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EFFECTS OF *DAUCUS CAROTA* SEED EXTRACT ON REPRODUCTIVE HORMONES AND ESTROUS CYCLE IN ALBINO MICE

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ABSTRACT: The use of plants for contraception and abortion is recorded throughout history, with contemporary reports from India and many other countries. *Daucus carota* is one of the several plants traditionally used for birth control. The present work was undertaken to evaluate the antifertility effect of the seeds of the plant by carrying out pharmacological studies with the methanolic extract of the seeds. The seeds of *Daucus carota* were extracted with methanol. The dried methanol extract was administered orally to an experimental group of Swiss albino mice for 21 days. The duration of the phases of the estrous cycle, changes in the levels of reproductive hormones (LH, FSH, prolactin, estradiol, and progesterone) and the number of litters obtained were monitored and compared with the control group of the animals. *Daucus carota* seed extracts prolonged the estrous cycle of the experimental group of animals with a significant increase of the duration of the diestrus phase. The number of litters produced by the experimental animals was significantly less. Moreover, the extract caused an alteration in the hormone levels with significant lowering of serum estradiol level. The present study indicates that the seed extract of *Daucus carota* may have a reversible antifertility effect and can impair the oogenesis.

INTRODUCTION: Regulation of fertility through varied products of plant origin is a new approach in the field of contraceptive development [1]. To avoid the side effects of available steroidal contraceptives and intrauterine devices, efforts have been made towards the development of new contraceptive agents from plant extracts [2, 3]. Use of medicinal plants is more prevalent among the rural population of India with the lower economic background, who has a strong faith in herbal medicines.

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Daucus carota is an umbelliferous plant with a tuberous taproot commonly distributed all over the world. Besides being used as food, these plants are included in the list of medicinal plants with anti-implantation (interceptive) activity with 100% antifertility effect [4-7].

The use of carrot seed for contraception and abortion is reported throughout European history with contemporary reports from the United States and India. Among the rural population from different parts of India, dry seeds of carrot are taken orally by women for their reputed efficiency in intercepting early pregnancy. *In-vivo* and *ex-vivo* studies suggested the antifertility action of the seeds with effect on the estrous cycle and anti-progestogenic activity [8-10].

Kaliwal and his group reported an abortifacient effect of carrot seed extract and its reversal and maintenance of implantation by progesterone in albino rat [11]. Bhatnagar (1995) reported a dose-dependent antifertility effect, especially as postcoital contraceptive effects of alcoholic extract of the *Daucus carota* Linn. seed in rats. Their study demonstrated that the seed extract at a lower dose has anti-implantation activity, whereas higher doses caused fetus resorption [12-13]. Though, the use of carrot seeds as antifertility agents has been reported by many workers from different geographical and ethnic locations of the World from time to time, the effect of this seed on endocrine function about estrus cycle and fertility status is still unclear. Hence, an attempt has been made to explore and document the effect of *Daucus carota* seed extract on serum levels of female reproductive hormones along with estrous cycle and fertility outcome in terms of the number of litters in female albino mice.

MATERIALS AND METHODS:

Animals: 20 adult female Swiss albino mice weighing between 20 to 25 g in the age group of 3 to 4 months were randomly selected from the animal house of the Zoology department of Gauhati University, Guwahati, Assam (India) for use in the experiment with approval of the Ethical Committee of the University (Letter no: IAEC/PER/2011-12/413 dated 20th May/2011). All the animals were acclimatized in the animal house for 4 weeks and fed on a balanced and nutritious animal diet and plenty of water before the experiment. All possible measures were taken to minimize pain or discomfort to the mice. The experiments were conducted by international standards on animal welfare and local and national regulations.

Collection of Sample and Extraction Procedure: The seeds of *Daucus carota* were collected from growers in the surrounding areas of Guwahati, India. Powdered seeds were extracted using methanol in a Soxhlet extractor. The methanol extract was then concentrated in a rotary vacuum evaporator to yield a thick and viscous liquid. This was further dried in a hot water bath to remove the methanol completely. This dry residue was used as the methanol extract for the phytochemical and pharmacological tests after reconstituting in distilled water.

Phytochemical Screening: Qualitative tests were performed with the methanolic extract to detect the presence of steroids (Salkowski test), flavonoids (Shinoda test), alkaloids (Wagner's test) and polyphenols (Ferric chloride test) following standard methods previously reported [14-15].

Design of Animal Experiment: The randomized and acclimatized group of 20 animals was randomly subdivided into 2 different groups of 10 animals in each group. The group of animals receiving normal standard diet and the vehicle (distilled water) without any treatment was designated as the control group. The group receiving an oral dose of 300 mg of methanol extract of carrot seeds (in distilled water) per kg body weight daily for 21 consecutive days was the experimental group.

Study of the Estrous Cycle: The estrous cycle was studied by examining the vaginal smear of the animals. The stages of the estrous cycle and their duration were studied following previously reported procedure [16].

