

HIBISCUS ROSA-SINENSIS LINN: ITS EFFECT ON β -GLUCURONIDASE IN THE UTERUS OF OVARIECTOMIZED RATS

A. O. PRAKASH, SANGEETA SHUKLA and R. MATHUR
School of Studies in Zoology, Jiwaji University, Gwalior 474011, India

ABSTRACT

The effect of ethanolic (50%) and benzene extracts of *Hibiscus rosa-sinensis* Linn has been studied on the activity of β -glucuronidase enzyme in the uterus of rats. The administration of extracts alone increased the enzyme activity marginally in the ovariectomized rats, however, in intact animals the activity was decreased. When the extracts were administered in combination with estradiol dipropionate, a significant decrease in the activity was observed. Their combined treatment with progesterone did not show significant change as compared to progesterone alone treated group. Similarly these extracts when applied to estradiol plus progesterone-treated rats, no significant change was observed over the combined effect of estradiol and progesterone. The decrease in the activity of the enzyme is attributed to estrogen antagonistic action of the extracts.

INTRODUCTION

FLOWERS of *Hibiscus rosa-sinensis* Linn have been reported to possess significant antifertility activity in female albino rats¹⁻⁵. The ethanolic and benzene extracts of these flowers reduce significantly uterine glycogen contents of adult rats⁶ and have significant antiestrogenic activity.⁷ β -glucuronidase is a lysosomal enzyme and has been reported to be sensitive towards estrogenic action⁸. In view of the antiestrogenic activity of the extracts of *H. rosa-sinensis*⁷, the present study is concerned with the effect of the ethanolic (50%) and benzene extracts on the activity of β -glucuronidase enzyme in uterus of ovariectomized rats.

MATERIALS AND METHODS

The collection of flowers of *H. rosa-sinensis* Linn and their extracts were prepared in 50% ethanol and benzene separately as described earlier⁹. A standard dose of 200 mg/kg body wt of each extract was prepared and administered orally as this dose has been reported as minimum effective dose (MED) in adult rats³⁻⁴. Similarly estradiol dipropionate (EDP) and progesterone (P) were prepared in olive oil and administered subcutaneously.

Colony bred female adult albino rats (160 \pm 10 g) maintained under uniform husbandry conditions were selected for the present study. These were ovariectomized under light ether anaesthesia and after 16 days these were divided into different groups of 6 each and were treated differently as shown in table 1. In addition

to these ovariectomized rats, 3 sets of five each of intact rats were also taken. One set served as intact control and two sets were considered for extracts treatment as shown in table 1. After 24 hr of the last treatment, the rats were killed. Both the uterine horns from each of the rats were excised, freed from adhering tissues and weighed quickly nearest to 1 mg. The weighed tissues were homogenised and the activity of β -glucuronidase was determined biochemically¹⁰. The results were statistically analysed using analysis of variance and (standard error of difference) S.Ed was considered to calculate the level of significance.

RESULTS

When the cyclic intact rats were ovariectomized, there was a significant decrease in the activity of β -glucuronidase in the uterus (table 1). The results also showed that estradiol dipropionate (EDP) when administered to ovariectomized rats, the activity of β -glucuronidase was increased significantly (group 3 vs 2 $P < 0.001$), however, progesterone (P) did not provoke any significant change (group 4 vs 2 $P > 0.05$). The combined treatment of EDP and P to ovariectomized rats decreased significantly the EDP induced increase in the β -glucuronidase activity (group 5 vs 3 $P < 0.001$). Both ethanolic (50%) and benzene extracts when applied to intact rats, decreased significantly the enzymic activity (groups 6 and 11 vs 1 $P < 0.02$); however, the administration of these extracts to ovariectomized rats increased the activity of β -glucuronidase enzyme (group 7 and 12 vs 2 $P < 0.02$). Furthermore, the administration of these extracts to

Table 1 Effect of 50% ethanolic and benzene extracts of *Hibiscus rosa-sinensis* Linn. on the activity of β -glucuronidase enzyme in the uterus of ovariectomized (ovx) rats. (Values are mean \pm S.E. Number of rats used are in parentheses)

