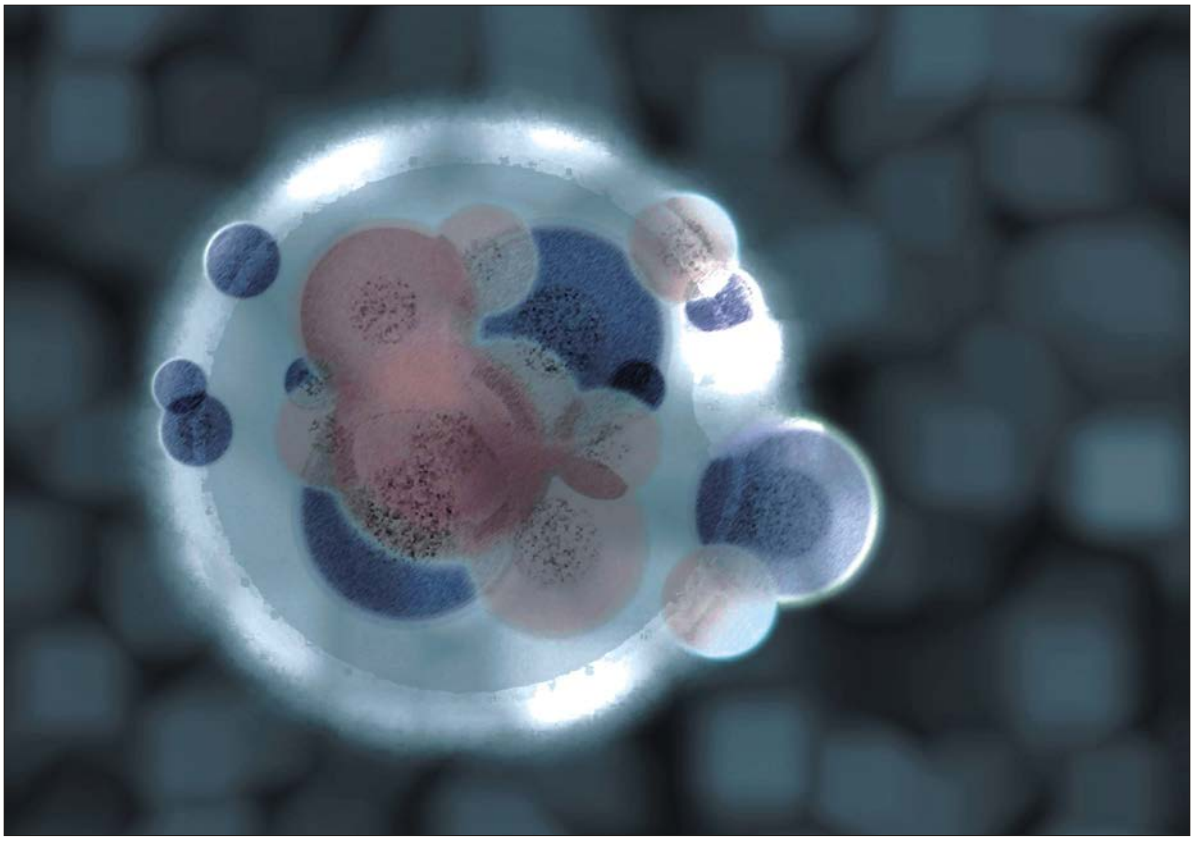

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Studies on Spermatotoxic Effect of Ethanolic Extract of *Capparis aphylla* (Roth)

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Abstract: Ethanol extract of *Capparis aphylla* was evaluated for possible spermatotoxic effect in 90 days old male rat. The ethanol extract at the doses of 50, 100 and 200 mg kg^{-1} of body weight was administered intra peritonially for 55 days. The analysis consists of counts, motility and abnormalities of the cauda epididymal sperm adapting light microscopy. The fertility of the treated rats was reduced drastically. The sperm concentration in the epididymis and sperm motility decreased, whereas sperm abnormalities increased in particular sperm abnormalities like flexed head, detached head and coiling of end tail. In extract treated rat the duration of sperm motility reduced with respect to the increased dose level. The results indicate disruption of the spermatogenic as well as androgenic compartment of the testis by the ethanolic extract of *C. aphylla*. The results also reflect an alteration of epididymal function towards the post-testicular sperm maturation processes by *C. aphylla*.

