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Research Article

EFFECTS OF A PROPRIETARY BLEND RICH IN GLYCOSIDE BASED STANDARDIZED FENUGREEK SEED EXTRACT (IBPR) ON INFLAMMATORY MARKERS DURING ACUTE ECCENTRIC RESISTANCE EXERCISE IN YOUNG SUBJECTS

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ABSTRACT

Objective: To assess the efficacy of a proprietary blend rich in glycoside based standardized fenugreek seed extract (400 mg) and minor quantities of curcumin and cinnamon (25 mg each) supplementation (IBPR) on inflammatory markers related to skeletal muscle soreness using double-blind placebo control, parallel design.

Methods: A total of 20 healthy non-resistance trained young male and female subjects were assigned to ingest either IBPR or matching placebo for 14 days before the eccentric exercise bout. Subjects were instructed to perform 24 sets with 10 eccentric knee extensor repetitions (with one leg at 30°/s on an isokinetic device). Subjects had their blood drawn at baseline, immediately post, 1 hr, 3 hrs, and 24 hrs post-eccentric exercise. Efficacy in terms of serum levels of anti-inflammatory cytokines interleukin-10 (IL-10), pro-inflammatory cytokines (IL-1rα, IL-1β, IL-6, and tumor necrosis factor) and safety in terms of kidney function (blood urea nitrogen (BUN), serum creatinine, BUN to creatinine ratio), and differential leukocyte count were measured. The data of each parameter were analyzed by two-way repeated measure ANOVA.

Results: Significant time-dependent effects were observed in IL1β, IL6, and creatinine values from baseline whereas significant treatment dependent effect was seen in IL-1rα. IBPR was found to be safe and well tolerated.

Conclusion: IBPR supplementation showed a significant anti-inflammatory efficacy on eccentric exercise-induced inflammatory markers of skeletal muscle soreness in non-resistance trained subjects.

Keywords: Fenugreek glycosides, Inflammation markers, Eccentric exercise.

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INTRODUCTION

Resistance exercise (RX) promotes muscle strength, physical performance, and healthy living [1]. There are two phases (concentric and eccentric) of muscle contraction during RX. During the eccentric phase of muscle contractions, skeletal muscles generate higher forces. Eccentric muscle actions are essential in performing normal daily activities and physical activities such as stair climb and descent, body transfers, and balance tasks [2]. Eccentric actions occur during sports activities such as running and walking. Eccentric strength is also necessary for retaining functional independence with aging [1].

Eccentric exercise is linked to several health-promoting adaptations. For example, chronic eccentric exercise elicits many beneficial effects, such as favorable changes in blood lipid profile [3,4], improved oxidative stress status [5], and improved insulin sensitivity [6]. Eccentric exercise also has beneficial effects on inflammatory status. Although the eccentric exercise-induced muscle micro damage acutely up-regulates proinflammatory/pro-oxidant agents (e.g., increased interleukin [IL-6]), chronically over a longer term, it down-regulates proinflammatory/pro-oxidant agents and up-regulates anti-inflammatory/antioxidant agents (e.g., increased IL-10; [7]). Eccentric exercise causes greater increases in muscle strength and the muscle soreness as compared to concentric exercise due to the greater muscle hypertrophy that follows eccentric training compared to concentric [8,9].

Preclinical studies showed the evidence of anti-inflammatory activity in decreasing inflammatory cytokine levels associated with fenugreek seed extract [10,11]. Fenugreek (*Trigonella foenum-graecum*) is a leguminous, annual plant found in India and North Africa. Fenugreek seed is part of diets of various countries and is now cultivated worldwide. It is a source of many natural products with health benefits [12]. Many natural products extract or powders that are formulated for medicinal and supplementation applications contain fenugreek seeds and leaves [13]. Fenugreek extract and supplementation has been studied extensively in human and animal models. Defatted fractions of fenugreek seeds (high in fiber content and containing steroidal saponins) are reported to lower blood glucose and plasma glucagon concentrations after 8 days of consumption in dogs [14]. Other investigations are utilizing human participants on fenugreek supplementation (daily doses of 1-25 g/day) by diabetic patients showed positive glucose regulation responses [15,16]. Anti-inflammatory activity of many glycosides from various plant extracts has been reported [17-19]. Therefore, it was thought worthwhile to explore a glycoside based standardized fenugreek seed extract against inflammatory markers in healthy human subjects during eccentric exercise using double-blind placebo control study design.

