



ABORTIFACIENT EFFICACY OF *TRICHYSPERMUM AMMI* SEED EXTRACT IN FEMALE ALBINO RATS.

Vandana Deshmukh¹, Varsha Zade^{2*}

- 1 Vandana Deshmukh, Research student, Government Vidarbha Institute of Sciences and Humanities, Amravati, India.
- 2 Dr. Varsha S. Zade, Associate Professor, Government Vidarbha Institute of Sciences and Humanities, Amravati, India.

Abstract: Objective: To study the potential abortifacient activity of the *Trichyspermum ammi* seed extract (aqueous, alcoholic and petroleum ether) in female albino rats.

Methods: Pregnant rats weighing 120 to 200 gm were randomized into 10 groups. Rats were laprotomised on 10th day of pregnancy and live fetuses were observed in both the horns of the uterus. Rats in group 1 (control) were orally administered, with 0.5 ml of distilled water once daily while those in group 2 to 10 (experimental groups) were administered 100, 200 and 400 mg/kg body weight doses of aqueous, alcoholic, ether extract of the *Trichyspermum ammi* seed respectively. The doses were administered from day 11th to 15th of pregnancy of rats and then the animals were allowed to go full term. **Results:** All the three extracts of *Trichyspermum ammi* seed exhibited abortifacient activity (33.61- 100%). The % of abortion was found to be highest (100%) with 400 mg/kg dose of alcoholic and ether extract of *Trichyspermum ammi* seed. There was reduction in the level of FSH and LH hormone, while level of estrogen increased however there was slight decrease in the level of progesterone hormone. The estrous cycle in rats treated with aqueous, alcoholic and ether extract reduced duration of estrous and metestrous phases and on the other hand it was also characterized by a prolongation of the diestrous phase. **Conclusion:** The study has provided evidence for the abortifacient activity of all the three extracts of *Trichyspermum ammi* seed. However the abortifacient properties were found to be more pronounced at dose of 400, 400 and 100 mg/kg body weight aqueous, alcoholic and ether extract respectively.

Keywords: Abortifacient activity, *Trichyspermum ammi*, Post Implantation loss, Resorption, Estrous

For Correspondence:

zvarsha27@gmail.com

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Introduction:

India within a few year of time span will be the leading country as far as the population growth is concerned. Since the population is rising tremendously, this may affect drastically the economic growth of India. Family planning has been promoted through several methods of contraception, but due to side effects produced

by the use steroidal contraceptive [1] and use of the abortifacient drugs, there is need of a drug which is effective with lesser side effects.

Antifertility agents obtained from indigenous medicinal plants would be of immense benefit especially to the inhabitants of developing countries, since the cost of these drugs would be within their means [2]. The antifertility plant with estrogenic property can directly influence pituitary action through peripheral modulation of lutenizing (LH) and Follicle stimulating hormones (FSH) by decreasing the secretion of these hormones and blocking ovulation [3].

Trachyspermum ammi, commonly known as ajowan or ajwain,[4] bishop's weed,[5] ajowan caraway, carom seeds, or thymol seeds, or vaamu in Telugu or omam (ஔமம்) in Tamil, is a plant of India, Pakistan and the Near East whose seeds are used as a spice.[6]*Trachyspermum ammi* originated in the Middle East, possibly in Egypt, and the Indian subcontinent, but also in Iran and Afghanistan.. In India, the major producers are the states of Rajasthan and Gujarat, with Rajasthan producing about 90% of India's total output. *Trachyspermum ammi* is traditionally believed to be a digestive aid. ^[citation needed]

The seeds contain 2–4.4% brown colored oil known as ajwain oil. The main component of this oil is thymol, which is used in the treatment of gastro-intestinal ailments, lack of appetite and bronchial problems. The oil exhibits fungicidal, [7] antimicrobial [8] and anti-aggregatory effects on humans. [9] Ajwain is a ^{traditional} potential herb and is widely used for curing various diseases in humans and animals. The fruit possesses stimulant, antispasmodic and carminative properties. It is an important remedial agent for flatulence, atonic dyspepsia and diarrhea.[10] The seed of ajwain is bitter, pungent and it acts as anthelmintic, carminative, laxative, and stomachic. It also cures abdominal tumors, abdominal pains and piles.[11] Seeds contain an essential oil containing about 50% thymol which is a strong germicide, anti-spasmodic and fungicide. Thymol is also used in toothpaste and perfumery.[12] The present

work was under taken to evaluate the abortifacient role of *Trichyspermum ammi* seeds in abino rat.