Key words: *Capparis aphylla*, sperm abnormalities, sperm count, sperm motility, cytoplasmic droplet (CD), testis, epididymis

INTRODUCTION

Several plant products inhibit male and female fertility and may be developed into contraceptives. Even though, many indigenous plants have been shown to prevent the birth, only few plants have so for been investigated for artifertility activity. Various medicinal plant extracts have been tested for their antifertility activity both in male and female (Kamboj, 1988). Some of these plants had spermicidal effects, other caused reduction in the sperm counts and altered the mobility of the sperms. Some of them caused testicular change and altered hormone levels (Bhargava, 1984; Reddy *et al*., 1997).

Capparis aphylla (*C. decidua*) Roth (Capparidaceae) is a much branched shrubs without leaves or with very small leaves. Flowers are orange red in colour. *C. aphylla* is seen in South west, North west India and Tirunelveli district in Tamilnadu, India. Bark of this plant is used as diaphoretic, whole plant is used in cough and asthma, fruits used in cardiac trouble. The plant consists capparine, capparilline, capparinine, n-pentacosane, n-triacontanol, β-sitosterol and 1-stachydrine, root bark contains spermidine alkaloid-capparisinine. This is the first attempt in antifertility research, on this plant (Asokkan *et al*., 1992).

MATERIALS AND METHODS

Plant material: Whole plants of *Capparis aphylla* were collected from Tirunelveli district of Tamilnadu, India. Taxonomic identification was made from botanical survey of medicinal plant unit, Government Siddha Medical College, Government of India, Palayamkottai, Tamilnadu, India. The whole plant was dried at room temperature, powdered by the mechanical grinder, sieved and stored for further used. The powder was soxhlated with 90% ethanol at 39°C. The extract was filtered and concentrated to dry mass by vaccum distillation. The semi dried material was diluted with n-butanol and water 1:1, the n-butanol soluble material was separated by separating funnel, dried under room temperature and powdered for further use.

Animal: Three months old Wistar strain male albino rat of 200 g body weight, was procured from the Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. Rats were fed with standard pelled feed from Gold Mohur Laboratory animals feeds, Bangalore, India and water *ad libitum*. The experiment was performed under the guidance of the Ethical Committee, Annamalai University, Annamalai Nagar. The animals were housed in polypropylene cage under control environmental condition with provision of 12 h light and 12 h dark.

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Animal experimental model: The animals were divided into 4 groups and treated as follows.

Group I: Control group I consists of 6 rats and received only Phosphate Buffer Saline (PBS) through i.p for 55 days.

Group II: Experimental group II consists of 15 rats, received 50 mg kg^{-1} body weight of ethanolic extract suspended in phosphate buffer saline (PBS) through i.p for 55 days.

Group III: Group III consists of 15 rats, received 100 mg kg^{-1} body weight of ethanolic extract suspended in phosphate buffer saline (PBS) through i.p for 55 days.

Group IV: Group IV consists of 15 rats, received 200 mg kg^{-1} of body weight of ethanolic extract, suspended in phosphate buffer saline (PBS) through i.p for 55 days.

Group V: Six rats from each group (II-IV) were left for recovery studies over a period of next 55 days (from 56th to 110th day). All spermatological parameters were repeated.

