Effect of Benzene Extract of *Hibiscus Rosa Sinensis* on the Estrous Cycle and Ovarian Activity in Albino Mice

D. Rama Krishna MURTHY, C. Madhusudana REDDY, and Saraswati B. PATIL*

Department of Studies in Zoology, Gulbarga University, Gulbarga-585 106, India.

Received October 21, 1996; accepted March 3, 1997

The benzene extract of *Hibiscus rosa sinensis* flowers was administered intraperitoneally at the dose levels of 125 and 250 mg/kg body weight to adult mice and resulted in an irregular estrous cycle with prolonged estrus and metestrus. An increase in the atretic follicles and the absence of corpora lutea indicate the antiovarian effect of the extract. The extract also showed estrogenic activity in immature mice by early opening of the vagina, premature cornification of the vaginal epithelium and an increase in uterine weight. Therefore the antiovarian effect may be due to an imbalance in the hormonal environment, as there may be an increase in the endogenous secretion of estrogen by atretic follicles, and also to the estrogenicity of the flower extract.

Key words *Hibiscus*; benzene extract; ovary; estrous cycle

The use of various parts of *Hibiscus rosa* (*H. rosa sinensis*) in medications as an antifertility agent has been a long-time rural folk practice in India.^{1,2)} This was also mentioned by Bhavamishra during the 18th century in *Yoniogadhikar*, in the 70th chapter of *Bhavaprakash*. He stated that a woman would never get pregnant if she consumed during her menses, a preparation made from the flowers of *H. rosa sinensis* and fermented rice broth, along with old jaggery (product of sugarcane juice). Recent studies using a systematic scientific approach have also shown that various extracts of *H. rosa sinensis* flowers have antifertility potency in female rats.^{1–4)} Therefore, in the present study, efforts have been made to test the effect of the benzene extract of these flowers on the estrous cycle in mice, as benzene extract has been shown to have the most potent antifertility effect out of four extracts tested in our laboratory.⁵⁾ Emphasis has been placed on studying the antiovarian effect of the extract, as its antiovarian property is the most important step in the contraceptive nature of the compound. The biological nature of the extract has also been studied.

MATERIALS AND METHODS

Flowers of *H. rosa sinensis* were collected from the wild during September of 1995. They were shade dried, powdered and Soxhleted with benzene solvent. The benzene extract was concentrated under reduced pressure and a controlled temperature (50–60 °C). 500 g of the dried powder of *Hibiscus* flower yielded 5.6 g of a brownish greasy extract. The greasy benzene extract thus obtained was dissolved in double refined groundnut oil according to the two doses fixed, during earlier studies in our laboratory, at 125 and 250 mg/kg body weight.⁵⁾

Female Swiss strain albino mice, 60 d old, were purchased from the National Institute of Nutrition (NIN), Hyderabad. The animals were acclimatized to laboratory conditions for 8 d. They were fed a diet formulated by CFTRI and water *ad libitum*. Care was taken to regulate the room temperature at 25 °C (± 2 °C). Animals weighing 25–30 g were selected for the experiments.

To study the effect of the extract on the estrous cycle,

animals with regular cycles were selected at estrous phase and grouped for treatment. The treatment was given intraperitoneally for 15 d to cover three regular estrous cycles. A vaginal smear from the experimental animals was observed every morning and the body weight was recorded every day. On autopsy, 24 h after the last treatment, the ovaries and uteri were dissected out, freed from extra depositions and weighed. Tissues were fixed in Bouin's fluid for histological studies. One ovary from each animal was processed for biochemical analysis such as cholesterol⁶⁾ and ascorbic acid.⁷⁾

To test the estrogenicity of the extract, immature female mice, aged 27–30 d, were selected. They were divided into four groups and treated for 5 d as follows. Group 1: 0.1 ml groundnut oil as a vehicle. Group 2: 250 mg benzene extract/kg body weight dissolved in 0.1 ml groundnut oil. Group 3: 20 μ g ethinyl estradiol dissolved in 0.1 ml groundnut oil. Group 4: 250 mg benzene extract/kg body weight + 20 μ g. Ethinyl estradiol dissolved in 0.1 ml groundnut oil.

At autopsy on the 6th day, the state of the vagina was observed and the vaginal smear was tested. After autopsy, the uteri were dissected and weighed. All the values were statistically analyzed and means of the different groups were compared using Student's *t* test. The values were judged as significant if $p < 0.05$.

