

Research Paper

Efficacy of Furosap™, a novel *Trigonella foenum-graecum* seed extract, in Enhancing Testosterone Level and Improving Sperm Profile in Male Volunteers

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Abstract

Background: Dietary fiber rich fenugreek (*Trigonella foenum-graecum*) seeds have exhibited cardioprotective, hypolipidemic and other health benefits. Furosap (FS), an innovative, patented, 20% protodioscin-enriched extract was developed in our laboratory from fenugreek seeds. This study examined the free and total testosterone levels, sperm profile and morphology, sexual health, mood and mental alertness, and broad spectrum safety parameters of FS in 50 male volunteers following supplementation over a period of 12 weeks.

Methods: Institutional Review Board (IRB) and other regulatory approvals were obtained for our study. This one-arm, open-labelled, multi-center study was conducted in 50 male volunteers (age: 35 to 65 years) over a period of 12 weeks to determine the efficacy of FS (500 mg/day/subject) on free and total testosterone levels, sperm profile, sperm morphology, libido and sexual health, mood and mental alertness, and broad spectrum safety parameters.

Results: Free testosterone levels were improved up to 46% in 90% of the study population. 85.4% of the study population showed improvements in sperm counts. Sperm morphology improved in 14.6% of volunteers. Majority of the subjects enrolled in the study demonstrated improvements in mental alertness and mood. Furthermore, cardiovascular health and libido were significantly improved. Extensive safety parameters were evaluated which included blood chemistry data. No significant changes were observed in serum lipid function, cholesterol, triglyceride, HDL and LDL levels, hemogram (CBC), hepatotoxicity and nephrotoxicity.

Conclusion: Overall, the results demonstrate that FS, enriched in 20% protodioscin, is safe and effective in attenuating testosterone levels, healthy sperm profile, mental alertness, cardiovascular health and overall performance in human subjects.

Key words: Fenugreek seed extract (Furosap™); Protodioscin; Testosterone; Sperm profile; Mental alertness; Mood; Cardiovascular health; Safety

Introduction

Fenugreek (*Trigonella foenum-graecum*, family Fabaceae) is a very popular leguminous annual plant grown extensively in the Indian sub-continent, China,

Iran, Egypt, Turkey, France, Spain, North Africa especially Morocco, and Argentina, while the largest producer is India (1-4). Fresh and dried leaves, twigs,

roots, sprouts, microgreens and the cuboid-shaped, yellow- to amber-colored seeds are extensively used in vegetable dishes, lentil soups and pickles, while the seeds and especially the roasted seeds are used as a flavor-enhancing spice in the Indian subcontinent and Middle Eastern countries (2,5-8). Fenugreek plants especially fenugreek seeds are rich in soluble fibers, and extensively used in Ayurvedic and Unani medicines for anti-inflammatory, anti-diabetic, antiseptic, aphrodisiac, women's health and diverse health benefits for centuries (1-3,5-12).

Fenugreek contains approximately 28% mucilage, 5% stronger-smelling, bitter fixed oil, rich in phosphates, lecithin and nuclealbumin, considerable amounts readily absorbable iron in an organic form, as well as trigonelline, choline, biotin, inositol, vitamin A, vitamin B1, vitamin B2, vitamin B3, vitamin B5, vitamin B6, vitamin B9, vitamin B12, and vitamin D, diosgenin, diosgenin- β -D-glucoside, vitexin, vitexin-7-glucoside, yamogenin and vicenin. Fenugreek seeds contain no essential oil and its characteristic flavor due to trace amounts of an extremely powerful odorant 4,5-dimethyl-3-hydroxy-2[5H]-furanone, known as fenugreek lactone (1,8,10-14).