Method of Estimation of Hormones: About 400 microlitres of blood samples were collected from the caudal vein of the experimental animals at different stages of the estrous cycle for hormonal assay. The serum 17-OH progesterone, FSH, LH, prolactin, and estradiol levels were measured using ELISA microwell kit (Transasia).

Statistical Analysis: The data obtained were statistically analyzed and expressed as Mean $\pm$ SEM. Statistical analysis of the variance between control and the experimental value was done using student's t-test [17].

RESULTS:

Phytochemicals in the Extract: The preliminary screening of the extract showed the presence of alkaloids, steroids and high amounts of polyphenols and flavonoids.

Effect of Seed Extract on the Serum Hormones and Estrous Cycle: The results obtained in the present study after treatment of mice with the seed extract are presented in **Table 1** and **Table 2**. The analysis revealed a differential impact on the hormones studied at different stages of the estrous cycle. Overall lowering in the levels of mean serum

estrogen, FSH and LH were observed in the treated group of female albino mice during the study period as compared to the control group. LH was significantly lowered (P<0.05) in pro estrous and apparently lowered in the other stages (P>0.05). A highly significant decrease in FSH content was observed in the diestrus (P<0.05) and estrous stage (P>0.05) in the extract administered animals.

The trend was accompanied by significantly lower (P<0.05) estradiol level in estrous stage with a simultaneous apparent decrease was observed in all the stages of the cycle. Prolactin level was elevated (P>0.05) throughout the cycle with significant (P<0.05) elevation in the diestrus stage. No significant (P>0.05) change was observed in the progesterone level with the treatment of seed extract.

In the treated group the duration of the total estrous cycle in days was significantly increased (P<0.01) to 6.84±0.58 against 4.7±0.2 of the control group. Duration of estrous days was observed to be minimally increased (P>0.05) to 1.10±0.2 from 0.98±0.16 but the duration of diestrus days was significantly increased (P<0.01) to 4.29±0.46 against 1.94±0.25 days in the control group. These results are similar to those reported in the earlier work of the authors [18].

However, these changes in the estrous cycle were found to regress on withdrawal of treatment although the reversal is not completed up to the levels of the control group during the period of the experiment (21 days). Highly significant (P<0.001) decrease in the number of litters was observed from 7.8±0.49 in control to 2.4±0.32 in the treated group. On the withdrawal of treatment, the number of litters increased to 5.6±0.46. None of the litter of treated mice showed any physical abnormality indicating the safe nature of the seed extract.

TABLE 1: EFFECT OF METHANOLIC SEED EXTRACT OF *D. CAROTA* ON THE ESTROUS CYCLE OF MICE FOR 21 DAYS AND NUMBER OF LITTERS PRODUCED IN DIFFERENT GROUPS OF MICE

Groups	Duration of the estrous cycle (days)	Duration of different phages of the estrous cycle (days)				No. of litters
		Proestrus (days)	Estrus (days)	Metestrus (days)	Diestrus (days)	
Control	4.72 ± 0.20	0.94 ± 0.12	0.98 ± 0.16	0.86 ± 0.14	1.94 ± 0.25	7.8 ± 0.49
300 mg/kg bw/d	6.84** ± 0.58	0.80 ± 0.24	1.10 ± 0.20	0.65 ± 0.10	4.29** ± 0.46	2.4*** ± 0.32
Post treatment of 300 mg/kg bw/d	4.96 ± 0.36	0.86 ± 0.16	1.18 ± 0.14	0.70 ± 0.18	2.22 ± 0.28	5.6* ± 0.46

N=10, data are mean ± S.E.M. *p<0.05, **p<0.01, ***p<0.001. Here bw/d=body weight per day.

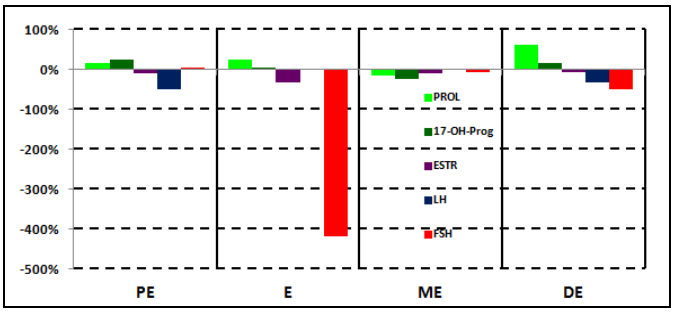

FIG. 1: PERCENT DEVIATION OF HORMONE LEVELS IN DIFFERENT STAGES OF ESTROUS CYCLE

DISCUSSION: One of the most prominent effects of *Daucus carota* seed extract observed in the present study was the negative impact on the pituitary-gonadal axis. This seed extract was found to induce hypogonadotropic hypogonadism coupled with decreased estradiol concentration in the experimental group, and that was observed to be highest during estrous stage with more than 400% depletion in FSH content in association with 50% depletion in estradiol content **Fig. 1** without any significant deviation in 17-OH-progesterone. Increased prolactin and suppressed FSH secretion in the treated mice during the study were observed to have an impact in significant (P<0.01) prolongation of the estrous cycle (44.9%), specifically affecting the diestrus stage 120.13%, **Fig. 2**. Major disturbance in the FSH secretion with significant decrease (P<0.05) of this hormone along with estradiol during the estrous stage of the cycle with the seed extract treatment may be related with the observed impairment of oogenesis resulting highly significant decrease (P<0.001) in number of litters after treatment with the seed extract with dose of 300 mg per kg body weight per day.

