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CLINICAL TRIAL REPORT

A Randomized, Placebo-Controlled, Double-Blind Study of *Phyllanthus Amarus* in Combination with Whey Protein Isolate on Skeletal Muscle Strength in Active Males

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Purpose: Athletes often use dietary supplements to enhance their exercise performance. This study investigated the additive effects of *Phyllanthus amarus* (EB-PA) and whey protein isolate (WPI), compared to WPI alone or placebo on skeletal muscle strength in active males.

Participants and Methods: A total of 121 healthy male participants, aged 20 to 35 years and engaged in moderate physical activity, were randomly assigned to one of three groups: placebo group (Microcrystalline cellulose [MCC] capsule and maltodextrin sachet), WPI group (WPI [40 g] and MCC capsule], or EB-PA + WPI group (WPI [40 g] and EB-PA capsule [500 mg]). All participants completed resistance training twice weekly for 30 days. The primary outcome was muscle strength, assessed by 1-repetition maximum (1-RM) for the upper and lower body. Secondary outcomes included muscle endurance, muscle flexibility, muscle mass, and grip strength.

Results: At the end of study, combining the EB-PA with WPI showed significantly improved in both upper (18.02%) and lower body (17.62%) 1-RM strength compared to WPI [upper (9.24%) and lower body (9.02%)] as well as placebo [upper (4.39%) and lower body (3.39%)]. The EB-PA + WPI group achieved a 22.89% significant increase in muscle endurance compared to 11.47% increase in the WPI group. Additionally, statistically significant enhancements were observed in muscle flexibility and grip strength in the EB-PA + WPI group versus the other groups ($p<0.05$).

Conclusion: Combination of EB-PA and WPI after 30 days significantly enhanced muscle strength, endurance, flexibility, and grip strength in active males. This suggests the EB-PA enhances muscle strengthening effect of protein supplements. EB-PA was safe throughout the study.

Keywords: *phyllanthus amarus*, protein, muscle strength, flexibility, 1-RM

Introduction

Muscle strength and endurance are critical components of athletic training. Athletes often consume high doses of protein supplements to enhance muscle strength. However, consuming high amounts of protein over time has been associated with several metabolic and clinical complications. Among the most commonly reported negative effects of prolonged high protein intake are kidney damage, persistent dehydration, and reduced bone density.[1]

Whey protein is distinctive in its digestion and absorption processes compared to other sources of dietary protein. Whey protein is rapidly absorbed, resulting in an increased delivery of amino acids to tissues and a more pronounced stimulation of protein synthesis, ultimately leading to a higher net protein retention in the body.[2,3] Additionally, whey protein exhibits high solubility and can be easily mixed with various liquids. Due to these characteristics, whey protein is

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considered an optimal nutritional supplement for consumption prior to, during, or following physical exercise or athletic performance.[4] A systematic review by Stark M. et al,[5] concluded that it takes 10–12 weeks to significantly improve muscle strength with an optimal dose of whey protein.

Herbal supplements with anti-inflammatory and antioxidant properties, such as *Zingiber officinale, Phlebodium decamanum*, and *Curcuma longa*, are known to help reduce muscle damage and pain caused by inflammation and oxidative stress after resistance exercise.[6] Studies showed: (1) *Zingiber* reduces muscle pain post-exercise;[7] (2) *Phlebodium* alleviates oxidative stress and inflammation in athletes;[8] (3) *Curcuma longa* helps prevent Delayed-Onset Muscle Soreness (DOMS) and enhances recovery.[9]

Phyllanthus amarus Schumacher & Thonn (*P. amarus* Schum & Thonn), a plant from the Phyllanthaceae family, is recognized for its diverse health benefits, which include but is not limited to anti-inflammatory, hematological, and immunological properties.[10–12] Recent studies have demonstrated that *P. amarus* possesses significant hepatoprotective properties,[13] supporting the maintenance of optimal liver function, which is essential for the metabolic processes involved in muscle growth.[14] Research suggests that the tannins and corilagin found in *P. amarus* exhibit potent anti-inflammatory and antioxidant effects.[15] These properties may help mitigate muscle damage and oxidative stress, facilitating faster recovery and promoting muscle growth.[16] Consequently, *P. amarus* is hypothesized to enhance muscle health by improving liver function, reducing systemic inflammation, and promoting protein synthesis through liver-mediated pathways. However, further experimental and clinical studies are necessary to elucidate the molecular mechanisms underlying the effects of the EB-PA extract on muscle strength and mass.

Additionally, in two previous clinical studies conducted by Roengrit T et al[17,18] the effects of *P. amarus* powder on oxidative stress, muscle damage, leukocyte counts, inflammation, and muscle soreness were evaluated following a single bout of moderate and high-intensity exercise. In both studies, participants were administered two capsules of *P. amarus* or a placebo control 20 minutes prior to cycling, followed by four capsules on the same day post-exercise and six capsules per day for the subsequent two days. The results demonstrated that acute supplementation with *P. amarus* enhanced antioxidant status following moderate-intensity exercise and mitigated oxidative stress and muscle soreness induced by high-intensity exercise.

In a proof-of-concept study conducted by Enovate Biolife, various extracts [EB-MS-01 (300 mg Microcrystalline cellulose (MCC) without exercise), EB-MS-02 (300 mg MCC with exercise), EB-MS-03 (500 mg *Phyllanthus amarus* with exercise), EB-MS-04 (250 mg *Cinnamomum verum* with exercise), and EB-MS-05 (350 mg *Ocimum sanctum* + *Rosmarinus officinalis* with exercise)] were screened for their effectiveness in enhancing skeletal muscle mass and strength. Participants consumed two Investigational Products (IP) capsules before breakfast, and 24 gm whey protein with breakfast and dinner.[19] The IP intake ceased for responders with a ≥10% increase in muscle strength, as assessed by 1-repetition maximum (1-RM) at day 28, while the remaining participants continued the study until day 56. The study revealed that *P. amarus* extract showed a 13.05% increase in 1-RM, whereas those in the EB-MS-02, EB-MS-04 and EB-MS-05 groups only experienced an increase of 9.65%, 12.78%, and 9.39%, respectively. EB-MS-03 group also showed a meaningful decrease in body fat, visceral fat, android fat and an increase in lean body mass. This study concluded that *P. amarus* extract with whey protein, increased muscle strength compared to whey protein alone.

Though *P. amarus* has long been used for its liver-protective and anti-inflammatory effects, this study is the first to use it in sports nutrition, specifically for its ability to increase muscle strength when combined with whey protein. This creates new opportunities for natural performance enhancing supplements. Based on these findings, the current study was designed as a randomized, placebo-controlled, double-blind study to confirm the additive effect of EB-PA (*P. amarus*) in exercising individuals.

Material and Methods

Ethical Considerations

This study was conducted in compliance with the International Conference on Harmonization (ICH) recommendation on Good Clinical Practice (GCP) - E6 (R2), 2016, National Ethical Guidelines for Biomedical and Health Research involving Human Participants, 2017 and Declaration of Helsinki. The ethical approval for the conduct of the study at three sites was provided by the independent ethics committee - Altezza Institutional Ethics Committee (Reg. No. ECR/1457/Inst/MH/2020). The eligible participants were enrolled in the study only after providing voluntary, written informed consent for participation. The study was registered with the NIH ClinicalTrials.gov (Identifier: NCT06127849, Submitted on: 06/11/2023) and Clinical Trials Registry, India (Registration Number: CTRI/2023/11/060027, Registered on: 20/11/2023). The CRO monitored and audited the study to ensure the study protocol and ICH-GCP compliance. The study conformed to the Consolidated Standard Reporting of Trials (CONSORT) guidelines.

Study Design

This randomized, double-blind, placebo-controlled clinical study was designed to evaluate the additive effects of EB-PA supplementation with whey protein on muscle strength over 30 days. The study was conducted between December 2023 and May 2024 at three sites in Maharashtra, India.

Participants

The study included young exercising males aged 20 to 35 years and BMI ranging from 22 to 29.9 kg/m² with moderate physical activity levels, as assessed by the International Physical Activity Questionnaire (IPAQ-SF). Individuals with 1-RM strength defined by upper body ≥ 25 kg and lower body ≥ 100 kg were included in the study. Eligible participants had a history of at least one month of endurance training and were required to abstain from caffeinated products and intense strength exercises 24 hours prior to the exercise study visit were included in the study. Additionally, individuals with thyroid-stimulating hormone (TSH) levels between 0.4 and 4.9 mIU/L were included.

Individuals who consumed whey or other supplemental proteins within the last 3 months were excluded from the study. Those with a weight fluctuation exceeding 4.5 kg within the past 3 months were also excluded. Additional exclusion criteria included presence of chronic diseases, hypertension, type I or type II diabetes mellitus and any history of renal, hepatic, endocrine, biliary, gastrointestinal, pancreatic, or neurological disorders.

