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ORIGINAL ARTICLE

WILEY

Testofen® (Fenugreek extract) increases strength and muscle mass compared to placebo in response to calisthenics. A randomized control trial

Amanda J. Rao[1,2] | **Alistair R. Mallard**[3] | **Ross Grant**[2,4,5]

[1]Faculty of Health Sciences, University of Sydney, Sydney, NSW, Australia

[2]Australasian Research Institute, Sydney Adventist Hospital, Wahroonga, NSW, Australia

[3]School of Human Movement and Nutrition Sciences, University of Queensland, Brisbane, QLD, Australia

[4]School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia

[5]Sydney Adventist Hospital Clinical School, University of Sydney, Sydney, NSW, Australia

Correspondence
Amanda J. Rao, School of Medicine, The University of Sydney, Sydney, NSW, Australia.
Email: amanda@rdcglobal.com.au

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This randomised, placebo controlled, double-blind study aimed to examine changes in muscular strength and endurance, body composition, functional threshold power, and sex hormones in response to an 8-week calisthenic programme with daily supplementation with Testofen® (Fenugreek extract) or a placebo. A total of 138 male participants (25-47yrs) were enrolled and randomized to three equal groups: 600 mg Testofen®/day, 300 mg Testofen®/day or placebo. Muscle strength and endurance, functional threshold power, body composition, and sex hormones were measured at baseline, weeks 4 and 8. Participants completed a whole-body calisthenic programme three times a week. All groups improved their maximal leg press from baseline to 8 weeks, however, both Testofen® treated groups improved more than placebo ($P < .05$). The 600 mg group showed decreases in body mass of 1.2 kg, −1.4% body fat and an increase in lean mass (1.8%) at 8 weeks. The 600 mg group also demonstrated an increase in testosterone concentration from baseline to 8 weeks. This study indicates that Testofen® may be an effective ergogenic aid for individuals wanting to rapidly improve their exercise performance capabilities and body composition above and beyond that of calisthenic exercise alone.

KEYWORDS

body composition, ergogenic aid, Fenugreek, functional threshold power, nutraceutical, resistance training, strength

1 | INTRODUCTION

Males who engage in regular exercise often seek the use of herbal formulations to positively influence muscle growth, aerobic capacity, stamina, and overall performance.[1,2] Formulations marketed toward males focus on testosterone enhancers in light of testosterone's known androgen-regulating physiological function, specifically, alterations in body composition, increased energy and muscle strength.[3]

Physical activity, specifically resistance training, acutely impacts protein synthesis and maintenance or improvement of muscle mass.[4] As a result, increased muscle mass improves exercise performance and is associated with raised basal metabolic rate, leading to increased fat oxidation.[5] Furthermore, regular exercise has a favorable effect on cardiovascular health (insulin sensitivity, lipid profile), mental health (mood, cognition, sleep), and appetite regulation.[5-8]

Trigonella foenum-graceum (fenugreek) is an annual plant in the Fabaceae family and both the leaves and seeds are used in cooking and traditional Ayurvedic medicine. Fenugreek contains over 100 phytochemical constituents, including furostanol saponins and steroidal saponins, and has multiple health applications such as reducing menopausal symptoms, decreasing fasting blood glucose, HbA1c, insulin, total cholesterol, and triglycerides.[9-12]

Testofen® is a unique extract of fenugreek, standardized to a 50% matrix of saponin glycosides, it has been previously

studied for its beneficial effects on male physiology, specifically sexual function and symptoms of androgen decline in older males.[13,14] Based on these studies, it is thought that the mechanism of action of *Testofen*® may be its ability to increase the release of free testosterone from sex hormone-binding globulin (SHBG).

