

# Single-blind placebo-controlled clinical study to evaluate the efficacy of *Sida cordifolia* Linn. in treatment of semen disorders (*Sukra Kshaya*) using semen and hormonal analysis

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## ABSTRACT

**Background:** *Sida cordifolia* Linn. is a well-known medicinal plant which exhibits anti-inflammatory, antioxidant, and sexual properties. It shows good promise in the treatment of male sexual disorders during its pre-clinical studies. **Objective:** The objective of the study was to assess the spermatogenesis action of aqueous extract of roots of *S. cordifolia* Linn. in clinical trials. **Materials and Methods:** The study uses qualitative criteria such as primary and secondary symptoms, and quantitative investigations such as hematological investigations, hormonal analysis, and semen analysis for assessing the therapeutic efficacy of research formulation through placebo-controlled clinical trials on 50 males having lack of sexual desire and non-satisfactory sexual life. **Results:** Very high inhibition was noticed in respect of primary symptoms such as lack of libido, difficulty in ejaculation or little amount of semen, as well as secondary symptoms such as nausea, body ache, headache, indigestion, loss of appetite, and general weakness in the research group. Lack of any adverse changes in hematological parameters (blood sugar, hemoglobin, ESR, RBC, and WBC) and biochemical parameters (bilirubin, protein, SGPT, SGOT, and ALP) indicates the non-toxic nature of research formulation. The hormonal levels registered a significant increase during the clinical study in research group, especially the testosterone level (8.53%). Semen quality evaluated through sperm count, motility, and morphology showed a significant improvement in research group, suggesting that administration of research drug in cases of stress-related sexual problems protected healthy cells by reduced generation of ROS and helped maintain quality parameters of spermatozoa during spermatogenesis. **Conclusion:** The research formulation made from roots of *S. cordifolia* Linn. shows good and significant ( $P < 0.05$ ) therapeutic efficacy through inhibition of primary and secondary symptoms and enhancement in hormonal and seminal parameters, validating its spermatogenesis effect without any toxic or adverse effects.


**KEY WORDS:** Clinical study; *Sida cordifolia*; Semen disorder; Herbal

## INTRODUCTION

Infertility is a serious problem for any couple. Most studies suggest that problems with the male account for about 40%

of all infertility cases, while couples with problems with both partners account for another 20%. The basic test to evaluate fertility in males is the semen analysis.

During spermatogenesis, male gamete cells called spermatozoa are created from spermatogonia in favorable environmental and thermal conditions. This delicate process happening under the right hormonal conditions and resulting in the production of testosterone can be adversely affected by dietary habits, exposure to heavy metals or radiation, etc.<sup>[1-3]</sup> In Ayurveda, the term *sukradhatu* means the substance

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which is produced after a long process of conversion of all other *dhatu*s responsible for semen production. Abnormal decrease in *shukradhatu* can be observed in the form of erectile dysfunction, difficult ejaculation or little quantity of ejaculate, general weakness, persistent weakness, etc. Many single herbs and combinations of medicinal plants are mentioned in the Ayurvedic texts for enhancement of semen quality.<sup>[4-6]</sup>

*Sida cordifolia* Linn. is a well-known medicinal shrub which finds mention in several Ayurvedic texts. It is a small tree found throughout tropical India and comes under Malvaceae family. While its leaves are chordate-oblong or ovate-oblong, the fruits have twin awanish carpels and the roots are grayish-yellow with bitter taste having secondary minor lateral extensions.<sup>[7-9]</sup> The Ayurvedic medical practitioners use it for treatment of cardiac ailments, muscular pain, joint problems, wound healing, urinary infections, dermal conditions, etc. Its therapeutic properties include antioxidant, anti-inflammatory, anti-microbial, analgesic, diuretic, and rejuvenator on account possibly of the presence of compounds such as vasicinone,  $\beta$ -sitosterol, stigmaterol, vasicinol, and N-methyl tryptophan in its roots and seeds. The leaves of this medicinal plant contain both ephedrine and pseudoephedrine.<sup>[10-12]</sup>

This clinical trial was undertaken to evaluate the spermatogenesis action of *S. cordifolia* Linn. root aqueous extract through placebo-controlled trials conducted on male human subjects having lack of sexual desire and non-satisfactory sexual life after getting significant *in vitro* reproductive effect and spermatogenesis along with non-toxic effect and significant pharmacological activity in the experimental male rat models. The spermatogenesis action of this research drug was established using qualitative criteria such as primary and secondary symptoms, and quantitative investigations such as hematological investigations, hormonal analysis by ELISA methods, and semen analysis, each patient enrolled after informed consent and approval of the Institutional ethical committee for a clinical trial on human subjects.