However, the increase in the number of liters on withdrawal of treatment **Table 1** suggests that the antifertility effect produced is reversible.

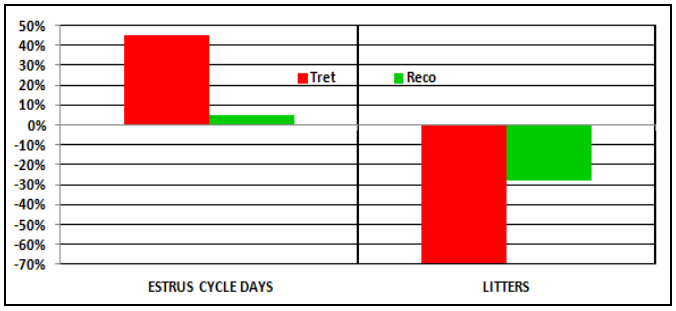

FIG. 2: PERCENT DEVIATION OF DURATION OF STAGES OF ESTROUS CYCLE (DAYS) FROM NORMAL CONTROL

The observed prolongation of the duration of the estrous cycle with significantly increased (P<0.001) diestrus stage along with higher prolactin during estrous and diestrus stage may be a contributory factor to the lowered implantation.

The apparent increase of 17-OH-progesterone in contrast to decreased estradiol may also have some impact on oogenesis as well as implantation [19].

TABLE 2: HORMONE LEVELS IN VARIOUS GROUPS OF ANIMALS DURING THE STUDY

Different hormones	Groups	Stages of the estrous cycle			
		Proestrus	Estrus	Metestrus	Diestrus
LH mIU/ml	Control	9.82 ± 1.16	4.46 ± 1.06	0.72 ± 0.19	0.86 ± 0.19
	300 mg/kg bw/day	5.86* ± 0.70	4.10 ± 0.62	0.62 ± 0.14	0.52 ± 0.10
FSH mIU/ml	Control	7.31 ± 1.52	8.96 ± 0.99	3.45 ± 0.71	4.45 ± 0.56
	300 mg/kg bw/day	7.74 ± 1.20	5.20* ± 0.64	3.20 ± 0.50	2.16** ± 0.42
Prolactin ng/ml	Control	30.62 ± 4.11	25.71 ± 2.33	12.10 ± 0.93	15.20 ± 1.96
	300 mg/kg bw/day	35.40 ± 6.30	32.20 ± 4.50	10.20 ± 1.64	24.60* ± 4.25
Estradiol pg/ml	Control	815.22 ± 108.29	710.08 ± 89.02	284.10 ± 44.24	273.51 ± 35.68
	300 mg/kg bw/day	742.10 ± 80.64	475.40* ± 42.10	256.50 ± 42.00	250.10 ± 25.10
17 OH Progesterone ng/ml	Control	10.20 ± 0.51	12.80 ± 1.21	16.42 ± 1.30	21.50 ± 1.32
	300 mg/kg bw/day	12.50 ± 1.20	15.12 ± 1.28	12.50 ± 1.21	25.10 ± 2.30

N=10, data are mean ± S.E.M. *p<0.05, **p<0.01. Here bw/d=body weight per day.

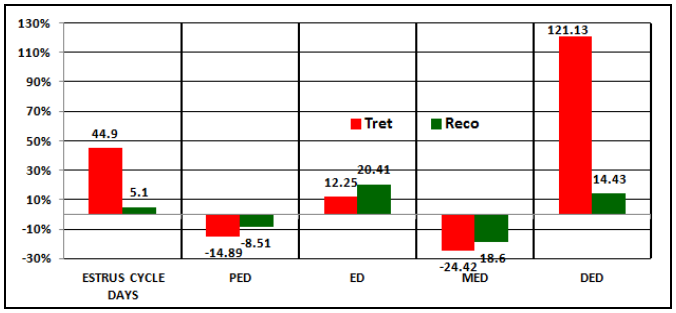

FIG. 3: PERCENT DEVIATION OF DURATION OF ESTROUS CYCLE AND NUMBER OF LITTERS FROM NORMAL CONTROL

The concerted analysis of the results of the cytological, hormonal and reproductive components **Table 1** and **Table 2**, **Fig. 3** in the present study revealed that methanol extract of *Daucus carota* seed exhibits the reversible antifertility effect.

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CONFLICT OF INTEREST: The authors declare no conflict of interest associated with this study.

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