Interventions

The *P. amarus* extract was prepared from the aerial parts of the plant using a hydroalcoholic extraction method. The extract was standardized for bitters, tannins, and corilagin, with the supplement containing 17% bitters, 17% tannins, and 3% corilagin. The content of the capsules was verified by an independent third-party lab.

The whey protein used in the supplement contained 92.50% protein content, which was verified through UV spectrophotometry. Additionally, the whey protein was flavored with vanilla. Participants were randomly assigned to one of three groups: The placebo group received an MCC capsule (500 mg) and a maltodextrin sachet (20 g); the WPI group received WPI (40 g) and an MCC capsule; and the EB-PA + WPI group received WPI (40 g) and an EB-PA capsule (500 mg) (Table 1). The dose regimen involved taking one sachet of WPI (20 g) or Maltodextrin (20 g) twice daily (morning and evening) with 200–300 mL of water and one capsule of the study product 30 minutes after WPI in the morning. Participants who had more than 90% of the prescribed regimen were considered IP-compliant. Any concomitant medications were recorded in both the source documents and the Electronic Case Report Form (e-CRF).

Table 1 Study Products Details

Groups	Study Products	Ingredients
Group 1	**Placebo**	MCC capsule (500 mg) and maltodextrin sachet (40 g)
Group 2	**WPI**	WPI (40 g) and MCC capsule (500 mg)
Group 3	**EB-PA + WPI**	WPI (40 g) and EB-PA capsule (500 mg)
Dose regimen	One sachet of WPI or Maltodextrin twice daily (morning and evening) and one capsule 30 minutes after WPI in the morning	

Randomization was performed using StatsDirect Software version 3.1.17 with a 1:1:1 allocation ratio for the placebo, WPI and EB-PA + WPI groups. The randomization chart was secured, saved, and maintained in the electronic trial master file with restricted access to only designated personnel. The participants, the research team, and the investigator were blinded to the study product allocation. To preserve the blinding, the investigational products and the placebo capsules were matched for size, shape, color, and texture and packed in identical packaging.

Study Conduct

During the screening visit, the study procedures were clearly explained to the participants in a language they fully understood. Following this, the participants signed and dated the informed consent. Demographics and baseline characteristics [weight, height, body mass index (BMI)], clinical examination, and histories related to exercise, diet, medical conditions, and medications were recorded. Fasting blood samples were collected for fasting blood glucose (FBG) and thyroid stimulating hormone (TSH) analysis. For every visit, participants were instructed to visit the clinical site after overnight fasting. Participants were screened based on their physical activity and 1-RM strength test for the upper and lower body.

To determine the 1-RM for both upper and lower body, a resistance training exercise protocol was implemented. In the exercise protocol, participants began with a warm-up that included 8 inward and 8 outward hip rotations (each side), 10 deep reverse lunges to knee raise, and 10 squats, followed by 1–2 minutes of rest. For the upper body 1-RM testing, they bench pressed 50% of their body weight (including the barbell) for 4 ± 1 repetitions, with weight increases of 10 kg if the Visual Analogue Scale - Fatigue (VAS-F) score was ≤ 6, or 5 kg if the VAS-F score was ≥ 7. The maximum weight lifted for 4 ± 1 repetitions was recorded, with 3 ± 1 minutes of rest between sets. For lower body 1-RM testing, participants leg pressed 70% of their body weight for 4 ± 1 repetitions, following similar weight adjustment criteria and rest periods as for upper body 1-RM. Baseline weights were rounded up to the nearest higher weight divisible by 5 kg. A total of 2 sessions of body weight training exercise for each major muscle group were performed each week (Table 2). At the randomization visit (day 0), participants were randomized based on their ability to lift at least 70% of their 1-RM, as assessed during the screening visit. At day 0 and day 30, muscle strength and endurance, muscle flexibility, grip strength, and muscle mass were measured using the 1-RM test, V sit and reach test, hand grip dynamometer, and dual-energy X-ray absorptiometry (DEXA), respectively. Additionally, the sustained effect of EB-PA on maintaining upper and lower body 1-RM from Day 30 to Day 37 was also determined.

Outcome Measures

The primary outcome of the study was to assess the impact of EB-PA on muscle strength using the 1-RM for the upper and lower body. 1-RM is the gold standard for determining muscle strength and has good to excellent test-retest reliability.[20] The heaviest weight the participants can lift for 4 ± 1 repetitions is noted. 1-RM is determined using the formula: Weight ÷ [1.0278 - (0.0278 × Number of repetitions)].[21] The weight represented here is the weight lifted.

Secondary outcomes included assessments of muscle endurance, muscle flexibility, muscle mass, and grip strength. Muscle endurance was calculated using the formula: (load × number of repetitions for upper body exercise) + (load × number of repetitions for lower body exercise), with exercises performed at ≥ 70% of the screening 1-RM. Muscle flexibility, specifically for the lower back and hamstring muscles, was evaluated using the V sit-and-reach test. DEXA

Table 2 List of Resistance Training

Muscle Group	Set 1	Set 2
Legs	Squats, Back Lunges Heel Raises, Hip Bridging	Side Lunges, Walking Lunges, Toe Raises, Single-Leg Deadlifts
Press	Push-up, Squats	Scapular Protraction Retraction Reverse lunges
Pull	Towel Row	Seated Wall Angles

measured changes in fat-free mass (FFM), lean muscle mass (LMM), and android fat. Hand grip strength was assessed with a hand grip dynamometer. The sustained effect on muscle strength was measured by comparing lower and upper body 1-RM values from day 30 to day 37. Safety was monitored by recording vital signs (blood pressure and pulse rate), the effect on the digestive system, and adverse events throughout the study. Adverse events were recorded immediately in the source document after and in the appropriate adverse event form of the electronic case report form (e-CRF).

Quality Assurance

The study was monitored and audited by the Contract Research Organization, Vedic Lifesciences (Mumbai, Maharashtra, India) to ensure compliance with the study protocol and with the ICH-GCP E6 (R2) guidelines.

Statistical Analysis

Considering a difference of approximately 10% in the increase of 1-RM between the WPI + EB-PA and WPI alone, with a probability of 80% of the study detecting an intervention difference at a two-sided 0.05 significance level, a minimum of 40 participants/ arm were estimated to be analyzed. Accounting for 13% dropouts and withdrawals, a total of 46 participants were planned to be enrolled and randomized into 3 groups in the ratio of 1:1:1.

The endpoint data sets were visually assessed for normality. All continuous variables were summarized by presenting the number of subjects, mean, standard deviation, median, 95% CI of mean, minimum, and maximum. Categorical variables were presented as frequencies and percentages. For continuous data, a *t*-test was used, whereas Chi Square/ fisher's exact test was used for categorical variables to compare the baseline demographic data between treatment groups. ANOVA was used to evaluate the difference in the three groups, whereas the change in endpoint variables from baseline to post-baseline visits among the groups was analyzed by analysis of covariance (ANCOVA) with treatment as a factor and Baseline assessment as a covariate. All statistical tests were performed with a significance level of 0.05 using the R/R Foundation for Statistical Computing, Vienna, Austria (https://www.R-project.org/version4.0.5) and XLSTAT Statistical and Data Analysis Solution, New York, USA (https://www.xlstat.com./version2021.3.1).

Results

Out of 166 screened participants, 138 participants met the eligibility criteria and were randomized into the study. Of these, 121 participants completed at least one dose and one follow-up study visit and were considered as the Full Analysis Set (FAS) population. The detailed study participant disposition is presented in Figure 1.

Demographic Characteristics of Participants

The enrolled participants had a mean age of 24.98 years with an average BMI of 25.08 kg/m^2 and an average waist circumference of 92.64 cm. The demographics and baseline characteristics of the enrolled participants are summarized in Table 3.

Muscle Strength

Lower Body 1-RM

At day 30, a significant increase in lower body 1-RM strength was observed in the three study groups compared to baseline. The EB-PA + WPI group exhibited a greater improvement in the lower-body strength compared to the other two groups. Specifically, the change in the EB-PA + WPI group [20.00 (10.12) kg] was two-fold compared to the WPI group [10.56 (7.15) kg] and almost seven-fold than the placebo group [3.81 (5.39) kg]. Furthermore, a statistically significant difference was observed in the EB-PA+ WPI group when compared with both the placebo and WPI groups ($p<0.0001$ each) (Table 4 and Figure 2).

Upper Body 1-RM

At the end of the study, all groups demonstrated a significant increase in upper body 1-RM strength compared to baseline. A noteworthy improvement was exhibited in the EB-PA+ WPI group with a twofold increase in the 1-RM [11.63 (7.85) kg] compared to the WPI group [5.97 (3.75) kg] and almost four-fold than the placebo group [2.62 (4.31) kg].

