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Research Article

# A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY ON STANDARDIZED FENUGREEK SEED EXTRACT COMPOSITION FOR ENDURANCE ENHANCEMENT IN RECREATIONALLY ACTIVE YOUNG SUBJECTS

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**ABSTRACT**

**Objective:** The objective of the study is efficacy and safety evaluation of 8-week supplementation with FEΔE (fenugreek seed extract composition, standardized to 4-hydroxyisoleucine, trigonelline, and select glycosides) on endurance capacity of recreationally active young male subjects, with randomized and double-blind design.

**Methods:** The 153 male participants were randomized equally into three groups and received either 300 mg or 600 mg of FeΔE capsules or a matching Placebo. Each participant performed endurance exercise training 4 times per week and visited the study center on the day of recruitment (baseline) and end of week-4 and week-8. The efficacy outcome measures were endurance (overall, cardiovascular, respiratory, and metabolic), power, work, physical and central fatigue, and stress, whereas safety outcomes were adverse events monitoring, compliance, and biochemical laboratory measurements.

**Results:** FEΔE supplementation (but not Placebo) showed statistically significant beneficial changes in overall (increased time to exhaustion and total distance run), respiratory (increased oxygen consumption), metabolic (increased metabolic equivalent and decreased non-esterified fatty acids levels), physical fatigue (decreased Wingate fatigue index), central fatigue (reduced visual analog score), and mental endurance (decreased mental domain score of "Multidimensional Fatigue Symptom Inventory-Short Form" during within the group (vs. baseline) comparisons. The safety outcome measures did not show difference between groups (FEΔE vs. Placebo).

**Conclusion:** Eight weeks of FEΔE supplementation in recreationally active participants resulted in comprehensive endurance enhancement, including respiratory, metabolic, and mental endurance. FEΔE supplementation was found to be safe without serious adverse events.

**Keywords:** Standardized extract, Fenugreek seeds, Endurance, Exercise performance, Energy balance.

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## INTRODUCTION

Endurance capacity is essential indicator of fitness which includes capacity of circulatory and respiratory systems [1]. Performance during distance events (e.g., running, swimming, and biking) largely depends on aerobic endurance capacity [2]. Long-distance exercise requires a steady stream of endogenous biomolecules such as adenosine triphosphate (ATP) to maintain optimal performance [3]. To sustain the required ATP resynthesis rates, alternative metabolic pathways in the lungs, heart, and brain must be activated [3].

During prolonged endurance exercise, coordination between various organs and systems is imperative to optimize ATP production by facilitating oxygen transport through the heart, enabling oxygen intake through the lungs, supporting oxygen diffusion to skeletal muscles, driving motivation and reward processes in the brain, regulating fat mass and body composition, and facilitating muscle contraction [4]. Numerous interventions are available for increasing metabolic, cardiorespiratory, mental, and skeletal muscle endurance. However, there is a demand for holistic interventions that are effective and safe for improving overall endurance.

In recent years, fenugreek seeds have gained attention for their potential in exercise performance and sports nutrition [5]. For centuries, the brownish-yellow seeds of fenugreek have been utilized as a spice and medicine [6]. Fenugreek seeds are rich in nutrients, including proteins, fibers, and a variety of minerals and have traditionally been used to treat a range of conditions, including diabetes and cardiovascular disease [7].

A recent systematic review detailed the role of fenugreek seeds as a natural supplement for athletes, especially as an ergogenic aid [8]. Fenugreek supplementation improves lipid profile in obese females performing endurance exercise [9]. Fenugreek seed extracts have been shown to improve strength [10], anaerobic exercise [11], and threshold power [12] with body fat reduction [12,13], suggesting comprehensive endurance enhancement potential.

Bioactive compounds isolated from fenugreek seeds, such as branched-chain amino acids (BCAA) [14-16], alkaloid such as trigonelline [17], soluble fibers [13], and glycosides [18,19] are known to improve endurance parameters. In addition, BCAA reduces the accumulation of serotonin, limiting the development of central fatigue during endurance events [20-22]. Trained male participants showed enhanced rate of post-exercise resynthesis of glycogen after consumption of an oral glucose drink containing 4-hydroxyisoleucine (4-HI), a main BCAA in fenugreek seeds [23]. Fenugreek supplementation also increases creatine delivery without requiring carbohydrates [24]. In addition, safety of standardized fenugreek seed extract based on 4-HI with trigonelline (IDM01) and glycosides (SFSE-G) during toxicology studies in rats without mutagenicity is reported [25,26]. Therefore, glycosides, trigonelline, and 4-HI containing fenugreek seed extract have potential to contribute toward endurance enhancement.

Several studies have suggested efficacy of fenugreek extract supplementation for benefits toward several physiological systems and processes related to metabolic, cardiorespiratory, skeletal muscle, and mental endurance [5]. Fenugreek supplementation has been reported to reduce muscle soreness and improve muscle recovery in humans [18]. Recently, specific fenugreek seed extract composition (standardized to 4-HI, trigonelline, and glycosides) was reported to enhance endurance capacity in animals subjected to treadmill endurance exercise [27]. However, clinical evidence for comprehensive endurance enhancement effects in the exercising human population is needed. Consequently, this study was undertaken with objective of the efficacy and safety evaluation of oral supplementation with standardized fenugreek seed extract composition capsules (coded as FEΔE) in combination with endurance exercise for 8 weeks. The overall endurance capacity, cardiorespiratory endurance, muscle strength and power, metabolism, and fatigue-related parameters of healthy young men during exercise were assessed.

## METHODS

### Design of study

The randomized, double-blind design and 8-week supplementation period was followed for the study. The study approved the study protocol (No. 2018000672), was approved by ethics committee of study center, and complied with the "Declaration of Helsinki." The study was registered with the "Australian and New Zealand Clinical Trial Registry" (ACTRN12618001356257).

### Participants

One hundred and ninety-one healthy, recreationally active male participants from Southeast Queensland, Australia, were screened based on the following inclusion and exclusion criteria. Participants who provided written informed consent were included if they were male, aged between 18 and 40 years old, had a body mass index between 18.5 and 25 kg/m$^2$, participated in recreational exercise (e.g., light to moderate jogging) for a minimum of 2 days per week, had daily dietary habits (i.e., no prescribed, slimming, vegan, and macrobiotic diet), agreed to maintain diet and exercise program, and not to use other supplements. Participants were excluded if they were female (due to the potential interference of the female hormonal cycle on the effectiveness of fenugreek), were taking any prescribed anticoagulation therapy, were active smokers, used nicotine, had a history of alcohol or drug abuse, had an allergy to active or placebo formula or ingredients, had an unstable or severe illness, mood disorder (assessed using the "Hamilton Depression Inventory" [28]), insomnia, clinically significant acute or chronic inflammation, connective tissue disease, arthritis, or any history of infection in the month, was working in night-shifts or not having normal sleep, neurological disorder(s), and participated in other studies in the past 3 months, before the study. After obtaining consent, the suitability of participants as per the "Exercise and Sports Science Australia Adult Pre-exercise Screening System" [29] and the "Physical Activity Readiness Questionnaire" [30] was determined before evaluation for inclusion.

