Effects of *Shatavari* (*Asparagus racemosus*) root powder of Sri Lankan origin on Seminal Fluid Parameters; A double-blind placebo-controlled randomized clinical study

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Abstract

Male factor infertility is a multifactorial disorder that affects a significant percentage of infertile couples; however, many of them remain untreated. In recent years, considerable numbers of infertile men have pursued 'herbal remedies' as an effective treatment. Among 'herbal remedies', *Shatavari – Asparagus racemosus* Willd is recommended for male sub-fertility in Ayurveda medicine. The effect of *Shatavari – Asparagus racemosus* root powder of Sri Lankan origin was compared with a placebo for the male reproductive potential. The study included 150 subfertile men with Oligospermia, Asthenozoospermia, Teratozoospermia, and Oligoasthenoteratozoospermia (OAT) who were randomized to receive Shatavari 24 g/day (group T) or a similar regimen of placebo (group C) for 6 months, administered daily in the morning before breakfast with cow's ghee. The two groups were compared for changes in semen parameters. At the end of the study, statistically significant improvements were observed in the Shatavari-admitted group in semen parameters (volume, sperm concentration, motility, and morphology). At the end of the trial, patients in group T had a mean volume of 2.69 ± 0.14 mL, concentration of 53.28 ± 5.50 million/mL and rapid motility of 26.75 ± 1.68% which was statistically significant from the mean of volume 2.10 ± 0.12 mL, concentration of 32.09 ± 2.88 million/mL and rapid motility on 16.84 ± 1.63% in the placebo group ($p < 0.001$). Normal sperm morphology was 41.12 ± 3.00% and 32.87 ± 2.71% in groups T and C, respectively ($p < 0.001$). Shatavari statistically significantly improved semen parameters in sub-fertile men with decreased seminal parameters.

Keywords: *Shatavari*, male reproductive potential, Seminal Fluid parameters

Introduction

Sub-fertility is both a clinical and a public problem, affecting the life of the couple, the healthcare services, and the social environment. Subfertility affects an estimated 15% of couples globally, and males are found to be solely responsible for 20-30% of cases, contributing to 50% of cases overall[7]. A decrease in the quality and quantity of sperm is one of the main factors responsible for reducing male reproductive potential. A wide range of strategies has been implemented to address this issue. Treatment of human chorionic gonadotropin and follicle-stimulating hormones and assisted reproductive technologies, including intrauterine insemination (IUI), *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), etc., are examples. However, high expenditure, invasive, and may increase the risk of birth defects, the whole world is seeking effective natural remedies to enhance male reproductive potential. According to Ayurveda properties of the *Shatavari* root enhance male reproductive potential. Classical compendia of Ayurveda mention that Shatavari has properties of *Shukrala* (Increasing sperm concentration), *Shukra pravartaka* (Increasing

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motility), *Shukra shodhana* (Purifying action of seminal fluid), *Rasayana* (Rejuvenating action), and *Balya* (Promoting strength)[2,5]. The roots of *A. racemosus* have been extensively used by Ayurveda physicians in Sri Lanka and India for treating enhanced seminal parameters. However, the effects of *A. racemosus* on enhancing the seminal parameters have not yet been proven scientifically. Recently published research reports revealed some beneficial effects of both alcoholic and aqueous extracts of the *Shatavari* roots on aphrodisiac activity[10]. Thus, the present study aims to evaluate the effect of *A. racemosus* on male reproductive potential with respect to seminal fluid parameters to demonstrate the scientific validity of the usage *in vivo*.

Materials and methods

Study design

This study was a randomized double-blind clinical trial conducted in the gynecology clinic of the National Ayurveda Teaching Hospital, Borella, Sri Lanka. The ethics approval for this clinical trial was obtained from the Research Approval Committee of the Faculty of Graduate Studies at the University of Colombo (FGS/ERC/2016/22). The study was conducted adhering to good clinical practice guidelines. Written informed consent was obtained from each participant before conducting the trial. The participants were given sufficient time to ask questions and decide whether they wished to participate in this study or not.

Study Population

The sample size was calculated with the main outcome parameters as the enhancement of concentration, motility, and morphology of the seminal fluid (each group, 75+, loss to follow-up 10).

Sample size

150, Convenient Sampling

Randomization Technique

The eligible males were identified, and they were randomly allocated to the test group or the placebo group. Envelopes were made, including groups by using computer-generated random sampling (SPSS), and were kept with the medical officer in the clinic who delivered the drug and placebo.

Both the test drug and the placebo were prepared, with the test drug labeled as A and the placebo as B. An equal number of each was kept with the medical officer, who delivered the drugs without revealing the meaning of the labels. A separate register was maintained by the medical officer providing the drugs, noting the name and label of each. The register was stored in a secure location and only reviewed during data analysis and interpretation.

