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EXPERIMENTAL STUDIES ON *ARTEMISIA VULGARIS* – A POSSIBLE ANTIFERTILITY DRUG

A. NARWARIA[1], R.L. KHOSA[1] and S.K. DHAR[1]

Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi 221 005[1], India.

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ABSTRACT: *The effect of alcoholic extract of the aerial parts of Artemisia vulgaris Linn on estrous cycle and implantation, in female albino rats, was studied. The drug induced an irregular estrous cycle with random disappearance of estrous phase and increase in the number of metestrus phases within the estrous cycle, observed for the total test period of 18 days. It exhibited 80% anti-implantation activity without showing any gross malformations in pups delivered, possibly due to its non-toxic nature even at the high dose of 3000 mg/kg p.o. These facts suggest the drug to possess some antifertility effect.*

INTRODUCTION

Artemisia vulgaris Linn.; family – Compositae, commonly known in Hindi as *Nagadouna*[1], and in Tamil as *Mashibattiri* or *Machipatri* is used in the Indian system of medicine as an emmenagogue, antihelmintic, antiseptic, antispasmodic, stomachic and in the treatment of respiratory and nervous diseases[1]. Some chemical work on this plant is already on record[2,3,4]. In the present study the alcoholic extract of the aerial parts of the plant was tested for its antifertility effect by studying its effect on the estrous cycle and implantation in female albino rats.

MATERIALS AND METHODS

Sample preparation: Properly identified plants of *Artemisia vulgaris* Linn. Collected from Ranikhet region of U.P. (India) were air dried, powdered coarsely, defatted with petroleum ether (60 – 80^{0}C), and then thoroughly extracted with alcohol in a soxhlet apparatus. The extract was dried and made into a suspension with distilled water using 0.5% carbpxymethyl cellulose as suspending agent.

Study of the effect of the drug on estrous cycle: Eight adult, healthy, female albino rats of Charles. Foster strain weighing between 150 – 160 g were acclimatized to animal house conditions for a week before starting the experiment. All rats were fed on standard pellet diet (Lipton India Ltd.) and water *ad libitum*. The rats were divided into two groups (Test groups and the Control group), each group containing a set of four animals. Suspension of the alcoholic extract (75 mg/kg) was fed orally through a rubber catheter once daily throughout the duration of the experiment (18 days) to the test animals individually whereas the animals of the control group received distilled water (1 ml) containing carboxymethyl cellulose (0.5%) as a placebo. The changes in the various phases within the estrous cycle were carefully monitored by vaginal smear

method[5], and observations presented in Table I.

TABLE – I

Occurrence of different phases in estrous cycle observed for all the animals in control and test group, for the total test period of 18 days

Phase	In control group	In test group	Disappearance Index*
Estrous	22	08	+63.64
Metestrus	07	15	-114.28
Diestrus	28	35	-25.00
Proestrus	15	14	+6.67

$$\text{*Disappearance Index of a particular phase} = 100 - \frac{\text{(Number of times a particular phase within the estrouscycle was observed for all the test animals during total duration of the experiment)}}{\text{(Number of times the same phase was observed all the control animals during the same duration)}} \times 100$$

Monitoring the effect of the drug on implantation: Adult albino rats of Charles Foster strain (150 – 160 g), acclimatized to animal house conditions for a week before strating the experiments, were fed a standard pellet diet (Lipton India Ltd) and water *ad. Libitum.* The estrous cycle in female rats was monitored by the vaginal smear method[5], and animals were kept in polypropylene cages with male rats of known fertility on the evening of proestrus. Presence of copious spermatozoa in the vaginal smear taken the following morning was considered as day 1, of pregnancy. The female rats were then divided into control and treated groups having five rats in each group. The test samples in the concentrations of 400 mg/kg p.o. and 800 mg/kg p.o. were administered to the pregnant rats, through a rubber catheter, once a day from day 1 to day 10 of pregnancy. The control group received 1 ml of distilled water containing 0.5% CMC. Laparotomy was performed on day 11 under light ether anesthesia and the number of implantation sites in the uterine horns was recorded. Any animal with, at least, one normal foetus was considered as pregnant. Following the operation, the animals were sutured as usual and were returned to their respective cages. After parturition, if it occurred, the number of litters was counted. The delivered pups were observed for, at least, one month for any gross malformations. The results are presented in Table II.