### Sample size

The sample size for this 3 groups (two FEΔE and one Placebo) study was calculated considering type II or type I error, i.e., 20% (β=0.20, power=80%) and 5% (α=0.05), respectively, and input data for $VO_{2max}$ from relevant research publications [31]. To detect an increase of 5% in $VO_{2max}$ from post-endurance training values (56.8±6.3 mL/kg/min) for 8 weeks, 153 participants were recruited (including 40% dropout allowance over a minimum of 36 participants) in each of three treatment groups (51 subjects in each group).

### Blinding

The study was performed using 3 (two FEΔE and one Placebo) groups of subjects for 8 weeks. Once enrolled, the participants were randomly allocated (1:1:1 ratio) using a computer-generated randomization code to either (a) Placebo, (b) 300 mg FEΔE (FEΔE-300), or (c) 600 mg FEΔE (FEΔE-600). Neither the participant nor the investigator knew the allocated treatment (double-blind). Participants' baseline characteristics (demographic and clinical) were recorded at baseline, following randomization, before starting their assigned treatment.

### Intervention

The investigational product (IP) contained an active ingredient (a powder of standardized fenugreek seed extract composition) which was provided by Indus Biotech, Ltd. (Pune, India). The final investigational products were made as capsules of FEΔE (available in market as Enducor) in two doses, FEΔE-300 and FEΔE-600 (containing 300 mg and 600 mg, respectively), and matching Placebo capsules (containing maltodextrin) provided by the manufacturer (AVS Nutrition, NSW, Australia). The composition of active ingredient of FEΔE capsules was 17.70% of 4-HI, 24.86% of trigonelline, and 20.74% of select glycosides (Vicenin 1, isoorientin, Vicenin 3, schaftoside, Vicenin 2, isovitexin, orientin, vitexin, isoschaftoside, vitexin-2-o-rhamnoside, and steroidal saponins) as characterized by HPLC by previously reported methods [27].

The doses of FEΔE (300 mg and 600 mg) were calculated based on the reported effective doses in animals. The 30 and 60 mg/kg doses significantly enhanced the endurance capacity and cardiorespiratory performance of rats during treadmill endurance exercise [27]. Therefore, the "human equivalent dose" (HED) for an average human weight of 60 kg was derived from inputs of doses (30 and 60 mg/kg in rats) in the formula recommended by "USFDA guidance for industry" [32], as 290.32 mg (rounded to 300 mg) and 580.64 mg (rounded to 600 mg) per day.

### Study procedures and outcome measures

At baseline, week-4, and week-8, participants attended clinic at similar times to ensure consistency in all assessments. Before each visit, the participants adhered to a fasting state, did not consume coffee, tea, or other stimulants for 2 h, and were requested to avoid moderate-to-high-intensity exercise for 24 h before each visit. The participants underwent demographic assessments (age, waist and hip circumference, height, weight), endurance parameters for cardiorespiratory function, muscle strength and power, metabolism, and fatigue, questionnaires for fatigue, quality of life (Baseline, week-4 and 8) and cortisol (salivary and plasma) levels, and biochemical assessments (blood) at Baseline and week-8 as illustrated in Table 1.

Details of the outcome measurements (efficacy and safety) with the associated methods, machines, and parameters are presented (Table 1).

### Statistical analysis

All recruited participants (n=153) were considered for statistical analysis of the intent-to-treat (ITT) population. The sphericity assumption test was performed using Mauchly's test. The Greenhouse–Geisser correction was used for significance, wherever appropriate. Effect of time, treatment, and time-treatment interaction, considering baseline measurements as covariates, was analyzed with repeated measures analysis of covariance, resulting in estimated marginal means at weeks-4 and week-8. Each baseline characteristics and efficacy outcome measure related to endurance (overall, cardiovascular, respiratory, metabolic), power, work, fatigue scores (Borges, Visual Analog Scale [VAS], and multidimensional fatigue symptom inventory-short form [MFSI-SF]), biochemical parameters related to stress, and organ function tests were expressed as mean ± standard deviation, tested for normality using Shapiro-Wilk test and analyzed by paired "t" test. Moreover, a pairwise comparison of each efficacy outcome measure was performed within the groups (vs. baseline) and between the groups (FEΔE vs. Placebo). Data on compliance with supplementation and exercise (number of participants compliant and non-compliant) were evaluated using "Pearson's Chi-square test" with continuity correction. Statistical significance at a 5% level was considered for analysis using SPSS Statistics for Windows (version 26.0; IBM Corp, Armonk, NY).