Inclusion and exclusion criteria

Inclusion criteria for recruitment of the male participants

- 25 to 45 years of age
- Semen parameters – men with Oligoasthenoteratozoospermia (OATS) syndrome, oligozoospermic, asthenozoospermic, and teratozoospermic abnormal semen parameters – total motile sperm count < (1.5 x 15 x 35) (volume x concentration x motility) as per reference ranges of the 5th edition of WHO laboratory manual for the examination and processing of human semen, 2010.

Exclusion criteria

- Patients with a history of chronic illnesses such as diabetes mellitus, hypertension, renal and liver dysfunction, fatty liver, hyperlipidemia, gall bladder stones, genetic disorders, and testicular atrophy.
- Patients on chemotherapy and any hormonal treatments.
- Men having a history of any surgeries for reproductive tract disorders.
- Varicocele, hydrocele, and testicular pathology

Data Collection and Intervention

A validated questionnaire collected data

Intervention

Group A - The test group was treated with 24 g *Shatavari* root powder with 10 mL of cow's ghee in the morning before breakfast.

Group B – The Control group was treated with a placebo, a form of bolus of 24 g powder of brown rice with 10 mL of cow's ghee in the morning before breakfast.
Treatment period – 6 months
Follow-up period – 6 months

Collection and Preparation of the test drug and placebo

Collection of Asparagus racemosus

Roots of *A. racemosus* of Sri Lankan origin were collected from Hambantota, Anuradhapura, and Colombo districts in Sri Lanka.

***Authentication of the Asparagus racemosus* plant**

The curator of the botanical garden in Peradeniya identified and authenticated plant material of Sri Lankan origin. A specimen was deposited at the National Herbarium, Botanical Garden, Peradeniya, Sri Lanka.

Preparation of a research drug for a clinical study

Ingredients of the research drug

Root powder of *A. racemosus* – 24 gm Cow's ghee – 10 mL. Dose of the research drug and *Anupana* were selected according to Kashyapa Samhita[8].

Preparation of root powder of Asparagus racemosus

The roots were de-stoned and cleaned with running tap water, followed by rinsing with deionized water, and cut into small pieces. Fine powder was made and packed in 24g sterilized vacuum sachet packets under hygienic conditions.

Cow's ghee

Highland cow's ghee was purchased and packed as 10 mL weight sterilized vacuum sachet packets under hygienic conditions. Specification of "Highland" cow's ghee was taken from MILCO Pvt Ltd, Sri Lanka.

Preparation of a placebo for a clinical study

Red rice was purchased from the Sri Lankan market and washed with running tap water, followed by rinsing with deionized water, and dried in direct sunlight. The fine powder was made and 24 g weight, sterilized, and vacuum sachet packets were packed under hygienic conditions.

Red rice powder and *Shatavari* root powder had almost the same appearance; therefore, the researcher could not identify a placebo from the trial drug.

Instructions for the participants in the clinical study

Instructions were given to the participants to make a bolus of one sachet packet mixed with 10 mL of cow's ghee, which was taken before breakfast.
red as statistically significant.

Efficacy Assessment

Seminal fluid analysis

Seminal fluid analysis (SFA) was done before treatment, after three months of treatment, and end of the treatment. During the follow-up period, SFA was analyzed twice with a gap of three months. Semen collection, analysis, and interpretation were carried out as per WHO guidelines[12].

Methodology to enhance compliance

All participants were asked to visit the clinic every 14th day. Telephone contacts, SMS, and emails were used as reminders.

Safety analysis

Each patient underwent the hematological investigations (full blood count (FBC), aspartate aminotransferase (ALT), alanine aminotransferase (AST), serum creatinine, glomerular filtration rate (GFR), urine full report (UFR) and USS of the abdomen and scrotum before and after the treatment. The vital signs were measured and recorded in a patient diary at each visit. The safety endpoints were considered as the number and proportion of patients withdrawing from treatment early for safety reasons, changes in hepatic and renal safety parameters, and the number and proportion of patients experiencing adverse effects.

Statistical analysis

The results of experiments were expressed using mean ± standard error of the mean. Differences

between the two groups were compared by using Student's t-test, and statistical comparisons between experimental groups were made by analysis of variance and Tukey's posttest, as offered by Minitab 18 Stat version (Minitab, Inc., USA)

Statistical significance was determined as $P < 0.05$.