Previous research supports the use of Fenugreek supplementation for promoting lean body mass, cardiovascular health, and enhancing endurance capacity associated with increased levels of free testosterone[15,16] These studies have demonstrated increases in muscle strength and repetition to failure and key determinants of strength development in resistance training.[16] However, recent research suggests that there is no causal relationship between circulating or localized testosterone concentration and changes in muscle hypertrophy.[17,18]

The aims of this study were to examine changes in body composition, muscular strength/endurance, functional threshold power, and sex hormones in response to an 8-week bodyweight resistance training programme with daily supplementation of two different doses of *Testofen*® or a placebo. We hypothesized that all participants would experience significant improvements in body composition, muscular performance, and functional threshold power following the bodyweight resistance training programme, and those participants who were supplemented with *Testofen*® would experience enhanced training adaptations. It was also hypothesized that a 600 mg dose per day would elicit greater responses than a 300 mg dose per day.

2 | METHODS

This study was a double-blind, randomized, placebo-controlled trial of 8-week treatment duration conducted in Brisbane, Australia. It was approved by Bellberrry Human Research Ethics Committee (HREC 2016-04-307) and registered on the Australian and New Zealand Clinical Trials Registry (ACTRN12616000938404). All participants were provided a full explanation of the study, an information brochure and each participant provided informed consent prior to participation.

Two hundred and eight individuals were screened for the study, of which 138 met eligibility criteria, were enrolled and were randomized to one of three treatment groups—600 mg *Testofen*® daily, 300 mg *Testofen*® daily or placebo. Ninety-eight participants completed the study. Thirteen individuals were lost to follow up and four declined to continue in the study due to non–study-related events. Twenty-three participants declined further participation after the initial testing (Figure 1). Baseline participant characteristics are listed by group in Table 1.

Participants were included if they were males between 25 and 47 years old, had a BMI between 18.5 and 29.9, and were non-smokers. Participants were undertaking regular cardiovascular exercise including but not limited to cycling, swimming, and walking not more than five times weekly, but were not doing any resistance training. Exclusion criteria included any clinically significant medical conditions, consumption of any dietary supplements in the last 3 months, known hypersensitivity to herbal drugs/nutritional supplements/foods, substantial alcohol consumption (21 or more standard alcoholic beverages per week), drug use, prolonged (≥6 weeks) medication with corticosteroids, antidepressants, anticholinergics, or any other drugs that may have an influence on the outcome of the study, severe pulmonary dysfunction, history of orthopedic injuries or surgery in the past 6 months, or other confounding conditions.

The primary outcomes of this trial were changed in muscle strength (as measured by 1-repetition maximum [RM] leg press and 1-RM bench press) and muscular endurance (as

