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Effects of seabuckthorn juice consumption on erythropoietin production, fatigue, and quality of life in women: a randomized, placebo-controlled, double-blind, parallel-group study

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ABSTRACT

Background: Seabuckthorn (*Hippophae rhamnoides* L.) has attracted interest for mitigating transient fatigue, but clinical evidence remains limited. Although a few preliminary reports suggest potential benefits for fatigue and quality of life, high-quality clinical data are scarce: prior studies have often been small, nonrandomized or open-label, short in duration, and used heterogeneous endpoints with a risk of bias, and preregistered RCTs are rare. This study aimed to address these gaps.

Objective: To evaluate the effects of seabuckthorn juice on fatigue and quality of life (QOL) in premenopausal women and to explore a mechanistic link via erythropoietin (EPO) induction.

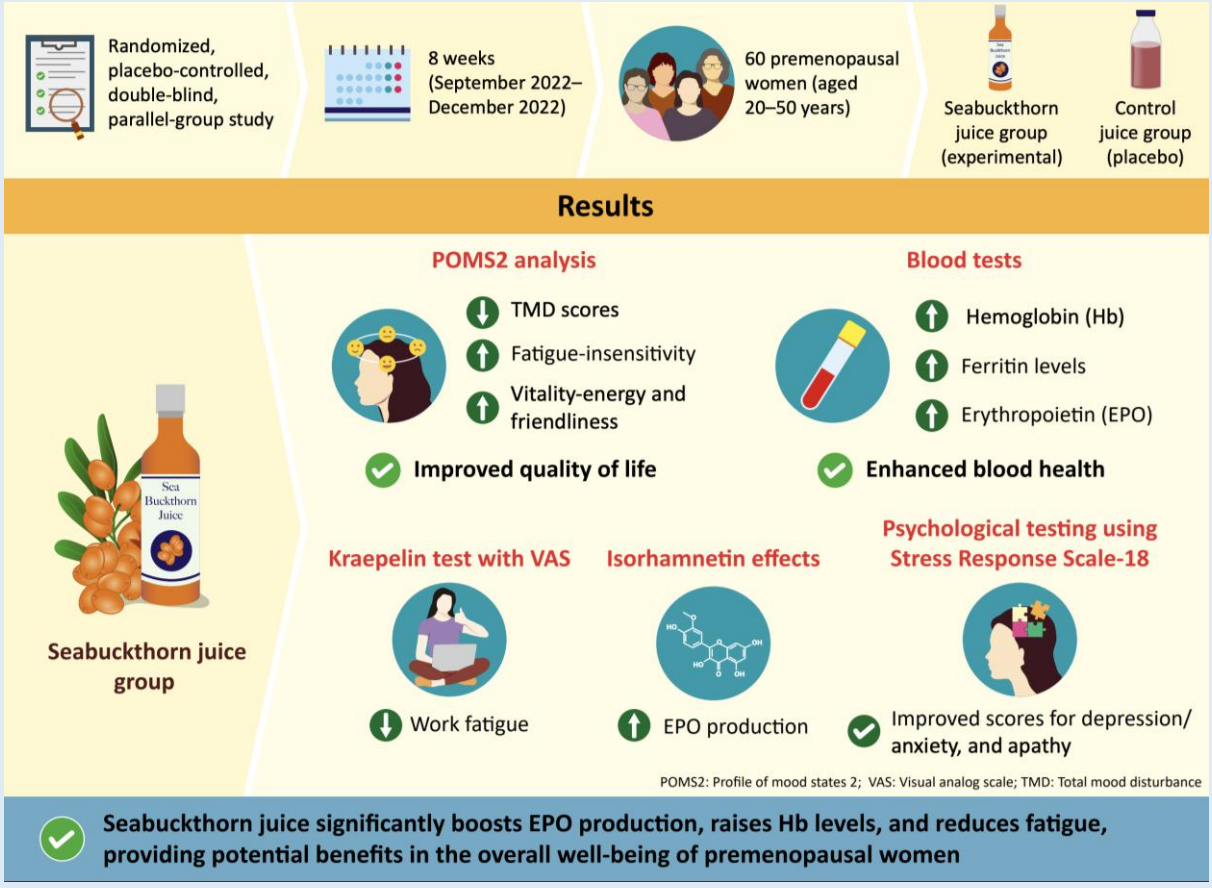
Methods: We conducted an 8-week, randomized, double-blind, placebo-controlled, parallel-group trial to evaluate the anti-fatigue and quality-of-life effects of seabuckthorn juice. Sixty healthy premenopausal women (20–50 years) with stable menstrual cycles, desk-bound jobs, and transient fatigue with decreased vitality were randomized 1:1 to

