

Effects of *Chorophytum borivilianum* and *Tribulus terrestris* Extract on Sperm count, Motility and Sex Hormones in Adult Male Rats

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ABSTRACT

Effect of ethanol extract of *Chorophytum borivilianum* and *Tribulus terrestris* was observed in male albino rats. The relative weight of the testis and epididymis were increased. The epididymal sperm count, motility and sperm abnormality were increased significantly ($P < 0.01$) in treated rats. The results of the hormonal assay showed that increased serum levels of LH and testosterone and also increase sperm count and motility compared to control. The results of the present study concluded that, ethanol root extract of *Chorophytum borivilianum* and *Tribulus terrestris* enhanced sperm concentration, motility and testosterone which might produce positive result in the male fertility enhancement.

Keywords: *Chorophytum borivilianum* and *Tribulus terrestris*, testosterone, fertility, FSH

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INTRODUCTION

Traditional herbs represent an extra ordinary reservoir of active ingredients which are still present in about 25% of all prescriptions of modern medicines. *Chlorophytum borivilianum* has therapeutic application in Ayurvedic system of medicine. Generally, it is considered very good to increase general body immunity. Its aphrodisiac properties have proved very much useful for the people suffering from Erectile Dysfunction and to increase male potency. It has spermatogenic property and helpful in curing impotency as they are rich in glycosides. Its roots are widely used for various therapeutic applications in the Ayurvedic and Unani systems of medicine [1-3]. It is known to cure many physical illness and weaknesses. Ancient records describe various medicinal properties of *T. terrestris* as a popular source to cure variety of different disease conditions in China, India, and Greece. The plant is used directly as a herb or as a main component for production of a number of medicines and food supplements such as for physical rejuvenation, therapy for the conditions affecting liver, kidney, cardiovascular system and immune systems. Also it is used as a folk medicine for increased muscle strength, sexual potency and in treatments of urinary infections, heart diseases and cough. It is considered invigorating stimulant, aphrodisiac, and nutritive [8-11].

Chorophytum borivilianum and *Tribulus terrestris* was designed to investigate the fertility enhancement effect in adult male rat.

MATERIAL AND METHODS

Plant Materials

Chorophytum borivilianum and *Tribulus terrestris* powder was purchased from Ayurvedic Store, Agra.

Preparation of plant extract

The whole plant of *Chorophytum borivilianum* and *Tribulus terrestris* were dried separately under shade and then powdered with a mechanical grinder to obtain a coarse powder, which were then subjected to extraction in a Soxhlet apparatus using ethanol. The ethanol extract was concentrated in a rotatory evaporator. The concentrated ethanol extract of whole plant of *C. borivilianum* and *T. terrestris* were used for fertility enhancement activity.

Animals

Normal healthy male Wistar albino rats (180-240g) were used for the present investigation. Animals were housed under standard environmental conditions at temperature (25±20C) and light and Dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan Lever Ltd., Mumbai, India) and water ad libitum.

EXPERIMENTAL DESIGN

The male rats were divided into 4 groups consisting of 5 animals.

Group I: Rats received normal saline daily for 15 days, orally. (Normal control).

Group II: Rats received ethanol extract of whole plant of *C. borivilianum* and *T. terrestris* at the dose of 100mg/kg body weight daily for 15 days. Group III: Rats received ethanol extract of whole plant of *C. borivilianum* and *T. terrestris* , at the dose of 250mg/kg body weight daily for 15 days.

Group IV: Rats received ethanol extract of whole plant of *C. borivilianum* and *T. terrestris* , at the dose of 500mg/kg body weight daily for 15 days.

After 24 hours of last treatment, the final weight was recorded and the animals were sacrificed by decapitation. Blood was collected. Sera were separated by centrifugation at 3000 rpm for 10 minutes and stored at 20oC until used for various biochemical assays. Then testis, epididymis, vas deferens, seminal vesicle and ventral prostate were dissected out, trimmed off extraneous and weighed accurately on torsion balance. The organs weights were expressed in terms of mg/100g body weight.

