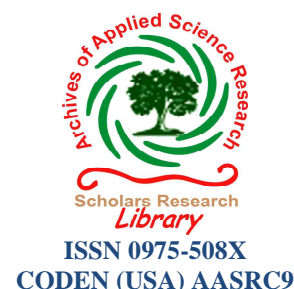




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Reversible contraceptive efficacy and safety evaluation of ethanolic extract of *Tinospora cordifolia* in animal model

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ABSTRACT

The purpose of present study was to evaluate the testes and epididymides mediated reversible contraceptive efficacy of ethanol extract of *Tinospora cordifolia* stem at the dose level of 400mg/kg. b.wt./rat/day. Oral treatment of 60 days caused significant reduction in number of spermatozoa in cauda epididymides as well as in testis with a decreased in the motility of sperm collected from cauda epididymides. The weight of testis, epididymis and seminal vesicle was also significantly reduced in case of treated animals when compared to control. Moreover, *Tinospora cordifolia* extract did not alter the hematological parameters, which shows its nontoxic nature. All these parameters were reversible after withdrawal of the treatment. In conclusion ethanol extract of *Tinospora cordifolia* stem have reversible contraceptive activity in male rats.

Key words: *Tinospora cordifolia*, sperm density, sperm motility, antifertility, reversible contraceptive efficacy.

INTRODUCTION

The increase in population especially of almost all developing countries is currently undergoing rapid transformation and the growths are unprecedented, a rate of three percent annually say, is not uncommon, implies a Population of twice the current size in less than twenty five years.

Of all the species, only man can control his fertility by interfering with the biological consequences (of sexual intercourse) at any one of several steps in the process of conception, fetal development and birth [1]. Various methods of preventing birth control have long been known and practiced. Some of them are celibacy, coitus interrupts, male sterilization through

castration, rhythm or natural method and in some societies marriage at late age of women as given by [2].

Current methods of contraception result in an unacceptable rate of unwanted pregnancies and having side effect also. Thus there is a need to replace these agents by safe and effective agents such as plant based contraceptive agents. The search for an effective, safe and reversible male antifertility agent with minimum side effect remains a challenge. To date, a number of plants with antifertility effects have been screened [3, 4, 5]. *Tinospora cordifolia* is a large, glabrous, deciduous climbing shrub belonging to the family Menispermaceae [6]. It is distributed throughout tropical Indian subcontinent and China, ascending to an altitude of 300 m. In Hindi language, the plant is commonly known as Gilroy, which is a Hindu mythological term that refers to the heavenly elixir that have saved celestial beings from old age and kept them eternally young. Dry barks of *Tinospora cordifolia* has anti-spasmodic, antipyretic [7], anti-allergic [8], anti-inflammatory [9, 10] and anti-leprotic [11] properties. Antifertility effect of *Tinospora cordifolia* has also been reported [12]. In our laboratory the antifertility effect of *Tinospora cordifolia* stem being investigated to explore the possibility of contraceptive efficacy emphasizing reversible fertility after withdrawal of the extract.

MATERIALS AND METHODS

Animal model

Colony bred, healthy adult male albino rats of the wistar strain, weighing between 260±15 g were used. The rats were housed in plastic cage under standardized conditions (12h light/ 12h dark). Standard rat feed and tap water provided *ad libitum*. Body weight of each animal in all groups was measured weekly to see the possible weight loss throughout the experiments. Indian national sciences academy, New Delhi [13] guidelines were followed for maintenance and use of the experimental animals.

Plant material

Stems of *Tinospora cordifolia* were collected from local growers of different parts of Haryana, Punjab and Uttar Pradesh states of India.

Preparation of extract

Fresh and healthy stems of *Tinospora cordifolia* were shade dried, powdered and subject to soxhlet extraction with 70% ethanol [14]. The ethanol was evaporated under reduced pressure to obtain the crud extract. The extracts so obtained was weighed and preserved at 4⁰ C in airtight bottles until further use. Extract was dissolved in distilled water in desired concentrations for oral administration.

Dose and duration of treatment:

The male rats of the experimental group were divided in to two groups. First group of 10 rats served as vehicle treated control, while other group of 10 rats, were treated with ethanol extract of *Tinospora cordifolia* stem at the dose level of 400 mg/kg. b.wt./rat/day. The second group was subdivided in two groups IIA and IIB, at the 60th day of experiment group IIA rats were autopsied while IIB group rats were allowed to recover for another 60 days without any drug administration.

Autopsy Schedule:

The rats were autopsied within 24h of the last dose and at the end of withdrawal period, under ether anesthesia. The testes, epididymides, seminal vesicle, ventral prostate were excised dissected and freed of fat, blood vessels and weighed. Tissues were fixed in Bouin's fluid, dehydrated in ethanol, cleared with xylene and embedded in paraffin wax. Sections were cut at 6µm, stained with Harris hematoxylin and eosin for histopathological examination.

Fertility test:

Mating tests were performed before the commencement of the treatment and at the end of the experiment between 55 to 60 days. The male rats were cohabited with proestrus females at a ratio of 1:2. The presence of sperm in vaginal smear in the next morning was considered positive matings. The inseminated females were separated and numbers of litters were recorded.