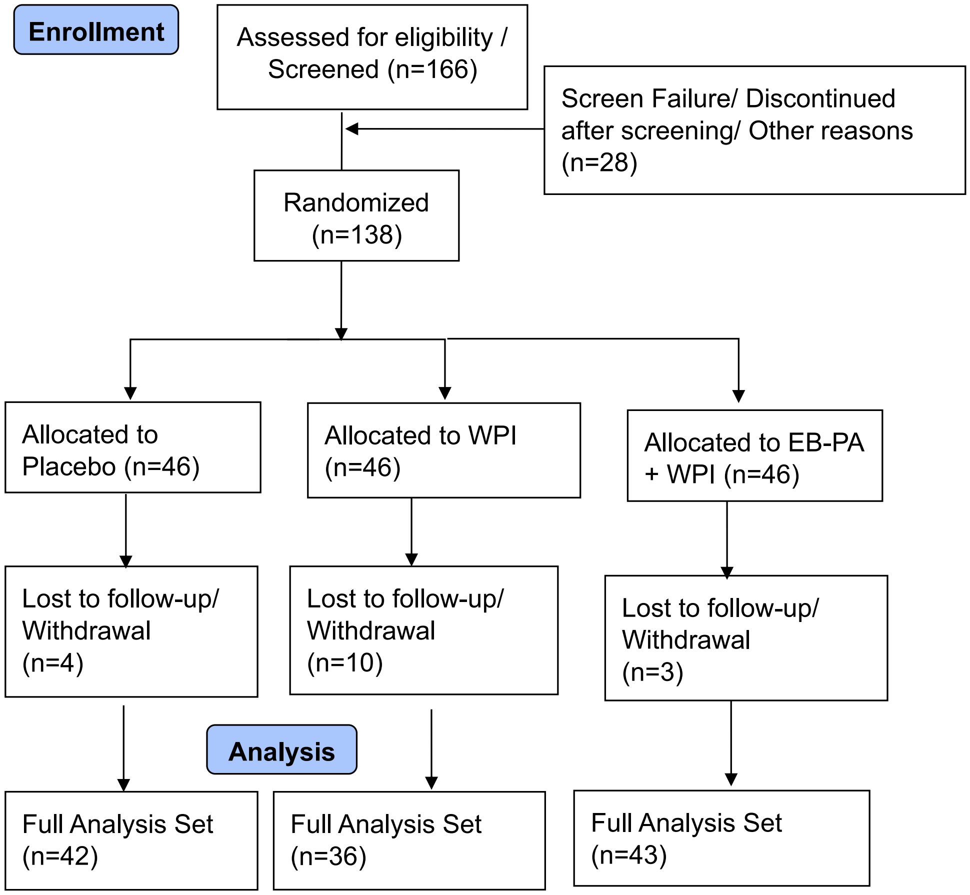

Figure 1 Participant disposition.

Additionally, significant differences were observed in the EB-PA+ WPI group compared to both the placebo and WPI groups ($p<0.0001$ each) (Table 5 and Figure 3).

Composite Body Strength

The composite body strength was calculated by summing the upper and lower body 1-RM values at days 0, 30 and 37. At day 30, mean change was significantly higher in the EB-PA+ WPI group [32.09 (15.36) kg] compared to both the placebo group [6.43 (7.83) kg] and the WPI group [15.97 (9.70) kg]. Similarly, at day 37, the EB-PA+ WPI group experienced a significantly higher mean strength increase [28.84 (16.86) kg] compared to the placebo group [3.45

Table 3 Demographic and Baseline Characteristics

Parameter	Statistics	Placebo (N=46)	WPI (N=46)	EB-PA + WPI (N=46)	P-Value
Age (Years)	**Mean**	24.39	25	25.54	0.4931 (A)
	SD	4.7	4.75	4.46	
	Median	23	23	24.5	
	Minimum	20	20	20	
	Maximum	35	35	34	

(*Continued*)

Table 3 (Continued).

Parameter	Statistics	Placebo (N=46)	WPI (N=46)	EB-PA + WPI (N=46)	P-Value
Height (meter)	**Mean**	1.69	1.7	1.69	0.9386 (A)
	SD	0.06	0.07	0.06	
	Median	1.7	1.7	1.7	
	Minimum	1.52	1.55	1.6	
	Maximum	1.82	1.84	1.84	
Weight (kg)	**Mean**	71.86	72.4	72.05	0.9575 (A)
	SD	8.87	9.72	8.47	
	Median	71.3	70.85	70.35	
	Minimum	52.1	57	57.5	
	Maximum	91.55	96.6	92.3	
BMI (kg/m^2)	**Mean**	25.06	25.09	25.1	0.9962 (A)
	SD	2.22	2.06	2.31	
	Median	24.57	24.64	24.83	
	Minimum	22.2	22.01	22.15	
	Maximum	29.72	29.51	29.79	
Waist Circumference (cm)	**Mean**	92.15	92.59	93.19	0.7593 (A)
	SD	7.02	6.43	6.66	
	Median	94.3	95	95	
	Minimum	72	78	74	
	Maximum	103	102.1	102	

Notes: For continuous variables p-values were calculated using ANOVA (A).
Abbreviations: BMI, Body mass index; SD, Standard deviation; N, Number of participants.

(7.03) kg] and the WPI group [12.36 (10.72) kg]. Notably, at day 30 and day 37, the EB-PA+ WPI group exhibited a statistically significant difference in strength compared to the placebo group and WPI group ($p<0.0001$) (Table 6).

Muscle Endurance

Muscle endurance demonstrated significant improvements from baseline at the end of the study in all the three groups. However, thirty days' supplementation with EB-PA+ WPI resulted in a much greater increase in the exercise volume with a mean change of 196.88 (162.50) kg as compared to both the placebo group [68.93 (115.37) kg] and the WPI group [102.94 (142.23) kg]. This increase in the EB-PA+ WPI group was statistically significant when compared to both the placebo ($p=0.0001$), and WPI ($p=0.0201$) groups (Table 7).

Muscle Flexibility

Flexibility of the lower back and hamstring muscles was assessed by the V-sit and reach test. At day 30, there was an increase in muscle flexibility compared to baseline in all the groups. Among the three groups, the EB-PA+ WPI group demonstrated a greater improvement in muscle flexibility with a mean change of 1.42 (1.24) cm compared to the placebo group and the WPI group wherein mean changes of only 0.46 (1.10) cm, 0.91 (1.18) cm, respectively, were observed. No

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Table 4 Lower Body 1-RM

Parameter	Categories	Placebo (N=42)			WPI (N=36)			EB-PA + WPI (N=43)			p-Value
		Mean (SD)	**95% CI**	**Min, Max**	**Mean (SD)**	**95% CI**	**Min, Max**	**Mean (SD)**	**95% CI**	**Min, Max**	
Achieved 1-RM_Lower Body (kg)	**Day 0**	112.50 (10.02)	(109.38, 115.62)	95.00, 135.00	117.08 (16.05)	(111.65, 122.51)	100.00, 175.00	113.49 (9.91)	(110.44, 116.54)	95.00, 140.00	[a] 0.2246 (A)
	Day 30	116.31 (9.44)	(113.37, 119.25)	100.00, 135.00	127.64 (15.09)	(122.53, 132.75)	100.00, 180.00	133.49 (14.62)	(128.99, 137.99)	100.00, 175.00	[a]<0.0001 (A)
	[b]p-value vs baseline	<0.0001 (t)			<0.0001 (t)			<0.0001 (t)			
	Change from baseline at day 30	3.81 (5.39)	(2.13, 5.49)	0.00, 25.00	10.56 (7.15)	(8.14, 12.97)	0.00, 25.00	20.00 (10.12)	(16.89, 23.11)	0.00, 45.00	[c]<0.0001(A)
	[d]p-value vs EB-PA + WPI	<0.0001 (T)			<0.0001 (T)						

Notes: [a]p-values were calculated using ANOVA Test (A). [b]p-values were calculated using paired *t*-test (t) comparing baseline to post-baseline assessment within each treatment group. [c]p-values were calculated using ANCOVA with treatment as factor and Baseline value as covariate. [d]p-values comparing each treatment group to the EB-PA + WPI group was calculated using Dunnet's *T*-test (T) with Analysis of Covariance (ANCOVA).
Abbreviations: C.I., Confidence Interval; SD, Standard deviation; n, Number of participants.

