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Original article

Beneficial effects of fenugreek glycoside supplementation in male subjects during resistance training: A randomized controlled pilot study

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Abstract

Purpose: To evaluate the efficacy and safety of the glycoside fraction of fenugreek (*Trigonella foenum-graecum*) seeds (Fenu-FG) on physiological parameters related to muscle anabolism, androgenic hormones, and body fat in healthy male subjects during an 8-week resistance training program using a prospective, randomized, double-blind, placebo controlled design.

Methods: Sixty healthy male subjects were randomized to ingest capsules of Fenu-FG (1 capsule of 300 mg, twice per day) or the matching placebo at a 1:1 ratio. The subjects participated in a supervised 4-day per week resistance-training program for 8 weeks. The outcome measurements were recorded at recruitment (baseline) and at the end of the treatment (8 weeks). The efficacy outcome included serum testosterone (total and free) levels, muscle strength and repetitions to failure, metabolic markers for anabolic activity (serum creatinine and blood urea nitrogen), and % body fat. The standard safety measurements such as adverse events monitoring, vital signs, hematology, biochemistry, and urinalysis were performed.

Results: Fenu-FG supplementation demonstrated significant anabolic and androgenic activity as compared with the placebo. Fenu-FG treated subjects showed significant improvements in body fat without a reduction in muscle strength or repetitions to failure. The Fenu-FG supplementation was found to be safe and well-tolerated.

Conclusion: Fenu-FG supplementation showed beneficial effects in male subjects during resistance training without any clinical side effects.

Keywords: Anabolic; Androgenic; Fenugreek seeds; Glycosides; Randomized controlled study; Resistance training

1. Introduction

Athletes use many nutritional supplements to improve their performance regardless of nutritional status.[1] Therefore, interest in safer nutritional therapies and supplements for muscle building and performance enhancement is rising.

All performance enhancing supplements are regulated by the World Anti-Doping Code as defined by the World Anti-Doping Agency.[2–5] Many traditional herbal medicines are being investigated as safer alternatives for their nutritional benefits and performance enhancement. However, they need to be scientifically evaluated for their efficacy and safety in the relevant populations using standardized procedures such as randomized double-blind placebo controlled studies.[6,7]

Fenugreek, *Trigonella foenum-groecum* L. (Fabaceae) seeds extract is a component of many nutritional dietary products that are recommended for athletes and exercising male subjects. Fenugreek seeds, a spice and food grain, has traditional history of medicinal use in the management of type 2 diabetes mellitus in Egypt, Southern Europe, India, Asia, and North Africa.[8] Fenugreek seeds extract is certified as a GRAS (generally recognized as safe) item under clause §182.20 (essential oils, oleoresins and natural extractives including distillates) by the US Food and Drug Administration.

Traditionally, fenugreek seeds have been reported to be useful in hormonal regulation, in particular for male impotence and as a galactagogue in lactating mothers.[9] In India, ground fenugreek seeds mixed with jaggery are recommended for females after childbirth for their anabolic effects to develop and

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strengthen muscles.[10] Recent studies on fenugreek seeds extracts support their effectiveness in promoting lean body mass, and lowering cholesterol.[11] Fenugreek extract is reported to enhance endurance capacity and the utilization of fatty acids as an energy source in male mice.[12] One recent study on fenugreek extract reported fat reducing effects greater than placebo in young, healthy resistance-exercising males.[13] These effects are purported to be mediated through an aromatase and 5α reductase inhibition, thereby increasing total testosterone levels by blocking its conversion to estrogen and dihydrotestosterone, respectively.[13] Increased testosterone levels are known to increase muscle size and strength in men[14] with downstream benefits on body weight, body fat, muscle size, strength, libido, energy, and mood.[15,16] Increased total testosterone levels could potentially affect serum free/bioavailable testosterone concentrations, resulting in escalated delivery and use by muscle cells to enhance protein synthesis, thus positively influencing strength and body fat. However, direct evidence for the androgenic effects of fenugreek seeds extract or its components in clinical practice is lacking.