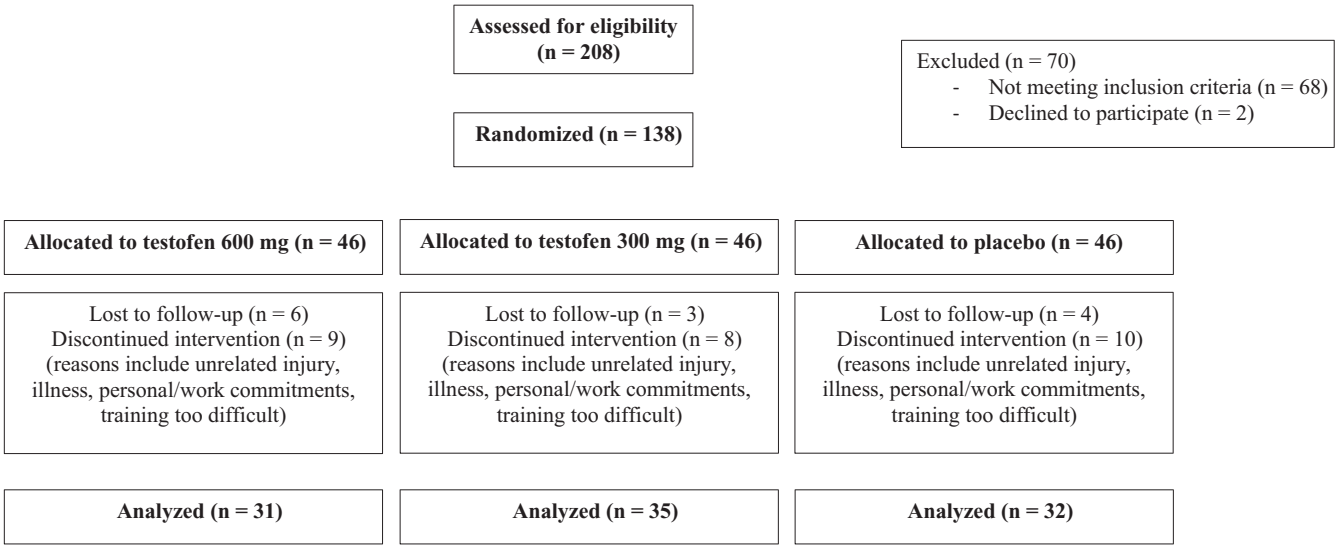

FIGURE 1 Patient flow diagram

TABLE 1 Participant baseline characteristics, mean ± SD

	Testofen® (600 mg)	Testofen® (300 mg)	Placebo
Participants (n)	31	35	32
Age (years)	37.1 ± 6.4	37.2 ± 5.9	37.5 ± 6.0
BMI (kg/m^2)	25.2 ± 2.9	25.7 ± 3.6	25.7 ± 2.9
Leg press 1-RM (kg)	187.9 ± 35.4	180.1 ± 41.3	183.1 ± 34.5
Bench press 1-RM (kg)	71.4 ± 12.8	66.6 ± 17.3	68.9 ± 15.3
FTP (Watts)	218.0 ± 46.3	237.4 ± 47.3	234.6 ± 45.5

Note: N.B.

Abbreviations: 1-RM, one-repetition maximum; BMI, body mass index; FTP, functional threshold power.

measured by 80% of 1-RM leg press reps to fatigue and 80% of 1-RM bench press reps to fatigue).

Secondary outcomes included: Body composition (lean muscle mass, fat mass, and % body fat), body mass index (BMI), weight, functional threshold power (FTP), plasma creatine kinase (CK), lactate dehydrogenase (LDH), testosterone, free testosterone, sex hormone-binding globulin, oestradiol, and safety.

The study comprised of three treatment arms randomized to equal numbers (n = 46), to each of which the investigators and participants were blinded. The three treatment arms included a 600 mg/day, 300 mg/day, and a placebo group. All capsules were matched for capsule size, shape, and appearance. The active treatment was standardized *Trigonella foenum-graecum* seed extract, *Testofen*®. The extract was supplied by Gencor Pacific Ltd. and the placebo product contained maltodextrin, all trial products were manufactured in a TGA licenced facility by BlueGum Pharmaceuticals. Each study product was consumed twice per day, one with a morning meal and one with an evening meal.

Participants were required to undertake an exercise training programme consisting of body weight resistance exercises for major muscle groups both in the upper and lower body. Participants undertook three at home training sessions per week during weeks 1-3 and 5-7 and 2 sessions during assessment weeks 4 and 8. All exercise sessions were provided in detail to the participant with instructional videos for each session supplied. Prior to each session, the participants completed a comprehensive 10-minute warm-up including aerobic exercises and dynamic stretches. An example exercise prescription programme is listed in Table 2. Participants recorded their number of exercises completed at each session in an exercise diary.

Prior to baseline testing, the participants were asked to refrain from strenuous exercise for 48 hours. During the baseline visit participants who had passed screening against the inclusion and exclusion criteria attended the exercise clinic between 6 AM and 5 PM to complete measures of height and weight. Participants then completed a light self-selected pace 10-minute warm-up on a bicycle ergometer. Following this, participants completed a 20-minute FTP test on a bicycle attached to a Wahoo Kickr® Power Trainer (Wahoo Fitness) and wore a heart rate monitor (Polar Electro Inc). Each participant was instructed to perform the highest possible mean power output for the duration of the test. Standardized verbal encouragement was provided to each participant and water could be consumed ad libitum. FTP was determined as the mean power output over the test duration.

