

EFFECTS OF GLYCOSIDES BASED FENUGREEK SEED EXTRACT ON SERUM TESTOSTERONE LEVELS OF HEALTHY SEDENTARY MALE SUBJECTS: AN EXPLORATORY DOUBLE BLIND, PLACEBO CONTROLLED, CROSSOVER STUDYMAHESH MOKASHI¹, RENU SINGH-MOKASHI², VISHWARAMAN MOHAN², PRASAD THAKURDESAI²¹Samiksha Hospital, 501-B, 2nd floor, Rasta Peth, Pune, Maharashtra, India, ²Department of Scientific affairs, Indus Biotech Private Limited, 1, Rahul Residency, Off Salunke Vihar Road, Kondhwa, Pune-411 048, India. Email: prasad@indusbiotech.com

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ABSTRACT

Objective: To evaluate acute effects of IND9 supplementation on serum testosterone levels in healthy sedentary male subjects. **Methods:** The study was designed as randomized, double blind, placebo controlled, two period, crossover study with 7 days of washout period using single study center. Sixteen healthy male subjects were randomized and received single dose of 600 mg (two capsules of 300 mg) of either IND9 or matching placebo capsules during each of the 2 study periods of 10 h each. Blood samples were collected three times at 3 h, 7 h and 10 h. The outcome measures were measurement of serum free testosterone (mFT) and total testosterone (TT), calculated levels of free testosterone (cFT), bioavailable testosterone (BT) levels and safety parameters. **Results:** During the study period, significant time-dependent interactions were found for mFT and cFT levels (within Placebo and IND9 supplemented arms), BT levels (within IND9 but not in Placebo arm) and TT levels (none of the arms). Two-way ANOVA of data of change from baseline at 10 h showed no significant interactions between the treatments and periods (absence of crossover effect) for all measures. Pairwise comparisons between change from baseline data (at 10 h) by unpaired 't' test showed significant increase in TT, BT and cFT but not in mFT levels in IND9 arm as compared to respective levels in placebo arm. The supplementation of IND9 and placebo was found to be safe and well-tolerated. All values were found within physiological limits. **Conclusion:** Acute administration of IND9 capsule supplementation to sedentary males showed potential androgenic benefits with good safety profile.

Keywords: Fenugreek seed extract, serum testosterone, Bioavailable, healthy sedentary subjects**INTRODUCTION**

Hypogonadism has been assumed to be the important factor in the development of a condition variably termed partial androgen deficiency in the ageing male, androgen deficiency /decline in the ageing male, late onset hypogonadism or testosterone deficiency syndrome (TDS) [1]. These conditions are associated with a wide range of clinical symptoms ranging from loss of muscle mass, loss of muscular strength, reduced bone mineral density and a decrease in general well-being, depressed mood with mild cognitive impairment.

Testosterone (T), a potent anabolic hormone, plays an important role in antagonizing catabolic stress from daily physical challenges. Because of its hydrophobic nature, most of the circulating T is bound to plasma proteins, including sex hormone-binding globulin (SHBG) and albumin. The SHBG-bound fraction is biologically inactive because of the high binding affinity with T, whereas the albumin-bound T is readily dissociable and thus bioavailable as is the small percentage of FT that is normally circulating in the blood [2]. In middle-aged men, there is a rise in SHBG levels, which results in a more substantial age dependent decline in FT than TT [3, 4]. Existing data suggests a cause-effect relationship between low serum T levels and sexual dysfunction [5]. Moreover, a causal relationship between obesity or metabolic syndrome and sexual dysfunction also exists. [6-8]

In recent years, there has been significant interest in plant based natural medicines that are traditionally used to improve sexual function and performance. However, only few natural therapies have undergone evidence based scientific scrutiny and need to be scientifically evaluated for their efficacy and safety in relevant populations using standardized procedures. One such potential medicinal plant is Fenugreek (*Trigonella foenum-graecum* L.). Fenugreek seed, a spice and food grain, has traditional history of medicinal use in Egypt, Southern Europe, India, Asia, and North Africa [9].

Fenugreek seed extract is a component of many nutritional dietary products that are recommended for athlete and exercising male subjects. Fenugreek seed extract is reported to enhance endurance capacity and utilization of energy in male mice [10]. Furthermore, fenugreek derived products including extracts have been explored successfully for many exercise physiology applications involving healthy volunteers and patients [11, 12] including anabolic [13] and androgenic activities [14]. Safety of fenugreek seeds in human has also been established in many clinical trials and reviews [11-13]. Because of excellent safety profile, fenugreek seeds extracts are certified as GRAS (Generally recognized as safe) item under clause §182.20 (Essential oils, oleoresins and natural extractives including distillates) by US Food and Drug Administration (US FDA).

