Furosap, a novel Fenugreek seed extract improves lean body mass and serum testosterone in a randomized, placebo-controlled, double-blind clinical investigation

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ABSTRACT

Background: The Indian spice fenugreek (Trigonella foenum-graecum) has been credited with numerous health benefits in cardiovascular disorders, metabolic syndrome, inflammatory conditions, glucose-insulin regulation, and sports performance. Previous studies from our laboratories demonstrated that fenugreek seed extract improved glucose tolerance and insulin sensitivity, augmented serum testosterone level, and improved cardiovascular functions. The aim of this study is to examine the efficacy of Furosap, a novel fenugreek seed extract enriched in 20% protodioscin, on exercise performance.

Methods: This randomized, double-blind, placebo-controlled, clinical study was conducted in forty healthy male athletes (n = 40) over a period of 12 consecutive weeks. Subjects were given either placebo or Furosap capsules (250 mg/day b.d.) and serum samples were used to assess serum total testosterone level and C-reactive proteins (CRP) at baseline and at the end of 12-weeks of treatment. Body fat mass, lean mass, fat mass, fat-free mass, grip strength, upper and lower body strength, maximal graded exercise stress using a digital hand dynamometer, dual-energy X-ray absorptiometry (DEXA), force plate, and treadmill with open-circuit spirometry were assessed at the baseline and at the end of 12-weeks of treatment.
Results: Furosap supplementation significantly increased mean lean body mass and fat-free mass compared to subjects receiving placebo. Additionally, Furosap-treated subjects elevated serum testosterone levels. Furosap supplemented subjects also exhibited a tendency towards lowering blood pressure during exhaustion. No adverse reports were reported.

Conclusions: Given improvement of lean body mass and serum total testosterone following intervention with Furosap, Furosap likely has benefits for exercise endurance and sports medicine.

Keywords: Fenugreek seed extract, safety, body mass, fat-free mass, blood pressure, muscle strength

BACKGROUND
Fenugreek (Trigonella foenum-graecum Linn. (Family: Fabaceae) is a popular spice and herb in Asian culinary dishes and a well-recognized traditional medicine. Fenugreek seeds and seed powder are used in diverse cooking recipes condiments and bread. The green leaves have been used in salad, curry powders, flavor dishes, pastes, seasonings, and various vegetable preparations for thousands of years. Fenugreek is one of the oldest medicinal plants, with its beneficial effects being cited in Ayurveda and traditional Chinese medicine. The phytochemical constituents in fenugreek seeds include fiber, 4-hydroxyisoleucine, steroidal saponins, protodioscin, glycosides, alkaloids, polyphenols, flavonoids, antioxidants, lipids, carbohydrates, amino acids, and hydrocarbons. Extensive pre-clinical and clinical investigations have demonstrated its broad spectrum pharmacological, medicinal, and therapeutic properties including antioxidant, anti-diabetic, anti-hyperlipidemic, anti-inflammatory, antifungal, antibacterial, and galactogogue. Furthermore, our previous studies have demonstrated its beneficial effects is sports nutrition, muscle building, and exercise.

A broad spectrum of scientific data and systematic reviews demonstrate that fenugreek seed extract attenuates mental alertness and mood as well as serum lipid function and lowers fasting and post-prandial blood glucose levels, in addition to glycosylated hemoglobin [1-6]. In another set of investigations, fenugreek seed extract improved serum testosterone level, exercise performance, and endurance capacity and lowered hyperglycemia in a variety of rodent models and humans [7-10]. Another group of investigators exhibited an elevated muscle glycogen synthesis after exhaustive exercise in athletes following supplementation of fenugreek seed extract [11]. Taylor et al. demonstrated supplementation of fenugreek in conjunction with creatinine increases creatinine uptake, muscular strength, endurance and anaerobic capacity in resistance trained men [12]. Similar observations were noted by Lewing et al. in another clinical study [13]. Fenugreek supplementation improved both upper and lower-body strength and body composition in resistance-trained men in a placebo-controlled, double-blind study [14].

