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Safety and Efficacy of Furosap®, a Patented *Trigonella foenum-graecum* Seed Extract, in Boosting Testosterone Level, Reproductive Health and Mood Alleviation in Male Volunteers

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ABSTRACT

Background: The medicinal herb fenugreek (*Trigonella foenum-graecum*) seeds, fortified with dietary fibers and furostanolic saponins including protodioscin, have demonstrated a significant contribution to human health. In our laboratories, Furosap®, a patented 20% protodioscin-enriched extract was developed from fenugreek seeds.

Objective: In an open-label, one-arm, single-center longitudinal study, we examined the safety and efficacy of Furosap® on free and total testosterone levels, fasting blood sugar, blood pressure, sperm count, motility and morphology, dihydroepiandrosterone sulfate (DHEA-S), sexual health, reflex erection, mood alleviation, mental alertness, and total blood chemistry analyses over a period of 12 weeks in healthy male volunteers.

Methods: Institutional Ethics Committee approvals and Clinicaltrials.gov registration were obtained. Effect of Furosap® (500 mg/day) was examined of free and total testosterone levels, sperm count, motility and morphology, sexual health, mood and mental alertness, and total blood chemistry analyses in 100 healthy volunteers (age 35–60 Y) over a period of 12 consecutive weeks.

Results: No changes were observed in body weight and BMI. Both systolic and diastolic blood pressure, and DHEA levels significantly decreased. Free and bound testosterone levels improved significantly at 12 weeks of treatment. Sperm motility significantly increased at 8- and 12-weeks of treatment, while abnormal sperm morphology significantly decreased at 12-weeks of treatment. Mental alertness, mood, and reflex erection score significantly alleviated. An age-induced increasing effect was observed. Furthermore, cardiovascular health and libido significantly improved. Blood chemistry analyses exhibited broad spectrum safety. A decreasing trend was observed in total cholesterol, triglycerides, and VLDL levels, while an increasing trend was observed in HDL level at 12 weeks of treatment. LDL level decreased significantly at 12-weeks of treatment. No adverse events were observed.

Conclusion: Results demonstrate that Furosap® is safe and effective in improving testosterone levels, cardiovascular health, healthy sperm profile, mental alertness in human male volunteers.

Abbreviations: ALP: Alkaline phosphatase; ALT/SGPT: Alanine aminotransferase/Serum glutamic pyruvic transaminase; AST/SGOT: Aspartate aminotransferase/Serum glutamic oxaloacetic transaminase; BMI: Body mass index; BUN: Blood urea nitrogen; DBP: Diastolic blood pressure; DHEA-S: Dihydroepiandrosterone sulfate; FBS: Fasting blood sugar; 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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Introduction

The legendary leguminous annual medicinal herb Fenugreek (*Trigonella foenum-graecum*, family Fabaceae) is cultivated and grown extensively in India, China, Egypt, Turkey, Iran, Spain, France, Morocco, and Argentina (1–4). The fenugreek seeds, yellow- to amber-colored cuboid-shaped seeds, fresh and dried leaves, twigs, roots, and sprout are widely used in spice blends and flavoring agent in diverse culinary preparations and soups (2–6). Both fenugreek seeds itself and the roasted seeds, are widely used in foods and beverages (1–4).

Dietary soluble fiber enriched fenugreek seeds are traditionally used for diabetics, women's health, and to boost lactation in breast-feeding women in both Ayurvedic and Unani traditional medicines, as well as for

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This study was registered at clinicaltrials.gov (NCT02702882).

anti-inflammatory, immune competence, antiseptic, aphrodisiac, and diverse health benefits for centuries (3–5,7). In Ayurveda, it is highly recommended to boost vitality, vigor, immunity, endurance, and sports performance, as well as improve digestion and prevent stomach disorder (3,5–11).

From the compositional analyses, fenugreek is fortified with approximately 5% stronger-smelling oil, 28% mucilage, trigonelline, choline, biotin, inositol, vitamins A, B1, B2, B3, B5, B6, B9, B12, and D, an organic form of bioavailable iron, phosphates, lecithin and nucleoalbumin, diosgenin, diosgenin-βD-glucoside, vitexin, vitexin-7-glucoside, yamogenin, vicenin, and fenugreek lactone (4,5-dimethyl-3-hydroxy-2[5H]-furanone) (2,3,8,12–16).

