THE INFLUENCE OF *ARTOBOTRYS ODORATISSIMUS* LINN. EXTRACTS ON PHOSPHATASE ACTIVITY IN THE UTERUS OF RATS

A. O. PRAKASH*

School of Studies in Zoology,
Jiwaji University, Gwalior 474 002

Summary: The effect of 50% ethanolic and benzene extracts of *Artobotrys odoratissimus* Linn., a potent antiestrogenic plant on uterine acid and alkaline phosphatase activity has been studied in adult rats. Both the extracts decreased the alkaline phosphatase activity in the uterus of intact and ovariectomized rats ($P<0.001$) but elevated the acid phosphatase activity ($P<0.001$). 50% ethanolic extract acts at low dose levels; however its activity decreases as the dose is increased. Benzene extract showed more consistent dose-response relationship.

Key words : antiestrogenic indigenous plants *Artobotrys odoratissimus* Linn.
uterine phosphatases activity

INTRODUCTION

Antifertility activity of *Artobotrys odoratissimus* Linn. (Vern. Harachampa, Katchampa) reported by Chakrabarti *et al.* (2) has been corroborated in rats (19). Ethanolic and benzene extracts (50%) of fresh green leaves of this plant disrupt the normal oestrous cycle (19) and also significantly prolong the duration of diestrus stage as checked with vaginal smear in rats (20). Furthermore, both these extracts have been reported to possess significant antiestrogenic activity when tested in immature female rats (21). In view of its antiestrogenic mode of action (21) the present investigation has been undertaken to determine their effect on acid and alkaline phosphatase activity in the rat uterine tissue as these parameters are sensitive to oestrogenic action.

MATERIALS AND METHODS

Ethanolic and benzene extracts (50%) of fresh green leaves of *A. odoratissimus* Linn. were obtained by soxhlet apparatus. The extracts were evaporated to dryness and macerated with gumacacia suspended in distilled water at 3 different doses i.e., 75, 150 and 300 *mg/kg* as described earlier (19). Each dose was administered orally once a day by an intragastric catheter.

Colony-bred Swiss adult female albino rats (3-4 months old, 165 ± 15 *g*) were maintained under uniform controlled condition and selected as described earlier (19).

---

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

The selected rats were divided into 2 groups; the first group consisted of intact rats whereas the other consisted of rats which had undergone bilateral ovariectomy under light ether anaesthesia 16 days before. Both intact and ov riectomized rats were randomly separated into 4 groups and were treated in the same manner as described earlier (19). The rats were killed by ether anaesthesia in diestrus stage and 48 hr after the last dose i.e. on 8th, 14th and 20th day. The uteri were carefully dissected out, freed from surrounding fat, blotted on filter paper and weighed quickly to the nearest 0.1 *mg* on single pan balance. The weighed piece of uterine tissue was deep-frozen for 48 hr, homogenized and suitable aliquots were processed for biochemical estimation. For acid and alkaline phosphatase estimation, the method outlined by Hawk *et al.* (11) was adopted with some necessary modifications. To 4 *ml* solution of sodium β glycerophosphate buffer (buffer of pH 5 for acid phosphatase and buffer of pH 9.2 for alkaline phosphatase), 0.5 *ml* of tissue homogenate was added and incubated for 60 min in a water bath maintained at 37 C. After 1 hr the reaction was stopped by 0.5 *ml* of 30% Trichloracetic acid, the test tube was centrifuged, and suitable aliquot was withdrawn. Colour was developed by adding 2.5% ammonium molybdate and 1-2-4 aminonaphthol sulphonic acid. Control was run simultaneously along with a blank solution. A standard curve of phosphorus was used for the calculations. The results were statistically analysed using analysis of variance. Significance between 2 groups was determined by the method of least significant difference. A P value of 0.05 or less was considered to be significant.

RESULTS

1. *Acid phosphatase activity :*

Table I gives the effect of *A. odoratissimus* Linn. extracts on uter ne acid phosphatase activt y in rats.

Ethanolic extract (50%) when fed to intact and ovariectomized rats at 75 *mg/kg* dose for 18 days elicited a significant increase in acid phosphatase activity of uterine ti sue ($P<0.001$). Similarly, 150 *mg/kg* dose for 12 days in both type of rats elevated the activity ($P<0.01$ and <0.001). Highest dose of ethanolic extract was not effective.