Results and observations

An epidemiological study encompassed age, religion, education, socio-economic status, occupation, dietetic pattern, addictions, BMI, sub-fertility history, sexual history, and history of environmental and occupational factors that affect reproductive potential, and the factors were non-significant in both groups. Therefore, participants of both the trial and control groups had similar backgrounds in the present study sample.

Liquefaction time, viscosity, appearance, and pH were almost similar in both groups before and after the treatments, and after the follow-up period. Liquefaction time was normal within 60 minutes, appearance was opaque, and pH was alkaline. According to trial group data, viscosity was normal in 85% of cases 1% of cases reported low viscosity and 15% of cases were in high viscosity before treatment. After 3 months of treatment, 11% in the trial group had high viscosity and 4% of cases had low viscosity. After the end of the treatment, all cases were reported to have normal viscosity in the trial group. Considering the viscosity of the control group 7% of cases were in high viscosity, 1% of cases were low viscosity and 92% of cases were normal. After the end of the six months, 94% of cases were in the normal category.

Trial group seminal fluid analysis is shown in Table1. Seminal fluid analysis, before, end of the treatment and end of the follow up period of the trial group is shown in Table 2.

At present study volume, concentration, motility and morphology were mainly considered and compared before the treatment, end of the treatment and after the follow-up period between trial and control groups. Seminal fluid analyses were taken after 3 days of abstinence. Before the treatment mean volume of trial group participants was 2.38 mL and it was increased to 2.5 mL and 2.69 mL after three months and the end of the treatment, respectively. After the follow-up period volume of sperm of participants in the trial group was 2.55 mL; however, this difference was insignificant. Tables 1 and 2 revealed that concentration also significantly increased before and after treatment. Further, that increment remained for another 6 months of the follow-up period.

Sperm motility and reproductive potential have a positive relationship. For fertility, it is required to have rapid motility of more than 25%. Tables 1 and 2 revealed that the mean value of rapid motility of the trial group was 11.23 at the beginning of the treatment of *Shatavari* root powder. It increased up to 17.68 and 26.75 after 3 months of duration and end of treatment, respectively. The difference was highly significant. Though there was no significant difference during the follow-up period, it was observed that rapid motility was maintained beyond the normal limit. The percentage of slow motility also significantly increased before treatment and at the end of the treatment. The mean value of slow motility was 18.34, 20.45, and 21.15 before the treatment, at the end of the treatment and end of the follow-up period. There was no significant difference shown between before and after treatment and after the follow-up period. However, the percentage of immotile sperm was dramatically reduced in the trial group between before and after the treatment. Reduction needed to be maintained during the follow-up period, and the percentage of immotile sperm was almost the same as at to end of the treatment i.e., 30.16%.

According to tables 1 and 2, the mean value of normal morphology was 29.91%. It was expressively increased at the end of the treatment, i.e., 41.12%. And abnormal morphology percentage pointedly reduced from 69.97% to 58.29% during 6 months of the treatment period and it persisted same another 6 months of the follow-up period.

Seminal fluid analysis of the control group is shown in Table 3. Seminal fluid analysis, before, end of the treatment and end of the follow up period of control group is shown in Table 4. Table 5 is shown the

seminal fluid analysis of Trial Group (TG) vs Control Group (CG).

Table 1: Seminal fluid analysis of Trial group

Parameter	Mean Values of before treatment (Mean ± SE)	Mean Values of after 3 months of treatment (Mean ± SE)	Mean Values of end of the treatment (Mean ± SE)	Mean Values of after 3 months of follow up (Mean ± SE)	Mean Values of end of the follow up period (Mean ± SE)	T value of before & after 3 months of Treatment	T value of after 3 months & end of the Treatment	T value of end of the treatment & after 3 months of Follow up	T value of after 3 months of Follow up & end of the follow up	Probability value of before & after 3 months of Treatment	Probability value of after 3 months & end of the Treatment	Probability value of end of the treatment & after 3 months of Follow up	Probability value of after 3 months of Follow up & end of the follow up
Volume	2.38 ± 0.14	2.50 ± 0.14	2.69 ± 0.14	2.44 ± 0.12	2.55 ± 0.11	-1.13	-2.19	2.69	-1.32	0.262	*0.032	**0.009	0.192
Concentration	31.60 ± 3.66	37.61 ± 3.48	53.28 ± 5.50	50.58 ± 4.72	53.70 ± 3.88	-3.42	-5.03	0.96	-1.12	**0.001	**0.000	0.340	0.268
Rapid Motility	11.23 ± 1.34	17.68 ± 1.30	26.75 ± 1.68	27.16 ± 1.64	29.52 ± 1.63	-5.14	-5.92	-0.35	-1.82	**0.000	**0.000	0.726	0.073
Slow Motility	18.34 ± 11.49	20.15 ± 12.61	20.45 ± 1.17	21.40 ± 1.22	21.15 ± 1.15	-1.02	-0.22	-0.67	0.21	0.309	0.831	0.502	0.834
Non-Motile	18.79 ± 1.58	19.99 ± 1.33	18.07 ± 1.11	18.51 ± 1.04	19.20 ± 1.12	-0.88	1.59	-0.38	-0.66	0.381	0.116	0.701	0.510
Immotile	50.44 ± 2.57	42.20 ± 2.31	34.57 ± 2.09	32.99 ± 1.98	30.16 ± 1.93	3.44	4.06	0.84	1.81	**0.001	**0.000	0.401	0.070
Normal Morphology	29.91 ± 3.04	35.52 ± 2.90	41.12 ± 3.00	39.41 ± 3.04	41.97 ± 2.96	-2.71	-3.29	1.01	-1.61	**0.008	**0.002	0.314	0.113
Abnormal Morphology	69.97 ± 3.03	64.48 ± 2.90	58.75 ± 2.99	60.45 ± 3.03	58.29 ± 2.95	2.64	3.38	-1.01	1.35	*0.010	**0.001	0.314	0.181

