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EFFECT OF CARROT SEED EXTRACT (DAUCUS CAROTA) ON OVARIAN GROWTH, IMPLANTATION AND PREGNANCY IN ALBINO RATS

General Introduction

Fertility and contraception are of vital importance in human and animal reproduction. It has been reported that some pasture plants exhibit antifertility activity, causing temporary or permanent infertility in livestock and domestic animals (Kirtikar and Basu, 1925; Bennett et al., 1946; Henshaw, 1953; Nadakarni and Nadakarni, 1954; Chopra and Chopra, 1955; Chopra et al., 1956, 1958; Noble, 1959; Moule, 1961; Moule et al., 1953; Bickoff, 1968; Khanna and Choudhury, 1968; Khanna et al., 1969; Farnsworth et al., 1975; Briggs and Christie, 1977). The use of plant extract to induce changes in human fertility and to avoid unwanted pregnancies has been practiced in many Asian countries (Casey, 1960; De Laszlo and Henshaw, 1954; Shutt, 1975; Briggs and Christie, 1977). There is considerable controversy regarding the antifertility effects of the plant material, which may interfere in nutrition, or may be due to general toxicity, estrogenic activity, anticonvulsant or anticonvulsant effect (Adler and Trainin, 1960; Stob et al., 1962; Shutt, 1967; Perel and Lindner, 1970; Perry et al., 1970; Munshi et al., 1972; Munshi and Rao, 1972; Kallela, 1973a, 1973b; Madoyan et al., 1973; Briggs and
A variety of drugs have been demonstrated to have postcoital antifertility activity in laboratory animals. These include estrogens (Burdick and Pincus, 1935; Driesbach, 1959; Greenwald, 1959; Deanesley, 1963; Wotiz and Seublinsky, 1971); progestogens (Chang, 1964; Edgren, 1969); androgens (Desaulles and Krahenbuhl, 1964; Edgren, 1969; Naqvi and Warren, 1971); and corticoids (Fraser et al., 1954); nonsteroidal estrogens (Lerner et al., 1958, 1963; Emmens, 1965; Morris and Van Wagenen, 1966; Humphrey, 1968); antiestrogens (Lerner et al., 1958, 1963; Martins et al., 1963; Callantine et al., 1966; Morris et al., 1967; Harper and Walpole, 1967); anti-gonadotrophins (Harper, 1964; Pincus, 1965) and anti-LH (Hayashida and Young, 1963; Loewit et al., 1969; Madhwaraj and Moudgal, 1970; Munshi et al., 1972); antimetabolites such as aminopterin (Murphy, 1965); 6-diazo-5-oxo-L-norleucine, alkylating agents such as chlorambucil and triethylenethiophosphoramide (Robson, 1959; Murphy, 1965); antimitotic compounds such as colcemic (Adams et al., 1961; Ferin, 1963); ergot alkaloids such as ergotoxin (Shelesnyak, 1955); and ergocornine (Shelesnyak, 1957) and prostaglandins (Outlenceht et al., 1969; Nutting and Cammarata, 1969; Kirtan et al., 1970).

It has been shown that dry plants, alcoholic,
petroleum ether or aqueous extracts of pine needles, paraguayan weeds, Butea frondosa, ergotoxin alkaloids, Embelia ribes, Embelin, Cuminum seeds, Carrot seeds (Daucus carota), Hyptis and Triandrea leaves, Hibiscus flowers or Acoutobrys are known to cause disruption of estrous cycle, gonadal atrophy, inactivation of ovarian response to gonadotrophins, estrogenic or antiestrogenic, antigonadotrophic effects, inhibition of implantation and termination of gestation in rodents particularly in rats and mice (Planas and Kue, 1968; Khurana et al., 1969; Razdan et al., 1969; Garg and Garg, 1971; Chow et al., 1972; Radhakrishnan and Alum, 1975; Chandrashekar, 1976; Sharma et al., 1976; Kholkute and Udupa, 1976; Rathinam et al., 1976; Prakash and Mathur, 1977; Prakash, 1978, 1979). Many of these plants and seeds contain nonsteroidal phytoestrogens or compounds mimicking estrogens (Briggs and Christie, 1977). In India, carrot seeds have been used to prevent unwanted pregnancies. It has been stated by Sharma et al., (1976) that alcoholic extract of carrot seeds is known to have uterotrophic activity and inhibits implantation in mice, probably, due to its estrogenic activity. However, Briggs and Christie (1977) have rightly pointed out that no detailed investigations on the antifertility effects of these plant materials have been carried out in a systematic way. Therefore the present study is designed to investigate in detail the antifertility effect of carrot seed extract in albino rats. It consists of 4 chapters namely,
Chapter I  Effect of carrot seed extract or estradiol-17β on estrous cycle and ovarian compensatory hypertrophy in albino rats.

Chapter II  Inhibition of implantation by carrot seed extract or by estradiol-17β and its rectification by progesterone in albino rats.

Chapter III  Maintenance of implantation or delayed implantation by progesterone in carrot seed extract treated albino rats with or without ovaries.

Chapter IV  Interruption of pregnancy by carrot seed extract and its maintenance by progesterone in albino rats.

Materials and Methods

Nulliparous female albino rats of inbred Holtzman strain, 75 to 85 days old, weighing 150 to 170 gm, showing regular estrous cycle were used in these experiments. All experimental rats were maintained in individual cages with Hindustan lever rat pellets and water ad libitum, at a room temperature of 27°±1°C with a lighting schedule of 14 hours of day light. They were autopsied by cervical dislocation. Ovaries, uterus and adrenals were dissected out free from
adherent tissues and weighed to the nearest miligram in the torsion balance. Ovaries were fixed in Bouin's fluid, sectioned at 10 μ thickness and stained in Harris' haematoxylin-eosin for histological observations.

Powdered carrot seeds (Daucus carota) was subjected to soxhlet extraction by petroleum ether at 40° to 60°C. The ether of the extract was evaporated using a hot water bath under reduced pressure. One kg of carrot seeds yields 60 to 80 ml of extract (oil).

Chapter I

The effect of carrot seed extract or estradiol-17β on estrous cycle and ovarian compensatory hypertrophy were studied.

I Estrous cycle

Normal cycling rats were injected subcutaneously with 0.2 ml of carrot seed extract/100 gm body weight for 15 days. In another experiment the rats were bilaterally ovariectomized on the day of estrus. They were treated with 0.2 or 0.6 ml of carrot seed extract or 1 μg of estradiol-17β/100 gm body weight for 15 days. Olive oil treated normal or ovariectomized rats served as controls. Vaginal smears were recorded every morning to determine the estrous cycle.
II Ovarian compensatory hypertrophy

Female albino rats were hemi-spayed by removing the right ovary at estrus under light ether anesthesia in semi-sterile conditions, 0.2 ml of carrot seed extract or 10 μg of estradiol-17β/100 gm body weight/day was administered subcutaneously for 15 days. Olive oil treated sham operated and hemispayed rats served as controls. All rats were autopsied 24 hours after last injection. A total of 55 rats with a minimum of 5 per group were used.