## MATERIALS AND METHODS

### Collection and Identification of Plants

*S. cordifolia* Linn. roots were procured from reputed herb supplier and authenticated by Botanical Survey of India, Howrah, India (REF./NO. BSI/CNH/SF/Tech./2016). All the equipment, testing kits, reagents, consumables,

and chemicals used for hematological, biochemical, and semen analysis were purchased from reputed suppliers of Kolkata.

### Preparation of Extracts

The aqueous extract of the root of *S. cordifolia* Linn. was prepared following the guidelines of Ayurvedic pharmacopeia for identification and standardization. One part coarse powder of research drug was boiled with four parts of distilled water until the quantity was reduced to one fourth. The residual quantity was filtered and concentrated using the lyophilizer instrument and stored in the dried form for preparation of zero size capsule having an average weight  $558.2 \pm 3.60$  g and dark brown color. During standardization, the capsules had an average disintegration time of 2 min 48 s and average dissolution time of 30 min.

### Selection of Subjects

The clinical study was conducted in the OPD of IPGAER Kolkata using male human subjects of 21–60 years' age group who had given informed consent following the guidelines of ICMR on biomedical research. Sixty male patients suffering from semen disorder (Sukra Dosh) over the past 6 months or more were selected after a general examination, of which 50 patients finally completed this study after the prescribed study period of 90 days. Due permission and approval were granted by the Ethical Committee of the Institute vide its memo no. SVSP/PG/363/2013 dated 22 March 2013 before undertaking this study.

The inclusion criteria included a history of infertility for the past 3 years, lack of sexual desire, difficulty in ejaculation or less quantity of ejaculate, painful coitus, or erectile dysfunction. Patients having a history of congenital deformity in genitals, malignancy, major surgery, serious hepatic, cardiac or kidney diseases, as well as chronic fever were not included in this study. All subjects were randomly allocated to two groups, as given below in Table 1. They were further advised to take plenty of water, avoid spicy food, alcohol and smoking, and take proper sleep.

### Diagnosis

The determination of spermatogenesis was done through the evaluation of the subjective and objective parameters, including semen analysis as well as physical examination and history of each patient.

**Table 1:** Treatment group allocation and drug protocol

Group	No. of patients	Drug used in capsule form	Oral dose prescribed after meal with water
Group A (control group)	25	Powder of rice (Placebo)	60 mg/kg bodyweight
Group B (research group)	25	Extract of <i>Bala</i> root powder	60 mg/kg bodyweight

## Evaluation of Subjective Parameters

### Primary symptoms

- History of infertility for the past 3 years
- Lack of libido/lack of sexual desire
- Difficulty in ejaculation of semen
- Ejaculating little quantity of semen after painful coitus
- Getting tired easily even after little exertion
- Impotence or erectile dysfunction.

### Secondary symptoms

- General weakness
- Headache and stress
- Loss of appetite
- Lack of sleep
- Constipation.

The severity of these physical symptoms was evaluated using an arbitrary grading scale (0–20% (+), 20–40% (++) , 40–60% (+++), 60–80% (++++), and 80–100% (+++++)) before and after the study.

## Assessment of Objective Parameters

Evaluation of objective parameters of each patient such as estimation of hematological parameters, liver function test, hormonal tests, routine urine tests, and semen analysis was done pre and post-treatment during this study. Semen analysis done in the reputed laboratory under expert supervision after 2–4 days of abstinence included physical examination (color, volume, viscosity, liquefaction time, etc.), microscopic examination (sperm agglutination and count), motility report (at 1<sup>st</sup> h and 3<sup>rd</sup> h), sperm morphology (normal and abnormal), and presence of other cells (round, epithelial, and RBC). Standard sperm morphology was indicated by the smooth oval head (5–6  $\mu$ m length, 2.5–3.5  $\mu$ m width), acrosome consisting of around 40–70% of anterior head, axial 1 $\mu$ m wide mid-piece, and around 45  $\mu$ m long straight thin tail. Sperm motility is assessed in an arbitrarily graded scale from 0 to 4 where 0 represents no movement and 4 means extremely fast forward movement while forward progression is represented by values above 2.<sup>[13-15]</sup>

The values of these parameters, as given in Table 2, were considered as normal during semen analysis.

## Statistical Tools Used

All obtained data values were expressed as mean  $\pm$  SEM. Statistical Package for the Social Sciences (SPSS) for Windows, version 10.0.7 (SPSS Inc., Chicago, IL) was utilized for undertaking all the necessary statistical calculations using this data. Results were considered as significant if the obtained *P*-value was below 0.05.

