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Effects of gypenoside L-containing *Gynostemma pentaphyllum* extract on fatigue and physical performance: A double-blind, placebo-controlled, randomized trial

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Abstract

This study was conducted to investigate the effect of *Gynostemma pentaphyllum* extract containing gypenoside L (GPE) on improving the cognitive aspects of fatigue and performance of the motor system. One hundred healthy Korean adults aged 19–60 years were randomized to the treatment (GPE for 12 weeks) and control groups, and efficacy and safety-related parameters were compared between the two groups. Maximal oxygen consumption (VO_2 max) and O_2 pulse were significantly higher in the treatment group than in the control group ($p = 0.007$ and $p = 0.047$, respectively). After 12 weeks, the treatment group showed significant changes such as decreases in the levels of free fatty acids ($p = 0.042$). In addition, there were significant differences in the rating of perceived exertion (RPE) ($p < 0.05$) and value of temporal fatigue between the treatment and control groups on the multidimensional fatigue scale ($p < 0.05$). Moreover, the level of endothelial nitric oxide synthase (eNOS) in the blood was significantly higher in the treatment group than in the control group ($p = 0.047$). In summary, oral administration of GPE has a positive effect on resistance to exercise-induced physical and mental fatigue.

KEYWORDS
clinical trial, cognitive fatigue, exercise performance, *Gynostemma pentaphyllum*, gypenoside L

1 | INTRODUCTION

Physical and mental health can be maintained by increasing motor performance and ability to balance through muscular strength and endurance, which is defined as the maximum force that can be endured by a muscle group (Caspersen et al., 1985). Although steady endurance exercise is suggested as an efficient activity to maintain health, exposure to reduced physical activity, fatigue, and lethargy due to the development of material civilization, accumulation of wealth, and unhealthy lifestyle behaviors is common (Baker et al., 2014; Eime et al., 2013; Malm et al., 2019; McPhee et al., 2016). In addition, it is difficult to lead a healthy life because of rampant environmental pollution and constant mental stress (Cianconi et al., 2020; Manisalidis et al., 2020). Therefore, not only elite athletes, but also

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the general public and non-elite amateur athletes have shown continuous interest in exercise to improve metabolic and immune functions, control body weight, relieve stress, and prevent various diseases (Piermattéo et al., 2020).

Muscle fatigue from indiscriminate exercise performance is caused by depletion of energy sources, accumulation of fatigue-related substances in muscles and blood, imbalance in homeostasis, and dysfunction of neuromodulation (Finsterer, 2012; Nikolaidis et al., 2012; Taylor et al., 2016; Wan et al., 2017). An imbalance between adenosine triphosphate (ATP) breakdown and resynthesis or between oxygen requirement for glycolysis and intramuscular oxygen content is related to lactate production, which increases hydrogen ion concentration and decreases blood pH (Sahlin, 1986; Wan et al., 2017). In addition, ammonia, which is produced as a result of fatigue, is known to stimulate the activity of phosphofructokinase, inhibit the citric acid cycle and gluconeogenesis, and affect lactate production and pH by reducing mitochondrial oxidation (Westerblad et al., 2002). Furthermore, it has been reported in several studies that increased levels of circulating ammonia during prolonged exercise can influence muscle metabolism and central fatigue (Nybo et al., 2005; Wilkinson et al., 2010). Previous studies on prevention of fatigue and improvement of exercise performance suggest that training methods, sports equipment, and dietary supplements are factors that improve exercise performance. Studies related to exercise plus nutritional supplements have shown that consumption of beverages containing glucose and fructose improves anaerobic power and that of a solution containing glucose and electrolyte decreases blood levels of lactate and ammonia and prolongs exercise duration (Demura et al., 2010; Hummer et al., 2019; Martinez et al., 2016). In addition, a decrease in blood levels of fatigue-related substances and an increase in exercise duration were reported when a carbohydrate and electrolyte mixture was orally administered (Linseman et al., 2014; Nassis et al., 1998).

Aerobic exercise through continuous muscle contraction affects mitochondrial respiration control, sarcoplasmic reticulum function, and lipid peroxidation, and increases intracellular free radical production and oxidative stress (Powers et al., 2011; Steinbacher & Eckl, 2015). This oxidative stress can be reduced by improving tissue defenses by promoting a cascade reaction of the antioxidant enzyme system and ingestion of antioxidants with radical-quenching potential (Dumanovic et al., 2021; Pizzino et al., 2017). In addition, repetitive muscle contraction due to exercise activates AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), which is known to regulate mitochondrial biosynthesis, energy homeostasis, body temperature, and glucose metabolism (Hardie, 2014; Hardie & Ashford, 2014; Thomson, 2018). Moreover, recent studies have focused on various materials used as food ingredients that can activate AMPK/PGC-1α pathway of muscles without inducing tolerance or side effects (Jeong et al., 2012; Zhang et al., 2019).

With regard to physical exercise and skeletal muscle fatigue, in vivo experiments and clinical trials have shown that the use of food supplements such as polysaccharides, anthocyanins, ginsenosides, and catechins from botanical extracts can ameliorate exercise performance and recovery (Furst & Zundorf, 2014; Yarahmadi et al., 2014). *Gynostemma pentaphyllum* (Thunb.) is a traditional medicinal herb containing a group of triterpene saponins called gypenosides, which are very similar to ginsenosides from the *Panax* species, but the triterpene saponin content of *G. pentaphyllum* is several times higher than that of *Panax ginseng* (Kim & Han, 2011). *G. pentaphyllum* shows a higher triterpenoid content, faster growth rate, and higher number of harvests than the *Panax* species, which is advantageous, and this medicinal plant is a promising alternative source for ginsenoside production. Previous studies have revealed that *G. pentaphyllum* shows antioxidant, anti-inflammatory, neuroprotective, and anti-cancer properties (Xie et al., 2010). In particular, gypenoside L, the main active constituent in *G. pentaphyllum*, is known to have a remarkable antioxidant capacity (Wang et al., 2018). Despite its beneficial effects on muscle fatigue, as reported based on in vitro and in vivo studies (Kim, Jung, Jeon, Kim, Hong, et al., 2020; Kim, Jung, Jeon, Kim, Oh, et al., 2020), there have been no clinical trials on its effects on exercise-induced changes in blood and biochemical parameters of fatigue and physical performance.

