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SPERMICIDAL ACTIVITY OF *ELETTARIA CARDAMOMUM* AND *CUMINUM CYMINUM* SEED EXTRACTS AND ASSESSMENT OF SPERM FUNCTION IN ALBINO RATS

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ABSTRACT: The spermicidal activity of *Elettaria cardamomum* and *Cuminum cyminum* seed extracts have been studied to evaluate the effective concentration to immobilize and kill 100% of sperm within 20 s, and the sperm function of rat is studied to find the effect on sperm membrane integrity. Sander and Cramer's test was used to study the spermicidal activity of *Elettaria cardamomum* and *Cuminum cyminum*. Sperm viability and sperm morphology were studied using Eosin-Nigrosin staining technique. Stability of the extracts was studied by storage of extracts at –20 °C. Under the test conditions, minimum effective spermicidal concentrations for *Elettaria cardamomum* and *Cuminum cyminum* seeds extract are 1 mg/million sperm and 5 mg/million sperm respectively. The effect of the extract on morphology and viability of sperm was also studied, and no change was observed in the morphology of head, midpiece, and tail and no viable sperm seen. The effect of different concentrations of extracts of *Elettaria cardamomum* and *Cuminum cyminum* on percentage motility of the sperm was also studied. With an increase in concentration, there is a linear decrease in percentage motility, becoming zero at 1mg and 5 mg dose respectively within 20 s. The effectiveness of lyophilized aqueous extract of cardamom and cumin seed extract does not change with time after storage in the deep fridge at -20 ºC for 4 months.

INTRODUCTION: Several methods have been used for the induction of infertility over a long period including chemical, hormonal and immunological approaches. Medicinal plant products have a long history of their use in India as well as other countries. Fertility regulating plants and plant products has been used as contraceptives to limit the family size.

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However, studies have been carried out using *Triptergium willfordi* in China and neem oil in India to find the potentiality for anti-fertility/ spermicidal activity. Plant products have been used for pregnancy interruption in traditional medicine worldwide. It has been reported several plants as more active anti-fertility agents however documented experimental data on anti-fertility plants are very meager [1, 2, 3]. It is therefore of our interest to investigate the spermicidal activity of *Elettaria cardamomum and Cuminum cyminum* seed extracts in Albino rats. Research on Indian plants with anti-fertility activity has been reviewed by Reviewer [4, 5]. Isolated and independent studies by different investigators and institutes on these

topics have been continued. Twenty-eight plants and about the same number of isolated materials have been reported to have shown anti-fertility activity [1].

Chaudhury and Haq, on the other hand, have listed plants with more than 60% anti-fertility activity in species like *Aristolochia indica, Curcuma longa, and Embelia ribes* [6, 7]. Kamboj and Dhawan have listed 16 plants as more active anti-fertility agents *viz. Abrus precatorius, Aristolochia indica, Datura quercifolia, Daucus carota, Hibiscus rosasinensis, Embelia ribes*, and *Polygonum hydropiper* [8]. The juice of fresh green leaves of *Azadirachta indica* was believed to suppress "Kam Vasana" (Desire for sex). It was consumed by sanyasees in shrines and the pupils studying in Gurukul for the same purpose. Deshpande *et al.,* have studied the anti-fertility activity of *Azadirachta indica* leaves in male mice [9, 10].

Freshly prepared water extract of crushed green leaves of *Azadirachta indica* was orally fed to mice every day for 1 month to study its effect on male reproduction function. It was observed that control mice showed 100% fertility rate. In *Azadirachta indica* treated animals, the anti-fertility effect was 80%. The *Azadirachta indica* leaves have shown reversible male anti-fertility activity. A gradual recovery was observed in both the histological and biochemical parameters after 8, 16 and 24 days of cessation of the treatment. The results suggest a possible reversible antiandrogenic property of the leaves of *A. indica* in male Albino rats [11, 12].

Two plants, *Carica papaya* seed and p-coumaric acid *Aristolochia indica* plant [13, 14] have been reported to reduce the fertility of male mice or rats without affecting spermatogenesis. *Embelia ribes* berries [15] were also found to affect adversely semen quality and quantity with a fall of testosterone levels in bonnet monkeys. Lohiya and colleagues [16] at the University of Rajasthan, Jaipur have performed, experiments with several plant products using small laboratory animals and non-human primate species. Using purified gossypol acetic acid alone and in combination with potassium chloride, the anti-fertility action, and possible hypokalemia were tested in adult male langurs for 120 days. The treatment resulted in severe oligospermia, with impairment of sperm motility. The functions of accessory glands and libido, however, remained unimpaired. The complete reversal of these changes was noted after 90 to 105 days of withdrawal of treatment leading the investigators to suggest that while oligospermia, achieved was reversible; the hypokalemic response of langurs is similar to human and not related to the impurity of gossypol. Upadhyay *et al.* observed a long-term contraceptive effect of single intra-vas administration of neem oil in male rats. The anti-fertility effect was observed for 8 months and was found to be an alternative approach to vasectomy [17].

