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Original Article

Herbal extract (*Cervus elaphus* Linnaeus, *Angelica gigas* Nakai, and *Astragalus membranaceus* Bunge) ameliorates chronic fatigue: A randomized, placebo-controlled, double-blind trial

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ABSTRACT

Background: Chronic fatigue syndrome (CFS) reduces the health-related quality of life in the working-age population; however, studies have rarely investigated this group. A mixture of Cervus elaphus Linnaeus, Angelica gigas Nakai, and Astragalus membranaceus Bunge (CAA) may be an effective anti-fatigue supplement. However, few clinical trials have explored the anti-fatigue effects of herbal medicines in human participants. Therefore, this study aimed to investigate the effects of the CAA herbal complex on muscle fatigue and endurance capacity in a randomized, placebo-controlled, double-blind trial.

Methods: In an 8-week trial, 80 patients with chronic fatigue symptoms were randomly assigned to the CAA (43.5 ± 1.2 years) or placebo group (41.8 ± 1.3 years). Fatigue and cardiorespiratory endurance were measured at baseline, interim, and post-intervention. Fatigue-related blood biomarkers were assessed before and at the end of the intervention.

Results: A significant improvement in overall fatigue scores was observed on the fatigue severity scale ($p = 0.038$), multidimensional fatigue inventory ($p = 0.037$), and 24-hour visual analog scale ($p = 0.002$) in the CAA group compared to those in the placebo group. Fatigue improvement was observed in the CAA group, as well as physiological variables, such as increased maximal exercise time to exhaustion ($p = 0.003$), distance until exhaustion ($p = 0.003$), and maximum oxygen consumption ($p = 0.039$).

Conclusion: CAA positively and significantly affected fatigue and cardiorespiratory endurance in patients with chronic fatigue, suggesting the potential use of herbal supplements for treating chronic fatigue.

Trial registration: Clinical Research Information Service (CRIS, https://cris.nih.go.kr/): KCT0005613.

1. Introduction

Fatigue is a subjective sensation encompassing physical, cognitive, and emotional features, characterized as physical and mental tiredness. It can impair physiological functionality and lead to adverse effects on quality of life (QoL).[1] Chronic fatigue syndrome (CFS) is defined as persistent fatigue lasting six months or longer.[2] CFS affects individuals of all ages, with two peak onset periods: 10–19 and 30–39 years, with an average onset age of 33 years.[3,4] The prevalence of CFS increases with age, rising from 0.7% in people aged 18–39 to 2.0% and 2.1% in those aged 50–59 and 60–69, respectively, before declining in those aged 70 and older.[5] CFS considerably affects work and family life and causes a substantial reduction in health-related QoL among the working-age population[6,7]; however, studies have placed little emphasis on this group.[8]

Furthermore, the pathophysiology of CFS is unclear. CFS is associated with viral infections, intracellular bacteria, environmental factors, and immune system disabilities.[9] However, several approaches have been proposed to alleviate the different types of CFS. The quality of

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evidence-based nutritional intervention (vitamin C, vitamin B complex, sodium, magnesium, zinc, coenzyme Q10) for addressing CFS has remarkably improved.[10,11]

Nutritional interventions are crucial for treating individuals with CFS because they support essential cellular functions involved in energy production and metabolism. Combining essential nutrients and enzymes into one intervention could effectively manage fatigue by enhancing these processes.[12] Patients with CFS often report a lack of energy, mental exhaustion, poor muscle endurance, delayed recovery, and nonrestorative sleep.[13] Many of these patients use nutritional supplements, and many have benefited from dietary modification.[14] Several commercially available supplements for CFS improve liver function or the central nervous system.[15,16] Studies typically focus on single nutrients; however, multi-nutrient treatments could benefit healthy individuals and patients with conditions such as fibromyalgia and other fatigue-related illnesses.[13] In addition, concerns regarding gastrointestinal issues have increased the need to develop safe supplements using natural products.

Cervus elaphus Linnaeus, *Angelica gigas* Nakai, and *Astragalus membranaceus* Bunge (CAA) are widely used in traditional medicine to prevent and treat various diseases and enhance health.[17-19] Recent studies have explored their potential mechanisms and pharmacogenetics; however, clinical research remains limited, and the overall anti-disease activity of these substances is unclear. *Cervus elaphus* Linnaeus has been extensively investigated withinin vitro and in vivo studies and has potential anti-fatigue, anti-cancer, and antioxidant properties.[17,20-22] However, most studies used crude deer antler base extracts, either alone or in combination with other herbs, often with simple experimental designs. Animal and cell-based studies suggested that *Angelica gigas* Nakai has various pharmacological properties, including anti-cancer, neuroprotective, and anti-inflammatory effects.[23-26] *Astragalus membranaceus* Bunge has neuroprotective and anti-inflammatory effects in rodent models and cell-based studies.[27–29]

The combination of CAA effectively alleviates fatigue.[20,22] CAA decreases intracellular reactive oxygen species by approximately 20 times in cell culture studies.[20] In exercised mouse models, CAA was an effective ergogenic and anti-fatigue supplement. It increased the maximum running time compared to the exercise control group, decreased serum lactate dehydrogenase levels by approximately 40%, and significantly boosted proliferator-activated receptor gamma coactivator 1-alpha protein expression.[20] Furthermore, CAA-treated mice had longer treadmill running times than the control group. Additionally, fatigue-related biochemical markers, including lactate dehydrogenase (approximately 30%), creatine kinase (approximately 20%), and proinflammatory cytokines interleukin (IL)-1β (approximately 10%) and IL-6 (approximately 10%), were significantly reduced in CAA-treated mice compared to controls.[22] These results indicate that CAA can be used as an efficient anti-fatigue and ergogenic supplement. Because existing literature primarily focuses on animal and in vitro studies, this study aimed to validate the effects of the CAA herbal complex on muscle fatigue and endurance capacity in human participants. The objective of this study was to provide scientific evidence to support the use of CAA in improving physical performance and combating fatigue in humans.

2. Methods

2.1. Participants and ethical approval

This study was approved by the Institutional Review Board of Seoul National University (IRB No. 2011/002–020) and conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. All participants provided written informed consent. The protocol of this trial has been registered at Clinical Research Information Service (CRIS, https://cris.nih.go.kr/): KCT0005613.

The sample size was determined according to previous studies[30,31] that established the impact of supplement consumption on exercise performance and fatigue levels. The minimum required sample size for this study was calculated using G*Power 3.1 to ensure statistical robustness, which was 64 participants, with 32 participants in each group. Considering an anticipated exclusion rate of 20%, 80 participants with chronic fatigue were recruited for this study.