The purpose of this randomized, double-blind, placebo-controlled clinical trial (No. 2019-10-023) was to evaluate the effect of *G. pentaphyllum* extract containing gypenoside L (GPE) under non-exercise conditions on the improvement of physical performance. The duration of treatment with GPE (once a day) was 12 weeks, and the functional properties of the substance were evaluated by comparing various parameters between time points and between the two groups.

2 | MATERIALS AND METHODS

2.1 | Materials

GPE was prepared by BTC Corporation (Ansan, Korea) to investigate the ergogenic effect of gypenoside L-enriched extract under non-exercise on exercise performance. To describe briefly, the dried leaves of *G. pentaphyllum* were extracted separately with hot water and 50% EtOH aqueous solution; these extracts were combined, concentrated, and filtered. Total gypenoside L, gypenoside LI, and ginsenoside Rg3 contents of the extracts were measured using liquid chromatography–mass spectrometry. A Thermo U3000-LTQ XL ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA), equipped with an electrospray ionization (ESI) mass source was used. Gypenoside L, gypenoside LI, and ginsenoside Rg3 were dissolved in methanol and prepared using an HSS T3 C18 column (2.1 mm I.D. × 150 mm L., 2.5-μm particle size, Waters, Milford, MA). The mobile phase A was 0.1% formic acid in deionized water and B was 0.1% formic acid in acetonitrile. The gradient for elution was as follows: 5%–100% B for the run time from 0 to 15 min, followed by a linear gradient from 5 min of 100% B. The flow rate was 0.5 mL/min. The total run time was 20 min. Mass spectrometric detection was operated using the ESI mode. The setting of operating parameters was as follows: spray voltage +5 kV; ion transfer capillary temperature 275°C; nitrogen sheath gas 35; and auxiliary gas 5 arb. The mass spectrometry experiments were controlled by the Xcalibur system (version 2.2 SPI.48; Thermo Fisher Scientific).

2.2 | Participants and study design

Participants were healthy Korean adults aged 19–60 years, and the study period was from February 2020 to November 2020. Participants who met the following criteria were included: (1) females and males (19 ≤ age ≤ 60 years); (2) a participant who agreed to an exercise stress test; and (3) a participant who voluntarily decided to participate in the study and agreed to comply with the precautions. Participants with the following conditions were excluded: (1) abnormalities in the results of the exercise stress test; (2) grade 1 or grade 5 in the standard five stages of maximal oxygen consumption (VO_2 max) according to the age at the time of the screening tests; (3) body mass index (BMI) <18.5 kg/m^2 or ≥35 kg/m^2; (4) presence of clinically severe cardiovascular, endocrine, neuropsychiatric, musculoskeletal, gastrointestinal, inflammatory, hematological, and/or neoplastic diseases; (5) use of drugs or health-functional foods related to exercise performance within 3 months before the study's screening tests; (6) administration of ergogenic aids within 2 weeks before the screening tests; (7) intake of anti-psychotic agents within the previous 3 months; (8) presence of alcoholism or substance abuse; (9) a history of renal failure, heart failure, myocardial infarction, or stroke; (10) participation in another clinical trial within 3 months before the screening tests; (11) detection of aspartate aminotransferase (AST) or alanine transaminase (ALT) level >3 times the upper limit or serum creatinine level >2.0 mg/dL in the diagnostic test; (12) presence of other reasons of ineligibility as determined by the investigators. A total of 100 participants who provided written consent were included in the study; 50 each were assigned to the control (placebo) and treatment (GPE) groups.

According to the approved protocol (Institutional Review Board No. 2019-10-023), this was a single-center, randomized, double-blind, placebo-controlled study. After screening (up to 4 weeks), all participants who signed an informed consent document were registered on the basis of the inclusion criteria and randomized for the administration of placebo or treatment. Randomization was used in the double-blind stage to avoid bias when assigning subjects to test groups, to evenly balance the characteristics of subjects for each test group, and to increase the effectiveness of statistical comparisons between test groups. Participant blocks of certain sizes were used for block randomization, and randomization sequences were concealed from all participants and investigators for the double-blind study until the end of the study. Of a total of 100 participants, 93 completed the study according to the protocol; two had withdrawn, and five were excluded. Moreover, those who showed abnormal creatine kinase (CK) levels and weight and those who were determined to be outliers using the two standard deviations method were excluded from the analysis. The study was approved by the Inha University Hospital Institutional Review Board (December 23, 2019) in Korea and was conducted in accordance with the ethical standards of the Declaration of Helsinki (1964).

2.3 | Treatment

The content of gypenoside L and ginsenoside Rg3 were 18.46 ± 0.13 mg/g and 1.61 ± 0.01 mg/g in the GPE (Figure 1). The participants were administered the capsules containing GPE (test product) or microcrystalline cellulose (placebo) as capsules once a day for 12 weeks. Each capsule contained excipients such as cyclodextrin, magnesium stearate, and silicon dioxide accounting for a total product of 700 mg (450 mg of GPE; Table S1).

2.4 | Physiological assessment

All parameters were measured at baseline and after 12 weeks of treatment. Blood parameters of exercise performance were measured at 3 time points (pre-exercise, post-exercise, and recovery), and each at baseline and after 12 weeks. In particular, post-exercise cardiorespiratory responses, biochemical parameters of fatigue, and exercise responses were measured and compared at baseline and 12 weeks, respectively. Physical characteristics, including height (cm) and weight (kg), were measured using a body composition analyzer (X-ScanPlus II, Jawon Medical Co., Ltd., Korea), and these parameters were used to calculate BMI (kg/m^2). In addition, systolic and diastolic blood pressures and pulse were assessed. The participants' metabolic equivalent of task (MET) was assessed using the Global Physical Activity Questionnaire (GPAQ). Cardiopulmonary responses, namely, VO_2 peak, O_2 pulse, and maximal heart rate (HR_{max}) were measured on a motorized treadmill (Q-Stress® Cardiac Stress Testing System, Hillrom, Chicago, IL) using a modified Bruce protocol (Bruce et al., 1973). All participants underwent simultaneous analysis of pulmonary ventilation and expired gases using a metabolic analyzer, 12-lead electrocardiographic recordings (Mason–Likar modified system), and blood pressure measurement through sphygmomanometry. After the participants were placed in a resting state, blood samples were collected for a fatigue-related blood test, which was subsequently conducted. A treadmill test started at 1.7 mph at 10% incline for 3 min; the speed was increased by 0.8–0.9 mph and the inclination by 2% every 3 min thereafter. The exercise was performed for 3 min at a time according to the slope and speed set for each step, up to stage 5, for a maximum of 15 min, but when the anaerobic threshold (AT) was reached, the exercise stress test was terminated. The AT was measured using the equipment at the point where anaerobic metabolism started as exercise performance increased, and the number of seconds was calculated and analyzed. In addition, the ratings of perceived exertion (RPE) scale, visual analog scale (VAS), and multidimensional fatigue scale (MFS) were used to assess exercise response. To investigate a participant's sensations and feelings of physical stress and fatigue during physical activity, a subjective estimate was made using Borg's 15 RPE scale (Borg, 1970). The VAS was used to measure fatigue at one time