**Table 1: Details of efficacy and safety outcome measures**

| Outcome measure, method, and machine | Process and parameters |
|---|---|
| Efficacy (Overall endurance)<br>Method: Modified Balke protocol [33]. | Machine: Treadmill with controlled by software (SentrySuite™; Vyaire Medical, Chicago, IL, USA)<br>Process: 5 min acclimatization→3 min walk (1% grade, speed - 4 km/h) → 6 min (5% grade, speed↑0.5 km/h every min) → 10% grade, speed↑0.5 km/h every min→exhaustion.<br>Duration (min) → ↑ 5 min/2 weeks, intensity → ↑ 5%/2 weeks, sessions→3 during visits else 4.<br>Parameter: Time to exhaustion (min), Total distance run (m) |
| Efficacy (Cardiovascular endurance)<br>Method: Modified Balke protocol [33]. | Machine: Continuous heart rate monitoring for short-range telemetry (Polar Electro S610; Kempele, Finland)<br>Parameter: Systolic and Diastolic blood pressure (mmHg), HR at GET1, HR at GET2, Maximal HR (beats/min) |
| Efficacy (Respiratory Endurance)<br>Method: Modified Balke protocol [33]. | Machine and Process: Oro-nasal mask (Model 7940; Hans Rudolph, Kansas, MO, USA) → turbine flow sensor (Vyaire Medical, Chicago, IL, USA) → Digital volume transducer (Vmax® Encore system, CareFusion, Yorba Linda, CA, USA)<br>Parameter: Maximal RER, $VO_2$ at GET1 and GET2 (ml/kg/min), $VO_2$ max (ml/min/kg), Velocity at GET1 (km/h), Velocity at GET2 (km/h), Maximal velocity $vVO_2$ max (km/h) |
| Efficacy (metabolic endurance)<br>Method: Blood withdrawal → immediately centrifuged → serum vacutainer → incubated (30 min at room temperature) → centrifuged (10 min, 2700×g, 4°C). | Machine: EDTA vacutainer (BD, Plymouth, UK), Central laboratory (Cardinal Bioresearch Pty Ltd, Slacks Creek, QLD, Australia)<br>Parameter: NEFA (mmol/l) and fasting glucose (mmol/l) |
| Efficacy (metabolic endurance, power and work)<br>Method: Wingate test [34]. | Machine: Friction braked cycle ergometer (Ergomedic 874E; Monark Vansbro, Sweden)<br>Process: 30 min rest→Wingate test→3 min warm-up at 60 W power output→workload adjusted (0.075×body mass) → participants cycled at maximal cadence for 30 s.<br>Parameter: Maximal MET, total peak power, resting mean power (W/kg), mean power (W), peak power (W), total work (Joules), Wingate fatigue index (%) |
| Efficacy (Fatigue)<br>Method: Borgs 6–20 scale [35] → Perceived exertion VAS [36] → Pain MFSI-SF [37] → Fatigue | Process and parameter: Measurement of scores on each item and total scores |
| Efficacy (Stress) and Safety (Biochemistry)<br>Method: Blood withdrawal → immediately centrifuged → serum vacutainer → incubated (30 min at room temperature) → centrifuged (10 min, 2700×g, 4°C). | Machine: EDTA vacutainer (BD, Plymouth, UK), Central laboratory (Cardinal Bioresearch Pty Ltd, Slacks Creek, QLD, Australia)<br>Parameter: Plasma cortisol levels, Liver Function (ALT and AST), Kidney Function (Creatinine), Electrolytes (Sodium and potassium) |
| Efficacy (stress)<br>Method and process: Saliva collection → centrifuged (2 min, 1000×g, room temperature). | Machine; Salivette® cotton swabs (Sarstedt, Nümbrecht, Germany).<br>Parameter: Saliva cortisol level |
| Safety (adverse events)<br>Method: As per CTCAE criteria | Process and parameter: Classification of AEs |
| Safety (Compliance for investigational product, IP)<br>Method: Weekly contact and remaining capsules count after 8 weeks | Process and parameter: % compliance |
| Safety (exercise)<br>Method: Physical exercise tracking system (Strava, San Francisco, CA, USA) | Process and parameter: % compliance |

GET1: First gas exchange threshold, GET2: Second gas exchange threshold, $VO_2$ max: Maximal oxygen uptake, RER: Maximal respiratory exchange ratio, $VO_2$: Oxygen uptake, $vVO_2$ max: Maximal velocity, EDTA: Ethylenediaminetetraacetic acid, NEFA: Non-esterified fatty acids, Maximal MET: "Maximal metabolic equivalent", VAS: Visual analog scale, MFSI-SF: "Multidimensional fatigue symptom inventory-short form," ALT: Alanine transaminase, AST: Aspartate transaminase, CTCAE: "Common terminology criteria for adverse events," HR: Heart rate

## RESULTS

### Demographics

A study flowchart with participant's number (screened, randomized, followed up, and analyzed) is presented in Fig. 1. A total of 191 potential participants were screened, 38 excluded (35 did not match inclusion criteria and 3 did not consent to participation). As a result, 153 participants formed the ITT population which undergone random allocation three groups (1:1:1 ratio): Placebo, FEΔE-300, or FEΔE-600 (i.e., 51 participants per group), of which 5, 5, and 6 participants, respectively, did not consume the intervention because they either withdrew consent or were lost to follow-up. Rest of the participants were considered as modified ITT (mITT). Ninety-nine participants (34 from the Placebo, 32 from the FEΔE-300, and 33 from the FEΔE-600) completed all study procedures (per-protocol population). Demographic characteristics were measured at baseline (Table 2). No significant differences were observed in the baseline characteristics values between the groups, suggesting a uniform distribution of participants as indicated in Table 2.

### Effects on overall endurance (time-to-exhaustion and total distance run)

The results of the 8-week supplementation with FEΔE indicated a significant enhancement in overall endurance (Table 3), as indicated