seabuckthorn juice or a matched control juice. The primary endpoint was Profile of Mood States 2 (POMS2) scores. Secondary endpoints included hematologic indices (hemoglobin [Hb], ferritin, iron-binding capacity), serum erythropoietin (EPO), visual analog scale (VAS) fatigue after a Kraepelin test load, and Stress Response Scale-18 (SRS-18). A mechanistic in-vitro substudy tested isorhamnetin (a seabuckthorn flavonol) for EPO induction in HepG2 cells.

Results: Compared with placebo, seabuckthorn juice significantly improved POMS2 outcomes at week 8, showing lower Total Mood Disturbance, fatigue-related scores, higher vitality/energy, and friendliness subscales. Hematologic endpoints favored seabuckthorn: ferritin, Hb, and EPO were significantly higher at weeks 4 and 8, with greater changes from baseline versus placebo. VAS-rated fatigue after the Kraepelin task was significantly reduced in the seabuckthorn group at weeks 4 and 8. In vitro, isorhamnetin increased EPO production in HepG2 cells, supporting biological plausibility.

Conclusion: Eight weeks of seabuckthorn juice consumption improved mood-related fatigue metrics and quality of life, enhanced blood health via increased EPO and Hb, and reduced work-related fatigue in premenopausal women; in-vitro findings suggest isorhamnetin-mediated EPO induction as a potential mechanism.

Keywords: saji; sea buckthorn; QOL; POMS2; Hb; erythropoietin



Graphical Abstract: Effects of seabuckthorn juice consumption on erythropoietin production, fatigue, and quality of life in women

INTRODUCTION

In recent years, IT equipment used in offices has become increasingly well-developed, and the use of information terminals, such as personal computers and tablets, has become an essential part of the work process in most industries. In addition, with the rapid spread of smartphones and the development of social networking services, many individuals are exposed to various information throughout the day, creating several opportunities for exposure to mental stress. According to a 1999 survey by the Ministry of Health, Labor, and Welfare, 60% of the Japanese population (47.2 million people) reported experiencing fatigue. Half of them (29.6 million people) had chronic fatigue for more than 6 months. As these figures indicate, Japan is a fatigue-prone country, and with the aging population, the number of people who experience fatigue is projected to increase [1-3]. In subsequent surveys, the proportion of people who experienced fatigue did not change significantly. In a 2018 survey of 1,000 Tokyo businesspeople, the proportion of those who said they "do not feel tired after a night's sleep" was "very applicable" at 27.5% and "somewhat applicable" at 42.5%, with approximately 70% of the respondents [4]. The causes of fatigue in daily life can be broadly classified into two categories: physical and mental. Physical fatigue results from housework, labor, and exercise, whereas mental fatigue is caused by office work and studying [5]. The chronicity of this type of fatigue is thought to lead to abnormalities in the body's regulatory functions. In particular, abnormalities in the autonomic nervous system and immune and endocrine regulatory functions have been observed, which are believed to lead to a disease called chronic fatigue syndrome [6,7]. Fatigue is known to manifest easily and is closely related to stress [8,9]. It is important to alleviate fatigue to reduce the decline in performance and improve quality of life[10]. Therefore, it is essential to address this issue in daily life through diet rather than solely relying on drug treatments. Although active disease treatment, such as drug therapy, is necessary to cure chronic fatigue, most of the fatigue experienced by people today is transient or can be alleviated by addressing the underlying causes. These causes often include work-related stress caused by the increased complexity of information due to the spread of IT devices and the accumulation of mental stress. Transient fatigue due to these tasks has been confirmed by the intake of various food-derived ingredients. For instance, γ-Aminobutyric acid (GABA) was found to be effective in a placebo-controlled, randomized, double-blind, crossover study of mentally stressful workloads (two trials) using the Uchida-Kraepelin test for computational tasks. GABA was reported to significantly alter the subjective assessment measure (visual analog scale; VAS) of fatigue in mentally stressful workloads [11].