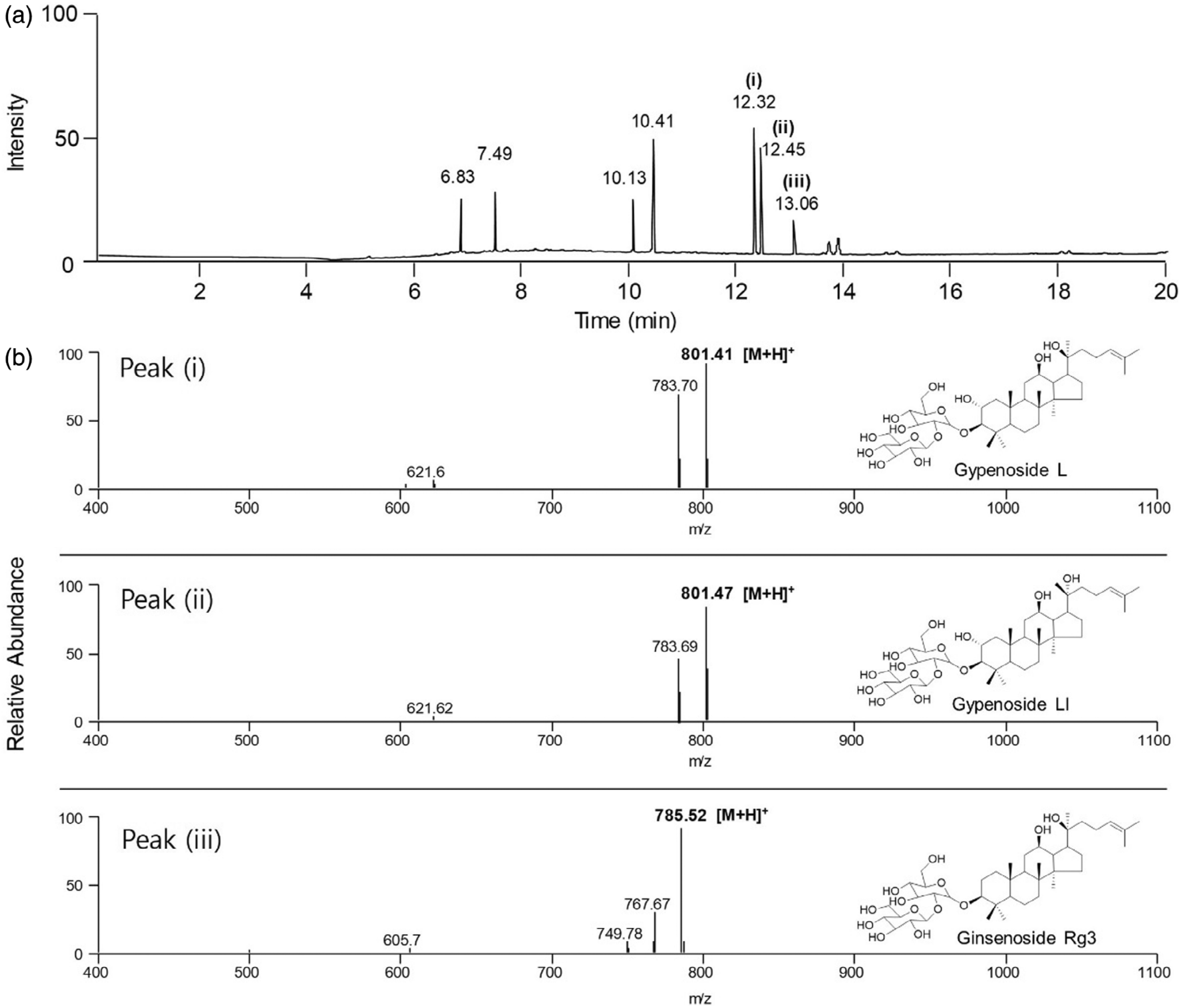

FIGURE 1 High-performance liquid chromatography chromatogram (a) and liquid chromatography–mass spectrometry spectra (b) at ESI mass spectrometry; Fragment of as noted peak (i), (ii), and (iii) with retention time 12.32, 12.45, and 13.06 min determining gypenoside L, gypenoside LI, and ginsenoside Rg3 with 801.41, 801.47, and 785.52 $[M + H]^+$, respectively.

point by marking directly on a 10 cm long straight line. MFS is a questionnaire consisting of 19 items describing symptoms of fatigue based on the Fatigue Assessment Inventory (FAI) of Schwartz et al. (1993) and the participant was asked to respond to the objective fatigue level. Body muscle mass was measured using dual-energy X-ray absorptiometry (DXA/Discovery DXA system, Hologic, Marlborough, MA).

2.5 | Assessment of efficacy and safety parameters

Blood parameters of physical activity and exercise performance were analyzed in Green Cross Laboratories (Yongin, Korea) using serum samples (Roche Diagnostics, Switzerland), and biochemical parameters of fatigue, namely, serum levels of myoglobin, interleukin (IL)-6 (Quantikine HS human IL-6 immunoassay, R&D systems, Inc., MN), total antioxidant status (Rel Assay Diagnostics, Turkey) and endothelial nitric oxide synthase (eNOS; RayBio® Human eNOS ELISA kit, Raybiotech, GA), were also examined using enzyme-linked immunosorbent assay and colorimetric analysis. Safety biomarkers were analyzed using the cobas 8000 c702 modular analyzer (Roche Diagnostics), ADVIA 2120i hematology system (Siemens, Germany), and TBA-C8000 chemistry analyzer (Toshiba Medical Systems Ltd., Japan).