RESULTS AND DISCUSSION

The use of plant preparations for pregnancy interruption has been in practice since ancient times in India. There are many reviews available on the antifertility activities of medicinal plants.^{8–10)} The antifertility effect may be due to antiovarian, antiimplantation or abortifacient activities. To quote a few, the seeds of *Albizia lebbek*,¹¹⁾ the roots of *Polygonum hydropiper*¹²⁾ and many other plants have been reported for their antiovarian activity. Similarly, the leaves of *Ocimum sanctum*,¹³⁾ seed oil of *Daucus carota*,¹⁴⁾ leaves of *Mentha arvensis*,¹⁵⁾ rhizomes of *Curcuma longa*¹⁶⁾ and many more have shown antiimplantation activity. The abortifacient activity of the entire plant of *Achyranthes aspera*,¹⁷⁾ leaves

* To whom correspondence should be addressed.

of *Anans cornosus*,¹⁸⁾ seeds of *Pisum sativum*,¹⁹⁾ and the unripe fruit of *Carica papaya*²⁰⁾ have also been investigated. In this experiment, the antiovarian effect of the flower extract of *H. rosa sinensis* has been tested by studying its effect on the estrous cycle, as well as on ovarian and uterine components.

Estrous Cycle (Tables 1—3) Administration of the benzene extract of *H. rosa sinensis* flowers showed a dose dependent increase in the duration of estrus and metestrus and a decrease in the duration of diestrus and proestrus, significantly, during the experimental period. The prolonged estrus observed indicates the mild estrogenicity of benzene extract. The decrease in the number of Graafian follicles may be due to the non-availability of sufficient pituitary gonadotrophins for folliculogenesis.²¹⁾ However, the absence of corpora lutea and an increase in the number of atretic follicles in the ovaries of the experimental mice

indicates the absence of ovulation, suggesting that the critical balance of pituitary gonadotrophins²²⁾ is disturbed due to treatment with the extract. As atretic follicles contribute to estrogen synthesis, mice treated with this benzene extract may have developed cornification of vaginal epithelial cells and prolonged estrus. A significant increase in cholesterol in the ovaries of the treated animals indicates the non-availability of pituitary gonadotrophins for steroidogenesis.^{23,24)} An increase in ovarian ascorbic acid also indicates the depletion of pituitary LH.²⁵⁾

The increase in uterine weight and thickness supports the estrogenic nature of the extract. Therefore, the estrogenic activity of the extract has been tested and confirmed.

Estrogenic Activity (Table 4) When the benzene extract of *H. rosa sinensis* was administered to the prepubertal mice for 5 d, with or without ethinyl estradiol, premature opening of the vagina was produced, along with cornification of the vaginal epithelium and an increase in the weight of uterus in all the animals.

Earlier studies have shown that the postcoital effectiveness of plant agents is roughly parallel to their estrogenicity.²⁶⁾ Therefore, the antifertility effects of various extracts of *H. rosa sinensis* which have been reported so far^{15,27)} may be due to estrogenic activity of the flower.

Table 1. Effect of Benzene Extract (BE) of *H. rosa sinensis* on Duration of Different Phases of Estrous Cycle

Treatment	Estrus (d)	Metestrus (d)	Diestrus (d)	Proestrus (d)
Control (8)	6.60 ± 0.92	3.00 ± 0.45	5.20 ± 2.02	0.80 ± 0.20
125 mg BE (8)	6.80 ± 2.02	5.40 ± 1.22	3.40 ± 1.21	0.20 ± 0.19
250 mg BE (8)	8.80 ± 0.58	5.60 ± 0.41*	1.60 ± 0.40**	0.00 ± 0.00**

Dose: mg/kg body weight, duration: 15 d. * $p < 0.01$; ** $p < 0.001$ when compared to control group. M ± S.E. = arithmetic mean ± standard error. Number in parenthesis denotes the number of mice.

Table 2. Ovarian Changes Due to Administration of Benzene Extract (BE) of *H. rosa sinensis*

Treatment	Weight (mg/100 g body wt.)	No. of Graafian follicles	No. of Corpora lutea	No. of Atretic follicles	Cholesterol (μg/mg)	Ascorbic acid (μg/mg)
Control (8)	25.80 ± 2.35	3.00 ± 0.12	3.20 ± 0.09	3.08 ± 0.10	12.76 ± 0.79	0.66 ± 0.03
125 mg BE (8)	21.00 ± 2.62	3.12 ± 0.34	1.80 ± 0.30*	3.84 ± 0.21	21.38 ± 0.37**	0.88 ± 0.14
250 mg BE (8)	20.40 ± 3.40	2.18 ± 0.29	0.00 ± 0.00**	5.32 ± 0.18*	22.20 ± 0.34**	0.92 ± 0.14

Dose: mg/kg body weight, duration: 15 d. * $p < 0.01$; ** $p < 0.001$ when compared to control group. M ± S.E. = arithmetic mean ± standard error. Number in parenthesis denotes number of mice.