Furosap, a novel natural extract from fenugreek seeds developed in our laboratories, is enriched in 20% protodioscin. Protodioscin exerts its androgenic effects by augmenting androgen receptors levels in cells and improving the body’s sensitivity to androgens like testosterone and DHT [15]. Protodioscin has also been shown to trigger the release of NO in corpus cavernosum tissue and enhance levels of testosterone, dihydrotestosterone, and dehydroepiandrosterone [16].
Previous studies in our laboratories have demonstrated that fenugreek saponins have broad spectrum safety profile, and improved glucose homeostasis, insulin signaling and tolerance, and reduced hepatic fat accumulation in high-fat diet-fed mice [17]. Concurrently, a multi-centered, placebo-controlled clinical investigation conducted in our laboratories demonstrated how the fenugreek seed extract reduced glycosylated hemoglobin and improved metabolic parameters in subjects with type-2 diabetes [18]. In a double-blind, placebo-controlled trial, we reported how fenugreek saponins improved HbA1c and decreased serum isoprostane levels in obese, insulin-resistant subjects (unpublished studies) [19]. Previous single-arm, open-labelled investigations using a novel fenugreek seed extract enriched in 20% protodioscin (Furosap, 500 mg/day) in 50 male volunteers over a period of 12 consecutive weeks demonstrated its broad-spectrum safety and its efficacy at boosting serum free testosterone levels, healthy sperm profile, mental alertness, cardiovascular well-being, mood, libido, and overall performance [20,21].

The investigation evaluated the benefits of supplementation of a novel fenugreek seed extract enriched in 20% protodioscin (Furosap) on lean body mass, fat-free mass, serum testosterone level, systolic and diastolic blood pressures, cardiorespiratory endurance, and muscle strength in healthy human subjects.

MATERIALS AND METHODS

Ethical Approval
The study design, recruitment, and methods were performed in compliance and accordance to the ICH guidelines for Good Clinical Practices (GCP), including the archiving of essential documents, and per international standards guaranteed by the Declaration of Helsinki and its subsequent amendments. The University of Wyoming’s Institutional Review Board (IRB) approval was obtained from this investigation (Protocol #20170118SN01429).

All subjects were provided with a consent form and provided sufficient information for subjects to make an informed decision about their participation in this study. The consent form was submitted with the protocol for review and approved by the IRB for the study. The formal consent of a subject using the IRB-approved consent form was obtained before the subject is submitted to any study procedure. Consent form was signed by the subject or a legally accepted representative. An investigator-designated research professional obtained the consent. Informed consent was obtained during the primary screening. Subjects also completed a brief health questionnaire. Patient confidentiality was strictly maintained.

Study Design
Furosap, a Novel Trigonella foenum-graecum Seed Extract: A novel, patented Trigonella foenum-graecum seed extract (color: off white to yellow powder, enriched in 20% protodioscin (Furosap, US Patents# US 8,217,165 B2; US 8,754,205 B2) enriched in 20% protodioscin (determined on anhydrous basis by HPLC). A proprietary extraction procedure was used to standardize Furosap, which is >95% water soluble and moisture content <5%. Both residual solvent and pesticide contents comply with USP38 requirements and the shelf-life is 2 years.
Subject Recruitment, Inclusion and Exclusion Criteria and Intervention Strategy: A total of 40 subjects (age: 24.02 ± 3.90 years) were screened for the clinical study on the basis of inclusion and exclusion criteria (Table 1) and randomly assigned (using a computer program) to receive either placebo or Furosap capsules (250 mg each, b.d., in the form of oral capsules) over a period of 12 consecutive weeks. Both placebo and Furosap capsules were prepared in a GMP-NSF certified facility (Cepham Inc., Somerset, NJ). All subjects were instructed to have a decent healthy lifestyle during this clinical investigation.