The advancement of women in society, particularly in developed countries, is progressing. Although women are expected to play an active role in society to a great extent, they often face various stressors as they are mainly responsible for housework and childcare due to their social roles. Prolonged stress can lead to physical and mental fatigue.

Seabuckthorn is a deciduous shrub of the genus Hippophae (*Hippophae rhamnoides* L.) in the family Elaeagnaceae. It is widely distributed in the temperate regions of Eurasia, and its fruits are eaten. The fruits are rich in nutrients and are known to contain vitamins, minerals, fatty acids, and the flavonoid isorhamnetin glycoside [12-16]. The vitamins found in fruits have also been reported to contain strong antioxidant vitamins, such as vitamin C [17] and vitamin E [18,19]. The fruits are often pureed and consumed as juices. Seabuckthorn has garnered attention for its potential anti-fatigue effects. Many users have reported experiencing anti-fatigue effects from seabuckthorn juice. These effects are thought to be due to the ingredients contained in the juice; however, there have been few studies on this subject. Therefore, we aimed to evaluate the potential of the ingredients in seabuckthorn juice to improve the temporarily impaired quality of life and blood health of working women as a secondary outcome of blood tests, given the many reports on its temporary reduction of fatigue, especially among female consumers. This study was conducted along with an EPO production enhancement study as a mechanism of action.

MATERIALS AND METHODS

Study Design: This was a randomized, double-blind, placebo-controlled, parallel-group trial. The clinical trial was conducted from September to December 2022 at the Shinanokai Left Moncho Clinic.

The study protocol was approved by the Independent Ethics Committee of the Kobuna Orthopedic Clinic on September 8, 2022 (approval number: MK-2209-04) and registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR; UMIN000048729). The study was conducted in compliance with the ethical principles of the Declaration of Helsinki and the ethical guidelines for epidemiological research (notified by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour, and Welfare). Written informed consent was obtained from all participants involved in the study.

Sample Size: Prior to conducting this study, a preliminary examination of the primary endpoint (daily fatigue) was conducted using the Quality of Life Questionnaire. The results revealed a decrease in the overall score by six points. Therefore, it was determined that consumption of the test food would result in a decrease of four points with a standard deviation of three points compared with the placebo. The sample size was calculated with an assumed 80% power, resulting in 25 subjects per group. In addition, to account for the possibility of dropouts, a minimum of 30 participants per group was planned.

Table 1. The nutritional composition of test foods.

Nutrition Facts	Placebo juice	Seabuckthorn juice
Energy (kcal)	19kcal/100g	51kcal/100g
Water (g)	94.1g/100g	87.8g/100g
Protein (g)	0.2g/100g	0.8g/100g
Fat (g)	0.0g/100g	0.9g/100g
Sugar (g)	5.0g/100g	9.7g/100g
dietary fibre (g)	0.1g/100g	0.5g/100g
isorhamnetin ((aglycone equivalent))	-	3.0mg/100g

Test Food: The study participants were divided into two groups based on the test foods: one consuming fruit juice made of pureed seabuckthorn fruit provided by Yuhangren Japan Co., LTD., and the other consuming a control food containing no seabuckthorn fruit. The nutritional composition of the test foods is shown in Table 1. The subjects were also given 30 ml of seabuckthorn juice or control food per day, with seabuckthorn juice containing 0.93 mg/30 ml of isorhamnetin as aglycone.