Table 2: Seminal fluid analysis of Trial group, before, end of the treatment and end of the follow up period

Parameter	Mean Values of before treatment (Mean ± SE)	Mean Values of end of the treatment (Mean ± SE)	Mean Values of end of the follow-up (Mean ± SE)	T value of before treatment & end of the Treatment	T value of before treatment & end of the Follow-up	The probability value of before treatment & end of the Treatment	Probability value before treatment & end of the follow-up
Volume	2.38 ± 0.14	2.69 ± 0.14	2.55 ± 0.11	-2.83	-3.12	**0.006	**0.003
Concentration	31.60 ± 3.66	53.28 ± 5.50	53.70 ± 3.88	-6.48	-6.37	**0.000	**0.000
Rapid Motility	11.23 ± 1.34	26.75 ± 1.68	29.52 ± 1.63	-7.80	-9.42	**0.000	**0.000
Slow Motility	18.34 ± 11.49	20.45 ± 1.17	21.15 ± 1.15	-1.23	-1.64	0.223	0.104
Non-motile	18.79 ± 1.58	18.07 ± 1.11	19.20 ± 1.12	0.47	-0.28	0.643	0.784
Immotile	50.44 ± 2.57	34.57 ± 2.09	30.16 ± 1.93	5.48	7.16	**0.000	**0.000
Normal Morphology	29.91 ± 3.04	41.12 ± 3.00	41.97 ± 2.96	-4.80	-4.40	**0.000	**0.000
Abnormal Morphology	69.97 ± 3.03	58.75 ± 2.99	58.29 ± 2.95	4.80	4.20	**0.000	**0.000

Table 3: Seminal fluid analysis of Control group

Parameter	Mean Values of before treatment (Mean ± SE)	Mean Values of after 3 months of treatment (Mean ± SE)	Mean Values of end of the treatment (Mean ± SE)	Mean Values of after 3 months of follow up (Mean ± SE)	Mean Values of end of the follow up period (Mean ± SE)	T value of before & after 3 months of Treatment	T value of after 3 months & end of the Treatment	T value of end of the treatment & after 3 months of Follow up	T value of after 3 months of Follow up & end of the follow up	Probability value of before & after 3 months of Treatment	Probability value of after 3 months & end of the Treatment	Probability value of end of the treatment & after 3 months of Follow up	Probability value of after 3 months of Follow up & end of the follow up
Volume	2.48 ± 0.13	2.27 ± 0.12	2.10 ± 0.12	2.06 ± 0.11	1.84 ± 0.12	1.82	1.33	0.31	1.69	0.074	0.187	0.760	0.096
Concentration	45.56 ± 4.43	37.21 ±.19	32.09 ± 2.88	26.09 ± 2.48	22.24 ± 2.30	2.45	1.83	2.29	1.93	0.017	0.072	*0.025	0.057
Rapid Motility	24.89 ± 2.27	21.57 ± 1.99	16.84 ± 1.63	16.28 ± 1.39	16.45 ± 1.50	2.15	2.73	0.37	-0.12	0.035	**0.008	0.715	0.909
Slow Motility	19.01 ± 1.20	19.56 ± 1.12	20.76 ± 1.20	21.52 ± 1.18	20.03 ± 1.09	-0.50	-0.98	-0.58	1.29	0.621	0.329	0.567	0.201
Non-motile	18.73 ± 0.99	21.75 ± 0.97	21.84 ± 0.98	23.76 ± 0.96	25.99 ± 1.07	-2.77	-0.09	-1.86	-1.92	**0.007	0.929	0.068	0.059
Immotile	37.89 ± 2.49	37.19 ± 1.92	40.68 ± 1.87	38.24 ± 1.82	37.36 ± 1.95	0.37	-1.97	1.20	0.47	0.715	0.053	0.234	0.637
Normal Morphology	28.31 ± 2.84	29.71 ± 2.77	32.87 ± 2.71	32.96 ± 2.82	33.51 ± 2.98	-0.69	-1.98	-0.08	-0.49	0.494	0.052	0.939	0.623
Abnormal Morphology	71.69 ± 2.85	70.29 ± 2.77	67.25 ± 2.72	67.04 ± 2.82	66.41 ± 2.97	0.69	1.85	0.18	0.57	0.494	0.068	0.862	0.572