Materials and Methods

Collection of plant material

The seeds of *Trichyspermum ammi* plant were collected from Shendurajanaghat of Amravati district during seedling period of September to December, identified and authenticated by experts from Botanical Survey of India, Pune where, a voucher specimen of the plant has been deposited in the herbarium of the department. (Accession No. VD –2).

Procurement and rearing of experimental animal

Healthy wistar strain female albino rats of about two month old and weighing 120- 200 g were procured from Sudhakarrao Naik Institute of Pharmacy, Pusad (Maharashtra). The rats were housed in polypropylene cages and maintained under environmentally controlled room provided with a 12:12 hour light and dark cycle approximately at 25 °C. They were fed on pellets (Trimurti Lab Feeds, Nagpur) and tap water ad libitum. The rats were allowed to acclimatize to laboratory environment for 15 days before experiment. Experimental protocols were subjected to the scrutinization and approval of Institutional Animal Ethics Committee registration number 1060/ac/07/CPCSEA (IAEC/7/2009)].

Preparation of extract

The of seed *Trichyspermum ammi* were collected, shade dried, powdered and subjected to soxhlet extraction successively with distilled water, ethanol, and petroleum ether. The extract was evaporated to near dryness on a water bath, weighed and kept at 4 °C in refrigerator until further use.

Phytochemical screening

The presence of various plant constituents in the plant extract was determined by preliminary phytochemical screening as described by Thimmaiah. [13]

Acute toxicity study

Healthy female albino rats were starved for 3- 4 h and subjected to acute toxicity studies as per Organization of Economic Co-operation

and Development (OECD) guidelines [14] No: 423 and a highest doses up to 4000 mg/kg of aqueous, Alcoholic & petroleum ether extract was selected for treatment. The rats were observed continuously for 2 hrs for behavioural, neurological and autonomic profile and next 24 and 72 hrs for any lethality or death. Extract of different doses was administered and animal were observed continuously for behavioral, neurological and autonomic profiles and for 24 and 72 hrs for any lethality or Death. [15]

Abortifacient activity

Caged with males of proven fertility in the ratio of 2:1, in the evening and examined the following day for the evidence of copulation. Rats exhibiting thick clump of spermatozoa in their vaginal smear were separated and that day was designated as day 1 of pregnancy. These rats were randomly distributed into 13 groups, 1 control group and 12 experimental groups of 6 animals each. On the plant extracts were tested in female albino rats for abortifacient activity as per Khanna et al [16]. The female rats in proestrous phase were the day 10 of pregnancy animals were laprotomised under light ether anesthesia using sterile conditions. The two horns of uteri were examined to determine the implantation sites. There after the abdominal wound was sutured in layers. The extract to be tested then fed to operated pregnant rats i.e. aqueous extract, alcoholic extract, ether extract at doses of 100, 200, 400 mg/kg body weight *Trichyspermum ammi* seed (one tenth of the highest tolerable dose) once daily by an intragastric (i. g.) soft rubber catheter from day 11 up to the 15th day of pregnancy. The animals were allowed to go full term. After delivery the pups were counted and the abortifacient activity of extract was evaluated. The following parameters were computed: number of live and dead fetuses; % survival ratio= (number of live fetus/number of live+ dead fetus) ×100; resorption index= (total number of resorption sites/total number of implantation sites) ×100; postimplantation loss= (number of implantations-number of live fetuses/ number of implantations) ×100. The variations in birth weight of litters and gestation period between

control and experimental animal were also determined to check the abortive effect of *Trichyspermum ammi* seed extract [17].

