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PERIODICITY OF ESTROUS CYCLE IN ALBINO RATS; RESPONSE TO SOME CRUDE DRUG COMBINATIONS

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ABSTRACT: *The extracts of bark, leaves and stem of A. indica, fruits of P. longum, berries of E. officinalis and seeds of G. indicum were prepared using different solvents. Three different combinations of these extracts were tried on the female albino rats for their effect on the estrous cycle. The combination consisting of alcoholic extracts of leaves and stem of A. indica, fruits of P. longum, berries of E. officinalis and seeds of G. indicum has exhibited considerable effect on estrous cycle by prolongation of diestrous phase.*

INTRODUCTION

Natural plant products are being used as contraceptives in Indian medicine since olden days. The antifertility activity of berries of *E. Ribes* (1 -3), fruits of *P. Longus* (4 – 6), aerial parts of *A. Indica* (7 – 9), (+) Gossypol (10) is reported earlier. The estrous cycle in mice (11) and rats (12, 13) has been exhaustively studied. The antifertility and antimotic effects of crude drug combinations have been reported earlier by the authors (14 – 17). The effect of *E. Officinalis* on cholesterol induced antherosclerosis in rabbits has been studied (18). Even though, the antifertility activity has been tested for the individual crude drugs, their effects in combinations have been rarely reported. Hence, in the present study an attempt is made to observe the effects of the combinations of crude drug extracts on the estrous cycle of female albino rats.

EXPERIMENTAL

Preparation of crude drugs combinations:

The ethanolic extracts of the powdered leaves, bark and stem of *Azadirachta indica* (Meliaceae), fruits of *Piper longum* (Piperaceae), berries of *Emblica offcinalis* (Euphorbiaceae) and seeds of *Gossypium indicum* (Malvaceae); methanolic extract of seeds of *G. indicum* ; benzene extract of leaves of *A. indica* and solventether extract of berries of *E. officinalis* were obtained by maceration technique. The pet. Ether (60 – 80^{o}C) extracts of powdered seeds of *G. indicum* and fruits of *P. longum* were prepared by successive solvent extraction. Different combination of the extracts were prepared as follows:

Combination (A) : Ethanolic extracts of leaves, stem and bark of *A. indica* in equal proportion; ethanolic extract of fruits of *P. longum* and methanolic extract of seeds of *G. indicom* (2 : 1 : 1).

Combination (B): Ethanolic extracts of leaves and stem of *A. indica* in equal proportion; ethanolic extract of fruits of *P. longum*, berries of *E. officinalis*, and seeds of *G. indicum* (1: 1: 1: 1).

Combination (C): Benzene extract of leaves of *A. indica;* solvent ether extract of berries of *E. officinalis;* pet. Ether extract of seeds of *G. indicum* and fruits of *P. longum* (1:1:1:1).