Beside the broad spectrum antioxidant efficacy of fenugreek seeds, these seeds also demonstrated significant benefits in diverse inflammatory responses including diabetic, hypercholesterolemia, polycystic ovary syndrome, gastric ulcer and hyperthyroidism, while few studies demonstrated its efficacy in sports nutrition and exercise in mice and humans. Ikeuchi et al. (2006) assessed the dose-dependent efficacy of fenugreek seed extract (0, 150 or 300 mg/kg body weight) in male mice on endurance capacity in a swimming model over a period of 4 weeks (15). The fenugreek seed (300 mg/kg body weight) administration caused a significant increase in swimming time and the results demonstrated that this improvement in swimming endurance is caused by the increase in utilization of fatty acids as an energy source. Another independent study by Arshadi et al. (2015) evaluated the efficacy of fenugreek seed (0, 0.8 or 1.6 g/kg body weight) extract in combination with swimming exercise compared to glibenclamide in type 2 diabetic male rats (16,17). Researchers concluded that fenugreek seed consumption, along with swimming exercise, induced a therapeutic effect on the improvement of diabetic parameters including plasma insulin, HOMA-IR, plasma leptin and adiponectin (16,17).

In a placebo-controlled, double blind study in 49 resistance-trained male volunteers, Poole et al. (2010) assessed the effect of fenugreek supplementation (500 mg/day) on strength, body composition, muscle

endurance, power output and hormonal profiles over a period of 8 weeks in a structured resistance training program (18). Results demonstrated that fenugreek can significantly increase upper- and lower-body strength, reduce body fat and improve overall body composition. Furthermore, the fenugreek supplement non-significantly impacted muscular endurance, hormonal concentrations and hematological variables. The authors also conducted broad spectrum safety parameters and no toxic manifestations were observed (18).

In the present investigation, we evaluated the efficacy of a novel, patented fenugreek (*Trigonella foenum-graecum*) seed extract enriched in 20% protodioscin (FS, Furosap™, US Patents# US 8,217,165 B2; US 8,754,205 B2) (19,20) to boost free and total testosterone levels, sperm profile and morphology, sexual health, mood and mental alertness, and broad spectrum safety parameters in 50 male volunteers (Age: 35-65 years) over a period of 12 weeks.

Materials and Methods

The study design, recruitment and methods were performed in compliance and accordance with the ICH guidelines for Good Clinical Practices (GCP), including the archiving of essential documents, and per international ethical standards guaranteed by the Declaration of Helsinki and its subsequent amendments.

Ethical Approval

Ethical Approval and Consent to Participate: Institutional Ethical Board for Medical Research and Institutional Ethics Committee (IEC) from the Ethical Board for Medical Research of Saroj Hospital & Maternity Center (Kanpur Road, Lucknow, Uttar Pradesh, India) approved this Clinical Study (Reference# EBMR/2014/07/28/01 dated July 28, 2014). The study was conducted in Saroj Hospital & Maternity Center (Kanpur Road, Lucknow, Uttar Pradesh, India). This study was also registered at clinicaltrials.gov (NCT02702882).

All subjects were provided a consent form and provided sufficient information for subjects to make an informed decision about their participation in this study. This consent form was submitted with the protocol for review and approved by the IEC for the study. The formal consent of a subject using the IEC-approved consent form was obtained before the subject is submitted to any study procedure. Consent form was signed by the subject or legally accepted representative and the investigator-designated research professional obtained the consent. Patient's confidentiality was strictly maintained.

Study Design

Subject Recruitment and Inclusion and Exclusion Criteria: The subjects were screened for the clinical study on the basis of the inclusion/exclusion criteria (Table 1) and fifty male subjects were enrolled after a systematic screening (Table 2). All subjects were given Furosap (FS, 1 capsules of 500 mg each/day after breakfast over a period of 12 consecutive weeks, Batch #FUP0814).

Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria	
1.	Agrees to written as well as audio-visual informed consent.
2.	Ability to understand the risks/benefits of the protocol.
3.	Male between 35-65 years of age.
4.	Diagnosed with Symptomatic hypogonadism.
Exclusion Criteria	
1.	Uncooperative Subjects.
2.	Impaired hepatic function indicated by serum GOT/GPT >2.5 times the upper limit of normal.
3.	Patients suffering from coronary artery disease (CAD) and allied complications.
4.	Abnormal liver or kidney function tests (ALT or AST > 2 times the upper limit of normal; elevated creatinine, males > 125 µmol/L
5.	History of malignancy.
6.	History of hypersensitivity to any of the investigational drugs.
7.	Receiving any other testosterone booster therapy/medication/supplement within the last 2 months.
8.	History of coagulopathies (clotting and bleeding).
9.	High alcohol intake (>2 standard drinks per day).
10.	History of psychiatric disorder that may impair the ability of subjects to provide written informed consent.
11.	Any medical condition, where the investigator feels participation in the study could be detrimental to the subjects overall well-being.