Table 1. Inclusion and Exclusion Criteria

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
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<tbody>
<tr>
<td>• Subjects who agree to written as well as audio-visual informed consent</td>
</tr>
<tr>
<td>• Subjects with the ability to understand the risks/benefits of the study protocol</td>
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<tr>
<td>• Healthy male human subjects 18-30 years of age.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Exclusion criteria</th>
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<tbody>
<tr>
<td>• Subjects who were uncooperative</td>
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<tr>
<td>• Females were excluded from the study</td>
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<tr>
<td>• Subjects with chronic illnesses (diabetes, cardiovascular disease, etc.)</td>
</tr>
<tr>
<td>• Those on prescription medication or supplements</td>
</tr>
<tr>
<td>• Any conditions that prevent the subject from participating in physical activities.</td>
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</table>

Subjects and Regulatory Approvals: A subset of this sample (n = 26) was used for the cardiorespiratory endurance testing. Participants were recruited through voluntary response to a flyer displayed throughout the community in Laramie, WY (Albany County). Females were excluded from the study, in addition to subjects with chronic illness or those on prescription medication or supplements. Participants were also required to be free from acute injury or illness, physical impairment or cardiometabolic disease that would preclude them from participating in these studies. Informed consent was obtained from the participants after discussing with them the study details and the extent of their individual involvement in the study. The University of Wyoming’s Institutional Review Board approved this protocol (protocol # 20170118SN01429).

Initial Assessments: Height, body weight, and waist and hip circumferences were carefully measured. Body mass index (BMI) was calculated using the formula BMI = body weight (kg)/height² (m²), waist to hip ratio (WHR) was assessed using the formula waist circumference (cm)/hip circumference (cm). Blood pressure and heart rate were carefully recorded using an Omron HEM-907XL Digital Blood Pressure Monitor (Omron Healthcare Company, Kyoto, Japan).

Lean Mass, Fat Mass and Bone Mineral Mass: All subjects used a GE Lunar iDXA densitometer equipped with a software, version Encore 2005, 9.15.010 (GE Healthcare, Madison, WI, USA) to assess lean, fat, android, gynoid and bone mineral mass composition [22].
Endurance studies. Open-circuit spirometry was used to assess oxygen consumption, CO₂ production and metabolic parameters, VO₂max, respiratory exchange ratio (RER), anaerobic threshold (AT), and ventilatory equivalents for oxygen (VE/VO₂) and carbon dioxide (VE/VCO₂). A total of 26 subjects completed maximal graded exercise tests (GXT) on a treadmill using open-circuit spirometry. Both heart rate and blood pressure were assessed prior to exercise, during the GXT and recovery. The GXT began at the self-selected speed and 0% grade. Grade was increased 1-2% every 2 minutes until volitional fatigue. Criteria for achievement of maximal/peak oxygen consumption (VO₂max) were: 1) RER at or near 1.15; 2) plateau in heart rate near age-predicted maximal heart rate following an increase in workload; 3) rating of perceived exertion at or above 17; 4) a plateau in oxygen consumption with increasing workload.

Body and Hand-Grip Strength. Maximum push-up test for upper body strength and countermovement jump test for lower body strength were assessed using two force plates (Bertec Corporation, Columbus, OH, USA). Hand-grip strength was determined using a DHD-1 Digital Hand Dynamometer, SH1001 (Saehan Corporation, South Korea) [23].

Clinical Chemistry. After overnight fasting, blood samples (2 x 10 mL each) were drawn at the Wyomed Laboratory (Laramie, WY) into Vacutainer tubs and used for clinical chemistry and testosterone assays.

Adverse events monitoring: Adverse events were monitored during week 1, week 2, and at the end of each month. Participants were encouraged to contact the investigator of any additional effects when it happened. Any adverse event reported was documented when reported.

Statistical analysis. Body mass, hand-grip tests, and clinical chemistry were compared using unpaired t-test. Two-way repeated-measures ANOVA was used to study changes in endurance studies. Changes in mean baseline values between groups was analyzed using R version 3.1.3. Data are represented as means ± SEMs and significance assigned at p<0.05. Power calculations for the determination of required sample sizes were not performed as this was a pilot study and due to the lack of preliminary data for estimating variability.

RESULTS
All subjects signed the informed consent forms. A total of thirty-five subjects completed this clinical investigation. However, there were no treatment-related adverse effects reported by the subjects who dropped out. A total of 24 subjects (twelve from each group) were enrolled in the endurance test. Two subjects had to be excluded, one with an abnormal EKG and the other whose face mask slipped off during the procedure.