Sperm density and motility:

Sperm motility was assessed in cauda epididymides and testis [15]. Spermatozoa samples were obtained by the cauda epididymal puncture in physiological saline (0.9% NaCl). Sperm density and motility were determined by using light microscope.

Toxicology Investigation:

Blood samples of each animal were collected by cardiac puncture. Total RBC, WBC, haematocrit, haemoglobin values were recorded.

Statistical analysis

The data are expressed as mean± SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) and the comparisons between the group were done by Student's t-test. Difference were considered to be statistically significant when $p < 0.001$.

RESULTS**Body and organ weights:**

The oral administration of *Tinospora cordifolia* extract to male rats for 60 days did not cause any significant change in the body weight of treated rats as well as in recovery group. Further, results revealed that the weight of the reproductive organ of the treated rats decreased in comparison to the control group. However, organ weights, after withdrawal of drug, were found to be measured significantly (Table 1).

Table 1: Effect of ethanolic stem extract of *Tinospora cordifolia* on the body weight, organ weight in rats

Treatment	Body weight (g)	Organ weight (mg/100g. b. wt.)			
		Testis	Epididymides	Seminal vesicle	Ventral prostate
Group I control	265.4±5.8	1567.8±20 ^a	467.2±9 ^a	780.1±4 ^a	385.3±10 ^a
Group IIA	270.8±6.3	928.8±12 ^b	338.5±8 ^b	585.3±8 ^b	325.8±8 ^b
Group IIB	280.4±4.8	1420.3±19 ^a	428.8±5 ^a	730.4±15 ^c	380.7±9 ^c

Values are in mean ± SEM (n=10); $p \leq 0.01$

Histopathology:

In histopathological examination, control group shows normal size of seminiferous tubule with all successive stages of spermatogenesis and lumen is filled with sperm. In case of treated rats

with extract, regresses size seminiferous tubule was observed with less number of spermatozoa in lumen. The recovery group seminiferous tubular size restored almost normal and sperm was also observed in lumen.

Sperm density, motility and fertility:

The treated rats showed significant reduction in the sperm concentration of testes and cauda epididymides. The sperm motility of the cauda epididymides was also reduced significantly. *Tinospora cordifolia* stem extract treatment reduced, the fertility of male rats by 0% with 60 days drug treatment, where control animals exhibited normal fertility. The most significant observation was the return of fertility after 60 days of withdrawal of the drug (Table 2 and 3).

Table 2: Effect of ethanolic stem extract of *Tinospora cordifolia* on the sperm motility and sperm density in rats

Treatment	Sperm motility (%) (cauda epididymides)	Sperm density (million/ml.)	
		testis	cauda epididymides
Group I control	72.05±5 ^a	5.9±0.5 ^a	70.2±4 ^a
Group IIA	41.3±2 ^b	2.2±0.2 ^b	14.5±3 ^b
Group IIB	69.2±2 ^a	5.3±0.3 ^a	63.9±4 ^c

Values are in mean ± SEM (n=10); p≤0.01

Table 3: Effect of ethanolic stem extract of *Tinospora cordifolia* on the percent fertility in females mated with rats

Treatment	no. of mated males/females	Percent fertility
Group I	10/20	100
Group IIA	10/20	0
Group IIB	10/20	100

Toxicological investigation

Total RBC, WBC, haematocrit and hemoglobin did not showed appreciable changes throughout the course of investigation (Table 4).

Table 4: Effect of ethanolic stem extract of *Tinospora cordifolia* on the blood parameters in male rats

Treatment	RBC (million/mm ³)	WBC(-/mm ³)	Haematocrit (%)	Haemoglobin (g%)
Group I	5.2±0.3	8721.3±105	12.3±1	43.8±1.5
Group IIA	5.5±0.4	8620.2±100	12.8±1	41.7±2
Group IIB	5.1±0.5	8763.6±109	11.7±1	42.5±1.3

Values are in mean ± SEM (n=10), p≤0.01, values are non significant compared with group I control.

DISCUSSION

The present study with extract of *Tinospora cordifolia* suggested a possible role of this plant as a potential agent in the field of male fertility regulation. Treatment with stem extract was highly effective in producing reversible function sterility. Weight reduction of the reproductive organ of the treated male rats clearly indicate that the drug caused structural and functional alteration in the testis, epididymides, seminal vesicle and ventral prostate and also lowered the testosterone as these organs are androgen-dependent [16]. Decreased number of spermatozoa or reduced androgen production may affect the level of sialic acid in testes. The reduced sialic acid content might alter the structural integrity of acrosomal membrane, ultimately affects the metabolism, motility and fertilizing capacity of spermatozoa [17], which could not penetrate the cervical

mucus and thus failed to fertilize the ova [18, 19]. All these factors thus brought about functional sterility in the extract treated rats. However, the induced infertility was completely reversed after withdrawal of treatment of another period of 60 days. Also no apparent abnormality was observed in the litters delivered by the females mated with the males of recovery group. Non-toxicity of extract of *Tinospora cordifolia* is further supported by the data obtained after examination of hematological parameters which remain unaltered.

CONCLUSION

It can be concluded that ethanol extract of *Tinospora cordifolia* stem possible exert a reversible antifertility effect mediated through testes and/or epididymis, without any adverse toxicological effects.

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