A number of preclinical and clinical investigations established the versatile benefits of fenugreek seeds as an antioxidant, anti-inflammatory, antidiabetic, antihyperlipidemic, anti-obesity, anticancer, antifungal, antibacterial, and galactogogue, as well as for diverse medicinal and pharmacological benefits (2,5–9). Research studies have also demonstrated its significant benefits in gastric ulcer, hypercholesterolemia, hyperthyroidism, polycystic ovary syndrome (PCOS), enhanced sports and exercise performance in animals and humans (2,5). In male mice, Ikeuchi et al. (17) assessed the dose-dependent efficacy of a standardized fenugreek seed extract (p.o.) in a swimming model for a period of 4 weeks. Interestingly, remarkable improvement in swimming time and endurance was reported at a 300 mg/kg body weight dose, which was explained by an enhanced utilization of fatty acids as an energy source. In a separate dose-dependent study, Arshadi et al. (2015) determined the comparative efficacy of fenugreek and glibenclamide in a swimming exercise model in type 2 diabetic male rats (18,19). These investigators exhibited that beside an alleviation in swimming exercise, potential improvement was observed in plasma insulin, plasma leptin, HOMA-IR, and adiponectin (18,19).

In an 8-weeks placebo-controlled, double-blind study in 49 resistance-trained male athletes, Poole et al. assessed the efficacy of fenugreek seed extract (500 mg/day) on body strength, power output, muscle endurance, body composition, and hormonal profiles in a structured resistance training setting (20). Significant increases of upper- and lower-body strength, improvement of overall body composition, and a significant reduction in body fat were observed. Fenugreek seed extract significantly increased upper- and lower-body strength, reduced body fat and improved overall body composition, while non-significantly enhanced muscular endurance, hormonal concentrations and normalized hematological indices (20).

The authors determined the broad-spectrum safety, while no toxic manifestations were reported (12). The authors assessed the efficacy of Furosap® (500 mg/kg/day) in an open-labelled, one-arm, single-center study in 50 males (age: 35–65 Y) over a period of 12 consecutive weeks on multiple parameters and demonstrated the efficacy of Furosap® in boosting free testosterone, healthy sperm profile, mental alertness, mood alleviation, reproductive well-being (21,22). Guo et al. conducted a randomized, placebo-controlled, and double-blind investigation on Furosap® (250 mg b.i.d.) in 40 male athletes over a period of 12 consecutive weeks (23). Furosap® significantly increased mean lean body mass and fat free mass, elevated serum testosterone levels, and exercise endurance, as well as a tendency of lowering blood pressure during exhaustion. No adverse events were reported (23).

In this longitudinal study, we evaluated the broad spectrum safety and efficacy of a unique, patented fenugreek (*Trigonella foenum-graecum*) seed extract fortified in 20% protodioscin (Furosap®) US and European Patents# US 8,754, 205 B2; US 8,217,165 B2; EP 2285821 B1) (24–26) to enhance free and total testosterone levels, sperm count, motility and abnormal sperm morphology, reproductive health, mood alleviation and mental alertness, in 100 male volunteers (Age: 35–60 years) over a duration of 12 consecutive weeks.

Materials and methods

Study design

This study recruitment and procedures were conducted in compliance and accordance with ICH guidelines for *Good Clinical Practices* (GCP), complying with all necessary guidelines, and maintaining all necessary documents, strictly following the international ethical standards guaranteed by the Declaration of Helsinki and its subsequent amendments. Institutional Ethics Committee (IEC) approval was obtained from King George's Medical University, Lucknow, India [Registration No ECR/262/Inst/UP/2013 accredited by the Drugs Controller of India (DCGI), for this Investigation entitled "Assess the efficacy of Furosap®: a testosterone booster supplement, in human volunteers: An add-on study" (Protocol number: CR-TEST-01/7-14, study initiation date: Oct 30, 2014 and completion date: Feb 27, 2017). The patient consent form was also approved by the Institutional Ethics Committee. This study also obtained www.clinicaltrials.gov registration #NCT02702882. All subjects duly signed the informed consent after explaining the details of the study protocol and the details of the subject's participation. All subjects also completed a brief health questionnaire during enrollment. During primary screening, all informed consent was obtained. All these were meticulously conducted before the inclusion of the study participants and commencement of the study. Subject confidentiality was strictly vigiled. Adverse event monitoring was strictly enforced.