Table 2. Demographic and Baseline Characteristics of the Subjects

	Age (years)	Height (cm)	Weight (kg)	Body Mass Index (BMI) (kg/m ²)	Systolic Blood Pressure (SBP) (mm Hg)	Diastolic Blood Pressure (DBP) (mmHg)	Pulse (per minute)
Mean	43.08	166.16	70.38	25.46	124.00	79.65	77.53
Standard Deviation	7.35	4.93	12.18	4.13	9.40	6.53	6.40
Minimum	35.00	151.00	27.40	10.98	104.00	64.00	56.00
Maximum	61.00	176.00	91.60	31.90	160.00	90.00	96.00

Study Compliance: Allocation of FS was done by the site staff only. Distribution of the product was maintained in the IP accountability log provided by the sponsor. Each entry was maintained separately with the date/signature of the principal investigator & study coordinator. The person responsible for the distribution of the product had also signed on the IP accountability log. The accountability log must be produced by principal investigator or study coordinator at the time of audit. All concomitant prescription medications taken during study

participation were recorded on the case report forms (CRFs). Medications to be reported in the CRF were concomitant prescription medications, over-the-counter medications (OTC) and non-prescription medications taken at the time of adverse events (all grades) too.

Assay Kits and Equipment

Free testosterone was measured using a Dia Sources' ELISA kit (catalog#CAN-FTE-260) purchased from Krishgen Biosystems, Mumbai, India, and total testosterone was assessed using an automated bidirectionally interfaced Chemiluminescent Immunoassay (CLIA) from Siemens Health Care Pvt Ltd, Mumbai, India. Dehydroepiandrosterone sulfate (DHEA-S) was assessed using the Cobas Electrochemiluminescence Immunoassay (ECLIA) (catalog# 03000087122) kit purchased from Roche Diagnostics India Pvt Ltd, Mumbai, India. Hemoglobin level was evaluated using a Sysmex fully automated bidirectional analyzer (SYSMEX XN-1000) purchased from Transasia Bio Medicals Ltd, Mumbai, India, and fasting blood glucose (FBS) levels were assessed using photometry technology (Agappe Diagnostics Ltd, Mumbai, India). Aspartate Aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), blood urea nitrogen (BUN), cholesterol, triglycerides, high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL), total leukocytes count (TLC), neutrophils, lymphocytes, monocytes, eosinophils and basophils in Central Processing Lab (CPL, a division of Thyrocare, Mumbai, India) and Regional Processing Lab (RPL, a division of Thyrocare, Mumbai, India). Sperm count, sperm motility and abnormal sperm morphology were assessed in Nigam Pathology (Lucknow, India).

Efficacy Assessment: The efficacy of FS was evaluated on these fifty volunteers at baseline, at the end of 4- and 8-weeks, and at the end of 12 weeks. Table 3 exhibits the different parameters measured at these time points.

Adverse Events: Subjects were advised to record adverse events (if any) during the duration of the study. At each visit, the subjects were asked if they have experienced any uncomfortable problems or difficulties. Thus, adverse event reporting was strictly enforced.

Statistical Analysis

Data is expressed as mean \pm SD (Standard Deviation) or SEM (Standard Error Mean). The baseline characteristics were compared with the outcome following completion of the dosing period.

All parametric and non-parametric assessments were conducted. Wilcoxon signed-rank test, a nonparametric test equivalent to the dependent t-test, was used for assessing mental alertness, mood, reflex erection and overall performance.