The inclusion criteria for this study were as follows: (1) agreed to participate in this study and provide written consent; (2) healthy men and women aged 30–59 years, with a score of 28 or higher on the fatigue severity scale (FSS)[32,33] and a body mass index (BMI) of 18.6–29.9 kg/m^2.

Exclusion criteria were as follows: (1) individuals with high maximal oxygen consumption (VO_2max), defined as being in the top 50% of the second grade by age and sex[34]; (2) excessive alcohol consumer: men > 210 g/week (approximately 3.5 bottles of soju/week), women > 140 g/week (approximately 2.5 bottles of soju/week); (3) smokers, drug addicts; (4) insomnia; (5) had the following diseases (including past history) or are taking related medicines: hyperlipidemia, asthma, diabetes, bronchitis, anemia, thyroid diseases, cardiovascular diseases (hypertension, and stroke), kidney diseases, liver diseases, musculoskeletal disorders, nervous system diseases, menstrual irregularity, bleeding diseases (for women), anticoagulant disease; (6) surgical procedures within 6 months of the first visit; (7) continuously consumed herbal medicine, health supplements or an injection which may affect fatigue (anti-fatigue) within 1 month of the first visit; (8) hypersensitivity to CAA ingredients or control supplements or who have experienced a severe food allergic reaction; (9) pregnant, lactating, or planning to become pregnant within 3 months; (10) had participated in another clinical trial within 1 month of the first visit or plan to participate in another clinical trial during this human application study period; (11) attended high intensity exercise within the last 3 months; and (12) unable to use smart phone.

2.2. Study design

The participants of this double-blind, randomized, placebo-controlled, and parallel clinical trial were required to visit the laboratory four times. During the initial visit (screening, visit 1), participants were asked to sign a consent form after receiving a full explanation of the study. Eligibility was determined by measuring VO_2max using a maximal cycle ergometer test. After a 2-week run-in period, participants who met the inclusion criteria were then randomly assigned to the CAA or placebo group using a “computer-generated random list” created by an independent third party who was not involved in the study during the second visit (visit 2).

A random number was assigned to each visitor during their second visit, with separate sequences for females and males. Block randomization was performed with a block size of four to achieve a 1:1 ratio between the CAA and placebo groups. Participants and researchers remained unaware of the assigned treatment groups until the completion of the study.

2.3. Intervention

All participants were instructed to consume CAA (500 mg) or a placebo in the form of a concentrated pill once daily throughout the 8-week trial period. Additional pills were provided to account for missed doses or late visits. Participants were advised to maintain their regular diet and lifestyle but were instructed to avoid consuming health supplements and herbal medicines. Additionally, they were asked to refrain from injections (such as vitamins or placenta), which aid in fatigue recovery. Participants were recommended to follow a consistent menu the day before each visit and abstain from alcohol and caffeine intake for 24 h before the visit. Furthermore, participants were instructed to engage in strenuous exercise for 48 h before each visit. The participants were encouraged to report any adverse events that they experienced. The researchers conducted interviews with the participants to verify the occurrence of any such events.

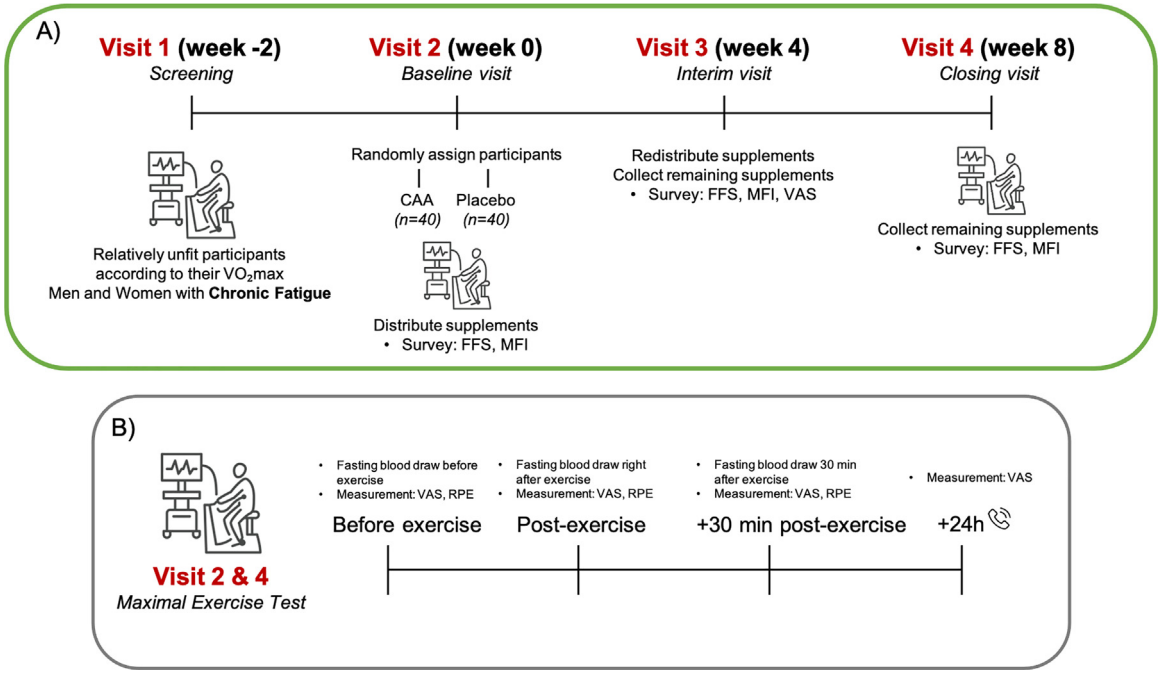

Fig. 1. Study design for a 10-week and 8-week of *Cervus elaphus* Linnaeus, *Angelica gigas* Nakai, *and Astragalus membranaceous* Bunge (CAA) extract consumption compared with placebo supplements in adults with chronic fatigue; A) Study flow for 10 weeks with four visits. B) Details of maximal exercise test and measurements for Visit 2, and 4 include assessments of the fatigue severity scale (FSS), multidimensional fatigue inventory (MFI), visual analog scale (VAS), and rating of perceived exertion (RPE).

2.4. Measurements

Various assessments, including body composition, vital signs, dietary intake, muscle and cardiorespiratory endurance (cycle ergometer test, except at interim visit [visit 3]), fatigue level (FSS, and multidimensional fatigue inventory [MFI] at each visit, visual analog scale [VAS] except at visit 3); physical activity level questionnaire (global physical activity questionnaire [GPAQ]); blood biomarkers (except at visit 3); and monitoring of adverse events, were conducted at each visit (Fig. 1).