Physiological beneficial properties of wide range of plant derived glycosides have also been reported and reviewed extensively [15]. The potent androgenic activities are among the major health benefits associated with natural glycosides [16, 17].

The beneficial effects of a commercially available glycosides based standardized fenugreek seed extract (Testosurge) has been reported on muscular strength, improved body composition, and male hormonal profiles (serum testosterone levels) in resistance-trained men with excellent safety profile [14]. Recently, non-clinical safety profile of glycosides based standardized extract from fenugreek seed has been reported [18].

The correlation between serum T levels and resistance training or exercise is complex. The available evidences suggested that intensity and duration of exercise can alter serum T levels in complex manner. On one hand, serum T levels were virtually unaffected by 12-weeks of strength training in young and elderly subjects is reported [19]. On the other hand, few reports suggested that exercise can result in increased levels of circulating T [20, 21]. Acute exercise were found to increase the expression of FT and dihydrotestosterone (DHT) (bioactive metabolite of T) in the skeletal muscles in both male and

females suggesting that exercise stimulates local bioactive androgen metabolism leading to differential T levels depending on exercise amount or duration [22]. Therefore, effects of bioactive compounds need to be evaluated separately in sedentary and exercising population. However, such study of glycosides based fenugreek seed extract in sedentary (non-exercising) male subjects is lacking. Therefore, it was thought worthwhile to explore potential of glycosides based standardized fenugreek seed extract (IND9) for its possible androgenic effects in healthy sedentary male subjects.

METHODS

Participants

Sixteen healthy and non-exercising male subjects were enrolled in the study. Inclusion criteria consisted of healthy volunteers aged 18-45 years with normal health status on the basis of clinical and laboratory examination and willing to sign the written informed consent form. Subjects were excluded on the basis of any condition which in the opinion of the investigator makes the subject unsuitable for inclusion, obvious medical disorder on the basis of medical history, physical examination reveals any abnormality, obvious male sexual dysfunction, known hypersensitivity to herbal drugs/nutritional supplement/ foods, subjects who is consuming/ has received any medication during last 30 days that can have impact on male sex hormones, chronic alcoholics, drug abusers and participation in any other clinical trial during last 30 days. The study protocol was assessed and approved by the Independent Human Ethics Committee

Experimental design

This objective of the present study was to explore effects of acute (single dose) administration of IND9 supplementation on serum testosterone levels in sedentary male subjects. The study was performed as randomized, double blind, placebo controlled, two period (10 h each), crossover design with 7-days washout period using single study center (Samiksha Hospital, Pune). The overall design and conduct of the study in a form of flow-chart in Figure 1.

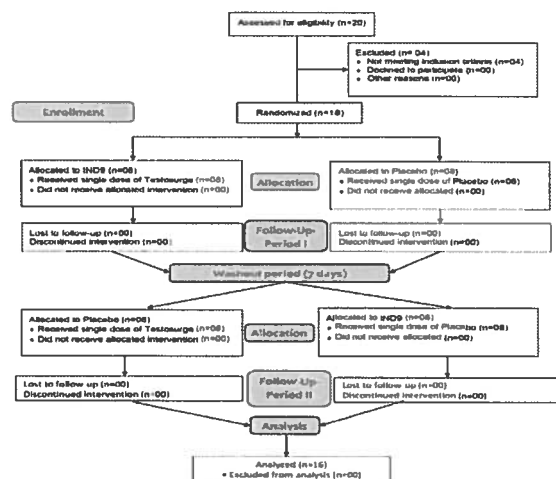


Fig.1: Study Flowchart (CONSORT diagram)

Screening and randomization

On day 0 of the study (i.e. period I), subjects were admitted to the site at 6.00 pm. Potential subjects were screened and requested to attend an information session. They were informed about the trial process and requested to provide consent for trial participation. This trial, being an exploratory study, no statistical method is applied to determine the sample size. A total of 16 healthy volunteers (on the basis of medical history and clinical examination) who met all inclusion and none of the exclusion criteria were included in the

study. Demographic data, medical history, physical examination, vital signs and medications history (one month) were recorded in case report forms (CRFs) for consenting subjects.