As participants had recently completed the FTP testing a light warm-up was completed on the 45° leg press with no weight on the sled. Participants then stretched major muscle groups of the lower limbs followed by 10 repetitions of leg press at 50% of estimated 1-RM and 2 minutes rest. The weight on the leg press was then increased to approximately 70% of 1-RM and participants completed 4-6 repetitions followed by 2 minutes rest. Weight was added onto the leg press to approximately 90% of estimated 1-RM and participants completed one repetition followed by 2 minutes rest. Weight was then increased to 100% of estimated 1-RM and participants attempted to complete a repetition. If successful weight was increased, and if unsuccessful weight was decreased. Participants were given up to seven attempts to achieve 1-RM. Participants were rested for 3 minutes in between attempts. Once 1-RM testing was complete participants rested for 5 minutes. At the completion of this resting period, the participants completed one set of 80% of 1-RM leg press for as many reps as possible. The participants again rested for a further 5 minutes before an identical protocol was followed for bench press.

Within a week from baseline testing participant's fasted (10 hours) blood was collected at an accredited local pathology laboratory (Queensland Medical Laboratory) for analysis, and a fasted (10 hours) whole-body DXA (Dual-energy X-ray Absorptiometry scan) was completed at an RANZCR radiology clinic. Serum and plasma were analyzed for CK, LDH, testosterone, free testosterone, sex hormone-binding globulin, and oestradiol. After completion of all baseline measures, participants were instructed to take the allocated product according to the directions prescribed.

At the mid-point (week 4) of the study, participants completed all baseline measures except pathology and DXA. At the completion of the study (week 8), an assessment identical to that undertaken at baseline was conducted. Participants were asked to refrain for consuming any additional dietary supplements and were to continue their habitual diet for the duration of the study. At both time points (weeks 4 and 8), participants were interviewed and asked to provide details regarding any lifestyle changes (diet, exercise, and medication) in addition to subjective changes in exercise performance and any adverse effects (including change in libido and mood). Participants were also monitored for compliance with the protocol by a combination of telephone and email communications in addition to during each scheduled session.

TABLE 2 Example exercise session

Week	Session	Exercise	Repetitions	Time limit	Notes
1	1	Bodyweight squat	10	10 min	Superset exercises to complete as many rounds as possible in the time limit
		Push up	10		
		Sit up	10		
	2	V sit up	20	10 min	Superset exercises to complete as many rounds as possible in the time limit
		Burpees	10		
		Knees to upper arm (plank position)	10 each leg		
	3	Walking lunges	10 each leg	15 min	Superset exercises to complete as many rounds as possible in the time limit
		Sit up	10		
		Superman hold	5 (10 s each)		
		U sit	15		
		Bear crawl	20 m		
2	1	Jumping lunges	10	8 min	Superset exercises to complete as many rounds as possible in the time limit
		Bear crawl	10 m		
		Backwards bear crawl	10 m		
	2	Push up complex	5	5 min	Superset exercises to complete as many rounds as possible in the time limit
		Side raises (left)	5		
		Leg raises	10		
		Side raises (right)	5		
		Sit up	50	-	Complete at the end of previous superset
	3	Burpee back roll	1	12 min	Superset exercises to complete as many rounds as possible in the time limit
		Shuttle run	10 m		

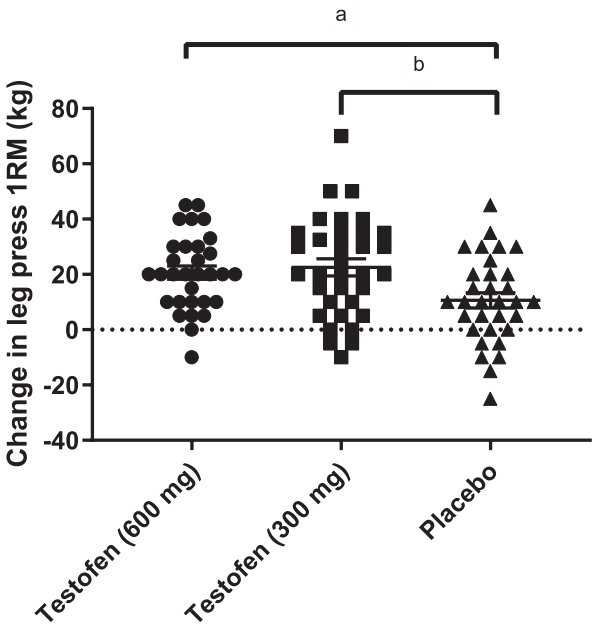

FIGURE 2 Change in leg press one-repetition maximum from baseline to week 8. Data are presented as mean ± SDN.B.
a = significant difference between Testofen (600mg) and placebo,
b = significant difference between Testofen (300 mg) and placebo

Randomization of the products was performed by a third party using Random Allocation software, Research Randomiser, version 1.0, May 2004. The investigational products were in opaque trial product containers that were identical in function and appearance to maintain blinding of both participants and investigators.