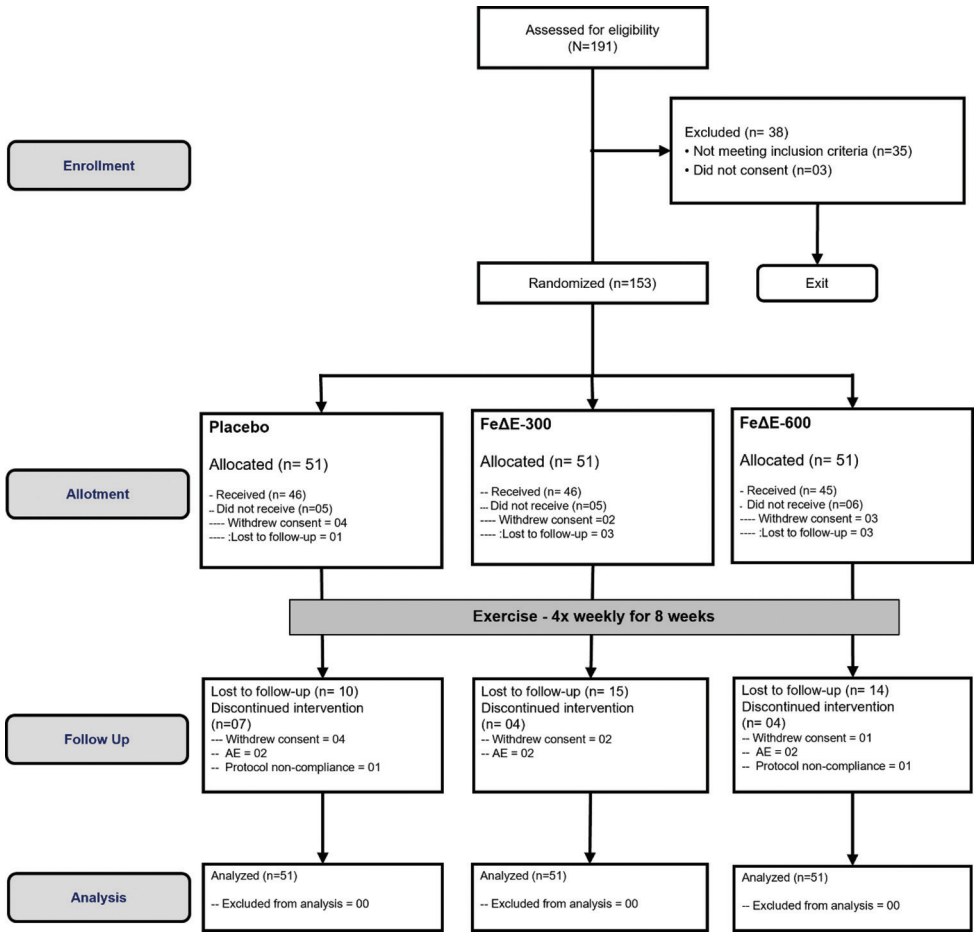


**Fig. 1: CONSORT flow diagram**

**Table 2: Summary of baseline characteristics**

| Parameters | Placebo | FEΔE-300 | FEΔE-600 |
|---|---|---|---|
| Age (years) | 30.70±5.80 | 29.45±6.08 | 30.80±5.90 |
| Height (m) | 1.80±0.07 | 1.79±0.07 | 1.79±0.06 |
| Weight (kg) | 78.07±9.79 | 76.93±9.81 | 78.12±1.49 |
| Body mass index (kg/m$^2$) | 24.15±2.16 | 23.97±2.33 | 24.21±2.34 |
| Waist circumference (cm) | 85.89±6.71 | 85.80±7.19 | 84.49±6.41 |
| Hip circumference (cm) | 99.41±5.69 | 98.441±5.56 | 99.52±5.68 |
| Waist-hip ratio | 0.86±0.04 | 0.87±0.05 | 0.85±0.039 |

Numbers in the parenthesis: dosage in mg/kg/day, Data as Mean±Standard deviation. No significant difference between the groups (paired t-test); n=51 participants in each group (mITT population)

by the increased time to exhaustion and total distance run at week-8 (vs. baseline) for both FEΔE-300 ($p<0.05$) and FEΔE-600 ($p<0.01$). However, significant differences (vs. Placebo) in time to exhaustion and total distance run between the treatment groups were not found, regardless of the dose of FEΔE administered.

**Effects on cardiovascular endurance**

Data related to the effects on cardiovascular endurance are shown in Table 3. None of the tested doses of FEΔE showed significant differences in cardiovascular endurance-related parameters (except diastolic blood pressure at week-4) between groups (vs. Placebo) at baseline, week-4, or 8. The HR at GET1 in FEΔE-300 at week-8 (but not at week-4) showed a significant ($p<0.01$) decrease at 8 weeks (vs. baseline). Supplementation with FEΔE-600 showed in a significant decline of maximal HR ($p<0.05$) at week-4 (vs. baseline), with the absence of significant differences at week-8. Placebo-supplemented participants did not show significant differences in within-group comparisons.

**Effects on respiratory endurance related parameters**

Respiratory and metabolic endurance-related parameters are shown in Table 4. The FEΔE-300 supplemented group had no significant differences within the group, at week-4 or week-8 (vs. baseline) in respiratory endurance-related parameters (Maximal RER, $VO_2$ at GET1, $VO_2$ at GET2, $VO_2$ max, Velocity at GET1, Velocity at GET2, or Maximal velocity $vVO_{2\ max}$) at week-4 or week-8 except for Velocity at GET2 when a significant increase ($p<0.05$) was observed within the group (week-8 vs. baseline). In addition, a significant increase within the group (vs. baseline) was found in FEΔE-600 supplemented group in $VO_2$ at GET1 at week-8 ($p<0.05$), $VO_{2\ max}$ at week-4 and week-8 ($p<0.05$ and $p<0.01$), Velocity at GET2 at week-8 ($p<0.05$), and maximal velocity $vVO_{2\ max}$ at week-4 and week-8 ($p<0.05$ and $p<0.01$). However, parameters related to respiratory endurance showed no significant differences between the groups (vs. Placebo).

**Effects on metabolic endurance-related parameters**

Supplementation with FEΔE-600 resulted in a significant enhancement in maximal MET within the group (vs. baseline) at week-4 ($p<0.05$) or week-8 ($p<0.01$), as presented in Table 4. However, the FEΔE-300 supplemented group did not show significant differences in MET between week-4 and week-8 within the group (vs. baseline). However, supplementation with FEΔE-600 significantly increased MET levels within the group (vs. baseline) at week-8. FEΔE-300 and FEΔE-600 showed a decrease in non-esterified fatty acids (NEFA) at week-8 ($p<0.01$) (vs. baseline). Placebo did not have significant differences within the group at week-4 or week-8 (vs. baseline) in measured

**Table 3: Effect of FEΔE on overall and cardiovascular endurance-related parameters**

| Parameters | Visit | Placebo | FEΔE-300 | FEΔE-600 |
|---|---|---|---|---|
| Overall endurance | | | | |
| Time to exhaustion (min) | Baseline | 13.24±1.88 | 12.72±2.13 | 12.95±1.73 |
| | Week-4 | 13.78±1.90 | 13.13±1.98 | 13.34±1.83 |
| | Week-8 | 14.51±1.85 | 13.77±2.29* | 13.60±1.89** |
| Total distance run (m) | Baseline | 2283.83±419.81 | 2173.70±466.05 | 2218.38±384.72 |
| | Week-4 | 2406.01±429.01 | 2260.44±447.48 | 2304.12±412.05 |
| | Week-8 | 2568.20±430.92 | 2407.65±529.45* | 2363.20±431.02** |
| Cardiovascular endurance | | | | |
| Systolic blood pressure (mmHg) | Baseline | 125.93±9.11 | 123.55±10.71 | 123.10±11.06 |
| | Week-4 | 125.11±8.27 | 122.43±11.99 | 121.68±10.65 |
| | Week-8 | 123.71±9.96 | 122.39±10.68 | 121.69±12.72 |
| Diastolic blood pressure (mmHg) | Baseline | 75.99±7.84 | 74.00±8.57 | 73.78±8.85 |
| | Week-4 | 74.83±7.51 | 74.89±7.42 | 71.58±7.51# |
| | Week-8 | 75.16±8.24 | 72.48±6.02 | 72.77±7.99 |
| HR at GET1 (beats per min) | Baseline | 126.62±12.91 | 123.55±15.12 | 126.35±17.77 |
| | Week-4 | 128.45±13.27 | 121.29±24.81 | 125.25±12.61 |
| | Week-8 | 123.60±14.12 | 120.03±27.78** | 123.18±13.56 |
| HR at GET2 (beats per min) | Baseline | 149.30±13.23 | 145.39±12.90 | 146.54±13.59 |
| | Week-4 | 149.37±12.32 | 145.15±10.73 | 147.10±11.53 |
| | Week-8 | 147.31±13.41 | 145.30±14.13 | 150.04±10.40 |
| Maximal HR (beats per min) | Baseline | 182.71±13.42 | 185.06±9.62 | 181.62±9.60 |
| | Week-4 | 183.03±9.07 | 181.87±9.50 | 179.95±8.47* |
| | Week-8 | 182.56±8.12 | 181.31±10.93 | 181.03±8.18 |