300 *mg/kg* of benzene extract administered for 6,12 and 18 days to intact rats evoked a significant increase in uterine acid phosphatase activity ($P<0.02$; <0.01 and <0.001 respectively). The effect was highly significant in ovariectomized rats where 300 *mg/kg* dose administered for any of the durations, caused a significant increase in the activity ($P<0.001$).

2. *Alkaline phosphatase activity :*

Table II summarizes the results. Alkaline phosphatase activity in the uterus of intact rats exhibited a marked decrease when 50% ethanolic extract was administered at

TABLE I : Effect of *A. odoratissiums* Linn. extracts on uterine acid phosphatase activity in rats.
(Values are means ±S.E. expressed as *mg*/100 *g* tissue/hr. Numbers of rats used are given in parentheses)

Extract	*Dose mg/kg/day*	*Treatment period (days)*		
		6	12	18
		A. Intact rats		
Control (vehicle only)	—	96±3.87 (10)	100±3.56 (10)	96±3.87 (10)
50% ethanolic extract	75	96±4.28 (6)	101±4.98 (6)	155±4.44*(8)
	150	97±3.99 (8)	122±3.49**(9)	114±9.24 (6)
	300	96±4.28 (9)	104±3.07 (8)	99±4.25 (10)
Control (vehicle only)	—	93±3.87 (10)	99±3.85 (10)	94±3.56 (10)
Benzene extract	75	92±4.02 (10)	95±4.39 (10)	103±2.06 (10)
	150	97±3.95 (10)	97±4.62 (8)	98±4.62 (8)
	300	119±4.58***(8)	127±4.62**(10)	144±4.62*(10)
		B. Ovariectomized rats		
Control (vehicle only)	—	92±4.26 (6)	89±3.60 (6)	83±2.57 (6)
50% ethanolic extract	75	101±5.45 (6)	91±5.03 (6)	179±8.33* (6)
	150	95±4.08 (6)	152±3.84*(6)	96±5.74 (6)
	300	94±6.25 (6)	87±8.33 (6)	83±2.79 (6)
Control (vehicle only)	—	93±9.72 (6)	86±3.57 (6)	91±2.84 (6)
Benzene extract	75	99±3.84 (6)	90±4.56 (6)	98±6.18 (6)
	150	101±7.09 (6)	96±3.95 (6)	83±3.53 (6)
	300	123±5.01*(6)	128±7.15*(6)	139±4.16*(6)

P values versus control: *<0.001; **<0.01; *** <0.02

75 *mg/kg* for 18 days and 150 *mg/kg* for 12 days ($P<0.001$ and <0.01). The action was more marked in ovariectomized rats ($P<0.001$).

The benzene extract administered to intact and ovariectomized rats at 300 *mg/kg* dose at every duration produced a significant decrease in uterine alkaline phosphatase activity ($P<0.001$ and <0.05).

TABLE II: Effect of *A. odoratissimus* Linn. extracts on uterine alkaline phosphatase in rats.
(Values of means ±S.E. expressed as *mg*/100 *g*. tissue/hr. Numbers of rats used are given in parentheses)

Extract	*Dose mg/kg/day*	*Treatment period (days)*		
		6	12	18
		A. Intact rats		
Control (vehicle only)	—	604±8.75 (10)	608±7.92 (10)	610±10.47(10)
50% ethanolic	75	615±11.07 (6)	609±11.39 (6)	511±10.37*(8)
	150	612±10.36 (8)	536±7.90**(9)	630±12.12 (6)
	300	609± 9.68 (9)	606±5.76 (8)	608±13.43(10)
Control (vehicle only)	—	613±9.86 (10)	622±9.83 (10)	603±11.56(10)
Benzene	75	641± 7.86 (10)	659±9.22 (10)	452±5.38*(10)
	150	615±11.07 (10)	600±7.61 (8)	484±11.38*(8)
	300	524±11.48* (8)	489±6.93*(10)	429±5.01*(10)
		B. Ovariectomized rats		
Control (vehicle only)	—	455±17.43 (6)	462±12.15 (6)	437±11.41 (6)
50% ethanolic	75	465±22.86 (6)	303±17.88*(6)	246±15.11*(6)
	150	446±19.48 (6)	294±17.38*(6)	404±27.55 (6)
	300	455±19.59 (6)	494±14.43 (6)	440 ±15.61 (6)
Control (vehicle only)	—	562±12.54 (6)	560±10.71 (6)	548±10.72 (6)
Benzene	75	550±23.01 (6)	529±21.14 (6)	557±15.62 (6)
	150	580±14.20 (6)	532±11.71 (6)	536±14.38 (6)
	300	510±19.14***(6)	384±11.49*(6)	392±11.48*(6)