However, a glycoside based standardized fenugreek seed extract has the characteristic smell of fenugreek seeds. Therefore, to maintain the double-blind, placebo control design of the study, minor quantities of curcumin and cinnamon (25 mg) were added to make a proprietary blend, IBPR. These added quantities of curcumin [20] and cinnamon [21] were below reported efficacious doses and durations against inflammatory markers and so nonactive. The objective of the study was to evaluate the anti-inflammatory effect of IBPR supplementation, a

proprietary blend rich in glycoside-based standardized fenugreek seed extract (400 mg) and minor quantities (25 mg each) of curcumin and cinnamon against eccentric exercise-induced inflammatory markers non-resistant healthy subjects.

METHODS

Participants

A total of 20 non-resistance-trained (<6 months) male and female participants assigned to ingested either IBPR, (n=10, age: 21±2.8 years, height: 174±10 cm, weight: 77±20 kg) or matching placebo (n=10, age: 20±1.9 years, height: 175±14 cm, weight: 89±20 kg) participated in this study. Following types of subjects were excluded from participation in this study: (a) With any metabolic disorder (known electrolyte abnormalities, heart disease, arrhythmias, diabetes, thyroid disease, or hypogonadism), (b) with a history of hypertension, hepatorenal, musculoskeletal, autoimmune, or neurologic disease, (c) those taking thyroid, hyperlipidemic, hypoglycemic, antihypertensive, or androgenic medications, and (d) if they have taken ergogenic levels of nutritional supplements that may affect muscle mass (e.g., creatine, beta-hydroxy beta-methylbutyric acid or anabolic/catabolic hormone levels androstenedione, and dehydroepiandrosterone) within 6 months before the start of the study. Institutional review board approved the study protocol as per human subjects Guidelines of University of Mary Hardin-Baylor University, USA. The subjects meeting eligibility criteria were informed of the requirements of the study and sign the statements of informed consent in compliance with the human subjects Guidelines of University of Mary Hardin-Baylor University, USA, and the American College of Sports Medicine, USA.

Supplementation

Subjects were randomized into either active supplementation (IBPR) or matching placebo. IBPR is a proprietary blend rich in glycoside based standardized fenugreek seed extract (400 mg) and minor quantities of curcumin and cinnamon (25 mg each). Subjects were randomly assigned to ingest 450 mg of IBPR or matching placebo. Subjects were ingested 2 capsules of assigned treatment for 14 days before the eccentric exercise. The supplements were prepared in capsule form and packaged in plastic bottles for double-blind administration by Indus Biotech Private Limited, Pune, India. Research assistants at the study center monitored the compliance.

Experimental design

The study was conducted as a double-blind, placebo-controlled clinical trial using parallel groups matched based on free fat mass. The independent variables were the nutritional supplements. Blood profiles including; inflammatory markers (IL-6, IL-1β, IL-10, IL-1rα, and tumor necrosis factor [TNF-α]), markers of muscle soreness [creatinine, blood urea nitrogen (BUN) to creatinine ratio], and safety parameters (neutrophils, eosinophils, basophils, lymphocytes, and monocytes) were measured. Subjects were randomly placed into one of two groups: IBPR or Placebo.

Entry and familiarization session

Subjects with interest of participation in this study were interviewed on the phone to determine they qualify to participate in this study and were invited to attend an entry/familiarization session. During this session, subjects signed informed consent statements and complete personal and medical histories. Subjects were undergone a general physical examination to check that they meet eligibility criteria. Subjects meeting entry criteria were familiarized to the study protocol via a verbal and written explanation outlining the study design. Subjects were given an appointment time to perform baseline/pre-supplementation assessments.