Fenugreek seeds are rich in steroidal compounds like glycosides and saponins including diosgenin, yamogenin, gitogenin, tigogenin, and neotigogens. Diosgenin is an important precursor for the synthesis of a number of sex hormones.[17] Diosgenin, a steroidal sapogenin, is reported to augment overall weight and muscle growth in rats.[18] Moreover, diosgenin is reported to improve glucose metabolism by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues.[19] We have previously shown the efficacy of the glycoside fraction of fenugreek seeds (Fenu-FG) on testosterone levels in immature castrated male rats.[20] Recently, the excellent safety profile of sub-chronic (90-day) administration of Fenu-FG without any effects on body weight has been demonstrated in rats.[21]

Taking clues from both traditional and modern literature regarding the androgenic potential and safety of fenugreek glycosides, the present pilot study investigated furostanol glycosides-based fenugreek seeds extract (Fenu-FG) supplementation using a prospective, randomized, double blind, placebo-controlled design in healthy male subjects during an 8-week resistance training programme.

2. Materials and methods

2.1. Study design and protocol

This study was designed as a prospective, double-blind, randomized, placebo controlled study in male subjects and conducted using good clinical practice and ethical guidelines of Helsinki Declaration. The study protocol was assessed and approved by the Independent Institutional Human Ethics Committee. Inclusion criteria were the male healthy volunteers aged between 18 and 35 years, with normal health status based on clinical and laboratory examination, willing to sign the written informed consent form, and trained for resistance exercise at least for 1 month. The exclusion criteria were the subjects with any one of the following: (1) Subjects with elevated resting heart rate (>100 beats per min) or blood pressure (BP) (systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg); (2) Subjects with history of medical or surgical events that may affect the study outcome or place the subject at risk, including cardiovascular disease, gastrointestinal problems, metabolic, renal, hepatic, neurological or active musculoskeletal disorders; (3) Subjects with history of orthopaedic injury or surgery within the last year; (4) Subjects with known hypersensitivity to herbal drugs/nutritional supplement/foods; (5) Subjects who were consuming/have received any performance enhancing therapy during last 2 months; (6) Subjects undergoing any weight loss or diet plan during the trial period; (7) Chronic alcoholics; (8) Drug abusers; (9) Subjects who participated in any other clinical trial during last 30 days and simultaneous participation in another clinical trial; and (10) Subjects with any condition which in the opinion of the investigator makes the subject unsuitable for inclusion.

2.2. Screening and familiarization (Visit 1)

Potential participants (subjects) that were believed to meet eligibility criteria during Visit 1 (screening visit) were then invited to attend an entry/familiarization session. During this session, they signed informed-consent statements and completed personal and medical histories. Subjects meeting entry criteria were familiarized with the study protocol via a verbal and written explanation outlining the study design. This included describing the training program, familiarizing subjects with the tests to be performed. Then the baseline assessments such as medical history, demography physical examination, laboratory investigations, clinical examination were performed and recorded in case report forms (CRFs) for the subjects who consented for the study.

Then subjects reported to the human performance laboratory for baseline assessments for height (cm), body weight (kg), heart rate (bpm), respiratory rate (per min), body temperature (°F), systolic blood pressure (mmHg), and diastolic blood pressure (mmHg). Body mass index (BMI) was calculated as per formula: Weight (kg)/Height^2 (m). The lean body mass (kg) was calculated as per James's formula for male[22] as: 1.10 × Weight (kg) − 128 × Weight^2 (kg)/100 × Height^2 (m).

Demographic and clinical characteristics of all recruited subjects, intent-to-treat population, are listed by group in Table 1. At baseline, subjects were found uniform with no statistical significance between the treatment groups with respect to demographic (age, weight, height) and physiological characteristics (heart rate, respiratory rate, body temperature, BMI). Fifty-five out of the 60 recruited subjects (Fenu-FG = 29, Placebo = 26) consumed at least 1 dose of treatment were considered as "per protocol", or "PP population". Five subjects (Fenu-FG = 1, Placebo = 4) were dropped out of study for reasons not related to treatment (such as inconvenience or moved out of the area or not adhering to the training protocol).

2.3. Randomization and baseline assessments visit (Visit 2)

Sixty eligible subjects were randomized to receive 1 of the 2 treatments, namely Fenu-FG (1 capsule, 300 mg, twice a day)

Table 1
Demographic and baseline characteristics of subjects (mean ± SD).

