Proc. Indian natn. Sci. Acad., B 45 No. 4 pp 327-331 (1979)

Protein Concentration in Rat Uterus under the Influence of *Hibiscus rosa-sinensis* Linn. Extracts

ANAND O PRAKASH*
School of Studies in Zoology, Jiwaji University, Gwalior 474 002

(Received 28 March 1979; after revision 16 July 1979)

Effect of total 50% ethanolic and benzene extracts of *Hibiscus rosa-sinensis* Linn. on protein content and related parameters of the uterus of adult rats, was studied. Both extracts significantly reduced the uterine fresh weight but increased the protein content, non-protein nitrogen titre and total solid matter of the rat uterus markedly. The effect depends upon the dose and the period of treatment. Effect is highly significant when these extracts are administered at higher dose for longer duration. Of the two, benzene extract seems to be more potent. The observed changes are due to the antiestrogenic nature of the extracts.

Keywords: **Antifertility activity, Hibiscus rosa-sinensis, protein / contents, albino rats, adult rats**

Introduction

Flowers of *Hibiscus rosa-sinensis* Linn. have been reported to possess significant antifertility activity in female albino rats (Batta & Santhakumari 1971) and this has been confirmed by Kholkute and Udupa (1974), Prakash and Mathur (1976a, b) and Kholkute et al. (1977). A benzene extract has been reported to disrupt the normal oestrous cycle in rats and reduce the ovarian, uterine and pituitary weight significantly (Kholkute et al. 1976.) In view of strong antiestrogenic activity of these extracts (Kholkute & Udupa 1976) the effect of 50% ethanolic and benzene extracts of this plant on protein content and related parameters in the uterus of rat has been examined.

Materials and Methods

Ethanolic and benzene extracts (50%) of *H. rosa-sinensis* Linn. (flowers) were obtained by soxhlet apparatus as described earlier (Prakash & Mathur 1976b). The extracts were evaporated to dryness and three standard doses, 75, 150 and 300 mg/kg body weight were prepared as described earlier (Prakash & Mathur 1977).

Colony-bred Swiss adult female rats, 3-4 months old (165 ± 15g), were maintained under uniform conditions of light and temperature and were given 'Hindustan Lever' palleted diet and water *ad libitum*. The vaginal smear of each rat was examined daily for 18 days to select animals of regular cycles. The rats showing proestrus stage of oestrous cycle were selected and divided into four groups separately for each extract. Each dose was administered orally with the help of an intragastric catheter for 6 days (1 complete cycle), 12 days (2 complete

**Present address:* Division of Endocrinology, Central Drug Research Institute, Lucknow 226 001

cycles) and 18 days (3 complete cycles) to different groups of adult rats (tables 1–4). Control rats in each group received vehicle only in a similar manner. The vaginal smear of each rat was examined daily in the morning. The animals were killed with light ether anaesthesia in diestrus stage and 48 hr after the last dose, i.e. on 8th, 14th and 20th day respectively. The rats showing stages other than diestrus were not considered. The uteri were carefully dissected out, trimmed, blotted on filter paper and weighed immediately to the nearest 0.1 mg. The tissue was kept in deep freezer for 48 hr and then homogenized and suitable aliquots were processed for biochemical estimation of non-protein nitrogen according to Koch and McMeekin (1924). Protein content was estimated by evaluation of total nitrogen as described by Umbreit et al. (1957), and substracting the concentration of non-protein nitrogen. The figure was then multiplied by 6.25. For total solid matter, tissue homogenate (2 ml) was taken on a tared-weighed watch glass, evaporated to dryness over a boiling water bath. Complete dehydration was obtained in a desiccator for 48 hr till constant weight readings were obtained.

The results of each parameter were statistically analysed using method of variance. Significance between two groups was determined by the method of least significant difference. A P (probable) value of 0.05 or less was considered to be significant.

Results

Ponderal change (table 1)

Both the treatments showed decrease in the weight of uterine depending on the duration of treatment. The effect is relatively more pronounced both at 300 dose level and at 18 days duration (thus a dose of 300 when given for 18 days is the most effective). However, benzene extract is more potent than 50% ethanolic extract.