Spermatological studies: At the end of the treatment rats were anasthetised with MS222, dissected out the entire male reproductive system. The cauda epididymides were also dissected out, washed thoroughly in phosphate buffer saline (PBS), the organ was incised at several places so as to allow the semen to ooze out. The semen was sucked into a capillary tube upto 0.5 μL mark. On being transfer to an eppendorf tube, the semen was diluted with 99.5 μL of phosphate buffer solution. The sperm counts were made using Neubauer's chamber (Gopalakrishnnan *et al*., 1980) the duration of motility of the last motile sperm was determined using hanging drop preparation.

The sperm abnormalities were observed at different magnification [40x, 100x and 400x]. The data were calculated from the respective groups, mean and standard deviation were determined.

Sperm vitality test: A drop of 10% Nigrocin and 1% eosin Y were added with a drop of diluted semen. The mixture was examined under bright field microscope, counted in random selected optical fields, dead and live sperm percentage were calculated.

Statistical analysis: Statistical analysis was done by using student-t-test.

RESULTS

Spermatological studies in the control group shows 91% of spermatozoa possess normal morphology (Fig. 1). In the rat treated with ethanolic extract of *C. aphylla* 64±7% of group I (50 mg kg^{-1} body wt.) show normal morphology of sperm, 52±7% of group II (100 mg kg^{-1} body wt.) and 28±4% of group III (200 mg kg^{-1} body wt.) normal in morphology. The remaining sperms show abnormalities of different types (Table 1).

The following various abnormalities were observed. Ten percent of the spermatozova were flexed head, the head turn to the flagellum (Fig. 2-4). The detached head about 8% (Fig. 5 and 6). The major abnormalities were sticking or fusion of the spermatozoa at various point (Fig. 7-9), coiling of end tail about 40% (Fig. 10 and 11), germical epithelial cell mass containing cells in a attached manner or as a compact mass (Fig. 12 and 13), quite a few sperm retained the Cytoplasmic Droplet (CD) (Fig. 14).

In the sperm count of control rats about 23×10^6 sperm mL^{-1}, in group-II 21×10^6 sperm mL^{-1}, in group III 19.5×10^6 sperm mL^{-1}, in group VI 17.6×10^6 sperm mL^{-1} were observed (Table 1).

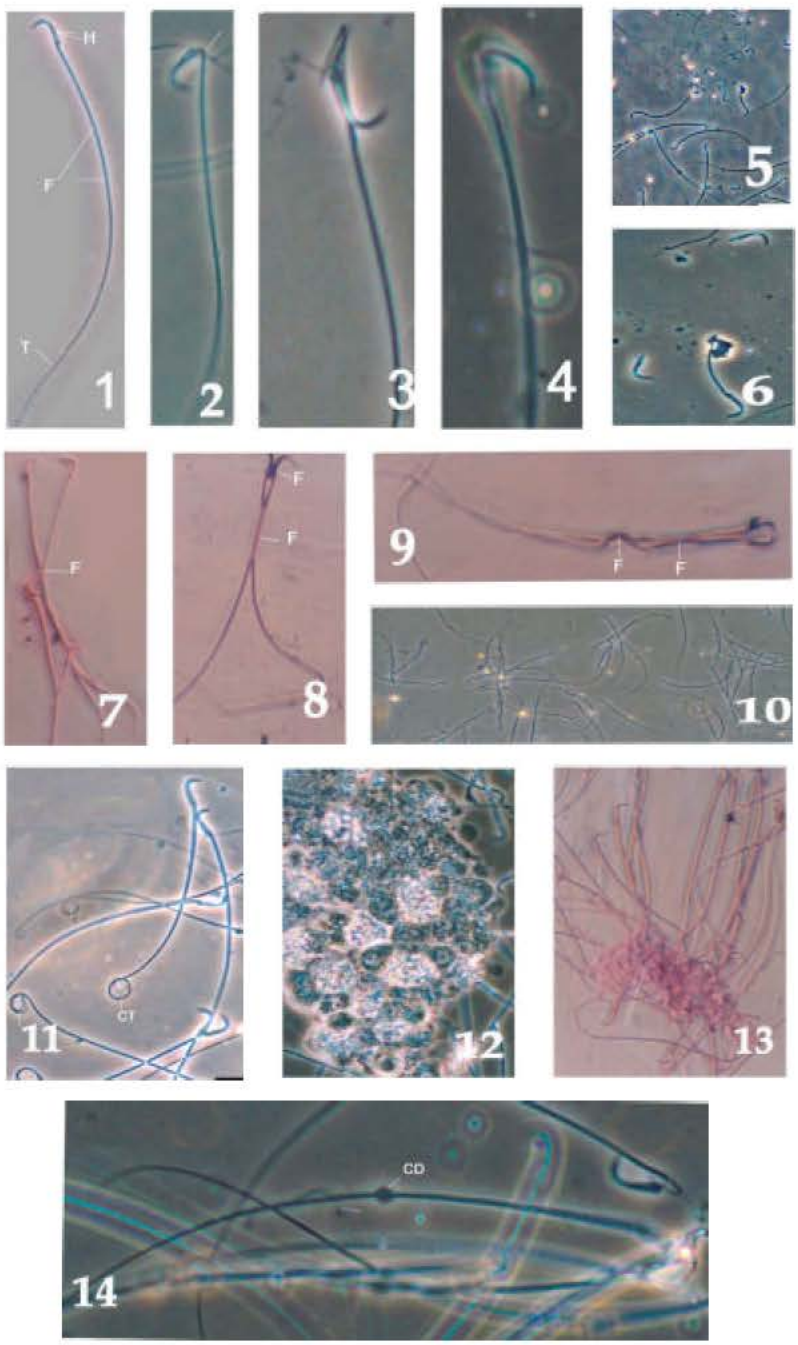
In the control rat of cauda epididymal sperm exhibited rapid and progressive motility and it was lasted for about 1 h 45 min, in the rat treated with ethanolic extract of *C. aphylla*, progressive 48 min (50 mg), sluggish 15 min (100 mg) and 200 mg treated rat sperm were not at all motile (Table 1).