Figure in the parenthesis indicates dosage in mg/day; Data are represented as Mean±standard deviation. Data were analyzed using repeated measures analysis of covariance using the baseline value as a covariate and paired t-test; # p<0.05 (vs. Placebo), *p<0.05 and **p<0.01 (vs. baseline). HR: Heart rate, GET1: Gas exchange threshold 1, GET2: Gas exchange threshold 2, $VO_2$: Oxygen uptake value, n=30–51 participants (mITT population)

**Table 4: Effect of FEΔE on respiratory and metabolic endurance-related parameters**

| Parameters | Visit | Placebo | FEΔE-300 | FEΔE-600 |
|---|---|---|---|---|
| Respiratory endurance | | | | |
| Maximal RER | Baseline | 1.37±0.19 | 1.40±0.16 | 1.40±0.15 |
| | Week-4 | 1.39±0.19 | 1.42±0.17 | 1.38±0.18 |
| | Week-8 | 1.35±0.15 | 1.44±0.16 | 1.37±0.17 |
| $VO_2$ at GET1 (mL/kg/min) | Baseline | 26.67±4.60 | 24.60±4.52 | 25.18±5.38 |
| | Week-4 | 26.56±4.74 | 26.20±4.20 | 26.20±4.69 |
| | Week-8 | 26.52±4.98 | 27.52±4.92 | 25.85±5.07* |
| $VO_2$ at GET2 (mL/kg/min) | Baseline | 36.13±5.64 | 33.38±6.20 | 34.36±5.55 |
| | Week-4 | 36.57±6.69 | 35.17±5.05 | 35.64±5.84 |
| | Week-8 | 37.53±5.47 | 35.65±5.82 | 36.39±4.89 |
| $VO_2$ max (mL/min/kg) | Baseline | 51.72±6.86 | 48.71±6.75 | 49.61±6.44 |
| | Week-4 | 52.31±7.76 | 50.80±7.02 | 51.12±6.40* |
| | Week-8 | 54.19±7.24 | 51.81±7.33 | 52.23±6.08** |
| Velocity at GET1 (km/h) | Baseline | 7.64±0.38 | 7.57±0.52 | 7.56±0.62 |
| | Week-4 | 7.66±0.70 | 7.69±0.43 | 7.71±0.41 |
| | Week-8 | 7.71±0.26 | 7.80±0.32 | 7.57±0.63 |
| Velocity at GET2 (km/h) | Baseline | 9.05±0.91 | 8.81±0.98 | 8.90±0.87 |
| | Week-4 | 9.28±0.99 | 8.10±0.94 | 9.06±0.88 |
| | Week-8 | 9.36±0.88 | 9.09±0.92* | 9.30±0.79* |
| Maximal velocity $vVO_2$ max (km/h) | Baseline | 11.72±0.94 | 11.38±1.01 | 11.50±0.89 |
| | Week-4 | 11.89±0.96 | 11.61±1.01 | 11.71±0.89* |
| | Week-8 | 12.17±1.08 | 11.91±1.13 | 11.88±0.96** |
| Metabolic endurance | | | | |
| Maximal MET | Baseline | 14.78±1.96 | 13.92±1.93 | 13.90±2.69 |
| | Week-4 | 14.94±2.22 | 14.51±2.01 | 14.61±1.83* |
| | Week-8 | 15.48±2.06 | 14.80±2.10 | 14.91±1.73** |
| NEFA (mmol/L) | Baseline | 0.42±0.28 | 0.40±0.18 | 0.48±0.33 |
| | Week-8 | 0.37±0.21 | 0.28±0.15** | 0.30±0.19** |
| Plasma glucose (mmol/L) | Baseline | 4.97±0.38 | 4.88±0.41 | 4.78±0.35 |
| | Week-8 | 4.89±0.54 | 4.60±0.86 | 4.68±0.56 |

Numbers in parentheses indicate dosage in mg/day; The data represented as mean±standard deviation; Data analysis with repeated measures analysis of covariance with baseline value as a covariate and paired t-test; n=30-51 participants (mITT population). *p<0.05 and **p<0.01 (vs. baseline), $vVO_2$ max: Maximal velocity, GET2: Gas exchange threshold 2, MET: Metabolic equivalent, NEFA: Non-esterified fatty acids, RER: Respiratory exchange ratio, GET1: Gas exchange threshold 1

metabolic endurance-related parameters. In addition, plasma glucose levels in the FEΔE-300 and FEΔE-600 showed no significant differences within the groups at week-8 (vs. baseline) or between the groups (vs. Placebo).

**Effects on power and strength parameters during wingate power test**

The power and strength parameter results are listed in Table 5. Supplementation with FEΔE-300 but not FEΔE-600 or Placebo had

**Table 5: Effect on power and strength parameters during wingate power test**

| Parameters | Visit | Placebo | FEΔE-300 | FEΔE-600 |
|---|---|---|---|---|
| Total peak power (W/kg) | Baseline | 9.44±1.30 | 9.21±1.54 | 9.52±1.50 |
| | Week-4 | 9.31±1.36 | 9.41±1.54 | 9.27±1.24 |
| | Week-8 | 9.42±1.35 | 9.28±1.42 | 9.50±1.32 |
| Resting mean power (W/kg) | Baseline | 7.63±0.97 | 7.97±1.03 | 7.94±0.91 |
| | Week-4 | 7.82±1.00 | 8.06±0.92 | 7.82±0.96 |
| | Week-8 | 7.78±0.93 | 7.82±0.99 | 7.79±0.88 |
| Mean power (W) | Baseline | 593.91±104.87 | 605.88±108.80 | 615.88±99.72 |
| | Week-4 | 607.59±102.17 | 611.93±103.46 | 612.47±101.79 |
| | Week-8 | 604.11±103.11 | 596.57±105.32 | 607.39±104.67 |
| Peak power (W) | Baseline | 738.16±142.10 | 705.62±162.97 | 739.02±142.44 |
| | Week-4 | 727.18±144.47 | 714.79±160.13 | 724.10±131.42 |
| | Week-8 | 730.54±132.35 | 710.3±149.18 | 744.29±155.40 |
| Total work (Joules) | Baseline | 17868.16±3135.68 | 18130.50±3247.58 | 18476.42±2991.70 |
| | Week-4 | 17833.57±4200.58 | 18357.77±3103.63 | 18340.27±3002.38 |
| | Week-8 | 18123.27±3093.15 | 17897.08±3159.45 | 18221.71±3140.24 |
| Wingate fatigue index (%) | Baseline | 39.00±16.40 | 38.86±20.15 | 41.12±14.90 |
| | Week-4 | 39.30±15.76 | 37.44±13.25* | 35.66±13.30 |
| | Week-8 | 41.44±15.44 | 37.15±17.11* | 39.26±12.72 |