Testing

Following entry and familiarization session, the subjects were informed about testing protocol and basic characteristics (age, height, and weight) and blood testing. Thigh mass was measured using previously established methods [22] and assessed for perceived muscle soreness. Subjects were instructed to warm-up briefly (10 minutes cycling at ~50 W). Then, they were asked to perform 24 sets of 10 eccentric knee extensor repetitions. They actively resisted against the lever arm of the Biodex, which forced their leg into flexion with one leg (1 minute recovery between sets) at 30°/s on a Biodex System - 3 (Biodex Medical Systems Inc., New York, USA). The eccentric exercise has been shown previously to decrease the range of motion and an increased inflammatory response that are hallmarks for muscle soreness and damage [23]. All subjects were seated during the exercise, and the dynamometer axis of rotation was oriented in alignment with their knee, while the force was applied below their knee with a padded lever arm. The point of contact between the subject's lower leg and the padded arm was at a fixed distance (relative to the subject's lower limb length), slightly above their ankle. The contractions were performed over a range of 60° (130°-70° of flexion, where 180° will be a full extension). A range of 60°, to achieve 70° of flexion, will be chosen so that the muscle will be exercised over a longer length, and result in greater muscle soreness than would be achieved over a smaller range, or at shorter muscle lengths [23]. The subjects were instructed to extend their knee as the lever arm of the Biodex was raised upward (no force required, i.e., passive mode), during the concentric movement and verbally encouraged to maximally resist the lowering lever arm of the Biodex, during the eccentric movement. Subjects will generate at least 50 Nm of torque to start and maintain the downward movement to the lever estimating eccentric contraction-induced muscle soreness. To further encourage maximal effort, a computer screen was provided subjects with a visual feedback of their force production throughout the exercise movement. Throughout the exercise protocol eccentric peak torque (Nm/kg) and total work (J/kg) data were monitored and collected for each set.

Assessments

Subjects were asked to fast overnight for 12 hrs, and approximately six teaspoons of fasting venous blood (30 ml) were withdrawn. Blood samples were obtained using standard phlebotomy procedures using standard sterile venipuncture of an antecubital vein by laboratory technician's trained in phlebotomy in compliance with guidelines established by the Texas Department of Health and Human Services, USA. Blood samples were taken pre-workout and immediately after the damaging bout, at 1 hr, 3 hrs, and 24 hrs post exercise. Serum and whole blood samples were collected, micro-centrifuged and assessed for anti-inflammatory cytokines (IL-10), proinflammatory cytokines (IL-1rα, IL-1β, IL-6, and TNF-α) using enzyme-linked immunosorbent assay techniques, and safety parameters of kidney function (BUN, creatinine, and BUN/creatinine ratio) and differential leukocyte count.

Statistical analysis

The data were represented as a mean ± standard deviation. The data of basic characteristics were analyzed by two-way ANOVA followed by Bonferroni's multiple comparisons test. The data of other assessments were analyzed by separate repeated measure two-way ANOVA for the time- or treatment-dependent significance. The $p<0.05$ was considered significant.

RESULTS

Basic characteristics

Basic characteristics of the participants in the group are represented in Table 1. There was no significant difference noted at baseline with respect to age (placebo - 20.0±1.9 years vs. IBPR 21.0±2.8 years), weight (placebo - 89.0±20.0 kg vs. IBPR - 77.0±20.0 kg), and height (placebo - 175.0±14.0 cm vs. IBPR - 174.0±10.0 cm).

Effects on anti-inflammatory cytokines, IL-10

The data for effects of supplementation on serum concentrations of the anti-inflammatory cytokine, IL-10 is presented in Table 2. Two-way ANOVA of data of IL-10 did not show time- or treatment-dependent effects. After 24 hrs, serum IL-10 concentration in IBPR group increased by 8.95% (pre-workout: 1.84 pg/ml to post-workout-24 hrs: 2.00 pg/ml)

whereas placebo showed increase of 1.32% (pre-workout: 1.14 pg/ml to post-workout-24 hrs: 1.15 pg/ml).

Effects on proinflammatory cytokines (IL-1rα, IL-1β IL-6, and TNF-α)

The data of effects of supplementation on serum concentrations of inflammatory cytokines such IL-1rα, IL-1β IL-6, and TNF-α are presented in Table 3.