TABLE – II

Effect of alcoholic extract of *Artemisia vulgaris* Linn, on implantation in albino rats

Sample	Dose mg/kg p.o	Total number of rats used	Number of rats without implantation	% Activity
Control	-	5	0	0.0
Alcoholic extract of *A. vulgaris* Linn	400	5	4	80.0
	800	5	4	80.00

Determination of estrogenic and anti-estrogenic activities of the drug :

Immature female albino rats, each weighing 40 – 45g, were ovariectomized one week prior to the date of experiment. The rats were then divided into the following groups comprising five rats each:

Group I : Treated with oestradio valereate (in groundnut oil) 0.1 µg/rat/day s.c

Group II : Treated with 0.05 ml of the oil only/rat/day s.c. (control).

Group III : Treated with test sample 400 mg / kg p.o

Group IV : Treated with oestradiol valereate 0.1 µg/rat/day in oil s.c. and test sample 400 mg/kg p.o

The treatment was given for three day ; 24 hours after the last treatment the rats were sacrificed, uteri were exercised quickly, cleared of the adhering tissues and weighed. The observations are presented in Table III.

TABLE – III

Weight of uteri of rats* in control and treated groups

S. No.	Treatment	Dose weight in grams	Mean body in mg (mean ± S.E)	Weight of uterus
1	Oil only	0.5 ml/rat/day	42.0	56.4 ± 1.2
2	Oestradiol valereate	0.1 µg/rat/day s.c.	41.0	308.7 ± 5.4
3	Test sample	400 mg/kg p.o	40.5	116.5 ± 5.9
4	Oestradiol valereate and test sample	0.1 µg/rat/day s.c and 400 mg/kg p.o	42.5	295.2 ± 6.8

* Number of animals used for each group in five

Determination of acute toxicity of the drug: 20 albino mice of either sex weighing 16-18 g each were randomly divided into control and treated groups having 5 animals

in each group. Test sample was administered to separate sets of mice in doses of 1000 mg, 2000 mg and 3000 mg per kg of body weight p.o. The control group received the vehicle only. The animals were observed for next 48 hours for mortality or any manifestation of signs of toxic symptoms.

RESULTS AND DISCUSSION

In rats, the estrous cycle extends for a period of about 5 days with its different phases changing over from estrous (9 – 15 hours) – mestestrus (10 – 14 hours) – diestrus (60 – 70 hours) to proestrus (9 – 12 hours) phase, estrous phase only being the heat period. While the animals of the control groups by and large, exhibited a normal estrous cycle, the animals of the test group showed a distorted picture. There was an appreciable increase in the number of metestrus and diestrus phases, little reduction in the number of proestrus phase but conspicuous absence of the estrous phase within the estrous cycle of the test group of animals, observed for the total test period of 18 days. The disappearance index of estrous, metestrus, diestrus and proestrus phases in test animals was about +63, -144, -25 and +7 respectively showing conspicuous absence of estrous phase during the test period. Since estrous phase within the estrous cycle is the only phase which permits mating of the animals, its continued absence in the test group animals could possibly suggest it to exhibit some antifertility effect.

Date presented in Table II indicates that the test samples exhibit a good degree of anti-implantation activity (80%) in the albino rats with no gross malformations having been observed in the pups. In acute toxicity studies no toxic symptoms or mortality were observed in mice upto 48 hours even at the high dose level of 3000 mg/kg p.o., which suggest that, LD_{50} value of the test sample to be more than 3000 mg/kg p.o.

The test sample shows a little estrogenic activity but is devoid of any anti-estrogenic activity (Table III). Further work is in progress.

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