Sperm count

Sperm count was carried out by using Neubauer's haemocytometer as described by Zaneveld and Pelakoski [10].

Sperm motility and abnormality

After anaesthetizing the rats, the caudal epididymis was then dissected. An incision (about 1mm) was made in the caudal epididymis and drops of sperm fluid were squeezed onto the microscope slide and 2 drops of normal saline were added to mobilize the sperm cells. Epididymal sperm motility was then assessed by calculating motile spermatozoa per unit area.

Morphology (abnormality) was evaluated on sperm from the caudal epididymis. The total morphological abnormalities were observed as described by Linde *et al* [9].

Hormonal Assay

Blood removed from the rats by intracardiac method. Blood was centrifuged at 3000 rpm to separate the serum for the measurement of testosterone was done by using Enzyme Immunoassay Method (EIA). The EIA kit was obtained from Immunometrics (London, UK).

Statistical Analysis

Data were expressed as Mean ± SEM. Student's t test was used for statistical comparison.

RESULTS

Body and reproductive organ weight

The administration of ethanol extract of whole plant of *C. borivilianum* and *T. terrestris* to rats did not cause any significant change in the body weight (Table 1) and on the libido of treated rats; whereas, weights of testis and other accessory sex organs were increased significantly ($p < 0.05$) (Table 1). Among the accessory sex organs, a significant weight gain was seen in the caput and caudal epididymal segment. Slight decrease was observed in vas deferens (VD) seminal vesicle (SV) and prostate.

Sperm count and sperm motility

Sperm motility and sperm density in caudal epididymis, significantly increased (Table 2) and the increase was IV) severe in whole plant extract of *C. borivilianum* and *T. terrestris* (Group followed by whole plant extract of *C. borivilianum* and *T. terrestris* (Group III) and the same trend was seen in the caput epididymal sperm density when compared to control (Group I).

Sperm abnormality

Sperm abnormality in caput and caudal region was not affected by ethanol extracts of *C. borivilianum* and *T. terrestris* whole plant and ($p < 0.05$) (Table 2). Among the studied concentrations, 300 mg/kg concentration of extract of *C. borivilianum* and *T. terrestris* have shown significant and improved the sperm morphology.

REPRODUCTIVE HORMONE PROFILE

Serum testosterone level

The ethanol extract of whole plant of *C. borivilianum* and *T. terrestris* (100, 200 and 300 mg/kg body weight) repeated treatment daily for 14 days caused a significant increase in the serum level of testosterone in male rats (Table 3).

Serum luteinizing hormone (LH) level

Repeated treatment of male rats with the *C. borivilianum* and *T. terrestris* extracts for 14 days caused a dose related increase in the serum level of LH (Table 3). The level of increase was statistically significant (Group IV) ($p < 0.05$).

Table 1: Effect of *C. borivilianum* and *T. terrestris* whole plant extract on the Body and Reproductive organ weight of adult male albino rats

Treatment Groups	Body wt(gm)		Testis (gm)	Epididymis (mg)	
	Before	After		Caput	Cauda
Group-I	199.35±6.12	211.61±5.72	2.064±0.11	242.16±3.93	274.52±5.67
Group-II	180.24±3.54	199.54±5.56	1.893±0.34	204.21±2.83	280.19±5.08
Group-III	218.34±5.90	234.63±5.14*	2.434±0.28**	258.71±3.94**	312.59±6.23*
Group-IV	209.19±3.73	245.51±4.12**	2.601±0.74**	264.94±4.31**	324.11±5.19**

Each Value is SEM of 5 animals * $p < 0.05$; ** $p < 0.01$. Control vs Treated ns : not significant

Table 2: Effect of *C. borivilianum* and *T. terrestris* whole plant on the sperm concentration and motility in the epididymis of adult male albino rats

