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INVESTIGATION ON FAGONIA CRETICA –ITS EFFECT ON HORMONAL PROFILE AND IMMUNOMODULATION IN RATS

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***ABSTRACT**: The effect of alcoholic extracts of the aerial parts of fagonia cretica on estrous cycle and implantation in female albino rats was studied, It induced random omission of heat phase in eh estrous cycle and exhibited 100% anti implantation activity without exhibiting any toxic manifestation, However it was found to have some strong androgenic antiprogestational activity, and immunostimulating property.*

INTRODUCTION

Fagonia cretica (Family-Zygophy-llaceae) commonly known in Hindi as Damahan and in Sanskrit as Dusparsha is used in the Indian system of medicine as diuretic1, astringent1, in the treatment of asthma, tumours, urinary disorders, and as an emmenagogue2. Some chemical work on the plant has been reported 3,4,5. The present study deals with the effect of the alcoholic extract of the aerial parts of the plant on estrous cycle, hormonal profile and immunomodulation in mice.

MATERIALS AND METHODS

Sample Preparation: Properly identified specimens of fagonia cretica, (aerial parts) were powdered and defatted with petroleum ether (60^o-80^o). After thoroughly extracting with alcohol in a soxhlet apparatus, the extract was dried and made into suspension with distilled water using carboxy methyl cellulose (CMC) (0.5%) as suspending agent.

Animals: Charles Foster albino rats of both sexes, inbred in the animal house, maintained in air conditioned room (25 $\pm 2^oC$) on a 14:10 hours light: dark cycle and the relative humidity of about 60% were used. They were fed on a standard pellet diet (Lipton Indian Ltd) with free access to water.

Effect on Estrous cycle: Eight adult healthy female rats (150-160g) were acclimatized to animal house condition for a week before starting of the experiment and divided into two groups (test group and control group) of 4 animals in each group. Suspension of alcoholic extract (100 mg/kg body weight) was fed orally through rubber catheter once daily throughout the duration of the experiment (18 days) to the test group of animals whereas the animals of the control group received distilled water containing 0.5% CMC as placebo. Changes in the phases of the estrous cycle were monitored carefully by vaginal smear method6 and the

disappearance indices of different phases were calculated[7] (Table I)

Effect on implantation: The female rats (150- 160 g each) were caged with male rats of known fertility on the evening of proestrus, the presence of copious spermatozoa in the vaginal smear in the following morning was counted as day 1 of the pregnancy. The suspension of the sample was administered to the pregnant rats orally from day 1 to day 10 of pregnancy. Laparotomy was per formed on day 11 under light ether anaesthesia and the number of implantation sits in the uterine horn was recorded. Animals with at least one normal foetus were considered as pregnant, the abdomen was sutured and the rats were returned to their cages, after parturition, the number of litters was counted the delivered pups were observed for at least, one month for any gross malformations. A group of pregnant rats which received vehicle only served as control. (Table II).

Estrogenic/Antiestrogenic activities:

These were determined on immature female rats (40-50g) animals were divided into four groups, each group comprising five animals. The various groups received the treatment as follows:

Group I: Oestradiol valerate 0.1µg/rat/day s.c in groundnut oil (0.05ml).

Group II: 0.05 ml of groundnut oil s.c

Group III: Suspension of drug sample 250mg/kg p.o

Group IV: Oestradiol valerate 0.1 µg/rat/day s.c in groundnut oil and suspension of sample 250 mg/kg p.o

The treatment was given for 3 days and 24 hours after the last treatment, the rats were sacrificed. The uteri excised, cleared of the adhering tissues and weighed. (Table III).

Progestational/ antiprogestational activities:

These were assessed by the pregnancy maintenance test in pregnant rats overiectomized on the 12th day of pregnancy[8] Overiectomized rats which bore normal implantation were regrouped into four groups of six rats in each group and treated according to the following schedule:

Group I: 0.05ml groundnut oil/rat days s.c

Group II: Progesterone 2 mg/rat/day s.c in groundnut oil.