2.4.1. Demographic characteristics and anthropometric measurements

Body weight, skeletal muscle mass, and fat mass were assessed using bioelectrical impedance analysis with an InBody720 (InBody, Seoul, Republic of Korea). Blood pressure, pulse, and body temperature were recorded after a 10-minute rest upon arrival at the laboratory. All measurements were consistently performed by the same researcher using the same machine at identical time points during each visit.

2.4.2. Dietary intake, physical activity, and sleep duration

Between each visit, the participants' dietary intake (total calories, carbohydrates, fats, proteins, and sodium) was monitored using a mobile application on three representative days (two weekdays and one weekend day). The GPAQ was used to assess physical activity levels, and average sleep time was monitored through self-reporting.

2.4.3. Fatigue level

The fatigue level was measured using the FSS, MFI, and VAS. The FSS is a nine-item scale that reflects the severity of fatigue and its effect on an individual's activities and lifestyle. Answers were scored on a seven-point scale where 1 = strongly disagree and 7 = strongly agree. The total score was calculated by adding all the scores of the sub-items, with a higher total score indicating a higher level of fatigue. MFI comprises 20 statements, rated on a five-point Likert scale from "yes, that is true" to "no, that is not true." It includes five subscales that measure general fatigue, physical fatigue, mental fatigue, reduced activity, and reduced motivation. The subscale scores range from 4 to 20, with higher scores indicating increased fatigue in each domain. The total score was calculated to represent the overall fatigue across different aspects. VAS used a 100 mm line with anchors at both extremes denoted as "no fatigue" and "severe fatigue." The VAS questionnaire was administered during visits 2 and 4, and it included exercise assessments. Measurements were taken at various time points: pre-exercise, immediately post-exercise, 30 min post-exercise, and 24 h post-exercise. Participants marked a point on the 100 mm line to indicate the severity of their fatigue based on their perceived state.

2.4.4. Blood test

Blood samples were collected under 12 h of fasting conditions. Blood tests, including lactate, lactate dehydrogenase, creatine kinase, creatinine, blood urea nitrogen, myoglobin, inorganic phosphate, ammonia, and glucose, were conducted before the maximal cycle ergometer test immediately after and 30 min post-exercise during visits 2 and 4.

2.4.5. Muscle and cardiorespiratory endurance

Muscle and cardiorespiratory endurance were evaluated using a gas analyzer (Quark CPET, COSMED, Rome, Italy) with a cycle ergometer (Corival CPET, LODE, Groningen, The Netherlands). A modified Åstrand protocol was used for testing. Before the test, participants were outfitted with a respiratory mask strapped to their faces and a heartbeat strap (Polar H10, Polar, USA). They engaged in a 1-min warm-up by paddling at 40–50 rpm and maintained a pace of 60–70 rpm during the test. The intensity of the test was gradually increased to 30 W in 2-minute increments. The rated perceived exertion (RPE) was measured every minute using a Borg Scale rating of 6–20. The test was stopped when the participants satisfied the following criteria: 1) inability to maintain at least 60 rpm for > 10 s, 2) RPE > 17, and 3) exhaustion (could not continue). When the test stopped, the total time to stop (time until exhaustion), VO_2max, maximal heart rate, total exercise distance, and anaerobic threshold levels were measured. Participants were instructed

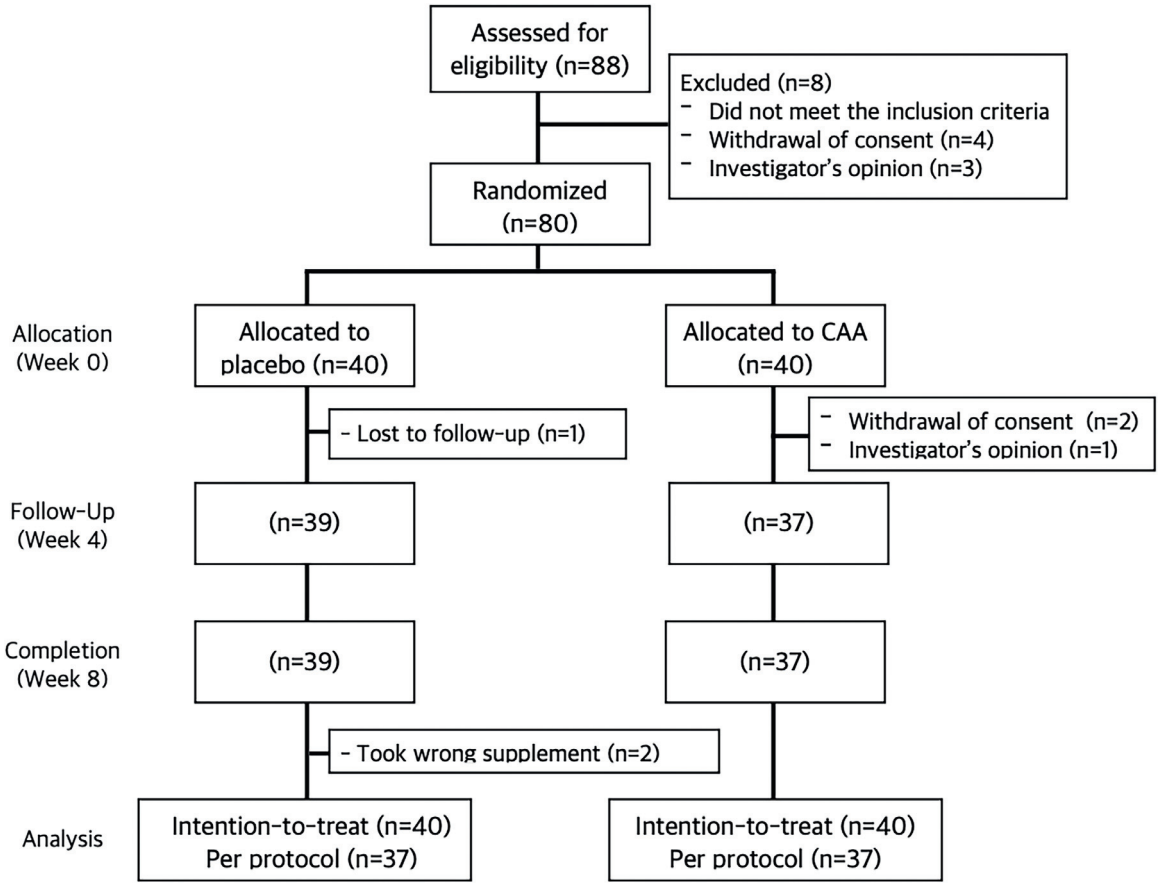

Fig. 2. CONSORT diagram for flow of subjects through the study; CAA, a mixture of *Cervus elaphus* Linnaeus, *Angelica gigas* Nakai and *Astragalus membranaceus* Bunge.

to report dizziness, illness, or inability to continue the ergometer test to ensure safety during fasting.