Chapter II

Inhibition of implantation by carrot seed extract or by estradiol-17β and its rectification by progesterone in albino rats were studied. Adult female nulliparous rats were mated with fertile males at proestrus or early estrus and those rats showing sperms in the vaginal smears on the following day were selected for experimentation and that day was designated as day 1 of pregnancy (Cochrane and Meyer, 1957).

Experiment I Probe experiment

0.4 or 0.6 ml of carrot seed extract/100 gm body weight was administered subcutaneously once or twice a day from days 1 to 7 of pregnancy.
Experiment II

To study the temporal effect, the effective dose of carrot seed extract (0.6 ml) was administered from days 1 to 7, 1 to 5, 1 to 3, 3 and 4 or day 3 of pregnancy only.

Experiment III

Graded doses of carrot seed extract ranging from 0.2 to 0.8 ml/100 body weight were administered once a day from days 1 to 7 of pregnancy.

Experiment IV

To study the temporal effect of estradiol-17\beta on inhibition of implantation 1 \mu g of this estrogen in 0.2 ml of olive oil was administered subcutaneously from days 1 to 7, 3 and 4, day 3 or 4 only.

Experiment V

Graded doses of estradiol-17\beta from 0.1 to 1 \mu g/100 gm body weight in 0.2 ml of olive oil were administered subcutaneously on day 3 only of pregnancy.

Experiment VI

To maintain implantation in the carrot seed extract treated pregnant rats, graded doses of progesterone from 2 to 8 mg/100 gm body weight was administered subcutaneously
along with 0.6 ml of carrot seed extract/100 gm body weight from days 1 to 7 of pregnancy.

**Experiment VII**

Similarly in this experiment 1 µg of estradiol-17β/100 gm body weight was administered on day 4 of pregnancy only while 8 mg of progesterone/100 gm body weight was given from days 4 to 7 of pregnancy.

All rats were autopsied on day 8 by cervical dislocation. A total of 155 rats with a minimum of 5 per group were used.

**Chapter III**

Maintenance of implantation or delayed implantation by progesterone in carrot seed extract treated albino rats with or without ovaries were studied.

**Experiment I**

To find out the crucial day of ovariectomy which prevents implantation and to maintain the blastocysts in a viable condition by progesterone, bilateral ovariectomy was performed on day 2 or 3 of pregnancy. 4 or 8 mg of progesterone/100 gm body weight in 0.2 ml of olive oil was administered from days 2 or 3 through 7 of pregnancy and autopsied on day 8. The results indicated that the crucial day of
ovariectomy was day 2 of pregnancy but not day 3 as it showed implantations on day 8 while all rats ovariectomized on day 2 with progesterone treatment showed no implantations.

Experiment II

In ovariectomized rats, graded doses of carrot seed extract from 0.2 to 0.6 ml/100 gm body weight was administered concurrently with 8 mg of progesterone/100 gm body weight subcutaneously from days 2 to 7 of pregnancy and autopsied on day 8.

Experiment III

In ovariectomized rats, graded doses of progesterone from 2 to 8 mg/100 gm body weight was administered subcutaneously from days 2 to 7 of pregnancy along with 0.2 ml of carrot seed extract/100 gm body weight and autopsied on day 8.

Experiment IV

To study the temporal effect of carrot seed extract in ovariectomized rats, 0.2 ml of this extract was administered from day 2, 3, 4 or 5 through 7 of pregnancy. However, 8 mg of progesterone was administered from days 2 to 7 along with carrot seed extract.
Experiment V  Delayed implantation

In normal mated rats 0.6 ml of carrot seed extract/100 gm body weight was administered from days 1 to 7 of pregnancy and laparotomized on day 8 to note the implantation sites. They were administered with 2, 4 or 8 mg of progesterone/100 gm body weight till day 15 and autopsied on day 16 of pregnancy to note the implantation sites.

Experiment VI

Mated albino rats were ovariectomized on day 2 and 4 mg of progesterone was administered from days 2 to 7 of pregnancy to maintain the blastocyst in a viable condition. The animals were laparotomized on day 8 to note the implantation sites. Rats without implantation were treated with 0.2, 0.4 or 0.6 ml of carrot seed extract with 3 mg of progesterone/100 gm body weight from days 8 to 15 and autopsied on day 16 of pregnancy. In all these experiments suitable olive oil treated controls were maintained. A total of 120 rats with 5 per group were used.

Chapter IV

Interruption of pregnancy by carrot seed extract and its maintenance by progesterone in albino rats were studied.
Experiment I

Graded doses of carrot seed extract from 0.2 to 0.8 ml/100 gm body weight were administered subcutaneously from days 7 through 19 or till abortion.

Experiment II

To study the temporal effect, the effective dose of carrot seed extract (0.2 ml) was administered from days 7, 9, 13 or 15 through 19 of pregnancy.

Experiment III

To maintain pregnancy in the carrot seed extract treated pregnant rats 0.2 ml of carrot seed extract with 8 mg of progesterone/100 gm body weight was administered subcutaneously from days 7 to 13, 7 to 15 or 7 to 19 of pregnancy. In another group the same dose of carrot seed extract was administered from days 7 to 13 and progesterone from days 7 to 19.

Experiment IV

Effect of graded doses of progesterone from 2 to 8 mg/100 gm body weight was administered subcutaneously concurrently with 0.2 ml of carrot seed extract/100 gm body weight from days 7 to 19 of pregnancy.
In all experiments olive oil treated pregnant rats served as controls. All rats were autopsied by cervical dislocation on day 20 of pregnancy. A total of 85 rats with a minimum of 5 per group were used.

RESULTS AND DISCUSSION

Chapter I

Effect of carrot seed extract or estradiol-17β on estrous cycle and ovarian compensatory hypertrophy in albino rats

I. Effect of carrot seed extract or estradiol-17β on estrous cycle and organ weights in normal and ovariectomised rats (Tables 1.1, 1.2, 1.2a; Graphs 1 & 2 plate I)

The usual method employed to determine the estrogenic activity of unknown compound or extract is to determine the vaginal cornification or increase in the uterine weight in normal or ovariectomized rats or mice (Allen and Doisy, 1923; Rubin et al., 1951; Jacob and Morris, 1969). Normal or olive oil treated rats have approximately 3 cycle in 15 days but with 0.2 ml of carrot seed extract treatment to normal rats there was cessation of estrous cycle with continuous diestrus for 14 or 15 days with concomitant decrease in the ovarian and uterine weight (Tables 1.1, 1.2a). Furthermore the adrenals are significantly heavy. It has been stated
by Shutt (1976) that non-steroidal estrogens can cause infertility in domestic animals by causing hormonal imbalance affecting the cycling rats. Leavitt and Wright (1965) has also recorded that coumestrol inhibits gonadotrophins in rats.

