

ORIGINAL RESEARCH ARTICLE

Antifertility Effect Of *Trachyspermum ammi* (Linn) Sprague Fruits On Male Rats

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ABSTRACT

Trachyspermum ammi (Linn.) Sprague fruits have traditionally been used in India as medicinal plant for the treatment of various diseases in both humans and animals such as spermicidal activity. *Trachyspermum ammi* is a small plant belongs to the family of umbelliferae rich in volatile oil (thymol). In the present study ethanolic extract of *Trachyspermum ammi* fruits are tested on male reproductive system. Animals are pretreated with ethanolic extract of *Trachyspermum ammi* at four different doses such as 100mg/kg, 200mg/kg and 400mg/kg for a period of 60 days with the recovery group animals for 120 days at the dose of 400mg/kg. Parameters such as testes weight, sperm count, sperm motility, sperm morphology and histopathological examination of the testis are carried out. The study revealed that the drug posses significant male anti-fertility effect dose dependently. The recovery group reverts back the elevated parameters by increasing the decreased testis weight, sperm count, sperm motility, decrease in production of abnormal sperms and restoring the cellular pattern of the testis. These findings indicate that *Trachyspermum ammi* fruit extract is a very good choice of male anti-fertility activity drug which can be formulated as a male contraceptive formulation.

Key Words: *Trachyspermum ammi*, Umbelliferae, Thymol and Anti-fertility.

INTRODUCTION

Trachyspermum ammi (Linn) Sprague, well known member of the umbelliferae family was found to be throughout India. In India the fruits were used for stimulant and carminative properties and was regarded as an antispasmodic. It is an important remedial agent for flatulence, atonic dyspepsia, and diarrhea (Bentely and Wrimen., 1999).

Fertility regulation comprising contraception and management of infertility forms an important component of reproductive health (Allag. I.S and Rangari. K., 2002). As per the WHO statistics, India stands second among the world countries with a total

Population share of 17.15% next to China, just below a margin of 2.5%. The biggest curse to poor countries is uncontrolled growth of population resulting poverty. Over 70% of 6.2 billion-world populations live in poor countries alone. Family

planning program failure has become joke to youth for naughty entertainment. It has failed to serve its purpose due to inefficient implementation or communication in poor countries. Sex education should be developed in a pattern that family planning becomes boon for them instead of sin. This can be achieved by promoting the need and necessity of family planning devices such as contraceptives and others.

With a view of this, the commonly available drug ajowan's (*Trachyspermum ammi*) role in utilization as a male antifertility drug has been studied here on the basement of available traditional literatures.

2.0 MATERIALS AND METHODS

2.1 Animals:

Male wistar rats (175-225g) of approximately same age used in the present studies were

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procured from Central Animal facility, SAstra University, Thanjavur, India. The animals were fed with standard pellet diet and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours in darkness and light. The animals were acclimatized to the laboratory condition for a period of one week before starting the experiment. The experiment protocols were approved by Institutional Animal Ethics Committee after securitization. The animal received the drug treatment by oral gavage tube.

2.2 Preparation of the extract:

Air dried fruits of *Trachyspermum ammi* were pulverized and extracted with 95% ethanol in a Soxhlet extractor. The extract was concentrated in a rotary flash evaporator to yield a residue. The percentage yield of the extract was found to be 06.20% w/w.

2.3 Phytochemical evaluation:

Trachyspermum ammi alcoholic extract was subjected to qualitative analytical test for the detection of various chemical constituents viz. Alkaloids, steroids, carbohydrates, fixed oils, glycosides, tannins, proteins, saponins and flavonoids.

2.4 Pharmacological evaluation:

2.4.1 Acute toxicity study:

The dose fixed for the study is based on OECD 425 guidelines. Based on this one medium, low and high doses were selected for the study as 100mg/kg b.wt, 200mg/kg b.wt, 400mg/kg b.wt and a recovery group with 400mg/kg b.wt.