Estrous cycle

The aqueous (400 mg/kg), alcoholic (400 mg/kg) and ether extract (100 mg/kg body weight of *Trichyspermum ammi* seed extract was found to be active amongst the three treatments in antifertility testing of. Hence it was subjected to a detailed investigation for study of estrous cycle. The studies were conducted on adult female rats (140-180 gm) for 30 days. To study the estrous cycle pattern, animal showing regularity in the normal cycle were separated and chosen for further studies. Those animals showing normal estrus cycle were divided in two groups of 6 animals each; Group I- control, received distilled water (Vehicle) and Group II-III, IV treated, received aqueous, alcoholic, extract at dose of 400 mg/kg body weight and 100 mg/kg body weight of ether dose. Vaginal smear using saline solution were taken twice daily during the entire treatment period, observation of the vaginal opening and the cell type obtained in a vaginal smear was also done. The duration of estrous cycle together with that of various phases was determined [18-20].

Effect on hormonal level

The aqueous, alcoholic, ether extract of *Trichyspermum ammi* seed was found to be most active amongst the four treatments in the abortifacient testing. Hence rats treated with aqueous extract were subjected to a detailed investigation for the study of hormonal assay. Sexually experienced female albino rats were divided into 4 groups of 6 animals each; Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Group 2- 4 received suspension of the aqueous, alcoholic, ether extract of *Trichyspermum ammi* seed orally at the dose 400, 400 and 100 mg/kg, daily for 21 days respectively. After 21 days of treatment all the control and experimental groups of female rats sera were analyzed for estrogen, progesterone, luteinizing and follicle stimulating hormone level with Accu Lite master CLIA VAST Enabled kit by

Chemiluminescence immunoassay (CLIA) method with semi automated Chemiluminescence analyzer and autoplex- A processor for CLIA [21- 22].

Hormonal assay

The animals were anesthetized with ether; blood samples were collected directly from their hearts and centrifuged at 2000-3000 rpm for 15 minutes at 4°C. Serum samples were stored at -20°C until assayed for FSH. Serum concentrations of FSH were measured by the chemiluminescence immunoassay (CLIA) method.

Statistical analysis

All the data are expressed as mean \pm SEM (Standard error). Statistical analysis was done by Student's t-test and one way ANOVA [23].

Results

Phytochemical Screening:

Preliminary phytochemical screening of the *Trichyspermum ammi* seed extract revealed the presence of alkaloids, flavonoids, steroids, tannins and anthraquinone where as saponines and simple phenolics were not detected (Table-1).

Acute Toxicity Study:

Clinical toxicity symptoms such respiratory distress, salivation, weight loss and appearance of hair as well as maternal mortality were not detected. No lethality or behavioral changes were observed 7 days after the treatment of the rats with the doses 4000 mg/kg body weight. Hence, 1/10th of this dose i.e. 400 mg/kg body weight, ten times less than 4000 mg/kg was used as maximal dose for the rest of the experiments.

Anti-Fertility Activity:

The administration of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight of the aqueous, alcoholic, petroleum ether extract of *Trichyspermum ammi* seed also induced vaginal opening in experimental extract resulted in 100% abortion. The aqueous, extract administration of 100 mg/kg, 200 mg/kg 400 mg/kg body weight showed 34.61%, 41.37% and 70% fetus abortion respectively. The alcoholic extract administration of 100 mg/kg,

200 mg/kg 400 mg/kg body weight showed, 33.92%, 86.79% and 100% fetus abortion respectively. Whereas administration of 100 mg/kg of petroleum ether extract showed 100% fetus abortion. The percent resorption index increased from zero in the control animals to 70% and 100% in 400 mg/kg body weight of aqueous and alcoholic extract treated animals and 100mg/kg body weight in ether extract dose.