Recruitment of subjects

All subjects were recruited strictly following the inclusion and exclusion criteria (Table 1). Subject demographics are shown in Table 2. A total of 100 subjects were recruited and directed to take one Furosap® capsule (Batch# FUP0914 and FUP0615) (500 mg/day p.o.) after breakfast during a

Table 1. Inclusion and exclusion criteria.

Inclusion criteria
1. Agrees to sign written and audio-visual informed consent.
2. Fully understand the risks and benefits of the study
3. Male Subjects (age: 35–65 Y)
4. Subjects diagnosed with symptomatic hypogonadism.
Exclusion criteria
1. Unwilling/uncooperative subjects
2. Subjects were excluded who had impaired hepatic functions i.e., SGOT/SGPT > 2.5 X
3. Subjects who were suffering from coronary artery disease
4. Subjects who were exhibiting abnormal hepatic or kidney function (ALT or AST > 2 X greater than normal value); elevated creatinine, males > 125 µmol/L or 1.4 mg/dl
5. Subjects who had cancer and suffering from malignancy.
6. Hypersensitivity to the investigational supplement
7. Subjects who had used any known testosterone booster/medication/supplement for the last 2 months.
8. History of blood coagulation and bleeding (coagulopathies)
9. Incidence of high alcohol intake (more than 2 standard drinks/day).
10. Psychiatric disorder/disability provide signed informed consent.
11. Existing medical condition of the participant which may be detrimental to the study and subject's overall well-being.

Table 2. Subject demographics.

Parameters	Data (mean + SD)
Age (mean)	35–60 Y
Height (mean)	155 – 198 cm
BMI (mean)	23.37 ± 3.67 kg/m^2
Pulse Rate	71.28 ± 2.22 per minute

period of 12 consecutive weeks. Subjects were directed to store the investigational product in a cool and dark place.

Protocol compliance

Nurses involved in this study handed out the Furosap® over a period of 4 weeks prior to each month. A logbook was maintained, which was regularly supervised by the principal investigator. The nurses who handed over the samples strictly maintained the IP accountability logbook, where every single entry was maintained after due endorsement of the principal investigator & study coordinator. Nurses who distributed the sample also signed the logbook. All preparatory actions were undertaken for technical audit.

Assay procedures

i. Free testosterone level was monitored using a Dia Sources' ELISA kit (catalog#CAN-FTE-260; Krishgen Biosystems, Mumbai, India), and total testosterone level was determined utilizing an automated bidirectionally interfaced Chemiluminescent Immunoassay (CLIA) from Siemens Health Care Pvt Ltd, Mumbai, India.
ii. Dehydroepiandrosterone sulfate (DHEA-S) was evaluated using Cobas Electrochemiluminescence Immunoassay kit (ECLIA) (catalog# 03000087122; purchased from Roche Diagnostics India Pvt Ltd, Mumbai, India).
iii. Hemoglobin level was determined using a Sysmex fully automated bidirectional analyzer (SYSMEX XN-1000; Transasia Bio Medicals Ltd, Mumbai, India), while fasting blood glucose (FBS) levels were monitored 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).
iv. Sperm count, sperm motility and abnormal sperm morphology were assessed in Nigam Pathology (Lucknow, Uttar Pradesh, India).

Study assessment

Efficacy of Furosap® was assessed in 100 healthy male volunteers at baseline, 4-, 8-, and 12 weeks of treatment. Table 3 exhibits the various parameters assessed.

Adverse event monitoring

All subjects were requested to record and report any uncomfortable problems or difficulties during the study period daily due to supplementation. For any serious adverse events, subjects were asked to contact the principal investigator immediately. Adverse event monitoring was strictly enforced.

Compliance

Enrolled subjects were monitored and contacted regularly on their mobile phones and email to ensure compliance. All subjects were required to bring back their used study supplement bottles as is every 15 days. Fresh supplement bottles were issued every 15 days. Everything was meticulously recorded to monitor compliance. Adverse event monitoring was strictly enforced.