Two neem oil derivatives, *viz.* sodium nimbinate [18] and sodium nimbidinate [19] have been found to possess weak spermicidal action *in-vitro*. Undiluted neem oil was found of possessing strong spermicidal action (within the 30 s) against rhesus monkey and human spermatozoa *in-vitro*. 3 mg of neem leaf extract, when treated with human spermatozoa kills 100% of sperm within the 20 s [20]. *Praneem polyherbal* cream has been developed, which has the synergistic spermicidal concentration for praneem (25%), reetha saponins (0.05%) and quinine hydrochloride (0.34%) and was found at this concentration to result in 100% immobilization of sperm within the 20 s [21].

The study was carried out to evaluate the effective concentration of aqueous extract of old and tender *Azadirachta indica* (neem) leaves to immobilize and kill 100% human spermatozoa within 20 sec. Under the test conditions, minimum effective spermicidal concentrations for tender and old leaf extracts were 2.91 ± 0.669 mg/million sperm and 2.75 ± 0.754 mg/million sperm respectively [21]. An aqueous decoction of *Chenopodium* seeds (CAD) was assessed for its sperm-immobilizing and contraceptive efficacy in laboratory animals. The minimum effective concentration of CAD that induces instantaneous immobilization of rat spermatozoa in vitro was 2mg/mL [22].

The leaf extract of *Cestrum parqui* was examined for its effects on sperm motility *in-vitro*. The maximal spermicidal effect was observed with a 250 μg/mL dose of extract [23]. It is our interest to investigate the spermicidal activity of *Elettaria cardamomum and Cuminum cyminum* seed extracts in albino rats. The spermicidal activity of *Elettaria cardamomum and Cuminum cyminum* seeds extract

is studied in albino rats. The dose and time dependent study of spermicidal activity is done to find a minimum concentration of extract required to kill 100% of spermatozoa within 20 s.

MATERIALS AND METHODS:

Materials: *Elettaria cardamomum* and *Cuminum cyminum* seeds from the local market.

FIG. 1: CUMIN SEED

FIG. 2: CARDAMOM SEED

Animal Model: All the experiments are carried out using sexually mature (60 days old) male rats of proven fertility as an experimental model. Rats (200-220g) were maintained under light and temperature control (complying with standard husbandry conditions) with food and water *ad libitum* and were acquired from our institute's animal house facility. All experiments were performed following the guidelines formulated by the animal ethics committee of our institute [24].

Preparation of Aqueous Extract: Cardamom / cumin seeds were taken and soaked in distilled water. Ground the cardamom/cumin seed in the grinder/mixer using distilled water for proper solubility separately. Filtered the ground seed extract with the help of cotton gauze. The filtrate was centrifuged at 3000 rpm for 20 min. After centrifugation, the pellet was discarded, and the supernatant was washed with 1:1 chloroform. The supernatant was kept at -20 °C for freezing and lyophilized at -40 °C for 15 days to get the extract in the powder form.

Preparation of Different Doses of Cardamom and Cumin Seed Extract:

A: At first saline of 0.9% NaCl has been prepared by dissolving 0.9 gm of NaCl in 100 ml distilled water.

B: Now lyophilized Cardamom and Cumin seed extracts in powder form were weighed in a different amount, *i.e*., 0.5 mg, 1 mg, 2 mg, 3 mg, 4 mg and 5 mg.

C: These weighed extracts were dissolved in 1 ml of incubated physiological saline. This was stored in the deep fridge at -20 °C.

D: The epididymis is separated from the rat and punctured to collect the sperms in incubated physiological saline. One million sperm was added to each of the above mixtures.

Spermicidal Activity Study: The present study was carried out to evaluate the effective concentration of aqueous extract of Cardamom/ cumin seed extract to immobilize and kill 100% rat sperm within 20 s. Sander and Cramer's method was used to study the spermicidal activity of *Elettaria cardamom / Cuminum cyminum* seed extract. The spermicidal activity was determined using Sander and Cramer method which measures the minimum concentration of spermicidal agent required to kill 100% sperm within 20 s test ingredients of various concentration (0.5 mg, 1 mg, 2 mg, 3 mg, 4 mg, 5 mg,) were mixed with sperm suspension containing 1 million sperm.