There was a decrease in litter size with increase in the dose of the plant extract of *Trichyspermum ammi seed* extract in all the treatment groups. The litter size of control group rats was the highest (6.86 \pm 0.50). The litter body weight recorded in animals administered with alcoholic, aqueous and ether extract of *Trichyspermum ammi seed* were not significantly different from control. The AGD to CRL ratio of the litter of rats dosed with various plant extract at doses of 100, 200 and 400 mg/kg body weight of aqueous, alcoholic, petroleum ether extract were significantly similar to that of control group. Similarly, the total body length of litters at day 1 of birth also did not vary significantly from that of control. When the sex ratios of litter were determined it was found that the male sex was dominant to female sex. The gestation period did not show any variation in extract treated group of animals as compared to control group (Table 3).

Effect of Extract on the Estrous Cycle of Rats

The present study indicated that the aqueous, alcoholic, ether extract of *Trichyspermum ammi* seed were responsible for the anti fertility effect. Rats showing routine estrous cycle were used. Treatment of rats with *Trichyspermum ammi* seed extracts for 5 days prolonged the estrous cycle significantly compared to control as indicated in Table 2. The estrous cycle in rats treated with aqueous, alcoholic, ether extract reduced duration of proestrus and estrous phases and on the other hand it was also characterized by a prolongation of the metestrus and diestrus phase.

From the above observation it is seen that administration of extract of *Trichyspermum*

ammi seed caused suppression of the estrous phase in female albino rats in a dose dependent, reversible manner different phases of estrous cycle are shown in. figure

Hormonal assay

In the present study after the administration of aqueous, alcoholic, and ether extract of *Trichyspermum ammi* seed at dose of 400mg/kg, 400mg/kg and 100mg/kg body weight respectively, significant alteration was accounted in the concentration of steroid hormones such as serum estrogen, progesterone, LH, FSH. Administration of rats with aqueous, alcoholic and ether

extract of *Trichyspermum ammi* seed at a dose of 400mg/kg, 400mg/kg and 100mg/kg body weight respectively showed a reduction in significant level of LH and FSH as compared to control rat. On the other hand, it was observed that the level of estrogen increased and there was a slight decrease in the progesterone hormone in the extract treated animals (table-5). This results suggest that high dose of estrogen disproportionate to progesterone leads to resorption of fetuses, this effect may due to the imbalance of estrogen and progesterone level.

Table 1. Phytochemical profile of *Trichyspermum ammi* seed extract

Name of the plant	Alkaloids	Simple Phenolics	Steroids	Anthraquinone	Flavonoids	Tannins	Saaaponins
<i>Trichyspermum ammi</i>	+	-	+	+	+	+	-

+ Present, - Absent

Table 2. The effect of aqueous, alcoholic, ether seed extract of *Trichyspermum ammi* seed on fertility of rats when fed orally from day 11 to 15 of pregnancy.

Treatment groups (dose, mg/kg body wt)	Sample size	No. of foetus observed in individual rats on day 10	No. of rats delivered (litter size)	No. of resorption in individual rats	No. of resorption (mean±SE)	Abortifacient activity (%)	
Control	Vehicle	6	6(10,8,8,8,9,12)	6(10,9,8,8,9,12)	0,0,0,0,0,0	0	Nil
Aqueous Extract of <i>Trichyspermum ammi</i> seed	100	6	6(6,9,10,6,7,11)	6(4,6,8,7,4,5)	2,3,3,3,3,4	3±0.63	34.61%
	200	6	6(8,11,9,12,10,8)	6(4,6,5,7,7,5)	4,5,4,5,3,3	4±0.36	41.37%
	400	6	6(6,8,7,10,10,9)	6(2,3,2,33,2)	4,5,5,7,7,7	5.83±0.54**	70%
Alcoholic extract of <i>Trichyspermum ammi</i> seed	100	6	6(6,9,7,12,12,10)	6(3,6,6,7,7,8)	3,3,1,5,5,2	3.16±0.65	33.92%
	200	6	6(9,9,7,8,10,10)	2,1,0,1,2,1	7,8,7,7,8,9	7.66±0.33***	86.79
	400	6	6(7,6,8,8,9,12)	00,0,0,0,0,0	7,6,8,8,9,12	8.33±0.84	100%
Petroleum ether extract of <i>Trichyspermum ammi</i> seed	100	6	6(6,4,9,9,8,7)	0,0,0,0,0,0	6,4,9,9,8,7	7.16±0.79	100%

Values are expressed as mean ± S.E. for six albino rats in each group. P values: * < 0.05, ** < 0.01, *** < 0.001, When compared between group, ns = non significant

Table 3. Effect of aqueous, ethanol, ether and extract of *Trichyspermum ammi* seed on fertility of female albino rats.