In bilaterally ovariectomised rats, 0.2 ml of carrot seed extract did not elicit a vaginal response, thus showing continuous diestrus. But the uterine weight was slightly heavier, when compared to oil treated ovariectomized controls. However, with 0.6 ml of carrot seed extract, there was prolonged estrus with short diestrus and a significant increase in uterine weight. Similarly with 1 μg of estradiol-17β, a potent estrogen, there was continuous estrus with a balloon shaped uterus. These results indicated that large doses of carrot seed extract in ovariectomized rats did show estrogentic activity but low doses were ineffective. It was stated that carrot seed extract contained coumerin, a non-steroidal estrogen, as per the analysis by Seifert et al., (1968). In all carrot seed extract treated rats the adrenals were heavy.

II. Effect of carrot seed extract or estradiol-17β on ovarian hypertrophy in hemispayed albino rats (Tables 1.3, 1.3a; Graph 2; Plate II)

Unilateral ovariectomy was always accompanied by compensatory hypertrophy of the remaining ovary in rats.
mice, hamsters and guinea pigs (McLaren, 1963, 1966; Joshi and Lohseetwar, 1973; Edgren and Peppler, 1975). The general contention was that after hemispaying there was an increase in the secretion and release of pituitary gonadotrophins due to reduction in estrogen secretion thus causing the hypertrophy of the remaining ovary (Grady and Greenwald, 1969; Benson et al., 1969; Welschen, 1970, 1972; Howald et al., 1974). This had been questioned as there was no increase in the serum or pituitary gonadotrophins after hemicastration (McLaren, 1963, 1966; Edgren et al., 1963; Joshi and Lohseetwar, 1973).

However, Greenwald (1968) and Peppler (1972) were of the opinion that the mechanism of ovarian compensatory hypertrophy was related more to the time of exposure to the available gonadotrophins for a single ovary.

Estrogens were known to be potent blockers of ovarian hypertrophy in hemispayed rats, probably inhibiting the secretion and release of pituitary gonadotrophins or insensitizing the remaining ovary directly (Heller et al., 1942; Peterson et al., 1969; Diasouza and Rao, 1969). In the present study the percent hypertrophy of the remaining ovary in hemispayed controls was 46.8. Carrot seed extract or estradiol-17β completely inhibited ovarian compensatory hypertrophy thus reducing the size of the ovary when compared to sham operated or hemispayed controls (-12.4 or -10.5 respectively; Tables 1.3, 1.3a; Figs. 1 to 4). This indicated that the
carrot seed extract appeared to be a potent inhibitor of ovarian growth just like estradiol-17β. However, in carrot seed extract treated rats, the uterine weight was considerably reduced, while in estradiol-17β treatment it was considerably increased. Besides, carrot seed extract treated rats showed continuous diestrus, but estradiol-17β treatment there was continuous estrus. The ovary of carrot seed extract treated hemispayed rats, though small, showed a few large corpora lutea and small developing follicles (Fig. 3). But in estradiol-17β treated rats there were a few graafian follicles and corpora lutea (Fig. 4). This indicated that the antiovaryan activity of carrot seed extract might not be comparable to that of estradiol-17β. Therefore, in low doses carrot seed extract might inhibit gonadotrophin secretion resulting in the reduction of the ovarian weight and in the uterine weight with cessation of estrous cycle. Leavitt and Wright (1965) were of the opinion that coumestrol, a plant estrogen, inhibited gonadotrophin secretion. No doubt estradiol-17β also inhibited gonadotrophin secretion (Bradbury, 1947; Callantine et al., 1966). But it stimulated the uterus and maintained continuous estrus. Therefore further experiments seemed to be necessary to determine whether plant estrogens were antigonadotrophic or not.
Chapter II

Inhibition of implantation by carrot seed extract or by estradiol-17β and its rectification by progesterone in albino rats

I. Inhibition of implantation by carrot seed extract in albino rats (Tables 2.1, 2.1a; Plate III)

Inhibition of implantation by chemicals, hormones or uterine devices may be due to several factors, such as blastototoxic effect, tubal locking, expulsion of blastocyst, anti-histamine reaction, hostile uterine endometrium and its milieu, cessation of estrogen surge or imbalance in the progesterone-estrogen ratio (Burdick and Pincus, 1935; Cochrane and Meyer, 1957; Greenwald, 1959; Deanesly, 1963; Shelesnyak et al., 1963; Prasad et al., 1965; Chang and Yanagimachi, 1965; Prasad and Kalra, 1967; Wotiz and Seubelinsky, 1971). In the probe experiment 0.4 or 0.6 ml of carrot seed extract was administered subcutaneously once or twice a day from days 1 to 7 of pregnancy. The results indicated that in controls, pregnancy was maintained in all rats, showing an average of 9.4 implantations with 9 to 10 corpora lutea, thereby indicating that the preimplantation loss was only 2.1% (Table 2.1; Figs 1 & 2). With 0.4 ml of carrot seed extract once a day, 3 out of 5 rats showed inhibition of implantation thus the preimplantation loss was 57.1%.
However, with 0.6 ml of carrot seed extract once a day or 0.4 ml twice a day none of the mated rats had any implantations while the ovaries showed 9 to 10 corpora lutea, thus resulting in a preimplantation loss of 100%. Therefore the effective dose of carrot seed extract to inhibit implantation was 0.6 ml/100 gm body weight (Tables 2.1, 2.1a; Figs 3 to 6).

II. Temporal effect of carrot seed extract on implantation in albino rats (Tables 2.3, 2.3a; Graph 3; Plate IV)

0.6 ml of carrot seed extract/100 gm body weight was administered from days 1 to 7, 1 to 5, 1 to 3, 3 and 4 and day 3 of pregnancy only. Olive oil treated pregnant rats served as controls. The results indicated that in controls, all rats were pregnant on day 8 with 9 to 10 implantation sites and with 10 to 11 corpora lutea resulting in the preimplantation loss of 6.3% (Table 2.3a; Fig. 1). With 0.6 ml of carrot seed extract from days 1 to 7 of pregnancy, all mated rats showed no implantation sites with the result that the preimplantation loss was 100%. Administration of the same dose of carrot seed extract from days 1 to 5, 1 to 3, 3 and 4 or day 3 only seemed to be not very effective in inhibiting implantation, as almost all rats were pregnant at autopsy showing 6 to 10 implantation sites with 3 to 11 corpora lutea and the preimplantation loss ranged from 8.5 to 26.2%. There was no significant change in uterine or
ovarian weight.