The mixture was observed under the microscope for the 20 s at 10X and read for the motile sperm. If any motile sperm were seen, the concentration was recorded as a "fail." A two-hundred-fifty microliter of baker buffer was added to all the mixture that passed the test and incubated at 37 °C for at least 60

min. The solution was slowly vortexed and observed again after 60 min for the presence of any motile sperm.

The concentration at which it was tested was recorded as effective if both tests indicated the absence of motile sperm. The endpoint was the lowest concentration of the *Elettaria cardamom*/ cumin seed extracts that caused complete immobilization of all the sperm within the 20 s of mixing. The dose - and time-dependent study for the spermicidal activity was done using the above test. The effect of different concentrations of both cardamom and cumin seed extract on percentage motility was studied [25].

Sperm Viability Test: Sperms were mixed with both cardamom and cumin seed extract separately for 20 s. Sperm viability was checked using Eosin-Nigrosin technique given below. Unstained spermatozoa were counted as live and stained were counted as dead spermatozoa [26].

Eosin-Nigrosin Staining Technique: One drop of above-treated sperm mixed with 2 drops of 1% EosinY. After 30 s, 3 drops of 10% Nigrosin solution was added. A drop of treated sperm-Eosin-Nigrosin mixture is placed on a clear microscope slide; allowed to dry and observed under microscope [26].

Sperm Morphology: Sperm morphology of treated sperm was studied under the microscope using EosinY and Nigrosin staining method as described above. A drop of sperm- eosin-nigrosin mixture treated with both Cardamom and Cumin seed extract was examined separately at 400X under phase contrast microscope to record any change in morphology of the sperm [27].

Hypo-Osmotic Swelling Test: A hypo-osmotic swelling solution was prepared with sodium citrate 0.735% and fructose 1.351% in distilled water. One hundred microliters of sperm preparation were added and mixed with a pre-warmed 1 ml hypo-osmotic swelling solution and incubated at 37 ºC for 30 min. Swelling as indicated by various types of coiling of the sperm tail (100 random sperm) was examined using a phase contrast microscope [27].

Stability Test: The effectiveness of cardamom and cumin seed extract during storage time was studied. The extract was stored in a deep fridge at –20 °C, for 4 months and the spermicidal activity of cardamom and cumin seed extract was studied using Sander and Cramer method.

RESULTS: The spermicidal activity of graded doses of *Cuminum cyminum* and *Elettaria cardamomum* seed extract was studied *in-vitro* using albino rat sperm. The results of the Sander-Cramer test showed potent spermicidal activity for both seed extract. The minimum effective concentration of aqueous cumin seed extract required to kill 1 million sperm in the 20 s, was 5 mg whereas the minimum effective concentration of aqueous cardamom seed extract required killing 1 million sperm in 20 s, 1 mg was observed **Table 1** and **2**.

TABLE 1: MINIMUM EFFECTIVE CONCENTRATION (MEC) OF AQUEOUS EXTRACT OF CUMIN SEED EXTRACT REQUIRED FOR KILLING 1 MILLION SPERM IN 20 s

Sample no.	Semen sample sperm count (mill/ml)	Amount of semen taken Containing 1 million Sperm (µL)	% Motility	MEC (mg)	MEC (mg) mean
1	20	50	50%	5 mg	
2	25	40	75%	5 mg	
3	22	45	60%	5 ng	
4	25	40	85%	5 mg	
5	20	50	50%	5 mg	5 mg
6	25	40	75%	5 mg	
7	40	25	85%	5 mg	
8	32	31.	55%	5 mg	
9	40	25	60%	5 mg	
10	40	25	50%	5 mg	

TABLE 2: MINIMUM EFFECTIVE CONCENTRATION (MEC) OF AQUEOUS EXTRACT OF CARDAMOM SEED EXTRACT REQUIRED FOR KILLING 1 MILLION SPERM IN 20 s

Sample no.	Semen sample sperm count (mill/ml)	Amount of semen taken Containing 1 million Sperm (µ L)	% Motility	MEC (mg)	MEC (mg) mean
1	25	40	55%	1 mg	
2	30	33	70%	1 mg	
3	35	28	90%	1 mg	
4	40	25	80%	1 mg	
5	23	43	60%	1 mg	1 mg
6	25	40	50%	1 mg	
7	30	33	75%	1 mg	
8	40	25	60%	1 mg	
9	40	25	80%	1 mg	
10	30	33	75%	1 mg	

Sperm Motility and Viability: The effect of different concentrations of *Elettaria cardamomum* & *Cuminum cyminum* seed extract on percentage motility of 1 million sperm after exposure to different doses for 20 s has shown that with an increase in concentration, there is a linear decrease in percentage motility. Approximately 1 mg and 5 mg of an extract of *Elettaria cardamomum* & *Cuminum cyminum* respectively are required to immobilize and kill 100% of 1 million sperm within 20 s the viability of sperm was studied using Eosin-Y and Nigrosin staining technique. It was found that crude extract kills 100% of 1million sperm within 20 s **Fig. 3** and **Fig. 4**.