2.6 | Statistical analyses

Data are expressed as mean ± standard deviation (SD), and differences in each group between timepoints (baseline and at 12 weeks) were assessed using an independent *t*-test and a paired *t*-test. The χ^2

test was used to check whether two categorial variables were related to each other. The level of significance was set at $p < 0.05$, using the Statistical Analysis System (Version 9.3, SAS Institute, Cary, North Carolina, NC).

3 | RESULTS

3.1 | Demographic characteristics of the participants

As shown in Table 1, among the study participants, there were 88 men (control group: 40; treatment group: 48) and 12 women (control group: 10; treatment group: 2); there was a statistically significant difference in sex between the two groups ($p = 0.014$). This finding showed that this sex distribution may affect any interaction with the results we obtained. Although we performed a repeated measures ANOVA using sex as a covariate, there were no statistically significant differences between sex and any variables. However, there was a statistically significant difference in average height between the two groups ($p = 0.048$). There were no statistically significant differences in body weight, BMI, BMR, SBP, DBP, and pulse between the control and treatment groups. The average quantity of alcohol consumed by the 70 participants with an alcohol drinking habit (control group: 33; treatment group: 37) was 6.18 ± 4.74 units/week (where one unit equals 10 mL or 8 g of pure alcohol). The 26 smokers (control group: 12; treatment group: 14) included in this study, on average, smoked 8.42 ± 3.35 cigarettes/day; there was no significant difference between the two groups with regard to smoking or alcohol consumption.

3.2 | Effects of GPE on parameters of exercise performance

We confirmed that there were no statistically significant differences between gender and all variables. In all data from Table 2 to the following, statistical significance was confirmed only in males, excluding a small number of female participants. When comparing the baseline with 12 weeks, no statistical changes were found in body weight, BMI and BMR in the two groups (Table S2). Regarding the MET value, when the baseline and 12 weeks were compared, a significant change was observed in the treatment group, but there was no statistically significant difference between the two groups (Table S3). As shown in Table 2, in the treatment group, the post-exercise levels of lactic acid, ammonia, lactate dehydrogenase (LDH), and free fatty acid in the blood serum were significantly higher after 12 weeks of treatment than at baseline ($p < 0.05$). Moreover, in the recovery period after 30 min of exercise, the level of free fatty acids in the treatment group was significantly higher after 12 weeks of treatment than at baseline ($p = 0.008$). The post-exercise blood glucose level of the treatment group was significantly lower after 12 weeks than at the baseline ($p = 0.045$), and the pre-exercise, post-exercise, and recovery period ALT levels were significantly lower after 12 weeks of treatment than at baseline ($p = 0.038$, $p = 0.049$, and $p = 0.032$ respectively). The blood levels of phosphorus showed a tendency to increase in both groups after exercise, and the control group ($p = 0.014$) showed a significant increase compared with the treatment group ($p = 0.054$). There were no significant differences in the blood parameters between the treatment and control groups at the pre-exercise, post-exercise, and recovery timepoints.

3.3 | Effects of GPE on cardiopulmonary responses

As shown in Table 3, the VO_2 max value was significantly higher in the treatment group ($p = 0.010$) after 12 weeks of treatment than at baseline, but there was a significant decrease in the placebo group ($p = 0.045$). In addition, VO_2 max was significantly different between the two groups ($p = 0.007$). The O_2 pulse value showed a tendency to increase in the treatment group and decrease in the placebo group compared with the baseline after 12 weeks; there was a statistically significant difference between the two groups ($p = 0.047$). There was

TABLE 1 Demographic characteristics of the participants.

	Control group ($n = 50$)	Treatment group ($n = 50$)	Total ($n = 100$)	p-value[1]
Sex (male/female)	40/10	48/2	88/12	0.014[2]
Age (years)	31.62 ± 7.00	32.06 ± 8.18	31.84 ± 7.58	0.773
Height (cm)	172.28 ± 6.69	174.92 ± 6.48	173.60 ± 6.68	0.048
Weight (kg)	73.24 ± 11.02	74.58 ± 10.63	73.91 ± 10.79	0.536
BMI (kg/m^2)	24.59 ± 2.80	24.37 ± 3.19	24.48 ± 2.99	0.715
BMR (kcal)	1479.04 ± 162.76	1538.20 ± 135.42	1508.62 ± 151.90	0.051
SBP (mmHg)	122.58 ± 10.11	124.20 ± 13.08	123.39 ± 11.66	0.490
DBP (mmHg)	78.60 ± 9.21	78.58 ± 10.07	78.59 ± 9.60	0.992
Pulse (times/min)	73.96 ± 11.62	75.76 ± 10.90	74.86 ± 11.25	0.426
Alcohol (*n*, %)	33 (66.0%)	37 (74.0%)	70 (70.0%)	0.383[2]
Smoking (*n*, %)	12 (24.0%)	14 (28.0%)	26 (26.0%)	0.648*

Note: Values are presented as the mean ± standard deviation. *p-values* were determined using [1]independent *t*-test or [2]Chi-square test.
Abbreviations: BMI, body mass index; BMR, basal metabolic rate; DBP, diastolic blood pressure; SBP, systolic blood pressure.

TABLE 2 Effects of *Gynostemma pentaphyllum* extract on blood parameters related to exercise performance.