2.5. Compliance with supplements

Adherence was defined as a threshold of > 80% for supplement intake. Adherence was quantified using the following formula: adherence = (number of supplements ingested/prescribed number of supplements) × 100.

2.6. Statistical analyses

An intention-to-treat analysis that included all randomly assigned participants was performed. Additionally, a per-protocol analysis was conducted on participants who adhered to the study guidelines (e.g., compliance with the consumption of the test or control foods) and completed all visits. The data that constituted a non-regular distribution were converted to a normal distribution and analyzed. Participant characteristics at baseline were compared using Student's *t*-test for continuous variables and the chi-square test for categorical variables. Compliance between groups was compared using the Student's *t*-test. Continuous variables were analyzed using a linear mixed-effects model to investigate the effects of group, week, and group × week. Categorical variables were analyzed using Fisher's exact test for comparisons between groups and McNemar's test for comparisons within each group. Continuous variables are presented as means and standard deviations. Categorical variables are presented as the number of participants. Data were analyzed using SAS v. 9.4 (SAS Institute, Cary, NC, USA), and statistical significance was set at $p < 0.05$.

3. Results

3.1. Recruitment

This study followed the design shown in Fig. 2. Participants who met the selection criteria were randomly allocated to the placebo or CAA group, with 40 participants per group. However, during the intervention, three participants from the placebo group were excluded (one because of loss to follow-up and two because of ineligibility caused by taking the wrong supplements while living together). Additionally, three participants from the CAA group were excluded (two withdrew consent, and one was deemed ineligible by the researcher). Therefore, 74 participants were included in the study (placebo: 41.8 ± 1.3 years; CAA: 43.5 ± 1.2 years).

3.2. Baseline characteristics

The baseline characteristics included age, sex, and medical history. No significant differences were observed in the fatigue scores. No significant differences regarding the other variables were observed between the groups at baseline (Table 1).

3.3. Compliance with supplements

The compliance rates of the supplements were as follows: 100.2 ± 1.0 (%) in the placebo group and 101.9 ± 1.1 (%) in the CAA group, in which some of the participants overtook the extra pills. The overall compliance rates for both groups were consistently > 100%, with no significant intergroup differences.

Table 1
Baseline characteristics between placebo and CAA groups.

Variables	Placebo (n = 40)	CAA (n = 40)	*p*-value
Age (year)	41.8 ± 1.3	43.5 ± 1.2	0.347
Sex (male / female)	11 / 29	11 / 29	1.000
Fatigue Severity Scale	38.9 ± 1.2	40.9 ± 1.4	0.323
Menstruation (Yes / Done / NA)	22 / 7 / 11	20 / 9 / 11	0.863
Alcohol drinker (Yes / No)	11 / 29	14 / 26	0.547
Body mass index (kg/m^2)	23.5 ± 0.5	22.9 ± 0.5	0.743
Recommended Food Score	21.4 ± 1.4	19.9 ± 1.6	0.480
Sleeping Time (min/day)	423.8 ± 9.6	420.0 ± 8.7	0.817
VO_2max (ml/kg/min)	25.7 ± 0.7	23.9 ± 0.8	0.651

All data are presented in mean ± standard error. CAA, a 500 mg mixture of *Cervus elaphus* Linnaeus, *Angelica gigas* Nakai, *and Astragalus membranaceus* Bunge; NA, not applicable; VO_2max, maximal oxygen consumption.

3.4. Dietary intake and physical activity

The dietary intake and physical activity levels were consistent with the data presented in Table S1. Dietary intake results indicated no significant differences between the placebo and CAA groups regarding total caloric intake, carbohydrates, proteins, and fats over the 8 weeks of intervention. Similarly, no significant differences were observed in the responses to the recommended food score questionnaire or in physical activity levels between the two groups.

3.5. FSS

The FSS results are shown in Fig. 3 and Table S2. A significant between-group difference was observed between the placebo and CAA groups regarding the total score at visit 4 ($p = 0.028$). The CAA group showed a significant between-group reduction in "My fatigue prevents sustained physical functioning" (item 6) at visit 4 ($p = 0.040$). "Fatigue interferes with carrying out certain duties and responsibilities" (item