Table 3. Assessment of Efficacy

Time Interval	Clinical Examination
At Baseline	Body Mass Index (BMI)
	Free testosterone (pg/ml)
	Total testosterone (ng/dl)
	DHEA-S levels
	Fasting blood sugar (FBS)
	Fasting lipid profile (total cholesterol, LDL, HDL, triglycerides, VLDL)
	Liver function tests (AST, ALT, ALP)
	Hemogram
	Semen examination (sperm count, sperm motility, sperm morphology)
	BMI (Kg/m ²)
At the End of 4- and 8 Weeks	Fasting lipid profile (total cholesterol, LDL, HDL, triglycerides, VLDL)
	Semen examination (sperm count, sperm mobility, sperm morphology)
	BMI
	Free testosterone (pg/ml)
	Total testosterone (ng/dl)
	DHEA-S levels
	Fasting blood sugar (FBS)
	Fasting lipid profile (total cholesterol, LDL, HDL, triglycerides, VLDL)
	Liver function tests (AST, ALT, ALP)
	Hemogram
End of Study (12 Weeks)	Semen examination (sperm count, sperm mobility, sperm morphology)
	BMI
	Free testosterone (pg/ml)
	Total testosterone (ng/dl)
	DHEA-S levels
	Fasting blood sugar (FBS)
	Fasting lipid profile (total cholesterol, LDL, HDL, triglycerides, VLDL)
	Liver function tests (AST, ALT, ALP)
	Hemogram
	Semen examination (sperm count, sperm mobility, sperm morphology)

Results

Effect of Furosap (FS) Treatment on Free Testosterone and Total Testosterone

Subjects were treated with FS over a period of 12 consecutive weeks. Free testosterone and total testosterone were measured at baseline and at the end of 12 weeks of treatment (Table 4). Free testosterone level increased by approximately 1.47-fold (p value = 0.0004**), whereas the total testosterone levels increased by 1.08-fold (p-value = 0.164ns) (Table 4).

Table 4. Free testosterone and total testosterone levels following supplementation of FS

Parameters	Time	Mean + Standard Deviation (SD)	p-value
Free Testosterone (pg/ml)	Baseline (day 0)	8.17 + 5.04	0.0004**
	On Completion (12 weeks)	11.97 + 5.65**	
Total Testosterone (ng/dl)	Baseline (day 0)	405.19 + 156.95	0.164ns
	On Completion (12 weeks)	436.34 + 189.94	

Data are expressed as mean \pm SD. **Significant reduction; ns = not significant

Sperm Count (millions/ml), Sperm Motility (%) and Abnormal Sperm Morphology (%) following Supplementation of FS over a Period of 12 Consecutive Weeks

Sperm count (millions/ml), sperm motility (%) and abnormal sperm morphology (%) were evaluated at baseline, after 4 weeks, after 8 weeks and after 12 weeks following supplementation of FS (Table 5). Sperm count and sperm motility were significantly increased at the end of 4-, 8- and 12-weeks of FS treatment, while abnormal sperm morphology (%) reduced at all these time point. Although, abnormal sperm morphology (%) was reduced at 4-weeks post-treatment, however, it was not significant. However, abnormal sperm morphology (%) was significantly reduced both at 8- and 12-weeks post-treatment, respectively (Table 5).

FS-Induced Effects on Dehydroepiandrosterone Sulfate (DHEA-S), Fasting Blood Sugar (FBS) and Total Leukocyte Count (TLC) in Human Subjects

DHEA-S, FBS and TLC levels were measured at baseline and following treatment with FS) over a period of 12 weeks. No significant changes were observed (Table 6).

Table 5. Sperm count, sperm motility and abnormal sperm morphology at baseline, 4-weeks, 8-weeks and 12-weeks of FS treatment

Parameters	Pair #1		Pair #2		Pair #3	
	Baseline (Mean \pm SEM)	After 4 Weeks (Mean \pm SEM)	Baseline (Mean \pm SEM)	After 8 Weeks (Mean \pm SEM)	Baseline (Mean \pm SEM)	After 12 Weeks (Mean \pm SEM)
Sperm Count (millions/ml)	35.13 + 2.79	48.90 + 23.19	35.13 + 2.79	86.16 + 13.70	35.35 + 2.84	88.31 + 3.18
p-value	0.001**		0.001**		0.0002**	
Sperm Motility (%)	35.79 + 2.77	45.73 + 3.19	35.79 + 2.77	67.35 + 2.59	35.92 + 2.82	74.11 + 2.13
p-value	0.022*		0.0003**		0.0003**	
Abnormal Sperm Morphology (%)	42.46 + 2.83	39.38 + 2.95	42.46 + 2.83	21.88 + 2.16	42.09 + 2.86	15.40 + 1.61
p-value	0.472 ns		0.0003**		0.0002**	

Data are expressed as mean \pm SEM. *,**Significant reduction; ns = not significant.