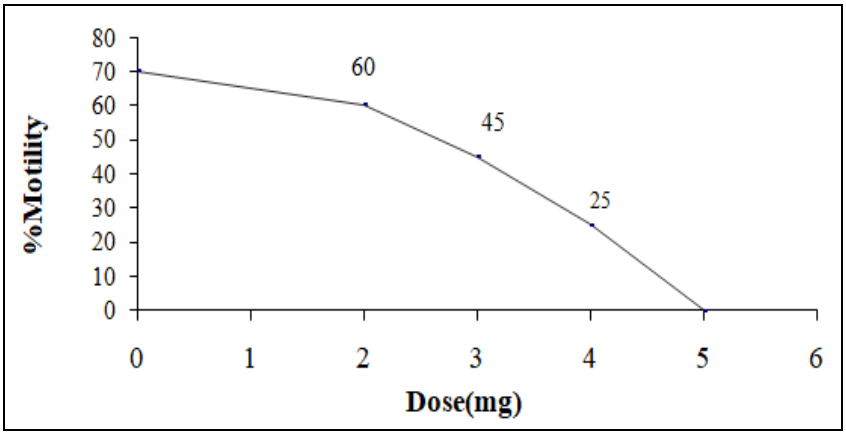

FIG. 3: EFFECT ON PERCENT MOTILITY OF 1 MILLION SPERM AFTER EXPOSURE TO DIFFERENT DOSES OF AQUEOUS EXTRACT OF CUMIN SEED FOR 20 s

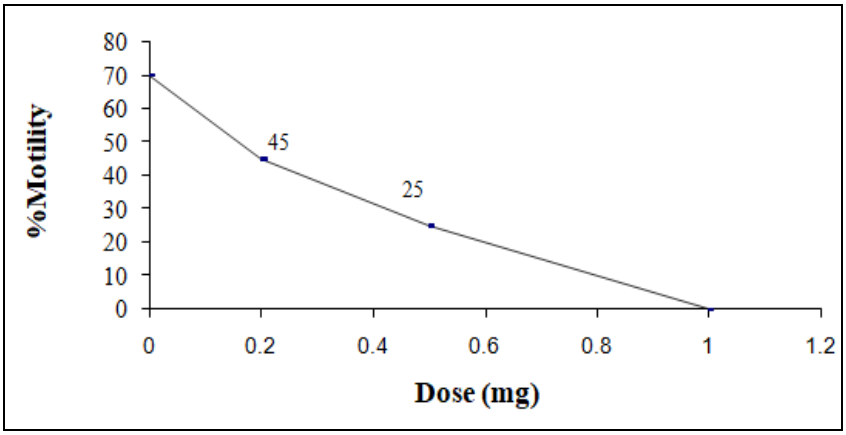

FIG. 4: EFFECT ON PERCENT MOTILITY OF 1MILLION SPERM AFTER EXPOSURE TO DIFFERENT DOSES OF AQUEOUS EXTRACT OF CARDAMOM SEED FOR 20 s

Sperm Morphology: The morphological study of sperm was done using Eosin-Nigrosin stain, and no morphological changes were found in the sperm head, mid-piece or tail when compared with untreated sperm.

Hypo-Osmotic Swelling Test: Hypo-osmotic swelling response in sperm was reduced from 70 percent to about 45% immediately after immobilization with *Elettaria cardamomum* & *Cuminum cyminum* seed extracts.

It was further reduced to 20% by 30 min. and after 60 min no hypo-osmotic swelling response was observed in any of the immobilized sperm **Fig. 5**.