All data analyses were completed using GraphPad Prism (version 7.04 for Windows, GraphPad software). Normality testing of data using the Shapiro-Wilks test was performed. Outliers were identified using the Grubbs test and removed if necessary. Linear mixed models were used for repeated measures. Change variables were analyzed using ordinary one-way analysis of variance. Bonferroni post-hoc analysis was performed to correct for multiple comparisons. Significance was assumed when $P < .05$. A sample size of 36 per group was calculated based on the power to detect a change of 5% change in leg/bench press 1-RM. Effect size: 0.56, Alpha error prob: 0.05, Power 0.8.

3 | RESULTS

There were no significant differences between the active treatments and placebo groups for age, anthropometric measures, pathology, lifestyle factors, or exercise

TABLE 3 Strength and body composition measures, mean ± SD

	600 mg (n = 31)		300 mg (n = 35)		Placebo (n = 32)	
	Baseline	**Week 8**	**Baseline**	**Week 8**	**Baseline**	**Week 8**
1-RM leg press (kg)	187.9 ± 35.4	208.6 ± 33.5[a, b]	180.1 ± 41.3	206.3 ± 47.9[a, b]	183.1 ± 34.5	193.8 ± 33.7[a]
1-RM bench press (kg)	71.4 ± 12.8	76.5 ± 16.4[a]	66.6 ± 17.1	69.9 ± 17.4[a]	68.9 ± 15.3	73.0 ± 14.6[a]
Weight (kg)	83.7 ± 9.1	82.5 ± 9.1[a, b]	82.1 ± 10.9	82.1 ± 11.5	83.0 ± 10.1	83.8 ± 10.3
Lean mass (%)	77.1 ± 5.7	78.7 ± 6.1[a, b]	77.2 ± 5.4	77.4 ± 5.5	76.8 ± 5.0	76.6 ± 5.9
Fat mass (%)	19.1 ± 5.9	17.8 ± 6.3[a]	19.1 ± 5.7	19.0 ± 6.1	19.4 ± 5.3	19.8 ± 6.0

Note: N.B.

Abbreviations: 1-RM, one-repetition maximum.

[a]Significant difference within group from baseline.

[b]Significant difference from placebo group at week 8.

measurements at baseline (Table 1). Exercise session attendance was 89% for all three trial groups, and there were no significant differences between groups for exercise session attendance.

All three groups improved their leg press 1RM from baseline to 8 weeks (Figure 2). However, both active groups (600 mg and 300 mg) significantly improved leg press 1RM ($P < .05$) more than placebo (Table 3; 20.66 ± 13.5 kg, 22.57 ± 17.9 kg, and 10.63 ± 15.6 kg, respectively). There were also significant increases ($P < .05$) from baseline in bench press 1RM for all three groups (4.2 kg, *Testofen*® 600 mg, 3.5 kg, *Testofen*® 300 mg, and 4.1 kg placebo), however, there was no significant between-group differences (Table 3). There was a significant positive correlation ($r = .30$, $P < .05$) between change in leg press 1RM and bench press 1RM from baseline to week 8 in all groups.

The 600 mg group was the only group to have a significant decrease in body mass, losing on average 1.2 kg over the 8 weeks ($P < .05$) compared with 0.0 kg and a 0.8 kg gain in the 300 mg and placebo groups, respectively (Table 3). Along with this decrease in absolute body mass the 600 mg group also had a significant increase in % lean mass compared to 300 mg and placebo (Table 3). The 600 mg group had an increase of 1.8% ($P < .05$) compared with 0.5% in both the 300 mg and placebo groups. Complementing an increase in % lean mass, the 600 mg group was the only group to have a significant decrease from baseline in % fat mass at −1.4%, with the 300 mg and placebo groups −0.46% and −0.49%, respectively, however, there was no between-group difference for % fat mass (Table 3).