		Control group				Treatment group				
	Time point	Baseline	12 weeks	Diff	*p*-value[1]	Baseline	12 weeks	Diff	*p*-value[1]	*p*-value[2]
Lactic acid (mmol/L)	Pre-exercise	1.06 ± 0.33	1.01 ± 0.38	−0.05 ± 0.09	0.560	1.13 ± 0.41	1.03 ± 0.31	−0.10 ± 0.08	0.206	0.413
	Post-exercise	8.57 ± 2.36	8.70 ± 2.61	0.13 ± 0.59	0.824	8.54 ± 2.67	9.73 ± 2.54	1.19 ± 0.58	0.034*	0.234
	Recovery (after 30 min)	2.63 ± 0.98	2.41 ± 1.07	−0.22 ± 0.24	0.357	2.35 ± 0.10	2.69 ± 1.44	0.34 ± 0.28	0.240	0.999
Ammonia (μg/dL)	Pre-exercise	36.54 ± 8.94	37.92 ± 9.51	1.38 ± 2.15	0.523	36.46 ± 8.26	38.24 ± 9.40	1.77 ± 2.00	0.379	0.935
	Post-exercise	94.29 ± 31.41	101.80 ± 44.41	7.51 ± 9.19	0.417	89.67 ± 39.12	107.15 ± 36.65	17.49 ± 8.58	0.045*	0.954
	Recovery (after 30 min)	45.89 ± 10.25	47.49 ± 10.83	1.6 ± 2.52	0.528	48.25 ± 11.39	49.10 ± 10.26	0.85 ± 2.42	0.727	0.256
Lactate dehydrogenase (IU/L)	Pre-exercise	161.54 ± 20.30	161.09 ± 18.62	−0.46 ± 4.66	0.922	165.24 ± 18.13	163.34 ± 19.70	−1.90 ± 4.18	0.650	0.339
	Post-exercise	179.11 ± 25.23	181.91 ± 22.17	2.8 ± 5.68	0.623	180.00 ± 23.04	190.20 ± 31.13	10.20 ± 6.12	0.038*	0.281
	Recovery (after 30 min)	163.62 ± 20.06	161.09 ± 16.43	−2.53 ± 4.45	0.571	166.31 ± 20.05	165.12 ± 19.85	−1.19 ± 4.35	0.785	0.283
Free fatty acid (μEq/L)	Pre-exercise	538.86 ± 179.10	583.09 ± 206.30	44.23 ± 46.19	0.342	508.05 ± 185.26	554.82 ± 176.26	46.77 ± 40.95	0.257	0.339
	Post-exercise	714.97 ± 178.21	698.82 ± 199.88	−16.15 ± 45.93	0.726	647.66 ± 189.48	723.40 ± 206.31	75.75 ± 43.75	0.042*	0.505
	Recovery (after 30 min)	561.15 ± 160.96	584.32 ± 197.88	23.18 ± 43.75	0.598	501.46 ± 159.07	589.00 ± 173.22	87.53 ± 36.73	0.008**	0.339
Phosphorus (mg/dL)	Pre-exercise	3.35 ± 0.45	3.46 ± 0.43	0.12 ± 0.10	0.266	3.22 ± 0.32	3.30 ± 0.39	0.08 ± 0.08	0.232	0.031
	Post-exercise	3.83 ± 0.44	4.04 ± 0.41	0.21 ± 0.10	0.014*	3.77 ± 0.38	3.95 ± 0.41	0.18 ± 0.09	0.054	0.273
	Recovery (after 30 min)	3.27 ± 0.39	3.32 ± 0.41	0.05 ± 0.09	0.583	3.19 ± 0.37	3.20 ± 0.43	0.02 ± 0.09	0.858	0.120
Creatine kinase (U/L)	Pre-exercise	130.52 ± 48.12	128.03 ± 43.97	−2.49 ± 11.35	0.827	129.00 ± 63.63	143.79 ± 77.37	14.79 ± 15.46	0.342	0.480
	Post-exercise	139.50 ± 46.95	137.34 ± 40.94	−2.16 ± 11.01	0.845	142.29 ± 69.21	159.71 ± 82.82	17.43 ± 16.65	0.298	0.241
	Recovery (after 30 min)	132.45 ± 47.27	125.73 ± 37.21	−6.73 ± 10.47	0.523	132.26 ± 62.78	146.86 ± 76.66	14.60 ± 15.29	0.343	0.286
Glucose (mg/dL)	Pre-exercise	96.33 ± 5.40	97.28 ± 6.30	0.94 ± 1.38	0.497	98.54 ± 6.26	98.35 ± 6.41	−0.193 ± 1.44	0.893	0.102
	Post-exercise	106.11 ± 8.67	104.22 ± 7.96	−1.89 ± 1.93	0.331	108.64 ± 12.62	105.13 ± 13.48	−3.51 ± 2.78	0.045*	0.330
	Recovery (after 30 min)	96.22 ± 7.16	95.19 ± 7.23	−1.03 ± 1.70	0.546	97.89 ± 8.20	97.97 ± 7.29	0.08 ± 1.78	0.964	0.071
Aspartate transaminase (IU/L)	Pre-exercise	17.00 ± 3.22	17.50 ± 3.59	0.50 ± 0.83	0.547	19.49 ± 4.73	20.09 ± 5.48	0.61 ± 1.13	0.593	0.001
	Post-exercise	19.41 ± 3.64	20.32 ± 3.87	0.91 ± 0.91	0.321	21.90 ± 5.19	22.28 ± 4.35	0.38 ± 1.07	0.727	0.002
	Recovery (after 30 min)	17.68 ± 3.30	17.88 ± 3.40	0.20 ± 0.81	0.801	19.58 ± 4.70	19.45 ± 3.73	−0.13 ± 0.95	0.895	0.007
Alanine transaminase (IU/L)	Pre-exercise	19.49 ± 7.72	19.37 ± 6.26	−0.11 ± 1.68	0.946	23.73 ± 11.55	20.32 ± 8.16	−3.41 ± 2.24	0.038*	0.067
	Post-exercise	21.03 ± 7.76	21.09 ± 6.94	0.06 ± 1.76	0.974	25.45 ± 11.88	22.31 ± 8.53	−3.14 ± 2.31	0.049*	0.054

TABLE 2 (Continued)

	Time point	Control group Baseline	12 weeks	Diff	p-value[1]	Treatment group Baseline	12 weeks	Diff	p-value[1]	p-value[2]
	Recovery (after 30 min)	19.46 ± 7.43	19.03 ± 6.18	−0.43 ± 1.63	0.794	23.48 ± 10.87	20.19 ± 7.93	−3.28 ± 2.13	0.032*	0.056
Creatine (mg/dL)	Pre-exercise	0.34 ± 0.12	0.36 ± 0.16	0.03 ± 0.03	0.412	0.33 ± 0.16	0.43 ± 0.17	0.02 ± 0.04	0.686	0.583
	Post-exercise	0.41 ± 0.14	0.46 ± 0.18	0.05 ± 0.04	0.167	0.43 ± 0.20	0.48 ± 0.23	0.05 ± 0.05	0.330	0.510
	Recovery (after 30 min)	0.36 ± 0.12	0.38 ± 0.15	0.02 ± 0.03	0.547	0.37 ± 0.17	0.38 ± 0.19	0.01 ± 0.04	0.804	0.926

Note: Values are presented as mean ± standard deviation ($n = 31–46$). p-values were determined by [1]paired t-test or [2]an independent t-test. Symbols indicate significant differences at *$p < 0.05$; **$p < 0.01$.

