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Clinical spermatogenic assessment of aqueous extract of Yashthimadhu (*Glycyrrhiza glabra* Linn.) in the treatment of male reproductive disorders

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Abstract
Background: *Glycyrrhiza glabra* Linn. has been mentioned in Ayurvedic texts for its anti-inflammatory, antioxidant and sexual properties. It shows good promise in the treatment of male sexual disorders during its pre-clinical studies.
Objectives: To assess the spermatogenesis action of aqueous extract of roots of *Glycyrrhiza glabra* Linn. in clinical trials.
Materials and Methods: The study uses qualitative criteria such as primary & 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.
Conclusion: The research formulation made from roots of *Glycyrrhiza glabra* 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.

Keywords: clinical study, *Glycyrrhiza glabra*, semen disorder, Ayurvedic

1. Introduction

A medicinal plant is having potent therapeutic efficacy in any part which contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. These herbal medicines being used for several centuries have the advantage of being effective, inexpensive, safe and readily available. Currently, extraction and development of several drugs and chemotherapeutics from these plants have been widely observed. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have the potential to reduce risk of diseases by scavenging free radicals.

Spermatogenetic failure, including azoospermia and oligospermia, is one of the important causes of male infertility. Several conditions can interfere with spermatogenesis and reduce sperm quality and production. Many factors such as drug treatment, chemotherapy, toxins, air pollution and insufficient intake of vitamins may have harmful effects on spermatogenesis and sperm normal production [1-3]. It has also been reported that using antioxidants and vitamins A, B, C, and E in daily diet can protect sperm DNA from free radicals. Most plant extracts have major bioactive components including phenolic compounds (phenols, sterol, lignans and flavonoids), vitamins (B1, B2, B3, B6, C and E), folic acid, bio-trace elements (Ca, Mg, P, Zn, K, Cu and Fe), most of the essential amino acids, volatile oils, polyphenols and saponins that may have positive effect on spermatogenesis, sperm parameters (sperm motility, count and viability); increasing Leydig cell counts, seminiferous tubule diameters, decreasing abnormal sperms and improving histopathological recovery and sexual stimulation (erection, intromission and ejaculatory latency) [4, 11].

Various herbs have been used by different medical systems of treatment in several pharmaceutical forms to treat conditions of male infertility or for treatment of reproductive disorders. In Ayurveda, many medicinal plants have properties of rejuvenator, antioxidant and tonic which have been used for the treatment of male reproductive disorders since ancient times [9]. *Glycyrrhiza glabra* Linn. (Yashthimadhu) is one such important rejuvenator and immunomodulatory plant which has been selected to evaluate its pre-clinical and clinical activities in respect of spermatogenesis.

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. The plant attains a maximum height up to 2 m. The underground stem grows horizontally up to 2 m length, highly branched consisting of short tap root with large number of rhizomes. The diameter of the root varies from 0.75 to 2.5 cm, grey-brown exterior and yellow interior. Externally, it is longitudinally wrinkled with patches of cork. It has a characteristic pleasant sweet

taste. Flowering & fruiting is from August to February. 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. Glycyrrhizic acid is not a glycoside since it yields on hydrolysis one molecule of Glycyrrhetic acid and two molecules of Glycuronic acid but no sugar. Glycuronic acid is, however, very closely related to the hexose sugars, and Glycyrrhetic acid has a haemolytic action like that of the saponins [12, 14]. Liqourice 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 relaxant, 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 [15, 17].

The aim of the present study was to establish the spermatogenesis action of aqueous extract of roots of *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 criteria such as primary & secondary symptoms, 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.

2. Materials & Methods

2.1. Collection & identification of plant materials

The roots of Glycyrrhiza glabra Linn. (Synonym: Yashthimadhu) were purchased from crude drug supplier of Katwa Chowrasta, Burdwan district and authenticated by the Research Officer, Botanical Survey of India, Howrah, India (REF./NO.2, BSI/CNH/SF/Tech./2016).

The chemicals, reagent and testing kits for haematological, biochemical, hormonal and semen analysis were purchased from the reputed suppliers of Kolkata following the Institutional norms.

2.2. Preparation of Extracts

The aqueous extract of the root of Yashthimadhu was prepared following the guidelines of Ayurvedic pharmacopeia for identification & 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 lypholizer instrument and stored in the dried form for preparation of zero size capsule having average weight 550.2 ± 2.50 gms and brown colour. During standardization, the capsules had an average disintegration time of 3 minute 28 seconds and average dissolution time of 30 minutes.