Parameters								
Treatment Groups		Gestation period (days)	Litter size (No.)	Litter body Weight (gm)	Total body length of litter at 1 st day of birth (mm)	Sex ratio of live fetuses (Male/female)	% Viable fetuses	% Fetuses resorptions
Control Group-I (Vehicle)		22.64±0.01	6.86±0.50	5.73.46±0.06	59.9 ±0.01	24/21	100	0
Aqueous Extract of <i>Trichyspermum ammi</i> seed	Group-II 100 mg/kg B.W	22.83±0.01 ^b	6.64±0.40 ^b	5.05±0.01 ^a	49.05±0.009 ^c	22/21	65.39	34.61
	Group-IV 200 mg/kg B.W	22.44±0.01 ^a	7.01±0.01 ^b	4.89±0.01 ^a	54.04±0.01 ^c	20/22	58.63	41.37
	Group-II 400 mg/kg B.W	22.34±0.01 ^b	6.25±0.01 ^c	4.24±0.01 ^c	53.44±0.01 ^c	18/17	30	70
Alcoholic Extract of <i>Trichyspermum ammi</i> seed	Group-II 100 mg/kg B.W	22.64±0.39 ^c	5.55±0.01 ^b	5.34±0.01 ^b	60.64±0.009 ^b	21/21	66.08	33.92
	Group-III 200 mg/kg B.W	22.55±0.01 ^c	5.26±0.01 ^a	6-06±0.01 ^a	59.50±0.01 ^b	15/12	13.31	86.79
	Group-IV 400 mg/kg B.W	0	0	0	0	0	0	100
Ether Extract of <i>Trichyspermum ammi</i> seed	Group-II 100 mg/kg B.W	0	0	0	0	0	0	100

values are expressed as mean ± S.E. for six albino rats in each group. P values: a=* < 0.05, b=** < 0.01, c=*** < 0.001, When compared with control, ns= non significant

Table 4. Effect on estrous cycle of female albino rats after the administration of aqueous (400mg/kg), alcoholic (400mg/kg), and ether (100 mg/kg) extract of *Trichyspermum ammi* seed

Phases	Proestrous phase (days)	Estrous phase (days)	Metaestrous phase (days)	Diestrous phase (days)	Estrous cycle (days)
Vaginal opening/ cell type obtained in a vaginal smear	25% to 40% / Epithelial cells only	Above 70% / Few cornified cells	50% to 70% / Cornified cells plus many leukocyte	50% to 70% / Leukocytes plus epithelial cells	
Group- A- Control	0.59±0.01	0.65±0.01	0.59±0.01	0.65±0.01	4.42±0.68
Group-B Aqueous Extract400mg/kg	0.44± 0.09***	0.54± 0.08*	0.74± 0.01***	2.57± 0.01**	4.29± 0.01***
Group-C ether Extract 400 mg/kg	0.51± 0.09**	0.58± 0.01***	0.74± 0.08**	3.60± 0.01***	5.42± 0.01**
Group-D Petroleum Extract 100mg/kg	0.53± 0.09***	0.59± 0.02***	0.69± 0.04**	3.09± 0.01***	4.90± 0.01

Values in Mean±S.E. (Standard error), n=6, *P<0.05, **P<0.01, ***p<0.001, when compared with control.

Table 5 Effect of with aqueous, alcoholic and ether extract of *Trichyspermum ammi* seed extract at dose 400, 400 mg/kg (aqueous, alcoholic)and 100mg/kg(ether) on hormone profile of female albino rats.