Table 4: Seminal fluid analysis of before, end of the treatment and end of the follow up period of the Control group

Parameter	Mean Values of before treatment (Mean ± SE)	Mean Values of end of the treatment (Mean ± SE)	Mean Values of end of the follow up (Mean ± SE)	T value of before treatment & end of the Treatment	T value of before treatment & end of the Follow up	Probability value of before treatment & end of the Treatment	Probability value of before treatment & end of the follow up
Volume	2.48 ± 0.13	2.10 ± 0.12	1.84 ± 0.12	3.18	4.34	**0.002	**0.000
Concentration	45.56 ± 4.43	32.09 ± 2.88	22.24 ± 2.30	3.78	5.76	**0.000	**0.000
Rapid Motility	24.89 ± 2.27	16.84 ± 1.63	16.45 ± 1.50	4.31	4.04	**0.000	**0.000
Slow Motility	19.01 ± 1.20	20.76 ± 1.20	20.03 ± 1.09	-1.23	-0.61	0.222	0.541
Non-motile	18.73 ± 0.99	21.84 ± 0.98	25.99 ± 1.07	-2.44	-5.31	*0.017	**0.000
Immotile	37.89 ± 2.49	40.68 ± 1.87	37.36 ± 1.95	-1.27	0.19	0.207	0.846
Normal Morphology	28.31 ± 2.84	32.87 ± 2.71	33.51 ± 2.98	-2.00	-2.03	*0.049	*0.046
Abnormal Morphology	71.69 ± 2.85	67.25 ± 2.72	66.41 ± 2.97	1.92	2.06	0.059	*0.043

Table 5: Seminal fluid analysis of the Trial Group (TG) vs Control Group (CG)

Parameter	Mean Values of before treatment (Mean ± SE) - TG	Mean Values of before treatment (Mean ± SE) -CG	Mean Values of after 3 months of treatment (Mean ± SE) - TG	Mean Values of after 3 months of treatment (Mean ± SE) - CG	Mean Values of end of the treatment (Mean ± SE) - TG	Mean Values of end of the treatment (Mean ± SE) - CG	T value of before Treatment	T value of before & after 3 months of Treatment	T value of after 3 months & end of the Treatment	Probability value of before Treatment	Probability value of before & after 3 months of Treatment	Probability value of after 3 months & end of the Treatment
Volume	2.38 ± 0.14	2.48 ± 0.13	2.50 ± 0.14	2.27 ± 0.12	2.69 ± 0.14	2.10 ± 0.12	-0.52	1.28	3.20	0.601	0.204	**0.002
Concentration	31.60 ± 3.66	45.56 ± 4.43	37.61 ± 3.48	37.21 ± 3.19	53.28 ± 5.50	32.09 ± 2.88	-2.43	0.08	3.42	*0.016	0.932	**0.001
Rapid Motility	11.23 ± 1.34	24.89 ± 2.27	17.68 ± 1.30	21.57 ± 1.99	26.75 ± 1.68	16.84 ± 1.63	-5.18	-1.63	4.23	**0.000	0.105	**0.000
Slow Motility	18.34 ± 11.49	19.01 ± 1.20	20.15 ± 12.61	19.56 ± 1.12	20.45 ± 1.17	20.76 ± 1.20	-0.37	0.32	-0.18	0.709	0.750	0.855
Non-Motile	18.79 ± 1.58	18.73 ± 0.99	19.99 ± 1.33	21.75 ± 0.97	18.07 ± 1.11	21.84 ± 0.98	0.03	-1.07	-2.55	0.977	0.287	*0.012
Immotile	50.44 ± 2.57	37.89 ± 2.49	42.20 ± 2.31	37.19 ± 1.92	34.57 ± 2.09	40.68 ± 1.87	3.50	1.67	-2.18	**0.001	0.097	*0.031
Normal Morphology	29.91 ± 3.04	28.31 ± 2.84	35.52 ± 2.90	29.71 ± 2.77	41.12 ± 3.00	32.87 ± 2.71	0.38	1.45	2.04	0.701	0.149	0.149
Abnormal Morphology	69.97 ± 3.03	71.69 ± 2.85	64.48 ± 2.90	70.29 ± 2.77	58.75 ± 2.99	67.25 ± 2.72	-0.41	-1.45	-2.10	0.680	0.149	*0.037

