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Studies on the antifertility activities of the aqueous extract and aqueous suspension of Carrot (*Daucus carota* L.) seed powder through oral administration on Mice and Rat

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ABSTRACT

The aqueous extract and aqueous suspension of Carrot (*Daucus carota* L) seed powder were tested through oral administration on adult mice and rats, individually to find out effect in their estrus cycle.

There was significant change in estrus cycle in both kind of animals indicating the effectiveness of the oral administration of the afore mentioned two aqueous dosage forms. Prolonged oral administration of the aforementioned aqueous extract or suspension of seed powder did not show any adverse feature and / or abnormality of the organs like stomach, liver, kidney, uterus, and ovary of the treated animals on Histopathological examinations.

The estimation of Haematological profile, Hepatorenal functions and metabolism of the untreated as well as treated animals were found in line with each other even after prolonged oral treatment with the aforementioned formulations.

Thus, results of this study revealed that the aqueous extracts of *D. carota* L seeds and the aqueous suspension of seed powder show antisteroidogenic activity without any aparent adverse effect.

Key words: Carrot seed, antifertility, aqueous extract, antisteroidogenic activity, estrus cycle.

INTRODUCTION

Carrot is scientifically known as *Daucus carota* Linn, (family: Umbelliferae)[1,2,3]. It is an annual or biannual herb, found and cultivated throughout the temperate regions of the world. Though carrot is widely used as vegetable, yet different parts of this plant posses multifarious medicinal properties like, antiasthmatic, anticancer, anti-inflammatory, anthelmintic, and protection against Kidney disfunction, and Hepatic injury [4,5].

Carrot seeds are found to be used by certain Indian tribes for controlling their birth rate[6]. But, they used to consume the plant or its parts thereof as such. Anticipating that the active principles of the plant , if given directly will be more active, hence the seed of the plant was selected.

The seeds and their different extracts with various organic solvents such as Methanol, Petroleum Ether, B.P. 40°C- 60°C., etc., have been shown to possess antifertility activity[7,8,9].

Considering the present day population explosion *vis-à-vis* demand for contraceptive agents, various synthetic compounds , mainly hormonal products are available commercially in the market. Although these hormonal contraceptives are quite effective, but they are not devoid of hazards and side effects, some of which are : nausea, vomiting, libido, headache, mental depression, weight gain, etc., etc.

Moreover, synthesis and formulation of such drugs requires sophistication, skill and high degree of technology with precision, which renders the drug molecule costly.

In consideration of the aforesaid inconveniences of the synthetic drugs, it was thought imperative to use drugs from indigenous plant sources, which are devoid of all such side effects and cost factors.

In fact, from the literatures, it could be noted that indigenous plants used as antifertility agents are usually devoid of all these side effects. Moreover they are less expensive.

Daucus Carota seed is one of such indigenous sources which is being used by primitive tribes[6] as birth control agent.

It has already been mentioned that various organic solvent extracts have shown antifertility activities. But extraction of carrot seeds with organic solvents in large scale for commercial purpose is again hazardous and costly too considering the hazards of the solvents used in the cost intensive process and extraction Process-plant to be erected and installed.

On the basis of the above factors , the aqueous extract by decoction method and also aqueous suspension of Daucus Carota seed powder have been taken for their antifertility activity so that the processed materials are cheap and may be used by the mass at large.

MATERIAL AND METHODS

Materials

Daucus Carota seed, benzene, chloroform, acetic anhydride, diethyl ether, petroleum ether, conc. Sulphuric acid, conc. Hydrochloric acid, sodium hydroxide pellete, sodium bicarbonate, methyl alcohol, acetone (S.D.Fine Chemicals), butanol, silica gel G (BDH), glacial acetic acid(Ranbaxy Ltd, India), ammonium hydroxide, sodium sulphate, potassium iodide(E.Merck),bismuth nitrate, tartaric acid (Loba), iodine(Sarabhai M. Chern Ltd.), lead acetate(E.Merck), carbon di sulphide (Spectrachem, India), ferric chloride(S.D .Fine Chemicals), Benedict's quantitative reagent, Fehling's solution, phloroglucinol, Mayer's reagent, Dragendroff's reagent, Wagner's reagent, Hager's reagent, Ninhydrin (Tri-ketohydrindene hydrate), ethanolic α-naphthol (Molisch's reagent), potassium dichromate(Recens Chemical Industries,India).