Treatment Groups	Dose mg/kg	FSH(pg/ml)	LH(pg/ml)	Estrogen(pg/ml)	Progesterone(pg/ml)
Group I-Control	Vehicle	1.93 ± 0.05	0.41 ± 0.05	85.42 ± 0.06	57.33 ± 0.04
Group II - aqueous e xtract of <i>Trichyspermum ammi</i> seed	400mg/kg	1.87 ± 0.01	0.25 ± 0.01**	83.52 ± 0.03***	42.76 ± 0.03***
GroupIII- Alcoholic <i>Trichyspermum ammi</i> seed	400mg/kg	1.74 ± 0.03***	0.35 ± 0.02**	48.59 ± 0.05***	35.83 ± 0.03***
Group IV- Ether extract <i>Trichyspermum ammi</i> seed	100mg/kg	1.37 ± 0.01***	0.22 ± 0.02***	56.34 ± 0.02***	41.33 ± 0.02***

P values: *<0.05, **<0.01, ***<0.001, When compared with control, ns= non significance.

Discussion

Flavonoids have been reported to possess antifertility activity [24, 25, 26, 27, 28]. In the present study the antifertility activity of the extract may be due to the presence of flavonoids or other constituent in it. According to the literature, flavonoids and saponins are known to exhibit antifertility activity. [29, 30, 31] The *Trichyspermum ammi* seed extract displayed significant antifertility activity when compared with controls, indicating that flavonoids could be responsible for the activity.

Our result were corroborated with the findings of ethanolic extract of root powder of *Cassia occidentalis*, *Derris brevipes* variety *Brevipes* and *Justica simplex* which showed 100% abortifacient activity at 600 mg/kg body weight [32]. Alcoholic extract of *Plumeria rubra* at a dose of 200 mg/kg resulted in 100% abortifacient effect in female albino rats [33]. The present investigation revealed that the extract with aqueous, alcoholic and ether extract of *Trichyspermum ammi* seed extract at dose 400, 400 mg/kg (aqueous, alcoholic) and 100mg/kg(ether) exhibit abortifacient activity ranging from 70% to 100% and 100% respectively. Our result also corroborate with antifertility activity of methanolic extract of three varieties of *Ricinus communis* Linn^[34] and Similar finding were reported by Yakubu, et al [35] using *Senna alata* leaves.

The absence of clinical toxicity symptoms in the treated female rats such as tremors, weakness, and refusal of feeds, diarrhea, weight loss, hair loss, coma and death suggests that the extract was not clinically toxic to the female rats.

An estrous cycle is a rhythmic reproductive cycle in sexually matured female mammals and is influenced by the release of gonadotropin releasing hormone from the hypothalamus, gonadotropins from the pituitary gland and sex hormones from the gonads. While female cyclicity characterized by vaginal changes as observed in estrus cycle is an index of good functioning of the neuroendocrine – reproductive system and ovarian activity, loss of

normal estrus cycle indicates the disruption of ovarian progesterone and estrogen balance [36, 37, and 38]. The presence of particular cell types indicates the follicular and luteal phases of the reproductive cycle. Observation of vaginal smear to monitor the estrous cycle of albino rats also indicated an alteration of estrus cycle and disruption of ovarian endocrine function [39, 40]

In the present study, *Trichyspermum ammi* seed treated rats showed a decrease in the duration of proestrous and estrous and, while it increased the duration of metestrus and diestrus. It has been evaluated as presence of phytochemicals like tannin, flavanoids, saponins, steroids in the cynodon extract might be a contributing factor in disruption of estrous cycle [41]. Estrogenic chemicals are known to cause infertility by shortening the time of transport of egg, disrupting estrous cycle, lowering the plasma progesterone and decreasing pregnanediol which finally stops development of endometrium [42,43]

It is well known fact that estrogenic substances inhibit pregnancy by suppressing the level of both follicular stimulating hormone (FSH) and luteinizing hormone (LH), which in turn prevent the implantation. Estrogen and progesterone are the hormones responsible for histology and functional modifications of female genital tract [44].

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