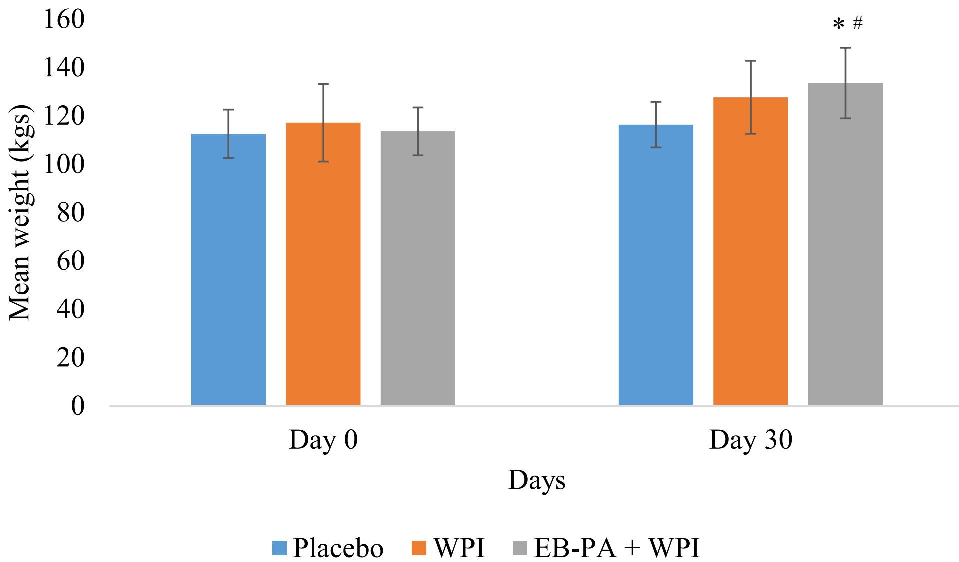

Figure 2 1-RM Lower body strength.
Note: * indicates statistically significant difference between placebo and EB-PA. (p=<.0001); # indicates statistically significant difference between WPI and EB-PA. (p=<.0001).

statistically significant difference was observed between EB-PA+ WPI and WPI group (p= 0.1275). However, a statistically significant difference was observed between the EB-PA+ WPI and Placebo group (p=0.0005) (Table 8 and Figure 4).

Muscle Mass

At day 30, the EB-PA + WPI group demonstrated increases in both LMM and FFM, with mean changes of 53.47 (813.22) g and 54.30 (803.28) g, respectively. In contrast, the placebo and WPI groups showed a reduction in LMM and FFM. These results indicate a positive trend in muscle mass gain with EB-PA + WPI, although the findings were not statistically significant. However, no significant impact of the IP was seen in the reduction of android fat (Table 9).

Grip Strength

Handgrip strength (HGS) reflects the power of the flexor muscles in the palmar, thenar, and hypothenar regions of the hand and is measured using a handgrip dynamometer. At the end of the study, hand grip strength increased in all the groups. Among these, the EB-PA + WPI group showed the most substantial improvement, with a mean increase of 5.12 (3.28) kg. This was significantly higher compared to the WPI [2.44 (2.50) kg] and the Placebo group [0.85 (2.97) kg]. Also, statistically significant differences were noted between the EB-PA + WPI and Placebo group (p<0.0001), as well as between the EB-PA + WPI and WPI groups (p=0.0002) (Table 10).

Sustained Effect

In this study, we hypothesized that the effects of the study products would be sustained for 7 days after the discontinuation of the IP. To assess this, the study products were discontinued from day 30, and their sustained effects were evaluated through day 37. As shown in Table 11, a decrease in both the upper and lower body strength was observed across all study groups. For upper body strength, the EB-PA + WPI experienced the least decline, followed by the placebo group, and then the WPI group. Specifically, the mean reductions in 1-RM for the EB-PA + WPI group was minimal at 0.47 (4.20) kg, compared to 1.81 (2.96) kg in the WPI and 1.07 (3.91) kg in the placebo group. In terms of lower body strength, the EB-PA + WPI group showed a reduction of only 0.93 (4.79) kg. In contrast, the placebo and WPI groups showed comparatively greater mean reductions of 1.81 (4.50) kg and 2.02 (6.15) kg, respectively. No statistically significant difference was observed between the EB-PA + WPI and WPI groups, as well as between the EB-

Table 5 Upper Body 1-RM

Parameter	Categories	Placebo (N=42)			WPI (N=36)			EB-PA + WPI (N=43)			p-Value
		Mean (SD)	**95% CI**	**Min, Max**	**Mean (SD)**	**95% CI**	**Min, Max**	**Mean (SD)**	**95% CI**	**Min, Max**	
Achieved 1-RM_Upper Body (kg)	**Day 0**	59.64 (11.17)	(56.16, 63.13)	40.00, 85.00	64.58 (10.58)	(61.00, 68.16)	45.00, 85.00	64.53 (11.74)	(60.92, 68.15)	40.00, 85.00	[a]0.0760 (A)
	Day 30	62.26 (11.80)	(58.58, 65.94)	40.00, 85.00	70.56 (10.40)	(67.04, 74.08)	50.00, 90.00	76.16 (10.63)	(72.89, 79.43)	50.00, 95.00	[a]<0.0001 (A)
	[b]p-value vs baseline	0.0003 (t)			<0.0001 (t)			<0.0001 (t)			
	Change from baseline at day 30	2.62 (4.31)	(1.28, 3.96)	0.00, 15.00	5.97 (3.75)	(4.70, 7.24)	0.00, 15.00	11.63 (7.85)	(9.21, 14.04)	−20.00, 25.00	[c]<0.0001(A)
	[d]p-value vs EB-PA + WPI	<0.0001 (T)			<0.0001 (T)						

Notes: [a]p-values were calculated using ANOVA Test (A). [b]p-values were calculated using paired *t*-test (t) comparing baseline to post-baseline assessment within each treatment group. [c]p-values were calculated using ANCOVA with treatment as factor and Baseline value as covariate. [d]p-values comparing each treatment group to the EB-PA + WPI group was calculated using Dunnet's *T*-test (T) with Analysis of Covariance (ANCOVA).
Abbreviations: C.I., Confidence Interval; SD, Standard deviation; n, Number of participants.

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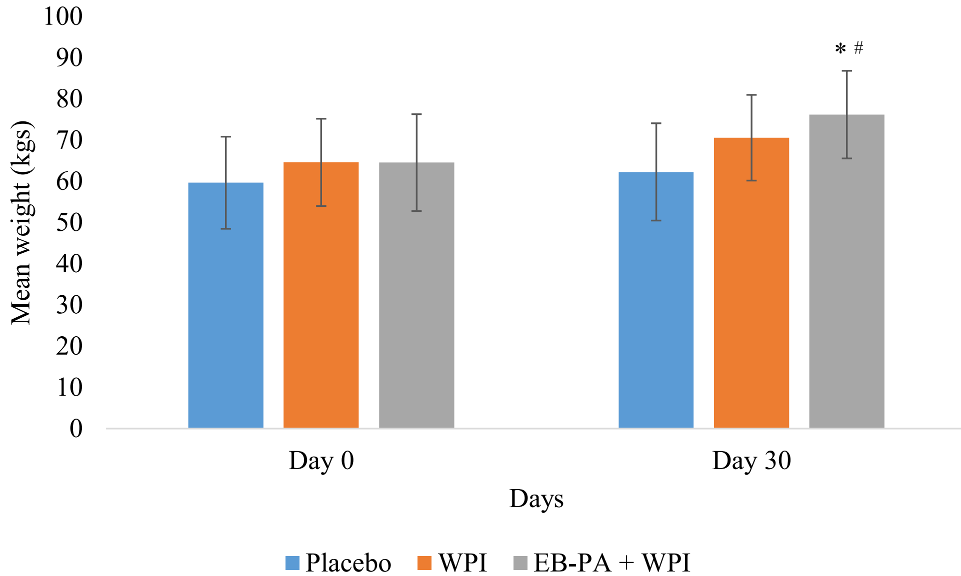

Figure 3 1-RM Upper body strength.
Note: * indicates statistically significant difference between placebo and EB-PA. (p=<.0001); # indicates statistically significant difference between WPI and EB-PA. (p=<.0001).

PA + WPI and placebo groups ($p > 0.05$) in both upper and lower body strength. This study suggests that the EB-PA + WPI group had a more sustained effect on both upper and lower body strength compared to the other groups.

Regarding muscle endurance, the WPI group experienced the greatest increase in endurance, showing a mean increase of 2.64 (131.10) kg. In contrast, the placebo group and the EB-PA + WPI group showed declines in endurance by 30.71 (130.45) kg and 77.67 (131.67) kg, respectively. There was no statistically significant difference was observed between the EB-PA + WPI and WPI groups, as well as between the EB-PA + WPI and placebo groups ($p > 0.05$). This suggests that WPI showed a slight improvement in muscle endurance, the EB-PA + WPI combination did not produce significantly different results compared to the other group (Table 11).