Parameter	Mean Values of after 3 months of follow up (Mean ± SE) - TG	Mean Values of after 3 months of follow up (Mean ± SE) - CG	Mean Values of end of the follow up period (Mean ± SE) - TG	Mean Values of end of the follow up period (Mean ± SE) - CG	T value of end of the treatment & after 3 months of Follow up	T value of after 3 months of Follow up & end of the follow up	Probability value of end of the treatment & after 3 months of Follow up	Probability value of after 3 months of Follow up & end of the follow up
Volume	2.44 ± 0.12	2.06 ± 0.11	2.55 ± 0.11	1.84 ± 0.12	2.35	4.45	*0.020	**0.000
Concentration	50.58 ± 4.72	26.09 ± 2.48	53.70 ± 3.88	22.24 ± 2.30	4.59	6.97	**0.000	**0.000
Rapid Motility	27.16 ± 1.64	16.28 ± 1.39	29.52 ± 1.63	16.45 ± 1.50	5.07	5.89	**0.000	**0.000
Slow Motility	21.40 ± 1.22	21.52 ± 1.18	21.15 ± 1.15	20.03 ± 1.09	-0.07	0.71	0.944	0.482
Non-Motile	18.51 ± 1.04	23.76 ± 0.96	19.20 ± 1.12	25.99 ± 1.07	-3.72	-4.37	**0.000	**0.000

Table 3 and 4 represented control group sperm analyzing data of the present study. Mean volume of control group before treatment was 2.48 mL. It was significantly reduced up to 2.1 mL and 1.84 mL at the end of the treatment and end of the follow up period. However, changes of volume were in normal limits. According to data sperm concentration also reduced from 45.56 million to 32.09 million during 6 months of treatment period and up to 22.24 million during 6 months of follow up period. Though above value different was significant statistically, all three were above to normal limit according to WHO. Control group of study participants were shown the decrease of rapid motility. At the beginning mean percentage of rapid motility was 24.89% and it was in minimum requirement of rapid motility. At the end of the treatment rapid motility was reduced 16.84% and it was remained in same level during the follow up period too. When considered slow motility, non-motility, normal morphology and abnormal morphology there were no any changes during the treatment and follow up period except nonmotile percentage at the end of follow up.

Table 5 shows the comparison of seminal fluid parameters between the trial and control groups. According to the data, it was revealed that the difference in volume was statistically significant at the end of the treatment and follow-up period. The

sperm concentration of the control group was higher than the trial group, and that increase was statistically significant. But, at the end of the treatment and during the follow-up period, that was reversed and concentration was higher in the trial group than the control group; the difference was statistically significant.

At the beginning of treatment mean rapid motility of the trial group was 11.23% and it was lower than the mean rapid motility percentage of the control group. Even though, in the trial group, rapid motility was increased remarkably and the mean percentage of rapid motility of the trial group at the end of treatment and end of follow-up period was 26.75 and 29.52, respectively. The difference was highly significant. There was no significant change in slow motility percentages between the groups of the study sample. Considered in non-motile sperm percentage, it was significantly increased in the control group at the end of the treatment and during the follow-up period. Immotile sperm percentage was dramatically reduced in the trial group and the difference was statistically significant. There was not much difference in normal and abnormal morphology between the trial and control groups (Table 6).

Table 6: Semen Classification of trial and control group participants before and after treatment and after follow up

Semen Classification	Before Treatment				After Treatment				After Follow-up			
	Trial		Control		Trial		Control		Trial		Control	
	No	%	No	%	No	%	No	%	No	%	No	%
Normal	02	2.7	25	33.3	38	50.7	12	16	51	68	09	12
Hypospermia	10	13.3	01	1.3	03	4	00	0	02	2.7	01	1.3
Hyperspermia	01	1.3	00	00	01	1.3	00	0	00	0	00	0
Oligozoospermia	16	21.3	06	8	08	10.7	20	26.7	05	6.7	22	29.3
Asthenozoospermia	35	46.7	32	42.7	21	28	35	46.7	13	17.3	31	4.3
Teratozoospermia	01	1.3	05	6.7	03	4	02	2.7	03	4	03	4
OATS	10	13.3	06	8	01	1.3	06	8	01	1.3	09	12