Characteristics	Fenu-FG ($n = 30$)	Placebo ($n = 30$)
Age (year)	23.21 ± 3.73	21.62 ± 3.96
Height (cm)	165.52 ± 7.02	168.35 ± 6.57
Weight (kg)	65.13 ± 8.51	63.21 ± 9.14
Heart rate (bpm)	76.20 ± 1.69	75.63 ± 2.16
Respiratory rate (per min)	17.17 ± 0.79	17.31 ± 0.71
Body temperature (°F)	97.93 ± 0.25	97.77 ± 0.50
Systolic blood pressure (mmHg)	113.10 ± 7.92	115.00 ± 6.93
Diastolic blood pressure (mmHg)	73.10 ± 7.92	75.23 ± 6.64
Body mass index (kg/m^2)	23.73 ± 2.32	22.36 ± 3.39
Lean body mass (kg)	51.68 ± 5.36	51.11 ± 5.35

Note: Intent-to-treat (ITT) population, data were analyzed by unpaired t test for each parameter.

or matching placebo in 1:1 ratio according to a computer-generated randomization list. Drop-outs were not replaced. Subjects were recruited at sites in city of Pune, India. Subjects were allocated a unique randomization number at randomization visit.

After the familiarization/practice session and having baseline assessments, recruited subjects were instructed to refrain from exercise for 48 h and fast for 12 h before randomization and baseline assessment visit (Visit 2) during which the baseline assessments for efficacy and safety outcome measure were done and recorded.

2.4. Outcome measures (Visit 2 and Visit 3)

The efficacy outcome measures of study were serum creatinine, blood urea nitrogen (BUN), skinfold thickness, % body fat, androgenic hormone profile (total and free testosterone levels in blood), muscular strength and repetitions to failure in bench press and leg press at baseline and end of 8 weeks of resistance training program.

The skinfold thickness measurement of subcutaneous tissue of abdominals (thigh), triceps, and chest were with the help of Lange skinfold caliper (Beta Technology, Santa Cruz, CA, USA) by well-trained site personal as per manufacture's instruction and body fat% was calculated. SKYNDEX speed rule which is part of the Lange's skinfold caliper, uses Jackson–Pollock formula to calculate body fat% from skinfold thickness measurement.[23]

2.5. The resistance training and measurements (Visit 2 and Visit 3)

The protocol outlined by the National Strength and Conditioning Association[24] and as described earlier[25] was used for resistance training and muscle strength measurement (1 repetition maximum (1RM) bench press and 1RM leg press). Briefly, each subject performed 1RM lifts on the isotonic bench press. Initially, the subjects were warmed up (2 sets of 8–10 repetitions at approximately 50% of anticipated maximum) on the bench press and then performed successive 1RM lifts starting at about 70% of anticipated 1RM and increased by 5 kg until they reached 1RM. The subjects were again allowed to rest and warm up by performing 2 sets of the bench press at 60% and 80% of the resistance. Then, the subject complete as many repetitions as possible with a resistance of 80% of their 1RM bench press. The subject rested for 10 min and then warmed up on the 45° leg press (2 sets of 8–10 repetitions at approximately 50% of anticipated maximum). The same procedure as that of 1RM bench press was adopted for 1RM leg press. The repetitions to failure in bench press and leg press was recorded from the number of maximum repetitions that the subject could complete with a resistance of 80% of their 1RM bench/leg press. All strength/exercise tests were supervised by lab assistants experienced in conducting strength/anaerobic exercise tests using standard procedures.

2.6. The safety outcome measurements

The safety outcome measures were vital signs (systolic and diastolic blood pressure, heart rate, respiratory rate, and body temperature) and the laboratory assessments namely hematology, biochemistry (serum creatinine, BUN), and hormonal profile (serum total testosterone and free testosterone) were performed on blood samples. Blood hematology parameters included hematological parameters such as red blood cells (RBC) count, hemoglobin, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total white blood cell (WBC) count, and platelet count. Biochemical parameters such as random glucose, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), and bilirubin (total, direct, and indirect).