The 600 mg was the only group to significantly increase FTP from baseline (Figure 3) with a 7.6-watt improvement

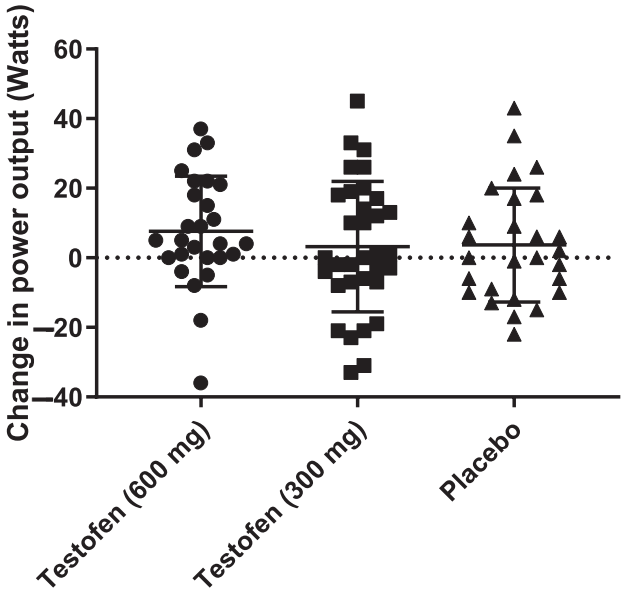

FIGURE 3 Change in functional threshold power output from baseline to week 8. Data are presented as mean ± SD

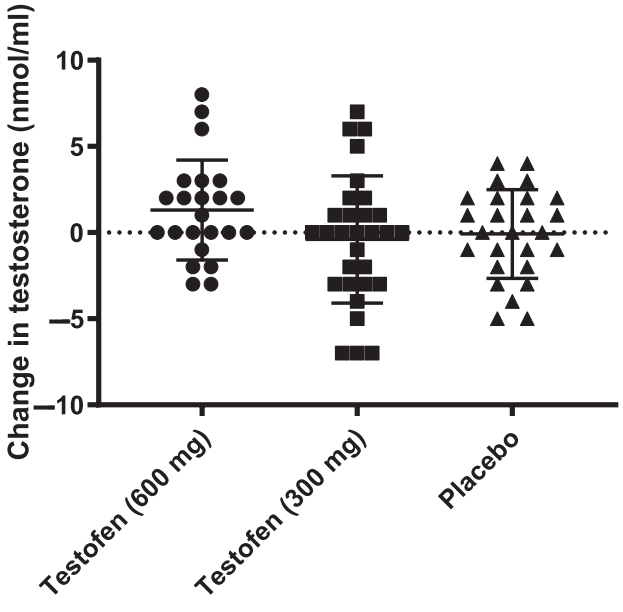

FIGURE 4 Change in testosterone from baseline to week 8. Data are presented as mean ± SD

TABLE 4 Sex hormones, mean ± SD

	600 mg (n = 23)		300 mg (n = 29)		Placebo (n = 26)	
	Baseline	**Week 8**	**Baseline**	**Week 8**	**Baseline**	**Week 8**
Testosterone (nmol/L)	17.2 ± 6.9	18.5 ± 6.7[a, b]	17.5 ± 4.5	17.1 ± 4.5	15.6 ± 5.1	15.5 ± 4.0
Free testosterone (nmol/L)	0.32 ± 0.06	0.32 ± 0.08	0.35 ± 0.10	0.34 ± 0.09	0.31 ± 0.08	0.30 ± 0.08
SHBG (nmol/L)	42.2 ± 23.3	45.9 ± 22.1	38.7 ± 9.4	37.5 ± 12.5	36.5 ± 13.8	37.4 ± 12.3
Estradiol (pmol/L)	106.3 ± 28.3	99.0 ± 38.1	116.6 ± 25.1	112.4 ± 25.9	99.1 ± 32.8	100.5 ± 27.7

Note: N.B

Abbreviation: SHBG, sex hormone-binding globulin.