Group No.	Treatment*	Uterine weight (mg/100g body wt)	μ g phenolphthalein released/mg tissue/hr	Statistical significance with respect to enzymic activity
1.	Intact control at diestrus stage	108.2 \pm 7.5	6.3 \pm 0.3 (5)	
2.	OVX control (vehicle only)	75.5 \pm 4.7	3.6 \pm 0.2 (6)	Vs 1 < 0.02
3.	OVX + EDP (SC) 1 μ g/rat/day for 7 days	265.6 \pm 6.8	12.9 \pm 1.1 (6)	Vs 2 < 0.001
4.	OVX + P (SC) 4 mg/rat/day for 6 days	135.7 \pm 8.3	4.3 \pm 0.3 (6)	Vs 2 > 0.05
5.	OVX + EDP + P (EDP & P as in groups 3 and 4).	210.5 \pm 6.7	6.4 \pm 0.5 (6)	Vs 3 < 0.001
A. 50% ethanolic extract				
6.	Intact + extract (oral) 200 mg/kg body wt/day for 5 days in the end	92.5 \pm 5.1	4.1 \pm 0.3 (5)	Vs 1 < 0.05
7.	OVX + extract (oral) 200 mg/kg body wt/day for 5 days in the end	103.5 \pm 8.3	5.1 \pm 0.4 (6)	Vs 2 > 0.05
8.	OVX + extract + EDP (extract and EDP as in groups 7 and 3)	175.5 \pm 11.6	7.3 \pm 0.6 (6)	Vs 2 < 0.02 Vs 3 < 0.05
9.	OVX + extract + P (extract and P as in groups 7 and 4)	127.1 \pm 7.1	4.9 \pm 0.3 (6)	Vs 4 > 0.05 Vs 7 > 0.05
10.	OVX + extract + EDP + P (extracts, EDP and P as in groups 7, 3 and 4)	203.2 \pm 13.1	6.9 \pm 0.4 (6)	Vs 5 > 0.05 Vs 7 > 0.05
B. Benzene extract				
11.	Intact + extract (Oral) 200 mg/kg body wt/day for 5 days in the end.	89.4 \pm 5.3	3.8 \pm 0.2 (5)	Vs 1 < 0.05
12.	OVX + extract(Oral) 200 mg/kg body wt/day for 5 days in the end.	110.5 \pm 8.8	5.4 \pm 0.3 (6)	Vs 2 > 0.05
13.	OVX + extract + EDP (extract and EDP as in groups 12 and 3)	163.3 \pm 9.2	6.8 \pm 0.4 (6)	Vs 3 < 0.01 Vs 12 > 0.05
14.	OVX + extract + P (extract and P as in groups 12 and 4)	148.4 \pm 10.6	5.4 \pm 0.3 (6)	Vs 4 > 0.05 Vs 12 > 0.05
15.	OVX + extract + EDP + P (extract, EDP and P as in groups 12, 3 and 4)	201.6 \pm 12.8	6.6 \pm 0.4 (6)	Vs 5 > 0.05 Vs 12 > 0.05

* Treatment (EDP = estradiol dipropionate; P = progesterone; Ext = extract).

ovariectomized rats significantly prevented the increase in enzymic activity induced by EDP (group 8 and 13 vs 3 $P < 0.001$); however, no significant change was observed as already induced by progesterone (groups 9 and 14 vs 4 $P > 0.05$).

ON

The presence of various lysosomal enzymes including β -glucuronidase in the uterus of adult rats has been studied by many authors¹¹⁻¹³. The activity of β -

β -glucuronidase enzyme is reduced significantly in the uterus of ovariectomized rats⁸, indicating its dependence on ovarian hormones, estrogen and progesterone. Later it was also reported that exogenous treatment of estrogen to ovariectomized rats restored the activity of the enzyme which was lost after ovariectomy¹⁴. Furthermore on the basis of histochemical studies it was seen that the activity of β -glucuronidase was reduced after ovariectomy but estrogen treatment restored the activity¹⁵. We also report in the present paper that ovariectomy leads to a significant decrease in the activity of β -glucuronidase in the uterus which is further restored with the administration of estradiol dipropionate; however progesterone does not seem to play any significant role. While describing the mechanism of action of estrogen and progesterone in relation to uterine β -glucuronidase activity it has been stated that though estrogen restored the activity of β -glucuronidase in the uterus of ovariectomized rats the effect is partially counteracted by the progesterone¹⁶. It is also observed that progesterone antagonized the β -glucuronidase activity induced by estradiol-17 β in ovariectomized mice¹⁷.

The present investigation reveals that both the extracts of *H. rosa-sinensis* Linn when administered *per se* do not affect the activity of β -glucuronidase in the uterus of ovariectomized rats but when these extracts are administered to EDP-induced rats, both extracts significantly reduced the enzymic activity when compared to EDP-induced groups. However, progesterone could not interfere in the process at any of the stages. These observations reveal that both these extracts antagonise the estrogenic action. Therefore, the decrease in activity of β -glucuronidase enzyme in the estradiol dipropionate induced rats may be due to their antiestrogenic activity. It is well established that lysosomal enzymes including β -glucuronidase are involved in the preparatory changes in the uterus for implantation¹⁸. High level of estrogen stimulates and enhances these preparatory changes. Therefore, it is expected that the antiestrogenic nature of the extracts of *H. rosa-sinensis* Linn decreases the activity of

β -glucuronidase enzyme which further interrupts the formation of implantation bed and thus the fertilized eggs are passed through the uterus without attachment.

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