Group III: Suspension of sample, 250 mg/kg p.o.

Group IV: Progesterone 2 mg/rat/day s.c in groundnut oil and sample suspension 250 mg/kg p.o.

Treatment was given from day 12 to day 19 of pregnancy. Autopsy of the rats was performed on day 20 and the number of live foetus was recorded. The results were expressed as the percentage of foetal survival. (Table IV).

$$\% \text{ Foetal Survival} = \frac{\text{No. of live foetus}}{\text{Total no. of implantation}} \times 100$$

Androgenic/ Antiandrogenic activities:

These were assessed in immature male rats (40-50g) divided into 4 groups of six rats in each, receiving the treatment as follows:

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Group I: 0.05ml groundnut oil s.c

Group II: Testosterone propionate 150 µg/rat/day s.c in groundnut oil.

Group III: Suspension of sample, 250 mg/kg p.o.

Group IV: Testosterone propionate150 µg/rat/day s.c in groundnut oil and sample suspension 250 mg/kg. p.o.

The Treatment was given for 7 days. The rats were sacrificed 24 hours after the last treatment. Ventral prostate and seminal vesicles were carefully removed from the animals and quickly weighted. The results are tabulated in table V.

Immunomodulation studies

For this, first the in vitro antibacterial effect of the suspension of the extract was per formed, the suspension was incubated with E.coli (3.8x108 CFU) in nutrient broth at the concentration of 1 mg 500 µg 250µg and 100 µg per ml.

After incubation at 37^{o} C, the cultures were diluted, uniformly plated on McConky Agar plates and the number of colonies was counted.

This was followed by conduction in Vivo studies on mice following, challenge by E.Coli. The suspension was administered to inbred mice (20-25g: 6-8 weeks) at a dose of 100 mg/kg once a day intra – peritonially for 7 days. After the last dose, a lethal dose of E.coli (3.8 x 108 CFU/mice) was administered and survival data was recorded (Table VI).

RESULTS AND DISCUSSION

It is evident from table I, that fagonia cretica induces as distortion in the regularity of the estrous cycle of the rats in which there is random omission of the heat period (estrous phase). Its disappearance index +53.33 which accounts for the reduction of the desire of the females to mate with makes. When administered in the dose of 250 mg/kg p.o it significantly acted as an anti-implantation agent (Table II).

Table III presents its estrogenic and antiestrogenic activities. The mean uterine weight of the control group was 54.85 ±1.2 mg/100gm body weight and estradiol valerate at dose of 0.1 µg/rat/day s.c in groundnut oil produced a very significant increase in the uterine weight compared to the control value. Sample suspension given by oral route at a dose of 250 mg/kg, failed to produce any significant change in the uterine weight indicating the absence of any estrogenic activity. Also, no significant change in the uterine weight was observed when sample suspension and estradiol valerate were given together as compared to the value obtained with estradiol valerate treatment alone, So any antiestrogenic activity was also ruled out.

The presence of any progestational activity is also lacking with sample suspension (Table IV) since the extract failed to sustain the pregnancy of he rats ovariectomized on day 12 of pregnancy. On the contrary the sample suspension seems to possess potent anti- progestational activity, since it nullified the effect of progesterone (Group IV). The foetal survival percent with progesterone is 91.1 whereas with the combination, it is 13.04% (Table IV).

The drug suspension has significant androgenic actively (Table V) as the weights of both seminal vesicles and ventral prostate in-creased in comparison to the control value. It does not seem to possess any

antiandrogenic activity as the values obtained by treatment with testosterone propionate were no significantly altered when the combination of the two was given.

The summarize suspension of alcoholic extract of fagonia cretica seems to possess strong antiprogestational activity with some androgenic activity.