Two-way ANOVA of data of IL-1rα showed significant treatment-dependent [F (1.76) = 11.81, p=0.001] but not time-dependent effects. Serum IL-1rα concentration in IBPR group decreased by 5.56% (pre-workout: 15.82 pg/ml to post-workout - 0 hr: 14.94 pg/ml) whereas placebo showed increase of 68.87% (pre-workout: 22.93 pg/ml to post-workout - 0 hr: 38.72 pg/ml). After 24 hrs of post-workout, IBPR group showed 13.33% decrease (pre-workout: 15.82 pg/ml to post-workout - 24 hrs: 13.71 pg/ml) whereas placebo group showed increase of 28.08% (pre-workout: 22.93 pg/ml to post-workout - 24 hrs: 29.37 pg/ml) in serum IL-1rα concentration.

Two-way ANOVA of data of IL-1β showed significant time-dependent (F [4,72] = 4.77, p<0.01) but not treatment-dependent effects. Serum IL-1β concentration in IBPR group increased by 12.90% (pre-workout: 0.31 pg/ml to post-workout - 0 hr: 0.35 pg/ml) whereas placebo showed increase of 21.2% (pre-workout: 0.33 pg/ml to post-workout - 0 hr: 0.40 pg/ml). After 24 hrs of post-workout, IBPR group showed 1.62% (pre-workout: 0.305 pg/ml to post-workout - 24 hrs: 0.31 pg/ml) increase whereas placebo group showed increase of 6.36% (pre-workout: 0.33 pg/ml to post-workout - 24 hrs: 0.35 pg/ml) in serum IL-1β concentration.

Two-way ANOVA of data of IL-6 showed significant time-dependent (F [4,72] = 4.033, p<0.01) but not treatment-dependent effects. Serum IL-6 concentration in IBPR group decreased by 1.6% (pre-workout: 2.19 pg/ml to post-workout - 0 hr: 2.15 pg/ml) whereas placebo showed increase of 43% (pre-workout: 1.73 pg/ml to post-workout - 0 hr: 2.50 pg/ml). After 24 hrs of post-workout, IBPR group showed 41% decrease (pre-workout: 2.19 pg/ml to post-workout - 24 hrs: 1.27 pg/ml) whereas placebo group showed increase of 8.3% (pre-workout: 1.73 pg/ml to post-workout - 24 hrs: 1.88 pg/ml) in serum IL-6 concentration.

Two-way ANOVA of data of TNF-α did not show time- or treatment-dependent effects. Serum TNF-a concentration in IBPR group increased by 11.9% (pre-workout: 2.04 pg/ml to post-workout - 0 hr: 2.28 pg/ml) whereas placebo showed increase of 5.14% (pre-workout: 2.45 pg/ml to post-workout - 0 hr: 2.57 pg/ml). After 1 hr of post-workout, IBPR group showed 4.67% decrease (pre-workout: 2.04 pg/ml to post-workout - 1 hr: 1.94 pg/ml) whereas placebo group showed increase of 1.3% (pre-workout: 2.45 pg/ml to post-workout - 1 hr: 2.48 pg/ml) in serum TNF-α concentration.

Effects on kidney function markers

The data of effects of IBPR supplementation on serum concentrations of kidney function markers, namely, BUN, serum creatinine, and BUN to creatinine ratio are presented in Table 4.

Two-way ANOVA of data of BUN did not show time- or treatment-dependent effects. Serum BUN concentration in IBPR group increased by 0.85% (pre-workout: 14.20 mmol/L to post-workout - 0 hr: 14.32 mmol/L) whereas placebo showed decrease of 6.64% (pre-workout: 15.00 mmol/L to post-workout - 0 hr: 14.00 mmol/L). After 24 hrs of post-workout, IBPR group showed 0.70% increase (pre-workout: 14.20 mmol/L to post-workout - 24 hrs: 14.30 mmol/L) whereas placebo group showed decrease of 6.00% (pre-workout: 15.00 mmol/L to post-workout - 24 hrs: 14.10 mmol/L) in serum BUN concentration.