Lean Mass, Fat Mass, and Fat distribution: DEXA analysis revealed that Furosap supplementation caused a significant increase in the mean lean mass and fat-free mass compared to the placebo group (Table 2). However, DEXA analysis exhibited no changes in tissue fat or fat mass following supplementation of Furosap. No changes were observed in BMI, android fat, gynoid fat or android to gynoid fat ratio (Table 2).
Serum Testosterone Level: A significant change in serum total testosterone level was observed in the Furosap-treated subjects compared to the group receiving placebo (Table 2).

Table 2. Baseline and 12-week GXT measures for placebo (N=11) and supplement (N=13) groups.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Supplement Baseline</th>
<th>12-weeks</th>
<th>Placebo Baseline</th>
<th>12-weeks</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting HR (bpm)</td>
<td>82±3</td>
<td>80±3</td>
<td>78±4</td>
<td>76±3</td>
<td>0.44</td>
</tr>
<tr>
<td>Maximal HR (bpm)</td>
<td>185±3</td>
<td>186±2</td>
<td>186±2</td>
<td>187±1</td>
<td>0.76</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>125±2</td>
<td>117±2</td>
<td>126±3</td>
<td>125±3</td>
<td>0.103</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>81±3</td>
<td>78±2</td>
<td>80±2</td>
<td>81±2</td>
<td>0.34</td>
</tr>
<tr>
<td>Total GXT Time (min)</td>
<td>9:10±0:5</td>
<td>9:24±0:5</td>
<td>9:54±0:4</td>
<td>9:54±0:4</td>
<td>0.60</td>
</tr>
<tr>
<td>VO₂max (ml/kg/min)</td>
<td>44.5±2.2</td>
<td>44.2±2.1</td>
<td>48.2±1.8</td>
<td>47.8±2.1</td>
<td>0.25</td>
</tr>
<tr>
<td>AT (ml/kg/min)</td>
<td>35.2±2.4</td>
<td>33.4±2.3</td>
<td>36.5±2.6</td>
<td>33.8±3.3</td>
<td>0.91</td>
</tr>
<tr>
<td>RER</td>
<td>1.16±0.05</td>
<td>1.15±0.01</td>
<td>1.16±0.01</td>
<td>1.16±0.02</td>
<td>0.40</td>
</tr>
<tr>
<td>VCO₂ (mL/min)</td>
<td>4,068±176</td>
<td>3,962±267</td>
<td>4,105±168</td>
<td>4,093±209</td>
<td>0.70</td>
</tr>
<tr>
<td>Vₑ/VCO₂</td>
<td>34.9±1.0</td>
<td>33.7±1.1</td>
<td>34.5±1.2</td>
<td>33.0±1.2</td>
<td>0.65</td>
</tr>
<tr>
<td>Vₑ/VO₂</td>
<td>40.3±1.1</td>
<td>38.7±1.1</td>
<td>39.4±1.5</td>
<td>38.5±1.5</td>
<td>0.94</td>
</tr>
</tbody>
</table>

*Two-way repeated measures analysis of variance: Group (placebo vs. supplement) X Time (baseline vs. 12-weeks) interaction. 1Two-tailed P-value. One-tailed P-value for difference between groups post-intervention (hypothesizing a supplement-induced reduction in SBP) was 0.051.

Endurance Test: Furosap treatment demonstrated a tendency of lowering blood pressure, based on the 8 mm of Hg change from baseline at 12 weeks. Table 3 exhibits the results of the endurance test. No baseline differences between placebo and treatment group was observed for resting heart rate, maximal heart rate, systolic blood pressure, diastolic blood pressure, total GTX time, VO₂max, anaerobic threshold, RER, VCO₂, Vₑ/VCO₂, and Vₑ/VO₂. Following 12-weeks of Furosap supplementation, no significant changes were observed in these parameters. Additionally, no changes in aerobic endurance (GXT Total Time or maximal oxygen consumption), metabolic parameters, and heart rate were observed.