Protein content (table 2)

Both the treatments showed increase in protein content of uterine depending on the dose and duration. The effect is more pronounced at 300 dose level and for 18 days duration. Benzene extract is more potent than the ethanolic extract when given for 12 and 18 days.

Table 1 *Effect of 50% ethanolic and benzene extracts of* H. rosa-sinensis *Linn. on uterine weight in adult rats (Values are mean ± S.E., expressed as mg/100g body weight. Number of rats used is given in parentheses)*

Extract	Dose (mg/kg/day)	Treatment period (days)		
		6	12	18
Control (vehicle only)	—	153.2±4.54 (5)	158.25±5.05 (4)	155.6±4.92 (5)
50% ethanolic	75	144.8±5.06 (5)	144.00±7.03 (5)	135.75±10.71 (4)
	150	140.00±7.07 (4)	142.25±2.87 (4)	131.00±12.44 (5)
	300	131.00±11.40 (5)	130.00±11.31 (5)	122.6±3.71[b] (5)
Control (vehicle only)	—	161.75±6.23 (4)	156.25±5.67 (4)	163.4±4.72 (5)
Benzene	75	143.75±4.34 (4)	143.5±5.44 (4)	131.75±10.43[c] (4)
	150	136.00∓4.18[d] (5)	134.25±2.62[d] (4)	130.00±9.72[c] (5)
	300	131.25±2.98[c] (4)	128.00±9.79[c] (4)	121.6±2.70[a] (5)

P values versus their respective controls: $a < 0.001$; $b < 0.005$; $c < 0.01$; $d < 0.02$

Table 2 *Effect of 50% ethanolic and benzene extracts of* H. rosa-sinensis *Linn. on uterine protein contents in adult rats (Values are mean ± S.E., expressed as mg/g tissue. Number of rats used is given in parentheses)*

Extract	Dose mg/kg/day	Treatment period (days) 6	12	18
Control (vehicle only)	—	122.8±3.11 (5)	127.5±2.08 (4)	124.6±2.96 (5)
	75	128.8±2.58 (5)	132.2±1.78 (5)	134.75±1.70[e] (4)
50% ethanolic	150	132.5±2.08[e] (4)	136.00±3.35[d] (4)	140.2±3.70[b] (5)
	300	137.8±1.92[c] (5)	143.4±2.70[b] (5)	158.2±2.38[a] (5)
Control (vehicle only)	—	124.5± (4)	125.00±2.16 (4)	123.8±3.03 (5)
	75	129.5±1.29 (4)	133.75±3.30[e] (4)	139.00±3.36[c] (4)
Benzene	150	132.8±1.92 (5)	137.00±2.16[d] (4)	142.2±1.92[b] (5)
	300	138.5±1.29[d] (4)	149.75±3.59[a] (4)	163.8±2.77[a] (5)

P values versus their respective controls: *a* <0.001; *b* <0.005; *c* <0.01; *d* <0.02; *e* <0.05

Non-protein nitrogen content (table 3)

A dose of 75 mg/kg body weight of the each extract shows no significant results, while higher doses, 150 and 300 mg/kg are effective in all schedules (vs control $P < 0.02$; < 0.01; < 0.005 and < 0.001), however, the effect is more pronounced when the extracts are administered for longer duration.

Total solids (table 4)

Both the extracts increase the total solid matter in the uterus of adult rat. Doses 75 mg/kg of 50% ethanolic extract when administered for 12 and 18 days increase the level significantly (vs control $P < 0.05$). Doses of 150 and 300 mg/kg for 18 days are most effective. Benzene extract also provokes a similar effect. Here also the 300 mg/kg

Table 3 *Effect of 50% ethanolic and benzene extracts of* H. rosa-sinensis *Linn. on uterine non-protein nitrogen contents in adult rats (Values are mean ± S.E., expressed as mg/g tissue. Number of rats used is given in parentheses)*