III. Effect of graded doses of carrot seed extract on implantation in albino rats (Tables 2.5, 2.5a; Graphs 3 & 4; Plates III & V)

To find out the effective dose, keeping the duration of treatment constant, 0.1 to 0.8 ml of carrot seed extract/100 gm body weight was administered subcutaneously to all mated rats from days 1 to 7 of pregnancy. Suitable controls were maintained. The controls showed 8 to 9 implantations with 9 to 10 corpora lutea resulting in a preimplantation loss 6.3% (Plate V, Figs. 1 & 2). 0.1 or 0.2 ml of carrot seed extract was not very effective in inhibiting implantation but the number of implantation sites was reduced, but the ovaries had 8 to 9 corpora lutea resulting in the preimplantation loss of 53.5% (Plate V, Figs. 3 & 4). 0.4 ml of carrot seed extract was partially effective in its antifertility activity wherein 3 out of 5 rats were pregnant showing 4 to 7 implantations (Plate V, Figs. 5 & 6). But 0.6 or 0.8 ml of carrot seed extract inhibited implantations in all the rats, despite the fact that the ovaries had 9 to 10 corpora lutea resulting in 100% preimplantation loss (Plate III, Figs. 5 & 6).
IV. Temporal and dose effect of estradiol-17β on implantation in albino rats (Tables 2.7, 2.7a, 2.8, 2.8a; Graph 5; Plate VII)

a) Temporal effect

The temporal and dose effect of estradiol-17β on implantation had been investigated to compare it with those of carrot seed extract treated rats. It had been stated that carrot seed extract contained phytoestrogen, coumarin, which might be responsible for inhibition of implantation. Administration of 1 μg of estradiol-17β/100 gm body weight was very effective in inhibiting implantations in all rats (Fig. 3). Therefore the same dose of this estrogen was administered from days 1 to 7, 3 and 4, day 3 or 4 only. Irrespective of the duration of treatment, estradiol-17β inhibited implantations in all rats even though the ovaries had 8 to 10 corpora lutea resulting in 100% preimplantation loss (Fig. 5).

b) Dose effect

The above experiment indicated that the crucial day to inhibit implantation by estradiol-17β was either day 3 or 4 or pregnancy. Therefore graded doses of 0.1, 0.25, 0.5, 0.75 and 1 μg of estradiol-17β/100 gm body weight was administered on day 3 only. The results indicated that 0.1 μg was not very effective in preventing implantations except for a slight reduction in the number of implantations, 0.25 or
0.5 µg was partially effective wherein 1 out of 5 mated rats was pregnant. Therefore the mean of implantation sites was reduced to 0.2 to 1, even though the ovaries had 3 to 9 corpora lutea. Therefore the preimplantation loss was 97.5 to 87.8% respectively. But 0.75 or 1 µg of estradiol-17β appeared to be an effective dose, wherein the implantations were completely inhibited in almost all rats (Figs. 1 & 3). Therefore estradiol-17β seemed to be a very potent estrogen to inhibit implantations. In case of carrot seed extract, prolonged treatment for 7 days was necessary to inhibit implantations. Therefore the carrot seed extract seemed to contain a weak phytoestrogen when compared to estradiol-17β. To compare the potency of carrot seed extract in relation to estradiol-17β, further investigations were necessary using low doses of estradiol-17β for prolonged period. Numerous investigations had been carried out to inhibit implantation by estrogens in rats, mice and rabbits (Burdick and Pincus, 1935; Greenwald, 1959, 1965; Desnesaly, 1961; Chang, 1966).

V. Maintenance of implantation by progesterone in carrot seed extract or estradiol-17β treated albino rats

a) Effect of graded doses of progesterone in the maintenance of implantation in carrot seed extract treated rats (Tables 2.11, 2.11a; Graph 6; Plate VI)

For normal implantation proper proportion of progesterone and estrogen was essential (Cochrane and Meyer, 1957).
The inhibition of implantation by carrot seed extract was attributed to the presence of phytoestrogen, coumarin. To maintain implantation in carrot seed extract treated rats, 2 to 8 mg of progesterone/100 gm body weight was administered along with 0.6 ml of carrot seed extract from days 1 to 7 of pregnancy. With 2 mg of progesterone 1 out of 5 rats maintained implantation. The ovaries had 9 to 10 corpora lutea of different sizes. Therefore the preimplantation loss was 77.6%. But in controls the preimplantation loss was only 6.2%. The size of the implantation sites was very small (Figs. 1-4). With 4 mg of progesterone 3 out of 5 carrot seed extract treated rats maintained pregnancy showing 4 to 5 implantations. With 6 or 8 mg of progesterone all carrot seed extract treated rats maintained implantations, each had 6 to 8 implantation sites. The ovaries were large with 8 to 11 corpora lutea resulting in a preimplantation loss of 13.6 or 17.5% respectively (Figs. 5 & 6). The implantation sites were quite large. This experiment indicated that the carrot seed extract, which contained phytoestrogen, caused imbalance in the progesterone-estrogen ratio, thus inhibiting implantation. This could be rectified by the administration of adequate quantity of progesterone.

b) Effect of progesterone in the maintenance of implantation in estradiol-17β treated rats (Tables 2.13, 2.13a; Graph 7; Plate VII)

If 0.75 μg of estradiol-17β administered on day 3 of
pregnancy inhibited implantations in almost all rats. But the same dose of this estrogen given on day 4 was unable to inhibit implantations in all rats, wherein 4 out of 5 rats showed implantation sites, though the number was considerably reduced. Therefore 0.75 μg of estradiol-17β was given on day 3 only with 8 mg of progesterone from days 3 to 7 of pregnancy. None of the rats could maintain implantations (Figs. 1 & 2).

If 1 μg of estradiol-17β was given along with 8 mg of progesterone from days 1 to 7 of pregnancy, the implantations could not be maintained (Figs. 5 & 6). However, if the same dose of estradiol-17β was given on day 4 only all rats showed no implantation sites, but with 8 mg of progesterone from days 4 to 7 of pregnancy, implantations could be maintained (Figs. 3 & 4). Therefore the crucial period for implantation lies between day 3 and 4 of pregnancy during which period there should be proper proportion of progesterone and estrogen for normal implantation. Any increase in the estradiol-17β caused expulsion of the blastocyst due to increased motility of uterus, while progesterone subdued this motility resulting in the occurrence of implantations. These experiments indicated that carrot seed extract contain extremely weak phytoestrogen, when compared to estradiol-17β.