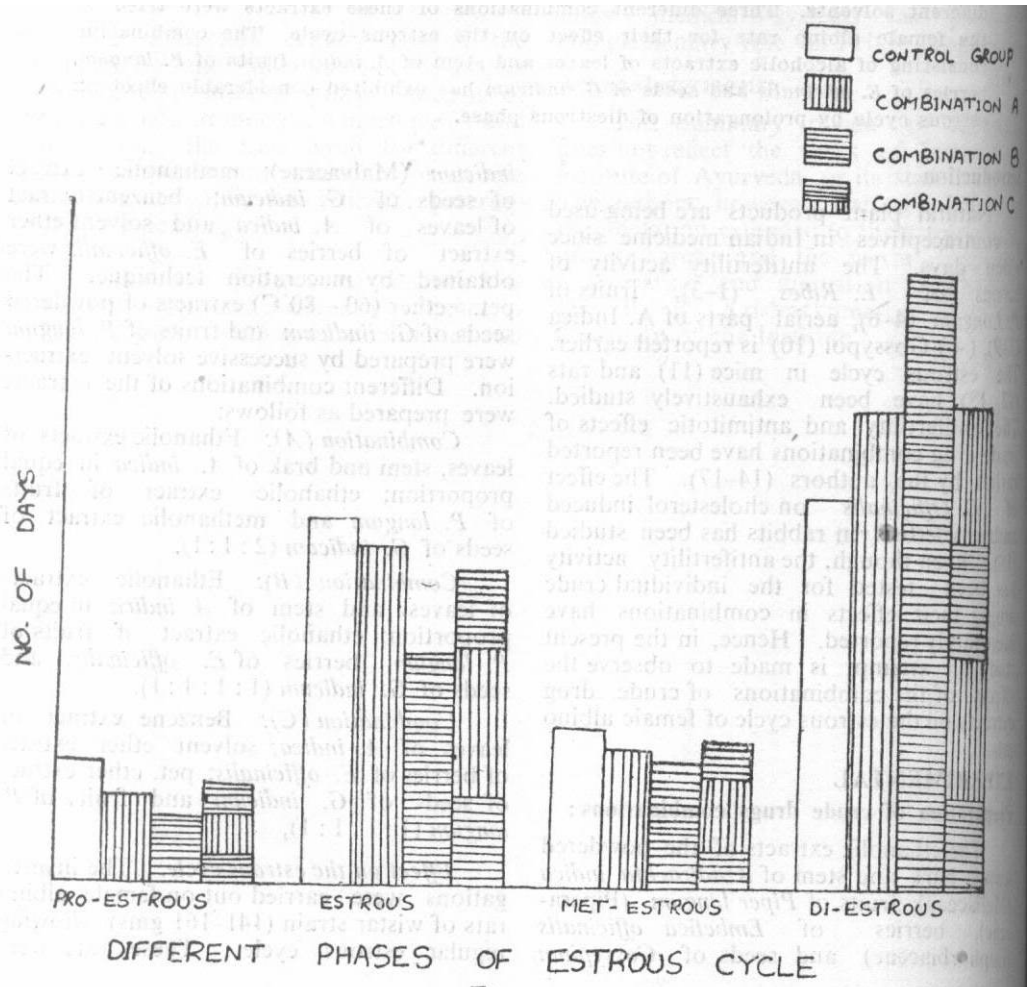
Effect on the estrous cycle: The investigations were carried out on female albino rats of wistar strain (141 – 161 gms) showing regular estrous cycle. These rats were grouped into four groups of 10 animals each, of which 3 groups were used for drug – treatment and the fourth group served as control. All the experiments were performed in triplicate at room temperature. The regularity of the estrous cycle was checked by taking the vaginal smears daily early in the morning, before drug – treatment. The vaginal smears were stained with Haematoxylin and Eosin and observed under microscope for various stages of the estrous cycle and its regular occurrence. The first three groups of rats with normal estrous cycle received the drug combinations (A), (B) and (C) orally at the dosage of 200 mg / kg in the form of suspension with 1% carboxymethyl cellulose (CMC) for 21 days, whereas the fourth group of rats received on 1% CMC (200 mg / kg).

From the very next day of the drug – treatment, the vaginal smears of the treated and control groups were observed under microscope to spot the different stages of estrous cycle. These observations have been made for 21 days to note four complete estrous cycles. The average period of each stage of estrous cycle was then computed.

RESULT AND DISCUSSION

The average duration of different phases of the estrous cycle is reported in Table – 1 and has seen show in Fig. 1. The combination has shown considerable effect on estrous cycle in terms of prolongation of diestrous phase of estrous cycle. The combinations (A) and (C) did not show any significant effect on any phase of estrous cycle. All the combinations, however, did not exhibit any adverse effect on the body weight of the animals.

Different stages of estrous cycle and their interconversions are governed by the synthesis of ovarian hormones, which are, in turn, controlled by the secretion of pituitary gonadotropins and hypothalamic releasing factors (19). Cornification of the vagina in the estrous cycle has been found to be caused by estrogen (20). Vaginal cornification has been induced when estrogen is applied to female mice (21, 22), rats (23) and immature rats (24). In the present investigation, combination (B) has prolonged the diestrous phase considerably as compared to the normal diestrous phase of estrous cycle. Increase in the length of diestrous phase of vaginal smear under the influence of combination (B) exhibits its weak estrogenic nature, may be due to an increase in the period of leucocytic stage. The exact mode of action of these combinations is being elucidated.



DIFFERENT PHASES OF ESTROUS CYCLE

Fig. I

TABLE – 1

Effect of some crude drug combinations on estrous cycle in Albino rats.

Group No.	No. of animals in each group	Combination	Route dose mg/kg	Average body Weight before treatment (Mean ± S. D)	Average body weight after treatment (Mean ± S. D)	Duration of stages of estrous cycle in days			
						Proestrous (Mean ± S. D)	Estrous (Mean ± S. D)	Metaestrous (Mean ± S. D)	Diestrous (Mean ± S. D)
1	10	(A)	Oral / 200	141.50 ± 16.65	133.80 ±14.53	1.80 ±1.42	6.80 ±1.42	2.80 ± 0.80	9.60 ± 2.48
2	10	(B)	Oral / 200	143.20 ± 13.32	138.00 ±12.59	1.40 ± 0.45	4.70 ± 1.32	2.60 ± 0.92	12.30 ± 2.46
3	10	(C)	Oral / 200	161.00 ±18.05	149.10 ±18.88	2.00 ± 0.82	6.30 ± 1.94	3.00 ± 0.84	9.70 ± 2.40
4	10	Control (1 % CMC)	Oral / 200	152.50 ±9.28	153.80 ±10.59	2.50 ± 0.44	7.40 ± 0.83	3.20 ± 0.82	7.90 ± 1.30

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