Preparation of aqueous extract of Daucus Carota seeds

Dry seeds of carrot were collected and identified by the division of Pharmacognosy in the Department of Pharmaceutical Technology, Jadavpur University, Kolkata, where voucher herbarium specimen is preserved (Gupta, Pharm 3/91). 700 Gm. of D. Carota seed was powdered in a grinder to about 60 mesh fineness. The powder was then mixed thoroughly with 11gm. (approx.-1.5% w/w) lime and then moistened with sufficient potable water and was kept overnight in a covered glass beaker.

The moist mass was boiled with sufficient potable water for about two hours. Clear filtrate was collected by centrifugation at 6000 r.p.m. for 15 mins. The filtrate was kept separately (filtrate-I) .

The residue was again boiled with sufficient potable water for about one hour, the clear filtrate was collected by centrifugation as before and mixed with filtrate-I.

The residue was once more boiled with sufficient potable water for half an hour and the clear filtrate was collected by centrifugation as before and mixed with previous combined filtrate.

The residue was discarded.

The combined filtrate , as mentioned above, was boiled to get a final concentrate volume of 300 ml. when cooled to room temperature & 1.5 gm. of Sodium Benzoate was added to this as preservative (0.5 % w/v of final volume).

Thus, from 700 gm. of D. Carota seed powder, concentrated aqueous extract obtained was 300 ml. for subsequent testings and oral administration to the animals. It was kept in refrigerator in a tightly closed glass reagent bottle (marked as DC).

Preparation of aqueous suspension of Daucus Carota seed powder

Sufficient quantity of D. Carota seed was taken in a grinder moistened with water and Propylene Glycol q.s. It was then wet grinded to prevent loss of volatile oil . Grinding was done for sufficient time to get the slurry with seed powder of about 60 mesh fineness. Further water was added alongwith Sodium Benzoate as preservative q.s., and Tween 80, q.s. to get a final aqueous suspension of D. Carota seed powder with 0.5% w/v of Tween-80 as well as 0.5% w/v. Sodium Benzoate, which contains 10 mg. of powder in 0.5 ml. of the suspension.This stock suspension of D. Carota seed powder was kept in refrigerator in a tightly closed glass reagent bottle (marked as DC-SUSP).

The presence of various active phytoconstituents (Steroids; Flavonoids; Alkaloids; Tannins; Saponins; Reducing sugars & Pentose) in DC as well as in clear supernatant of DC-SUSP were detected by various chemical tests.

Design of animal experiments

Adult female albino mice of swiss strain (20 ± 2 gm.of body weight) and matured albino rats of swiss strain (110 ± 10 gm. of body weight) were housed in separate animal cages and kept in a room with well ventilation and with 12 hours light and 12 hours dark cycle. The animals were given standard laboratory diet food (Food pellets, Hindustan Lever Ltd., India) and had free access to drinking water.

Two different samples of D. Carota seed (DC) & (DC-SUSP) were used as oral dosage form to see their antifertility activity by observing the estrus cycle and change if any in both mice as well as rats.

Matured female albino mice and rats were taken for experiments. Before the oral feeding of the aforementioned drugs to mice and rats were started, their normal estrus cycle were checked twice daily for three consecutive cycles.
30 mice and 18 rats were divided into three groups each containing 10 mice / 6 rats.

Treatment, animal, dosage of drug and duration of treatment

Each group of animals were kept in separate cages and treatments were designed as follows.

For mice

Group1:	Control group : Normal food and water was supplied.
Group2:	0.5 ml. DC-SUSP /20 gm. body weight/day , orally for 15 days, in addition to normal food and water.
Group 3:	0.25 ml. DC /20 gm of body weight/ day, orally for 15 days, in addition to normal food and water.

