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PERIODICITY OF OESTROUS CYCLE IN RATS: RESPONSE TO *ARTOBOTRYS ODORATISSIMUS* LINN. EXTRACTS

ANAND O. PRAKASH*

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

ABSTRACT

Fifty per cent ethanolic and benzene extracts of *Artobotrys odoratissimus* Linn. (fresh green leaves) have been investigated to know their effect on the duration of various stages of oestrous cycle in rats. The effect of the extracts on the duration of stages of oestrous cycle depends upon the dose and the period of treatment. 50% ethanolic extract, when administered at 75 mg/kg for 18 days, prolonged the length of leucocytic phase and lowered the period of cornified stage significantly (*vs.* respective control $P<0{\cdot}001$), but when administered at 150 mg/kg for the same period, it decreased the former and increased the latter. Dose 300 mg/kg is insignificant (*vs.* control $P > 0{\cdot}05$). Benzene extract showed a significant increase in the period of diestrus stage and a decrease in the duration of estrus phase (*vs.* respective control $P<0{\cdot}001$). However, maximum increase in the period of diestrus phase has been observed when it is applied at 75 mg/kg for 6 days (*vs. control* $P<0{\cdot}001$). The results adumbrate the weak estrogenic and antiestrogenic nature of 50% ethanolic extract while benzene extract may act as a strong antiestrognic agent which is yet to be elucidated.

INTRODUCTION

ARTOBOTRYS ODORATISSIMUS Linn. (local and Bengali — Katchampa; Sanskrit — Harachampa; Marathi — Hiruchampa; Bombay—Vilayati champa; Tamil—*Monoranjitam*; Telugu—Sempelanga, Monoraujitam), a member of family Annonaceae, has been reported to possess antifertility activity[1-7]. Its antifertility activity reported by Chakrabarty *et al.*[8] has been confirmed in albino rats[9]. 50% ethanolic and benzene extracts of its fresh green leaves, when administered to female albino rats, exhibited a property which disrupts the normal oestrous cycle[10]. As the fresh green leaves of this plant have been reported to effect on early pregnancy[9], and disrupts the normal oestrous cycle of rats[10], the present investigation was undertaken to assess the effect of its extracts on the duration of various stages of oestrous cycle in rats.

MATERIALS AND METHODS

Fresh green leaves of *A. odoratissimus* Linn., collected from V.C. High School, Gwalior (M.P.) in March, were soxhleted with 50% ethanol and benzene separately as described earlier[9,10].

Colony-bred Swiss adult female albino rats, 3-4 months old, weighing 165 ± 15 g were used. These animals were maintained under uniform controlled conditions of light and temperature and were given Hindustan Lever palleted rat diet and tap water. The vaginal smear of each rat was examined daily for 18–20 days to select animals showing regular cycle. The vaginal smear was taken carefully to avoid the chances of pseudopregnancy which occurred by the cervical stimulation. Rats of 5 and 6 days cycle length showing proestrus condition were selected in equal ratio for each set of experiment; however, the rats with intermittent stages between late 2nd diestrus-proestrus were also selected.

Each extract was separately macerated with gum-acacia and suspended in distilled water and administered at dose of 75, 150 and 300 mg/kg body weight. Each dose was administered orally by an intragastric catheter for 6 days (1 complete cycle), 12 days (2 complete cycles) and 18 days (3 complete cycles) to different batches of animals (Tables I and II). Controled rats in each batch received gum acacia suspension alone in a similar manner. Vaginal smear of each rat was examined daily in the morning and stages of oestrous cycle recorded. The record of vaginal smear was analysed statistically using '*t*' test and is presented in Tables I and II.

RESULTS AND OBSERVATIONS

(1) 50% *ethanolic extract*

Effect of 50% ethanolic extract on the duration of various stages of oestrous cycle depends upon the dose and the period of treatment. Dose 75 mg/kg, when administered for 18 days, prolonged the period of diestrus phase and lowered the duration of estrus stage significantly (*vs.* respective control $P < 0{\cdot}001$), but, when administered at 150 mg/kg for the same period (Table I), decreased the former and increased the latter (*vs.* respective control $P < 0{\cdot}05$ and $< 0{\cdot}02$). Treatment for 6 days with 75 mg/kg dose has resulted in the decrease of duration of diestrus phase (*vs.* control $P < 0{\cdot}05$) and increase in the period of metaestrus stage significantly (*vs.* control $P < 0{\cdot}001$),

* Present address: Division of Endocrinology, Central Drug Research Institute, Lucknow-226001.