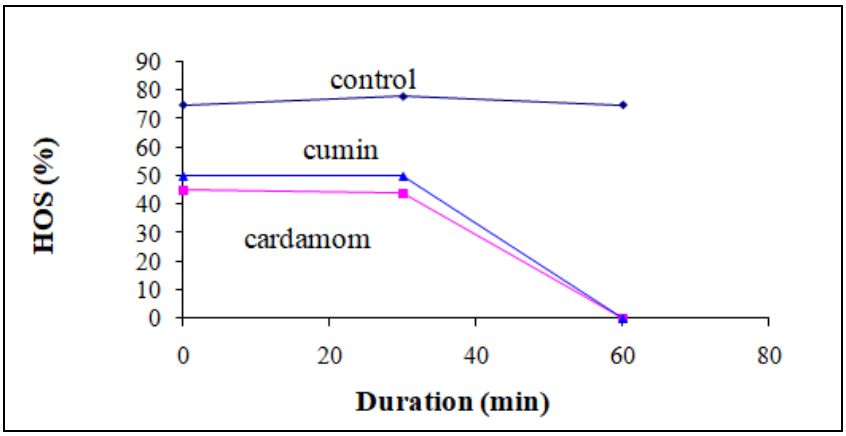

FIG. 5: HYPO-OSMOTIC SWELLING RESPONSE IN SPERM AFTER IMMOBILIZATION WITH *ELETTARIA CARDAMOMUM* & *CUMINUM CYMINUM*, UNLIKE VIABILITY HYPO-OSMOTIC SWELLING RESPONSE DECREASED TO ZERO IN SPERM IMMEDIATELY AFTER IMMOBILIZATION

Stability: It was observed that the activity of both *Elettaria cardamomum* and *Cuminum cyminum* remains the same, and the effect does not change with storage time of 4 months. This study demonstrates that aqueous extracts of *Elettaria cardamomum* and *Cuminum cyminum* have *a* potent spermicidal effect. Its minimum effective concentration attains 100% immobilization and killing of sperm within 20 s.

DISCUSSION: The result of the present study revealed that the aqueous extract of *Elettaria cardamomum* and *Cuminum cyminum* seed extract is a potent spermicide, which was demonstrated through a dose-dependent study on the effect on motility of spermatozoa and then confirmed by viability test. The aqueous extract of *Elettaria cardamomum* and *Cuminum cyminum* seed extract inhibited spermatozoal activity *in-vitro* at the concentration of 1 mg/mL and 5 mg/mL respectively for rat sperms.

No morphological changes were found in the sperm head, mid-piece and tail when compared with untreated sperm. Therefore, 100% killing of sperm may be due to blockage of some biochemical pathway like energy utilization, which would require further investigation. Sperm plasma membrane integrity, as determined by the hypo-osmotic swelling test, decreased instantaneously and also with the time after exposure to *Elettaria cardamomum* and *Cuminum cyminum.* This indicates that *Elettaria cardamomum and Cuminum cyminum* seed extract damages sperm plasma membrane physiology leading to alterations in other functional characteristics. The order of the effect of *Elettaria cardamomum and Cuminum cyminum* seed extract on sperm function was observed to be viability > hypo-osmotic swelling response as 100% sperm are killed within 20 s, followed by a drastic reduction in hypo-osmotic swelling and condition of no hypo-osmotic swelling response was observed at 60 min. The effectiveness of lyophilized aqueous extract of cardamom and cumin seed extract does not change with time after storage in the deep fridge at -20 ºC for 4 months [27, 28].

Existing methods of contraception are effective and probably underutilized for much of the developing world. A lack of basic education and access to health care services limits male involvement in family planning. For new contraceptive methods to have an impact on the worldwide problem of unintended pregnancies, they must be acceptable to

both men and women and increase the overall efficacy of use. It is heartening to see that traditional Indian plant medicine has now led to several therapeutically and industrially useful preparations and compounds which generates enough encouragement among the scientist in exploring more information about these medicinal plants. The global scenario is changing towards the use of nontoxic plant product having traditional medicinal use [29, 30, 23].

Vaginal contraceptives play an important role in limiting family size, especially in developing countries. To achieve this, many chemicals agents have been tried, but these methods are not popular, primarily due to high failure rates and due to side effects such as local reactions and systemic effects. Plant products as contraceptive will be more acceptable for economic reasons regarding self-reliance and possible practicability for a plant-based contraceptive approach in countries where population pressure is much high. Our studies are directed toward understanding the physiology of the epididymis to develop novel strategies for male contraceptive based on the inhibition of sperm maturation. However, a new method of contraception become available, family planning and public health experts must cautiously evaluate the impact of the new technology [20, 31].

CONCLUSION: The Cardamom and Cumin seed extract is hydrophilic, mixes immediately with water as well as body fluids and kills sperms within the 20s. The proposed spermicide can be a potent vaginal contraceptive and be formulated as a cream or a peccary. *Elettaria cardamomum* and *Cuminum cyminum* possess appreciable spermicidal potential, which may be explored as an effective constituent of vaginal contraceptive.

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CONFLICT OF INTEREST: Nil

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