Safety Outcomes

There was no untoward effect observed in the vitals of the enrolled participants throughout the study (Table 12).

Gastrointestinal adverse events were reported in all study groups, showing a comparable distribution among the placebo (n=14 participants), WPI (n=12 participants) and EB-PA + WPI arms (n=16 participants). In the EB-PA group, the majority of reported AEs were mild, self-limiting, and typically resolved within one to two days without significant intervention. A similar pattern was observed in the placebo and WPI groups, where AEs were predominantly transient and mild. Notably, the gastrointestinal AEs in the three groups were assessed as unlikely to be related to the IP. The uniformity of these mild gastrointestinal symptoms across all groups suggests that these events may be non-specific and possibly unrelated to EB-PA supplementation. The similarity in the resolution of symptoms across groups indicates that EB-PA demonstrates a safety profile similar to that of whey protein and placebo. These results suggest that EB-PA is well-tolerated in a healthy population, supporting its potential as a safe supplement for enhancing muscle strength (Table 13).

Discussion

Protein supplements are commonly used by athletes, active adults, and soldiers, who often believe that combining protein intake with exercise will enhance gains in lean mass and improve physical performance.[22] While several studies have shown that whey protein alone may not significantly boost muscle strength in the short duration,[23] it is still regarded as a safe protein source for muscle growth among athletes.[24] The primary hypothesis of the study was thus that EB-PA+ WPI would enhance muscle strength, as assessed by an increase in lower body and upper body 1-RM more than whey

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Table 6 Composite Body Strength

Parameter	Categories	Placebo (N=42)			WPI (N=36)			EB-PA + WPI (N=43)			[a]p-Value
		Mean (SD)	**95% CI**	**Min, Max**	**Mean (SD)**	**95% CI**	**Min, Max**	**Mean (SD)**	**95% CI**	**Min, Max**	
Composite body strength	**Day 0**	172.14 (16.72)	166.93, 177.35	145.00, 210.00	181.67 (21.11)	174.52, 188.81	150.00, 250.00	177.56 (16.59)	172.45, 182.67	145.00, 205.00	0.0694
	Day 30	178.57 (17.51)	173.12, 184.03	145.00, 210.00	197.64 (20.82)	190.59, 204.68	155.00, 255.00	209.65 (19.77)	203.57, 215.74	160.00, 260.00	<0.0001
	[b]p-value vs baseline	<0.0001			<0.0001			<0.0001			
	Change from baseline at day 30	6.43 (7.83)	3.99, 8.87	0.00, 40.00	15.97 (9.70)	12.69, 19.25	0.00, 35.00	32.09 (15.36)	27.36, 36.82	0.00, 65.00	<0.0001
	[c]p-value vs EB-PA + WPI	<0.0001			<0.0001						
	Day 37	175.60 (18.52)	169.82, 181.37	140.00, 210.00	194.03 (19.89)	187.30, 200.76	155.00, 250.00	206.40 (19.65)	200.35, 212.44	170.00, 260.00	<0.0001
	[b]p-value vs baseline	0.0028			<0.0001			<0.0001			
	Change from baseline at day 37	3.45 (7.03)	1.26, 5.64	−5.00, 25.00	12.36 (10.72)	8.73, 15.99	−15.00, 30.00	28.84 (16.86)	23.65, 34.03	0.00, 60.00	<0.0001
	[c]p-value vs EB-PA + WPI	<0.0001			<0.0001						

Notes: [a]p-values were calculated using ANOVA Test (A). [b]p-values were calculated using paired *t*-test (t) comparing baseline to post-baseline assessment within each treatment group. [c]p-values comparing each treatment group to the EB-PA + WPI group were calculated using Students *t* test (T).

Abbreviations: C.I., Confidence Interval; SD, Standard deviation; n, Number of participants.

Table 7 Muscle Endurance

Parameter	Categories	Placebo (N=42)			WPI (N=36)			EB-PA + WPI (N=43)			p-Value
		Mean (SD)	95% CI	Min, Max	Mean (SD)	95% CI	Min, Max	Mean (SD)	95% CI	Min, Max	
Muscle endurance	**Day 0**	846.79 (95.95)	(816.88, 876.69)	650.00, 1030.00	897.75 (152.59)	(846.12, 949.38)	660.00, 1375.00	859.98 (129.66)	(820.07, 899.88)	540.00, 1100.00	[a] 0.1952 (A)
	Day 30	915.71 (124.56)	(876.90, 954.53)	710.00, 1220.00	1000.69 (148.38)	(950.49, 1050.90)	680.00, 1250.00	1056.86 (165.66)	(1005.88, 1107.84)	755.00, 1425.00	[a]0.0001 (A)
	[b]p-value vs baseline	0.0004 (t)			0.0001 (t)			<0.0001 (t)			
	Change from baseline at day 30	68.93 (115.37)	(32.98, 104.88)	−135.00, 310.00	102.94 (142.23)	(54.82, 151.07)	−215.00, 390.00	196.88 (162.50)	(146.87, 246.89)	−100.00, 631.00	[c]<0.0001(A)
	[d]p-value vs EB-PA + WPI	<0.0001 (T)			0.0201 (T)						

Notes: [a]p-values were calculated using ANOVA Test (A). [b]p-values were calculated using paired *t*-test (t) comparing baseline to post-baseline assessment within each treatment group. [c]p-values were calculated using ANCOVA with treatment as factor and Baseline value as covariate. [d]p-values comparing each treatment group to the EB-PA + WPI group was calculated using Dunnet's *T*-test (T) with ANCOVA.

Abbreviations: C.I., Confidence Interval; SD, Standard deviation; n, Number of participants.

Table 8 Muscle Flexibility

Parameter	Categories	Placebo (N=42)			WPI (N=36)			EB-PA + WPI (N=43)			p-Value
		Mean (SD)	95% CI	Min, Max	Mean (SD)	95% CI	Min, Max	Mean (SD)	95% CI	Min, Max	
V sit and reach test	Day 0	2.48 (4.28)	(1.15, 3.82)	−5.00, 12.00	2.53 (4.12)	(1.13, 3.92)	−4.00, 14.00	2.00 (4.28)	(0.68, 3.32)	−9.00, 15.00	[a] 0.8213 (A)
	Day 30	2.94 (4.11)	(1.66, 4.22)	−5.00, 12.00	3.44 (3.96)	(2.10, 4.78)	−3.00, 15.00	3.42 (3.97)	(2.19, 4.64)	−10.00, 13.00	[a]0.8192 (A)
	[b]p-value vs baseline	0.0105 (t)			<0.0001 (t)			<0.0001 (t)			
	Change from baseline at day 30	0.46 (1.10)	(0.11, 0.80)	−2.00, 4.00	0.91 (1.18)	(0.51, 1.31)	−3.00, 4.00	1.42 (1.24)	(1.04, 1.80)	−2.00, 4.00	[c]0.0012 (A)
	[d]p-value vs EB-PA + WPI	0.0005 (T)			0.1275 (T)						

Notes: [a]p-values were calculated using ANOVA Test (A). [b]p-values were calculated using paired *t*-test (t) comparing baseline to post-baseline assessment within each treatment group. [c]p-values were calculated using ANCOVA with treatment as factor and Baseline value as covariate. [d]p-values comparing each treatment group to the EB-PA + WPI group were calculated using Dunnet's *T*-test (T) with Analysis of Covariance (ANCOVA).
Abbreviations: C.I., Confidence Interval; SD, Standard deviation; n, Number of participants.