Numbers in the parenthesis indicate dosage in mg/day; Data are represented as Mean±standard deviation. Data analyzed using repeated measures analysis of covariance using the baseline value as a covariate followed by paired t-test; No significant difference between groups; n=30–51 participants (mITT population)

significant ($p<0.05$) reduction of "Wingate fatigue index" at week-4 and week-8, (vs. baseline, within the group). However, no treatment group showed significant differences in the power and strength parameters between the groups (vs. placebo).

**Effects on fatigue symptoms and related parameters**

Data on fatigue and stress-related parameters measured as scores of perceived exertion on the Borgs- 6–20 scale, VAS, and MFSI-SF (general, physical, emotional, mental, vigor, and total) are shown in Table 6. None of the treatment groups, FEΔE-300 or FEΔE-600, showed significant differences (between groups) for any of the fatigue or stress-related parameters at either week-4 or week-8 (vs. Placebo), except for a significant decrease in the fatigue score (measured by VAS) within the group at week-4 (vs. baseline) in the FEΔE-300 group.

Significant differences were not found in the perceived exertion and VAS scores in the FEΔE-300 or FEΔE-300 supplemented groups (vs. Placebo) or within groups (at week-4 or week-8, vs. baseline). The FEΔE-600 showed a significant decrease in mental fatigue ($p<0.05$) at week-8 within the group (vs. baseline). However, the FEΔE-300 had a significant raise within the group in general ($p<0.05$) (week-8) and physical ($p<0.05$) (weeks 4 and 8) and total scores ($p<0.01$) (week-8) of the MFSI-SF questionnaire.

In addition, the stress-related parameters, namely plasma and salivary cortisol levels, in the FEΔE-300 or FEΔE-600 supplemented group did not show significant change between the groups either at week-4 or week-8 (vs. Placebo).

**Effects on safety outcome measures, compliance, and adverse events**

The participants had good compliance (>80%) with the investigational product (88.0% and 93.2%) and exercise (91.4% and 87.1%) for FEΔE-300 and FEΔE-600, respectively, with no statistical difference compared to Placebo (92.6% and 92.1%, respectively). The biochemical safety outcome measures performed at baseline and 8-weeks are depicted in Table 7. The data of representative safety outcome measures related to liver function (alanine transaminase and aspartate transaminase), kidney function (creatinine), and serum electrolyte levels (sodium and potassium) did not show significant differences between treatments (FEΔE-300 or FEΔE-600 vs. Placebo) or within groups (vs. baseline), except for significant decline ($p<0.05$) in potassium levels in Placebo group. All values were within normal physiological limits, indicating the safety of the treatments.

All treatments were well tolerated by most participants. Seven participants from Placebo, four participants from FEΔE-300, and four from FEΔE-600 discontinued the treatment and dropped out of the study for reasons such as withdrawal of consent, AEs, or protocol non-compliance, as shown in Fig. 1. A total of eight AEs (three in Placebo, three in FEΔE-300, and two in FEΔE-600, as described in Table 8) were observed. None of the AE were severe, but seven AEs of mild intensity and one AE of moderate intensity were found. The moderate-intensity AE (Achilles pain) subsided with rest and physiotherapy.

## DISCUSSION

The present study assessed the effect of FEΔE capsules at two doses (300 mg/day or 600 mg/day) with exercise training for 8-weeks on the endurance capacity of healthy young men using a gold standard design (randomized double-blind placebo-control). During the 8-week study period, FEΔE supplementation was well tolerated and safe, with comprehensive enhancement, including respiratory and metabolic endurance-related physiological benefits to participants. The FEΔE-supplemented group showed a higher time to exhaustion, shorter ventilatory threshold, and reduced mental fatigue during exercise without affecting vital blood biochemical parameters. Females were not included in this study because of the possibility of interference of hormonal changes during the menstrual cycle, which can affect exercise performance [39] and related outcome measures, such as muscle glycogen [40], muscle strength, and power performance [41].

This study implemented a comprehensive exercise regimen that included both aerobic and anaerobic schedules. Aerobic exercise generally lasts for an extended duration and aims to improve cardiorespiratory endurance by utilizing oxygen in the muscles during physical activity [1]. Conversely, anaerobic exercises, which are generally shorter in duration, are intended to increase muscle power, strength, and size without the need for oxygen [1].

Aerobic exercise with treadmill was performed using a modified version of the Balke protocol [33]. This protocol is widely recognized for improving cardiovascular fitness by progressively increasing exercise intensity [42]. Anaerobic training was performed using Wingate test with a cycle ergometer. Wingate test is a widely recognized technique for assessing an individual's anaerobic capacity and power output [43]. Wingate test is a straightforward and time-efficient method for assessing performance with brief yet intense cycling period against high resistance, which is effective for muscle power and size enhancement [43].