P values versus control : * <0.001; ** <0.01; *** <0.05

DISCUSSION

The role of two enzymes in the uterus is not perfectly understood. However, according to Novikoff (17) acid phosphatase is located mainly in the lysosomes of uterine tissue. The increased enzymatic activity signifies the disintegration of the complex organelle and the liberation of hydrolytic enzymes. Alkaline phosphatase is believed to be variously involved in the growth and secretory function of the tissue cells (10), the metabolism of carbohydrates (24), nucleoproteins and lipids (1) and nucleic acids (14). Increased

activity in the acid phosphatase enzyme has also been observed in the uterus and oviduct of women during secretory phase (4); such an increase in acid phosphatase may help to remove any debris or lysed cells.

Literature on the effect of active antifertility plants on acid and alkaline phosphatase activity in the uterine tissue is scanty (3,18,22). Ethanolic and benzene extracts (50%) of *A. odoratissimus* Linn. inhibited significantly the alkaline phosphatase activity of the uterus of both intact and ovariectomized rats but markedly increased acid phosphatase activity. This can change secretory functions by influencing permeability as also the uterine milieu required for the implantation of an egg.

An increase in the uterine acid phosphatase activity after estrogen treatment has been reported (5,6). Similar results in the uterus of adult ovariectomized rats have also been described (7,13). Although progesterone is considered to be responsible for the increase in acid phosphatase activity of uterine tissue in rats (8), no significant change has been observed in acid phosphatase activity in the uterus of ovariectomized rats treated with progesterone (12). It is possible that increased acid phosphatase activity in the uterus of both intact and ovariectomized rats after the treatment with *A. odcratissimus* Linn. extracts is due to their strong antiestrogenic action (21).

Inhibition of alkaline phosphatase in the uterus of ovariectomized mouse and rats after the injection of progesterone has been described (8). But progesterone has also been reported to increase the alkaline phosphatase activity in the uterus of rat (12). The diminution in the alkaline phosphatase activity in the uterus of intact and ovariectomized rats under the influence of 50% ethanolic and benzene extracts of *A. odoratissimus* Linn. (Tables I, II) may be due to the antiestrogenic activity of the extracts (21). The results on alkaline phosphatase activity tally with those of Pakrashi (18) on the fruit of *Ferula alliaceae* in adult rats.

With both the extracts, the effect was more pronounced in ovariectomized rats than intact ones which may be due to the fact that ovariectomized rats are free from endogenous ovarian hormones and whatever the changes in the uterine constituents, are only due to the exogenous hormonal treatment while in the intact rats endogenous hormones are also available which make the phenomenon intricate.

Enigmatically the effect of 50% ethanolic extract was more significant at low dose level. Low dose level of this extract may act as an antiestrogenic peak beyond which the activity diminishes as in the case with the synthetic hormones. However, no exact explanation can be offerred for this paradox. Similar paradoxical results have also been reported by others (9,15,16,23) while screening some indigenous plants for antifertitity activity.

ACKNOWLEDGEMENTS

The author is thankful to the Council of Scientific & Industrial Research, New Delhi for a grant and to Dr. Jagdish Bahadur, Professor & Head, School of Studies in Zoology, Jiwaji University, Gwalior for providing adequate facilities to present work. He is also indebted to Dr. R. Mathur, Research Supervisor, for valuable guidence and critical assessment of the manuscript.