For before treatment; Chi-Square value is 36.303 df, Asym. Significant (2 sided) 0.000

At the end of the treatment; Chi-Square value is 29.934 df, Asym. Significant (2 sided) 0.000

After follow up period; Chi-Square value is 54.201 df, Asym. Significant (2 sided) 0.000

According to seminal fluid analysis of the study sample, participants were categorized into 07 groups normal, hypospermia, hyperspermia, oligo-zoospermia, asthenozoospermia, teratozoospermia, and OATS syndrome. It was statistically significant in a normal category before the treatment between trial and control groups. In the trial group, there were 2.7% of participants belonged to a normal category, and it was 33.3% in the control group. Participants who suffered from *asthenozoospermia* were high in both groups, i.e., 46.7% and 42.7% in the trial and control groups, respectively. There were 21.3% of *oligozoospermic* clients in the trial group and 8% in the control group before the treatment. A total of 21.3% of clients suffered from OATS syndrome before the treatment.

At the end of the treatment highly significant difference was seen in normal participants between the two groups. There were 50.7% and 68% normal SFA seen at the end of the treatment and end of the follow-up period. It was only 16% and 12% in the control group. Hypospermia, oligospermia, asthenozoospermia and OATS percentages were also dramatically reduced in the trial group at the end of the treatment and it was maintained during the follow-up period. Considering the teratozoospermia category, 2 cases were increased in the trial group and 3 cases were decreased in the control group at the end of the treatment.

Discussion

Seminal fluid analysis can identify problems with the count of sperm (quantitative) and their overall quality (qualitative). Seminal fluid analysis remains the primary test for assessing male reproductive potential and evaluates certain characteristics of semen such as volume, pH, sperm count, motility, morphology and viability of sperm. To get a better sample, participants were asked to avoid ejaculation or abstinence for 3 days (72 hours) before the test. Semen was collected by masturbation and delivered within 30 to 60 minutes for testing. Participants were asked not to apply any cream or liquid before masturbation and they were directed to a reputed hospital to undergo their investigation to minimize lab technician error.

Semen viscosity, appearance, pH and volume were macroscopically examined. All the semen samples showed a normal appearance (grey-opalescent) and alkaline pH between 6 and 10. According to the results, 85% of cases had normal viscosity in the trial group and 92% in the control group. High viscosity was found in 14% of participants in the trial group. High-viscosity seminal fluid restricts sperm motility. *A. racemosus* root powder may lower the viscosity due to its *Pitta shamaka* property. This *Pitta shamaka* property maintains the *Drava guna* (liquidity), which is inherent with *Pitta dosha*. Hence, *Shatavari* may lower the high viscosity of the seminal fluid. Though the mean volume of seminal fluid of study participants was normal (1.5 mL) before, the end of the treatment and end of the follow-up period, a significant increment (from 2.38 ± 0.14 mL to 2.69 ± 0.14 mL: p; 0.009) was recorded in the trial group (table 1). This may reflect the enhancement of secretory activity of the glands of the accessory organs, mainly the prostate and seminal vesicles.

Sperm concentration, motility and morphology are initial characteristics that are examined microscopically. Sperm concentration should be more than 15 million/mL, at least 32% of all sperm should show progressive motility, the sum of progressive and non-progressive motility should be at least 40% and at least 4% of all sperm cells should have normal morphology, indicating a normal seminal fluid sample[12]. The *Shatavari* root powder-treated group showed a highly significant ($p \leq 0.001$) enhancement in sperm concentration at the end of the treatment period of six months as compared to the sperm concentration at the beginning and at the end of the treatment in the control group (table 2 and 4). The increase in sperm concentration was from 31.60 ± 3.66 × 10^6/mL to 53.28 ± 5.50 × 10^6/mL, corresponding to an increase of 146%. A significant increment ($p\leq 0.001$) of rapid motility was also recorded from 11.23 ± 1.34% to 26.75 ± 1.68% (table 1) after 6 months, in contrast to the value before treatment in the trial group and at the end of treatment in the control group i.e.,16.84 ± 1.63% (table 3). There was a dramatic enhancement of normal morphology documented in the *Shatavari*-treated group from 29.91 ± 3.04% to 41.12 ± 3.00%; p 0.002 at the end of the treatment. Though it was normal according to WHO, that difference was not significant at the end of the treatment, in contrast to the beginning in the control group.

According to the data of the study sample, semen was classified into seven groups: normal, *hypospermia*, *hyperspermia*, *oligozoospermia*, *asthenozoospermia*, *teratozoospermia* and OATS. There was a significant increase in normal seminal fluid percentage from 2.7% to 50.7% in the *Shatavari-treated* group at the end of treatment. The number of participants in the normal category decreased to 16% from 33.3% in the control group.