**Table 2:** Normal values of semen parameters

Parameter	Normal values
Semen volume	2–5 ml per ejaculation
Liquefaction time	20–30 min after collection
Sperm count	20 million/ml or more spermatozoa
Sperm shape (morphology)	More than 30% of the sperms have normal shape Kruger criteria: More than 14% of the sperms have a normal shape
Sperm movement (motility)	More than 50% of the sperms show normal forward movement after 1 hour
Semen pH	7.2–8.0
White blood cells	Not detected

## RESULTS

Analysis of the demographic parameters indicated that most of the subjects were in the 21–40 years' age group, 54% belonged to the rural areas, 47% were Muslims, 53% were married having 1–3 children, and 76% were non-vegetarian in food habits. During the study period of 90 days, except for 6.81% increase in the body weight of patients in the research drug-treated group, no significant changes occurred in the blood pressure, temperature, or pulse rate of the participants.

## Evaluation of Subjective Parameters

### Primary symptoms

Analysis of the results obtained during the study is shown in Table 3.

### Secondary symptoms

The evaluation of secondary symptoms before and after the study is detailed in Table 4.

## Evaluation of Objective Parameters

### Hematological parameters

The results of the hematological examination have been presented in Table 5.

### Liver function test

The results of various parameters relating to functioning of liver are outlined in Table 6.

### Hormonal study

The data obtained during the evaluation of the various hormonal parameters are given in Table 7.

## Routine Urine Tests

The results related to urine testing showed that no significant changes occurred after treatment and all the

**Table 3:** Percentage inhibition in primary symptoms during the study period

Primary symptoms	Group A			Group B		
	Before treatment	After treatment	% inhibition	Before treatment	After treatment	% inhibition
History of infertility for past 3 years	-	-	-	-	-	-
Lack of sexual desire	66	64	3.0	73	29	60.3
Difficulty in ejaculation	78	81	-3.9	82	22	73.2
Ejaculating little semen after painful coitus	76	72	5.3	81	28	64.2
Getting tired easily after little exertion	85	68	20.0	79	23	70.9
Impotence or erectile dysfunction	76	71	6.6	86	62	27.9
Absence or little amount of semen ejaculation	53	38	28.3	61	23	62.3

Mean values (n=25)

**Table 4:** Results obtained regarding the secondary symptoms in both groups

No of patients having secondary symptoms	Group A			Group B		
	Before treatment	After treatment	% inhibition	Before treatment	After treatment	% inhibition
Nausea	3	2	33	8	2	75
Body-ache	5	4	20	7	1	85
Headache	3	2	33	3	0	100
Indigestion	13	12	8	12	1	91
Loss of appetite	7	6	14	4	1	75
Weakness	11	10	9	9	1	88q

Mean values (n=25)

**Table 5:** Changes in hematological parameters during study

Hematological parameters	Group A			Group B		
	Before treatment	After treatment	% increase	Before treatment	After treatment	% increase
Blood sugar (mg/dl)	101.2±6.15	105.8±6.71	+4.55	109.7±10.96	110.9±20.66	+1.09
Hb (%)	13.30±0.22	13.10±0.19	-1.50	13.70±0.28	13.81±0.24	+0.88
ESR (mm)	20.0±1.77	19.5±1.57	-2.50	16.5±1.63	18.6±1.41	+12.73
RBC (millions/mm <sup>3</sup> )	4.78±0.11	4.74±0.10	-0.84	5.04±0.15	5.12±0.13	+1.59
WBC (thousand/mm <sup>3</sup> )	6.68±0.40	6.73±0.40	+0.75	8.07±0.58	8.17±0.53	+1.24

Values are expressed as mean±SEM (n=25)

**Table 6:** Results of liver function test

Liver function test (L.F.T.)	Group A			Group B		
	Before treatment	After treatment	% increase	Before treatment	After treatment	% increase
Bilirubin	0.76±0.14	0.74±0.11	-2.63	0.60±0.05	0.73±0.04	+21.67
Total protein	7.32±0.15	7.35±0.14	+0.41	7.24±0.15	7.57±0.13	+4.56
SGPT	32.9±3.73	28.3±2.00	-13.98	39.0±7.09	33.4±4.56	-14.36
SGOT	33.5±4.97	30.8±3.37	-8.06	29.2±2.91	32.4±2.01	+10.96
ALP	80.5±3.50	85.1±3.31	+5.71	88.0±7.78	98.3±5.33	+11.70

Values are expressed as mean±SEM (n=25). Bilirubin (mg/dL); Total protein (g/dl); SGPT (IU/L): Serum glutamic pyruvic transaminase; SGOT (IU/L): Serum glutamic-oxaloacetic transaminase; ALP: Alkaline phosphate (U/L)

parameters remained within normal ranges. In fact, values of some parameters which were found above normal during pretreatment period such as pus cell, RBC, protein, and WBC

in a few patients returned in the normal range after treatment, indicating the non-toxic and beneficial effect of the research drug upon the urogenital system.