Table 3. Uterine Changes Due to Administration of Benzene Extract (BE) of *H. rosa sinensis*

Treatment	Weight (mg/100 g body wt.)	Diameter (μm)	Thickness of endometrium (μm)	Thickness of myometrium (μm)
Control (8)	344.60 ± 61.85	78.79 ± 0.43	32.95 ± 0.59	5.86 ± 0.32
125 mg BE (8)	384.20 ± 56.87	87.08 ± 0.36	35.19 ± 1.83	6.28 ± 0.41
250 mg BE (8)	524.80 ± 42.34	93.70 ± 0.61*	38.98 ± 1.38*	6.74 ± 0.63*

Dose: mg/kg body weight, duration: 15 d. * $p < 0.01$ when compared to control group. M ± S.E. = arithmetic mean ± standard error. Number in parenthesis denotes the number of mice.

Table 4. Estrogenic Activity of Benzene Extract (BE) of *H. rosa sinensis*

Group	Treatment	Vaginal opening	Stage of estrous cycle on autopsy	Mean wt. of uterus ± S.E. (mg/100 g body wt.)
1	Control (5)	Not opened	—	556.88 ± 5.41
2	250 mg BE (5)	Opened	Estrus (only cornified cells)	723.67 ± 5.09**
3	20 μg ethinyl estradiol (5)	Opened	Estrus (only cornified cells)	673.85 ± 2.98*
4	250 mg BE + 20 μg ethinyl estradiol (5)	Opened	Estrus (only cornified cells)	775.00 ± 4.67**

Dose: mg/kg body weight, duration: 5 d. * $p < 0.01$; ** $p < 0.001$ when compared to control group. M ± S.E. = arithmetic mean ± standard error. Number in parenthesis denotes the number of mice.

REFERENCES

- 1) Kirtikar K. R., Basu B. D., "Indian Medicinal Plants," Lalit Mohan Basu, Allahabad, 1935.
- 2) Nadkarni A. K., Nadkarni K. M., "Indian Materia Medica," Popular Book Depot, Bombay, 1954, p. 1319.
- 3) Choudhary R. R., Haq M., *M. Bull. Medico. Ethmo. Bot. Res.*, **1**, 408 (1980).
- 4) Chopra R. N., Chopra I. C., Honda K. L., Kapur L. D., "Chopra's Indigenous Drugs of India," Academic Publishers, Calcutta, 1982, p. 679.
- 5) Murthy D. R. K., "Antifertility Activities of Hibiscus Rosa Sinensis in Female Mice," M. Phil. thesis, submitted to Gulbarga University, (1996).
- 6) Peters J. P., Vanstyke D. D., "Quantitative Clinical Chemistry," Vol. 1, Williams & Wilkins, 1946.
- 7) Roe J. H., Krether C. A., *J. Biol. Chem.*, **147**, 399 (1943).
- 8) Kamboj V. P., *Indian J. Med. Res.*, **87**, 336 (1988).
- 9) Bhargava S. K., "Fitoterapia" LIX, 163 (1988).
- 10) Chopra R. N., Nayer B. L., Chopra I. C., "Glossary of Indian Medicinal Plants," CSIR Publication, New Delhi, 1956, p. 31.
- 11) Vohora S. B., Khan M. S. Y., *Indian J. Pharm.*, **36**, 77 (1974).
- 12) Kapoor M., Garg S. K., Mathur V. S., *Indian J. Med. Res.*, **62**, 1225 (1979).
- 13) Moya M., Gonzalex C., Forero I. C., Abstract of Papers, 24th Meeting of the International Congress for Research on Medicinal Plants, 1976, p. 6.
- 14) Kamboj V. P., Dhawan B. N., *J. Ethnopharmacol.*, **6**, 191 (1982).
- 15) Batta S. K., Shanthakumari G., *Indian J. Med. Res.*, **59**, 777 (1970).
- 16) Bhaduri B., Ghose C. R., Bose A. N., Moza B. K., Basu V. P., *Indian J. Med. Res.*, **64**, 252 (1968).
- 17) Garg S. K., *Indian J. Med. Res.*, **64**, 1133 (1976).
- 18) A Treatise on Medicinal Uses of Plants by Indian Tribes of Nevada, United States Department of Agriculture, 1951.
- 19) Agarwal S. S., Ghatak N., Arora R. B., *Indian J. Pharm.*, **31**, 175 (1969).
- 20) Grahm R. C. B., Nable R. L., *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **11**, 58 (1952).
- 21) Richards J. S., *Physiol. Rev.*, **60**, 51 (1980).
- 22) Kobayashi F. K. H., Tamotsu M., *Endocrinol Japan*, **501**, (1969).
- 23) Mason N. R., Savard K., *Endocrinology*, **75**, 215 (1964).
- 24) Dey S. K., Sengupta J., Ghosh S., Deb C., *Endocrinology*, **70**, 758 (1972).
- 25) Krum A. E., Mons M. D., Bennet C. L., *Endocrinology*, **74**, 573 (1964).
- 26) Bhargava S. K., *Plant Medicinals et Phytotherapie*, **18**, 74 (1984).
- 27) Kholkute S. D., Udupa K. N., *J. Res. Indian Med. Yoga Horneopath*, **13**, 107 (1978).