For rats

Group 1:	Control group : Normal food and water was supplied.
Group 2:	1.5 ml .DC-SUSP/100 gm.of body weight /day, orally for 21 days, in addition to normal food and water.
Group 3:	0.75 ml. DC /100 gm of body weight/day, orally for 21 days, in addition to normal food and water.

Observation of estrus cycle during the treatment

During the entire period of treatment, the estrus cycle of each animal i.e. mice and rats of each group was checked twice daily at an interval of 8 hours by pipetting two drops of normal saline into and out of the vagina and placing on a glass slide in smear form for observation under microscope.

It was observed therefrom that, the estrus cycle was of regular pattern in control group before and during the treatment. Whereas the estrus cycles of the other treated animals became irregular from 7^{th} to 9^{th} day.

5 mice/ 3 rats were taken from each group and sacrificed by cervical dislocation after 24 hours of last dose of administration and 18 hours fasting. Rest 5 (five) mice/3 rats from each group were kept in separate cages to observe the restoration of the normal estrus cycle after discontinuing the drug and allowing normal food and water.

Blood samples were collected before sacrifice, in separate microcentrifuge tubes with closures containing sodium citrate as anticoagulant.

These blood samples were tested for Hematological profiles, Hepatorenal functions and metabolism and Histopathological observation of isolated organs (ovary, uterus, liver and kidney) of the untreated as well as treated animals.

Estimation of total cholesterol [44], total ascorbic acid content [45], Glucose-6- Phosphate Dehydrogenase activity [46]and Δ^5-3β- Hydroxy Steroid Dehydrogenase activity [47] in ovarian tissue of both treated and untreated animals were done.

RESULTS

The results are summarized in Tables 1 to 10

- The estrus cycle was of regular pattern in control group before and during the treatment. Whereas the estrus cycle of the other treated animals became irregular from 7th to 9th day (Tables 1-2).
- The weight of ovaries and uterus of the drug treated mice as well as rats decreased considerably (Tables 3-4).
- The results of Hematological, Hepatic and Renal parameters as obtained from the blood samples of both treated and untreated animals revealed similarity expressing no adverse effect on these parameters for both mice & rats after treatment of the animals with drug. (Tables: 7-10).
- The content of Cholesterol and Ascorbic acid of ovarian tissues had increased in the drug

Table 1: Effect of the formulations of *D. carota* seeds on the estrus cycle of matured female mice, after oral administration for 15 days

Treatment	Phase of estrus cycle during treatment days								
	7	8	9	10	11	12	13	14	15
1:Control	O/M	D/D	D/D	P/O	M/D	D/D	D/P	O/M	D/D
2: DC-SUSP(25 ml/Kg b.w.)	O/M	D	D	D	D	D	D	D	D
3: DC(12.5 ml/Kg b.w.)	D	D	D	D	D	D	D	D	D

Note : upto 6 days the estrus cycle was normal for all the groups. The table is from 7 th day showing the blocking of oestrus cycle at 'D' from 7 th day for 'DC', and from 8 th dayfor 'DC-SUSP'.
M = Metestrus; D = Diestrus; P = Pro-estrus; O = Estrus

Table 2: Effect of the formulations of *D. carota* seeds on the estrus cycle of matured female rats, after oral administration for 21 days

Treatment	Phase of estrus cycle during treatment													
	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1: Control	M/D	P/O	O/M	M/D	D/D	D/D	P/O	O/M	D/D	D/D	D/P	O/M	D/D	D/D
2: DC-SUSP (15 ml/Kg .w.)	M	D	D	D	D	D	D	D	D	D	D	D	D	D
3: DC (7.5 ml/Kg. b.w.)	D	D	D	D	D	D	D	D	D	D	D	D	D	D

Note : upto 7 days the estrus cycle was normal for all the groups. The table is from 8 th day showing the blocking of estrus cycle at 'D' from 8 th day for 'DC', and from 9 th day for 'DC-SUSP'.
M = Metestrus; D = Diestrus; P = Pro-estrus; O = Estrus.

treated animals. (Tables : 5-6)