**Table 7:** Results of hormonal parameters study

Hormonal parameters	Group A			Group B		
	Before treatment	After treatment	% increase	Before treatment	After treatment	% increase
T.S.H.	2.45±0.17	2.42±0.16	-1.22	2.32±0.22	2.36±0.17	+1.72
F.S.H.	7.79±1.57	7.77±0.75	-0.26	6.88±1.45	6.97±1.01	+1.31
L.H.	6.32±1.22	6.19±0.76	-2.06	6.06±0.75	6.26±0.60	+3.30
Testosterone	4.42±0.35	4.34±0.29	-1.81	4.34±0.35	4.71±0.36	+Q8.53

Values are expressed as mean±SEM (n=25). TSH: Thyroid-stimulating hormone, mcU/ml, FSH: Follicle-stimulating hormone: mIU/ml; LH: Luteinizing hormone: mIU/ml; Testosterone: ng/ml

**Table 8:** Results of analysis of seminal parameters

Seminal parameters	Group A			Group B		
	Pre-treatment	Post-treatment	% increase	Pre-treatment	Post-treatment	% increase
Physical examination						
Colour	Whitish grey	Whitish grey	-	Whitish grey	Milky white	-
Volume (ml)	1.5	1.5	0	1.5	2	33
Viscosity	Viscous	Viscous	-	Viscous	Viscous	-
pH value	7.8	7.6	-	7.5	7.8	-
Reaction	Alkaline	Alkaline	-	Alkaline	Alkaline	-
Liquefaction time (minutes)	20	23	15	20	30	50
Microscopic						
Sperm count (millions/ml)	68.6±6.10	69.4±4.74	+1.17	72.0±7.24	74.7±6.25	+3.75
(% Sperm morphology)						
Normal sperm	78.7±1.44	79.7±1.37	+1.27	77.4±1.14	82.0±1.39	+3.36
Abnormal sperm	21.3±1.44	20.3±1.37	-4.69	22.6±1.14	18.0±1.39	-11.50
(% Sperm motility)						
Rapid progressive	57.6±3.15	58.3±3.28	+1.22	50.5±4.01	54.5±3.96	+7.92
Slow progressive	13.0±0.92	13.5±1.16	+3.85	16.1±1.92	16.8±1.29	+0.62
Non-progressive	14.9±2.05	14.2±1.98	-11.41	14.8±2.22	12.2±2.06	-17.57
Immotile	14.5±2.66	15.0±2.44	+3.45	18.7±2.44	16.1±2.80	-8.56
Other cells per 100 sperm						
Round cells	2-3/H.P.F.	2-2/H.P.F.	-	2-4/H.P.F.	1-2/H.P.F.	-
Epithelial cells	1-2/H.P.F.	1-2/H.P.F.	-	1-2/H.P.F.	Nil	-
RBCs	1-2/H.P.F.	0-1/H.P.F.	-	Nil	Nil	-

Values are expressed as mean±SEM (n=25)

## Semen Analysis

Detailed analysis was performed in respect of the various parameters connected with semen and the results have been shown in Table 8.

## DISCUSSION

This clinical study evaluates the spermatogenesis effect of the research formulation prepared from the roots of *S. cordifolia* Linn. by assessing the changes occurring in the primary and secondary parameters as well as semen analysis, hematological investigations, urine analysis, and hormonal analysis vis-à-vis the control group after getting significant spermatogenesis action and non-toxic effect in animal models and standardization of this drug. Analysis of the curative

effect observed in respect of the primary symptoms indicates that while the very little impact was noticed in the control group, there was significant inhibition of these symptoms in case of the research group to the tune of 60.3%, 73.2%, 64.2%, 70.9%, and 62.3%, respectively, in respect of symptoms such as lack of sexual desire, difficult semen ejaculation, very less semen ejaculation, getting tired easily after limited exertion, and absence/little amount of semen ejaculation. In respect of secondary symptoms such as nausea, body-ache, headache, indigestion, loss of appetite, and general weakness, very high percentage of inhibition was observed ranging between 75% and 100% in the case of the research group while the control group showed very low values in this regard.