The lack of in vitro antibacterial activity and the positive in vivo protection afforded to the host by the pretreatment with the sample suspension (Table Vi) suggest that the drug possesses possible immuno-boosting property.

Judging from the aforesaid results it is clear that fagonia cretica has good antifertility potential in as much as it possesses strong anti-implantation, anti-progestational and some degree of androgenic activity. Immunoboosting property might be due to action of the drug at the level of macrophage lymphocyte or compliment activation to enable the host to develop resistance to biological challenge, However it needs to be investigated further.

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Table – 1

Occurance of different phases in estrous cycle observed for all the animals in control and test group for the total period of 18 days following treatment with alcoholic extract of fagonia cretica.

Phase	Control group	Test Group	Disappearance Index
Estrus	15	7	+53.33
Metestrus	14	16	-14.29
Diestrus	30	34	-13.39
Proestrus	13	15	-15.38

Table – II

Anti-implantation activity of alcoholic extract of Fagonia cretica in rats

Extract	Dose mg/kg p.o	No. Pregnant rats/ Total No. of rats	No. of implantation sits (Mean)	% Activity
Control	-	4/4	9,7,9,8,(8.0)	0.0
Alcoholic extract of F. cretica	250	0/4	0,0,0,0(0.0)	100.00

Table – III

Estrogenic / Antiestrogenic activity of alcoholic extract of fagonia cretica in immature female rats.

S. No	Treatment	Dose	Body Weight (gm)	Weight of uterus (mg/100g body weight)
1.	Control	0.05 ml groundnut oil	42,45,40,40,43	54.35 ±1.20

2.	Oestradiol valerate	0.1µg/rat/day s.c	40,45,47,40,42	302.6 ± 6.14
3.	Suspension of alcoholic extract of F. cretica	250 mg/kg. p.o.	45,45,40,46,40	58.43 ± 1.6
4.	Oestradiol valerate sample suspension	0.1µg/rat/day s.c +250 mg/kg. p.o.	40,43,45,42,45	310.4 ± 5.6

Table – IV

Progestational/Anti-progestational activity of alcoholic extract of Fagonia cretica in pregnant female rats overiectomized on day 12 of pregnancy.

S.No	Treatment	Dose	No. of Implantation sits (mean)	No. of Live fetuses (mean)	Fetal survival %
1.	Control	-	8,9,9,10,9(9)	0.0	0
2.	Progesterone	2µg/rat/day s.c	10,9,10,8,8(9)	8,8,9,8,8 (8.2)	91.1
3.	Suspension of alcoholic extract of F. cretica	250 mg/kg. p.o.	8,8,10,11,9,(9.2)	0,0,0,0,0 (0)	0
4.	Progesterone + Sample suspension	2µg/rat/day s.c +250 mg/kg. p.o.	9,9,11,9,8 (9.2)	3,1,0,0,2 (1.2)	13.04

Table – V

Androgenic/Anti-androgenic activities of alcoholic extract of Fagonia cretica.

S.No	Treatment	Dose	Weight mg/100g body weight	
			Seminal vesicles	Ventral prostate
1.	Control	Oil Only	24.3 ± 0.4	30.4 ± 0.6
2.	Testosterone progesterone	150µg/rat s.c	189.6 ± 2.8	123.6 ± 0.4
3.	Suspension of alcoholic extract of F. cretica	250 mg/kg. p.o.	86.2 ± 1.7	65.1 ± 0.7
4.	Testosterone progesterone + Sample suspension	150 µg/rat s.c +250 mg/kg. p.o.	203.7 ± 2.3	127.3 ± 1.9

Table – VI

Immunoprotective effect of alcoholic extract of Fagonia cretica in mice against E.coli challenge

Fraction	Dose of E.coli	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Control	3.6×10^8 CFU/mice	4	4	0	-	-	-	-	-
Suspension of alcoholic extract of F. cretica	3.6×10^8 CFU/mice	4	4	4	4	4	4	4	4

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