Postcoital contraception involves inhibition of fertilization, development of blastocyst, tubal locking of
blastocyst delayed tubal transportation, or expulsion of the blastocyst before implantation (Burdick and Pincus, 1935; Greenwald, 1959). Inhibition of implantation by chemicals and hormones may be due to their blastotoxic effect, delayed tubal transportation, expulsion of blastocyst from the uterus, antihistamine reaction, cessation of estrogen surge or imbalance in the progesterone and estrogen ratio (Cochrane and Meyer, 1957; Greenwald, 1959; Shelesnyak et al., 1963; Chang and Yanagimachi, 1965; Prasad et al., 1965). Estrogens, non-steroidal compounds with estrogenic effect, antiestrogens, antigonadotrophins, LH antisera will inhibit implantation (Burdick and Pincus, 1935; Greenwald, 1959; Hayashida and Young, 1963; Lerner et al., 1958, 1963; Emens, 1963; Harper, 1964; Pincus, 1965; Madwaraj and Moudgal, 1970; Munshi, 1972; Hair, 1977).

The use of plant extracts for contraception and abortion to avoid unwanted pregnancies has been practiced for centuries in India, China and Africa (Himes 1936; De Laszlo and Henshaw, 1954; Casey, 1960; Yuan Tien, 1965). It has been reported in Argentina and Australia, that many forage plants cause temporary or permanent infertility in domestic animals, particularly in sheep and cattle (Shutt, 1976). There is a paucity of information and investigation regarding the exact nature of antifertility activity of these plant extracts and many of them may contain toxic substances.
or weak estrogens (Briggs and Christie, 1977). It has been reported that alcoholic extract of seeds of Sutea fremdosa and carrot seed inhibit implantation in rats and mice and seem to have estrogenic activity (Razdan et al., 1969; Garg and Garg, 1971; Sharma et al., 1976). In the present experiments petroleum ether extract of carrot seeds requires prolong administration to inhibit implantation, while 1 dose of 1 μg of estradiol-17β on day 3 or 4 of pregnancy was sufficient enough to prevent nidation. This indicates that carrot seed extract contains a weak phytoestrogen, coumarin, (Seifert et al., 1968). If progesterone was given in adequate dose along with carrot seed extract, normal implantation was ensured. This indicates that carrot seed extract is neither blastotoxic nor causes expulsion of blastocyst from the uterus. Further this indicates that the inhibition of implantation by carrot seed extract may be due to the imbalance in the progesterone-estrogen ratio.

Chapter III

Maintenance of implantation or delayed implantation by progesterone in carrot seed extract treated rats with or without ovaries

I. Maintenance of implantation by progesterone in carrot seed extract treated ovariectomised rats

a) Crucial day of ovariectomy to inhibit implantation in rats (Tables 3.1, 3.1a)

According to Cochrane and Meyer (1957, 1963)
ovariectomy on day 3 of pregnancy in rats of Sprague-Dawley strain with 4 mg of progesterone inhibits implantation and the blastocysts were alive and viable. But in Holtzman strain of rat, ovariectomy on day 2 with 4 or 8 mg of progesterone inhibited implantation in almost all rats, while on day 3 it was not so effective, as implantations occurred in 4 out of 5 rats. Therefore the crucial day of ovariectomy for Holtzman strain of albino rats was day 2 of pregnancy.

b) Effect of graded doses of carrot seed extract with progesterone in the maintenance of implantation in ovariectomized rats (Tables 3.2, 3.2a; Graph 8; Plate VIII)

0.2 to 0.6 ml of carrot seed extract with 8 mg of progesterone/100 gm body weight was administered concurrently to ovariectomized rats from days 2 to 7 of pregnancy and autopsied on day 8. Suitable controls were used (Figs. 1 to 3). 0.2 ml of carrot seed extract along with 8 mg of progesterone in ovarian ablated rats could induce 4 to 6 implantation sites in all the rats (Fig. 4). With increase in the dose of carrot seed extract there was progressive inhibition of implantation inspite of 8 mg of progesterone treatment. Thus 0.4 ml of carrot seed extract with 8 mg of progesterone had reduced the number of implantation sites (Fig. 5). With 0.6 ml of carrot seed extract with 8 mg of progesterone, only 2 out of 5 rats showed implantation sites. Besides that in ovariectomized rats neither the carrot seed extract nor
progesterone could induce implantations (Figs. 1 to 3). A proper combination of progesterone and carrot seed extract ensured normal implantations. Increased dose of carrot seed extract had adverse effect on implantations. This indicated that a proper proportion of progesterone and estrogen was necessary for normal implantation. Further it indicated that carrot seed extract behaved like an estrogen.

It has to be noted that in normal rats the effective dose to inhibit implantation was 0.6 ml while 0.2 ml of carrot seed extract was not at all effective. In ovariectomized rats 0.2 ml of carrot seed extract with 8 mg of progesterone was sufficient enough to induce normal implantation. This further support the view that carrot seed extract behaved like an estrogen in inducing implantation in the absence of ovary, provided there was sufficient amount of circulating progesterone (Table 3.2b).

II. Effect of graded doses of progesterone in the maintenance of implantation in carrot seed extract treated ovariectomized rats (Tables 3.3, 3.3a; Graph 8; Plate IX)

Ovariectomy with or without carrot seed extract inhibited implantation in all rats (Figs. 1 & 2). In the previous experiment it has been shown for normal implantation in ovariectomized rats, 0.2 ml of carrot seed extract with 8 mg of progesterone was necessary. Therefore 2 mg of progesterone with 0.2 ml of carrot seed extract was unable to maintain
Implantations. 4 or 6 mg of progesterone was partially effective wherein 3 out of 5 mated rats showed implantations in left or right horn of the uterus (Figs. 3 & 4). However, 8 mg of progesterone was very effective in inducing 4 to 6 implantations in both horn of the uterus in all rats (Fig. 5). This clearly indicated that proper proportion of progesterone and estrogen has necessary. This also further corroborated that carrot seed extract might contain estrogen like substance.

III. Temporal effect of carrot seed extract on implantation in progesterone treated ovarectomized rats (Tables 3, 4, 3.4a; Graph 3; Plate X)

Implantation of blastocyst occurred in rats on day 3 or 4 postcoitally, when the uterus had undergone preimplantation changes by synergistic action of progesterone and estrogen (Prasad and Kalra, 1967; Prasad et al., 1968). It had been shown clearly that ovariecotmy on day 2 or 3 prevented implantation but not on day 5 because the estrogen surge had already occurred an essential factor for implantation (Shelosnyak et al., 1963). Therefore 0.2 ml of carrot seed extract was given with 8 mg of progesterone from day 2, 3, 4, 5 through 7 of pregnancy and autopsied on day 8 note the implantation sites.