Drop outs

All enrolled subjects successfully completed the study

Statistical analysis

All parametric and non-parametric assessments were determined. Data is expressed as mean ± SD (Standard Deviation). The baseline values were compared with the data at the completion of 4-, 8- and 12-weeks post- treatment, while t test

was employed to compare demographic and baseline variable across the treatment group. For comparing among the four groups [(i) placebo, (ii) treatment intervention, (iii) pre- (before), and (iv) post- (after)] a one-way analysis of variance followed by Tukey's post-hoc test was used. A p value of < 0.05 was considered a statistically significant difference. Effect size for all experiments were based on established data, our previous clinical studies (21,23), and published literature. A prior power analysis was performed for all experiments.

Results

A total of 100 healthy male subjects were enrolled and successfully completed the investigation over a duration of 12 consecutive weeks.

Body weight, BMI and blood pressure

No significant changes were observed in body weight and BMI (Data not shown). However significant decrease was observed in both systolic and diastolic blood pressures at the completion of 12 consecutive weeks (Table 4).

Free- and total testosterone

Both free- and total testosterone were determined at the baseline and at the completion of 12 consecutive weeks of treatment (Table 5). Free- and total testosterone levels escalated by approximately 1.73-fold (p value = 0.0004) and by 1.28-fold (p-value = 0.0003), respectively (Table 5).

Influence of Furosap® on sperm count (millions/ml), sperm motility (%) and abnormal sperm morphology (%) at the 0, 4, 8, and 12-weeks of treatment

Sperm count (millions/ml), sperm motility (%) and abnormal sperm morphology (%) data at the baseline (day 0), weeks 4-, 8-, and 12-weeks of Furosap® treatment are demonstrated in Table 6. Sperm count significantly increased following completion of 4-, 8- and 12-weeks of treatment, while sperm motility (%) significantly increased at 8- and 12-weeks post-treatment. Simultaneously, abnormal sperm morphology (%) decreased at all time points, but significantly decreased at 12-weeks post-treatment, respectively (Table 6).

Table 3. Efficacy at 0-, 4-, 8- and 12-weeks of treatment.

	Treatment period (in weeks)		
Baseline (0 week)	Week 4	Week 8	Week 12
Body mass index (BMI)(kg/m^2)	BMI (kg/m^2)	BMI (kg/m^2)	BMI (kg/m^2)
Free testosterone (pg/ml)	Fasting lipid profile (total cholesterol, LDL, HDL, triglycerides, VLDL)	Fasting lipid profile (total cholesterol, LDL, HDL, triglycerides, VLDL)	Free testosterone (pg/ml)
Total testosterone (ng/dl)	Semen examination (sperm count, sperm mobility, sperm morphology	Semen examination (sperm count, sperm mobility, sperm morphology	Total testosterone (ng/dl)
DHEA-S (µg/dL)			DHEA-S
Fasting blood sugar (FBS)			Fasting blood sugar (FBS)
Fasting lipid profile (total cholesterol, LDL, HDL, triglycerides, VLDL)			Fasting lipid profile (total cholesterol, LDL, HDL, triglycerides, VLDL)
Liver function tests (AST, ALT, ALP)			Liver function tests (AST, ALT, ALP)
Hemogram			Hemogram
Sperm count, sperm motility, and sperm morphology			Sperm count, sperm mobility, sperm morphology

Table 4. Systolic and diastolic blood pressure (mmHg) following supplementation of Furosap® over a duration of 12 consecutive weeks.

Parameters	Time	Mean ± SD	p-value
Systolic blood pressure (SBP) (mmHg)	Baseline (day 0)	113.83 ± 5.93	0.008**
	On completion (12 weeks)	112.10 ± 5.59**	
Diastolic blood pressure (DBP) (mmHg)	Baseline (day 0)	85.06 ± 7.16	0.0002**
	On completion (12 weeks)	81.51 ± 3.86**	

Data are expressed as mean ± SD. **Significant reduction.

Table 5. Free testosterone (pg/ml) and total testosterone levels (ng/dl) following 12 consecutive weeks of Furosap® supplementation.

Parameters	Time	Mean ± SD	p-value
Free testosterone (pg/ml)	Baseline day 0	11.28 ± 8.86	0.0004**
	Final 12 weeks	19.50 ± 9.73**	
Total testosterone (ng/dl)	Baseline day 0	386.79 ± 161.90	0.0003**
	Final 12 weeks	495.27 ± 144.03**	

Data are expressed as mean ± SD. **Significant change.