- The inhibition of activity of the two enzymes in the ovarian tissues of the drug treated animals were observed. (Tables : 5-6)
- No abnormality was observed in the organs such as ovary, uterus, kidney and liver of drug treated animals, histologically.
- No ulceration / errotion / inflammation was

Table 3: Effect of the formulation of *D. carota* seeds on weight of body, ovary and uterus of matured female mice, after oral administration for 15 days

Design of Treatment	Body Weight (g)		Wt after Treatment (mg)	
	Before Treatment	After Treatment	Ovary	Uterus
Group I(Control)	19.24±0.12	22.28±0.08	2.29±0.03	21.26±0.17
Group II(DC-SUSP 25ml/kg)	19.89±0.16*	23.02±0.15**	1.90±0.04**	16.14±0.05**
Group III(DC 12.5ml/kg) (Values are mean ±SEM)	21.10±0.12**	24.12±0.07**	1.95±0.03**	15.10±0.06**

n**=5 in each group**

* P<0.05 as compared with control ** P<0.001 as compared with control

Table 4: Effect of the formulation of *D. carota* seeds on weight of body, ovary and uterus of matured female rats, after oral administration for 21 days (Values are mean ±SEM)

Design of Treatment	Body Weight (g)		Wt after Treatment (mg)	
	Before Treatment	After Treatment	Ovary	Uterus
Group I(Control)	112.33±1.45	132.33±1.45	72.00±1.15	177.00±1.15
Group II(DC-SUSP 15ml/kg)	115.00±1.15	132.67±1.45	60.67±1.76*	120.67±0.68**
Group III(DC 7.5ml/kg)	112.33±1.45	133.00±1.53	52.67±1.45**	112.67±1.76**

n=3 **in each group**

* P<0.05 as compared with control ** *P*<0.001 as compared with control

Table 5: Effect of the formulation of *D. carota* seeds on cholesterol and ascorbic acid content and activities of enzymes eg. G-6-PDH and Δ^5 -3β-HSD in ovarian tissue of matured female mice after oral administration for 15 days

Design of Treatment	Cholesterol (µg/mg tissue)	Ascorbic acid (µg/mg tissue)	G-6-PDH activities (IU/mg tissue)	Δ^5 -3β-HSD activies (IU/mg tissue)
Group I(Control)	45.02±0.07	69.99±0.09	3.51±0.04	0.94±0.01
Group II(DC-SUSP 25ml/kg)	79.96±0.19**	86.17±0.09**	2.09±0.01**	0.70±0.01**
Group III(DC 12.5ml/kg) (Values are mean ±SEM)	90.01±0.07**	95.09±0.10**	1.39±0.01**	0.57±0.02**

n=5 in each group * P<0.05, ** P<0.001 as compared with control, the significance were determined by ANOVA followed by Dunnett's Test.

observed visually on the inner wall of oesophagus and stomach of the drug treated animals at the same time the inner mucosal lining thereon was also found absolutely intact. All the animals showed more or less regular estrus cycle from the tenth day of discontinuation of the drug treatment.

DISCUSSION

Antifertility agents produce their action in female by either prevention of ovulation or by affecting the ovarian activities and thereby uterine cycle[10,11,12,13]. Normally antifertility activity is measured in female rats/mice by observing their ovarian activity (estrus cycle)[14, 15], when they are sexually matured, amongst other parameters/ physiological functions. Disturbances at any stage of the oestrus cycle shows effect of drug on ovarian activity indicating that the drug may be considered as antifertility agent.

The data on the above score (Tables II & III) shows positive trend of the drug being considered as antifertility agent since it affects the ovarian cycle.