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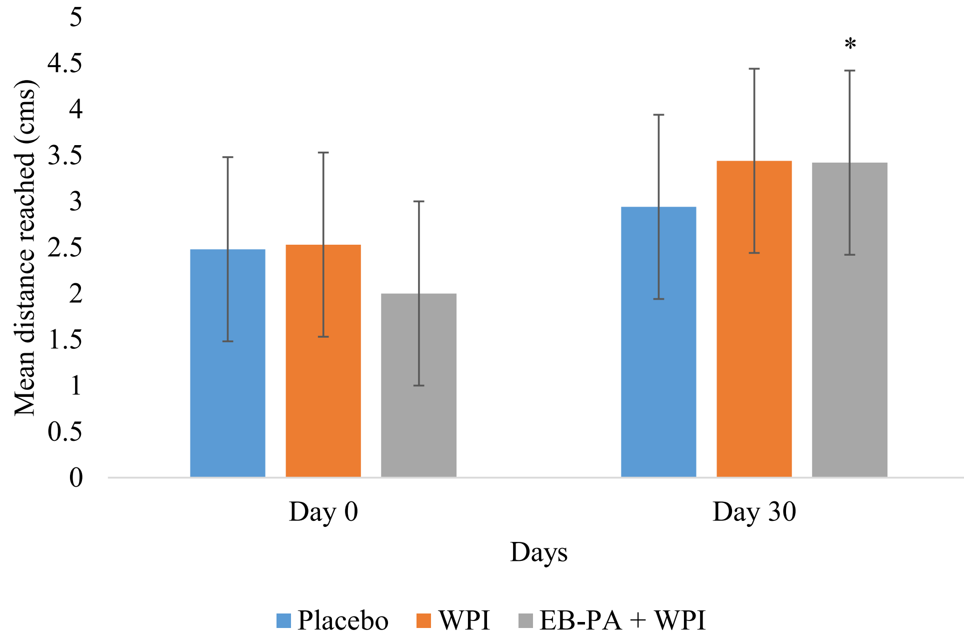

Figure 4 Muscle flexibility.
Note: * indicates statistically significant difference between placebo and EB-PA. (p=0.0005).

protein alone. The current study proved the superiority of EB-PA + WPI over WPI leading to significantly greater improvement in lower as well as upper body strength. 1-RM is a widely used measure in sports and exercise science for assessing maximal muscle strength and tracking an athlete's training progress.[22] In this study, participants in the EB-PA + WPI group experienced a twofold greater increase in upper body strength, with an 18.02% improvement compared to 9.24% in the WPI group. The EB-PA + WPI group also observed a 17.62% improvement in lower body strength, while the WPI group achieved a 9.02% increase. Additionally, at both day 30 and day 37, the EB-PA+ WPI group showed a statistically significant improvement in composite body strength compared to both the placebo and WPI groups ($p<0.0001$). These findings demonstrated that the EB-PA + WPI supplementation proved to be significantly efficacious in enhancing both lower and upper body 1-RM strength compared to the other groups, highlighting the superiority of this combination over protein alone. Similar results are in line with the known literature. In a clinical study conducted by Escalante G et al,[25] 8 weeks of phosphatidic acid supplementation, combined with resistance exercise training three days per week, resulted in a significant increase in 1-RM strength for both leg press and bench press exercises compared to a placebo group. Furthermore, in another clinical study, 8 weeks of amylopectin-chromium complex plus 15 g of whey protein led to greater relative 1-RM improvements (Squat: 1.32 kg/kg and bench press: 1.14 kg/kg) compared to 15 g or 30 g of whey protein alone.[26] These improvements in muscle strength also led to enhanced muscle endurance in the IP group. Muscle endurance is the ability of a muscle or muscle group to sustain repeated contractions against a load over an extended period.[27] The results indicated that the EB-PA + WPI group achieved twice the improvement in exercise volume, with a 22.89% increase, compared to the 11.47% increase in the WPI group. Furthermore, this change between the two groups was also statistically significant ($p=0.0201$). This finding is in line with previous research, which demonstrated that 10 weeks of creatine plus β-alanine supplementation significantly increased the mean training volume per week (reps × sets × load × number of sessions), leading to greater improvement in strength.[28] Additionally, when the study products were discontinued for 7 days to assess the sustained effects, all groups experienced a reduction in muscle strength. However, the EB-PA + WPI group had the smallest reduction, with decreases of 0.47 kg in upper body strength and 0.93 kg in lower body strength, compared to the WPI group, which showed reductions of 1.81 kg in both. This suggests that the EB-PA + WPI group had a more sustained effect on muscular strength compared to the other groups. Furthermore, for muscle endurance, WPI demonstrated a slight improvement compared to the other groups.

Table 9 Muscle Mass

Parameters	Categories	Placebo (N=42)			WPI (N=36)			EB-PA + WPI (N=43)			p-Value
		Mean (SD)	95% CI	Min, Max	Mean (SD)	95% CI	Min, Max	Mean (SD)	95% CI	Min, Max	
Lean body mass (g)	Day 0	49,298.21 (5960.98)	(47,440.64, 51,155.79)	38,975.00, 61,911.00	50,079.56 (5917.31)	(48,077.42, 52,081.69)	41,756.00, 64,573.00	48,737.30 (4826.39)	(47,251.96, 50,222.65)	40,281.00, 60,726.00	[a]0.5670 (A)
	Day 30	49,257.33 (5889.51)	(47,422.03, 51,092.63)	39,428.00, 62,683.00	49,892.08 (5675.89)	(47,971.64, 51,812.53)	42,980.00, 63,132.00	48,790.77 (4803.37)	(47,312.51, 50,269.03)	39,872.00, 60,756.00	[a]0.6718 (A)
	[b]p-value vs baseline	0.8108 (t)			0.4004 (t)			0.6686 (t)			
	Change from baseline at day 30	−40.88 (1099.44)	(−383.49, 301.73)	−2667.00, 1771.00	−187.47 (1321.26)	(−634.52, 259.58)	−3346.00, 2513.00	53.47 (813.22)	(−196.81, 303.74)	−2023.00, 1724.00	[c]0.7350 (A)
	[d]p-value vs EB-PA + WPI	0.9313 (T)			0.6535 (T)						
Fat free mass (g)	Day 0	51,948.48 (6118.85)	(50,041.71, 53,855.24)	41,380.00, 65,098.00	52,786.14 (6128.40)	(50,712.59, 54,859.69)	44,219.00,67,642.00	51,430.53 (5013.74)	(49,887.53, 52,973.54)	42,615.00, 63,558.00	[a] 0.5795 (A)
	Day 30	51,905.52 (6023.21)	(50,028.56, 53,782.49)	41,836.00,65,492.00	52,614.75 (5891.84)	(50,621.24, 54,608.26)	45,165.00, 66,298.00	51,484.84 (4969.49)	(49,955.45, 53,014.22)	42,193.00, 63,542.00	[a]0.6722 (A)
	[b]p-value vs baseline	0.7991 (t)			0.4293 (t)			0.6598 (t)			
	Change from baseline at day 30	−42.95 (1086.52)	(−381.53, 295.63)	−2661.00, 1757.00	−171.39 (1285.95)	(−606.49, 263.71)	−3267.00, 2390.00	54.30 (803.28)	(−192.91, 301.51)	−2005.00, 1737.00	[c]0.7648(A)
	[d]p-value vs EB-PA + WPI	0.9198 (T)			0.6875 (T)						
Android Fat (%)	Day 0	34.87 (13.66)	(30.62, 39.13)	10.10, 54.70	35.00 (8.14)	(32.24, 37.75)	16.80, 48.80	34.99 (9.31)	(32.13, 37.85)	14.50, 51.90	[a]0.9982 (A)
	Day 30	34.81 (13.98)	(30.45, 39.17)	7.50, 55.00	35.93 (8.33)	(33.11, 38.75)	15.00, 50.30	35.07 (9.21)	(32.23, 37.90)	14.80, 51.20	[a]0.8959 (A)
	[b]p-value vs baseline	0.8161 (t)			0.0881 (t)			0.7285 (t)			
	Change from baseline at day 30	−0.06 (1.71)	(−0.60, 0.47)	−3.80, 3.80	0.93 (3.18)	(−0.15, 2.01)	−3.20, 16.20	0.07 (1.40)	(−0.36, 0.50)	−2.60, 3.50	[c]0.1036 (A)
	[d]p-value vs EB-PA + WPI	0.9415 (T)			0.1512 (T)						

Notes: [a]p-values were calculated using ANOVA Test (A). [b]p-values were calculated using paired *t*-test (t) comparing baseline to post-baseline assessment within each treatment group. [c] p-values were calculated using ANCOVA with treatment as factor and Baseline value as covariate. [d]p-values comparing each treatment group to the EB-PA + WPI group were calculated using Dunnet's *T*-test (T) with Analysis of Covariance (ANCOVA).
Abbreviations: C.I., Confidence Interval; SD, Standard deviation; n, Number of participants.

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Table 10 Grip Strength

Parameter	Categories	Placebo (N=42)			WPI (N=36)			EB-PA + WPI (N=43)			p-Value
		Mean (SD)	**95% CI**	**Min, Max**	**Mean (SD)**	**95% CI**	**Min, Max**	**Mean (SD)**	**95% CI**	**Min, Max**	
Grip strength	**Day 0**	31.25 (6.14)	(29.34, 33.17)	21.67, 52.67	34.11 (7.26)	(31.66, 36.57)	21.67, 52.67	32.93 (7.63)	(30.58, 35.28)	20.00, 50.67	[a] 0.1984 (A)
	Day 30	32.10 (5.65)	(30.34, 33.86)	21.67, 52.00	36.56 (6.35)	(34.40, 38.71)	26.00, 53.00	38.05 (7.05)	(35.88, 40.23)	27.67, 53.33	[a]0.0001 (A)
	[b]p-value vs baseline	0.0705 (t)			<0.0001 (t)			<0.0001 (t)			
	Change from baseline at day 30	0.85 (2.97)	(−0.07, 1.78)	−5.00, 10.16	2.44 (2.50)	(1.60, 3.29)	−4.00, 8.33	5.12 (3.28)	(4.12, 6.13)	−4.00, 12.67	[c]<0.0001(A)
	[d]p-value vs EB-PA + WPI	<0.0001 (T)			0.0002 (T)						

Notes: [a]p-values were calculated using ANOVA Test (A). [b]p-values were calculated using paired *t*-test (t) comparing baseline to post-baseline assessment within each treatment group.[c]p-values were calculated using ANCOVA with treatment as factor and Baseline value as covariate. [d]p-values comparing each treatment group to the EB-PA + WPI group were calculated using Dunnet's *T*-test (T) with Analysis of Covariance (ANCOVA).
Abbreviations: C.I., Confidence Interval; SD, Standard deviation; n, Number of participants.