Table 6. Effect of Furosap® treatment on sperm count, sperm motility and abnormal sperm morphology at baseline (day 0), and weeks 4-, 8-, and 12-weeks post-treatment.

Parameters	Baseline (day 0) (Mean ± SD)	4 weeks (Mean ± SD)	8 Weeks (Mean ± SD)	12 Weeks (Mean ± SD)
Sperm count (millions/ml)	46.15 ± 27.56	51.63 ± 25.55**	56.10 ± 26.66**	62.22 ± 25.01
p-value	–	0.0001**	0.0003**	0.0002**
Sperm motility (%)	47.12 ± 23.48	48.26 ± 22.15	51.65 ± 22.52**	55.35 ± 21.98**
p-value	–	0.220 ns	0.003**	0.0003**
Abnormal sperm morphology (%)	32.79 ± 19.99	30.64 ± 18.59	29.46 ± 17.75	26.62 ± 17.13**
p-value	–	0.221 ns	0.133 ns	0.009**

Data are expressed as mean ± SD. **Significant Change; ns = not significant.

Table 7. Effect of Furosap® on dehydroepiandrosterone Sulfate (DHEA-S), fasting blood sugar (FBS) and total leukocyte count (TLC).

Parameters	Time	Mean ± SD	p-value
DHEA-S (µg/dL)	Baseline (day 0)	197.32 ± 74.39	0.003*
	Final 12 weeks	182.96 ± 65.13	
FBS (mg/dl)	Baseline (day 0)	106.95 ± 35.18	0.066 ns
	Final 12 weeks	102.51 ± 26.45	
TLC (x 10^3) (μl^{-1})	Baseline (day 0)	7.09 ± 2.18	0.239 ns
	Final 12 weeks	7.12 ± 2.15	

Data are expressed as mean ± SD. *Significant Change; ns = not significant.

Table 8. Effect of Furosap® on mental alertness at 0, 4-, 8- and 12-weeks of treatment.

		Improvement in mental alertness (%)			
Time period	Overall Score	Age (35–40 Y)	Age (41–50 Y)	Age (51–60%)	Overall
Mental alertness (Day 0)	5.22	–	–	–	–
Mental alertness (Week 4)	5.41	18.3%	23.10%	33.3%	21.5%
Mental alertness (Week 8)	5.94	55.4%	80%	66.7%	63.6%
Mental alertness (Week 12)	6.51	76.8%	92%	83.3%	81.8%

Influence of Furosap® on dehydroepiandrosterone sulfate (DHEA-S), fasting blood sugar (FBS) and total leukocyte count (TLC) in male subjects

DHEA-S, FBS and TLC levels were determined at baseline and 12-weeks of treatment. A significant reduction was observed in DHEA-S, however, no changes were observed in FBS and TLC (Table 7)

Effect of Furosap® on mental alertness, mood alleviation, reflex erection and overall performance

Mental alertness, mood alleviation, reflex erection and overall performance following treatment with Furosap® were assessed at baseline, weeks 4, 8 and 12 of treatment. Following supplementation of Furosap®, overall scores of mental alertness, mood alleviation, and reflex erection increased (Tables 8–10). Moreover, time- and age-dependent increases (%) in all these three factors were observed.

Influence of Furosap® on serum aspartate aminotransferase/glutamic oxaloacetic transaminase (AST/SGOT), alanine aminotransferase/glutamic pyruvic transaminase (ALT/SGPT), alkaline phosphatase (ALP) and blood urea nitrogen (BUN) levels at the initiation and completion of 12 consecutive weeks

Furosap® treatment caused significant decreases in serum AST/SGOT, ALT/SGPT, ALP or BUN levels at the completion of 12 weeks of treatment (Table 11). Significant changes were observed in these blood chemistry parameters, although, these values remained within the specified range.

Effect of Furosap® on cholesterol, triglycerides, serum HDL-C, LDL-C and VLDL-C

Significant decreases were observed in the total cholesterol (mg/dl) levels at all time points. A decreasing trend was observed in LDL-C levels at all time points, while significant reduction was observed at week 12 of treatment. On the contrary, a significant decrease was observed in the HDL-C level at 4 weeks of treatment, while time-dependent increases were observed at both 8- and 12-weeks in the HDL-C level at both 8- and 12-weeks of treatment, respectively, which is indeed a promising sign. However, no significant changes were observed in triglycerides, and VLDL-C levels (Table 12).