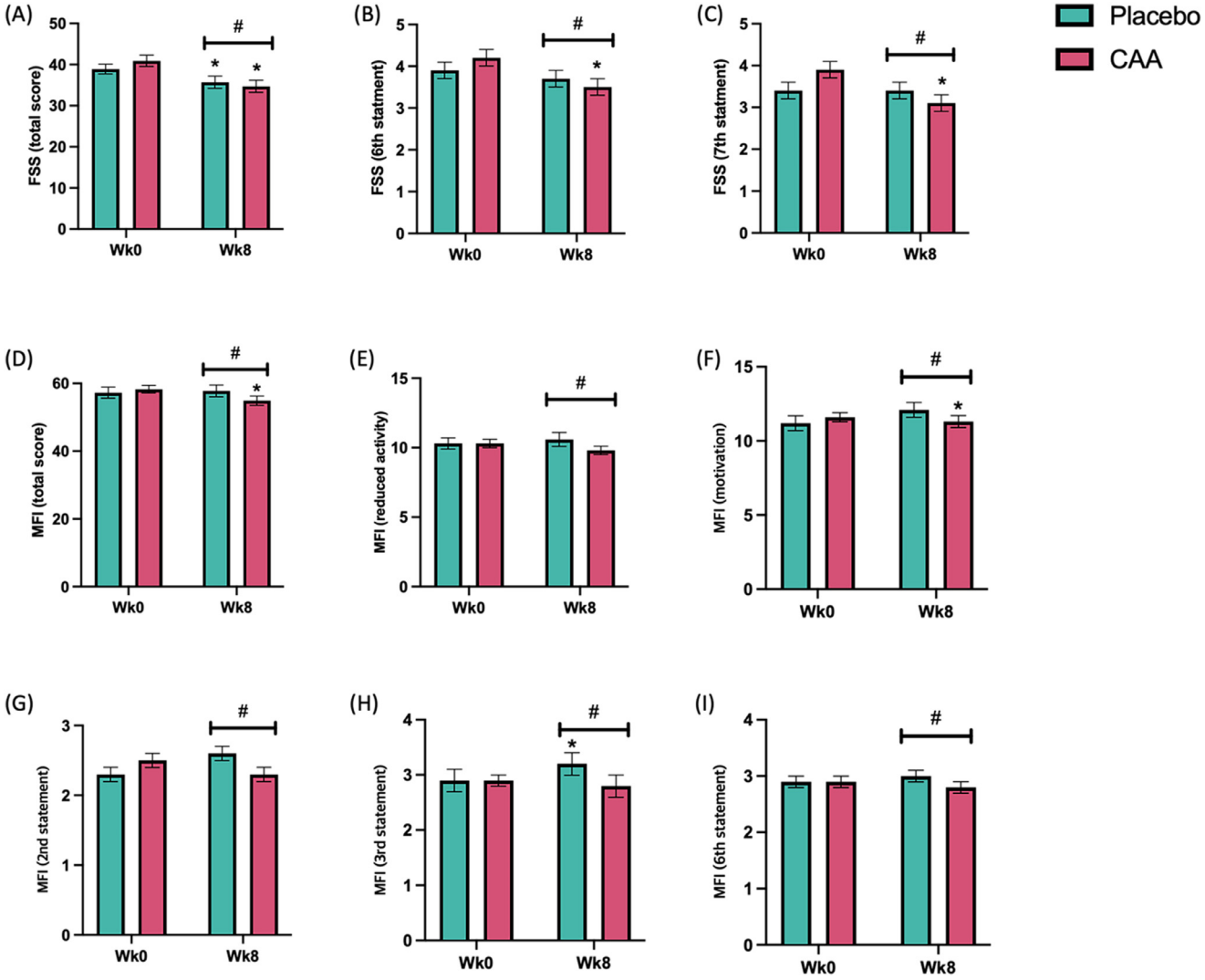

Fig. 3. Changes in fatigue severity (FSS) and multidimensional fatigue inventory (MFI) scores during the intervention. For the FSS, significant differences between groups are found in the total score (A), the sixth statement of the questionnaire, which indicates "My fatigue prevents sustained physical functioning" (B), and the seventh statement of "Fatigue interferes with carrying out certain duties and responsibilities" (C) after 8 weeks of consumption of CAA. For the MFI, significant differences between groups emerged in the following aspects: the total score (D), fatigue-related dimensions of "reduced activity"' (E) and "Motivation" (F), the second statement of MFI indicating "Physically, I feel only able to do a little" (G), the third statement, "I feel very active" (H), and the sixth statement "I think I do a lot in a day" (I) after 8 weeks of consuming CAA. ∗ denotes $p < 0.05$; a linear mixed-effect model adjusted with sleeping time at baseline is used to analyze the difference within each group. # denotes $p < 0.05$; between group *p*-value for group*time effect. Linear mixed-effect model adjusted with sleeping time to analyze the between group *p*-value for group*time effect. CAA, a 500 mg mixture of *Cervus elaphus* Linnaeus, *Angelica gigas* Nakai, *and Astragalus membranaceus Bunge*; W0, Week 0 stands for visit 2; W8, Week 8 stands for visit 4.

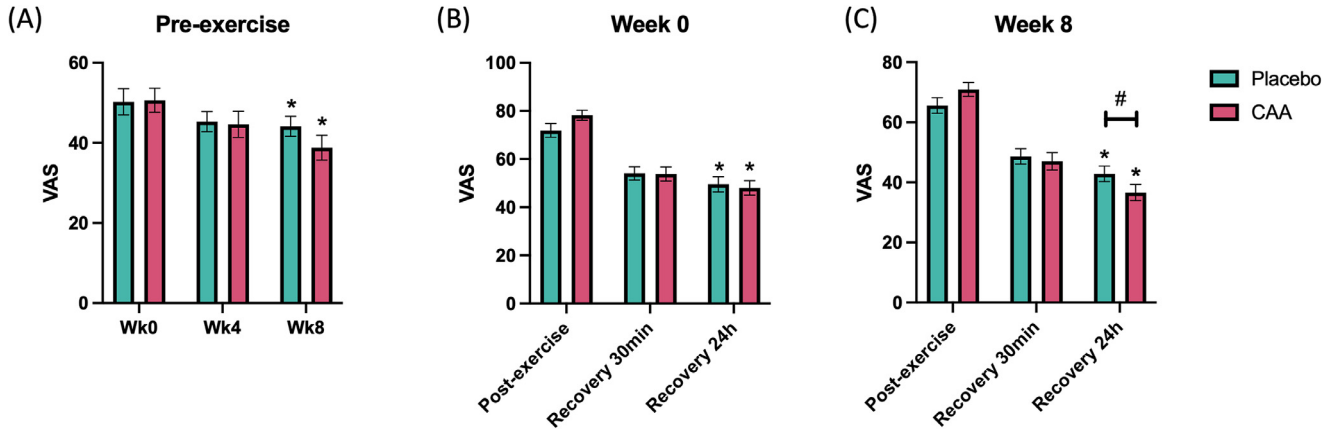

Fig. 4. The results of the visual analog scale (VAS) survey during the 8-week CAA intervention consumption. (A) No significant differences between the groups at pre-exercise during the whole intervention. While there are no differences at Visit 2 (B), a significant decrease is observed at 24 h (C). ∗ denotes $p < 0.05$; a linear mixed-effect model adjusted with sleeping time at baseline is used to analyze the difference within each group. # denotes $p < 0.05$; between group p-value for group*time effect. Linear mixed-effect model adjusted with sleeping time to analyze the between group p-value for group*time effect. CAA, a 500 mg mixture of *Cervus elaphus* Linnaeus, *Angelica gigas* Nakai, *and Astragalus membranaceus* Bunge. W0, Week 0 stands for visit 2; W4, Week 4 stands for visit 3; W8, Week 8 stands for visit 4.

7) also exhibited significant between-group differences at visits 3 and 4 ($p = 0.001$ for both visits). Additionally, a between-group difference was observed for "Fatigue is among my three most disabling symptoms" (item 8) at visits 3 ($p = 0.026$) and 4 ($p = 0.058$), respectively.

3.6. MFI

The MFI results are shown in Fig. 3 and Table S3. Significant improvements were observed in the total score and specific subfactors in the CAA group. The total score significantly decreased at visit 4 ($p = 0.037$). The sum of six items related to "general and physical fatigue" decreased at visit 4 ($p = 0.079$), the sum of four items pertaining to "reduced activity" significantly decreased at visit 4 ($p = 0.049$), and the sum of four items related to "motivation" significantly improved at visit 4 ($p = 0.044$).