TABLE I

Effect of 50% ethanolic extract of Artobotrys odoratissimus *Linn on duration of various stages of oestrous cycle in rats*

Dose mg kg body weight/ day	Treatment period (days)	Duration of stages of the oestrous cycle in days			
		Diestrus	Proestrus	Estrus	Metaestrus
Controls Vehicle only	6	3·50±0·15*	1·00±0·0	2·50±0·15	1·00±0 0
	12	6·25±0·26	2·00±0·12	4·41±0·27	1·33±0·14
	18	7·25±0·18	3·25±0·13	7·08±0·15	1·91±0·15
75	6	2·50±0·36[d]	1·20±0·14[e]	2·10±0·36[e]	2·20±0·43[a]
	12	5·70±0·98[e]	1·80±0·26[e]	5·40±0·91[e]	1·10±0·18[e]
	18	13·10±1·32[a]	1·80±0·37[a]	3·90±0·80[a]	1·10±0·24[e]
150	6	4·40±0·72[e]	0·70±0·16[e]	1·50±0·28[a]	1·40±0·47[e]
	12	5·50±0·65[c]	2·40±0·17[e]	4·30±0·41[e]	1·80±0·30[e]
	18	5·40±0·17[d]	3·00±0·22[e]	9·80±0·34[c]	1·80±0·14[e]
300	6	3·60±0·17[e]	1·10±0·10[e]	2·30±0·22[e]	1·00±0·27[e]
	12	7·10±0·74[e]	2·00±0·31[e]	3·40±0·55[e]	1·50±0·28[e]
	18	7·40±0·35[e]	3·00±0·15[e]	7·70±0·49[e]	1·90±0·24[e]

* Mean ± S.E. Number of rats per group is 12 in controls and 10 in all other cases.
Versus respective control P values : $a < 0·001$; $c < 0·02$; $d < 0·05$; $e > 0·05$.

TABLE II

Effect of benzene extract of Artobotrys odoratissimus *Linn. on duration of various stages of oestrous cycle in rats*

Dose mg/kg body weight/ day	Treatment period (days)	Duration of stages of the oestrous cycle in days			
		Diestrus	Proestrus	Estrus	Metaestrus
Controls vehicle only	6	3·50±0·20*	1·00±0·0	2·58±0·15	0·91±0·08
	12	6·16±0·21	1·91±0·08	4·66±0·23	1·25±0·13
	18	7·33±0·23	3·25±0·13	7·25±0·13	2·16±0·21
75	6	5·70±0·56[a]	0·70±0·16[e]	1·20±0·30[a]	0·40±0·17[a]
	12	8·80±1·10[b]	1·40±0·32[e]	2·60±0·67[a]	1·20±0·34[e]
	18	12·70±0·87[a]	1·90±0·15[a]	4·10±0·53[c]	1·30±0·16[d]
150	6	6·80±0·53[a]	0·30±0·16[a]	0·70±0·31[a]	0·20±0·14[a]
	12	9·80±1·05[a]	1·10±0·29[e]	2·20±0·53[a]	0·90±0·29[e]
	18	10·90±1·19[a]	2·60±0·35[e]	5·20±0·75[a]	1·30±0·27[d]
300	6	6·60±0·61[a]	0·40±0·17[a]	0·60±0·28[a]	0·40±0·17[a]
	12	7·30±0·52[e]	2·00±0·15[e]	3·50±0·32[d]	1·20±0·21[e]
	18	13·90±0·61[a]	1·60±0·17[a]	3·40±0·42[a]	1·10±0·24[a]

* Mean ± S.E. Number of rats per group is 12 in controls and 10 in all other cases.
Versus respective control P values ; $a < 0·001$; $b < 0·01$; $c < 0·02$; $d < 0·05$; $e > 0·05$

Dose of 300 mg/kg is not effective (*vs.* control $P > 0{\cdot}05$), *see* Table I.

(2) *Benzene extract*

Table II epitomized the effect of benzene extract of *A. odoratissimus* Linn. on duration of oestrous stages in rats. It provoked an orderly change in the periodicity of oestrous cycle stages. The length of diestrus stage has prolonged significantly at almost every dose and duration (*vs.* respective control $P < 0{\cdot}001$ and $< 0{\cdot}01$) except the dose 300 mg/kg at 12 days level where this increase is insignificant (*vs.* control $P > 0{\cdot}05$). Every dose is highly effective in the prolongation of diestrus stage when administered for 6 days (*vs.* respective control $P < 0{\cdot}001$) wherein dose 150 mg/kg showed maximum effectiveness. Highest degree in the prolongation of diestrus stage caused significant and insignificant suppression in the duration of other stages proestrus, estrus and metaestrus.

Discussion

The effect of 50% ethanolic and benzene extracts of *A. odoratissimus* Linn. (fresh green leaves) has been studied on the duration of oestrous cycle in adult rats. 50% ethanolic extract has prolonged the period of diestrus phase and estrus phase whereas benzene extract increased the periodicity of diestrus stage significantly (Tables I and II).