TABLE 3 Effects of *Gynostemma pentaphyllum* extract on cardiopulmonary responses.

	Control group Baseline	12 weeks	Diff	p-value[1]	Treatment group Baseline	12 weeks	Diff	p-value[1]	p-value[2]
Maximal treadmill test									
VO_2 max (mL/kg/min)	44.16 ± 3.21	43.32 ± 3.89	−0.84 ± 0.82	0.045*	42.23 ± 3.83	43.34 ± 3.88	1.10 ± 0.83	0.010*	0.007**
O_2 pulse (mL/beat)	18.70 ± 2.60	18.59 ± 2.70	−0.11 ± 0.62	0.861	18.03 ± 2.58	18.48 ± 2.32	0.46 ± 0.55	0.404	0.047*
HR_{max} (beats/min)	177.37 ± 11.33	175.06 ± 12.47	−2.31 ± 2.85	0.419	176.33 ± 11.32	176.81 ± 10.94	0.48 ± 2.40	0.842	0.468

Note: Values are presented as mean ± standard deviation ($n = 31–46$). *p-values* were determined using [1]paired t-test or [2]an independent t-test. Symbols indicate significant differences at *$p < 0.05$; **$p < 0.01$.

no significant difference between the HR_{max} values at baseline and the 12-week timepoint in each group and between the two groups. In addition, there were no significant changes in arm, leg, trunk, and total muscle mass before and after 12 weeks of treatment in either the control or the treatment groups, and no significant difference was found between the groups (Table S4).

3.4 | Effects of GPE on biochemical parameters of fatigue

Results related to biochemical parameters of fatigue are presented in Table 4. The cytokine IL-6, which is involved in the development of fatigue, did not show a significant difference between baseline and 12 weeks in the control group. With regard to myoglobin, a marker used to detect muscle damage and the overall antioxidant status of the body, there were no significant differences before and after *G. pentaphyllum* treatment in the treatment group or between the treatment and control groups. Further, the eNOS level before exercise did not show a significant difference between the baseline and 12 weeks in the two groups, and there was no difference between the two groups. However, the eNOS level was significantly higher in the treatment group than in the control group during the recovery period after 30 min of exercise ($p = 0.047$).

3.5 | Effects of GPE on mental fatigue

The RPE, AT, VAS, and MFS scores are shown in Table 5. Part of both groups passed stage 4, which corresponds to a speed of 4.2 mph at 16% incline, but failed to achieve stage 5, which corresponds to 5.0 mph at 18% incline. When comparing baseline and 12 weeks, the RPE values of the control group significantly increased from stage 2 to stage 4 ($p = 0.006$, $p = 0.004$, and $p = 0.003$, respectively). In the case of the treatment group, there was a significant change in stage 4 ($p = 0.049$), and there was a significant difference in stage 2 between the treatment and control groups ($p = 0.049$). The AT measured at baseline and at 12 weeks in each group did not differ significantly, and there was no significant change in comparison between groups. On the multidimensional fatigue scale, physical, temporal, and total fatigue of the control group were significantly higher after 12 weeks of treatment than at baseline ($p = 0.042$, $p = 0.038$, and $p = 0.047$, respectively). However, there was no significant change in the MFS score in the treatment group, and temporal fatigue was significantly lower in the treatment group than in the control group ($p = 0.049$).

3.6 | Safety parameters and adverse event of administration of GPE

Blood and urine sample analyses and adverse event monitoring were performed to assess safety in a total of 77 participants who provided

TABLE 4 Effects of *Gynostemma pentaphyllum* extract on biochemical parameters of fatigue.

	Control group				Treatment group				
	Baseline	12 weeks	Diff	*p*-value[1]	Baseline	12 weeks	Diff	*p*-value[1]	*p*-value[2]
Myoglobin (ng/mL)	23.12 ± 2.88	23.03 ± 3.47	−0.09 ± 0.77	0.808	25.00 ± 5.72	24.31 ± 4.34	−0.69 ± 1.14	0.547	0.420
Interleukin-6 (pg/mL)	1.31 ± 0.73	1.39 ± 0.77	0.08 ± 0.18	0.647	1.47 ± 0.80	1.20 ± 0.57	−0.27 ± 0.16	0.085	0.883
Total antioxidant status (mmol/L)	1.36 ± 0.11	1.34 ± 0.12	−0.01 ± 0.02	0.541	1.36 ± 0.15	1.35 ± 0.13	−0.01 ± 0.03	0.812	0.958
eNOS									
Pre-exercise	75.69 ± 70.60	61.94 ± 82.32	−13.75 ± 18.33	0.354	46.80 ± 60.52	61.85 ± 95.55	15.05 ± 18.11	0.409	0.262
Recovery (after 30 min)	81.50 ± 76.46	64.63 ± 84.19	−16.87 ± 19.22	0.292	49.01 ± 65.39	65.14 ± 99.24	16.13 ± 19.03	0.399	0.047*

Note: Values are presented as mean ± standard deviation ($n = 31–46$). *p-values* were determined by [1]paired *t*-test or [2]an independent *t*-test. Symbols indicate significant differences at $^*p < 0.05$.
Abbreviation: eNOS, endothelial nitric oxide synthase.

written informed consent (Table S5). There was a statistically significant difference in blood creatinine between the two intake groups ($p = 0.022$), but this change was not clinically significant within the reference range. In terms of other parameters, there was no statistically significant difference between the two groups. Although 24 adverse reactions were reported in 13 participants during the study period, all adverse reactions did not have a causal relationship with the administration of GPE, and the number of participants with adverse reactions was not significantly different between the two groups ($p > 0.05$).

4 | DISCUSSION

Although regular exercise is a behavioral factor that can modulate the endogenous antioxidant system and protect the body from oxidative damage, when the homeostasis of the endogenous antioxidant defense system and exercise-induced oxidative stress is unbalanced through acute and chronic physical activity, non-pathological conditions can be induced. Physical and mental fatigue are complex physiological phenomena, and the main causes of fatigue during exercise include deficiency of energy stored in muscles, difficulty in transmitting nerve impulses, and accumulation of metabolites generated during exercise (Enoka & Duchateau, 2008). To reduce the accumulated inflammatory, oxidative, and nitrosative stress through exercise, the ingestion or use of diets with antioxidant-rich materials that promote high antioxidant activity and can induce endogenous and exogenous antioxidants is currently suggested by various scientific studies (Yavari et al., 2015). However, further evidence from clinical trials on how the administration and application of antioxidant-rich substances may affect physical activity-related fatigue is required.