Test Participants: The participants were premenopausal women aged 20–50 years who had desk-bound jobs, were temporarily fatigued, and often lacked energy. Further selection criteria included: (1) BMI less than 30 kg/m^2, (2) relatively stable menstrual cycle, (3) written consent and voluntary participation in the study, and (4) ability to continue drinking seabuckthorn juice. In addition, the exclusion criteria were as follows: (1) Patients currently determined to have iron deficiency anemia and are being treated with medication; (2) those currently receiving medication for any disease; (3) those consuming foods or health foods containing ingredients of this test food; (4) those with irregular menstruation; (5) those with a history of serious diseases of the glucose metabolism, lipid metabolism, liver function, renal function, cardiac, cardiovascular, endocrine, immune, or nervous systems, or psychiatric disorders; (6) those with a history of abnormal laboratory values that would make participation in the study problematic; (7) those with a disease under treatment or a history of a serious disease that required medication; (8) subjects who may develop allergies related to the study; (9) subjects judged to be unsuitable as subjects based on the results of the lifestyle questionnaire; (10) subjects participating in other clinical studies at the start of this study; (11) pregnant or breastfeeding subjects or those who plan to become pregnant during the study period; (12) subjects who

were informed by the study investigator (or the study administrator) that they will not be able to participate in the study; and (13) those deemed unsuitable for participation in the study by the principal investigator (or the study investigator).

Test Method: The study was explained to the subjects, and consent was obtained during the preintake examination, after which each test was administered. The selected subjects were randomly assigned to two groups, and intake of the test foods was started. The subjects then visited the clinic 4 and 8 weeks after intake, and various tests were conducted.

Evaluation Items

Primary Endpoints: The primary endpoint was the POMS2 scores in the quality of life questionnaire. The POMS2 questionnaire was administered at the pre-intake, 4 weeks post-intake, and 8 weeks post-intake examinations.

Secondary Endpoints: The secondary endpoints included fatigue during mental workload by performing the Uchida-Kraepelin test at the time of the visit. In addition, Hb, ferritin, and serum iron (indicators of anaemia) and erythropoietin (EPO) levels were measured as blood test items. Hematology and blood biochemistry tests and confirmation of adverse events were conducted as safety evaluation items.

Mental Workload Assessed by Uchida-Kraepelin Test: The mental workload of the Uchida-Kraepelin test was based on the method of Kanehira et al [10]. After the patients had come to the hospital, they rested for 15 min, and then a subjective evaluation using the VAS was conducted. Subsequently, a calculation task using the Uchida-Kraepelin test was performed for 15 min. Immediately after this test, subjective evaluation using the VAS was conducted.

Fatigue Assessment using VAS: VAS is useful for the evaluation of the subjectivity of pain, as well as for self-assessment of fatigue [18]. In this study, subjective evaluation by VAS was conducted using a 100 mm line segment, with the left end as "the best sensation not felt at all" and the right end as "the worst sensation felt so." The subjects were asked to respond by filling in a diagonal line on the straight line for their current subjective state. The VAS questionnaire was administered before the Uchida-Kraepelin test and immediately after the calculation task.

Psychological Stress Response Scale (SRS-18): The SRS-18 was administered to assess psychological stress response. The SRS-18 was administered at the preintake, 4-week postintake, and 8-week postintake tests.

Statistical Analysis: Statistical analysis tests for efficacy evaluation were performed by Kansai University of Social Welfare and Science. Student's t-test was used to analyze between-group comparisons. The analysis method considered the normality of each data set but did not consider multiplicity, and the two-sided significance level was set at 5%. SAS 9.4 (SAS Inc.) was used as the statistical software for analysis.

Erythropoietin Production Promotion Test: The samples utilized isorhamnetin, narcissin (isorhamnetin glycoside), and the lyophilized seabuckthorn juice.

METHODS

The effect of EPO production was evaluated in a human hepatocellular carcinoma-derived cell line (HepG2 cells). Cells were pre-cultured in 10 cm diameter dishes in EMEM (containing 1 % penicillin-streptomycin and 10 % fetal bovine serum) until confluent. The cells were then washed with phosphate-buffered saline (PBS), resuspended in medium, seeded in 96-well plates at a concentration of 0.5×10^5 cells/well, and cultured overnight in a CO_2 incubator (37°C, 5% CO_2). After incubation, samples were added and incubated for another 24 h. The supernatant of the culture medium was collected, and the amount of EPO was measured using an Enzyme-linked Immunosorbent Assay kit for Erythropoietin (Cloud-Clone Corp, USA).