Treatment Groups	Sperm Concentration (Counts x 10 ⁶ mil)		Sperm Motility (FMI) @ (cauda)	Sperm Abnormality #	
	caput	cauda		Head (%)	Tail (%)
Group-I	293.63±6.91	349.54±8.39	168.51±7.55	5.23±0.11	9.14±0.84
Group-II	311.49±8.37ns	369.14±10.81ns	189.50±8.14ns	4.61±0.14	7.05±0.33
Group-III	324.11±9.23	398.56±8.94**↑	201.10±9.14**↑	4.83±0.24	7.84±0.74
Group-IV	339.63±10.66	454.16±9.36**↑	207.63±8.55**↑	4.03±0.26	5.88±0.16↓

Each Value is SEM of 5 animals * $p < 0.05$, ** $P < 0.01$ Control vs Treated:

↓-Significantly reduced; ↑ significantly increased

@: Motility is movement recorded after 5 min in suspension of caudal epididymal spermatozoa in phosphate buffered solution.

#: Expressed in percentage

Table 5: Effect of *C. borivilianum* and *T. terrestris* whole plant extract on the Sex hormone levels in male albino rats

Treatment Groups	Hormones	
	Testosterone (ng/ml)	LH (µIu/ml)
Group I	3.92±0.13	3.15±0.16
GroupII	4.13±0.22	5.32±0.15ns
GroupIII	4.64±0.56*	7.44±0.23*
Group IV	4.98±0.16*	8.14±0.67*

Each Value is SEM of 5 animals * $p < 0.05$, ** $p < 0.01$ Control vs Treated: ↓ significantly reduced; ↑ significantly increased.

DISCUSSION

In the present study, the weight of reproductive organs markedly increased. The weight and secretory functions of testes, epididymis, seminal vesicles, ventral prostate and vas deference are closely regulated by androgens. The drug may act on pituitary gland and increased main hormone of spermatogenesis.

The results presented in this work also show that the seminal vesicles weights were increased in adult male rats ingested *C. borivilianum* and *T. terrestris*. This increase in the accessory glands weights might suggest an increase in the pattern of testosterone secretion. Significant increase in the sperm motility of caudal epididymis was observed in treated group. This may be due to activity effects of *C. borivilianum* and *T. terrestris* on the enzymes of oxidative phosphorylation [1-3].

The ethanol extract of *C. borivilianum* and *T. terrestris* whole plant produced a significant increase in total sperm count and viable sperms. This may be as a result of the ability of the extract at the given doses, to either interfere with spermatogenetic process in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis [10]. The presence of mature sperm concentration was increased in the experimental rats treated with 500 mg/kg body weight *C. borivilianum* and *T. terrestris* whole plant extract. This suggests that the 500 mg/kg dose could influence the maturation of the spermatozoa in the male rats, which might also be a contributory factor to the increase in the mean total sperm count. In the present investigation the observed increase in the caudal epididymal sperm motility might be due to the androgen-stimulatory effect of the extract of *C. borivilianum* and *T. terrestris*. The increase in the caudal epididymis sperm counts in the treated animals substantiates the spermatogenic nature of the extract. The extract had a direct effect on the testes resulting in an increase in the number of spermatozoa and the increased level of testosterone production. Also, the extract had no spermatotoxic effect as previously indicated by Shah et al. [27]. Increase in LH concentration observed in the treated rats with 500mg/Kg of *C. borivilianum* and *T. terrestris* whole plant extract with confer the increase in testosterone concentration. This may be an indication that the *C. borivilianum* and *T. terrestris* has stimulatory effect on the hypothalamic- pituitary axis of the male rats [4-8]. In conclusion, these results proved that, long term *C. borivilianum* and *T. terrestris* ingestion produces increased effects on fertility on reproductive system in adult male rat. However, the exact mode of action requires further studies.

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