Table 1: Basic characteristics of the participants in the studied groups

Parameters	Placebo	IBPR	Significance
Age (years)	20.0±1.90	21.0±2.80	ns
Height (cm)	175.0±14.0	174.0±10.0	ns
Weight (kg)	89.0±20.0	77.0±20.0	ns

Data were represented as mean±SD. The data were analyzed by two-way ANOVA followed by Bonferroni's multiple comparisons test, ns: Not significant

Table 2: Serum concentrations of anti-inflammatory cytokines

Parameters	Time	Placebo	IBPR
IL-10 (pg/ml)	T1 (pre-workout)	1.14±0.61	1.84±1.64
	T2 (post-workout - 0 hr)	1.10±0.49	1.68±1.51
	T3 (post-workout - 1 hr)	1.10±0.44	1.71±1.41
	T4 (post-workout - 3 hrs)	1.23±0.45	1.82±1.40
	T5 (post-workout - 24 hrs)	1.15±0.44	2.00±1.47

Data were represented as mean±SD. Data were analyzed by two-way repeated measure ANOVA. There was no time- or treatment-dependent significance

Table 3: Serum concentrations of proinflammatory cytokines

Parameters	Time	Placebo	IBPR
IL-1rα (pg/ml)	T1 (pre-workout)	22.93±17.47	15.82±14.49
	T2 (post-workout - 0 hr)	38.72±23.11	14.94±13.70
	T3 (post-workout - 1 hr)	32.02±23.52	22.47±22.02
	T4 (post-workout - 3 hrs)	34.67±27.29	18.00±14.29
	T5 (post-workout - 24 hrs)	29.37±26.17	13.71±11.33
IL-1β (pg/ml)	T1 (pre-workout)	0.33±0.23	0.31±0.22
	T2 (post-workout - 0 hr)	0.40±0.28	0.35±0.23
	T3 (post-workout - 1 hr)	0.40±0.25	0.38±0.25
	T4 (post-workout - 3 hrs)	0.37±0.27	0.38±0.32
	T5 (post-workout - 24 hrs)	0.35±0.23	0.31±0.25
IL-6 (pg/ml)	T1 (pre-workout)	1.73±1.18	2.19±1.90
	T2 (post-workout - 0 hr)	2.50±1.53	2.15±1.29
	T3 (post-workout - 1 hr)	3.34±2.58	2.11±1.14
	T4 (post-workout - 3 hrs)	2.98±1.80	2.03±0.73
	T5 (post-workout - 24 hrs)	1.88±1.48	1.27±0.55
TNFα (pg/ml)	T1 (pre-workout)	2.45±0.80	2.04±0.46
	T2 (post-workout - 0 hr)	2.57±1.10	2.28±0.92
	T3 (post-workout - 1 hr)	2.48±1.26	1.94±0.68
	T4 (post-workout - 3 hrs)	2.65±1.21	2.26±1.01
	T5 (post-workout - 24 hrs)	2.77±1.46	2.27±1.43

Data were represented as mean±SD. Data were analyzed by two-way repeated measure ANOVA. IL-1rα - treatment-dependent significant effects but no significant time-dependent effects, IL-1β and IL6 - significant time-dependent effects but no treatment-dependent significant effects, TNF-α - no time- or treatment-dependent significant effects, TNF: Tumor necrosis factor

Two-way ANOVA of data of creatinine showed significant time-dependent ($F [4,72] = 3.22$, $p<0.05$) but not treatment-dependent effects. Serum creatinine concentration in IBPR group increased by 4.3% (pre-workout: 0.92 mg/dL to post-workout - 0 hr: 0.96 mg/dL) whereas placebo showed increase of 0.76% (pre-workout: 0.92 mg/dL to post-workout - 0 hr: 0.93 mg/dL). After 24 hrs of post-workout, IBPR group showed 0.87% decrease (pre-workout: 0.92 mg/dL to post-workout - 24 hrs: 0.91 mg/dL) whereas placebo group showed decrease of 1.3% (pre-workout: 0.92 mg/dL to post-workout - 24 hrs: 0.91 mg/dL) in serum creatinine concentration.

Two-way ANOVA of data of BUN to creatinine ratio did not show time- or treatment-dependent effects. BUN to creatinine ratio in IBPR group decreased by 0.4% (pre-workout: 15.7 to post-workout - 0 hr: 15.3) whereas placebo showed a decrease of 1% (pre-workout: 16.1 to post-workout: 15.1). After 24 hrs of post-workout, IBPR group showed 0.5% increase (pre-workout: 15.7 to post-workout - 24 hrs: 16.2) whereas placebo group showed decrease of 0.7% (pre-workout: 16.1 to post-workout - 24 hrs: 15.4) in BUN to creatinine ratio.

