC, Osinubi AA, Noronha CC, Okanlaorn AO (2008) Antifertility potential of Neem flower extract of adult female Sprague-Dawley rats. Afr Health Sci 8: 168-173.
17. Ahirwar D, Ahirwar B, Kharya MD (2010) Effect of ethanolic extract of *Jatropha curcus* seeds on estrous cycle of female albino rats. Scholars Research Library 2: 146-150.
18. Ganguly M, Borthakur M, Devi N, Mahanta R (2007) Effect of *Mimosa pudica* root extract on vaginal estrous and serum hormones for screening of antifertility activity in albino mice. Contraception 76: 482-485.
19. Bodhankar SL, Garg SK, Mathur VS (1973) Mechanism of action of Indigenous antifertility agents. In: Bull PG, India. pp 127-129.

20. Garg SK, Garg GP (1971) Antifertility screening of plant part VII effect of the five indigenous plants of early pregnancy in albino rats. Indian J Med Res 59: 302-306.

21. Prakash AO, Mathur R (1977) Antifertility investigation on the green leaves of *Artabotrys odoratissimus* linn in female albino rats. Probe 16: 155.

22. Dexit VP (1977) Effects of chronically administered on *Malva viscus conzatti* flower extract on the female genital tract of Gerbil, Merionis Hurrianae, Jerdon. Indian J Exp Biol 15: 1650-1652.

23. Edgren RA, Calhoun DW (1957) The biology of steroidal contraception. In: Edgren, RA (2nd eds). Contraception: The chemical control of fertility. New York, Marcel Deckker, USA, pp 23-68.

24. Prakash AO (1978) Antiestrogenic modes of action of leaf extract of *Artobotrys odoratissimus* linn. Indian J Exp Biol 16: 1214-1215.

25. Walaas O (1952) Effect of oestrogens on the glycogen content of the rat uterus. Acta Endocrinol 10: 175-192.

26. Guillemin R, Sakiz E (1963) Quantitative study of the response to LH after hypophysectomy in the ovarian Ascorbic acid depletion test. Endocrinology 72: 813-816.

27. Mason NR, Marsh JM, Savard K (1962) An action of gonadotrophin in vitro. J Biol Chem 237: 1801.

28. Wang C, Hsuch AJ, Erikson WGF (1979) Induction of functional prolactin receptors by follicle stimulating hormone in rat granulose cells in-vivo and in-vitro. J Biol Chem 254: 11330-11336.

29. Sharanabasappa A, Vijayakumar B, Saraswathi B (2002) Effect of *Momordica Charantia* seed extracts on ovarian and uterine activities in albino rats. Pharm Biol 40: 501-507.

30. Pal SS (1990) Fertility control of female through *Sesbania sesban* seeds. J Res Educ Indian Med 9: 27-32.