Cholesterol is an obligatory precursor for progestin biosynthesis in rat, mice, rabbit and bovine luteal tissue, has already been established[16-18]. Cholesterol content in

Ovarian tissues of rat and mice vary considerably with the reproductive cycle[19-21]. Cholesterol accumulation has been observed in the atretic follicles[22], and regressing corpora lutea[23]. Leutenizing hormone administration and simultaneous increase in ovarian steroidogenesis

Table 6: Effect of the formulation of *D. carota* seeds on cholesterol and ascorbic acid content and activities of enzymes eg. G-6-PDH and Δ^5 -3β-HSD in ovarian tissue of matured female rats after oral administration for 21 days (Values are mean ±SEM)

Design of Treatment	Cholesterol (µg/mg tissue)	Ascorbic acid (µg/mg tissue)	G-6-PDH activities (IU/mg tissue)	Δ^5 -3β-HSD activies (IU/mg tissue)
Group I(Control)	50.00±0.02	67.00±0.03	3.64±0.02	1.04±0.02
Group II(DC-SUSP 25ml/kg)	76.00±0.03**	78.39±0.02**	2.70±0.01**	0.78±0.01**
Group III(DC 12.5ml/kg)	88.01±0.04**	85.32±0.03**	1.80±0.01**	0.60±0.01**

n=3 in each group * P<0.05, ** P<0.001 as compared with control, the significance were determined by ANOVA followed by Dunnett's Test.

Table 7: Effect of the formulation of *D. carota* seeds on Haematological parameters of matured Female mice, after oral administration for 15 days (Values are mean±SEM)

Design of treatment	RBC (Count × 10^6/mm^3)	WBC (Count × 10^3/mm^3)	Hb (gm/dl)	ESR (mm/hr)	Clotting Time(sec)
Group I(Control)	6.47±0.04	4.77±0.03	13.21±0.07	1.69±0.01	34.96±0.20
Group II (DC-SUSP 25ml/kg)	6.64±0.04	4.80±0.01	13.09±0.04	1.59±0.0*	35.98±0.09 *
Group III(12.5ml/kg)	6.31±0.16	4.76±0.07	12.94±0.11	1.72±0.03	35.88±0.10*

n=5 in each group

P *<0.05 as compared with control*

Table 8: Effect of the formulation of *D. carota* seeds on Hepatorenal parameters of matured female mice, after oral administration for 15days.
(Values are mean ±SEM)

Design of Treatment	Plasma Cholesterol (mg/dl)	SGOT protein (IU/L)	SGPT (IU/L)	Serum Bilirubin (mg/dl)	Serum ALP (IU/L)	Blood Urea (mg/dl)	Creatinine (mg/dl)	Plasma (gm/dl)
Group I(Control)	31.20±1.07	21.02±0.13	45.20±0.24	034±0.01	125.80±0.58	33.92±0.12	0.35±0.01	5.00±0.02
Group II(DC- SUSP 25ml/kg)	28.98±0.16	20.28±0.22*	44.92±0.12	0.34±0.01	127.00±1.00	32.84±0.26*	0.32±0.01	5.07±0.03
Group III(DC 12.5ml/kg)	29.04±0.19	19.30±0.12**	44.42±0.21*	0.33±0.01	125.00±0.71	32.10±0.19**	0.33±0.01	5.14±0.03*

n=5 in each group
* P<*0.05 as compared with control*
** *P*<0.001 as compared with control

is associated with depletion of ovarian cholesterol and further, cholesterol ingestion causes stimulation of sexual activity[24].

The role of ascorbic acid in gonadal steroidogenesis has been explained by Deane[25] where the author has demonstrated that increased consentration of ascorbic acid occurs in the non functional or hypofunctioning ovaries.

Dey *et al*[26] noticed an increased concentration of cholesterol and ascorbic acid in the hypofunctioning ovaries.

Works relating to result of ovarian stimulation with some gonadotrophic hormones on ascorbic acid depletion from ovary[27,28], and result of reduction/abolition of gonadotrophic hormones secretion on atrophy of ovary[40] are also available. Thus, in the present investigation, the accumulation of cholesterol and ascorbic acid in the ovaries of the drug treated animal, indicate the hypofunction of the ovary (hypofunctioning of steroidogenic activity of the ovary).

Mckerns[29,30] have shown that gonadotrophins, through the activation of Glucose-6-Phosphate metabolism, increase the rate of production of NADPH, which is essential for the hydroxylation reaction in the formation of gonadal steroids from cholesterol in the ovarian tissue. Thus, G-6-P is an essential factor in ovarian steroidogenesis[31].