2.4.2 Screening of *Trachyspermum ammi* alcoholic extract for male anti-fertility effect:

Proven fertile male rats were taken and divided into 5 groups of 8 animals each. The animals received the following treatments.

Group1- Received only food and water.

Group2- Alcoholic extract of *Trachyspermum ammi* - 100mg/kg/day - for 60 days

Group3- Alcoholic extract of *Trachyspermum ammi* - 200mg/kg/day - for 60 days

Group4- Alcoholic extract of *Trachyspermum ammi* - 400mg/kg/day - for 60 days

Group5- Alcoholic extract of *Trachyspermum ammi* -400mg/kg/day - for 60 days, followed by recovery for 60 days (Recovery group).

2.4.3 Sperm motility and sperm count:

Sperm motility and sperm count were assessed using haemocytometer. 100mg of each tissue was minced in 1 ml of physiological saline. For sperm motility and sperm count, 1 drop of evenly mixed

sample was applied to haemocytometer with improved double Neubauer ruling. The counts of the 4 chambers were averaged (Rita de Cassia da S. et al.2000)

2.4.4 Sperm morphology:

1 drop of the sperm suspension is suspended in 5ml of phosphate buffered saline in a petridish and gently shaken for 15 min at 37°C. The suspension was passed through a nylon mesh to separate the tissue from sperm. The sperm isolated in this manner were examined under the microscope (Mortimer D.1994.).

2.4.5 Histological examination of Testis:

Testes were fixed by immersing Bouin's fixative, processed for histological section cutting, embedded in paraffin and 6µm sections were cut using a microtome and mounted on glass slides. Deparaffinized sections were stained with eosin and hematoxylin observed microscopically (Sujit K Bhowal et al.2008)

2.5 Statistical analysis:

Data's were expressed as means ± SEM and were analyzed using ANOVA. A "p" value < 0.05 was considered as statistically significant.

3.0 RESULTS AND DISCUSSION

Phytochemical evaluation of *Trachyspermum ammi* shows the presence of Carbohydrate, glycoside, proteins, volatile oils and Tannins.

The effect of alcoholic extract on testes was checked by taking the weights of both left and right testis. Both left and right testes were decreased in weight dose dependently, clearly marking the constriction of cellular pattern of the organ. The decreased organ weight exhibits a negative effect of the extract over the reproductive organ, which may accumulate towards the antifertility property. The results of the testes weight among the groups were shown in (Table 1) and (Fig 1).

Table 1: Effect of alcoholic extract of *Trachyspermum ammi* on testes weight

Group No.	Treatment	Weight of testes (gm/100gm body weight)
1	control	1.75 ± 0.0682
2	100mg/Kg	1.46 ± 0.1012
3	200mg/Kg	1.10 ± 0.0596***
4	400mg/Kg	0.86 ± 0.0229***
5	400mg/Kg(Recovery)	1.20 ± 0.0940***