Table 11 Sustained Effect

Parameter	Categories	Placebo (N=42)			WPI (N=36)			EB-PA + WPI (N=43)			p-Value
		Mean (SD)	95% CI	Min, Max	Mean (SD)	95% CI	Min, Max	Mean (SD)	95% CI	Min, Max	
Achieved_1-RM_Upper body (kg)	**Day 30**	62.26 (11.80)	(58.58, 65.94)	40.00, 85.00	70.56 (10.40)	(67.04, 74.08)	50.00, 90.00	76.16 (10.63)	(72.89, 79.43)	50.00, 95.00	[a]<0.0001 (A)
	Day 37	61.19 (12.44)	(57.32, 65.07)	40.00, 85.00	68.75 (9.81)	(65.43, 72.07)	50.00, 80.00	75.70 (9.79)	(72.68, 78.71)	50.00, 90.00	[a]<0.0001 (A)
	Change from day 30 to 37	−1.07 (3.91)	(−2.29, 0.15)	−15.00, 10.00	−1.81 (2.96)	(−2.81, −0.80)	−10.00, 0.00	−0.47 (4.20)	(−1.76, 0.83)	−10.00, 20.00	[b]0.0804 (A)
	[c]p-value vs EB-PA + WPI	0.1223 (T)			0.0726 (T)						
Achieved_1-RM_Lower Body (kg)	**Day 30**	116.31 (9.44)	(113.37, 119.25)	100.00, 135.00	127.64 (15.09)	(122.53, 132.75)	100.00, 180.00	133.49 (14.62)	(128.99, 137.99)	100.00, 175.00	[a]<0.0001 (t)
	Day 37	114.29 (10.45)	(111.03, 117.54)	95.00, 135.00	125.83 (15.14)	(120.71, 130.96)	100.00, 180.00	132.56 (14.94)	(127.96, 137.15)	100.00, 175.00	[a]<0.0001 (A)
	Change from day 30 to 37	−2.02 (6.15)	(−3.94, −0.11)	−20.00, 15.00	−1.81 (4.50)	(−3.33, −0.28)	−15.00, 10.00	−0.93 (4.79)	(−2.40, 0.54)	−15.00, 15.00	[b]0.3270 (A)
	[c]p-value vs EB-PA + WPI	0.2436 (T)			0.5286 (T)						
Muscle endurance	**Day 30**	915.71 (124.56)	(876.90, 954.53)	10.00, 1220.00	1000.69 (148.38)	(950.49, 1050.90)	680.00, 1250.00	1056.86 (165.66)	(1005.88, 1107.84)	755.00, 1425.00	[a]0.0001 (A)
	Day 37	885.00 (119.35)	(847.81, 922.19)	655.00, 1170.00	1003.33 (153.77)	(951.31, 1055.36)	765.00, 1370.00	979.19 (147.61)	(933.76, 1024.61)	705.00, 1300.00	[a]0.0006 (A)
	Change from day 30 to 37	−30.71 (130.45)	(−71.37, 9.94)	−375.00, 195.00	2.64 (131.10)	(−41.72, 47.00)	−315.00, 270.00	−77.67 (131.67)	(−118.20, −37.15)	−315.00, 315.00	[b]0.0211 (A)
	[c]p-value vs EB-PA + WPI	0.7908 (T)			0.0656 (T)						

Notes: [a]p-values were calculated using ANOVA Test (A). [b]p-values were calculated using ANCOVA with treatment as factor and Baseline value as covariate. [c]p-values comparing each treatment group to the EB-PA + WPI group was calculated using Dunnet's *T*-test (T) with Analysis of Covariance (ANCOVA).

Abbreviations: C.I., Confidence Interval; SD, Standard deviation; n, Number of participants.

Table 12 Vitals

Parameters	Categories	Placebo (N=42)			WPI (N=36)			EB-PA + WPI (N=43)			p-Value
		Mean (SD)	**95% CI**	**Min, Max**	**Mean (SD)**	**95% CI**	**Min, Max**	**Mean (SD)**	**95% CI**	**Min, Max**	
Pulse Rate (Beats/Min)	**Day 0**	74.33 (7.41)	(72.02, 76.64)	58.00, 88.00	75.61 (5.53)	(73.74, 77.48)	66.00, 88.00	75.53 (5.04)	(73.98, 77.09)	66.00, 84.00	[a] 0.5700 (A)
	Day 30	74.83 (5.78)	(73.03, 76.63)	64.00, 90.00	75.39 (3.12)	(74.33, 76.44)	68.00, 80.00	75.79 (4.81)	(74.31, 77.27)	66.00, 86.00	[a] 0.6507 (A)
	[b]p-value vs baseline	0.6108 (t)			0.7991 (t)			0.7998 (t)			
	Change from baseline at day 30	0.50 (6.32)	(−1.47, 2.47)	−11.00, 18.00	−0.22 (5.20)	(−1.98, 1.54)	−16.00, 10.00	0.26 (6.57)	(−1.77, 2.28)	−11.00, 18.00	[c]0.8177 (A)
	[d]p-value vs EB-PA + WPI	0.7657 (T)			0.8783 (T)						
	Day 37	74.48 (4.59)	(73.05, 75.91)	66.00, 84.00	74.42 (3.42)	(73.26, 75.57)	66.00, 82.00	74.53 (5.00)	(73.00, 76.07)	64.00, 86.00	[a]0.9931 (A)
	[b]p-value vs baseline	0.8980 (t)			0.2384 (t)			0.3225 (t)			
	Change from baseline at day 37	0.14 (7.18)	(−2.09, 2.38)	−16.00, 16.00	−1.19 (5.98)	(−3.22, 0.83)	−14.00, 10.00	−1.00 (6.55)	(−3.02, 1.02)	−16.00, 12.00	[c]0.9588 (A)
	[d]p-value vs EB-PA + WPI	0.9811 (T)			0.9870 (T)						
Systolic BP (mmHg)	**Day 0**	118.36 (7.04)	(116.16, 120.55)	100.00, 129.00	118.92 (5.72)	(116.98, 120.85)	108.00, 130.00	117.79 (6.76)	(115.71, 119.87)	100.00, 130.00	[a] 0.7500 (A)
	Day 30	119.24 (6.65)	(117.17, 121.31)	108.00, 131.00	119.14 (5.27)	(117.36, 120.92)	110.00, 130.00	118.44 (5.71)	(116.68, 120.20)	108.00, 130.00	[a]0.7994 (A)
	[b]p-value vs baseline	0.3697 (t)			0.8020 (t)			0.5903 (t)			
	Change from baseline at day 30	0.88 (6.29)	(−1.08, 2.84)	−13.00, 14.00	0.22 (5.28)	(−1.56, 2.01)	−16.00, 10.00	0.65 (7.87)	(−1.77, 3.07)	−18.00, 20.00	[c]0.8859(A)
	[d]p-value vs EB-PA + WPI	0.8422 (T)			0.9699 (T)						
	Day 37	119.81 (5.29)	(118.16, 121.46)	108.00, 129.00	121.28 (6.01)	(119.25, 123.31)	110.00, 130.00	120.37 (5.46)	(118.69, 122.05)	110.00, 128.00	[a]0.5092 (A)
	[b]p-value vs baseline	0.1468 (t)			0.0305 (t)			0.0235 (t)			
	Change from baseline at day 37	1.45 (6.36)	(−0.53, 3.44)	−10.00, 14.00	2.36 (6.28)	(0.23, 4.49)	−10.00, 18.00	2.58 (7.20)	(0.37, 4.80)	−12.00, 18.00	[c]0.5403 (A)
	[d]p-value vs EB-PA + WPI	0.7198 (T)			0.8682 (T)						

(*Continued*)

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Table 12 (Continued).