Extract	Dose mg/kg/day	Treatment period (days) 6	12	18
Control (vehicle only)	—	4.06±0.13 (5)	4.2±0.14 (4)	4.76±0.05 (5)
	75	4.2±0.15 (5)	4.44±0.18 (5)	4.67±0.02 (4)
50% ethanolic	150	4.2±0.18 (4)	4.65±0.10[d] (4)	5.18±0.08[d] (5)
	300	4.5±0.10[e] (5)	4.68±0.13[d] (5)	5.80±0.31[a] (5)
Control (vehicle only)	—	4.07±0.09 (4)	4.47±0.09 (4)	4.65±0.11 (5)
	75	4.42±0.22 (4)	4.62±0.09 (4)	4.72±0.09 (4)
Benzene	150	4.62±0.13[d] (5)	4.82±0.17[d] (4)	5.28±0.13[c] (5)
	300	4.7±0.18[b] (4)	5.27±0.09[a] (4)	6.18±0.14[a] (5)

P values versus their respective controls: *a* <0.001; *b* <0.005; *c* <0.01; *d* <0.02; *e* <0.05

Table 4 *Effect of 50% ethanolic and benzene extracts of* H. rosa-sinensis *Linn. on uterine total solid matter in adult rats (Values are mean ± S.E., expressed as g/100 g tissue. Number of rats used is gives in parentheses)*

Extract	Dose mg/kg/day	Treatment period (day)		
		6	12	18
Control (vehicle only)	—	29.00±1.58 (5)	28.5±1.29 (4)	28.6±0.89 (5)
50% ethanolic	75	33.002±1.58 (5)	34.0±1.58[e] (5)	35.75±1.70[e] (4)
	150	36.25±1.70[d] (4)	40.00±1.82[c] (4)	42.2±1.92[a] (5)
	300	39.40±1.94[c] (5)	42.60±1.94[a] (5)	43.2±1.30[a] (5)
Control (vehicle only)	—	30.25±1.70 (4)	29.5±1.29 (4)	29.00±1.58 (5)
Benzene	75	33.5±1.29 (4)	35.5±1.29 (4)	38.5±1.29[c] (4)
	150	35.00±1.58 (5)	38.5±1.29[c] (4)	40.00±1.58[b] (5)
	300	38.5±1.29[c] (4)	40.25±1.70[b] (4)	45.00±1.58[a] (5)

P values versus their respective controls: $a < 0.001$; $b < 0.005$; $c < 0.01$; $d < 0.02$; $e < 0.05$

dose is highly effective when administered for 18 days (vs control $P < 0.001$).

Discussion

Physiological and biochemical changes in the uterine tissue are governed by the ovarian hormones, estrogen and progesterone (Nalbandor 1970) which are in turn regulated by pituitary gonadotrophins and their releasing factors (McCann 1968). Research on biochemical constituents in the uterus under the influence of antifertility factors of plant origin is very limited (Chakaravarthi 1961, Pakrashi 1967, Raman et al. 1976 & Seshadri et al. 1978).

Synthetic estrogen and progesterone influenced the uterine ponderancy significantly (Lerner 1969) but no significant change is observed in uterine wet weight when an antiestrogenic plant material was administered to adult rat (Prakash & Mathur 1977). Present findings show that 50% ethanolic and benzene extracts of *H. rosa-sinensis* Linn. decreased the uterine weight of adult rats significantly (table 1). The results are in agreement with those of Kholkute et al. (1976).

Similarly, estrogen enhances the uterine protein concentration of ovariectomized rat (Telfer 1953, Davis et al. 1956 & Dutta et al. 1968). On the contrary, progesterone increases the total protein contents, non-protein nitrogen level and total solid matter of the uterus of ovariectomized rat (Dutta et al. 1968). In the present investigation both 50% ethanolic and benzene extracts of *H. rosa-sinensis* Linn. elevated the uterine protein concentration, non-protein nitrogen contents and total solid matter significantly (tables 2–4).

The inhibition in uterine fresh weight and enhancement in protein contents, non-protein nitrogen level and total solid matter in the uterus of adult intact rats treated with *H. rosa-sinensis* Linn. extracts may be due to the antiestrogenic nature of the extracts which has already been assayed by Kholkute and Udupa (1976). Interestingly, the effect is dose and duration-dependent as both the extracts reveal a clear-cut dose-response relation. Effect is more significant at higher doses. Benzene extract seems to be more potent.