All figures are mean ± SEM n=8

***p< 0.001 vs. control

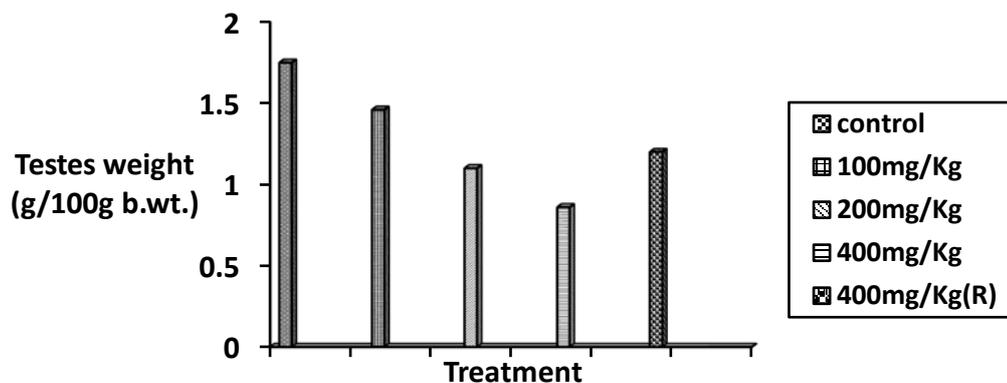


Figure 1: Effect of alcoholic extract of *Trachyspermum ammi* on testes weight

Assessment of alcoholic extract of *Trachyspermum ammi* over sperm count exhibits decrease in sperm count number as compared to the normal group. The results were exhibited in (Table 2) and (Fig. 2). The decrease in sperm count number may attribute towards the effect on the androgen binding protein (ABP) of sertoli cells, (Steinbergere., 1975) via FSH, there by interfering with sperm maturation and release.

Differentiation of primordial germ cells into spermatogonia and the consequent appearance of spermatogenic cycles are under the control of gonadotophin and testosterone, such control being possibly mediated by sertoli cells (Courot .M., 1984) which regulate cell cycle kinetics and influence both spermatogonia and preleptotene spermatocytes (Gasinsk A 1990).

Table 2: Effect of alcoholic extract of *Trachyspermum ammi* on Sperm count, Sperm motility and Sperm morphology

Group No.	Treatment	Sperm count (millions/ml)	Sperm motility	Abnormal sperms (%)
1	control	30.8333± 0.2386	66.166±2.167	0.37±0.0706
2	100mg/Kg	29.5416 ±0.6905	58.5±0.9916**	2.83±0.6009*
3	200mg/Kg	26.4166 ±0.6791***	49.833±0.9098***	4.42±0.2007***
4	400mg/Kg	22.2083 ±0.1357***	47.66±1.054***	10±0.5164***
5	400mg/Kg (Recovery)	25.5416 ±0.7171***	57±1.155***	5.67±0.8028***

All figures are mean ± SEM n=8

*p< 0.05 vs. control ; **p<0.01 vs. control ; ***p<0.001 vs. control

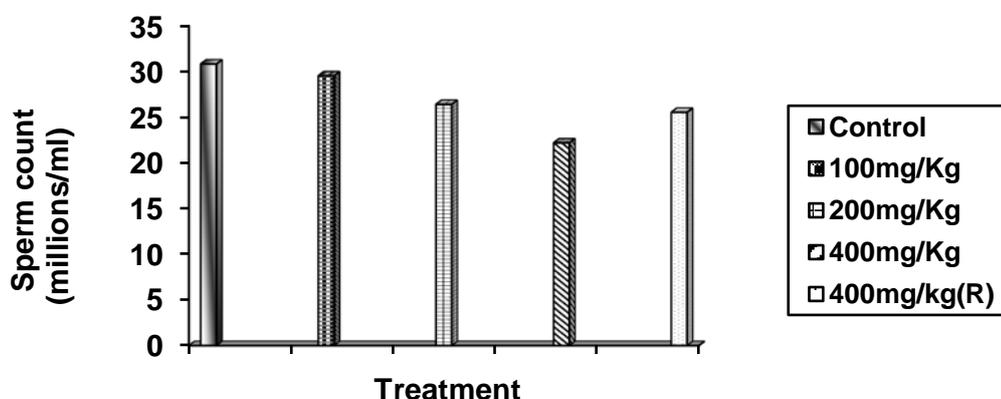


Figure 2: Effect of alcoholic extract of *Trachyspermum ammi* on sperm count

The reduction in sperm motility in cauda epididymis is of importance with regard to fertilization (Bedford JM 1983). The reduction in sperm motility clearly depicts the drug is of a

choice with antifertility potential. Alcoholic extract of the fruits of *Trachyspermum ammi* clearly lowers sperm motility level as compared to

the normal group. The results were tabulated in (Table 2) and (Fig 3) confirms the same.