Effects of neutrophils, lymphocytes, monocytes, eosinophils, basophils and hemoglobin levels following supplementation Furosap®

No significant changes were observed on all these parameters following 12 weeks of treatment (Table 13).

Discussion

Fenugreek, *Trigonella foenum-graecum* (family Fabaceae), a legendary Ayurvedic medicinal plant has been used as

Table 9. Effect of Furosap® on mood alleviation at 0-, 4-, 8- and 12 weeks of treatment.

		Improvement in Mood Alleviation (%)			
Time period	Score	Age (35–40 Y)	Age (41–50 Y)	Age (51–60%)	Overall
Mood alleviation (Day 0)	4.98	–	–	–	–
Mood alleviation (Week 4)	5.55	53.3%	65.4%	50%	57%
Mood alleviation (Week 8)	6.08	76.8%	100%	83.3%	84.1%
Mood alleviation (Week 12)	6.68	92.9%	100%	100%	95.5%

Table 10. Time-dependent effect of Furosap® on reflex erection at baseline, weeks 4-, 8- and 12- of treatment.

		Improvement in reflex erection (%)			
Time period	Score	Age (35–40 Y)	Age (41–50 Y)	Age (51–60%)	Overall
Reflex erection (Day 0)	4.72	–	–	–	–
Reflex erection (Week 4)	5.24	46.7%	46.2%	66.7%	47.3%
Reflex erection (Week 8)	5.85	73.2%	76%	100%	75%
Reflex erection (Week 12)	6.62	89.3%	92%	100%	90.9%

Table 11. Effect of Furosap® on serum aspartate aminotransferase/glutamic oxaloacetic transaminase (AST/GOT), alanine aminotransferase/glutamic pyruvic transaminase (ALT/GPT), alkaline phosphatase (ALP) and blood urea nitrogen (BUN) levels.

Parameters	Time point	Mean ± SD	p-value
AST/SGOT (U/L)	Baseline (day 0)	31.83 ± 9.57	0.0003*
	Final (12 weeks)	28.31 ± 6.82	
ALT/SGPT (U/L)	Baseline (day 0)	38.95 ± 18.14	0.0002*
	Final (12 weeks)	33.98 ± 14.52	
ALP (U/L)	Baseline (day 0)	97.22 ± 25.02	0.001*
	Final (12 weeks)	89.84 ± 23.13	
BUN (mg/dl)	Baseline (day 0)	12.02 ± 3.53	0.004*
	Final (12 weeks)	12.78 ± 3.53	

Data are expressed as mean ± SD. *Significant Change.

Table 12. Cholesterol, triglycerides, serum HDL-C, LDL-C and VLDL-C levels following treatment with FS (Furosap) over a period of 12 consecutive weeks.

Parameters	Baseline (Mean ± SD)	Week 4 (Mean ± SD)	Week 8 (Mean ± SD)	Week 12 (Mean ± SD)
Total cholesterol (mg/dl)	182.41 ± 31.57	176.34 ± 32.61	175.36 ± 27.46	175.24 ± 29.71
p-value	–	0.009**	0.007**	0.024*
Triglycerides (mg/dl)	151.14 ± 99.07	147.09 ± 79.94	143.73 ± 71.53	144.41 ± 70.22
p-value	–	0.64 ns	0.425 ns	0.562 ns
HDL-C (mg/dl)	44.85 ± 11.15	42.45 ± 9.47	43.87 ± 9.48	46.47 ± 10.03
p-value	–	0.02**	0.184 ns	0.105 ns
LDL-C (mg/dl)	106.21 ± 26.15	103.11 ± 24.77	102.98 ± 23.60	98.11 ± 21.62
p-value	–	0.095 ns	0.115 ns	0.000**
VLDL-C (mg/dl)	30.17 ± 19.84	29.67 ± 14.66	28.65 ± 12.54	28.50 ± 13.94
p-value	–	0.741 ns	0.307 ns	0.458 ns

Data are expressed as mean ± SD *, ** Significant Change; ns = not significant.

culinary purposes and novel foods, as well as for health promoting and therapeutic remedies for diverse degenerative diseases (1–4). It has been reported that fenugreek is one of the oldest medicinal plant in Ayurveda. In both Ayurvedic and Oriental traditional medicines, the seeds, leaves, and whole plant are used meticulously for diverse therapeutic purposes. In fact, the traditional medicines are now being utilized for basic medical needs by approximately two-thirds of global population (2–8). Currently, it became an extremely popular herb around the world as holistic and traditional medicine, as well as novel foods, spice, and diverse culinary practices (1–6,9–15).