Furosap (FS)-Induced Effects on Mental Alertness, Mood Alleviation, Reflex Erection and Overall Performance at Baseline, Week 4-, Week 8- and Week 12-Treatment

Mental alertness, mood alleviation, reflex erection and overall performance were assessed at baseline, week 4, week 8 and week 12 of treatment (Tables 7A-D). Wilcoxon signed-rank test, a nonparametric test equivalent to the dependent t-test, were used to assess the statistical significance. Significant changes were observed for all these parameters at all time points (Tables 7A-D).

Table 6. Dehydroepiandrosterone Sulfate (DHEA-S), Fasting Blood Sugar (FBS) and Total Leukocyte Count (TLC) following supplementation of FS at Baseline and 12 weeks of treatment

Parameters	Time	Mean \pm Standard Deviation (SD)	p-value
DHEA-S ($\mu\text{g}/\text{dL}$)	Baseline (day 0)	176.88 \pm 93.12	0.279ns
	On Completion (12 weeks)	167.31 \pm 88.41	
FBS (mg/dl)	Baseline (day 0)	133.36 \pm 78.70	0.983ns
	On Completion (12 weeks)	133.06 \pm 55.07	
TLC ($\times 10^3$) (μl^{-1})	Baseline (day 0)	7.52 \pm 1.87	0.454ns
	On Completion (12 weeks)	7.32 \pm 1.75	

Data are expressed as mean \pm SD. ns = not significant

Table 7A. Effect of FS on Mental Alertness at Baseline, Week 4, Week 8 and Week 12 of Treatment

	Valid N	Median	Minimum	Maximum	Wilcoxon Signed Ranks	p-value
Mental Alertness Baseline	43	5.00	4.00	7.00	4.69	0.0002**
Mental Alertness Week 4	48	6.00	4.00	7.00		
Mental Alertness Baseline	43	5.00	4.00	7.00	5.66	0.0002**
Mental Alertness Week 8	48	6.50	5.00	8.00		
Mental Alertness Baseline	43	5.00	4.00	7.00	5.79	0.0003**
Mental Alertness Week 12	47	7.00	6.00	8.00		

**Significant improvement

Effects on Serum Aspartate Aminotransferase/Glutamic Oxaloacetic Transaminase (AST/GOT), Alanine Aminotransferase/Glutamic Pyruvic Transaminase (ALT/GPT), alkaline phosphatase (ALP) and Blood Urea Nitrogen (BUN) Following treatment with FS over a Period of 12 Weeks

No significant changes were observed in serum AST/GOT, ALT/GPT, ALP or BUN levels following treatment with FS over a period of 12 weeks (Table 8).

Table 7B. Effect of FS on Mood Alleviation at Baseline, Week 4, Week 8 and Week 12 of Treatment

	Valid N	Median	Minimum	Maximum	Wilcoxon Signed Ranks	p-value
Mood Baseline	43	5.00	3.00	7.00	5.11	0.0003**
Mood Week 4	48	6.00	4.00	7.00		
Mood Baseline	43	5.00	3.00	7.00	5.57	0.0002**
Mood Week 8	48	7.00	5.00	8.00		
Mood Baseline	43	5.00	3.00	7.00	5.74	0.0001**
Mood Week 12	47	8.00	6.00	9.00		

**Significant improvement

Table 7C. Effect of FS on Reflex Erection at Baseline, Week 4, Week 8 and Week 12 of Treatment