REFERENCES

1. Atkinson, W.B. and H. Elftman. Mobilization of alkaline phosphatase in the uterus of the mouse by estrogen. *Endocrinology*, **40** : 30-36, 1947.
2. Chakrabarti, B., A. Chaudhury and P.R. Chaudhury. Antifertility effect of green leaves of *Artobotrys odoratissimus* Linn. *J. Ind. Med. Ass.*, **51** : 227-229, 1968.
3. Chakravarti, H.S. Inhibition of alkaline phosphatase activity of the endometrium of rabbit by oral administration of indigenous contraceptive drugs. *J. Ind. Med. Ass.*, **37** : 322-326, 1961.
4. Clyman, M.J. Electron microscopy of the human fallopian tube. *Fert. Steril.*, **17** : 281-301, 1966.
5. Davis, J.S., R.K. Mayer and W.H. McShan. The effect of aminoprotein and estrogen on the phosphate metabolism of rat uterus. *Endocrinology*, **59** : 505-515, 1956.
6. Dugan, F.A., B. Radhakrishnamurthy, R.A. Rudman and G.S. Berenson. Stimulation of synthesis of gloycoprotein enzymes of the rat uterus by estradiol. *J. Endocr.*, **42** : 261-266, 1968.
7. Dutta, I.C., J.N. Karkun and A.B. Kar. Studies on physiology and biochemistry of the cervix : Effect of estrogen and progesterone on the rat cervix. *Acta Biol. Med. Germ.*, **20** : 155-162, 1968.
8. Garcia-Bunnel, R. and D. Brandes. Lysosomal enzymes in human endometrium. *Am. J. Obstet. Gynaec.*, **94** : 1045-1055, 1966.
9. Garg, S.K. and G.P. Garg. Antifertility screening of plants Part VII - Effect of five indigenous plants on early pregnancy in albino rats. *Ind. J. Med. Res.*, **59** : 302-306, 1971.
10. Giering, J.E. and M.X. Zarrow. Change in uterine morphology and phosphatase levels induced by chronic stimulation with the ovarian hormones. *Acta Endocr. (Kbh.)*, **29** : 499-501, 1958.
11. Hawk, P.B., B.L. Oser and W.H. Summerson. Practical Physiological Chemistry, McGraw Hill Co. N.Y., 1954.
12. Hayashi, M. and W.H. Fishman. Enzymorphologic observations in the uterus and vagina of castrate rats receiving ovarian hormones. *Acta Endocr. (Kbh.)*, **38** : 107-120, 1967.
13. Karkun, J.N. and P.K. Mehrotra. Studies on physiology and biochemistry of female genital tract : Response to uterus, cervix and vagina of albino rats to cis - and trans - clomiphene in the presence or absence of estrogen. *Ind. J. Exp. Biol.*, **11** : 7-14 1973.
14. Kelsall, M.A. and E.D. Grabb. Lymphocytes and Mast cells. The Williams & Wilkins Co. Baltmore 1959.
15. Munshi, S.R. and S.S. Rao. Antifertility activity of an indigenous plant preparation (ROC-101) I - Effect on reproduction. *Ind. J. Med. Res.*, **60** : 1054-1060, 1972.
16. Munshi, S.R., T.V. Purandare and S.S. Rao. Biological activity of an indigenous plant preparation. *Ind. J. Physiol. Pharmac.*, **16** : 181-189, 1972.
17. Novikoff, A.B. In : The Cell. Vol. 2, pp. 423 (Ed. Brachet J. and Mirsky A.E. Academic Press Inc. N.Y. 1961).
18. Pakrashi, A. Endocrinoligical studies on plant producrts : Part V. Effect of Byakangelicin on female sex hormones and on fertility of rats. *Ind. J. Exp. Biol.*, **5** : 75-79, 1967.
19. Prakash A.O. and R. Mathur. Effect of *Artoboytrs odoratissimus* Linn. extracts on oestrous cycle body weight and uterine weight in albino rats. *Ind. J. Exp. Biol.*, **15** : 1038-1040, 1977.
20. Prakash, A.O. Periodicity of oestrous cycle in rats : Response to *Artobotrys odoratissimus* Linn. extracts. *Curr. Sci.*, **47** : 659-662 1978.
21. Prakash, A.O. Antiestrogenc mode of action of *Artobotrys odoratissimus* Linn. extracts (fresh green leaves). *Ind. J. Exp. Biol.*, **16** : 1214-1215, 1978.
22. Raman, G., S.R. Munshi and S.S. Rao. Alkaline phosphatase activity in the uterus of rats and guinea pigs fed an Ayurvedic antifertility agent. *Ind. J. Med Res.*, **64** : 959-962, 1976.
23. Razdan, K., K. Kapila and N.K. Bhide. Antifertility effect and some pharmacological action of *Butea frondosa* seed extracts. *Ind. J. Physiol. Pharmac.*, **13** : 239-249, 1969.
24. Walaas, O. Effect of oestrogens on the glycogen contents of the uterus. *Acta Endocr. (Copenh.)* **10** : 175-192 1952.