Parameters	Categories	Placebo (N=42)			WPI (N=36)			EB-PA + WPI (N=43)			p-Value
		Mean (SD)	95% CI	Min, Max	Mean (SD)	95% CI	Min, Max	Mean (SD)	95% CI	Min, Max	
Diastolic BP (mmHg)	**Day 0**	74.21 (7.59)	(71.85, 76.58)	60.00, 84.00	78.19 (5.14)	(76.46, 79.93)	66.00, 84.00	75.93 (6.72)	(73.86, 78.00)	60.00, 86.00	[a]0.0332 (A)
	Day 30	74.93 (6.85)	(72.79, 77.06)	60.00, 85.00	76.39 (5.38)	(74.57, 78.21)	68.00, 86.00	75.35 (6.44)	(73.37, 77.33)	60.00, 84.00	[a]0.5823 (A)
	[b]p-value vs baseline	0.4644 (t)			0.0336 (t)			0.3955 (t)			
	Change from baseline at day 30	0.71 (6.27)	(−1.24, 2.67)	−14.00, 12.00	−1.81 (4.90)	(−3.46, −0.15)	−12.00, 10.00	−0.58 (4.44)	(−1.95, 0.79)	−10.00, 10.00	[c]0.6123 (A)
	[d]p-value vs EB-PA + WPI	0.7439 (T)			0.9069 (T)						
	Day 37	74.88 (7.73)	(72.47, 77.29)	58.00, 86.00	75.25 (10.14)	(71.82, 78.68)	24.00, 84.00	74.56 (6.08)	(72.69, 76.43)	60.00, 84.00	[a]0.9298 (A)
	[b]p-value vs baseline	0.4076 (t)			0.0982 (t)			0.0982 (t)			
	Change from baseline at day 37	0.67 (5.16)	(−0.94, 2.28)	−12.00, 14.00	−2.94 (10.40)	(−6.46, 0.57)	−56.00, 12.00	−1.37 (5.84)	(−3.17, 0.42)	−14.00, 12.00	[c]0.4044 (A)
	[d]p-value vs EB-PA + WPI	0.5595 (T)			0.8621 (T)						

Notes: [a]p-values were calculated using ANOVA Test (A). [b]p-values were calculated using paired *t*-test (t) comparing baseline to post-baseline assessment within each treatment group.[c]p-values were calculated using ANCOVA with treatment as factor and Baseline value as covariate. [d]p-values comparing each treatment group to the EB-PA + WPI group were calculated using Dunnet's *T*-test (T) with Analysis of Covariance (ANCOVA).
Abbreviations: C.I., Confidence Interval; SD, Standard deviation; n, Number of participants.

Table 13 Adverse Events

Adverse Event	Placebo	WPI	EB-PA + WPI	Overall
	(N=46)	(N=46)	(N=46)	(N=138)
Heart burn	1	0	0	1
Abdominal Pain	9	2	8	19
Abdominal Rumbling	0	2	4	6
Abdominal fullness	1	0	0	1
Acidity	1	2	3	6
Belching	1	3	1	5
Bloating	5	5	9	19
Constipation	5	1	0	6
Diarrhea	1	3	1	5
Flatulence	1	3	7	11
Nausea	2	0	4	6
Postprandial fullness	4	5	8	17
Thigh Muscle pain	1	0	0	1

Notes: Percentages were calculated using respective column header count as denominator.
Abbreviation: N,Number of participants.

The current study also reports a noteworthy impact of EB-PA on muscle flexibility and grip strength. The sit-and-reach test is commonly used to evaluate flexibility in the hamstrings and lower back, which are important components of overall health-related fitness.[29,30] In the present study, at the end of the intervention, the EB-PA + WPI group had the greatest improvement in muscle flexibility with a 71% increase, which was significantly higher than the placebo (18.55%) and WPI (35.97%) groups. The difference between EB-PA + WPI and placebo was highly significant (p=0.0005), indicating that EB-PA + WPI was the most effective in enhancing muscle flexibility. This result is consistent with findings from a pilot study conducted by Enovate,[19] where 30 days of EB-PA supplementation resulted in a threefold mean increase in the muscle flexibility measured by the V Sit-and-Reach Test, with a 2.08 cm compared to 0.65 cm in the placebo group. Another research conducted by Oh et al,[31] reported a notable improvement in the muscle flexibility measured by the Chair Sit-and-Reach Test after 12 weeks of leucine-rich protein supplementation compared to a placebo. HGS training is conceivably of importance to enhancing the performance of a number of gross motor movement patterns in sports and athletic disciplines involving the hand.[32] According to the National Health and Nutrition Examination Survey (NHANES) 2011–2014, for adults aged 19+ years, intake of plant protein significantly increased grip strength with a p-value of 0.0007. While, in this study, the EB-PA + WPI group demonstrated a significant improvement in handgrip strength of 15.55%, compared to the WPI (7.15%) and the placebo group (2.72%). Statistically significant differences were found between the EB-PA + WPI group and both the placebo (p<0.0001) and WPI groups (p=0.0002), indicating that the EB-PA and whey protein was the most effective intervention for enhancing handgrip strength. This finding is in line with the previous research conducted by Rokkam MP et al,[33] which showed that 8 weeks of a standardized formulation of *Sphaeranthus indicus* flower head and *Mangifera indica* stem bark extracts increased handgrip strength to 46.10 kg, compared to 42.10 kg with a placebo.

Body composition is crucial for sports performance, with lower fat mass and higher FFM linked to improved physical performance. For high-level men's sports, evaluating FFM, especially muscle mass, is essential for enhancing performance.[34] Increases in lean body mass are also important for developing strength and power.[35] In this study, the EB-PA + WPI group showed considerable increases in both LMM and FFM, while the Placebo and WPI groups observed reductions in these parameters. A previous study showed that 12 weeks of whey protein combined with RET significantly increased lean body mass ($p = 0.015$),[36] whereas our 4-week study did not achieve statistical significance, likely due to the shorter duration. This finding aligns with research by Hoffman JR et al,[37] which demonstrated an increase of 2.6% in lean body mass after 8 weeks of phosphatidic acid supplementation combined with a 4-day-per-week resistance training program, compared to a 0.1% increase in the placebo group. Conversely, a study by McAdam et al[38] found no significant change in FFM after 8 weeks of whey protein supplementation combined with twice-daily physical fitness training in healthy male participants.

The present study had few limitations. Firstly, the short duration of the study may not have been adequate to fully evaluate the long-term effects and sustainability of the intervention. Secondly, the effect of the EB-PA alone group was not evaluated in this study. Thirdly, the study included only male participants, which limits the generalizability of the findings. The foundation for more focused research in female groups is laid by discussing the male cohort's results in light of potential mechanisms driving the botanical's interaction with whey protein. Indeed, the effectiveness and use of *P. amarus* as a supplement to promote muscle health may be impacted by physiological variations; however, this should not limit the use of this botanical to the male population alone. The results of this current study highlight *P. amarus's* potential for clinical applications to optimize muscle health, which calls for an extensive investigation to validate its advantages across a range of demographics, including EB-PA alone group, longer study duration and inclusion of female participants. Additionally, future studies should also assess the role of EB-PA in muscle recovery for longer periods and explore its potential in preventing muscle loss due to aging and assess the long-term safety of the supplement.

Conclusion

EB-PA + WPI supplementation was found to be safe and well-tolerated throughout the study. The results indicated that the addition of EB-PA to WPI, along with RET, resulted in significant improvements in both lower and upper body strength compared to WPI alone. Additionally, EB-PA + WPI enhanced muscle endurance, muscle flexibility, and improved handgrip strength compared to WPI alone. These findings suggest that EB-PA enhances the muscle strengthening effect of protein supplements. Therefore, we suggest that EB-PA can be utilized as an adjuvant in combination with WPI for active adults, including athletes, bodybuilders and gym-goers. However, larger sample size and longer duration study with diverse populations are needed to confirm these results.

Data Sharing Statement

The data presented in the study is available on reasonable request from the corresponding author with due permission from the sponsor.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted, and agree to be accountable for all aspects of the work.

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Disclosure

Rekha Patel (RP) is an employee of Enovate Biolife, but not involved in the study conduct, data management and statistical analysis. Therefore, it shows non-financial conflict of interest. RP also reports a pending all rights of patent assigned to OmniActive Health Technologies. Patent Cooperation Treaty (PCT) is also filled. Archana Gujja (AG) is affiliated with Vedic Lifesciences but not involved in the study conduct, data management and statistical analysis. Preeti Bawaskar (PB) and Anand Mahendra Prasad Yadav (AMPY) are affiliated with clinics that contributed to the study's conduct under the supervision of Vedic Lifesciences (Contract Research Organization), with incentives provided according to the terms of the clinical research agreement by Vedic Lifesciences. The authors report no other conflicts of interest in this work.

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