	Valid N	Median	Minimum	Maximum	Wilcoxon Signed Ranks	p-value
Reflex Erection Baseline	43	5.00	4.00	7.00	3.40	0.001**
Reflex Erection Week 4	48	6.00	4.00	7.00		
Reflex Erection Baseline	43	5.00	4.00	7.00	4.97	0.0002**
Reflex Erection Week 8	48	7.00	5.00	8.00		
Reflex Erection Baseline	43	5.00	4.00	7.00	5.57	0.0001**
Reflex Erection Week 12	47	8.00	6.00	9.00		

**Significant improvement

Table 7D. Effect of FS on Overall Performance at Baseline, Week 4, Week 8 and Week 12 of Treatment

	Valid N	Median	Minimum	Maximum	Wilcoxon Signed Ranks	p-value
Overall Performance Baseline	43	5.00	4.00	7.00	3.75	0.0002**
Overall Performance Week 4	48	6.00	5.00	8.00		
Overall Performance Baseline	43	5.00	4.00	7.00	5.35	0.0001**
Overall Performance Week 8	48	7.00	5.00	8.00		
Overall Performance Baseline	43	5.00	4.00	7.00	5.71	0.0002**
Overall Performance Week 12	47	8.00	6.00	9.00		

**Significant improvement

Effects on Cholesterol, Triglycerides, Serum HDL-C, LDL-C and VLDL-C

No significant changes were observed in cholesterol, triglycerides, serum HDL-C, LDL-C and VLDL-C levels following supplementation of FS over a period of 4-, 8- or 12-weeks of treatment (Table 9).

Effects of Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils and Hemoglobin Levels following Supplementation of FS

No significant effect of FS was observed on neutrophils, lymphocytes, monocytes, eosinophils and basophils. Although, a small decrease in the hemoglobin level was observed, however, the baseline and 12-weeks post-treatment hemoglobin levels lied within the normal range (Table 10).

Table 8. Serum aspartate aminotransferase/glutamic oxaloacetic transaminase (AST/GOT), alanine aminotransferase/glutamic pyruvic transaminase (ALT/GPT), alkaline phosphatase (ALP) and blood urea nitrogen (BUN) levels at baseline and 12-weeks of FS treatment

Parameters	Time	Mean \pm Standard Deviation (SD)	p-value
AST/GOT (U/L)	Baseline (day 0)	29.79 \pm 9.96	0.593ns
	On Completion (12 weeks)	31.00 \pm 13.79	
ALT/GPT (U/L)	Baseline (day 0)	42.14 \pm 22.61	0.487ns
	On Completion (12 weeks)	39.41 \pm 28.24	
ALP (U/L)	Baseline (day 0)	108.35 \pm 43.01	0.050*
	On Completion (12 weeks)	94.75 \pm 32.13	
BUN (mg/dl)	Baseline (day 0)	11.36 \pm 3.91	0.082ns
	On Completion (12 weeks)	12.39 \pm 4.32	

Data are expressed as mean \pm SD. *Significant reduction; ns = not significant

Table 9. Cholesterol, Triglycerides, Serum HDL-C, LDL-C and VLDL-C Levels following treatment with FS over a period of 12 weeks

Parameters	Pair #1		Pair #2		Pair #3	
	Baseline (Mean \pm SEM)	After 4 Weeks (Mean \pm SEM)	Baseline (Mean \pm SEM)	After 8 Weeks (Mean \pm SEM)	Baseline (Mean \pm SEM)	After 12 Weeks (Mean \pm SEM)
Cholesterol mg/dl	175.08 \pm 8.37	170.31 \pm 5.70	175.08 \pm 8.37	176.63 \pm 6.28	175.08 \pm 8.37	167.53 \pm 5.54
p-value	0.573ns		0.880ns		0.377ns	
Triglycerides (mg/dl)	152.59 \pm 12.82	158.53 \pm 11.07	152.59 \pm 12.82	176.34 \pm 11.50	152.59 \pm 12.82	161.93 \pm 13.40
p-value	0.561ns		0.048*		0.463ns	
HDL-C (mg/dl)	40.69 \pm 1.62	43.38 \pm 1.85	40.69 \pm 1.62	43.65 \pm 1.57	40.69 \pm 1.62	41.18 \pm 1.47
p-value	0.102ns		0.089ns		0.755ns	
LDL-C (mg/dl)	106.87 \pm 4.68	104.51 \pm 5.26	106.87 \pm 4.68	108.48 \pm 6.15	106.87 \pm 4.68	100.20 \pm 4.16
p-value	0.564ns		0.825ns		0.161ns	
VLDL-C (mg/dl)	33.77 \pm 3.61	31.90 \pm 2.25	33.77 \pm 3.61	35.25 \pm 2.30	33.77 \pm 3.61	32.37 \pm 2.68
p-value	0.583ns		0.683ns		0.704ns	

