

Implantation delay and nidation by progesterone in carrot seed (*Daucus carota*) extract treated albino rats

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MS received 4 July 1981; revised 19 March 1983

Abstract. In the present study the effect of petroleum ether extract of carrot seeds and progesterone on implantation have been investigated. 0.5 ml/100 g body weight of carrot seed extract was administered subcutaneously from days 1–7 of pregnancy and laparatomized on day 8. No implantation sites were observed. Thereafter graded doses of progesterone 2, 4 or 8 mg/100 g body weight was administered upto day 15 and autopsied on day 16. Two mg of progesterone did not show any effect. However, 4 mg of progesterone treated rats had smaller implantation sites compared to the controls whereas, 8 mg of progesterone was most effective with the result that in almost all the rats, delayed implantation was observed.

Keywords. Implantation; carrot seed extract; progesterone; albino rats.

1. Introduction

The use of plant extracts to induce changes in human fertility has been practised for centuries in many countries (Casey 1960). Many pasture plants exhibit antifertility activity, probably due to phytoestrogens in grazing live stock and domestic animals (Farnsworth *et al* 1975; Briggs and Christie 1977). It has been noted that any imbalance in the progesterone-estrogen ratio inhibits implantation in rats (Cochrane and Meyer 1957). Administration of antiestrogenic compounds or high dose of estrogens inhibits or delays implantations in rats (Cochrane and Meyer 1957; Chang and Yanagimachi 1965; Prasad and Kalra 1967). Many plant material or seed extracts are known to have estrogenic or antifertility activities (Kholkute and Udupa 1976; Sharma *et al* 1976). Alcoholic, aqueous or petroleum ether extract of carrot seeds prevent implantations in rats and mice, probably, due to its weak estrogenic activity as evidenced by luteotrophic uterine response (Garg and Garg 1971; Sharma *et al* 1976; Kaliwal and Rao 1977). The present study is designed to know the effect of progesterone in the antiimplantation activity of the carrot seed extract in the albino rats.

2. Materials and methods

Powdered carrot seed extract was Soxhleted with petroleum ether as described earlier (Kaliwal and Rao 1977, 1979). Colony bred, adult, nulliparous female albino rats of Holtzman strain, 75–85 day old with a body weight ranging from 130–170 g were used. After checking the rats for 3 regular estrous cycles, they were caged with males for mating. Mating was confirmed by the presence of sperms in the vaginal smears on the following morning designated as day 1 of pregnancy. Laparotomy was performed on day 8 of pregnancy by mid ventral incision under mild ether anaesthesia in semi-sterile condition.

In normal mated rats, 0.6 ml/100 g body weight of carrot seed extract was administered subcutaneously from days 1–7 of pregnancy, laparotomized on day 8 to note the number of implantations and autopsied on day 16. Thereafter in another group, 2, 4 or 8 mg of progesterone was administered till day 15. Suitable controls were maintained.

All the rats were autopsied on day 16 by cervical dislocation. Ovaries, uteri and adrenals were dissected out free from adherent tissues and weighed. Ovaries were processed for histological observations.

All the experimental rats were maintained in individual cages with Hindustan Lever rat pellets and water *ad libitum* at a room temperature of $27^{\circ} \pm 1^{\circ}\text{C}$ with a lighting schedule of 14 hr of day light. The results were analysed statistically using Student's 't' test.

3. Results

3.1 Delayed implantation (table 1)

Controls exhibited implantations on day 8 and small embryos on day 16. When carrot seed extract was given from days 1–7, there were no implantation sites on day 8. The second group treated with carrot seed extract and graded doses of progesterone from days 8–15 gave the following results: 2 mg of progesterone was ineffective resulting in 100% preimplantation loss. However, with 4 mg or 8 mg of progesterone almost all the rats had implantations on day 16. However, the total number and size of implantations was smaller in rats receiving 4 mg of progesterone.

3.2 Body and organ weights (table 2)

3.2.1 Body weights: Controls recorded a gradual and considerable increase in the body weight whereas, carrot seed extract treated rats showed a decrease. There was very little increase in the body weight in the carrot seed extract treated rats receiving progesterone.

3.2.2 Ovarian weights: Ovaries of controls weighed heavy and had well developed corpora lutea. In carrot seed extract treated rats with or without progesterone treatment, there was significant reduction in the ovarian weight ($P < 0.001$). The corpora lutea in these animals were small.