2.7. Supplementation/treatment protocol

The study medication (Fenu-FG capsules) was dispensed at randomization visit and baseline values of outcome measures were also recorded. Both Fenu-FG and the placebo product were enclosed in bottles containing capsules that were identical in function and appearance and individually coded. The active treatment product is capsules containing 300 mg of Fenu-FG supplied by manufacturer (Indus Biotech Private Ltd., Pune, India). The preparation and characterization of Fenu-FG (HPLC fingerprinting) is reported in earlier research papers.[20] Fenu-FG is also active component of marketed formulation, Testofen. The subjects were instructed to consume 1 capsule with water twice a day (20 min before breakfast and 20 min before dinner). The matching capsules containing di-calcium phosphate (IP grade) was used as placebo treatment. Both Fenu-FG and placebo were analyzed and complied with quality requirements related to microbial content or heavy metals. The treatment period was 8 weeks and subjects visited the study centers for screening, randomization (baseline), and end of treatment evaluations. Subjects were monitored for compliance with the protocol by a telephone or face-to-face communications from Visit 2 to the end of the 8-week treatment period (Visit 3).

2.8. Statistical analysis

The data of efficacy and safety outcome measures were analyzed using paired *t* test (within the treatment comparisons) or unpaired *t* test (between the groups comparisons) using SPSS for Windows Version 13.0 (SPSS Inc., Chicago, IL, USA). Date were considered significant when the $p < 0.05$.

3. Results

The data obtained from muscle strength and repetitions to failure measurements, serum total and free testosterone levels, anabolic activity related markers, skinfold thickness measurement and body fat calculations, and safety outcomes such as blood biochemical and hematological measurements are presented in Table 2.

3.1. Effect of Fenu-FG on muscle strength and repetitions to failure

At baseline, no statistical significant difference for 1RM-bench press, 1RM-leg press, repetitions to failure in bench press or leg press was found between the group (Fenu-FG *vs.* placebo). Similarly, no statistically significant difference was found in 1RM-bench press responses within the group (at the end of the study *vs.* baseline) or between the groups (Fenu-FG *vs.* placebo) at the end of the study. However, statistically significant increase in 1RM-leg press responses within the Fenu-FG (73.41 kg *vs.* 80.45 kg, $p < 0.001$) and placebo (74.73 kg *vs.* 86.85 kg, $p < 0.001$) group. The increase found in 1RM-leg press responses between the groups (Fenu-FG *vs.* placebo) was not statistically significant.

Statistically significant increase was found in repetitions to failure in bench press for Fenu-FG (7.21 *vs.* 8.79, $p < 0.001$)

Table 2
Effect of treatments on muscle strength, repetitions to fail, hormonal parameters; anabolic activity related markers and body fat parameters; safety parameters (serum biochemistry); safety parameters (hematology) (mean ± SD).

