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Original Research Article

Clinical evaluation of aphrodisiac activity of a novel ayurvedic formulation for treatment of male sexual disorders

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ABSTRACT

Background: *Sida cordifolia* Linn. and *Glycyrrhiza glabra* Linn. have been mentioned in Ayurvedic texts for their anti-inflammatory, antioxidant and sexual properties. After finding good results in treatment of male sexual disorders during pre-clinical studies, this clinical trial was taken up to assess the spermatogenesis action of aqueous extract of roots of these two plants.
Methods: The study used qualitative criteria such as primary and secondary symptoms and quantitative investigations such as haematological 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 haematological parameters (blood sugar, haemoglobin, ESR, RBC and WBC) and biochemical parameters (bilirubin, protein, SGPT and SGOT and ALP) indicate the non-toxic nature of research formulation. The hormonal levels registered a significant increase during clinical study in research group, especially the testosterone level (10.36%). 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.
Conclusions: The research formulation made from roots of *Sida cordifolia* Linn. and *Glycyrrhiza glabra* Linn. showed 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.

Keywords: Ayurvedic, Clinical study, *Glycyrrhiza glabra* Linn., *Sida cordifolia* Linn., Semen disorder

INTRODUCTION

Male reproductive capacity was found to be deficient in nearly 50% of infertile couples according to a study carried out by the World Health Organization in 1987. Sexual arousal is dependent on neural (sensory and cognitive), hormonal and genetic factors. Modern stressful life style has enhanced the number of persons

suffering from various forms of sexual dysfunction such as Oligozoospermia, sexual and ejaculatory dysfunction. Sexual dysfunction is an inability to achieve a normal sexual intercourse, including premature ejaculation, retrograded, retarded or inhibited ejaculation, erectile dysfunction, arousal difficulties (reduced libido), compulsive sexual behaviour, orgasmic disorder, and failure of de tumescence. Sexual dysfunction could be caused by various factors such as psychological disorders like anxiety, depression, stress, fear of sex, neurological disorders, stroke, cerebral trauma, Parkinson's disease and penile diseases like phinosis and peyronies, etc. Other organic causes include chronic renal failure, hepatic failure, multiple sclerosis, Alzheimer's disease, sleep apnea and chronic obstructive pulmonary disease. Chronic alcohol abuse and cigarette smoking also adversely affect sexual potency.[1-4] Although many synthetic drugs are available to treat these problems, some of their drawbacks include their high costs and serious adverse effects. Hence, the use of plant-based products to stimulate sexual desire and to enhance performance and enjoyment is almost as old as the human race itself. The quest for finding out active, natural principles and crude extracts of plants which have been useful in sexual disorders have the potential for improving sexual behaviour and performance and are helpful in spermatogenesis and reproduction. Accordingly, a number of herbal drugs have been validated for their effect on sexual behaviour and fertility and can therefore serve as the basis for identification of new chemical leads useful in sexual and erectile dysfunction.[5,6] An aphrodisiac is defined as any substance or drug that arouses the sexual instinct and increase libido. In Ayurvedic medicine, sukradhatu means the substance which is produced after a long process of conversion of all other dhatus responsible for semen production. The drugs which increase the quantity of semen or stimulate the production of semen, purify and improve the quality of semen, improve ejaculatory functions and drugs delaying the time of ejaculation or improving ejaculatory performance are called aphrodisiac. These have a long standing reputation as a cure for sexual dysfunction and have been used in numerous preparations for improving sexual performance and fertility especially in case of males.[7-11]

Sida cordifolia Linn. belonging to the Malvaceae family is one of the most useful medicinal plants in Ayurvedic literature. Also known as Bala, it is a small, erect, annual downy shrub.[12-14] It has been used as a cooling, astringent, aromatic, diuretic and tonic in Ayurvedic system of medicine for treatment of diseases like asthma, cough, fever, skin diseases, heart ailments, facial paralysis, muscle and joints pain, swelling, wounds, inflammation, urinary infection, lack of sexual desire and unwanted weight loss.[15-17] Its roots and seeds contain alkaloid ephedrine, vasicinol, vasicinone, β-sitosterol and stigmasterol and N-methyl tryptophan while the leaves of *Sida cordifolia* contain small amounts of both ephedrine and pseudoephedrine. Its pharmacological actions include hypoglycemic, wound healing, anti-microbial, antioxidant, anti-inflammatory, analgesic, adaptogenic and hepato-protective activities.[18-21]