Uterine and adrenal weights: In controls, the uterus was heavy due to the developing foetuses. In the carrot seed extract treated rats, the uterine weight was considerably reduced due to inhibition of implantation and resembled that of nonpregnant rats. The uterus of 2 mg progesterone treated rats was approximately similar with that of nonpregnant rats whereas, in those receiving 4 mg of progesterone, there was slight increase in the weight as the number and size of the implantation sites was smaller. But 8 mg of progesterone treated rats exhibited larger implantation sites, resulting in the increase in uterine weight.

The adrenal weights in all the treated groups exhibited increase when compared to the olive oil treated controls probably, due to stress.

Table 1. Delayed implantation by progesterone in carrot seed extract treated rats.

Group	Treatment	No. of rats				Implantation at						Preimplan- tation loss at autopsy (%)
		Pregnant at		Corpora lutea		Laparotomy		Autopsy		Mean M ± S.E.	Total	
		Mated	Auto- psy	Total	Mean M ± S.E.	Total	Mean M ± S.E.	Total	Mean M ± S.E.			
A	Olive oil (Control)	5	5	41	8.20 ± 0.58	41	8.20 ± 0.51	40	8.00 ± 0.45	40	8.00 ± 0.00	2.43
B	Carrot seed extract only Days 1-7	5	0	40	8.00 ± 0.32	0	0.0 ^a	0	0.0	0	0.0	100.0
C	Carrot seed extract + 2 mg progesterone Days 8-15	5	0	42	8.40 ± 1.08	0	0.0	0	0.0	0	0.0	100.0
D	" + 4 mg	5	0	44	8.80 ± 0.58	0	0.0	42	8.40 ± 0.40	42	8.40 ± 0.40	4.54
E	" + 8 mg	5	0	43	8.60 ± 1.08	0	0.0	38	7.60 ± 0.81	38	7.60 ± 0.81	11.62

Treatments:

Carrot seed extract from days 1-7 of pregnancy
Laparotomy on day 8
Progesterone— days 8-15
Autopsy—Day 16

Dose/100 g body wt/day
Olive oil
Carrot seed extract
Progesterone

-0.6 ml (Days 1-15)
-0.6 ml
-2-8 mg

$$\% \text{ Preimplantation loss} = \frac{\text{Total number of corpora lutea} - \text{Total number of implantations}}{\text{Total number of corpora lutea}} \times 100$$

M ± S. E = Arithmetic mean ± Standard error.

^a P < 0.001

Table 2. Effect of carrot seed extract and progesterone on body and organ weights in rats.

Group	Treatment	Increase in body weight (%) (g)	Organ weights (mg)/100 g body Wt. M ± S.E		
			Ovaries	Uterus	Adrenals
A	Olive oil (Control)	(5) 45.05	39.10 ± 1.02	5507.82 ± 608.20	26.49 ± 0.86
B	Carrot seed extract only Days 1-7	(5) 11.27	24.72 ± 1.65 ^c	149.19 ± 37.77	40.36 ± 3.44
C	Carrot seed extract + 2 mg progesterone Days 8-15	(5) 2.87	27.15 ± 1.92	105.59 ± 24.26 ^c	37.61 ± 3.29 ^a
D	" + 4 mg	(5) 6.18	25.32 ± 1.56 ^c	297.95 ± 39.92 ^c	36.26 ± 1.66 ^b
E	" + 8 mg	(5) 6.94	26.08 ± 0.76 ^c	968.27 ± 289.30 ^c	43.75 ± 10.08 ^b

Treatments:

Carrot seed extract from days 1-7 of pregnancy

Laparotomy on day 8

Progesterone from days 8-15

Autopsy → Day 16

Dose/100 g body wt/day

Olive oil

-0.6 ml (Days 1-15)

Carrot seed extract

-0.6 ml

Progesterone

Number in parentheses denotes the number of rats.

M ± S. E. = Arithmetic mean ± Standard error.

^aP < 0.05; ^bP < 0.01; ^cP < 0.001.

4. Discussion

Implantation is a critical phase in the ontogeny of eutherian mammals, involving regulated co-ordinated changes in the uterus, uterine fluids and the blastocyst in sequential order by synergistic action of estrogen and progesterone (Prasad and Kalra 1967). Inhibition of implantation by chemicals and hormones may be due to their blastotoxic effect, expulsion of blastocyst, antihistaminic reaction, delayed transportation of the blastocyst into the uterus, imbalance in the progesterone-estrogen ratio or cessation of estrogen surge on day 3 or 4 of pregnancy in rats (Cochrane and Meyer 1957; Shelesnyak *et al* 1963; Chang and Yanagimachi 1965; Prasad *et al* 1965).