3.7. VAS

The VAS survey results are shown in Fig. 4. No significant difference was observed between the control and test groups after 8 weeks before exercise (pre-exercise). However, improvements were observed during the recovery period. In the CAA group, no significant difference was observed at visits 2 and 4; however, a decreasing trend was observed 30 min after exercise cessation ($p = 0.059$) and a significant decrease at 24 h ($p = 0.002$). Both the placebo and CAA groups showed significant changes from visit 2 to 4.

3.8. Muscle and cardiorespiratory endurance

The results of cardiorespiratory endurance due to exercise load are shown in Fig. 5. Significant increases were observed in the time ($p = 0.019$) and distance covered until exhaustion ($p = 0.017$) in the CAA group. No difference was observed in the anaerobic threshold level; however, the VO_2max was significantly enhanced ($p = 0.039$). Significant differences within the CAA group were observed for the following parameters: time to exhaustion ($p = 0.002$), distance covered until exhaustion ($p = 0.003$), maximum heart rate ($p = 0.007$), and maximum respiratory quotient ($p = 0.043$).

3.9. Blood test

The results of biochemical marker assessments are presented in Table 2. Creatine kinase levels were significantly decreased in the control group ($p = 0.004$) when comparing the CAA group with the control group at baseline (pre-exercise). All other indicators showed no significant differences in any of the test groups compared with the control group. Myoglobin levels significantly decreased in the control group 30 min after exercise ($p = 0.020$) from the end of exercise (post-exercise) to the recovery period). In contrast, no significant change was observed in the CAA group, resulting in a significant difference between the two groups ($p = 0.010$). No other indicators showed significant differences between the CAA and control groups.

4. Discussion

This randomized, placebo-controlled, double-blind study is the first clinical trial to evaluate the effects of complex herbal medicine extracts (CAA) on fatigue levels and muscle and cardiorespiratory endurance in adults with CFS. The overall fatigue scores on the FSS, MFI, and 24-hour VAS in the CAA group improved significantly compared to the placebo group. Notably, consistency was observed between the fatigue improvement in the CAA group and physiological variables such as increased maximal exercise time to exhaustion, distance until exhaustion, and maximum oxygen consumption. These results suggest that the reported fatigue reduction aligns with the physiological markers during fatigue-inducing exercise, underscoring their resilience to exercise-induced fatigue in the CAA group and providing substantial support for the observed fatigue improvements in this group.

Cervus elaphus Linnaeus (comprising polypeptides, polysaccharides, and phospholipids[21,35,36]), *A. gigas* Nakai (including decursin, decursinol angelate, and essential oils[23,24]), and *Astragalus membranaceus* Bunge (containing astragaloside IV and polysaccharides[27,37]) have antioxidant effects. The consumption of CAA can enhance muscle endurance capacity and alleviate fatigue symptoms in mice.[20,26] These studies have also highlighted the potential of CAA as a highly effective supplement for improving performance and reducing fatigue by preventing inflammation through the inhibition of nuclear factor-κB activation. However, in this study, no significant changes were observed in the blood tests even though participants consumed the same CAA supplements as in previous CAA in vitro studies. These findings highlight the variability between animal and human studies and underscore the limited understanding of biomarkers associated with CFS in humans.[38]

Generalized abnormal muscle fatigue following mild activity is a primary CFS symptom.[39] Accordingly, this study observed that increased cardiorespiratory and muscle endurance capacities align with the anti-

Table 2
Changes in biochemical biomarkers during intervention.

Variables	Placebo (n = 40)	CAA (n = 40)	*p*-value[1]
Lactate (mg/dL)			
Pre-exercise			
Visit 2 (W0)	9.6 ± 0.7	9.7 ± 0.7	
Visit 4 (W8)	9.6 ± 0.7	10.7 ± 1.1	0.444
p-value[2]	0.969	0.300	
From post-exercise to recovery			
Visit 2 (W0)			
Post-exercise	44.4 ± 2.9	41.7 ± 3.0	
Recovery 30 min	23.4 ± 2.0	21.6 ± 1.9	0.783
p-value[2]	<0.001	<0.001	
Visit 4 (W8)			
Post-exercise	50.6 ± 3.2	50.0 ± 3.5	
Recovery 30 min	24.4 ± 2.0	25.6 ± 2.5	0.581
p-value[2]	<0.001	<0.001	
Lactate dehydrogenase (U/L)			
Pre-exercise			
Visit 2 (W0)	170.7 ± 4.9	170.7 ± 5.0	
Visit 4 (W8)	167.9 ± 4.5	162.7 ± 3.7	0.306
p-value[2]	0.443	0.031	
From post-exercise to recovery			
Visit 2 (W0)			
Post-exercise	180.4 ± 5.0	175.8 ± 3.8	
Recovery 30 min	174.5 ± 4.8	167.7 ± 3.6	0.535
p-value[2]	0.018	0.002	
Visit 4 (W8)			
Post-exercise	176.3 ± 4.4	173.3 ± 4.2	
Recovery 30 min	169.3 ± 4.8	161.6 ± 3.9	0.170
p-value[2]	0.004	<0.001	
Creatine kinase (U/L)			
Pre-exercise			
Visit 2 (W0)	131.5 ± 21.6	101.5 ± 10.3	
Visit 4 (W8)	88.0 ± 5.6	82.9 ± 4.6	0.239
p-value[2]	0.004	0.225	
From post-exercise to recovery			
Visit 2 (W0)			
Post-exercise	139.9 ± 22.9	104.1 ± 10.5	
Recovery 30 min	133.4 ± 21.8	102.3 ± 10.5	0.157
p-value[2]	0.007	0.481	
Visit 4 (W8)			
Post-exercise	94.1 ± 6.0	88.2 ± 5.0	
Recovery 30 min	89.4 ± 5.7	83.4 ± 4.6	0.992
p-value[2]	<0.001	<0.001	
BUN (mg/dL)			
Pre-exercise			
Visit 2 (W0)	12.5 ± 0.4	12.8 ± 0.5	
Visit 4 (W8)	12.2 ± 0.5	12.8 ± 0.5	0.703
p-value[2]	0.528	0.935	
From post-exercise to recovery			
Visit 2 (W0)			
Post-exercise	12.4 ± 0.4	12.8 ± 0.5	
Recovery 30 min	12.4 ± 0.4	12.8 ± 0.5	0.297
p-value[2]	0.056	0.675	
Visit 4 (W8)			
Post-exercise	12.3 ± 0.5	12.8 ± 0.5	
Recovery 30 min	12.2 ± 0.5	12.7 ± 0.5	0.775
p-value[2]	0.032	0.013	
Creatinine (mg/dL)			
Pre-exercise			
Visit 2 (W0)	0.78 ± 0.03	0.78 ± 0.02	
Visit 4 (W8)	0.78 ± 0.03	0.76 ± 0.03	0.218
p-value[2]	0.974	0.081	
From post-exercise to recovery			
Visit 2 (W0)			
Post-exercise	0.84 ± 0.03	0.82 ± 0.02	
Recovery 30 min	0.81 ± 0.03	0.80 ± 0.03	0.450
p-value[2]	<0.001	<0.001	
Visit 4 (W8)			
Post-exercise	0.84 ± 0.03	0.81 ± 0.03	
Recovery 30 min	0.82 ± 0.03	0.78 ± 0.03	0.307
p-value[2]	<0.001	<0.001	