Stages of oestrous cycle and their interconversions are governed by the synthesis of ovarian hormones which are in turn controlled by the secretion of pituitary gonadotropins and hypothalmic-releasing factors[11]. Cornification of the vagina in the oestrous cycle has been found to be caused by estrogen[12]. Vaginal cornification has been induced when estrogen is applied to spayed female mice[13,14], rats[15] and immature rats[16]. In the present experiment, 50% ethanolic extract has prolonged the period of estrus phase significantly when administered at 150 mg/kg for 18 days (Table I)

Inhibition of cornification of the vaginal epithelium of ovariectomized rodents administered estrogen is a most important criterion to detect the antiestrogenic nature of a compound[11]. Oral administration of an antiestrogenic compound to cyclic rats resulted in the cessation of the cycle in the diestrus stage[17,18] and decrease the per cent of cornified cells in the vaginal smear of the cyclic monkeys[18]. 50% ethanolic extract of *A. odoratissimus* Linn. in this investigation has increased the period of leucocytic phase significantly when administered at 75 and 150 mg/kg for 18 and 6 days respectively (Table I). Similarly, benzene extract has extended the period of diestrus stage at almost every dose significantly (Table II). It is very interesting to note that prolongation in the diestrus phase of the treated rat is not continuous for longer days (6-7) days in majority of the rats but still other rats exhibited a continuous diestrus stage, from day 1 to day 18 of treatment[10], which clearly ruled out the idea of pseudopregnancy wherein the diestrus stage is continued for 12–14 days in rats. Moreover, the effect of extracts depends upon the dose and the pericd of treatment. Every dose of benzene extract is highly significant in the prolongation of diestrus stage when administered for 6 days (Table II).

Increase in the length of diestrus and estrus stages of the vaginal smear under the influence of 50% ethanolic extract adumbrate its weak estrogenic and antiestrogenic nature whereas the benzene extract suggests its antiestrogenic nature, characterized by an increase in the period of leucocytic stage. But it would be unjustified to conclude the estrogenic and antiestrogenic nature of the extracts merely on the basis of vaginal smear record and without some other classical biological indices. The exact mode of action of these extracts is yet to be elucidated.

Furthermore, the chemical study of its various fractions is under progress to evaluate whether the antifertility activity of *A. odoratissimus* Linn. (fresh green leaves) is due to the chemical compounds like artobotryn and snaveolin, isolated by Santosh and Ryes[19], or due to some other active ingredients present in the crude extracts of this plant.

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DIFFERENTIAL EFFECTS OF ULTRASONICATION ON THE MYOFIBRILLAR AND MITOCHONDRIAL ATPase ACTIVITY OF "SLOW" AND "FAST" MUSCLES IN BIRDS AND MAMMALS

C. L. TALESARA AND VASDEV NARANG

Muscle Physiology and Histochemistry Unit, Department of Zoology, University of Delhi, Delhi–110007

STUDIES on myosin from fast (white) and slow (red) muscles of the same species have revealed strong evidence for their marked structural and physiological differences[1]. It has been reported that myofibrillar-ATPase activity increases on ultrasonication of the skeletal muscle myofibrils[2]. There are no particular data available which indicate the differential pattern of the effect of ultrasonication on myofibrillar and mitochondrial ATPase activity in different types of muscles in different animal species. Hence, in the present study, experiments were carried out on the heart, pectoralis and gastrocnemius muscles of the common blue rock pigeon (*Columba livia*), and on the heart, pectoralis and soleus muscles of the adult albino rat (*Rattus rattus*). The heart and soleus muscles represent the "slow" type and pectoralis and gastrocnemius the "fast" type. The differential effects of ultrasonication on both types of muscles in the two species are compared and their implications discussed.

The individual muscles were carefully dissected from the animals and stored in beakers, chilled in ice. The myofibrils were obtained basically by the method of Perry and Grey[3], and the mitochondrial fraction was isolated from the supernatant fraction by using the conventional centrifugation method. The myofibrillar and mitochondrial–ATPase activities were measured with and without ultrasonic treatment, the details regarding the ultrasonication method followed are described earlier[2]. In both (myofibrillar and mitochondrial) fractions Ca^{++} as well as Mg^{++}-activated–ATPase assays were carried out. ATPase assasy were carried out at pH 7·5 and 37° C. In the Ca^{++}–ATPase assay incubation medium, 40 mM Tris-HCl, 40 mM KCl, 10 mM $CaCl_2$, 0·2 ml of myofibrils/mitochondria and 3 mM of ATP were used, whereas in Mg^{++}–ATPase assay the concentration of $CaCl_2$ was 0·2 mM and in addition to this 3 mM of $MgSO_4$ solution was used, the rest of the reactants were the same in both the assays. The final volume of the reactants in both assays (Ca^{++} and Mg^{++}-activated) was 1·5 ml. The reaction was started with the addition of ATP and was stopped by the addition of 1·5 ml of 10% trichloroacetic acid. The amount of Pi liberated and protein content present were measured by the methods of Rockstein and Herron[4] and Gornall *et al.*[5] respectively. All the values of specific-ATPase activity were expressed as μ moles of Pi liberated/mg protein/min.

On the basis of results, it is clearly indicated (Table I) that myofibrillar–ATPase activity of skeletal muscles increases on ultrasonication, which is consistent with our earlier work[2]. This table also shows that the effect of ultrasonication is well marked in "fast" muscles (pectoralis major and gastrocnemius) as compared with that of the "slow" muscle (heart) in pigeon. There is almost no effect of ultrasonication on the mitochondrial–ATPase activity of the three muscles studied.

Table II shows the same pattern of results in rat muscles. Here the change in myofibrillar–ATPase activity of "fast" muscle (pectoralis major) is much more as compared to the change in "slow" muscles (heart and soleus) after ultrasonic irradiation.