Glycyrrhiza glabra Linn. also called Liquorice root belongs to the Fabaceae family. It is a perineal herb/sub-shrub found in the subtropical and temperate zones. Its underground stems and roots are used medicinally for treatment of cough, hyperacidity, skin and ophthalmic diseases and as a tonic, rejuvenator, demulcent, expectorant, etc. The chief constituent of liquorice is glycyrrhizin, which is present in the drug in the form of the potassium and calcium salts of glycyrrhizic acid. Liquorice also contains glucose (up to 3.8 per cent), sucrose (2.4 to 6.5 per cent), bitter principles, resins, mannite, asparagines (2 to 4 per cent) and fat (0.8 per cent). Its pharmacological activities are reported to be muscle depressant, anti-microbial, hypo-lipidaemic, anti-antherosclerotic, antiviral, hypotensive, hepato-protective, anti-exudative, spasmolytic, antidiuretic, antiulcer, anti-mutagenic, antipyretic, antioxidant, anti-inflammatory, anti-nociceptive and expectorant.[12-17, 22-27] The aim of the present study was to establish the spermatogenesis action of the aqueous extract of the combination Ayurvedic formulation called AFBY having equal amount of roots of *Sida cordifolia* Linn. and *Glycyrrhiza glabra* Linn. through placebo controlled clinical 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 by using qualitative and quantitative investigations such as haematological investigations, hormonal analysis by ELISA methods and semen analysis each patient enrolled after informed consent and approval of the Institutional ethical committee for clinical trial on human subjects.

METHODS

Collection and identification of plant materials

The roots of *Sida cordifolia* Linn. and *Glycyrrhiza glabra* Linn. were purchased from reputed crude drug supplier of Katwa Chowrasta, Burdwan district and authenticated by the Research Officer, Botanical Survey of India, Howrah, India (REF./NO. BSI/CNH/SF/Tech./2016). The chemicals, reagent and testing kits for haematological, biochemical, hormonal and semen analysis were purchased from reputed suppliers of Kolkata following the Institutional norms.

Preparation of extracts

The aqueous extract of the roots of *Sida cordifolia* Linn. and *Glycyrrhiza glabra* Linn. were prepared following the guidelines of Ayurvedic pharmacopeia for identification and standardization. One-part coarse

powder of research drug (containing equal amounts of both the herbs) 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 lypholizer instrument and stored in the dried form for preparation of zero size capsule having average weight 558.2±3.60 gms and dark brown colour. During standardization, the capsules had an average disintegration time of 2 minute 48 seconds and average dissolution time of 30 minutes. This research formulation used in this study was an Ayurvedic formulation called AFBY.

Selection of subjects

The clinical study was conducted in the OPD of IPGAER Kolkata after getting approval from the Institutional ethical committee (vide no. SVSP/PG/363/2013 dated 22.3.2013) using male human subjects of 21- 60 years age group who had given informed consent following the guidelines of ICMR on biomedical research. 70 male patients suffering from semen disorder (Sukra Dosh) over past 6 months or more were selected after general examination out of which 50 patients finally completed this study after the prescribed study period of 90 days. The inclusion criteria included history of infertility since last three years, lack of sexual desire, difficulty in ejaculation or less quantity of ejaculate, painful coitus or erectile dysfunction.

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 powder of Ayurvedic formulation (AFBY)	60 mg/ Kg bodyweight

Patients having history of congenital deformity in genitals, malignancy, major surgery, uncontrolled diabetes mellitus, severe hepatic or renal insufficiency, cardiovascular diseases, uncontrolled hypertension, or with previous history of cryptorchidism, varicocele and testicular hypertrophy and chronic fever were excluded from the 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 evaluation of the subjective and objective parameters including semen analysis as well as physical examination and history of each patient.

Evaluation of subjective parameters

Primary symptoms

History of infertility since last three 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, early ageing symptoms with dry skin and wrinkles on face and body, anemia, constipation. The severity of these physical symptoms was evaluated by 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 (TC, DC, Hb%, ESR, Blood sugar), liver function test (Bilirubin, SGOT, SGPT, Alkaline phosphate, Total protein), hormonal tests (FSH, LH, total testosterone and TSH), routine urine tests (protein, sugar, urobilinogen, phosphates, RBC, epithelial cells, pus cells, parasites and yeast cells) and semen analysis was done pre and post treatment during this study.