(*continued on next page*)

Table 2 (*continued*)

Variables	Placebo (n = 40)	CAA (n = 40)	*p*-value[1]
BUN/creatinine ratio			
Pre-exercise			
Visit 2 (W0)	16.6 ± 0.8	16.8 ± 0.8	
Visit 4 (W8)	16.1 ± 0.8	17.4 ± 0.8	0.250
p-value[2]	0.315	0.011	
From post-exercise to recovery			
Visit 2 (W0)			
Post-exercise	15.6 ± 0.7	15.9 ± 0.7	
Recovery 30 min	16.0 ± 0.8	16.4 ± 0.8	0.845
p-value[2]	<0.001	<0.001	
Visit 4 (W8)			
Post-exercise	15.1 ± 0.7	16.0 ± 0.7	
Recovery 30 min	15.5 ± 0.8	16.7 ± 0.7	0.161
p-value[2]	<0.001	<0.001	
Myoglobin (ng/dL)			
Pre-exercise			
Visit 2 (W0)	28.2 ± 2.5	33.2 ± 7.0	
Visit 4 (W8)	23.9 ± 0.9	21.8 ± 0.3	0.268
p-value[2]	0.425	0.438	
From post-exercise to recovery			
Visit 2 (W0)			
Post-exercise	29.2 ± 2.6	27.1 ± 3.2	
Recovery 30 min	27.6 ± 2.4	33.5 ± 7.5	0.161
p-value[2]	0.696	0.115	
Visit 4 (W8)			
Post-exercise	24.3 ± 0.9	22.8 ± 0.8	
Recovery 30 min	23.0 ± 0.6	23.0 ± 0.8	0.010
p-value[1]	0.002	0.584	
Inorganic phosphate (mg/dL)			
Pre-exercise			
Visit 2 (W0)	3.7 ± 0.1	3.8 ± 0.1	
Visit 4 (W8)	3.7 ± 0.1	3.7 ± 0.1	0.807
p-value[1]	0.566	0.364	
From post-exercise to recovery			
Visit 2 (W0)			
Post-exercise	3.9 ± 0.1	4.0 ± 0.1	
Recovery 30 min	3.5 ± 0.1	3.7 ± 0.1	0.02
p-value[1]	<0.001	<0.001	
Visit 4 (W8)			
Post-exercise	4.0 ± 0.1	4.0 ± 0.1	
Recovery 30 min	3.6 ± 0.1	3.6 ± 0.1	0.368
p-value[1]	<0.001	<0.001	
Ammonia (μg/dL)			
Pre-exercise			
Visit 2 (W0)	59.4 ± 7.9	51.2 ± 2.2	
Visit 4 (W8)	66.1 ± 12.0	62.1 ± 13.7	0.809
p-value[1]	0.521	0.778	
From post-exercise to recovery			
Visit 2 (W0)			
Post-exercise	82.2 ± 5.4	75.2 ± 5.2	
Recovery 30 min	55.9 ± 4.0	50.0 ± 3.4	0.881
p-value[1]	<0.001	<0.001	
Visit 4 (W8)			
Post-exercise	99.5 ± 7.1	87.8 ± 7.4	
Recovery 30 min	54.2 ± 2.9	67.0 ± 15.1	0.210
p-value[1]	0.001	0.138	
Glucose (mg/dL)			
Pre-exercise			
Visit 2 (W0)	89.5 ± 1.5	88.1 ± 1.1	
Visit 4 (W8)	88.5 ± 1.3	87.6 ± 1.2	0.171
p-value[1]	0.333	0.330	
From post-exercise to recovery			
Visit 2 (W0)			
Post-exercise	92.5 ± 1.7	90.7 ± 1.0	
Recovery 30 min	91.2 ± 1.6	91.9 ± 1.1	0.046
p-value[1]	0.144	0.169	
Visit 4 (W8)			
Post-exercise	90.8 ± 1.2	88.1 ± 1.3	
Recovery 30 min	91.4 ± 1.3	90.2 ± 1.3	0.174
p-value[1]	0.384	0.007	

All data are presented in mean ± standard error. CAA, a 500 mg mixture of *Cervus elaphus* Linnaeus*, Angelica gigas* Nakai*, and Astragalus membranaceus* Bunge; W0, Week 0 stands for visit 2; W8, Week 8 stands for visit 4

[1] *p*-value is presented as group*time effect. A linear mixed model is used to compare the changes for 8 weeks between the placebo and CAA groups.

[2] Linear mixed-effect model adjusted with sleeping time at baseline is used to analyze the difference within each group.