After recovery for 55/110 days of cauda epididymidal sperm count as well as motility recovery were found to be normal stages. However, the percentage of abnormal sperm slightly higher in the case of recovery for 55 days,

Table 1: Effect of ethanol extract of whole plant of *Capparis aphylla* on percentage of normal sperm, abnormal sperm, sperm count, sperm motility and type of movement of sperm (mean±SD, n = 6)

Groups	Normal sperm (%)	Abnormal sperm (%)	Sperm count × (10^6 sperm mL^{-1})	Duration of sperm motility	Type of movement
Group-I (PBS)	91	9	23.0	(1 h 45 min)	Rapid and progressive
Group II (50 mg kg^{-1} of b.wt., i.p) EECA	58±4**	41±6***	21.0**	(35 min)	Progressive
Group III (100 mg kg^{-1} of b.wt., i.p) EECA	49±4***	50±6***	19.5***	(12 min)	Sluggish
Group IV (200 mg kg^{-1} of b.wt., i.p) EECA	28±4***	71±6***	17.6***	Not motile	No movement

PBS = Phosphate buffer saline, b.wt. = Body weight, i.p. = Intraperitoneal, EECA = Ethanol extract of *C. aphylla*, **p<0.01; ***p<0.001 significantly different form phosphate buffer saline control



(H: Head; F: Flagellum; T: Tail)

Fig. 1 : A typical sperm of rat from a control animal. (X 400x), Fig. 2-12: Sperm form *C. aphylla*-treated rats
2-4 : Sperm with head flexed, tip of the head facing towards the flagellum. (Fig. 2 X 100x; 3,4 X 400x)
5 and 6 : Detached head. (Fig. 5 X 40x; 6X 100x)
7-9 : Fussion sperm. (Fig. 7 X 40x; 8, 9X 400x)
10 and 11 : Coiling of tail (Fig. 10 X 40x; 11X 400x)
12 and 13 : Compact mass of immature epithelial cell in the epididymal tract (X400x)
14 : Cytoplasmic droplet (CD) (X 400x)

but lesser than those without recovery. Yet sperm abnormalities decrease to insignificant level on recovery over a period of 110 days.