The enzymes present in the liver, such as SGPT, SGOT, and ALP, as well as the bilirubin level and protein content,

were evaluated during the study. No significant changes were observed in the hematological parameters (blood sugar, hemoglobin, ESR, RBC, and WBC) and biochemical parameters (bilirubin, protein, SGPT and SGOT, and ALP) during the treatment period in both the groups although some parameters registered a little increase but remained within normal values in the research group. Since these parameters are related to the toxicity or adverse effects of the administered drug, the obtained results indicate the non-toxic and non-adverse nature of the research formulation.

Efficient spermatogenesis is dependent upon synchronized actions of the two gonadotropins, FSH and LH. FSH is responsible for conversion of the spermatogonia into spermatozoa and plays a major role in the enhancement of fertility. LH levels are directly related to the production of testosterone in Leydig cells, which is the key hormone in the entire process.<sup>[1,16,17]</sup> The results of the hormonal analysis clearly suggest that very marginal changes were noticed in the control group in the level of the hormonal parameters. However, hormonal levels registered a significant increase during the study period in the research group, especially in the testosterone level (8.53%). This is quite significant as this hormone plays a pivotal role in the development of both prostate and testes and helps in the development of secondary sexual features like enhanced muscular structure and bone composition.<sup>[2,18,19]</sup>

Sperm morphology was studied using the count, motility, and other physical parameters before and after the treatment. During the study period, the sperm count in the research group increased by 3.75% while there was no appreciable increase in the control group. Similarly, the research group showed a very high increase in normal sperms (3.36%) and a decrease in abnormal sperms (11.50%) in comparison to the control group.

In terms of motility, the increase in rapid progressive sperms was only 1.22% in the control group and 7.92% in research group during the study period. At the same time, the number of non-progressive sperms decreased by 11.41% in the control group, while the decrease was 17.57% in the research group. The number of immotile sperms increased by 3.45% in the control group while it decreased by 8.56% in the research group. The volume of semen increased by 33% and its liquefaction time increased by 50 % in the research group during this study as compared to a very low increase in the control group.

All the sperm parameters exhibited very good enhancement during the study period in the research group in comparison to the control group which is further corroborated by the observed inhibition rates in primary and secondary symptoms in the study participants. The results clearly suggest that administration of research drug to subjects who are suffering from sexual problems probably due to various conditions of

stress (adverse action stress, bad food habits, exposure to harmful radiation, etc.) led to enhance the longevity of healthy body cells due to lower generation of ROS which is essential for maintaining the quality parameters of the spermatozoa during spermatogenesis.<sup>[3,20-22]</sup> The observed therapeutic effect of the research formulation could be primarily attributed to the sweet, cold potency, rejuvenator, tonic, antioxidant, anti-inflammatory, and immunomodulatory properties and presence of high concentration of phenolic and flavonoidic compounds in *S. cordifolia* Linn.

Many animal and human studies have reported that antioxidant supplementation produces a preventive effect on oxidative stress-induced decreased sperm count, motility, viability, mitochondrial function, DNA damage, and apoptosis.<sup>[23-26]</sup> Therefore, there is resurgence in undertaking research efforts relating to medicinal plants which are good sources of natural antioxidants. Many medicinal plants have been mentioned in the Ayurvedic textbooks for enhancement of *Sukradhatu* and these frequently mention *Bala* (*S. cordifolia*) which is particularly beneficial in diseases relating to the nervous and urinary systems. It is used in the treatment of inflammation and bleeding disorders since it has a cooling and astringent effect and is also very useful in ameliorating urinary problems.<sup>[10-12]</sup> Several previous *in vivo* and *in vitro* studies of aqueous extract of *Bala* in animal models have also reported good antioxidant action of this research formulation on the spermatogenesis process.<sup>[10-12]</sup>

The strength of this study arises from its comprehensive nature since it covers a wide range of subjective criteria and objective parameters for making a thorough assessment of the therapeutic efficacy of the research formulation. The number of patients and the diversity of their demographic characteristics has been highlighted above which could be a limiting factor since the same needs to further enhanced and made more broad-based and extensive before this formulation can be approved for clinical treatment.

## CONCLUSION

The clinical study strongly suggests that the research formulation shows good therapeutic efficacy in terms of inhibition of the primary and secondary symptoms and significant enhancement in the hormonal and seminal parameters, validating the spermatogenesis effect of the research drug formulated from the roots of *S. cordifolia* Linn. without any toxic or adverse effects.

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