In the present investigation, administration of petroleum ether extract of carrot seeds, inhibited implantation in almost all rats. This may be due to the imbalance in progesterone-estrogen ratio of 4000:1 as stated by Cochrane and Meyer (1957) in their delayed implantation experiments in rats. If exogenous progesterone was administered from days 8–15 to the carrot seed extract treated rats, all of them retained the capacity to implant and exhibited implantations on day 16. The size of the implantation sites was depended on the dose of progesterone. This may indicate that carrot seed extract may neither be blastotoxic nor it might have caused expulsion of blastocyst as in the case of estrogens (Burdick *et al* 1937; Morris *et al* 1967). Any imbalance in the hormonal ratio causes delay or inhibits implantation (Nutting and Meyer 1963; Prasad *et al* 1965). Therefore, the inhibition or delay of implantation by carrot seed extract might be due to an imbalance in the progesterone-estrogen ratio which could be reversed by the administration of exogenous progesterone. However, further investigation is necessary to elucidate the mechanism of action of carrot seed extract on the inhibition or delay in implantation in albino rats.

Acknowledgements

The fellowship to the first author (BBK) by UGC, New Delhi and the research facilities provided by Prof. V B Nadkarni, are gratefully acknowledged.

References

- Briggs M H and Christie G A 1977 Antifertility substances in plants; *Adv. Steroid. Biochem. Pharmacol.* **6** XI–XIX
- Burdick H O, Whitney R and Pincus G 1937 The fate of mouse ova, tube locked by injections of oestrogenic substances; *Anat. Rec.* **67** 5131
- Casey R C D 1960 Alleged antifertility plants of India; *Indian J. Med. Sci.* **14** 590
- Chang M C and Yanagimachi R 1965 Effect of estrogens and other compounds as a oral antifertility agent on the development of rabbit ova and hamster embryo; *Fertil. Steril.* **16** 281–291
- Cochrane R L and Meyer R K 1957 Delayed nidation in the rat induced by progesterone; *Endocrinology* **96** 155
- Farnsworth N R, Bingel A S, Cordell G A, Crane F A and Fong M H S 1975 Potential value of plants as sources of new antifertility agents-1; *J. Pharmacol. Sci.* **64** 535–598
- Garg S K and Garg G P 1971 Antifertility screening of plants (Part VII). Effect on pregnancy in albino rats; *Indian J. Med. Res.* **59** 307
- Kaliwal B B and Appaswamy Rao M 1977 Inhibition of implantation by carrot extract and its rectification in albino rats; *J. Karnatak. Univ. Sci.* **22** 167–172

- Kaliwal B B and Appaswamy Rao M 1979 Dose and durational effect of carrot seed extract on implantation in albino rats; *Comp. Physiol. Ecol.* **4** 92-97
- Khoikute S D and Udupa K N 1976 Effect of *Hibiscus rosa sinensis* on pregnancy of rat; *Plant Medica.* **29** 321-329
- Morris J M, Van Wagenen B, McCann T and Jacob D 1967 Compounds interfering with ovum implantation and development. II. Synthetic estrogens and antiestrogens; *Fertil. Steril.* **18** 18-34
- Nutting E F and Meyer R K 1965 *Implantation delay, nidation and embryonic survival in rats treated with ovarian hormones.* In *delayed implantation* (ed.) A C Enders (University of Chicago Press) p 233
- Prasad M R N, Kalra S P and Segal S J 1965 Effect of Clomiphene on blastocysts during delayed implantation in the rats; *Fertil. Steril.* **6** 101-105
- Prasad M R N and Kalra S P 1967 Mechanism of anti-implantation action of Clomiphene; *J. Reprod. Fertil.* **13** 59-66
- Sharma M M, Lal G and Jacob D 1976 Estrogenic and pregnancy interceptory effect of carrot, *Daucus carota* seeds; *Indian. J. Exp. Biol.* **14** 506-508
- Shelesnyak M C, Kraicer S P and Zeilmaker G H 1963 Studies on the mechanism of decidualization. I. The oestrogen surge of pseudopregnancy and progravity and its role in the process of decidualization; *Acta Endocrinol. Kbh.* **42** 225-232