2.3. Selection of subjects

The clinical study was conducted in the OPD of IPGAER Kolkata after getting approval from the Institutional ethical committee using male human subjects of 20 -60 years' age group who had given informed consent following the guidelines of ICMR on biomedical research. 60 male patients suffering from semen disorder & male reproductive disorders 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. 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.

Table 1: Treatment group allocation & 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 Yashthimadhu root	60 mg/ Kg bodyweight

2.4. 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.

2.5. Evaluation of subjective parameters

2.5.1. Primary Symptoms

a. History of infertility since last three years
b. Lack of libido/ lack of sexual desire
c. Difficulty in ejaculation of semen
d. Ejaculating little quantity of semen after painful coitus
e. Getting tired easily even after little exertion
f. Impotence or erectile dysfunction

2.5.2. Secondary symptoms

a. General weakness
b. Headache and stress
c. Loss of appetite
d. Lack of sleep
e. Early ageing symptoms with dry skin and wrinkles on face and body
f. Anemia
g. 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.

2.6. 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. Semen analysis done in the reputed laboratory of the Dravyaguna Vigyan of IPGAER Kolkata & from Probe Diagnostic Pvt. Ltd, Kolkata 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) [18-20]. The following values of these parameters were considered as normal during semen analysis (table 2):

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

2.7. 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 $p < 0.05$.

3. Results
Analysis of the demographic features indicated that majority of the subjects were young (25-50 years' age), 58% belonged to rural areas, 44% were Muslims, 32 % Hindus and 24% others, 63% were married having 1-3 children and 72% were non-vegetarian in food habits. During the treatment period of 90 days, no significant changes were noticed in the blood pressure, temperature or pulse rate of the participants.

3.1. Evaluation of subjective parameters
3.1.1. Primary Symptoms
The obtained results are shown in table 3.

Table 3: Percentage inhibition in primary symptoms during study period

Primary Symptoms	Group A			Group B		
	Pre-treatment	Post-treatment	% inhibition	Pre-treatment	Post-treatment	% inhibition
History of infertility since last three years	–	–		–	–	-
Lack of sexual desire	66	64	3.0	69	24	65.2
Difficulty in ejaculation	78	81	-3.9	80	27	66.3
Ejaculating little semen after painful coitus	76	72	5.3	81	31	61.7
Getting tired easily after little exertion	85	68	20.0	82	22	73.2
Impotence or erectile dysfunction	76	71	6.6	79	55	30.4
Absence or little amount of semen ejaculation	53	38	28.3	60	21	65.0

Mean values (n=25)

3.1.2. Secondary Symptoms
The evaluation of secondary symptoms before and after the study is detailed in table 4.

Table 4: Results obtained for the secondary symptoms in both groups

No of patients having Secondary Symptoms	Group A			Group B		
	Pre-treatment	Post-treatment	% inhibition	Pre-treatment	Post-treatment	% inhibition
Nausea	4	3	33	7	2	71
Body-ache	8	6	25	10	4	60
Headache	3	2	33	6	2	67
Indigestion	15	13	13	15	2	87
Loss of appetite	9	6	33	13	3	77
Weakness	15	10	33	16	4	75

Mean values (n=25)

3.2. Evaluation of objective parameters
3.2.1. Haematological parameters
The results obtained in respect of the various haematological parameters are shown in table 5.

Table 5: Changes observed in haematological parameters during study

Haematological Parameters	Group A			Group B		
	Pre-treatment	Post-treatment	% increase	Pre-treatment	Post-treatment	% increase

Blood Sugar (mg/dl)	101.2 ± 6.15	102.8 ± 6.71	+1.58	90.5 ± 2.35	95.5 ± 2.80	+5.52
Hb (%)	13.30 ± 0.22	13.38 ± 0.19	+0.60	14.24 ± 0.25	14.39 ± 0.26	+1.05
ESR (mm)	20.0 ± 1.77	19.5 ± 1.57	-2.50	18.4 ± 1.53	17.0 ± 1.03	-7.61
RBC (millions/mm^3)	4.78 ± 0.11	4.76 ± 0.10	-0.02	4.82 ± 0.12	4.98 ± 0.11	- 0.81
WBC (thousand/mm^3)	6.68 ± 0.40	6.73 ± 0.40	+0.75	6.86 ± 0.43	6.46 ± 0.36	- 5.83