Shatavari is comprised of a predominance of *Jala* and *Pritvi mahabhuta,* which are responsible for flourishing the body contents. Therefore, *Shatavari* increases the sperm too. By having this predominance of *Jal*a and *Pritvi* elements, *Shatavari* pacifies *Vata* and *Pitta* and increases *Kapha dosha*. Due to these physiological functions/attributes, *Shatavari* has an anabolic effect on the sperm. Due to the entire metabolic process described in Ayurveda, all digested food and medicinal substances ingested by humans are carried by the *Rasa dhatu*. As *Rasa dhatu* is the first *Dhatu* that is formed by the essence of *Ahara Rasa*, all the other *Dhatus* get their particular nourishment from this circulating *Rasa Dhatu* either directly or indirectly. *Shatavari*, the selected drug,

has the characteristic power of enhancing the quality of *Rasa dhatu* and *Shukra dhatu* by its *Madura rasa*, *Guru* and *Snigdha guna*, *Sheeta veerya* and *Madhura vipaka*. According to Ayurveda *Balya*, *Rasayana*, *Vajeekarana, Shukra janana, Shukra shodana* and *Shukra pravartaka* are the pharmacological properties of *Shatavari,* which strengthen *Sapta dhatu*[4]. Thus, improving reproductive function and general health and strength.

Samanya, Vishesa, Guna, Dravya, Karma, and *Samavaya* are the *Shad padartha* mentioned by *Charaka*. The treatments of Ayurveda are based on this theory of *Shad padartha*. Among them, *Samanya* and *Vishesa* are very useful in Ayurveda medicine. These six categories, *Shad padartha,* are seen to be of immense value in the applied aspect of treatment and also for maintaining health. The object of Ayurveda has been said to be maintaining homeostasis to the level of physiological equilibrium. *Samanya* and *Vishesa* are the dynamic forces that keep normal physiological conditions in the body. *Samanya* is the cause of the increase of all things at all times, whereas the application of this principle leads to enhancing the body elements. According to this theory, the pharmacological properties and functions of *Shatavari* in the aforementioned paragraph may increase the quality of semen.

Oxidative stress is one of the most common reasons for the destruction of sperm quality and the impairment of male reproductive potential. Low levels of reactive oxygen species (ROS) provide certain positive effects to sperm cells and enhance the ability to bind to the zona pellucida; high amounts of ROS alter the integrity of spermatozoa DNA and result in DNA fragmentation. Free radicals also alter the sperm structure and function, which impairs all semen parameters. The polyunsaturated lipid membrane of the mature spermatozoa is vulnerable to oxidation in the presence of ROS and causes impaired sperm morphology and motility. Antioxidants that are naturally found in semen act as free radical scavengers that help to overcome ROS. If endogenous antioxidants are not enough, exogenous antioxidant supplements can be useful to protect the sperm cell. Research studies have proven that *Shatavari* has an antioxidant action due to having various chemicals and their different pathways to combat free radicals[6]. Further, roots of *A. racemosus* are rich in vitamins C and E and trace minerals (zinc, copper, manganese, iron, cobalt, sodium, potassium, calcium, and lithium)[6, 10] help to enhance sperm quality. Animal studies have shown that *Shatavari* caused an increase in testicular size by 6.8 percent, possibly followed by an increase in spermatogenesis[9]. The size of the testis influences the total number of spermatozoa per ejaculate[11, 12]. Testicular size reflects the level of spermatogenic activity, which also affects sperm morphology[12]. The alleviating of oligospermia may be due to increasing or maintaining the actual size of the testis, and due to the spermatogenetic potential and aphrodisiac activity of *Shatavari,* which were demonstrated by previous researchers. The enhancement of semen quality and protecting the testis by improving antioxidant capacity might be useful to overcome fertility complications of diabetic individuals[1,3].

Conclusion

Shatavari root powder could improve the quantity and quality of semen in a statistically significant manner in comparison to the placebo, at the given dose of 24 gm/d with 10 mL cow's ghee before breakfast for six months.

The results suggested that the *Shatavari* root powder may be a new, auspicious therapeutic amalgamation that can be used to improve the male reproductive potential of sub fertile men. This spermatogenic and aphrodisiac property may be due to the bioactive compounds of *Shatavari* root. Hence, further investigations are warranted to confirm and elucidate the effect of *A. racamosus* on semen parameters.

Acknowledgment

University Grant Commission, Ward Place, Colombo 7, Sri Lanka, is acknowledged for financial assistance.

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