DISCUSSION

The present study indicates that *Capparis aphylla* treatment result in impairment of male fertility in the rat by affecting both spermatogenesis and cauda epididymal spermatozoa. Spermatogenesis, an sequential process of transformation of A_1 spermatogonia through a series of stages into the round spematids which involves cell division through mitosis as well as miosis (de Krester and Kan, 1994).

Lack of motility, decrease sperm count, increase incident of sperm abnormalities strongly point to a spermatotoxic effect of *C. aphylla* via epididymis, particularly tail coilly nature of the sperm suggested some biochemical changes in the sperm surface. Ethanolic extract of whole plant of *C. aphylla* at treated doses of 100 and 200 mg arrested normal spermatogenic cycles and showed increase sperm abnormalities.

The recovery of spermatogenesis after withdrawal of treatment from 55th to 110th days was clear by decrease relative percentage of abnormal sperm and increase motility of sperm. Ethanolic extract of *C. aphylla* to be deleterious to the fertilizing ability of sperm.

C. aphylla produces effect on various parameter would have resulted from the alteration in the epididymal milieu and reduction of sperm count might be due to the reduce output of spermatozoa from the testis.

The sperm have two principle attributes viz., motility, fertilizeability which are prerequisite for fertilization; any negative impact on the motility would seriously affect the fertilizing ability (Akbarsha *et al*., 2000, 2001). The sperm sample contains more than 20% of abnormal spermatozoa consider to be more infertile (Bauer *et al*., 1974). Motility of the sperm is due to flagellar beat which in turn is dependent on microtubular apparatus of the flagella (Eddy and O'Brein, 1994).

Sperms while leaving testis are not motile but show motility during their epididymal transit. The epididymis contribute to initiate motility by providing unique microenvironment along the length for the sperm to resist and secreting protein and some important compound which in one way or other are concerned with the initiation of sperm motility (Robaine and Hemo, 1988). It is reasonable to speculate that the active compound of *C. aphylla* makes access into epididymis and alters the epididymis in respect of its function towards initiation of sperm motility.

The breaking away of head from flagellum and flexion of head of the sperm appears to occur due to impact of active chemicals of *C. aphylla* at the neck or connecting piece of flagellum. The main component of connecting piece are the basal plate, capitulum and segmented columns. Trypsin treatment appears of cleave the head from the tail between capitulum and basal plate (Young and Cooper, 1983). Thus, it could be perceived that the ethanolic extract distrupts this protein also as much as distrupting tubulin, causing the breaking away of the head from flagellum. A less impact at this point would cause the head to flex, or flexion itself may be a step towards the breaking away (Nakai *et al*., 1992).

The cytoplasmic droplet (CD) is a smear of cytoplasm initially remains attached to the neck region and gradually shift its position to the end of the mid piece during epididymal transit of the sperm. The droplet is shed when the sperm leaves the corpus epididymis and when sperm arrives at the cauda there are devoid of droplet. The sperms which retain extra cytoplasm are inhibited in motility (Hermo *et al*., 1988; Keating *et al*., 1997). The retention of cytoplasmic droplet by cauda epididymal sperm of ethanolic extract of *C. aphylla* treated rat would be speculated as due to ethanolic extract treatment imparing the process of shedding of the cytoplasmic droplet.

Since the analysis of sperm parameter pertained to the cauda epididymal spermatozoa change indicates the effect of compound(s) at the level of sperm maturation. A decrease in the sperm count may be due to death and removal of the sperm during cauda epididymal stage. The impact on the sperm motility and morphology indicate the impairment of sperm processing in the epididymis.

The present study indicates that *C. aphylla* responsible for the aspects of male anti fertility effect and points to the prospective of this active compound(s) of *C. aphylla* in male contraceptive, which deserves further investigation.

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