**Table 6: Effect of FEΔE on fatigue and stress-related parameters**

| Parameters | Visit | Placebo | FEΔE-300 | FEΔE-600 |
|---|---|---|---|---|
| Fatigue-related | | | | |
| Perceived exertion score (Borgs 6–20 scale) | Baseline | 19.63±1.17 | 19.36±1.46 | 19.73±0.95 |
| | Week-4 | 19.61±0.93 | 19.58±1.15 | 19.85±0.49 |
| | Week-8 | 19.91±0.38 | 19.67±0.92 | 19.76±0.66 |
| Fatigue score (VAS) | Baseline | 39.44±15.90 | 40.67±19.20 | 40.32±14.47 |
| | Week-4 | 38.16±16.90 | 36.93±13.18* | 35.64±13.30 |
| | Week-8 | 41.84±14.62 | 37.30±16.76 | 38.67±13.33 |
| MFSI-SF scores | | | | |
| General | Baseline | 4.25±3.34 | 3.62±3.35 | 4.00±3.81 |
| | Week-4 | 4.05±3.42 | 3.93±3.56 | 4.72±3.30 |
| | Week-8 | 4.23±4.07 | 4.88±3.11* | 4.22±2.74 |
| Physical | Baseline | 1.90±1.98 | 1.62±1.58 | 1.86±1.99 |
| | Week-4 | 2.34±2.33 | 2.12±1.85* | 2.08±1.80 |
| | Week-8 | 2.10±2.43 | 2.79±2.23** | 1.84±1.69 |
| Emotional | Baseline | 1.96±2.47 | 1.92±3.00 | 2.25±2.90 |
| | Week-4 | 2.03±2.79 | 1.14±1.37 | 2.28±2.96 |
| | Week-8 | 1.27±1.41 | 1.47±2.00 | 1.66±2.01 |
| Mental | Baseline | 2.27±2.47 | 2.20±2.47 | 2.61±2.86 |
| | Week-4 | 1.66±2.12 | 1.86±2.35 | 2.23±2.71 |
| | Week-8 | 1.70±2.22 | 1.97±2.85 | 1.41±1.88* |
| Vigor | Baseline | 9.00±1.84 | 9.14±1.91 | 8.92±2.31 |
| | Week-4 | 8.63±1.78 | 8.50±2.24 | 8.95±1.92 |
| | Week-8 | 9.13±1.94 | 8.35±2.76 | 8.47±1.88 |
| Total | Baseline | 1.39±7.44 | 0.22±8.45 | 1.80±8.62 |
| | Week-4 | 1.45±9.03 | 0.55±7.15 | 2.36±8.72 |
| | Week-8 | 0.17±8.49 | 2.76±7.27** | 0.6±6.81 |
| Stress-related | | | | |
| Plasma cortisol (mmol/L) | Baseline | 382.73±142.93 | 404.72±122.65 | 340.14±131.06 |
| | Week-8 | 427.09±148.95 | 438.63±126.98 | 372.35±122.36 |
| Salivary cortisol (mmol/L) | Baseline | 10.49±7.47 | 10.79±9.59 | 7.96±5.29 |
| | Week-8 | 12.38±8.41 | 15.36±14.51 | 9.07±5.63 |

Numbers in parentheses: dosage in mg/day; Data as mean±standard deviation. Analysis with repeated measures analysis of covariance using the baseline value as a covariate and paired t-test; * p<0.05 and **p<0.01 (vs. baseline); n=30–51 participants (mITT population)

**Table 7: Effect of FEΔE on safety outcome measures-biochemistry**

| Parameters | Visit | Placebo | FEΔE-300 | FEΔE-600 |
|---|---|---|---|---|
| ALT (U/L) | Baseline | 27.10±7.40 | 27.60±7.60 | 27.60±7.00 |
| | Week-8 | 29.70±9.90 | 26.60±9.00 | 24.80±10.40 |
| AST (U/L) | Baseline | 29.49±18.86 | 28.67±8.90 | 25.42±7.35 |
| | Week-8 | 29.36±10.12 | 25.12±5.86 | 27.79±13.17 |
| Creatinine (μmol/L) | Baseline | 94.89±15.29 | 96.90±12.50 | 96.90±13.70 |
| | Week-8 | 95.8 0±19.20 | 95.60 a±18.60 | 92.10±20.10 |
| Sodium (mmol/L) | Baseline | 141.00±2.00 | 141.00±3.00 | 140.00±2.00 |
| | Week-8 | 141.00±4.00 | 141.00±3.00 | 140.00±3.00 |
| Potassium (mmol/L) | Baseline | 4.09±0.49 | 4.05±0.56 | 3.99±0.56 |
| | Week-8 | 3.82±0.53* | 4.03±0.62 | 4.14±0.66 |

Numbers in the parenthesis indicate dosage in mg/day; data are represented as Mean±standard deviation, ALT: Alanine transaminase, AST: Aspartate transaminase, data analyzed using paired t-test; * p<0.05 (vs. Baseline); n=29–35 participants (mITT population)

Endurance exercise is closely related to the production and utilization of ATP, which is the primary cellular energy source. Research has demonstrated that endurance training can cause adaptations in mitochondrial ATP production in human skeletal muscles [44]. Maintaining ATP levels during endurance exercise and sustaining muscle function requires energy regulation [45]. Fenugreek seed marker compound, trigonelline, stimulate biochemical processes in endogenous ATP production and protect vital organs such as lungs and heart from oxidative stress [17]. Furthermore, fenugreek seeds have potential mechanisms as hypoglycemic and hypolipidemic agents [46], muscle glycogen resynthesis [23], gut microbiota modulators [47], insulin resistance reducers, and anabolic hormone enhancers [48]. These mechanisms are believed to be behind the present study's observations of reduced fatigue scores on VAS and mental domain score in MFSI-SF and improved overall endurance performance.

The endurance-boosting effects of FEΔE in the present study may be largely attributed to its flavonoid glycosides content which can enhance endurance and reduce fatigue. In the past, flavonoid glycosides from other natural sources were reported to possess endurance-enhancing properties, including increasing $VO_{2max}$, [49] delaying muscle fatigue and promoting recovery [50-52], anti-inflammatory activity [53], enhanced mitochondrial biogenesis [54], and vasodilatory and nitric oxide-boosting effects [55].

The results of this study showed substantial decrease in blood NEFA levels with 8 weeks of FEΔE supplementation, which suggests better utilization of NEFA by skeletal muscles during endurance exercise. Reduced NEFA levels might minimize the need for muscle glycogen, delay fatigue, and enhance aerobic endurance [56,57]. The accelerated resynthesis of glycogen reduces dependence on additional muscle glycogen during endurance exercise, improves cardiorespiratory performance, and postpones muscle fatigue [58].

BCAA, like 4-HI, has been demonstrated to improve metabolic endurance by increasing free fatty acid oxidation and elevating creatine and ATP stores in muscles [59]. Furthermore, the present study showed unchanged glucose levels with FEΔE supplementation, which is consistent with glucose-dependent insulin secretion properties of 4-HI in maintaining blood glucose levels without causing hypoglycemia [60,61]. Previous research on 4-HI has also reported increased muscle glycogen resynthesis in trained male cyclists after 4-HI-rich fenugreek seed supplementation [23]. Fenugreek seed extracts rich in 4-HI and trigonelline have been reported to enhance the performance of males [8,24]. These findings emphasize the metabolic endurance-enhancing effects of FEΔE through increased glucose reuptake in skeletal muscles [62] and its abundance as a fuel to