Data are expressed as mean \pm SEM. *Significant reduction; ns = not significant

Table 10. Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils and Hemoglobin levels following supplementation of FS

Parameters	Time	Mean \pm Standard Deviation (SD)	p-value
Neutrophils %	Baseline (day 0)	61.25 \pm 6.86	0.043*
	On Completion (12 weeks)	63.65 \pm 7.70	
Lymphocytes %	Baseline (day 0)	30.56 \pm 6.36	0.815ns
	On Completion (12 weeks)	28.69 \pm 6.17	
Monocytes %	Baseline (day 0)	3.11 \pm 0.83	0.451ns
	On Completion (12 weeks)	3.08 \pm 0.74	
Eosinophils %	Baseline (day 0)	4.57 \pm 3.50	0.451ns
	On Completion (12 weeks)	4.08 \pm 4.30	
Basophils %	Baseline (day 0)	0.17 \pm 0.09	0.030*
	On Completion (12 weeks)	0.21 \pm 0.11	
Hemoglobin %	Baseline (day 0)	14.97 \pm 1.46	0.001**
	On Completion (12 weeks)	14.28 \pm 1.35	

Data are expressed as mean \pm SD. *,**Significant change; ns = not significant

Discussion

This study demonstrated that novel, patented, dietary rich fenugreek (*Trigonella foenum-graecum*) seed extract, Furosap (FS), enriched in 20% protodioscin extract is beneficial in significantly

enhancing free testosterone level, sperm count, sperm motility, mental alertness, mood, reflex erection and overall performance in human volunteers.

In both Ayurvedic and Chinese medicine, fenugreek leaves and seeds have long been known for the therapeutic efficacy in diabetes, muscle building and wrestling (1,7,21-25). A number of studies have demonstrated that fenugreek attenuated body weight gain and improved insulin sensitivity (11,26). The anti-diabetic efficacy of fenugreek seed extract was attributed due to the presence of furostanolic saponins and 4-hydroxyisoleucine (10,11,26). Hamden et al. (28) demonstrated that administration of fenugreek seed extract to diabetic rats significantly decreased the sperm shape abnormality and improved the sperm count. Furthermore, potential protective efficacy of fenugreek seed extract was observed on reproductive systems, as demonstrated by histological studies on testis and epididymis (28). An in vitro study was conducted by Tomcik et al. (29) which demonstrated that fenugreek seeds in combination with insulin significantly modulated

creatine content via a mechanism which is independent of the activity of sodium- and chloride-dependent creatine transporter, SLC6A8 (29). Aswar et al. (30) assessed the efficacy of fenugreek seed extract (10 mg/kg s.c. bi-weekly or 10 and 35 mg/kg body weight orally on immature castrated male Wistar rats. Some anabolic activity was observed in these animals without androgenic activity (30).

Several independent human studies were conducted in the recent past demonstrating the efficacy of fenugreek seeds in boosting both free- and total testosterone levels, sexual and physical health. A clinical investigation in forty nine resistance-trained male subjects demonstrated that fenugreek seed extract (500 mg/day) had a significant impact on both upper- and lower-body strength and body composition in a double-blind placebo-controlled study (31). Rao et al. (32,33) and Steels et al. (34) conducted three independent studies on their proprietary fenugreek extract in both male and female subjects and exhibited their efficacy on boosting testosterone levels. Steels et al. (34) conducted this a randomized, double blind and placebo controlled study in 60 healthy males (age: 25-52 years; daily dose of fenugreek seed extract: 600 mg) over a period of 6 weeks. The authors indicated that a positive effect was observed on the physiological aspects of libido, muscle strength and energy. The second study was conducted in 80 healthy menstruating women who reported low sexual drive (age: 20 to 49 years; dose: 600 mg/day), which demonstrated that fenugreek seed extract is beneficial for boosting sexual arousal and desire in women. The third randomized, double-blind, placebo-controlled study was conducted in 120 men (age: 43-70 years; daily dose of fenugreek extract: 600 mg) over a period of 12 weeks. Both free and total testosterone levels and sexual function increased significantly after 12 weeks of treatment (32).