Subjects were allocated a unique randomization number as per computer-generated randomization code which was available to investigator in case of serious adverse event (SAE) during the study. At time 8.00 pm (baseline), they were randomized to receive single dose of 600 mg (two capsules of 300 mg) in 1:1 ratio with 8 subjects each in IND9 or matching placebo supplementation arms. Subjects were not allowed to eat anything 1 h before and 2 h after consumption of the test supplementations.

The supplementation

After dispensing IND9 or matching placebo supplementations, baseline characteristics of subjects were recorded. Both IND9 and matching placebo products were enclosed in bottles containing capsules that were identical in appearance and individually coded. The IND9 and matching placebo (lactose, IP grade) capsules were manufactured and supplied by Indus Biotech Private Limited, Pune. Both IND9 and placebo were analyzed and complied with quality requirements related to microbial content and heavy metals.

Outcome measures

The primary efficacy outcome measures were serum free testosterone (directly measured (mFT) and calculated (cFT)). The secondary (exploratory) efficacy outcome measures were measured serum total testosterone (TT), and calculated bioavailable testosterone (BT) levels at baseline (8.00 PM) and 3 h (11.00 PM), 7 h (3.00 AM) and 10 h (6.00 AM) after baseline. Calculation of cFT and BT was based on measurements of TT, SHBG and albumin assays in serum and applying the established formula [23]. The calculation was performed using website calculator of International Society for the Study of the Aging Male (ISSAM). The website (URL: <http://www.issam.ch/freetesto.htm>) uses the association constants ($K_{SHBG} = 1 \times 10^9$ L/mol and $K_{albumin} = 3.6 \times 10^4$ L/mol) and considers average albumin concentrations equal to 4.3 g/dL in each sample.

Safety outcome measures such as vital signs and adverse events (AE). Subjects were asked to report at study center after 7 days of wash-out period (Period II). The procedure of period I was repeated with crossover supplementation schedule (reversed sequence of supplementation).

Statistics

The data was represented as mean \pm standard deviation (SD). The data of each parameter of demographic and baseline measurements was analyzed by unpaired 't' test to evaluate homogeneity between the subjects receiving different sequence namely sequence-AB (Active-Placebo) and sequence-BA (Placebo-Active). The data of serum testosterone (mFT, cFT, TT, and BT) values was analyzed by separate one-way repeated measure ANOVA to assess within the treatment effects. To assess crossover effects (interaction between two sequences), the data of change from baseline values for each type of serum T (mFT, cFT, TT, and BT) was analyzed by separate two-way ANOVA.

To assess between the group effects (IND9 vs placebo), the values of change of serum testosterone levels from baselines were compared by separate unpaired 't' tests for each time point (3 h, 7 h and 10 h). The P values less than 0.05 (two-sided) were considered significant.

Results

Demographics and baseline characteristics

Sixteen volunteers were enrolled and randomized between IND9 (n=8) and placebo group (n=8) per sequence. None of the subjects dropped out during the study. The unpaired 't' test showed uniformity in demography and baseline characteristics with no significant difference between sequence AB and BA with respect to age, weight, height, heart rate, respiratory rate, blood pressure (systolic and diastolic), body temperature and all efficacy outcome measures (Table 1).

Table 1. Demographic and baseline characteristics of study population

Characteristics	Sequence AB (n=8)	Sequence BA (n=8)	P value
Age (yrs)	24.50 ± 5.63	27.62 ± 4.40	0.237
Weight (kg)	63.59 ± 8.05	66.75 ± 8.18	0.449
Height (cm)	169.11 ± 6.58	164.75 ± 9.35	0.299
Heart Rate (bpm)	77.00 ± 1.51	76.25 ± 1.67	0.362
Respiratory rate (per min)	17.00 ± 0.76	17.63 ± 0.74	0.118
Systolic Blood pressure (mmHg)	113.75 ± 5.18	115.00 ± 9.26	0.744
Diastolic blood pressure (mmHg)	73.75 ± 5.18	75.00 ± 9.26	0.744
Body temperature (° F)	97.75 ± 0.46	97.63 ± 0.74	0.693
mFT (pg/ml)	11.59 ± 2.07	13.83 ± 6.46	0.365
cFT (pg/ml)	8.28 ± 1.95	8.99 ± 3.69	0.639
TT (ng/ml)	466.48 ± 133.08	435.70 ± 191.77	0.715
BT (ng/ml)	202.88 ± 45.45	228.38 ± 98.97	0.519

The data was presented as mean ± standard deviation (SD) and analyzed by unpaired "t" test between the sequences. FT - Free testosterone, TT - Total testosterone, BT - Bioavailable testosterone, mFT and cFT are serum free testosterone levels by direct measurement and calculated respectively.

