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EFFECT OF AZADIRACHTA INDICA LEAF EXTRACT ON OESTROUS CYCLE OF RATS

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ABSTRACT: *Effect of Azadirachta indica leaf extracts on duration of oestrous cycle of adult rats was studied. The extract (200 mg/kg) induced cornified phase of the cycle which persisted till the last day of treatment. Possible reasons behind the results are discussed.*

INTRODUCTION

Greater emphasis is being laid in recent years to find out safe and effective drugs to control human fertility in view of increasing pressure of population. Apart from findings of effective chemical drugs, plant drugs of folklore medicine are also watched carefully and extensively for their possible efficacy in this respect. Ethnopharmacol uses of *Azadirachta indica* (Meliaceae; Neem) leaves on oral antifertility and antiimplantation activity have been reported from different geographically distant areas[1-3]. It is well documented that antifertility agents acting on the ovarian-uterine axis certainly provoke changes in the pattern of reproductive cycles along with bio-chemical changes in uterine tissue[4-5]. Although antiimplantation and oral antifertility activity of A.indica leaves have been reported by several workers but its effect on the periodicity of oestrous cycle seems to be dubious. The present investigation has therefore been designed to study the same with a view to find out the possible hormonal dependent effect of *A.indica* leaf extract on the duration of various phases of the oestrous cycle in adult intact rats.

MATERIALS AND METHODS

Fresh matured leaves of *A.indica* were collected locally during Sept-Oct., 1992 and was botanically identified from the University of Kalyani, India. The air dried powder of the leaves of *A.indica* (1 Kg) was extracted by percolation at room temperature with 70 percent ethyl alcohol. The extract was concentrated under reduced pressure (bath temperature 50^0C) and finally dried in a vacuum desicator. The residue of *A.indica* (yield = 100g) was dissolved in propylene glycol at a concentration of 100 mg/ml and was used in experiments.

Colony bred Swiss adult female rats (6 – 8 weeks old), weighing 150 ± 10 g were maintained under controlled conditions of light (14h/24h) and temperature (23 ± 1^0C). Food pellets (Hindustan Lever Ltd., Bombay) and tap water was provided *ad libitum.* Animals showing three normal oestrous cycles were taken and *A.indica* leaf extract (200 mg/kg/day) was administered orally with the help of intragastric catheter to three different groups of rats for 6 days (1 complete cycle), 12 days (2 complete cycles) and 18 days (3 complete cycles) to different batches of animals of 6 each. Each

group having its own control that received equal volume of propylene glycol in peace of leaf extract and treated similarly. Vaginal smear using normal saline of each rat was recorded daily at a regular interval of 24h. At the end of the experiment, the record of the different stages of the oestrous cycle of each rat was analysed. As the majority of rats depicted prolongation in the estrus phase of vaginal smear, the animals from each group were sacrificed in estrus stage at 48^{th} after the last dose i.e. on 8^{th}, 14^{th} and 20 day respectively. Rats showing stages other than estrus were rejected. From the sacrified animal the uteri were dissected out, freed from adjacent mesentery and weighed. Suitable amount of each uterus was taken, weighed and biochemically analysed for glycogen (6), protein (7), acid and alkaline phosphatase activity (8).

Results were statistically analysed using students 't' test.

RESULTS

Table 1 shows that *A.indica* leaf extract (200 mg/kg) significantly lengthened the cornified phase of the oestrous cycle with a gradual increase from 6 to 18 days of treatment with simultaneous suppression in the duration of other stages like metaestrus and diestrus, proestrus phase was completely abolished.

Table 2 shows that *A.indica* leaf extract elicited a significant increase in the uterine glycogen and protein level in 200 mg/kg dose for 18 days. However, the uterine acid and alkaline phosphatase activity decreased significantly.

DISCUSSION

The changes in the vagina of normal animals believed to be due to fluctuations and interconversions of the female sex hormones, estrogen and progesterone, mainly synthesized in the ovary. The lever of these hormones is controlled by pituitary gonatotrophins and hypothalamic releasing hormones. The cornification in the vagina is mainly due to the level of stimulation of estrogen which acts directly on vaginal epithelium (9). In the present investigation *A.indica* leaf extract induced the cornified stage which persisted till the last day of treatment and points towards the possibility of estrogenic nature of the leaf extract.

Biochemical analysis revealed that *A.indica* leaf extract increased the uterine glycogen and protein level and decreased the acid and alkaline phosphatase activity significantly throughout experiments and corroborates the findings of Dutta et al (10) where same results were obtained after the injection.

Thus induction of persistence of cornification in the oestrous cycle due to the treatment of *A.indicia* leaf extract may be accounted for its estrogenic nature. This preliminary report may serve as the foot step on this aspect.

TABLE – 1

Effect of *A.indica* leaf extract on duration of various stages of oestrous cycle in rate

Duration of treatment (days)	Duration of stages of oestrous cycle in days (Mean ± S.E)			
	Proestrus	**Estrus**	**Metaestrus**	**Diestrus**
6	1.15 ± 0.12 (0.00 ± 0.00)	2.45 ± 0.30 (6.15 ± 0.32)	1.00 ± 0.15 (0.60 ± 0.16)	3.40 ± 0.12 (1.20 ± 0.15)
12	1.74 ± 0.25 (0.00 ± 0.00)	4.10 ± 0.22 (11.00 ± 0.65)	1.80 ± 0.25 (0.70 ± 0.09)	6.35 ± 0.42 (2.25 ± 0.15)
18	2.58 ± 0.36 (0.00 ± 0.00)	6.65 ± 0.63 (16.15 ± 0.92)	2.64 ± 0.66 (0.80 ± 0.12)	8.12 ± 0.32 (3.00 ± 0.16)

Values in the parenthesis indicate the values of the extract treated animals. N = 6. Animals were sacrificed 48h after last treatment and hence 2 additional days have been included in the analytical data.

TABLE – 2

Uterine glycogen, protein, acid and alkaline phosphatase level of *A.indica* leaf extract treated rats.

Parameter	Group	DAYS		
		6	**12**	**18**
Glycogen	Control	25.64 ± 1.25	27.02 ± 1.55	26.44 ± 1.17
(Micromoles glucose equivalent /g Tissue)	Treated	30.47 ± 2.12	37.64 ± 2.05**	41.04 ± 2.45***
Protein	Control	3.50 ± 0.02	3.72 ± 0.03	3.46 ± 0.02
(mg/g tissue)	Treated	3.74 ± 0.02***	4.87 ± 0.01***	6.25 ± 0.03 ***
Acid phosphatase	Control	2.15 ± 0.04	2.12 ± 0.02	2.16 ± 0.01

(K.A.U/G Tissue)	Treated	2.00 ± 0.03*	1.66 ± 0.02***	1.42 ± 0.02 ***
Alkaline phosphatase	Control	2.35 ± 0.01	2.32 ± 0.03	2.30 ± 0.02
(K.A.U / G Tissue)	Treated	2.05 ± 0.02 ***	1.72 ± 0.02 ***	1.55 ± 0.01***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ n=6

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