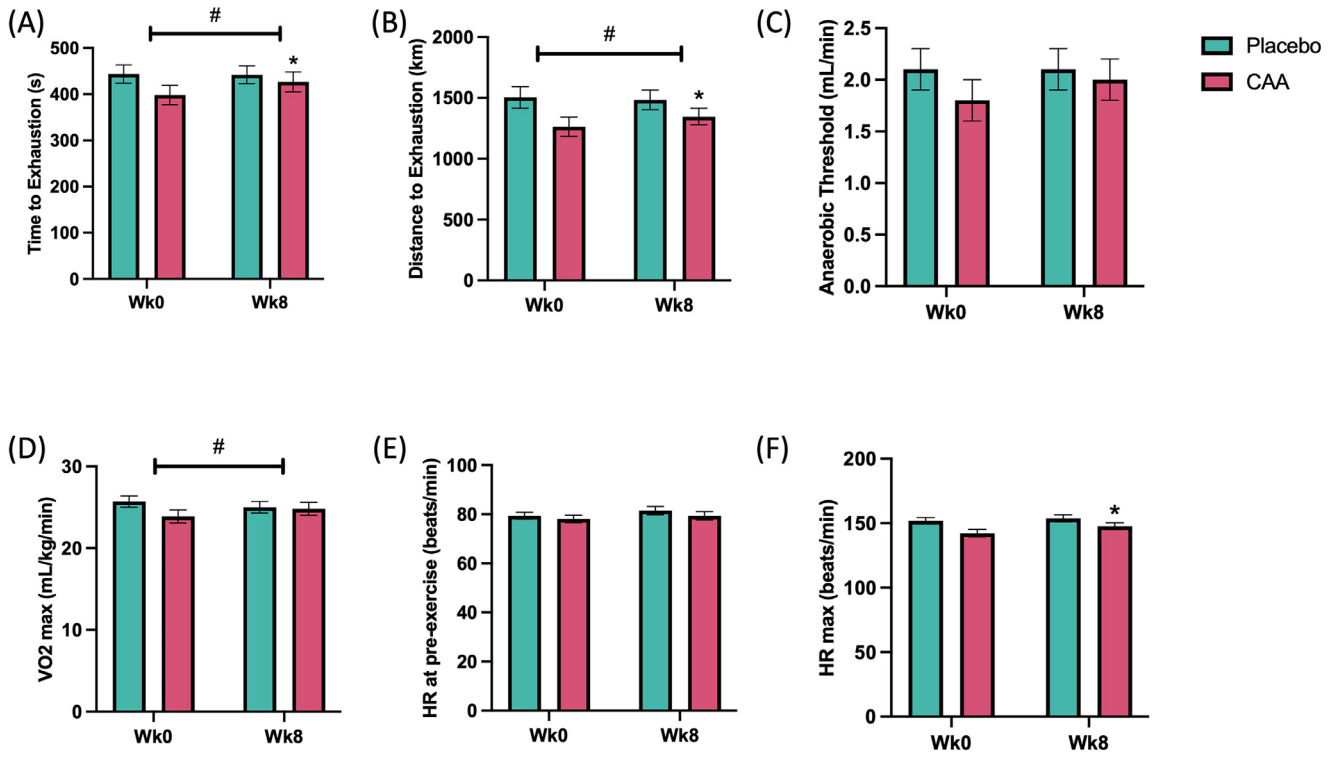

Fig. 5. Alternation in muscle and cardiorespiratory endurance after consumption of CAA for 8 weeks. Significant differences between groups are noted in both the duration of exercise (A) and the distance covered (B) until the point of exhaustion. While there are no significant differences observed between the groups in terms of the anaerobic threshold (C), there is a difference in VO_2max in both groups after consuming CAA (D). When comparing heart rates, there are no differences between groups observed at the pre-exercise stage (E), and maximal heart rate F). ∗, denotes $p < 0.05$; a linear mixed-effect model adjusted with sleeping time at baseline is used to analyze the difference within each group. #, denotes $p < 0.05$; between group p-value for group*time effect. Linear mixed-effect model adjusted with sleeping time to analyze the between group p-value for group*time effect. CAA, a 500 mg mixture of *Cervus elaphus* Linnaeus, *Angelica gigas* Nakai, *and Astragalus membranaceus* Bunge. W0, Week 0 stands for visit 2; W8, Week 8 stands for visit 4.

fatigue benefits of herbal medicines. The intake of *Cervus elaphus* Linnaeus in a mouse model improved exercise endurance, which suggested potent anti-fatigue qualities[40]; these results align with the physical performance enhancement results of the current study. In another study, *Angelica radix* improved exercise performance and reduced physical fatigue in adults.[41] Furthermore, a 6-week regimen of exercise training in combination with *Astragalus membranaceus* Bunge supplementation enhanced endurance capacity and increased hepatic and muscle glycogen content in trained mice.[42] These effects can be attributed to the forced exercise tests conducted in this animal model. In line with the outcomes reported by Huang et al., a significant increase was observed in the duration and distance covered until exhaustion during a treadmill running test.[20,22] Notably, a similar study observed a significant increase in lactate levels after the consumption of HemoHIM (a combination of *Angelica gigas* Nakai, *Cnidium officinale* Makino, and *Paeonia lactiflora* Pallas).[41] However, considering these differences is essential when interpreting these results because both studies used maximal exhaustion protocols. This discrepancy may be caused by the varied muscle endurance of the participants, potentially resulting in differences in lactate levels. In the present study, the CAA group demonstrated a significant increase in exercise distance and time to exhaustion. However, this group did not exhibit similar increases in lactate levels after maximal exercise, suggesting that CAA supplementation may affect muscular fatigue by maintaining high lactate levels during high-intensity exercise.

Oriental medicine has a positive impact on anti-fatigue outcomes with a particular emphasis on improving total FSS, which is primarily attributed to various active components.[43,44] *A. membranaceus* B. has notable anti-fatigue effects,[45] and this study also demonstrated significant improvements in subjective outcomes, including FSS, indicating the potential anti-fatigue effects of CAA supplementation.

However, the present study had several limitations. First, the study sample included a higher proportion of women than men, which may have affected the generalizability of the findings. Second, older populations with different characteristics were not assessed, which further limited the applicability of the results. Nonetheless, the sex ratio between the two groups was consistent, and the study's objectives were adequately addressed using the younger participants.

In conclusion, CAA has a significant effect on subjective fatigue scales, as well as muscle and cardiovascular endurance in individuals with chronic fatigue. The findings of this study support the use of herbal supplements in individuals with chronic fatigue. Future research should explore interventions across a broader range of doses and more diverse participant groups to better evaluate their efficacy. Applying the intervention to a wider and more varied sample could further enhance the generalizability of the results.

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Ethical statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Seoul National University (IRB No. 2011/002–020, 2021–10–25). Informed consent was obtained from all participants.

Data availability

The data that has been used is confidential and cannot be shared.

Declaration of competing interest

The authors have no conflict of interest to declare. The funders had no role in the study design, collection, analyses, interpretation of data, or in the decision to publish the results.

CRediT authorship contribution statement

SoYoung Ahn: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Parivash Jamrasi:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Byunggul Lim:** Investigation, Writing – review & editing. **Ji-won Seo:** Investigation, Writing – review & editing. **Xinxing Li:** Investigation, Writing – review & editing. **Shu Jiang:** Investigation, Writing – review & editing. **Yunho Sung:** Investigation, Writing – review & editing. **Seo Hyun Ahn:** Investigation, Writing – review & editing. **Chaeyoung Shin:** Investigation, Writing – review & editing. **Dongjin Noh:** Writing – review & editing. **Bora Jin:** Writing – review & editing. **Seonjoo Lee:** Writing – review & editing. **Ki Won Lee:** Writing – review & editing. **Jin Soo Kim:** Writing – review & editing. **Young Tae Koo:** Writing – review & editing. **Wook Song:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition, Supervision.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.imr.2024.101085.

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