Effect of treatments on serum testosterone levels (within the group)

The data of serum T levels at baseline, 3 h, 7 h and 10 h for IND9 and placebo is presented in Table 2. Moreover, the statistical measures obtained from separate one-way repeated measure ANOVAs (F, df and p-value) of each of outcome measure for IND9 and placebo are presented in Table 2.

Significant within the group (time dependent) difference in mFT levels was found within placebo (F = 5.11, df = 3, p = 0.003) or IND9 (F = 4.15, df = 3, p = 0.010). Significant within the group (time dependent) difference in cFT levels was found within placebo (F = 2.9, df = 3, p = 0.042) or IND9 (F = 4.26, df = 3, p = 0.009). However, no significant within the group (time dependent) difference was found in TT levels within placebo (F = 2.58, df = 3, p = 0.062) or IND9 (F = 2.14, df = 3, p = 0.104). Significant within the group (time dependent) difference was found in BT levels within IND9 (F = 2.97, df = 3, p = 0.039) but not within placebo arm (F = 2.05, df = 3, p = 0.116).

Table 2. Effect of treatments on serum testosterone levels (within the group effects)

Parameters	Treatment	Time from intervention				Statistics		
		Baseline	3 h	7 h	10 h	F	d.f.	P-value
mFT (pg/ml)	Placebo	13.6 ± 4.8	9.1 ± 2.6	11.5 ± 2.7	13.2 ± 3.8	5.11	3	0.003
	IND9	11.7 ± 2.6	9.2 ± 2.3	11.4 ± 3.2	13.5 ± 5.0	4.15	3	0.010
cFT (pg/ml)	Placebo	8.4 ± 3.0	7.0 ± 3.5	9.3 ± 2.1	9.7 ± 2.3	2.9	3	0.042
	IND9	7.2 ± 2.1	7.0 ± 2.4	9.1 ± 3.3	9.9 ± 2.8	4.26	3	0.009
TT (ng/ml)	Placebo	473.6 ± 185.3	361.9 ± 140.2	467.5 ± 133.7	501.1 ± 144.6	2.58	3	0.062
	IND9	405.5 ± 142.9	386.9 ± 149.3	439.8 ± 152.2	519.0 ± 189.3	2.14	3	0.104
BT (ng/ml)	Placebo	222.6 ± 81.9	180.8 ± 96.8	231.9 ± 54.3	242.1 ± 59.7	2.05	3	0.116
	IND9	184.5 ± 49.6	182.3 ± 67.0	218.6 ± 86.1	246.9 ± 77.7	2.97	3	0.039

The data was presented as mean ± standard deviation (SD) and was analyzed by one way repeated measure ANOVA for each parameter (within the treatments). FT - Free testosterone, TT - Total testosterone, BT - Bioavailable testosterone, mFT and cFT are serum free testosterone levels by direct measurement and calculated respectively.

treatments at corresponding time) * P < 0.05 as compared to values of placebo group at corresponding time period. FT - Free testosterone, TT - Total testosterone, BT - Bioavailable testosterone, mFT and cFT are serum free testosterone levels by direct measurement and calculated respectively.

Effects of treatments on change in levels at 10 h from baseline (between the groups)

The data of change from baseline values of serum testosterone levels is presented in Figure 2. Two-way ANOVA of change at 10 h from baseline data showed no significant interaction between the treatments and periods (crossover effect) for mFT (F = 1.386, df = 1, 31, p = 0.249), cFT (F = 0.132, df = 1, 31, p = 0.719), TT (F = 0.208, df = 1, 31, p = 0.652) and BT (F = 0.068, df = 1, 31, p = 0.796). The unpaired 't' test was used to compare changes from baseline between IND9 and placebo.

The mFT levels in IND9 supplemented subjects showed increase of 1.79 (± 5.65) whereas in placebo supplemented subjects showed decrease of 0.46 (± 4.24) with no significant difference between treatments. Increase in cFT levels showed by IND9 supplemented subjects (2.68 ± 2.14) were significantly (p = 0.038) higher than increase showed by placebo arm (1.22 ± 1.62). Increase in TT levels showed by IND9 supplemented subjects (113.55 ± 109.85) were significantly (p = 0.018) higher than increase showed by placebo arm (27.49 ± 83.65). Similarly, significant (p = 0.025) increase was found in BT levels in IND9 supplemented subjects (62.43 ± 56.78) as compared to increase showed by placebo supplemented subjects (19.5 ± 45.66).