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

3.2.2. Liver function Test

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

Table 6: Results of Liver Function Test

Liver Function Test (L.F.T.)	Group A			Group B		
	Pre-treatment	Post-treatment	% increase	Pre-treatment	Post-treatment	% increase
Bilirubin	0.76 ± 0.14	0.74 ± 0.11	-2.63	0.77 ± 0.12	0.66 ± 0.05	-14.15
Total protein	7.32 ± 0.15	7.35 ± 0.14	0.41	7.74 ± 0.12	7.85 ± 0.10	1.48
SGPT	32.9 ± 3.73	28.3 ± 2.00	-13.98	40.6 ± 8.49	30.5 ± 3.49	-24.88
SGOT	33.5 ± 4.97	30.8 ± 3.37	-8.06	35.6 ± 4.80	26.8 ± 1.83	-24.86
ALP	80.5 ± 3.50	85.1 ± 3.31	5.71	70.6 ± 3.25	73.2 ± 2.74	3.68

values are expressed as mean ± SEM (n=20). [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)]

3.2.3. Hormonal Study

The data obtained during evaluation of the various hormonal parameters are given in table 7 and depicted in figure 1.

Table 7: Results of Hormonal parameters study

Hormonal Parameters	Group A			Group B		
	Pre-treatment	Post-treatment	% change	Pre-treatment	Post-treatment	% change
T.S.H.	1.45 ± 0.18	1.43 ± 0.13	-1.38	2.82 ± 0.53	2.95 ± 0.25	+4.61
F.S.H.	4.99 ± 1.36	4.86 ± 0.62	-2.61	6.39 ± 0.69	6.65 ± 0.45	+4.03
L.H.	4.42 ± 1.24	4.71 ± 0.59	+2.06	6.11 ± 0.60	6.35 ± 0.45	+3.93
Testosterone	3.42 ± 0.42	3.64 ± 0.38	+1.81	4.29 ± 0.42	4.73 ± 0.24	+10.36

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)]

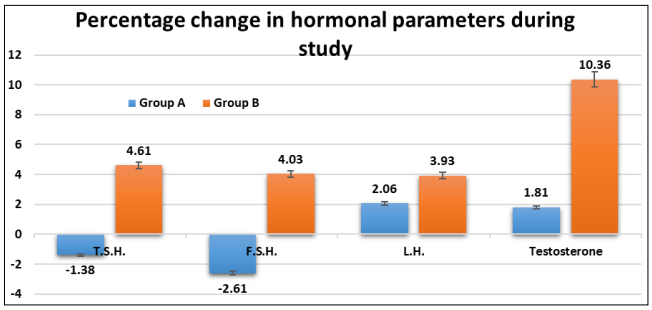

Fig 1: Changes observed in hormonal parameters during treatment

3.2.4. 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 during the study period.

3.2.5. Semen analysis

Detailed analysis was performed in respect of the various parameters connected with semen and the results have been shown in table 8 and figure 2.

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	Milky white	Milky white	–	Milky white	Milky white	–
	Volume (ml)	1.5 ml	1.5 ml	0	1.5 ml	2 ml	33
	Viscosity	Viscous	Viscous	–	Viscous	Viscous	–
	pH value	7.5	7.6		7.5	7.7	
	Reaction	Alkaline	Alkaline	–	Alkaline	Alkaline	–
	Liquefaction time (minutes)	18 minutes	22 minutes	22.22	20 minutes	30 minutes	50
Microscopic	Sperm count (millions/ml)	68.6 ± 6.10	69.4 ± 4.74	1.17	84.9 ± 7.12	86.9 ± 6.78	2.36
(%) Sperm Morphology	Normal Sperm	78.7 ± 1.44	79.7 ± 1.37	1.27	76.3 ± 0.97	78.2 ± 1.35	2.49
	Abnormal Sperm	21.3 ± 1.44	20.3 ± 1.37	-4.69	23.7 ± 0.97	21.8 ± 1.35	-8.02
(%) Sperm Motility	Rapid progressive	57.6 ± 3.15	58.3 ± 3.28	1.22	51.4 ± 4.12	55.5 ± 4.25	7.98
	Slow progressive	13.0 ± 0.92	13.5 ± 1.16	3.85	16.1 ± 1.81	16.2 ± 1.33	0.62
	Non-progressive	14.9 ± 2.05	13.2 ± 1.98	-11.41	17.5 ± 2.33	14.1 ± 1.67	-19.43
	Immotile	14.5 ± 2.66	15.0 ± 2.44	3.45	15.0 ± 2.33	13.2 ± 3.46	-12.0
Other Cells per 100	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.	–