A significant number of studies have demonstrated the anabolic potential of fenugreek seed extract in boosting testosterone levels, muscle building and sexual health. Our product development was dedicated to come up with a natural protodioscin-enriched extract of fenugreek seeds. It is very important to emphasize that protodioscin-enriched botanical extract are the prime source for boosting free and total testosterone levels in both animals and humans (25). Our novel invention crystallized the development of a state-of-art fenugreek seed extract enriched in approximately 20% protodioscin. Our preliminary study was provided on dose-determination, which provided us the daily effective dose of 500 mg/day. In the present study, we focused to determine the efficacy of our novel,

patented fenugreek-seed extract, enriched in 20% protodioscin, on boosting free and total testosterone levels, sperm count, sperm motility, sperm morphology and other allied parameters.

Conclusions

The results of our investigations demonstrated that supplementation of Furosap (FS)(500 mg/day), a novel, patented, dietary fiber rich *Trigonella foenum-graecum* seeds extract enriched in 20% protodioscin, to 50 male volunteers (age: 35-65 years) over a period of 12 consecutive weeks. Free testosterone level increased significantly by approximately 1.47-fold (p value = 0.000**), whereas the total testosterone levels increased by 1.08-fold, which was not significant. Statistically significant increases were observed in sperm count and sperm motility at 4-, 8- and 12-weeks of FS treatment, while, a statistically significant decrease in abnormal sperm morphology was observed. A non-significant decrease in abnormal sperm morphology was observed at 4-week post-treatment, however, significant decreases in abnormal sperm morphology were observed at both 8- and 12-weeks of treatment. This was a very enlightening factor along with a significant increase in free testosterone level. Furthermore, mental alertness, mood alleviation, reflex erection and overall performance were assessed at baseline, week-4, week-8 and week-12 of treatment, and significant alleviation was observed in all these parameters at all time points. Extensive blood chemistry and lipid profile were assessed in our investigation, which demonstrated the broad spectrum safety of FS. Cardiovascular health and profile was also significantly improved. Future studies are in progress to demonstrate its extensive use in muscle building, sports nutrition and exercise, and to unveil the molecular mechanism of action.

Abbreviations

ALP: Alkaline phosphatase
ALT/GPT: Alanine aminotransferase/Glutamic pyruvic transaminase
AST/GOT: Aspartate aminotransferase/ Glutamic oxaloacetic transaminase
BMI: Body mass index
BUN: Blood urea nitrogen
CAD: Coronary artery disease
DBP: Diastolic blood pressure
DHEA-S: Dihydroepiandrosterone sulfate
FBS: Fasting blood sugar
FS: Furosap
HDL-C: High density lipoprotein
LDL-C: Low density lipoprotein

ns: Not significant
 SBP: Systolic blood pressure
 TLC: Total leukocyte count
 VLDL: Very low density lipoprotein

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Consent to Publish

All authors have read, consented and approved the final manuscript for publication. This manuscript doesn't contain any individual person's data.

Availability of Data and Material

AM and NV have appropriately stored all the data in their Laboratories Storage Facility in Kings Georges Medical University, Lucknow, Uttar Pradesh, India, and SHK Diabetic Clinic & Research Center, Lucknow, Uttar Pradesh, India.

Conflict of Interest

AM is the principal investigator and NV is the co-principal investigator, organized, coordinated the study and analyzed the data. MB and HGP served as consultants and coordinated in writing the manuscript and coordinated by AM, NV and DB. DB is the chief scientific officer of Cepham, Inc., and AS is the president of Cepham Inc. AM, NV, MB, HGP and DB have no competing interests. AS being a PhD in Biochemistry took interest in reviewing the final report.

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