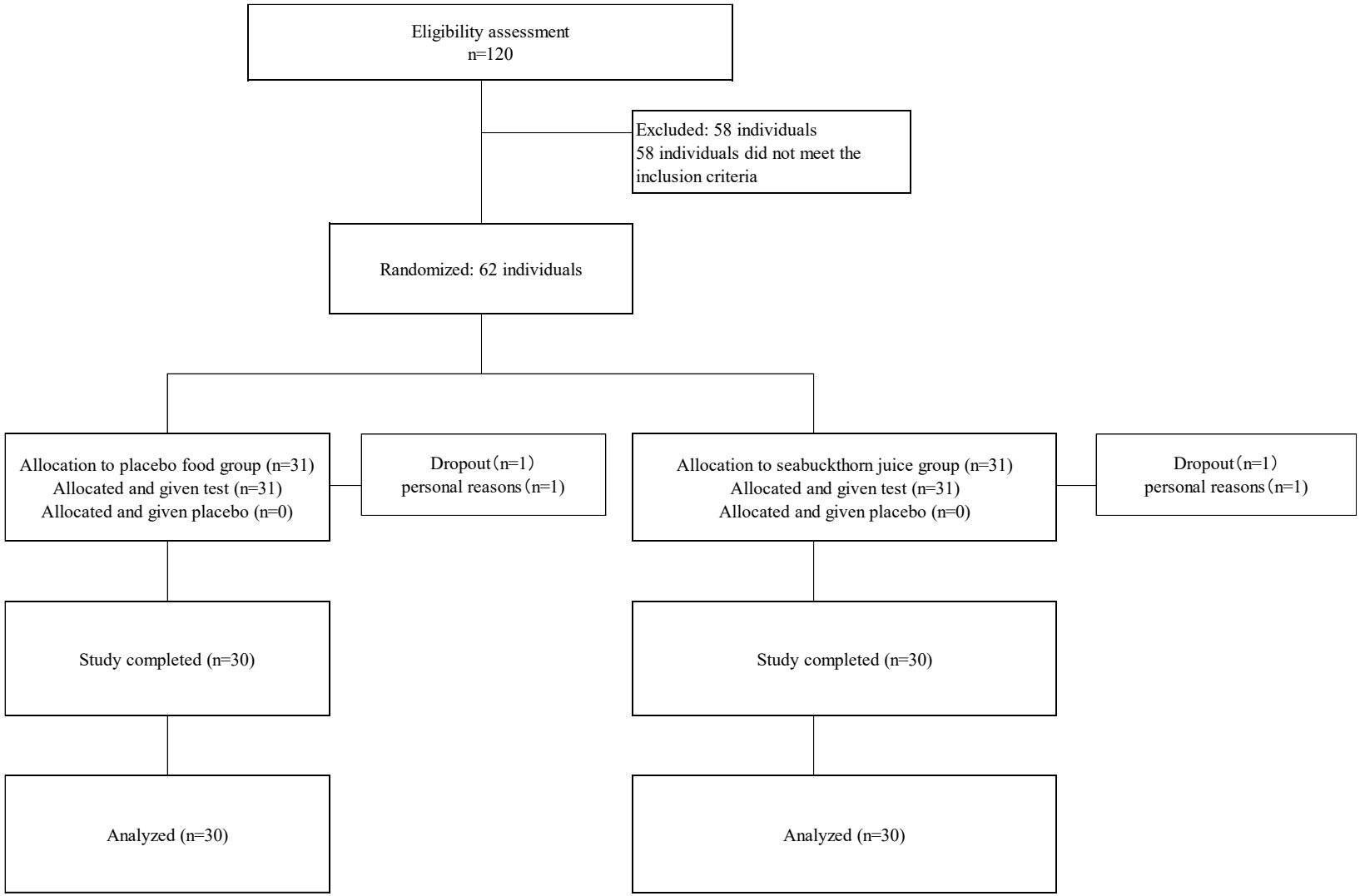


Figure 1. Subject selection flow

The subject selection flow is shown in Figure 1. The study was conducted with 120 subjects for the SCR examination; 62 subjects who met the inclusion criteria and did not meet the exclusion criteria were selected. After selection, 31 subjects each were assigned to two groups using the stratified block randomization method so that there were no significant differences in age or QOL survey results at the time of SCR. One participant in each group dropped out of the study for personal reasons, and 60 participants (30 in the seabuckthorn juice group and 30 in the placebo juice group) were included in the analysis. However, one of the 30 subjects in the seabuckthorn juice group failed to come to the hospital 8 weeks after intake; therefore, the blood test for this patient was treated as a missing value. Outcomes other than those of blood tests (VAS, POMS2, and SRS-18) were obtained. Table 2 shows the subjects' demographics (e.g., age).

Table 2. Participants' demographics and Baseline of POMS2, Fatigue VAS Score and SRS-18 Total Score.

	Group	N	Average	±	SD	p-value
Age	Seabuckthorn juice group	30	36.47	±	5.76	0.7326
	Placebo group	30	37.00	±	6.26	
POMS2 TMD Score	Seabuckthorn juice group	30	24.1	±	20.9	0.5624
	Placebo group	30	21.0	±	20.3	
Fatigue VAS score	Seabuckthorn juice group	30	3.83	±	2.30	0.5989
	Placebo group	30	4.18	±	2.77	
SRS-18 Total Score	Seabuckthorn juice group	30	16.7	±	12.6	0.6725
	Placebo group	30	15.3	±	12.3	