Table 2: Normal values of semen parameters.

Parameter	Normal values
Semen volume	2-5 ml per ejaculation
Liquefaction time	20-30 minutes 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	No white blood cells or bacteria are detected

Semen analysis (Table 2) was done in the reputed laboratory under expert supervision after 2- 4 days of abstinence included physical examination (colour, volume, viscosity, liquefaction time, etc.), microscopic examination (sperm agglutination and Count), motility

report (at 1st hour and 3rd hour), sperm morphology (normal and abnormal) and presence of other cells (round, epithelial and RBC).[28-30]

Statistical analysis

Individual parameters were expressed as mean±SEM. The Statistical Package for the Social Sciences (SPSS) for Windows, version 10.0.7 (SPSS Inc., Chicago, IL) was used for all calculations and statistical analysis. Statistical significance was benchmarked at $p<0.05$.

RESULTS

Analysis of the demographic features indicated that majority of the subjects were young (23-47 years' age group), 66% belonged to the rural areas, 42% were Muslims while 38% were Hindus, 62% were married having 1-3 children and 66% were non-vegetarian in food habits. During the study period of 90 days, except for 8.92% 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 are shown in Table 3 and secondary symptoms before and after the study are detailed in Table 4.

Evaluation of objective parameters

Haematological parameters: The results obtained in respect of the various haematological parameters are shown in Table 5.

Table 3: Percentage inhibition in primary symptoms during study period.

Primary symptoms	Pre-treatment	Post-treatment	% inhibition	Pre-treatment	Post-treatment	% inhibition
	Group A			Group B		
History of infertility since last three years	–	–		–	–	-
Lack of sexual desire	65	62	4.62	71	28	60.56
Difficulty in ejaculation	72	75	- 4.17	79	24	69.62
Ejaculating little semen after painful coitus	77	73	5.19	81	29	64.20
Getting tired easily after little exertion	81	69	14.81	79	25	68.35
Impotence or erectile dysfunction	78	72	7.69	82	63	23.17
Absence or little amount of semen ejaculation	58	46	20.69	61	22	63.93

Mean values (n=25)

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

No of patients having secondary symptoms	Pre-treatment	Post-treatment	% inhibition	Pre-treatment	Post-treatment	% inhibition
	Group A			Group B		
Nausea	3	2	33	6	2	67
Body-ache	5	4	20	5	1	80
Headache	3	2	33	2	0	100
Indigestion	13	12	8	12	10	17
Loss of appetite	7	4	43	5	1	80
Weakness	11	10	9	13	5	62

Mean values (n=25)

Table 5: Changes in haematological parameters during study.

Haematological parameters	Pre-treatment	Post-treatment	% increase	Pre-treatment	Post-treatment	% increase
	Group A			Group B		
Blood sugar (mg/dl)	101.2±6.15	105.8±6.71	4.55	100.3±6.42	105.5±8.57	5.18
Hb (%)	13.30±0.22	13.32±0.19	0.04	13.80±0.25	13.67±0.23	-0.94
ESR (mm)	20.0±1.77	19.5±1.57	-2.50	18.4±2.32	20.0±2.28	8.70
Rbc (millions/mm^3)	4.78±0.11	4.76±0.10	-0.02	4.86±0.10	4.91±0.11	1.03
Wbc (thousand/mm^3)	6.68±0.40	6.73±0.40	0.75	7.13±0.39	6.70±0.29	-6.03

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

Table 6: Results of liver function test.

Liver function test (L.F.T.)	Pre-treatment	Post-treatment	% increase	Pre-treatment	Post-treatment	% increase
	Group A			Group B		
Bilirubin	0.76±0.14	0.74±0.11	-2.63	0.65±0.09	0.66±0.10	1.54
Total protein	7.32±0.15	7.35±0.14	+0.41	7.66±0.10	7.89±0.10	3.00
SGPT	32.9±3.73	28.3±2.00	-13.98	35.3±2.49	33.2±2.87	-5.95
SGOT	33.5±4.97	30.8±3.37	-8.06	32.3±2.02	31.1±2.54	-3.72
Alp	80.5±3.50	85.1±3.31	+5.71	79.2±3.84	79.5±4.07	0.38

values are expressed as mean±SEM (n=25). (bilirubin (mg/dl); total protein (gm/dl); sgpt (iu/l): serum-glutamic-pyruvic-transaminase; sgot (iu/l): serum-glutamic-oxaloacetic-transaminase; alp: alkaline phosphate (u/l))

Table 7: Results of hormonal parameters study.