The results indicated that there were no implantations in ovarectomized rats treated with either carrot seed extract or progesterone from days 2 to 7 of pregnancy.
However, with 0.2 ml of carrot seed extract along with 3 mg of progesterone administered from days 2 to 7 of pregnancy almost all rats showed 4 to 6 implantation sites (Fig. 1). If the same dose of carrot seed extract and progesterone given from day 3, 4, 5 through 7 of pregnancy there was partial maintenance of implantation wherein 4, 3, 2 out of 5 rats had implantation sites respectively. The number of implantations gradually reduced depending upon the duration of treatment (Figs. 2 to 4). This experiment indicated that carrot seed extract given continuously from the day of ovariection along with progesterone could maintain nidation in all rats similar to those of normal rats.

IV. Delayed implantation by carrot seed extract followed by graded doses of progesterone in rats (Tables 3.5, 3.5a, Graph 9; Plate XI)

In Chapter II, it was shown that in normal rats, carrot seed extract, if administered from days 1 to 7 of pregnancy, inhibited implantation. If progesterone was given concurrently, the implantations were ensured. In this chapter, carrot seed extract and progesterone, given concurrently, can maintain implantations even in ovariecctioned rats, if administered from days 2 to 7 of pregnancy. Still the question arises whether the inhibition of implantation by carrot seed extract may still be due to several factors such as delayed transportation of blastocyst or blastotoxic, or expulsion.
of the blastocyst from the uterus or imbalance in the progesterone-estrogen ratio. To investigate this, delayed implantation was induced both in normal and ovariectomized rats.

Delayed implantation was induced in normal rats by administering 0.6 ml of carrot seed extract from days 1 to 7 of pregnancy and checked for implantation on day 8. Generally, almost all rats did not show implantations on day 8. These rats were treated with 2, 4 or 8 mg of progesterone from days 8 to 15 and autopsied on day 16. Suitable olive oil treated or carrot seed extract treated mated rats served as controls.

The results indicate that in the olive oil treated controls, all mated rats showed implantations at laparotomy; on day 16 there were small embryos (Fig. 1). When carrot seed extract was given from days 1 to 7 of pregnancy, there was no implantations on day 8. From days 8 to 15 no treatment was given and the rats were autopsied on day 16. There were no implantations in any of these rats (Fig. 2). When 2 mg of progesterone was given from days 8 to 15 in carrot seed extract treated rats from days 1 to 7 there were no implantations (Fig. 3), indicating thereby that the amount of progesterone needed was insufficient. However, with 4 or 8 mg of progesterone given from days 8 to 15, there were implantations in all rats on day 16, each showing 8 to 9
implantations. In rats, treated with 4 mg of progesterone the size of the implantation sites were smaller than those of 8 mg progesterone treated rats (Figs. 4 & 5). This clearly indicated that carrot seed extract was not blastotoxic nor caused expulsion of the blastocyst from the uterus, because treatment of progesterone afterwards caused implantations. Therefore, the inhibition of implantation by carrot seed extract in normal rats appeared to be due to an imbalance in the progesterone-estrogen ratio as evidenced in tables 3.5, 3.5a (Graph 9).

V. Delayed implantation by progesterone and carrot seed extract in ovariec tomized rats (Table 3.7)

In the previous experiment, implantation, which was inhibited by early administration of carrot seed extract, could be induced when progesterone was given later in intact rats (Table 3.5, 3.5a). In ovariec tomized rats concurrent administration of carrot seed extract and progesterone had ensured normal implantations on day 8 (Tables 3.2, 3.2a; Plate VIII).

The phytoestrogens, particularly coumestrol and genistein, showed estrogenic activity, but failed to induce implantation of dormant blastocyst in mated ovariec tomized progesterone treated rats in experimentally delayed nidation (Perel and Lindner, 1970). Therefore it became essential to test whether carrot seed extract induces implantation in
ovariectomized progesterone treated mated rats. Mated rats were ovariectomized on day 2 with 4 mg of progesterone administered from days 2 to 7 of pregnancy. The animals were laparotomized on day 8 to record the number of implantations. Rats, which did not show any implantations, were treated with 0.2, 0.4 or 0.6 ml of carrot seed extract with 8 mg of progesterone from days 8 to 15 and autopsied on day 16. The results indicated that 0.2, 0.4 or 0.6 ml of carrot seed extract with 8 mg of progesterone administered from days 8 to 15 in ovariectomized rats was unable to induce implantation of dormant blastocyst (Table 3.7). However, 0.2 ml of carrot seed extract with 8 mg of progesterone administered from days 2 to 7 of pregnancy induced implantations in ovariectomized rats (Experiment II). But it was unable to induce implantation if it was given after day 8. Probably, the carrot seed extract may contain a weak nonsteroidal phytoestrogen incapable of inducing implantation of dormant viable blastocyst in delayed implantation experiments. No definite conclusion can be drawn from this experiment because very few rats were used. However, it was interesting to note that, just like coumestrol and genistein (phytoestrogens), the carrot seed extract was also incapable of inducing implantation of dormant blastocyst (Perel and Lindner, 1970). Further experiments seemed to be necessary to elucidate this problem.
The antifertility effect of plant materials has been attributed to phytoestrogens, particularly coumestrol and genistein, which had estrogenic activity and are incapable, of inducing implantation in ovariectomized progesterone treated rats, in delayed implantation experiments. It was interesting to note that alcoholic extract of carrot seeds, which exhibits uterotrophic effect, characteristic of estrogen, inhibited implantation in rats and mice (Garg and Garg, 1971; Sharma et al., 1976). In high concentration a weak plant estrogen can exert a significant estrogenic effect in the animal and can produce a hormonal imbalance even though their activity is only $10^{-3}$ to $10^{-5}$ times the estrogenic activity of estradiol. Plant estrogens could also act as anti-estrogens (Shutt, 1976).

Treatment of progesterone or carrot seed extract from days 2 to 7 of pregnancy in ovariectomized rats failed to induce implantation. But 0.2 ml of carrot seed extract with 8 mg of progesterone from days 2 to 7 of pregnancy induced implantation in all the mated rats. However, an increased dose of carrot seed extract (0.6 ml) with 8 mg of progesterone maintains implantation only in a few mated rats each showing 1 or 2 implantations. This indicated that proper proportion of progesterone and carrot seed extract was necessary for implantation. It had been well documented that implantation required proper proportion of progesterone and estrogen ratio.
Any imbalance in the progesterone-estrogen ratio inhibited implantations (Cochrane and Meyer, 1957; Nutting and Meyer, 1963; Callantine et al., 1966; Prasad and Kalra, 1967). This indicated that carrot seed extract mimicked an estrogen in inducing implantation in progesterone treated ovariectomized rats. The temporal effect of carrot seed extract on implantation in progesterone treated ovariectomized rats indicated clearly that administration of carrot seed extract and progesterone from days 2 to 7 of pregnancy maintains implantation in almost all ovariectomized rats. But the same proportion of progesterone and carrot seed extract given from days 3, 4, 5 through 7 progressively reduces the number of mated rats becoming pregnant with reduced implantation sites. Probably these weak phytoestrogens could induce implantations only, if the uterus was extremely sensitive enough to react to such weak estrogens probably, on day 3 or 4. Therefore these phytoestrogens might not be so effective in inducing implantation after day 5 when the uterus might not be that sensitive.