Sperm	RBCs	nil	nil	–	1-2/H.P.F.	0-1/H.P.F.	–

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

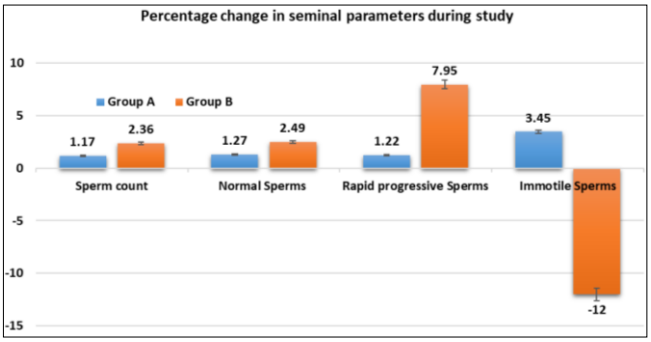

Fig 2: Changes in seminal parameters during treatment

4. Discussion

Evidence-based research in Ayurveda is receiving larger acceptance in India and abroad. Use of plants as a source of medicine has been an ancient practice and is an important component of the health care system in India. In India, about 70 percent of rural population depends on the traditional Ayurvedic system of medicine. Spermatogenic activity could be impaired due to several reasons such as reduced sertoli cell proliferation, testicular dysgenesis syndrome, lifestyle effects such as scrotal heating, obesity, smoking, alcohol and drugs and exposure to environmental chemicals in adulthood [21, 23]. Although various chemical drugs are available to treat infertility, researchers are looking for drugs with less adverse effect and toxicity which are available in traditional medicine.

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 the 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 *Glycyrrhiza glabra* Linn. extends to the nervous, circulatory, and urinary systems. It has a diuretic effect, is useful in urinary problems and is also used in 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 *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 therapeutic 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 65.23%, 66.3%, 61.7%, 73.2% and 65.0% 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, bodyache, headache, indigestion, loss of appetite and general weakness, very high percentage of inhibition was observed ranging between 67% to 87% in case of the research group whereas substantially low rates were observed in case of the control 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 while all the parameters 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 [1, 24]. The results of the hormonal analysis clearly suggest 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 (10.36%). Testosterone hormone plays a key role in the development of male reproductive tissues such as testes and prostate, as well as promoting secondary sexual characteristics such as increased muscle and bone mass.

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 noticeably higher in the research group (2.36%) as compared to the control group. Similarly, the increase in normal sperms (2.49%) and the decrease in abnormal sperms (8.02%) 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 7.98% 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 19.43% in the research group. The number of immotile sperms increased by 3.45% in the control group while it decreased by 12% 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 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 [25, 30]. The observed therapeutic effect of the research formulation could be primarily attributed to the sweet, cold potency, rejuvenator, tonic, antioxidant, anti-inflammatory & immunomodulatory properties and presence of high concentration of phenolic and flavonoidic compounds such as glycyrrhizin, glycyrrhizinic acid and glabridin in *Glycyrrhiza glabra* Linn.

5. Conclusion

The clinical study indicates that the research drug formulation shows very good therapeutic efficacy in terms of inhibition of primary and secondary symptoms and highly significant ($p < 0.05$) increase in the hormonal and seminal parameters, validating the spermatogenesis effect of the research drug formulated from the roots of *Glycyrrhiza glabra* Linn. without any toxic or adverse effects.