Acknowledgements

I am thankful to the Council of Scientific & Industrial Research, New Delhi for a grant. I am indebted to Dr Jagdish Bahadur, of the

School of Studies in Zoology, for providing me the necessary laboratory facilities and to Dr R Mathur, Research Supervisor for the critical assessment of the manuscript.

References

Batta, S K and Santhakumari G 1971 The antifertility effect of *Ocimum sanctum* and *Hibiscus rosa-sinensis*; *Indian J. Med. Res.* **59** 717

Chakravarti H S 1961 Inhibition of alkaline phosphatase activity on the endometrium of the rabbit by oral administration of indigenous contraceptive drug; *J. Ind. Med. Assoc.* **37** 322

Davis J S, Meyer R K and McShan W H 1956 The effect of aminopterin and estrogen on the phosphatase metabolism of rat uterus; *Endocrinology* **59** 505

Dutta I C, Karkun J N and Kar A B 1968 Studies on physiology and biochemistry of the cervix: Effect of estrogen and progesterone on the rat cervix; *Acta biol. med. germ.* **20** 155

Kholkute S D and Udupa K N 1974 *J. Res. Ind. Med. Yoga & Homeo.* **9** 99 (Cited by S D Kholkute et al. 1977 Studies on the antifertility potentiality of *Hibiscus rosa-sinensis. Planta Medica* **31** 35)

———1976 Antiestrogenic activity of *Hibiscus rosa-sinensis* Linn. flowers; *Indian J. exp. Biol.* **14** 175

Kholkute S D, Chatterjee S and Udupa K N 1976 Effect of *Hibiscus rosa-sinensis* Linn. on oestrous cycle & reproductive organs in rats; *Indian J. exp. Biol.* **14** 703

Kholkute S D, Mudgal V and Udupa K N 1977 Studies on the antifertility potentiality of *Hibiscus rosa-sinensis* Linn; *Planta Medica* **31** 35

Koch F C and McMeekin 1924 A new direct nesslerization micro-kjeldahl method and the modification of the nessler folin reagent for ammonia; *J. Amer. Chem. Soc.* **46** 2066

Lerner L J 1969 The biology of non-steroidal antifertility agents; in *Contraception: Chemical control of fertility* ed Daniel Lednicer, pp. 161 (New York: Marcel Dekker Inc.)

McCann S M 1968 in *Pharmacology of Reproduction*, ed Diczfalusy (N. Y. Pergamon)

Nalbandov A V 1970 Reproduction in female mammals & birds; in *Reproductive Physiology* ed A V Nalbandov (Bombay: D B Taraporewala Sons & Co Pvt Ltd) 119

Pakrashi A 1967 Endocrinological studies on plant products: Part V—Effect of byakangelicin on female sex hormones & fertility of rats; *Indian J. exp. Biol.* **5** 75

Prakash A O and Mathur R 1976a Effect of oral administration of *Hibiscus rosa-sinensis* on early and late pregnancy in albino rats; *J. Jiwaji Univ.* **4** 79

———and Mathur R 1976b Screening of Indian plants for antifertility activity; *Indian J. exp. Biol.* **14** 623

———and Mathur 1977 Effect of *Artobotrys odoratissimus* Linn. extracts on oestrous cycle, body weight and uterine weight in albino rats; *Indian J. exp. Biol.* **15** 1038

Raman G, Munshi S R and Rao S S 1976 Alkaline phosphatase activity in the uteri of rats and guineapigs fed an Ayurvedic antifertility agent; *Indian J. Med. Res.* **64** 959

Seshadri C, Suganthan D and Santhakumari G 1978 Biochemical change in the female genital tract & liver of mated rats treated with embelin, a non-hormonal oral contraceptive agent; *Indian J. Pharmacol.* **10** 89

Telfer M A 1953 Influence of estradiol on nucleic acids, respiratory enzymes and the distribution of nitrogen in the rat uterus; *Archs. Biochem.* **44** 111

Umbreit W W, Burris R J and Stauffer J F 1957 *Manometric Techniques* (Manneapolis: Burgess Publishing Co.)