Table 3. Effect of Furosap treatment over a period of 12-consecutive weeks on serum testosterone, lean body mass and fat free mass

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Baseline (0 week)</th>
<th>Final Visit (12 weeks)</th>
<th>Change (PL)</th>
<th>Change (Furosap)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Testosterone</td>
<td>608 ± 50</td>
<td>545.6 ± 59</td>
<td>-12.8</td>
<td>124*</td>
</tr>
<tr>
<td>Lean Body Mass (g)</td>
<td>59604 ± 3107</td>
<td>59858 ± 1573</td>
<td>-545.5</td>
<td>429.3*</td>
</tr>
<tr>
<td>Fat-Free Mass (g)</td>
<td>62829 ± 1650</td>
<td>63161 ± 3246</td>
<td>-532.1</td>
<td>485.5*</td>
</tr>
</tbody>
</table>
Changes at three months compared to baseline (pre-treatment) of serum total testosterone level in the placebo and fenugreek seed extract enriched in 20% protodioscin (Furosap) (A), *p<0.05, n = 35 per group).

Changes from baseline (pre-treatment) of lean body mass and fat-free mass following three months of intervention with the placebo or fenugreek seed extract enriched in 20% protodioscin (Furosap) as assessed by whole-body DEXA measurements. p<0.05 versus placebo, mean ± SEM, n= 35.

Strength Assessment. No significant changes were observed in jump height, grip-test, peak jump force, and peak push-up forces in the subjects taking placebo or Furosap (data not shown).

Blood Chemistry: No significant changes were observed from baseline in the fasting glucose, AST, ALT, serum creatinine, insulin or total cholesterol levels between the placebo and Furosap group.

Adverse effects. No adverse events were reported.

DISCUSSION

Trigonella foenum-graecum (Fenugreek), a novel medicinal plant, originated from Indian sub-continent and used as an herb, spice, salad, vegetable, and condiment in Southeast Asia. The medicinal use of both leaves and seeds has been recommended in Ayurveda and Chinese traditional medicines. A large number of pre-clinical, clinical, pharmacological, and mechanistic studies were conducted on fenugreek seeds to unveil the mechanism of action and its extensive use in the nutraceuticals market worldwide [24-28].

These beneficial effects of fenugreek have been attributed to the presence of a diverse chemical entities present in fenugreek seeds [24, 29, 30]. Fenugreks seeds exhibited the ability to inhibit both cholesterol absorption in the intestine and cholesterol production by the hepatic tissue [29-31]. Fenugreek seeds also demonstrated the ability to lower triglyceride and LDL levels. Fenugreek seeds are enriched in soluble dietary fiber, furostanolic saponins, protein, potassium, niacin, vitamin C, 4-hydroxyisoleucine, lysine and numerous amino acids, L-tryptophan, diosgenin, yamogenin, tigogenin, and neotigogenin [27-33]. However, the chemical component mediating the reported beneficial effects of fenugreek remains unknown. These diverse chemical entities in fenugreek seeds and its extensive health benefits that include anti-diabetic, anti-hepatotoxic, anti-inflammatory, anti-obesity, sports performance, and sexual health modulating activities [28-35] encouraged us to test a novel fenugreek formulation enriched with 20% protodioscin on exercise tolerance and related cardiometabolic parameters.

Saponins, the most abundant class of phytochemicals present in fenugreek, belong to either the furostanol steroidal saponin class or the spirostanol saponin class [35-37]. The safety, efficacy, and pharmacokinetic profile of furostanolic saponins are very encouraging in the fields of cardiometabolic endurance, sports performance, and its anabolic potential in boosting testosterone levels. Broad spectrum safety studies in our laboratories demonstrated the extensive safety of Furosap. Our previous studies demonstrated the ability of Furosap in testosterone boosting, mood and mental alertness, sperm profile, morphology and motility, libido, and sexual health [17-21, 38]. Additionally, we noted the superior pharmacokinetic profile of furostanolic saponins [39], broad-spectrum safety profile, and the magnificent efficacy in improving metabolic profile in both
obese animals [38] and diabetic subjects [18]. In light of these effects, we studied the role of furostanolic saponins on cardiometabolic endurance.