Hormonal parameters	Pre-treatment	Post-treatment	% increase	Pre-treatment	Post-treatment	% increase
	Group A			Group B		
T.S.H.	2.45±0.17	2.42±0.16	-1.22	2.85±0.52	2.98±0.34	4.56
F.S.H.	7.79±1.57	7.77±0.75	-0.26	7.16±0.94	7.85±0.74	9.64
L.H.	6.32±1.22	6.19±0.76	-2.06	6.14±0.71	7.23±0.80	17.69
Testosterone	4.42±0.35	4.34±0.29	-1.81	4.32±0.38	4.93±0.38	14.12

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		Pre-treatment	Post-treatment	% increase	Pre-treatment	Post-treatment	% increase
			Group A			Group B	
Physical examination	Colour	Milky white	Milky white	–	Milky white	Milky white	–
	Volume (ml)	1.5 ml	1.5 ml	–	1.5 ml	2 ml	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 minutes	22 minutes	10	21 minutes	30 minutes	43
Microscopic	Sperm count (millions/ml)	68.6±6.10	69.4±4.74	1.17	87.1±7.04	90.8±4.92	4.25
(%) sperm morphology	Normal sperm	78.7±1.44	79.7±1.37	1.27	77.1±1.14	80.0±1.39	3.76
	Abnormal sperm	21.3±1.44	20.3±1.37	-4.69	22.9±1.14	20.0±1.39	-12.66
(%) sperm motility	Rapid progressive	57.6±3.15	58.3±3.28	1.22	46.3±3.63	51.7±4.10	11.66
	Slow progressive	13.0±0.92	13.5±1.16	3.85	22.6±2.34	22.9±1.72	1.33
	Non-progressive	14.9±2.05	13.2±1.98	-11.41	17.2±2.31	12.9±2.26	-25.00
	Immotile	14.5±2.66	15.0±2.44	3.45	13.9±2.06	12.5±2.29	-10.07
Other cells Per 100 sperm	Round cells	1-2/h.p.f.	1-2/h.p.f.	–	3-4/h.p.f.	1-2/h.p.f.	–
	Epithelial cells	Occasional	Occasional	–	1-2/h.p.f.	1-2/h.p.f.	–
	Rbcs	Nil	Nil	–	1-2/h.p.f.	0-1/h.p.f.	–

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

Liver function test

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

Hormonal study

The data obtained during 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 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, WBC, etc, in a few patients returned in the normal range after treatment indicating the non-toxic and beneficial effect of the research drug upon the uro-genital system.

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

Sexual dysfunction is a serious medical symptom that occurs in 10-52% of men and 25-63% of women. ED, the main reason of male impotence, is considered as one of the most important public health problems, since it affects an estimated 20-30 million men. ED is defined as the consistent inability to obtain or maintain an erection for satisfactory sexual relations. Penile erection occurs as a result of smooth muscle relaxation in the penis mediated by a spinal reflex and involves central nervous processing and integration of tactile, olfactory, auditory, and mental stimuli. The reflex involves both autonomic and somatic afferents and modulated by supraspinal influences peripherally. Drugs affecting sexuality can either act on the central nervous system (Brain) and/or on the peripheral nervous system. Drugs affecting the brain and presumably sex centers are generally attributed with an increase or decrease in sexual arousal.[31-32] Drugs that affect peripheral nerves will not affect arousal directly but may affect sexual function. However, most of the available drugs and treatments have limited efficacy, unpleasant side effects, and contraindications in certain disease conditions. Hence, the search for natural supplement from medicinal plants is being intensified probably because of its fewer side effects, its ready availability and less cost. A variety of botanicals are known to have a potential effect on the sexual functions, supporting older claims and offering newer hopes.