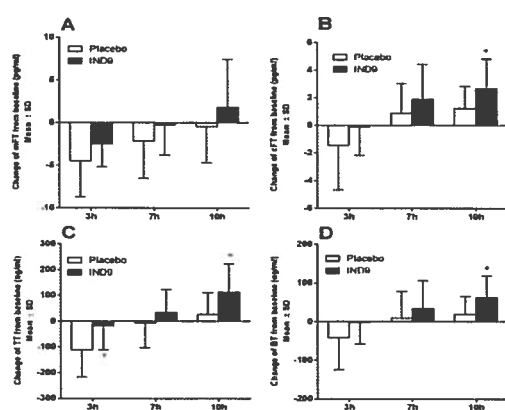


Fig.2. Effects of treatments on change in levels from baseline (between the group effects). (A) mFT (B) cFT (C) TT and (D) BT. The data for each parameter was presented as mean ± SD and analyzed by unpaired "t" test (comparisons between the

SAFETY OUTCOME MEASURES

The supplementation of IND9 and placebo was found to be safe and well tolerated without any AEs during the study.

DISCUSSION

It is well recognized that circulating concentrations of testosterone in all forms (TT, BT and FT) are characterized by a diurnal rhythm, with highest levels in the morning and a nadir in the late afternoon [24]. Therefore, we have evaluated effects of acute administration of IND9 at 8.00 pm, when testosterone levels were at its nadir. The measurements were done during 10 h period with last measurement at 6.00 am (when testosterone levels were at the peak).

In the present study, significant increase in T levels (FT, TT and BT) on acute administration of IND9 supplementation as compared with placebo group was observed. There was no significant crossover effect (treatment X sequence effect) found which further substantiate efficacy of IND9 on testosterone levels.

It is important to confirm low T concentrations in men with an initial T level in the mildly hypogonadal range, because 30% of such patients may have a normal T level on repeat measurement [25]. Also, 15% of healthy young men may have a T level below the normal range in a 24-h period [26]. These variations make it difficult to draw conclusions based on direct T measurement alone. Therefore, FT levels were calculated based on reported method [23]. In the present study, acute administration of IND9 supplementation showed statistically significant increase in cFT but not in terms of mFT. These results are attributed to the large variations in mFT in healthy young men as reports earlier [26].

Serum total T concentrations, representing the sum of unbound and protein-bound testosterone in circulation. Most of the T is bound to SHBG or albumin with only 0.5–3% of circulating T is unbound or "free" [27, 28]. The term BT refers to FT plus albumin-bound T. BT reflects the view that albumin-bound testosterone is readily dissociable and thus bioavailable. Calculated BT is the reported as the best marker for the androgen status in males [29]. Therefore, increased FT and BT levels by IND9 in the present study might be mediated through displacement of T from binding domains of SHBG thereby making it bioavailable. However, more studies will be needed for confirmation.

In the present study, single dose of IND9 capsule supplementation was found to increase BT as compared to placebo group. In the past, 8-week supplementation of fenugreek seed extracts showed to increase strength and body composition by increasing TT and BT with decreased serum estradiol levels in resistance-trained men [30]. These effects are purported to be mediated through increasing BT levels via blocking conversion of T to estrogen and dihydrotestosterone (DHT). Further, increased BT levels are suggested to be responsible for increased protein synthesis and strength. The results of present study provided additional support to androgenic activity profile of fenugreek seed extract in sedentary male.

Recently, hypogonadism was found to increase risk of CVD. On one hand, low T levels not only correlate significantly with CVD risk factors, CHD events and mortality independent of age [31]. On the other hand, lower T and SHBG levels are reported to have inverse relationship with markers of metabolic syndrome such as triglycerides, HDL-cholesterol and hypertension in young male population [32]. Furthermore, strong link between abnormal lipid profile and CVD risk is well established. In increased T levels shown in the present study suggest possible beneficial effects of IND9 in reducing CVD risk in aging males with hypogonadism. However, detailed study of IND9 in aging male will be required to evaluate this hypothesis.

The acute administration of IND9 was well tolerated and showed excellent safety profile with no adverse events during the study. Furthermore, all T levels were found to be within physiological limits.

CONCLUSION

In conclusion, acute administration of IND9 capsule supplementation showed promising androgenic activity with good safety profile in healthy sedentary male subjects.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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