*In vitro* and *in vivo* studies have demonstrated that protodioscin may exert a relaxation effect on the cavernous smooth muscle in a concentration-dependent manner and that the mechanism included a reaction involving the NO/NOS pathway in the corpus cavernosum endothelium. The traditional understanding of action of NO in the male reproductive organ is that NO is constitutively produced and released from autonomic nerve terminals and endothelial cells in corporal tissue. NO diffuses locally into adjacent smooth muscle cells and binds with intracellular guanylate cyclase, which serves as a physiological "receptor" [40]. This binding induces a conformational change in guanylate cyclase, activating the enzyme so that it catalyzes the conversion of guanosine triphosphate to cGMP. cGMP then operates through a cGMP-dependent protein kinase to regulate the contractile state of the corporal smooth muscle [41].

A significant concentration-dependent increase in intracavernous pressure was observed in accordance with the increasing dosage of the *T. foenum-graecum* extract. The oral administration of a *T. foenum-graecum* extract in clinical practice is expected to improve sports nutrition performance and increased erectile function. Although further clinical research is needed, fenugreek seed extract shows promise as an erectogenic agent [41-45].

Previous studies have shown that fenugreek can alter body composition. Salgado et al. demonstrated that oral administration of a standardized *Tribulus terrestris* extract enriched in natural protodioscin increased lean body mass and fat free mass [46]. Another clinical investigation by Hwang et al. [47] demonstrated how supplementation of L-citrulline, a novel enhancer of athletic performance, also boosts lean body mass [47].

The majority of testosterone is converted to androsterone, the active metabolite, which is eventually conjugated with glucoronic acid or sulphuric acid and excreted as a 17-keto-steroid metabolite. However, since this is not the major source of 17-ketosteroid, simple measurement of this compound in the urine does not provide an accurate picture of testes steroid production. Moreover, this measurement would also not be able to detect the small amount of testosterone converted into a specific form of androgen called dihydrotestosterone (DHT) in specific target tissues [42]. In the majority of target cells, some testosterone is enzymatically converted into DHT by the microsomal enzyme 5-alpha-reductase [42]. Similar to testosterone, DHT is then bound by an intracytoplasmic receptor protein specific for it. After the DHT-protein complex formation, the bound hormone is transported into the nucleus. There the protein complex undergoes a conformational transformation, which involves chromatin binding. This transformation results in mRNA syntheses and in syntheses of cytoplasmic proteins, which leads to cell growth and other secondary effects mediated by androgens [42]. Furthermore, testosterone and other anabolic-androgenic steroids enhance athletic performance in men and women not only through its long-term anabolic actions but also through rapid effects on behavior [48]. A positive testosterone boost can directly translate in directly translating or improving or enhancing athletic performance to be successful in sports [48].

In the current study, compared to the placebo, significant increases in lean-body mass, fat-free mass and total serum testosterone levels were observed following supplementation of Furosap over a period 12 consecutive weeks. Total serum testosterone levels, a lack of which is an indicator of hypogonadism, is in dynamic equilibrium with its corresponding free form in the serum. Elevated
serum testosterone level is also considered as a marker of strength, vitality, and vigor. Thus, an increase in the total testosterone levels in Furosap supplemented subjects suggest that the supplement may possess significant anabolic activity.

In our study, Furosap did not alter baseline aerobic endurance, upper and lower body strength, grip strength, and total fat mass. The investigation was conducted in healthy, young men rather than older individuals or diabetic subjects, which may explain the lack of significant changes in terms of aerobic endurance. In contrast to older subjects, the compensatory factors in young healthy individuals are stronger and helps maintain homeostasis under stressful conditions. Accordingly, a comparatively lower dose of Furosap was used in the current study and did not subject the volunteers to resistance-training unlike some of the previous studies reported with fenugreek seed extract. Neither did the intervention result in changes to ALT, AST, or serum lipid levels (data not shown), which further substantiate the broad-spectrum safety of Furosap.

Overall, the present investigation demonstrates that Furosap supplementation improves lean body mass and fat-free mass, and serum testosterone levels in healthy young men, which further substantiates its potential benefit in sports nutrition, muscle building, and exercise.

List of Abbreviations: CRP, C-reactive proteins; DEXA, dual-energy X-ray absorptiometry; BMI, body mass index; WHT, waist to hip ratio; RER, respiratory exchange ratio; AT, anaerobic threshold; VE/VO2, ventilatory equivalents for oxygen; GHT, graded exercise tests; DHT, dihydrotestosterone.

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