Effects on differential leukocyte count

The data of effects of supplementation on differential leukocyte count are presented in Table 5. Two-way ANOVA of data of none of the differential leukocyte count (neutrophils, eosinophils basophils lymphocytes, and monocytes) showed time- or treatment-dependent effects.

DISCUSSION

The objective of this study was to evaluate the efficacy of IBPR supplementation on inflammation associated with an acute physical activity such as eccentric exercise in non-resistance trained young subjects. The effect of 14-day intake of 450 mg IBPR showed a reduction in the inflammatory markers induced by eccentric knee extensor repetitions in non-resistance young subjects. The major component of IBPR is derived from fenugreek seed extract and has the characteristic

Table 4: Parameters of kidney function parameters

Parameters	Time	Placebo	IBPR
BUN (mmol/L)	T1 (pre-workout)	15.00±5.98	14.20±4.08
	T2 (post-workout - 0 hr)	14.00±5.01	14.32±3.56
	T3 (post-workout - 1 hr)	14.10±6.12	13.70±3.89
	T4 (post-workout - 3 hrs)	14.55±6.00	14.45±3.62
	T5 (post-workout - 24 hrs)	14.10±4.91	14.30±2.83
Serum Creatinine (mg/dL)	T1 (pre-workout)	0.92±0.19	0.92±0.20
	T2 (post-workout - 0 hr)	0.93±0.22	0.96±0.18
	T3 (post-workout - 1 hr)	0.93±0.21	0.92±0.20
	T4 (post-workout - 3 hrs)	0.95±0.20	0.99±0.20
	T5 (post-workout - 24 hrs)	0.91±0.19	0.91±0.19
BUN to creatinine ratio	T1 (pre-workout)	16.1±3.93	15.7±4.00
	T2 (post-workout - 0 hr)	15.1±2.89	15.3±3.20
	T3 (post-workout - 1 hr)	14.9±4.01	14.9±3.51
	T4 (post-workout - 3 hrs)	15.2±3.43	14.8±3.62
	T5 (post-workout - 24 hrs)	15.4±3.20	16.2±1.93

Data were represented as mean±SD. Data were analyzed by two-way repeated measure ANOVA. BUN and BUN to creatinine ratio - no time- or treatment-dependent significant effects, serum creatinine - significant time-dependent effects but no treatment-dependent significant effects. BUN: Blood urea nitrogen

Table 5: Differential leukocyte count

Parameters	Time	Placebo	IBPR
Neutrophil	T1 (pre-workout)	58.05±8.19	55.94±14.25
	T2 (post-workout - 0 hr)	55.38±19.38	66.63±9.11
	T3 (post-workout - 1 hr)	61.11±18.16	64.40±17.22
	T4 (post-workout - 3 hrs)	51.95±21.88	65.93±9.55
	T5 (post-workout - 24 hrs)	52.37±12.86	54.64±8.69
Eosinophil	T1 (pre-workout)	1.54±0.57	3.24±2.11
	T2 (post-workout - 0 hr)	1.50±0.59	2.42±2.07
	T3 (post-workout - 1 hr)	1.38±0.87	3.36±3.44
	T4 (post-workout - 3 hrs)	2.31±1.80	2.47±1.51
	T5 (post-workout - 24 hrs)	2.69±1.86	3.65±2.86
Basophil	T1 (pre-workout)	0.43±0.13	0.00±0.00
	T2 (post-workout - 0 hr)	0.40±0.23	0.30±0.13
	T3 (post-workout - 1 hr)	0.37±0.22	0.37±0.25
	T4 (post-workout - 3 hrs)	0.47±0.34	0.35±0.15
	T5 (post-workout - 24 hrs)	0.35±0.18	0.44±0.24
Lymphocyte	T1 (pre-workout)	33.18±7.52	30.92±7.09
	T2 (post-workout - 0 hr)	33.04±15.32	24.29±7.77
	T3 (post-workout - 1 hr)	30.97±16.41	27.09±12.94
	T4 (post-workout - 3 hrs)	37.02±16.78	26.24±8.65
	T5 (post-workout - 24 hrs)	36.99±11.21	35.17±7.21
Monocyte	T1 (pre-workout)	6.87±2.34	6.50±1.06
	T2 (post-workout - 0 hr)	6.95±2.88	6.06±1.15
	T3 (post-workout - 1 hr)	6.17±2.45	4.79±3.20
	T4 (post-workout - 3 hrs)	8.24±5.23	4.99±2.44
	T5 (post-workout - 24 hrs)	7.61±2.86	6.10±3.07