G. pentaphyllum, which is used as an herbal medicine, has an inhibitory effect on symptoms related to various diseases. Recent studies have reported that saponins, polysaccharides, flavonoids, and other chemicals have been reported to have antioxidant, anti-inflammatory, and anti-cancer properties (Razmovski-Naumovski et al., 2005; Yang et al., 2008). Moreover, Kim et al. reported that *G. pentaphyllum* activates the AMPK/p38/Sirtuin 1 signaling pathway, which activates PGC-1α, a major regulator of muscle differentiation and regulation of mitochondrial metabolism (Kim, Jung, Jeon, Kim, Oh, et al., 2020). In treadmill-trained mice, extract-induced activation of PGC-1α increases mitochondrial biosynthesis by increasing the expression of mitochondrial DNA and the nuclear respiratory factor 1 gene (Kim, Jung, Jeon, Kim, Hong, et al., 2020). Activated PGC-1α increases mitochondrial biosynthesis, glucose, and lipid metabolism, indicating high exercise performance and oxygen uptake capacity, and mitochondria and ATP are known determinants of VO_2 max (Calvo et al., 2008; Jacobs & Lundby, 2013; Marcuello et al., 2009; Radak et al., 2019; Toedebusch et al., 2016). VO_2 max is considered an important indicator of cardiorespiratory endurance and aerobic exercise capacity, and it is determined by cardiac output, blood oxygen carrying capacity, and tissue oxygen utilization (Leary & Wyndham, 1965; Vitacca et al., 2020). Although recent evidence

TABLE 5 Effects of *Gynostemma pentaphyllum* extract on exercise response.

	Control group				Treatment group				
	Baseline	12 weeks	Diff	*p*-value[1]	Baseline	12 weeks	Diff	*p*-value[1]	*p*-value[2]
RPE									
Stage 1	10.33 ± 1.07	10.50 ± 0.94	0.17 ± 0.24	0.4851	10.63 ± 1.01	10.40 ± 1.04	−0.23 ± 0.23	0.321	0.566
Stage 2	12.37 ± 0.80	12.86 ± 0.60	0.49 ± 0.17	0.006**	12.49 ± 0.85	12.53 ± 0.80	0.04 ± 0.19	0.836	0.049*
Stage 3	14.59 ± 0.80	15.16 ± 1.14	0.57 ± 0.23	0.004**	14.98 ± 1.21	15.02 ± 1.46	0.05 ± 0.30	0.870	0.527
Stage 4	16.97 ± 0.49	17.57 ± 0.94	0.60 ± 0.19	0.003**	17.11 ± 0.64	17.44 ± 0.93	0.33 ± 0.22	0.049*	0.941
Anaerobic threshold (s)	416.22 ± 83.74	411.95 ± 103.26	−4.27 ± 21.86	0.746	412.20 ± 78.99	420.77 ± 98.46	8.58 ± 19.71	0.539	0.869
VAS score	4.47 ± 2.06	4.53 ± 2.22	0.05 ± 0.49	0.915	4.79 ± 1.92	5.21 ± 1.79	0.42 ± 0.40	0.299	0.112
MFS									
General fatigue	29.56 ± 7.94	30.19 ± 7.83	0.64 ± 1.86	0.732	28.48 ± 6.70	29.08 ± 5.81	0.60 ± 1.40	0.668	0.338
Physical fatigue	25.36 ± 4.70	26.79 ± 4.26	1.42 ± 1.11	0.042*	25.85 ± 4.13	25.99 ± 4.54	0.14 ± 0.97	0.888	0.831
Temporal fatigue	21.03 ± 3.80	22.39 ± 4.00	1.36 ± 0.96	0.038*	21.61 ± 4.01	21.33 ± 3.35	−0.28 ± 0.82	0.733	0.049*
Total fatigue	74.78 ± 14.96	78.36 ± 14.84	3.58 ± 3.51	0.047*	75.83 ± 12.82	76.70 ± 12.12	0.86 ± 2.72	0.752	0.402

Note: Values are presented as mean ± standard deviation ($n = 31$–46). *p-values* were determined using [1]paired *t*-test or [2]independent *t*-test. Symbols indicate significant differences at $^*p < 0.05$ and $^{**}p < 0.01$.
Abbreviations: MFS, multidimensional fatigue scale; RPE, rating of perceived exertion; VAS, visual analog scale.

suggests the effects of *G. pentaphyllum* on the molecular and morphological aspects of muscle development and exercise performance, there have been no reports of clinical trial results related to the efficacy and safety of *G. pentaphyllum* extract.

Increased reactive oxygen species (ROS) production levels during exercise are associated with antioxidant defense, muscle insulin sensitivity, and regulation of mitochondrial biosynthesis and have a significant impact on the reduced risk of several diseases (Klemow et al., 2011). Although the adaptive response to ROS is an important mechanism mediating the beneficial effects of exercise, the accumulation of ROS leads to muscle dysfunction and chronic fatigue. A study by Alghadir et al. reported a positive correlation between α- and γ-tocopherols, total antioxidant capacity, and physical activity in a serum analysis conducted on 120 students (Alghadir et al., 2019). Cortisol levels decrease after exercise after the administration of large quantities of ascorbic acid supplements, and the increased exercise-induced oxidative stress is associated with changes in the concentration of a single antioxidant circulating in the blood (Peake, 2003).

In addition, herbal and traditional plant medicines containing antioxidants have been reported to improve exercise performance in a number of animal studies. Ginseng contains glycosylated steroid saponins and is known to help skeletal muscle mass, oxidative metabolism, and exercise endurance through in vitro and in vivo experiments (Jeong et al., 2019; Lee et al., 2018; Shin et al., 2020). Liang et al. reported that an increase in endurance time and a decrease in maximum mean blood pressure and VO_2 max occurs when a capsule containing *Panax notoginseng* extract (1350 mg/day for 1 month) was administered to untrained adults and endurance cycling exercise was performed (Liang et al., 2005).