There are many herbal drugs that have been prescribed for the treatment of semen disorders under the category of Vajikaran Rasayan in Ayurveda for their putative positive influence on sexual performance in humans. Many animal and human studies have reported that antioxidant supplementation produces preventive effect on oxidative stress induced decreased sperm count, motility, viability, mitochondrial function, DNA damage and apoptosis. Therefore, there is an increasing interest in natural antioxidants by using the medicinal and dietary plants, which are candidates for the prevention of oxidative damage. Many medicinal plants have been mentioned in the Ayurvedic text books for enhancement of Sukra dhatu. Among these, the rejuvenating action of Bala (*Sida cordifolia*) and Yashthimadhu (*Glycyrrhiza glabra*) extends to the nervous, circulatory, and urinary systems. Because of their diuretic effect, they are useful in urinary problems, inflammations and bleeding disorders being cooling and astringent.

This clinical study has studied the spermatogenesis effect of the research formulation prepared from the roots of *Sida cordifolia* Linn. and *Glycyrrhiza glabra* Linn. in comparison to the control group by assessing the changes occurring in the primary and secondary parameters as well as semen analysis, haematological investigations, urine analysis and hormonal analysis 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 very little impact was observed in case of the control group, there was significant inhibition of these symptoms in case of the research group to the tune of 60.6%, 69.6%, 64.2%, 68.35% and 63.9% respectively in respect of symptoms such as 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 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, while substantially low rates were observed in case of the control group, very high percentage of inhibition was observed ranging between 62% to 100% in case of the research group.

Analysis of the activities of some basic liver enzymes in the plasma or serum such as SGPT, SGOT and ALP are largely used along with Bilirubin and total protein content to indirectly assess the integrity of tissues after being exposed to any pharmacological agent. No significant changes were observed in the haematological parameters (Blood sugar, haemoglobin, 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.

The production of male gametes depends on the concerted action of the two gonadotropins FSH and LH on the testis. FSH is required for normal functioning of Sertoli cells, in which transformation of spermatogonia to spermatozoa occurs in testes while LH stimulates testosterone production from Leydig cells, not only required for normal spermatogenic process but also

essential for maintaining secondary sexual characteristics, libido, and anabolic actions. FSH acts synergistically with testosterone to increase spermatogenesis efficiency and fertility.[33-36] The results of the hormonal analysis clearly suggested that the level of all the hormonal parameters reduced a little in case of the control group. However, the hormonal levels registered a significant increase during the study period in case of the research group especially in case of the testosterone level (14.12%). Androgens play a crucial role in the development of secondary male sexual organs such as epididymis, vas deferens, seminal vesicle, prostate, and the penis. Furthermore, androgens are needed for puberty, male fertility, and male sexual function. Testosterone is the principal androgen secreted by the testes which is synthesized in the Leydig cells of the testes, stimulated by luteinizing hormone (LH). One of the principal effects of testosterone within the testes is the stimulation of spermatogenesis in seminiferous tubules.

The semen quality was evaluated in terms of sperm count, sperm motility, sperm morphology and physical attributes. During the study period, the increase in sperm count was found to be considerably higher in the research group (4.25%) as compared to the control group. Similarly, the increase in normal sperms (3.76%) and the decrease in abnormal sperms (12.66%) in case of the research group was substantially higher than in case of the control group. In terms of motility, the increase in rapid progressive sperms was only 1.22% in control group and 11.66% 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 25.00% in the research group. The number of immotile sperms increased by 3.45% in the control group while it decreased by 10.07% in the research group. The volume of semen increased by 33% and its liquefaction time increased by 43 % in the research group during this study as compared to very low increase in the control group.

All the sperm parameters showed a significant improvement in the research group during the study period as compared to the control group which is also corroborated by the observed inhibition rates in the 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.) counteracted and protected the healthy cells by reduced generation of the reactive oxygen species which is essential for maintaining the quality parameters of the spermatozoa during spermatogenesis.[37-40] 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 *Sida cordifolia* Linn. and *Glycyrrhiza glabra* Linn..

CONCLUSION

The clinical study strongly suggests that the research drug formulation exhibits very 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 *Sida cordifolia* Linn. and *Glycyrrhiza glabra* Linn. without any toxic or adverse effects. However, the efficacy of this formulation needs to be further validated and substantiated through more wide-based and extensive clinical trials.

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