Data were represented as mean±SD. Data were analyzed by two-way repeated measure ANOVA. None of the parameter (neutrophils, eosinophils basophils lymphocytes, and monocytes) has no time- or treatment-dependent significance

smell of fenugreek seeds. Therefore, curcumin and cinnamon are added in minor quantities (25 mg each) for blinding and placebo design. The doses and duration of curcumin [20] and cinnamon [21] are below reported efficacious doses and durations against inflammatory markers and so nonactive. Therefore, the anti-inflammatory efficacy that was observed in this study can be attributed to the major component, a glycoside based standardized fenugreek seed extract, which constitute 88.89% of IBPR. The IBPR supplementation was found to be safe and well tolerated for all subjects during this study. No adverse side effects were reported by any of the participants, nor were any clinical safety markers or hematological variables significantly altered ($p>0.05$) demonstrating supplement appears safe when taken over 14-day before exercise bout. These results are in line with reported safety profile of the glycoside based standardized fenugreek seed extracts in human subjects [24] as well as animals [25,26].

Following 14-day of supplement dosage, inflammatory markers were assessed after an exercise bout. The serum level of the inflammatory marker, IL6 in IBPR supplemented group was significantly less than placebo at 1 hr and 3 hrs post-exercise which supports anti-inflammatory properties of IBPR. IL-6 increases hepatic glucose production during exercise and lipolysis in adipose tissue [27]. The increase in IL-6 also enhances insulin action and sensitivity [28] unlike TNF-α-induced insulin resistance [29]. The absence of classical proinflammatory cytokines (TNF-α and IL-1β) in the exercise-induced cytokine cascade causes an increase of IL-6, IL-1rα, IL-19, and sTNF-R [30], creating an anti-inflammatory environment. It appears that exercise inhibits TNF-α directly by IL-6 [31] and indirectly via epinephrine [32]. The down-regulation of TNF-α induced by skeletal muscle-derived IL-6 may also participate in mediating the atheroprotective effect of physical activity [33]. Findings of this study of lowered numbers of leukocyte counts after eccentric exercise support the past report of exercise-induced lowering of blood leukocyte count during and following exercise in healthy and moderately fit males [34]. These findings were consistent also consistent with the published reports from animal studies in arthritic condition, where a reduction in an acute-phase inflammatory response, after daily intake of fenugreek seed extract was observed [35,36]. Considerable evidence supported the anti-inflammatory properties of fenugreek seed extract administration through decreased cytokine gene expression in Freund's complete adjuvant-induced arthritis in rats [37]. Fenugreek seed extract showed a significant reduction in proinflammatory cytokines *in vitro* [10]. This study supports probable mechanism of anti-inflammatory action of fenugreek seed extracts that is demonstrated in the previous reports [35-37].

The strengths of the study include homogenous population between the study group and a comprehensive assessment of subjective and objective variables before, during and after each exercise bout. We have observed increased trend for anti-inflammatory cytokine (IL-10) and decreased trend toward proinflammatory cytokines (IL-1rα, IL-1β IL-6, and TNF-α) with IBPR supplementation. However, the small sample size and large variability in the inflammatory cytokine responses may be limiting factors for statistical significance. This study was a comparison of acute exercise responses, where effects of training adaptations to the eccentric knee extensions were not seen. Because of the greater inflammatory response with eccentric knee extensions, participants transitions slowly with progressive loading, which is important for untrained persons, older adults or individuals with a recent musculoskeletal injury. Therefore, the data of this study are useful for future studies for exploration of IBPR as a dietary supplement for subjects with RX in a wide variety of populations.

CONCLUSION

The supplementation of IBPR, a proprietary blend rich in glycoside based standardized fenugreek seed extract, showed efficacy and safety in young subjects for the reduction of eccentric exercise-induced inflammatory markers.

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