In the present study, we investigated the effects of GPE on blood parameters related to exercise performance using serum biochemical analysis (Table 2). GPE administration regulated characteristic responses, such as changes in the levels of lactate, ammonia, free fatty acids, LDH, and ALT, caused by exercise. Assessment of biological changes, such as biomarkers of adenosine triphosphate (ATP) metabolism, oxidative stress, and inflammation, allows to understand individual responses of fatigue and physical performance (Finsterer, 2012). The biomarkers of muscle fatigue from ATP metabolism products such as lactate and ammonia are closely related to exercise intensity in healthy individuals regardless of age, and available free fatty acids from fat provide energy and alleviate fatigue after exhaustive exercise (Liu et al., 2014). During exercise, hydrolyzed free fatty acids are released into the circulation to provide energy for muscles for physiological activity. Chronic exercise increases NO bioavailability by promoting eNOS, a critical homodimeric enzyme (Forstermann & Sessa, 2012; Green et al., 2004; Mika et al., 2019). ALT reactions establish and maintain high concentrations of tricarboxylic acid cycle intermediates in the muscles during exercise and are required to meet the increased energy needs during exercise (Wagenmakers, 1998). In this study, it was demonstrated that lactate and ammonia levels in the treatment group increased and mental fatigue caused by exercise decreased, but in the case of curcumin, supplementation from natural products has been reported to have ergogenic functions in swimming or rat tests, as well as in the reduction of serum lactate and ammonia levels in male ICR mice (Huang et al., 2015). Furthermore, LDH is known to be involved in the conversion of pyruvate to lactic acid and to have a positive effect on sports performance, and LDH activity reportedly increases when ingesting red ginseng (Kim et al., 2016).

Although VO_2 max decreased in the placebo group compared with the baseline after 12 weeks, administration of GPE was reversely increased (Table 3). This study analyzed whether 12 weeks of GPE administration would improve the maximum aerobic capacity and delay fatigue during exercise in healthy but untrained participants. Fatigue can generally be evaluated by the analysis of VO_2 max to assess the ability of the heart and lungs to transport oxygen to the working muscles (Levine, 2008). Numerous studies have focused on natural products that increase VO_2 max and endurance capacity. In particular, administration of natural polyphenolic flavonoid substances has been reported to induce an apparent increase in the VO_2 max and particular endurance performance without exercise training (Davis et al., 2010; Malaguti et al., 2013).

The results obtained in this study suggest that GPE reduces the serum levels of IL-6 (Table 4). Chronic fatigue induced by the effects of increased levels of proinflammatory cytokines, including IL-1, IL-6, and tumor necrosis factor-α, on the central and autonomic nervous systems has also been associated with pain, behavioral symptoms, and depressive symptoms (Louati & Berenbaum, 2015). Furthermore, secretion of inflammatory markers such as IL-6, TNF-α, and C-reactive protein is involved in the development of the sensation of tiredness in many connective tissues (Grygiel-Gorniak & Puszczewicz, 2015). These observations would require the use of non-pharmacological treatments because of the potentially harmful side effects of pharmaceutical drugs to reduce the chronic inflammation that accumulates through exercise. Preclinical studies have shown that plant-derived anti-inflammatory compounds (curcumin, resveratrol, epigallocatechin-3-gallate, and quercetin) regulate proinflammatory signaling cascades, including the nuclear factor kappa light chain enhancer of activated B cell-, signal transducer and activator of transcription 1-, activator protein-1-, mitogen-activated protein kinase-, cyclooxygenase-, and lipoxygenase-pathways (de la Lastra & Villegas, 2005; Hamalainen et al., 2007; Hong et al., 2004; Khan et al., 2006; Kim et al., 2005; Lee et al., 2008; Surh et al., 2001; Yang et al., 2011). On the basis of this evidence, herbal medicine has been suggested to be effective in the removal of factors that cause fatigue.

Exercise responses, such as the RPE value, significantly changed in the treatment group administered GPE compared with that in the placebo group (Table 5). Mental fatigue is defined as a psychobiological condition that reduces cognitive activity due to prolonged exercise (Meeusen et al., 2020). RPE and MFS are commonly used for measuring an individual's mental fatigue during physical activity in clinical trials along with heart rate, blood pressure, and oxygen consumption (Jo & Bilodeau, 2021; Smets et al., 1995). Recent evidence has indicated that botanical extracts can contribute to a reduction in mental fatigue during endurance exercise. *Panax ginseng* has been shown to ameliorate the increase in subjective feelings of mental fatigue induced through continuous cognitive processing (Reay et al., 2006).

Results from clinical trials have also shown that *Eurycoma longifolia* Jack with quassinoids, has anxiolytic properties and positive effects on cycling and running endurance performances (Kiew et al., 2003; Muhamad et al., 2010; Ooi et al., 2001). In addition, the use of caffeine from plant species not only improves mental alertness, but also improves endurance, anaerobic performance, serum catecholamine levels, and immune responses (Sellami et al., 2014; Senchina et al., 2014; Yeomans et al., 2002).

A total of 100 participants were investigated between the two intake groups, and 24 adverse events were observed in 13 participants, but there was no causal relationship with GPE intake (Table S5). A clinical study reported the effect of *G. pentaphyllum* on body composition in overweight males and females ranging in dose 450 mg per two capsules suggesting a large margin of safety (Rao et al., 2022). The effects of *G. pentaphyllum* on anxiety levels in healthy Korean participants under chronic stress conditions have been investigated, and the efficacy and safety have been reported (Choi et al., 2019). Additionally, in animal studies, *G. pentaphyllum* was tested for acute oral toxicity and subchronic toxicity at doses of 1000 and 5000 mg/day, and it was reported that it did not cause mortality or any abnormalities (Chiranthanut et al., 2013).

5 | CONCLUSION

Our randomized controlled trial showed that 12 weeks of administration of GPE resulted in ergogenic properties that may improve both physical and mental performance. These results show that GPE induces changes in blood parameters in the recovery period after exercise when comparing baseline and 12 weeks of treatment. In addition, the group that was administrated GPE for 12 weeks showed changes in cardiopulmonary and exercise responses and eNOS levels compared with the control group. Taken together, our results demonstrate the potential of plant-derived extracts to mitigate the cognitive aspects of fatigue and improve the performance of the motor system from exercise-induced oxidative stress.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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