**Table 8: Effect of FEΔE on safety outcome measures - adverse events (AEs)**

| Adverse event | Severity | Action taken with IP | Causality | Relation to IP |
|---|---|---|---|---|
| Placebo | | | | |
| Urinating less than normal | Asymptomatic or mild | Permanently discontinued | Possible | No |
| Change in urine color | Asymptomatic or mild | Permanently discontinued | Possible | No |
| Drop in Libido | Asymptomatic or mild | None | Possible | No |
| FEΔE- 300 mg | | | | |
| Achilles pain | Moderate | Permanently discontinued | Probable (with endurance exercise schedule) | No |
| Neurological pain (migraine) | Asymptomatic or mild | Permanently discontinued | Unlikely (history) | No |
| Mild reflux | Asymptomatic or mild | None | Possible | No |
| FEΔE- 600 mg | | | | No |
| Increased bowel movements and flatulence | Asymptomatic or mild | Permanently discontinued | Probable (Not during treatment) | No |
| Large drop in libido | Asymptomatic or mild | Permanently discontinued | Possible (Present in Placebo group as well) | No |

Adverse events based on "Common Terminology Criteria for Adverse Events (CTCAE)" version 4.0 [38] according to severity: 1=asymptomatic or mild, 2=moderate, 3=severe, 4=life-threatening, and 5=death

generate ATP and enhance endurance.

This study observed a significant increase in MET after 8 weeks of supplementation with FEΔE. MET is a indicator of the cost of energy on physical activities, represented as a multiple of resting metabolic rate [63], and indicates oxygen consumption during an activity. Higher MET values indicate improved endurance [64] through enhanced oxygen uptake, increased oxygen availability to skeletal muscles, and improved oxygen efficiency [65]. Therefore, the present results of improved respiratory and metabolic endurance by FEΔE supplementation can be attributed to increased MET values through enhanced oxygen uptake [66].

Maintaining an adequate oxygen supply to the body is essential for preserving cardiorespiratory endurance, optimizing muscle performance and health. FEΔE supplementation showed a significant enhancement in oxygen-related physiological markers, including $VO_2$ max, $VO_2$ at GET1, velocity at GET2, and $vVO_2$ max. $VO_2$ max (the highest rate of oxygen consumption during intense exercise) and $vVO_2$ max (the velocity at which $VO_2$ max is achieved) are considered indicators of maximal aerobic capacity [67] and reflect the individual's ability to sustain high-intensity exercise [68]. The ventilatory anaerobic threshold (GET1) and second ventilatory threshold (GET2) were calculated as percentages of $VO_2$ max, which signifies the transition from moderate to heavy exercise intensity [69]. The positive effects of FEΔE supplementation suggest the crucial role of the lungs and oxygen delivery in improving time to exhaustion and reducing fatigue during endurance exercise, as observed in this study.

The enhancement of cardiorespiratory endurance-related parameters observed with FEΔE can be ascribed to the flavonoid glycoside content. A range of bioactive flavonoids derived from natural sources has been reported to enhance endurance and mitigate fatigue in a beneficial way. The fenugreek seed extracts were reported to significant improvement in lung function in animals [70] and human subjects during maximal graded exercise on a cycle ergometer [11] and exercising obese women with type-2 diabetes [9]. These improvements in lung function led to better oxygen delivery to the heart, resulting in improved cardiac performance, as observed in this study.

The effects of mental fatigue and psychological stress on endurance have been widely documented [71]. Eight weeks of supplementation with FEΔE-600 led to a significant improvement in the MFSI-SF score for the mental domains in this study, indicating a mental fatigue reduction and motivation-elevating effect of FEΔE, which, in turn, may have contributed to the overall endurance enhancement.

Trigonelline exerts dopaminergic effects in animals [46,72,73] and human studies [74]. The neurotransmitter, Dopamine, plays a significant role in reward-seeking behavior [75] including exercise motivation and endurance [76]. Hence, the dopaminergic action of trigonelline (a marker of FEΔE) is responsible for reduced mental fatigue (reduced mental domain score in the MFSI-SF) for overall endurance enhancement, as seen in this study.

Another marker compound of FEΔE is 4-HI. Fenugreek seed extract containing 4-HI is documented for stress relieving [77] and mood-enhancing properties [72] through an increase in central nor-epinephrine and decrease in serum cortisol levels in animal studies [77]. Cortisol, a hormone released in response to stress, has physiological and psychological effects on individuals engaged in physical activity and exercise [78] by decreasing mental fatigue [79] and increasing mental endurance [79,80]. In addition, an increase in cortisol concentration during exercise has been well documented [81]. Although the mental domain score in the MFSI-SF was reduced in the FEΔE-supplemented group, the plasma and salivary cortisol levels did not show significant changes within or between the groups (vs. Placebo).

Endurance exercises trigger the production of pro-inflammatory mediators, such as prostaglandins, kinins, and histamine, toward muscle fatigue and swelling [82]. The glycoside-based standardized fenugreek seed extract prevents the release of inflammatory markers in healthy male volunteers following eccentric exercise [18] and to improve muscle endurance in resistant male subjects [10]. Consequently, the improved overall endurance demonstrated by FEΔE in this research can be partially explained presence of flavonoid glycosides which was known for relief from muscle soreness [18]. Therefore, the overall endurance enhancement shown by FEΔE in this study can be partly attributed to the probable reduction in muscle soreness induced by the glycoside content.

All IPs (FEΔE or Placebo) were well tolerated by participants, except for a few instances of asymptomatic and mild AEs, as well as one moderate-intensity AEs that were not related to the study. The safety of FEΔE supplementation was further demonstrated by the results of biochemical outcome measures, which fell within physiological limits and showed no significant differences compared to the Placebo group.

The FEΔE-supplemented group demonstrated a trend toward improvement in many physical fatigue-related efficacy outcome measures without statistical significance between the groups. This may be attributable to several factors, such as shorter treatment period and higher Placebo effect. In addition, the limited number of participants and their diverse and wider physiological status with a non-uniform amount of exercise might have contributed to the large variability. Nevertheless, the prominent efficacy and safety of FEΔE supplementation in enhancing endurance justify more extensive clinical trials involving a larger number of participants or extended study periods.

## CONCLUSION

8-weeks of supplementation with FEΔE, a standardized fenugreek seed extract composition, to recreationally active subjects showed higher

time to exhaustion, shorter ventilatory threshold, enhance metabolic parameters, and reduced mental fatigue, indicating comprehensive endurance enhancement including physical, metabolic, and mental endurance, without compromising safety.

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**AUTHOR'S CONTRIBUTIONS**

PT and PD were involved in the conception and design of study. PT, PD, and MK were involved in drafting, revising, and approval of the manuscript. DV was involved in the analysis and interpretation of data, with drafting, revising, and approval of the manuscript.

**CONFLICT OF INTEREST**

PT, PD, and MK are employees of Indus Biotech Limited, Pune, India, but had no role in data collection and analysis of the study.

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