Fenugreek has been demonstrated to contain a diverse number of phytochemicals including saponins, steroids, steroidal saponins, alkaloids namely trigonelline, coumarins namely cinnamic acid and scopoletin, vitamins, polyphenols, flavonoids, lipids, structurally diverse amino acids, hydrocarbons, glycosides, carbohydrates, hydrocarbons, significant amount of fiber and micronutrients such as iron, manganese, and magnesium (1–4). A significant volume of preclinical and clinical investigations has established the versatile applications of fenugreek seeds as an antioxidant, insulin sensitizer, antidiabetic, galactagogue, anti-inflammatory, anticancer, antifungal, antibacterial, polycystic ovary

Table 13. Neutrophils, lymphocytes, monocytes, eosinophils, basophils and hemoglobin levels following supplementation of Furosap® over a period of 12 consecutive weeks.

Parameters	Time	Mean ± SD	p-value
Neutrophils %	Baseline (day 0)	60.10 ± 7.59	0.293 ns
	Final 12 weeks	61.22 ± 7.81	
Lymphocytes %	Baseline (day 0)	30.46 ± 6.86	0.480 ns
	Final 12 weeks	29.80 ± 7.37	
Monocytes %	Baseline (day 0)	4.07 ± 2.37	0.16 ns
	Final 12 weeks	4.93 ± 3.18	
Eosinophils %	Baseline (day 0)	4.97 ± 4.03	0.17 ns
	Final 12 weeks	3.82 ± 2.77	
Basophils %	Baseline (day 0)	0.23 ± 0.22	0.892 ns
	Final 12 weeks	0.23 ± 0.31	
Hemoglobin %	Baseline (day 0)	14.31 ± 1.47	0.239 ns
	Final 12 weeks	16.03 ± 13.5	

Data are expressed as mean ± SD. ns = not significant.

syndrome (PCOS), and as well as for muscle building, sports nutrition, wrestling, and exercise benefits (1–5,13–20,27,28). Moreover, researchers have unveiled a considerable volume of cellular and molecular pathologies for versatile therapeutic benefits (2,5).

In an in vitro model, Tomcik et al. (29) that fenugreek along with insulin can potentially improve the creatine content by an independent mechanistic pathology other than the activity of sodium-and chlorine-dependent creatine transporter, SLC6A8. Fenugreek has exhibited potential benefits in weight management and attenuating insulin sensitization (13,21,22,30), while its constituents, furostanolic saponins and 4-hydroxyisoleucine, have documented potential anti-diabetic benefits (2,12,31). It has been demonstrated that fenugreek can significantly modulate sperm shape abnormality and improving sperm counts in diabetic animals (32). Based on the histological studies on testis and epididymis these researchers also affirmed the potential benefits of fenugreek on reproductive systems. Aswar et al. (33) exhibited that oral or sub-cutaneous administration of fenugreek seed extract to immature castrated male rats exhibited potential anabolic activity in the absence of upsurge of androgenic activity.

In multiple clinical settings, fenugreek seed extract has exhibited the ability to potentiate both free- and total testosterone levels, sexual and physical health. In a double-blind placebo-controlled investigation in 49 resistance-trained males, fenugreek seed extract (500 mg/day) exhibited dramatic impact on both lower- and upper body strength and body composition (20).