Variable	Fenu-FG ($n = 29$)		Placebo ($n = 26$)	
	Baseline	End	Baseline	End
Muscle strength and endurance				
1RM-bench press (kg)	87.03 ± 29.48	88.24 ± 27.45	76.65 ± 22.57	82.77 ± 29.40
1RM-leg press (kg)	73.41 ± 18.90	80.45 ± 21.15***	74.73 ± 23.79	86.85 ± 26.03***
Repetitions to failure in bench press (*n*)	7.21 ± 2.68	8.79 ± 2.26***	7.12 ± 2.82	6.19 ± 1.59
Repetitions to failure in leg press (*n*)	8.79 ± 2.11	9.31 ± 2.32	10.04 ± 3.39	9.08 ± 2.51
Serum testosterone measurements				
Free testosterone (ng/dL)	17.76 ± 10.98	35.29 ± 15.01***	21.30 ± 12.24	31.70 ± 19.48**
Total testosterone (ng/dL)	404.95 ± 83.57	452.60 ± 107.87	387.52 ± 86.19	421.27 ± 93.36
Anabolic activity related markers				
Serum creatinine (mg/dL)	1.14 ± 0.17	0.94 ± 0.12***	1.06 ± 0.21	0.97 ± 0.13
BUN (mg/dL)	28.28 ± 4.69	25.62 ± 3.50***	26.62 ± 4.31	26.42 ± 4.24
Body fat parameters				
Skinfold thickness-tricep (mm)	7.48 ± 3.09	5.97 ± 2.33**	7.85 ± 2.44	7.20 ± 3.22
Skinfold thickness-thigh (mm)	9.83 ± 3.37	8.39 ± 3.47*	10.62 ± 3.15	9.57 ± 3.11
Skinfold thickness-chest (mm)	4.86 ± 2.58	3.33 ± 1.98**	5.08 ± 2.26	3.21 ± 1.48***
body fat%	6.30 ± 2.56	4.63 ± 2.49**	6.43 ± 2.34	5.15 ± 2.37
Safety parameters (serum biochemistry)				
Glucose-random (mg/dL)	87.66 ± 11.92	86.86 ± 8.66	90.27 ± 12.00	88.92 ± 7.04
AST (IU/L)	25.00 ± 9.47	23.72 ± 5.25	23.96 ± 5.16	23.00 ± 3.39
ALT (IU/L)	32.93 ± 13.61	30.83 ± 10.08	30.04 ± 8.86	28.12 ± 3.88
ALP (IU/L)	75.72 ± 18.06	78.45 ± 14.45	75.31 ± 12.56	80.73 ± 14.09*
Bilirubin (mg/dL)				
Total	0.95 ± 0.38	0.91 ± 0.37	0.90 ± 0.23	0.88 ± 0.12
Direct	0.40 ± 0.31	0.36 ± 0.30	0.38 ± 0.18	0.33 ± 0.11
Indirect	0.56 ± 0.10	0.55 ± 0.09	0.53 ± 0.09	0.56 ± 0.06
Safety parameters (hematology)				
RBC count (million/mm^3)	4.48 ± 0.23	4.49 ± 0.21	4.51 ± 0.44	4.53 ± 0.42
Hemoglobin (%)	13.36 ± 0.72	13.51 ± 0.66	13.46 ± 1.30	13.67 ± 1.24
HCT (%)	40.52 ± 2.13	40.79 ± 2.01	40.58 ± 3.70	41.19 ± 4.01
MCV (femtoliters/cell)	90.50 ± 2.60	88.70 ± 11.26	90.10 ± 2.15	90.97 ± 2.32
MCHC (g/dL)	32.97 ± 0.64	33.12 ± 0.55	33.16 ± 0.54	33.22 ± 0.78
Total WBC (mm^3)	9706.90 ± 1225.02	8562.07 ± 1301.49**	8903.85 ± 977.13	8376.92 ± 1449.77
Platelet count (per µL)	257,586.21 ± 55,085.98	260,068.97 ± 45,566.22	255,461.54 ± 4666.74	259,846.15 ± 47,848.67

Note: Per protocol (PP) population, data were analyzed by paired *t* test for each parameter.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared to baseline values of corresponding treatment.
Abbreviations: RBC = read blood cells; 1RM = 1 repetition maximum; BUN = blood urea nitrogen; ALT = alanine transaminase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; HCT = hematocrit; MCV = mean corpuscular volume; MCHC = mean corpuscular hemoglobin concentration; WBC = white blood cell.

while placebo group did not show such increase. The Fenu-FG treated group showed increase in repetitions to failure in bench press (by 1.59 ± 1.11) whereas placebo group showed decrease (by 0.92 ± 3.39). This difference (Fenu-FG = +1.59 *vs.* placebo = −0.92) between the groups was statistically significant ($p < 0.001$). In case of repetitions to failure in leg press, no statistically significant difference was found within the groups (end of the study *vs.* baseline) or between the groups (Fenu-FG *vs.* placebo).

3.2. Effect of Fenu-FG on serum testosterone levels

On 8 weeks of treatment, the levels of free testosterone was found to have steep (98.7%) increase from baseline (17.76 to 35.29 ng/dL, $p < 0.001$) in Fenu-FG group whereas placebo group showed moderate (48.8%) increase from 21.30 to 31.70 ng/dL ($p < 0.01$). The increase in free testosterone from baseline (Fenu-FG: 17.53 ± 8.55 ng/dL; placebo: 10.39 ± 14.71 ng/dL) was found significantly between the groups ($p < 0.05$).