In normal rats, if carrot seed extract was given from days 1 to 7 of pregnancy, it inhibited implantations as evidenced by laparotomy on day 8, wherein almost all rats did not have any implantations (Tables 3.5, 3.5a). If 8 mg of progesterone was administered from days 8 to 15 of pregnancy, all rats showed implantation sites on day 16. This indicated that the carrot seed extract was not blastotoxic, nor will it
cause expulsion of blastocyst from the uterus or tube locking. Therefore the inhibition of implantation by carrot seed extract might be due to an imbalance in the progesterone-estrogen ratio which could be counteracted by the administration of exogenous progesterone. The delayed implantation experiment, in progesterone treated ovariectomized rats, the carrot seed extract and progesterone given after day 7 of pregnancy were unable to induce implantation. Similar results have been obtained by Perel and Lindner (1970) wherein phytoestrogens such as coumestrol and genistein were unable to induce implantation of dormant blastocyst in progesterone treated ovariectomized rats. This had been attributed to the fact that endogenous estrogens and phytoestrogens might act on different cellular receptors of the uterus during implantation (Perel and Lindner, 1970; Shutt, 1976). The experiments described in chapter II and in this chapter indicated that the inhibition of implantation by carrot seed extract could be reversed, if adequate quantities of progesterone were given concurrently or after 7 days in normal rats in delayed implantation experiment. But in ovariectomized rats implantation could be ensured by administration of carrot seed extract and progesterone from days 2 to 7 but not after day 7 in progesterone treated ovariectomized rats in delayed experiment just like coumestrol and genistein. These experiments indicate that these weak nonsteroidal phytoestrogens were incapable of stimulating uterus of
decidualization, a prerequisite for implantation, after day 7 in ovariectomized mated rats, because the uterine sensitivity to estrogen and progesterone was considerably reduced. Therefore the carrot seed extract, which might contain a phytoestrogen (coumarin), was unable to induce implantation of dormant viable blastocysts after 7 days in progesterone treated ovariectomized rats.

Chapter IV

Interruption of pregnancy by carrot seed extract and its maintenance by progesterone in albino rats

I. Effect of graded doses of carrot seed extract on the interruption of pregnancy in rats (Tables 4.1, 4.1a; Graph 10; Plate XII)

In the present experiment 0.2 to 0.8 ml of carrot seed extract administered from days 7 to 13 of pregnancy caused abortion in almost all rats with profuse vaginal bleeding from days 10 to 13 of pregnancy (Figs. 2, 3 & 4). These rats showed normal implantations at laparotomy on day 8. Olive oil treated controls maintained pregnancy with 95% foetal survival when compared to carrot seed extract treated rats - wherein percent foetal survival was nil (Figs. 1 & 2). In carrot seed extract treated rats, the ovaries are slightly reduced in weight due to abortion or resorption (Figs. 5 & 6), the uterine weight was reduced considerably and the uterus
resembled that of nonpregnant rats except for placental scars.

II. Temporal effect of carrot seed extract on pregnancy in rats (Tables 4.3, 4.3a; Graph 10; Plate XIII)

The results indicated in the previous experiment that the effective dose of carrot seed extract causing abortion was 0.2 ml/100 gm body weight. In the present experiment the same dose of carrot seed extract was administered subcutaneously from days 7, 9, 11, 13 or 15 through 19 of pregnancy or till abortion. The results indicated that the effective period for causing abortion by carrot seed extract ranges from days 7 to 9 wherein abortion occurred in almost all rats. Olive oil treated controls maintained pregnancy with 95% foetal survival when compared to carrot seed extract treated rats wherein the percent foetal survival was nil (Figs. 1 & 2). Administration of carrot seed extract from day 9 onwards had partially effect wherein 3 out of 5 rats showed complete abortion with incipient placental scars (Fig. 2). The foetal survival was only 20%. The remaining had normal pregnancy. From days 11, 13 or 15 onwards, the carrot seed extract was not effective as the gestation was maintained in all rats and the percent foetal survival was ranging from 84.8 to 95.3% (Figs. 3, 4 & 5; Graph 10). Therefore the effective period during which the carrot seed extract had the maximum abortifacient effect ranged from days 7 to 9.
III. Temporal effect of progesterone in the maintenance of pregnancy in carrot seed extract treated rats (Tables 4.5, 4.5a; Graph 11; Plate XIV)

0.2 ml of carrot seed extract appeared to be abortifacient, if administered from day 7 of pregnancy onwards (Fig. 1). If 8 mg of progesterone was administered along with 0.2 ml of carrot seed extract/100 gm body weight from days 7 to 19 or 7 to 15, the pregnancy was maintained in all the rats, but a few showed one or two placentomas (Figs. 2 & 3). The percent foetal survival was 88.4 or 81.4 respectively. Administration of progesterone along with carrot seed extract from days 7 to 13 was partially effective in maintaining pregnancy wherein the foetal survival was 50% (Fig. 4). If the carrot seed extract was given from days 7 to 13 and progesterone was administered from days 7 to 19 there was slight improvement in the maintenance of pregnancy where 2 rats maintain pregnancy to a full term without any placentomas, the rest of the 2 rats had placentomas and live foetuses (Fig. 5). The percent foetal survival was 72.1.

IV. Effect of graded doses of progesterone in the maintenance of pregnancy in carrot seed extract treated rats (Tables 4.7, 4.7a; Graph 11; Plate XV)

In the previous experiment 8 mg of progesterone given from days 7 to 19 of pregnancy along with 0.2 ml of carrot seed extract maintained pregnancy in all the rats except for
a few placentomas. Therefore graded doses of progesterone from 2 to 8 mg were administered from days 7 to 19 along with 0.2 ml of carrot seed extract/100 gm body weight. The results indicated that even 2 mg of progesterone was able to maintain pregnancy in all rats but a few rats had one or two placentomas, with a percent foetal survival of 76.6. With 4 or 6 mg of progesterone, all the carrot seed extract treated rats maintained pregnancy to full term with 82 to 89% foetal survival. 8 mg of progesterone seemed to be an overdose wherein all carrot seed extract treated rats maintained pregnancy to a full term, but a few showed two to three placentomas (Fig. 5). The percent foetal survival was 88.4%. This indicated that the abortifacient effect of carrot seed extract could be counteracted by progesterone. It had been shown earlier that carrot seed extract seemed to have estrogenic activity due to the presence of coumarin a nonsteroidal phytoestrogen (Seifert et al., 1968). Therefore abortifacient effect of carrot seed extract might be due to its increased estrogenic activity, thus causing an imbalance in the endogenous progesterone-estrogen ratio. It might not be toxic as pregnancy could be maintained by progesterone in carrot seed extract treated rats.