Three independent clinical studies by Steels et al. (34) and Rao et al. (35,36) in both male and female subjects demonstrated the efficacy of fenugreek seed extract in boosting testosterone levels. A randomized, double-blind, placebo-controlled study (34) in 60 healthy males (age: 25–52 Y; daily dose: 600 mg; duration: 6 weeks) exhibited potential therapeutic benefits on libido, muscle strength, and energy. Rao et al. (35) determined the efficacy of 600 mg/day of fenugreek seed extract in 80 healthy menstruating female subjects (20–49 Y) in a randomized, placebo-controlled study over a period of 8 consecutive weeks and exhibited its efficacy in boosting sexual arousal and desire, as well as free testosterone and estradiol levels significantly increased. Independently, the same investigators investigated the efficacy of 600 mg fenugreek seed extract in a randomized, placebo-controlled study over a period of 12 weeks in 120 healthy male volunteers (age: 43–70 Y) and demonstrated that both free and total testosterone levels and sexual functions significantly increased (36). Rao et al. indicated that fenugreek seed extract is a safe and efficacious therapy for reducing androgen deficiency, and boosting serum free and total testosterone levels, as well as sexual competence middle-aged and elderly subjects (35,36).

Zameer et al. (37) demonstrated that in an in vitro model fenugreek exhibited selective cytotoxic activity against T-cell and B-cell lymphoma. In fact, the anticancer effect in T-cell lymphoma was mediated through induction of apoptosis. In cultured HL-60 human leukemia cells, protoodioscin-enriched fenugreek seed extract demonstrated potent growth-inhibitory effect (15). Induction of apoptosis in tumor cells was confirmed by morphological, flow cytometric and molecular analyses (15). Another in vitro study in A-549 human lung cancer cells (Verma et al) exhibited potent anticancer activity of fenugreek whole plant extract (38). Especially, fenugreek derived diosgenin inhibited the growth of cancer of cells along with potent down regulation of hTERT expression, and inhibition of telomerase activity (38,39). This effect significantly strengthens the potential of fenugreek seed extract in sports nutrition, exercise and muscle building.

Earlier clinical investigations in our laboratory in 50 male subjects (age: 35–65 Y) over a period of 12 consecutive weeks demonstrated that fenugreek seed extract, Furosap® enriched in 20% protodioscin treatment (500 mg/day), has broad spectrum safety and the ability to boost free testosterone level, healthy sperm profile, mental alertness, mood alleviation, reflex erection, cardiovascular health significantly (21,22). Guo et al. (23) conducted a randomized, placebo-controlled, double-blind study in forty young male athletes (age = 24.02 ± 3.9 Y) to assess the efficacy of Furosap® (250 mg bid/day) over a period of 12 consecutive weeks and demonstrated significant improvement in lean body mass, fat free mass, and serum testosterone level. The researchers concluded that Furosap® has the potential to boosts sports performance and exercise endurance (23).

This longitudinal investigation exhibited the efficacy of Furosap® (500 mg/day), enriched in 20% protodioscin, in 100 healthy male subjects (age = 35–60 Y) over a period of 12 consecutive weeks on multiple parameters including enhanced production of free and total testosterone levels, sperm profile, sperm morphology, reproductive health, mood alleviation, mental alertness, reflex erection, and reproductive health. Furthermore, both systolic and diastolic blood pressure, and DHEA-S significantly decreased at 12 weeks of treatment. Sperm motility significantly increased at 8- and 12-weeks of treatment, while abnormal sperm morphology significantly decreased at 12-weeks of treatment. Furthermore, cardiovascular health and libido significantly improved. Mental alertness, mood, and reflex erection score significantly improved. An age-induced increasing effect was observed. In addition, broad spectrum safety was affirmed from blood chemistry analyses. A decreasing trend was observed in total cholesterol, triglycerides, and VLDL levels, while an increasing trend was observed in HDL level at 12 weeks of treatment. LDL level decreased significantly at 12-weeks of treatment. No adverse events were observed.

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Authors' contributions

Dr. S.N. Sankhwar (SNS), Professor & Head, Department of Urology, King George's Medical University, Lucknow, Uttar Pradesh, India, conducted this investigation as the Principal Investigator with his esteemed clinical study team members in Kings George's Medical Center. Dr. Apul Goel, Professor, Department of Urology, King George's Medical University, served as the Co-Principal Investigator. Dr. Shreya Pant, MD, King George's Medical University, served as the research assistant in this project.

Disclosure statement

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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

SNS have appropriately stored all the data in their Laboratories Storage Facility in Kings Georges Medical University, Lucknow, Uttar Pradesh, India.

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