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7. References

1. Walker WH. Non-classical actions of testosterone and spermatogenesis. Philos. Trans. R. Soc. Lond. B. Biol. Sci, 2010; 365:1557-1569.
2. De Rooij DG, Grootegoed JA. Spermatogonial stem cells. Curr Opin Cell Biol. 1998; 10:694-701.
3. Li H, Chen Q, Li S. Effect of Cr (VI) exposure on sperm quality: human and animal studies. Ann. Occup. Hyg, 2001; 45:505-511.
4. Sharma PC, Yelne MB, Dennis TJ. Database on medicinal plants used in Ayurveda. Central Council for Research in Ayurveda & Siddha, Department of Indian system of medicine, Govt. of India, New Delhi, 2001.
5. Sastry JLN. Dravyaguna Vijnana, 1st ed, Chaukambha Orientalia, Varanasi, 2014, p.153.
6. Hegde PL, Harini A. A Textbook of Dravyaguna Vijnana, 1st ed. Chaukhamba Publications, New Delhi, 2014, p.903.
7. Sharma PV. Dravyaguna Vijnana, 2nd ed. Chaukhamba Bharati Academy, Varanasi, 2011, p.284.
8. Sukhdev. Prime Ayurvedic Plant Drugs, 2nd ed. Ane Books Pvt. Ltd, New Delhi, 2012, p.365.
9. Khojasteh SMB, Khameneh RJ, Houresfsnd M, Yaldagard E. A Review on Medicinal Plants Used for Improvement of Spermatogenesis. Biol Med (Aligarh), 2016; 8:292. doi:10.4172/0974-8369.1000292
10. Chatterjee A, Pakrashi SC. The treatise on Indian Medicinal Plants, Publications and Information Directorate, CSIR, New Delhi, 1992, Vol. 2 &3.
11. The Ayurvedic Pharmacopoeia of India, Ministry of Health and Family welfare, Department of Indian system of medicine & homoeopathy, Govt. of India, New Delhi, 2001; Part-I, Vol. I, 127-128.
12. Indhu BS, Shajahan MA, Hameed AS. Yashtimadhu (Glycyrrhiza Glabra) - A Review, Indian Journal of Applied Research. 2019; 9(12):64-65. DOI: 10.36106/ijar.
13. Korhalkar A, Deshpande M, Lele P, Modak M), Pharmacological Studies of Yashtimadhu (Glycyrrhiza Glabra L.) in various Animal Models - A Review, Global J Res. Med. Plants & Indigen. Med. 2013; 2(3):152-164.
14. Vispute S. *Glycyrrhiza Glabra* Linn. - "Klitaka": A Review. International Journal of Pharma and Bio Sciences. 2011; 2(3):42-51.
15. Nishant P. Visavadiya, Soni B, Dalwadi N. Evaluation of antioxidant and anti-atherogenic properties of Glycyrrhiza glabra root using in vitro models. International Journal of Food Sciences and Nutrition, 2009; 60(2): https://doi.org/10.1080/09637480902877998 (accessed 13 Aug 2009).
16. Sheshagiri S, Patel KS, Rajagopala S. Randomized placebo-controlled clinical study on enhancement of Medha (intelligence quotient) in school going children with Yashtimadhu granules. Ayu, 2015; 36(1): http://www. ayu journal. org/ text. asp?2015/36/1/56/169011 (accessed 4-Nov-2015)
17. Raveendra KR, Jayachandra, Srinivasa V, *et al*. An Extract of Glycyrrhiza glabra (GutGard) Alleviates Symptoms of Functional Dyspepsia: A Randomized, Double-Blind, Placebo-Controlled Study. Evidence-Based Complementary and Alternative Medicine, 2012; http://dx. doi. org/10. 1155/2012/216970 (accessed 11 April 2011).
18. WHO, Laboratory manual for the examination of human semen and sperm cervical mucus interaction," (4th ed). Cambridge, United Kingdom: Cambridge University Press, 1999.
19. Manual on Basic Semen Analysis 2002, Final version, NAFA and ESHRE-SIGA.
20. Irvine DS, Aitken RJ. Seminal fluid analysis and sperm function testing. Endocrinology & Metabolism Clinics of North America. 1994; 23(4):725-748.
21. Huckins C. The spermatogonial stem cell population in adult rats. I. Their morphology, proliferation and maturation. Anat Rec, 1971; 169:533-557.
22. Cooke HJ, Saunders PT. Mouse models of male infertility. Nat Rev Genet, 2002; 3:790-801.
23. Aladakatti RH, Jadaramkunti UC. In Vitro effect of azadirachtin-A, a tetranortritarpinoid of neem seed kernel, on rat spermatozoa. Int. Journal of Gen. Chem. 2015; 1(1):15-18.
24. Sairam MR, Krishnamurthy H. The role of follicle stimulating hormone in spermatogenesis: lessons from knockout animal models. Arch. Med. Res. 2001; 32:601-608.
25. Sikka SC. Role of oxidative stress and antioxidants in andrology and assisted reproductive technology.

Journal of Andrology. 2004; 25(1):5-18.
26. Bar-Chama N. Lamb DJ. Evaluation of sperm function. What is available in the modern andrology laboratory? Urologic Clinics of North America. 1994; 21(3):433-446.
27. De Rooij DG. Stem cells in the testis. Int J Exp Pathol, 1998; 79:67-80.
28. Aitken RJ, Baker MA. Oxidative stress and male reproductive biology. Reproduction, Fertility and Development. 2004; 16(5):581-588.
29. Zalata AA, Ahmed AH, Allamaneni SSR, Comhaire FH, Agarwal A, Relationship between acrosin activity of human spermatozoa and oxidative stress. Asian J Androl, 2004; 6:313-318.
30. Chandra AK, Chatterjee A, Ghosh R, Sarkar M. Effect of curcumin on chromium-induced oxidative damage in male reproductive system. Environmental Toxicology and Pharmacology. 2007; 24:160-166.