Brandau et al[32] have also shown that in bovine corporalutea, a close positive correlation exist between progesterone synthesis and the heightened activities of Δ^5 -3b-Hydroxy Steroid Dehydrogenase(HSD) and G-6-P in the luteal tissues. Knorr[33] established that Δ^5 -3β-HSD is an important enzyme in the production of steroid hormones. The presence of the enzyme suggests the steroidogenic activity of the tissue and estrogen synthesis in increased amount is associated with heightened Δ^5-3β-HSD and G-6-P-Dehydrogenase activities in the follicular granulose cells of the polycystic ovaries and in the ovaries of mature rats/ mice on the second day of Dioestrus[34,35].

Table 9 : Effect of the formulation of D. Carota seeds on Haematological parameters of matured female rats, after oral administration for 21days (Values are mean ±SEM)

Design of	RBC (Count × 10^6/mm^3)	WBC (Count ×10^3/mm^3)	Hb (gm/dl)	ESR (mm/hr)	Clotting Time(sec)
Group I(Control)	5.58±.04	3.66±.02	12.70±0.08	2.05±0.05	35.77±0.14
Group II(DC-SUSP 15ml/kg)	5.54±0.03	3.69±0.01	12.71±0.02	1.93±0.01	34.66±0.33
Group III (DC 7.5ml/kg)	5.48±0.06	3.72±0.04	12.46±0.20	1.85±0.03*	35.00±0.58

n=3 in each group

* P<0.05 as compared with control

** *P*<0.001 as compared with control

Table 10: Effect of the formulation of D. Carota seeds on Hepatorenal parameters of matured female rats, after oral administration for 21days (Values are mean ±SEM)

Design of Treatment	Plasma Cholesterol (mg/dl)	SGOT(IU/L)	SGPT(IU/L)	Serum Bilirubin (mg/dl)	Serum ALP (IU/L)	Blood Urea (mg/dl)	Creatinine (mg/dl)	Plasma protein (gm/dl)
Group I(Control)	44.68±0.33	41.10±0.21	56.10±0.21	0.36±0.01	171±2.08	24.66±0.88	0.43±0.01	6.67±0.06
Group II(DC-SUSP 15ml/kg)	45.66±0.33	41.33±0.88	58.53±0.26*	0.33±0.01	158.66±0.86*	27.00±0.58	0.45±0.02	6.19±0.02*
Group III(DC 7.5ml/kg)	45.00±0.57	40.50±0.28	53.67±0.44*	0.34±0.02	154.67±2.40*	26.33±0.67	0.35±0.02*	5.97±0.09**

n=3 in each group

* P*<0.05 as compared with control*

** *P*<0.001 as compared with control

Mazumder et al[36,37,38,39] established reduction of gonadal steroidogenesis in animals (mice and rats) due to inhibition of enzyme activities and accumulation of substrate (cholesterol and ascorbic acid) when treated with Methanol extract of some indigenous plants.

The present work indicated that the weight of the ovary and uterus of treated animals has reduced considerably (Tables 3-4). Further, biochemical results exhibited that while there is accumulation of the substrates i.e. ascorbic acid and cholesterol in the ovary and uterus. There is considerable reduction in the enzymatic activities (Δ^5-3β-HSD and G-6-P) with the administration of both DC & DC-SUSP.

It may therefore be concluded from the above observations that both DC & DC-SUSP produces inhibition of steroidogenesis due to accumulation of substrates and inhibition of enzyme activity . Therefore, DC and DC-SUSP may be considered as antifertility agents .

Presence of flavonoids & steroids amongst other phytoconstituents was found positive in DC. Since various flavonoids have been reported[41-43] to possess antifertility activity , the antisteroidogenic property of DC & DC-SUSP might be due to the presence of such compounds also,

After withdrawing drug treatment, the irregular oestrus cycle of both rats/mice reverted to normalcy from tenth day.

Biochemical parameters, Haematological profiles as well as Histopathological observations remained more or less normal in groups II & III, for both mice and rats.

It is therefore deduced that there is no permanent damage on the ovarian activity by the treatment with DC & DC-SUSP .

Further work along this line will hopefully generate low cost and effective therapeutic agent.

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