On 8 weeks of treatment, subjects from Fenu-FG and placebo groups showed mild but non-significant increased levels of total testosterone as compared with corresponding baseline values. The increase in total testosterone from baseline was also not significant between the treatment groups (Fenu-FG *vs.* placebo).

3.3. Effect of Fenu-FG on indirect markers of anabolic activity

Serum creatinine levels of subjects from Fenu-FG group showed significant ($p < 0.001$) reduction whereas serum creatinine values of placebo group showed non-significant reduction (within the groups). The decrease in serum creatinine between the groups, Fenu-FG (0.20 ± 0.10) and placebo (0.09 ± 0.23), was found significantly at $p < 0.05$.

The Fenu-FG treatment showed significant ($p < 0.001$) reduction within the group in BUN levels as compared with baseline values (25.62 *vs.* 28.28 mg/dL) whereas BUN level within the placebo group were not statistically significant. BUN level changes were not found statistically significant between the groups (Fenu-FG *vs.* placebo).

3.4. Effect of Fenu-FG on body fat

At the end of the study, subjects with Fenu-FG treatment showed significant decrease in thigh ($p < 0.05$), tricep ($p < 0.01$), and chest ($p < 0.01$) as compared with baseline values. Subjects in placebo group did not show such difference either in thigh or tricep skinfold thickness measurement. However, skinfold thickness of chest decreases significantly ($p < 0.001$) in placebo group during the study period. Percent body fat in subjects with Fenu-FG treatment showed significant ($p < 0.01$) reduction but not in placebo group. The decrease in skinfold thickness values or body fat between the groups (Fenu-FG *vs.* placebo) was not statistically significant.

3.5. Effect of Fenu-FG on safety parameters

No significant difference was found within the Fenu-FG and placebo group when compared with baseline values of biochemical parameters such as random glucose, AST, ALT, bilirubin (total, direct, and indirect) and hematological parameters such as RBC, hemoglobin, HCT, MCV, MCHC, and platelet count. However, values of ALP showed mild significant increase in subjects with placebo group ($p < 0.05$) whereas total WBC count decreased significantly in Fenu-FG group ($p < 0.01$). All values of biochemical and hematological parameters were within normal physiological range.

4. Discussion

Fenugreek is commonly used food ingredient in many parts of the world. Fenugreek glycosides including saponins, sapogenins (e.g., diosgenin), similagenin, savsalpogenin, and yuccagenin[26,27] are reported to be major components behind health benefits of fenugreek seeds.[28] The present study attempted as proof of concept evidence-based pilot study towards evaluation of traditionally reported androgenic and anabolic benefits of fenugreek glycosides supplementation on male subjects during resistance training program. The results of present clinical study demonstrated the efficacy of 8-week treatment of Fenu-FG offered beneficial effects in terms of repetitions to failure in leg press, free testosterone levels and serum creatinine as compared with placebo.

Testosterone, a male sex hormone (androgen) is one of the major hormones involved in anabolic effects (muscle mass, strength increase). Like most hormones, testosterone is supplied to target tissues in the blood where much of it is transported bound to a specific plasma protein, sex hormone binding globulin (SHBG). Testosterone can be measured as “free” (that is, bioavailable and unbound) or more commonly, “total” (including the percentage, which is chemically bound to SHBG and unavailable). The free and nonspecifically bound plasma hormone levels generally reflect the clinical situation more accurately than total plasma hormone levels.[29] Two major enzymes involved in testosterone metabolism are 5α-dihydrotestosterone[30] and aromatase (CYP19A1).[31] In the present study, Fenu-FG supplementation was found to increase levels of free testosterone without reduction in total testosterone. Our results are in agreement with the recent study where fenugreek seeds extract supplementation showed increase in serum testosterone levels in college aged men and perhaps acted as inhibitor of aromatase and 5α reductase[13] and increased free (i.e., bioavailable and unbound) testosterone by decreasing the metabolism of serum testosterone. It is important to note that the levels of total testosterone were unchanged between treatment and placebo and well within the physiological limits during the present study.