[a]Significant difference between baseline and week 8.

[b]Significant difference between groups.

(vs 3.2 watt and 3.7 watt, 300 mg, and placebo, respectively). There were no between-group differences identified.

The 600 mg group was the only group to significantly increase the serum testosterone levels by 1.3 nmol/mL after 8 weeks, whereas the 300 mg and placebo had small decreases from baseline (Figure 4). No other hormone data showed any significant changes (Table 4). All blood biochemical safety markers remained stable and within normal reference ranges.

No product-related adverse events were recorded. One serious adverse event was reported, an acute knee injury which occurred during the exercise training and was related to a pre-existing knee injury. Four non–product-related adverse events occurred, and each participant subsequently dropped out of the study. Reasons for dropout included thumb injury, ulcerative colitis, influenza, and non-specific injury.

4 | DISCUSSION

This study was designed to assess the effectiveness and dose response of *Testofen*® on muscle strength, aerobic fitness, and body composition in healthy exercising males. Our findings indicate that *Testofen*® is effective for increasing muscle strength, aerobic endurance, and improving body composition in conjunction with a bodyweight resistance exercise training programme conducted 3 times per week. An adherence rate of 89% for exercise session attendance, along with congruent increases in leg press, bench press, and changes in body composition by all intervention groups, indicates that the bodyweight resistance exercise regime was effective.

The higher dose (600 mg) group was the only intervention group that increased % lean mass (1.8%) and FTP (7.6 watts) and decreased their body weight (1.2 kg). This suggests a dose-response effect of *Testofen*® with an increasing dose corresponding with positive effects in body composition and aerobic fitness.

While testosterone concentration increased in the 600 mg group, oestradiol remained unchanged, consistent with previous studies on *Testofen*® at this dose.[13-14,16] In the 300 mg and placebo groups, testosterone remained unchanged which is also consistent with other studies conducted in similar populations on resistance training alone, where changes in muscle mass and strength have been seen without changes in serum testosterone levels.[18,19] Determination of localized changes in sex hormones and androgen receptor changes would have further improved this study, however, muscle biopsies were beyond the scope of this research.

The present study indicates that when used in conjunction with an effective exercise regime, a 600 mg/day dose has positive effects in enhancing leg strength, aerobic capacity, and body composition compared with placebo and a 300 mg/day dose in young to middle-aged males. Previous research on *Testofen*® in older men with androgen decline showed similarly increased levels of testosterone and a reduction in physical symptoms related to hypogonadism.[13] Our work supports the use of *Testofen*® as an adjunct to exercise training, and for managing sarcopenia in older populations. As testosterone is also important for muscle strength and performance in women, additional studies are warranted.

Compared with a placebo, 600 mg of *Testofen*® per day for 8 weeks significantly increased FTP (7.6 watts), lean mass (1.8%), leg press (20.66 kg), and serum testosterone (1.3 nmol/mL) and decreased fat mass (1.4%) from baseline. Sports competitors could supplement with a WADA approved herbal extract (*Testofen*®) to increase lean mass, leg strength, and endurance capacity while also decreasing fat mass. These desirable traits for performance can apply to many sports/activities and have the potential to enhance performance. It must be noted that this study was conducted with recreational athletes only where no control over diet was performed.

5 | CONCLUSION

Testofen® is an effective ergogenic aid for those wanting to rapidly improve their exercise performance capabilities and

body composition above and beyond that of resistance exercise alone.

5.1 | Perspective

Sports competitors are always looking for an approved competitive edge over their competition. While many nutraceuticals bolster claims about improving antioxidant defences and immunity these may not necessarily translate directly to performance. Increases in strength, aerobic capacity, and body composition above and beyond bodyweight training alone place the fenugreek extract, *Testofen*®, in a category not achieved by many ergogenic aids. While fenugreek and exercise research is still in its infancy, the results shown in this study support growing evidence that suggests that *Testofen*® can be a useful instrument in the toolbox of sports competitors.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID

Alistair R. Mallard https://orcid.org/0000-0002-7961-1458

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