Maintenance of pregnancy in rats is due to the co-ordinated secretion of the pituitary, ovarian and placental hormones. The hypophysis is indispensable for the first
half of pregnancy and thereafter hypophysectomy does not interrupt pregnancy, particularly in rats and hamsters (Pencharz and Long, 1931, 1933; Seley et al., 1933; Astwood and Greep, 1939; Averill, 1950; Ray et al., 1955; Alloiteau, 1957; Morishige and Rothchild, 1974). Maintenance of pregnancy by exogenous LH in hypophysectomized rats or its interruption in normal pregnant rats by using LH antisera indicates that LH seems to be the main luteotrophic substance in the early part of gestation (Alloiteau and Bouhours, 1965; Loewit et al., Moudgal, 1969; Madhwara j and Moudgal, 1970; Loewit, 1970; Yang, 1973). But other investigators claim that prolactin with FSH or estrone forms the luteotrophic complex essential for the maintenance of pregnancy in rats and hamsters (Lyons et al., 1943; Greenwald, 1967, 1973; Greenwald and Johnson, 1968). Studies of Morishige and Rothchild (1974) have clarified this controversial problem to some extent by showing that luteal progesterone secretion is sustained by prolactin up till day 7 whereas pituitary LH had successfully maintained pregnancy during the first half of gestation from day 8 onwards.

Though the pituitary is indispensable only during the early part of pregnancy, the ovaries are essential throughout gestation, as ovariectomy at any time of pregnancy causes abortion or foetal resorption (Harris and Pfiffner, 1929; Johnson and Challans, 1930; Astwood, 1941; Lyons, 1943;
Fraser and Alexander, 1954). Maintenance of pregnancy by daily administration of progesterone and estrogen in proper proportions to ovarieotomized rats indicated the importance of the ovary in producing the hormones essential throughout gestation (Lyons, 1943; Talwalker et al., 1966; Dickman and De Fee, 1967; Callard and Leathem, 1971; Dickman and Hart, 1972; Butterstein and Leathem, 1974). Any imbalance in progesterone and estrogen ratio, depending on the period of gestation, causes abortion in rats (Yochim and Zarrow, 1960). Administration of ethynyl estradiol causes abortion in rabbits (Parkes et al., 1929). All these indicate that progesterone with a small amount of estradiol-17β is primarily the pregnancy maintaining hormones.

Many plant material extracts (root, stem, flowers, leaves, seeds) seem to have abortifacient effects in animals (Casey, 1960; Kchouk and Chaoli, 1963; Ocampo, 1972; Chow et al., 1974; Briggs and Christie, 1977). This may be due to toxic substances or due to phytoestrogens particularly genistein and coumestrol (Chow et al., 1972; Shutt, 1976). These phytoestrogens may inhibit gonadotrophic secretion (Leavitt and Wright, 1965) or may displace estradiol from uterine receptor sites (Shutt, 1967; Perel and Lindner, 1970). It is known that carrot seed oil contains coumarin, a nonsteroidal phytoestrogen (Seifert et al., 1968). In the present experiments even 0.2 ml of carrot seed extract was sufficient
to cause abortion, if it was given from day 7 of pregnancy. However, it was not very effective in interrupting gestation, if it was administered from day 11 onwards.

From the dose and temporal effect of progesterone in carrot seed extract treated animals, it was evident that progesterone nullifies the abortifacient effect of carrot seed extract and maintains pregnancy. 4 to 6 mg progesterone with 0.2 ml of carrot seed extract from days 7 to 19 of pregnancy maintains the gestation in all rats. However, 2 mg of progesterone was partially effective, while 8 mg of progesterone appears to an overdose in the maintenance of pregnancy, in carrot seed extract treated rat. Therefore proper proportion of progesterone with carrot seed extract was essential for the maintenance of normal pregnancy. If an adequate dose of progesterone was given along with carrot seed extract the pregnancy was maintained. This indicates that the action of carrot seed extract in interrupting pregnancy might be due to several factors such as,

1. Antiestrogenic
2. Antiprostational
3. Antagonadotrophic activity
4. It may cause imbalance in the progesterone and estrogen ratio. It may not be antiestrogenic because the requirement of estrogen in the maintenance of pregnancy is considerably less. Besides, it exhibits estrogenic
activity. It has been stated that even slight increase in estrogenic activity may cause abortion (Parkes et al., 1929; Yochim and Zarrow, 1960). There is some possibility that it may be antiprostational probably, mediated through pituitary wherein it might inhibit gonadotrophins as suggested by Leavitt and Wright (1965). In the present experiments, administration of exogenous progesterone to carrot seed extract treated animals maintains pregnancy, thereby indicating that this carrot seed extract contains a weak estrogen which may cause imbalance in the progesterone and estrogen ratio thus causing abortion in normal rats. This can be rectified by the administration of progesterone. However, it cannot preclude the suggestion that it is not antiprostational or antigonadotrophic in its activity. This requires further investigation.

Conclusion

Many forage plants cause infertility in farm animals. Besides, plant and seed extract were known to cause abortion thus preventing unwanted pregnancies in human beings. But the exact mode of operandi to cause infertility activity was not elucidated.

Carrot seed extract if administered in high doses caused continuous estrus both in normal and ovariectomized rats. It also inhibited the ovarian hypertrophy in hemispayed
rats. Similar observations had been observed in estradiol treatment. This indicated that carrot seed extract contains a weak phytoestrogen coumarin.

Carrot seed extract inhibited implantation in mated rats provided they were given continuously from days 1 to 7 of pregnancy. But estradiol-17β, a potent estrogen, could inhibit implantation if given on day 3 or 4 of pregnancy. This inhibition of implantation could be rectified by the administration of 8 mg of progesterone in carrot seed extract or estradiol-17β treated animals, thereby indicating that inhibition of implantation by carrot seed extract or estradiol-17β was due to the imbalance in the progesterone-estrogen ratio.

In ovariectomized mated rats neither carrot seed extract nor progesterone could induce implantation. But 0.2 ml of carrot seed extract with 8 mg of progesterone given from days 2 to 7 of pregnancy maintained normal implantations. Delayed implantation in normal rats could be induced by giving carrot seed extract from days 1 to 7 of pregnancy followed by 8 mg of progesterone from days 8 to 15 wherein normal implantation occurred. But in ovariectomized rats delayed implantation could not be induced by carrot seed extract due to its having a weak phytoestrogen.
0.2 ml of carrot seed extract given from day 7 of pregnancy caused abortion which could be rectified by progesterone treatment. After day 11 of pregnancy, the carrot seed extract was not very effective in inducing abortions.