The relationship between serum testosterone levels and libido is well established.[32] The recent double-blind randomized placebo-control study demonstrated beneficial effects of fenugreek seeds extract powder enhancing male libido in healthy adult males.[33] In the present study, Fenu-FG supplementation was found to increase free serum testosterone levels as com-

pared with placebo group. Testosterone plays a key role in the development of bone mass, increased bone density and strength, stimulation of linear growth and bone maturation. Increased levels of free testosterone was found to be correlated well with indirect measures of anabolic activity of Fenu-FG supplementation observed during the study.

Muscle endurance refers to the ability of a muscle to perform a continuous effort without fatiguing (repetitions to failure). Cycling, step machines, and sit-up tests are often used to measure muscular endurance. A fenugreek seeds extract is reported to enhance swimming endurance capacity in mice *in vivo* perhaps by utilization of fatty acids as an energy source and spares glycogen.[12] In our study, increased number of repetitions to failure during resistance training was observed in subjects with Fenu-FG supplemented subjects whereas placebo supplemented subjects showed decrease. In addition, significant decrease in serum creatinine and BUN levels in Fenu-FG treated subjects is probable indicator of anabolic activity.

In the present study, significant reduction in body fat and skinfold thickness (thigh and tricep) was observed by 8 weeks of treatment of Fenu-FG with non-significant increase in muscle strength and significant increase in repetitions to failure. These physiological effects can be attributed to marker compounds, furostanol glycosides that are present in Fenu-FG supplementation. In the past, furostanol glycosides fraction of *Trigonella foenum-graecum* showed potent anabolic and fat burning activity in immature castrated male rats.[20] Further, role for furostenol glycoside from *Trigonella foenum-graecum* seeds especially diosgenin in hormone mediated activity pathways has also been reported.[34,35]

Diosgenin has various effects on cholesterol metabolism, one of the most important being the capacity to lower plasma cholesterol concentration in hypercholestemic animals.[36] The hypocholesterolaemic effect of diosgenin has been suggested to depend on its capacity to inhibit cholesterol absorption, increase biliary cholesterol secretion, increase faecal excretion of neutral sterols and thus to decrease liver cholesterol concentrations.[37] Further, fenugreek saponins are shown, at least partly, responsible for antihyperlipidemic effects in overweight men with erectile dysfunction[38] mediated through inhibition of fat accumulation and up-regulation of Low-density lipoprotein receptor.[39]

The results of earlier studies on fenugreek seeds extract[40] indicate that saponins present in fenugreek do not interact directly with cholesterol but have a strong inhibitory effect on bile salt absorption[40] in a quantitative manner. Other reports[41,42] of increased faecal weight and excretion of bile acids by fenugreek enriched diets support the notion. The blood and hepatic cholesterol lowering may be due to a subsequent increase in the conversion of cholesterol to bile acids by the liver. Taken together, observed benefits of Fenu-FG in the present study may lead to improved lipid profile through bile salt mechanism in exercising subjects as indicated by body fat improvement observed in the present study.

The resistance exercise is known for the beneficial effects on strength, endurance, and serum testosterone levels. The significance between the groups is only possible in case of excellent effect over and above the beneficial levels offered by exercise. In the present study, statistical significance was achieved between the groups for some of the outcome measures (serum free testosterone, serum creatinine, and repetitions to failure during leg press). In case of other outcome measures, Fenu-FG supplementation showed superior effects as compared with placebo without statistical significance between the groups (BUN, skinfold thickness, and body fat%). In these cases, extent of effects was perhaps not sufficient for statistical significance between the groups. Probably longer duration of study in more subjects could have shown statistical significance. Therefore, the present pilot study is indicative of beneficial effects of Fenu-FG supplementation to the resistance exercise.

Fenugreek is certified as GRAS ingredient in USA and so the risk of inherent toxicity is very low. The results of present study supported the safety of Fenu-FG on chronic administration. The level of blood biochemical and hematological parameters in Fenu-FG treated subjects were found within normal physiological range throughout the study period. No serious adverse event occurred during the study period. The treatment was found to be safe and well tolerated.

5. Conclusion

In conclusion, Fenu-FG supplementation showed promising anabolic and androgenic effects in male subjects during 8 weeks of resistance training program in a double-blind randomized placebo-controlled study without serious adverse events.

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