

Studies on Spermatotoxic Effect of Ethanolic Extract Of Root of “*Caesalpinia digyna* (Rottler)”

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Summary

Ethanolic extract of *Caesalpinia digyna* (Rottler) was evaluated for possible spermatotoxic effect in 90 days old male rat. The ethanolic extract at the doses of 50,100and200 mg kg⁻¹ of body weight was administered intra peritonally 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 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 *Caesalpinia digyna* (Rottler). The results also reflect an alteration of epididymal function towards the post-testicular sperm maturation processes by *Caesalpinia digyna* (Rottler).

Keywords: *Caesalpinia digyna* (Rottler), sperm abnormalities, sperm count, sperm motility, cytoplasmic droplet (CD), testis, epididymis

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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 far been investigated for their antifertility activity. Various medicinal plant extracts have been tested for their antifertility activity both in male and female (1). 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 (2).

Plant material

The Root of *Caesalpinia digyna* (Rottler) was purchased from Abhirami botanicals, Tuticorin, Tamilnadu, India. Taxonomical identification was made from botanical survey of medicinal plant unit, Government Siddha Medical College, Government of India. Palayamkottai, Tamilnadu, India. The root was dried at room temperature, powdered by the mechanical grinder, sieved and stored for further use. The powder was soxhlated with 90% ethanol at 60-70^o. The extract was filtered and concentrated to dry mass by vacuum 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 Wister strain male albino rat of 150-200 g body weight was procured from the Department of Pharmacology, Rural college of Pharmacy, Devanahalli, Bangalore Rural District, Karnataka, India. Rats were fed with standard pellet feed from Gold Mohr Laboratory animal's feeds, Bangalore, India and water *ad libitum*. The experiment was performed under the guidance of the Ethical Committee, Rural college of Pharmacy, Devanahalli. The animals were housed in polypropylene cage under control environmental condition with provision of 12 h light and 12 h dark.

Animal experimental model

The animals were divided into 4 groups and treated as follows.
Group I: control group I consists of 6rats and received only phosphate buffer saline (PBS) through i.p. for 55 days.
Group II: experimental groupie 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 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 56 to 110th days). All spermatological parameters were repeated.

Spermatological studies: At the end of the treatment rats were anesthetized with MS222, 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 μ L mark. On being transfer to an eppendorf tube, the semen was diluted with 99.5 μ L of phosphate buffer solution. The sperm counts were made using Neubauer's chamber (3) 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

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's-t-test.

Results

Spermatological studies in the control group shows 91% if spermatozoa possess normal morphology. In the rat treated with ethanolic extract of *Caesalpinia digyna* 56 \pm 2% of group I (50 mg kg-1 body wt.) show normal morphology of sperm, 47 \pm 4% of group II (100 mg kg-1 body wt.) and 20 \pm 62% of group III (200 mg kg-1 body wt.) normal in morphology. The remaining sperms show abnormalities of different types (table1).

The following various abnormalities were observed (Table 1). Ten percent of the spermatozoa were flexed head, the head turn to the flagellum. The detached head about 8%, coiling of end tail about 40%, germinal epithelial cell mass containing cells in a attached manner or as a compact mall, quite a few sperm retained the Cytoplasmic Droplet (CD).

In the sperm count of control rats about 23 \times 10⁶ sperm mL⁻¹, in group-II 20 \times 10⁶ sperm mL⁻¹, in group III 16 \times 10⁶ sperm mL⁻¹, in group VI 14 \times 10⁶ 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 hr 40 mins, in the rat treated with ethanolic extract of *Caesalpinia digyna*, progressive 30 min (50mg), sluggish 10 min (100 mg) and 200 mg treated rat sperm were not at all motile (Table 1).

After recovery for 55/110 days of cauda epididymal sperm count as will 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, but lesser than those without recovery. Yet sperm abnormalities decrease to insignificant level on recovery over a period of 100 days.

Table 1: effect of ethanol extract of root of *Caesalpinia digyna* on percentage of normal sperm, abnormal spam, sperm count, sperm motility and type of movement of sperm (mean± SD, n=6)

PBS: Phosphate buffer saline, b.w=body weight, i.p: intraperitoneal, EECD: Ethanol extract of *Caesalpinia digyna* (Rottler) *p<0.05. **p<0.01. ***P<0.001. Significantly different from phosphate buffer saline control.

Groups	Normal sperm (%)	Abnormal sperm (%)	sperm count ×(10 ⁶ sperm) (mL ⁻¹)	Duration of sperm motility (mins)	Type of movement
Group-I (PBS)	88	14	23	1 hr 40 mins	Rapid and progressive
Group-II (50 mg kg-1 of b.w., i.p)	56±2	43±7	20	30 mins	Progressive
Group III (1000 mg kg-1 of b.w., i.p)	47±4**	52±27**	16	10 mins	Sluggish
Group IV (200 mg kg-1 of b.w., i.p)	20±62***	78±28***	14	Not motile	No movement

Discussion

The present study indicates that *Caesalpinia digyna* - 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 A₁ spermatogonia through a series of stages into the round spermatids which involves cell division through mitosis as well as mitosis (4).

Lack of motility, decrease sperm count, increase incident of sperm abnormalities strongly point to a spermatotoxic effect of *Caesalpinia digyna* -via epididymis, particularly tail coilly nature of the sperm suggested some biochemical changes in the sperm surface. Ethanolic extract of whole plant of *Caesalpinia digyna* 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 *Caesalpinia digyna* to be deleterious to the following ability of sperm.

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

The sperm have two principal attributes viz., motility, fertilize ability which are prerequisite for fertilization; any negative impact on motility would seriously affect the fertilizing ability (5, 6). The sperm sample contains more than 20% of abnormal spermatozoa consider to be more infertile (7). Motility of the sperm is due to flagellar beat which in turn is dependent on microtubular apparatus of the flagella (8).

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 (9). It is reasonable to speculate that the active compound of *Caesalpinia digyna* - makes access into epididymis in respect of its function towards initiation of sperm motility.

The breakage away of head from flagellum and flexion of head of the sperm appears to occur due to impact of active chemicals of *Caesalpinia digyna* at the neck or connecting piece of flagellum. The main components 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 (10). 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 breading away (11).

The Cytoplasmic droplet (CD) is a smear of cytoplasm initially remains attached to the neck region and gradually shifts 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 droplets. The sperms which retain extra cytoplasm are inhibited in motility (12, 13). The retention of Cytoplasmic droplet by cauda epididymal sperm of ethanolic extract of *Caesalpinia digyna* treated rat would be speculated as due to ethanolic extract treatment impairment pf sperm processing in the epididymis.

The present study indicates that *Caesalpinia digyna* responsible for the aspects of male antifertility effect and points to the prospective of this active compound(s) of -- *Caesalpinia digyna* in male contraceptive, which deserves further investigation.

Acknowledgement

The authors sincerely thankful to our Secretary **Sri C.Srinivasa Baba**, Gokula Krishna College of Pharmacy, Sullurpet-524121, Nellore (DT) A.P, INDIA.

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