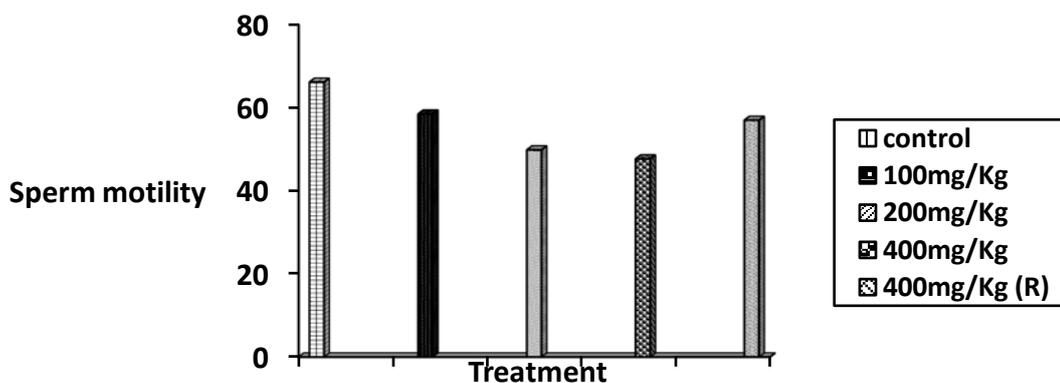


Figure 3: Effect of alcoholic extract of *Trachyspermum ammi* on sperm motility

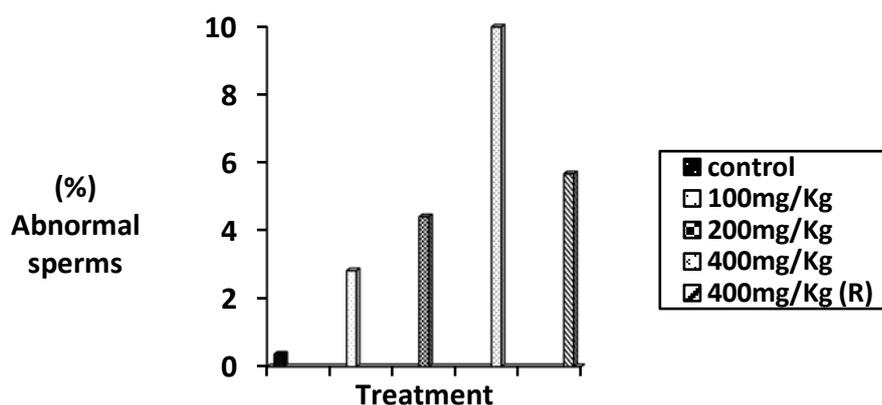


Figure 4: Effect of alcoholic extract of *Trachyspermum ammi* on sperm morphology

The occurrence of high number of abnormal sperm indicates interference with testicular spermatogenesis (Tulsiani DRP 1988). Potentiated increase of abnormal sperms exhibits the antifertility capacity of the drug. In the current study, alcoholic extract of *Trachyspermum ammi* seems to be with increased level of abnormal sperms, which may attribute it towards the antifertility property.

All the three groups (100mg/kg, 200mg/kg and 400mg/kg) of alcoholic extract of *Trachyspermum ammi* potentially exhibited antifertility property. At the same time, the recovery group (group 5) showed the revert back conditions of the antifertility property by means of increasing the elevated sperm count, sperm motility, testes weight and decreasing the abnormal sperms. The same were confirmed by means of histopathological studies of the testes.

CONCLUSION:

Trachyspermum ammi (Linn.) Sprague, an umbelliferous fruit that is used since long time as

a stimulant, stomachic, carminative and aromatic had been tried here to evaluate its antifertility potential. The alcoholic extract dose dependently produces the male anti-fertility by reduction in testes weight, number of sperms, sperm motility, but increased the production of abnormal sperms and altering the cellular pattern of testes. However the drug on withdrawal, the reproductive system reverts back to normal conditions by restoring the testes weight, sperm motility. Sperm number, sperm morphology and cellular pattern of testes. Thus we can conclude that, the drug *Trachyspermum ammi* can be a very good drug of choice for male antifertility effect and can tried out as a male contraceptive.

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