Primary Endpoint (POMS2 results)

Table 3. Results of POMS2.

	Week	Group	N	Average	±	SD	p-value
TMD Score	0W	Seabuckthorn juice group	30	24.1	±	20.9	0.5624
		Placebo group	30	21.0	±	20.3	
	4W	Seabuckthorn juice group	30	16.5	±	16.1	0.9332
		Placebo group	30	16.1	±	20.5	
	8W	Seabuckthorn juice group	30	7.4	±	13.4	0.0133
		Placebo group	30	18.1	±	18.6	
A-H	0W	Seabuckthorn juice group	30	5.5	±	3.8	0.7321
		Placebo group	30	5.1	±	4.5	
	4W	Seabuckthorn juice group	30	4.6	±	4.1	0.9241
		Placebo group	30	4.7	±	4.0	
	8W	Seabuckthorn juice group	30	4.0	±	3.2	0.3578
		Placebo group	30	4.9	±	4.5	
CB	0W	Seabuckthorn juice group	30	6.1	±	5.0	0.6172
		Placebo group	30	5.5	±	4.7	
	4W	Seabuckthorn juice group	30	4.5	±	3.4	0.7449
		Placebo group	30	4.8	±	3.7	
	8W	Seabuckthorn juice group	30	3.6	±	2.9	0.2222
		Placebo group	30	4.6	±	3.5	
DD	0W	Seabuckthorn juice group	30	4.4	±	4.7	0.7283
		Placebo group	30	3.9	±	4.9	
	4W	Seabuckthorn juice group	30	3.3	±	3.6	0.7016
		Placebo group	30	3.7	±	4.4	

	Week	Group	N	Average	±	SD	p-value
	8W	Seabuckthorn juice group	30	2.4	±	2.7	0.0813
		Placebo group	30	4.1	±	4.5	
FI	0W	Seabuckthorn juice group	30	9.9	±	10.1	0.4245
		Placebo group	30	8.3	±	4.1	
	4W	Seabuckthorn juice group	30	7.0	±	4.6	0.5521
		Placebo group	30	6.3	±	4.4	
	8W	Seabuckthorn juice group	30	4.4	±	2.8	0.0195
		Placebo group	30	6.5	±	3.9	
TA	0W	Seabuckthorn juice group	30	6.4	±	4.2	0.7382
		Placebo group	30	6.1	±	3.5	
	4W	Seabuckthorn juice group	30	5.0	±	2.7	0.4924
		Placebo group	30	5.6	±	4.2	
	8W	Seabuckthorn juice group	30	4.4	±	3.0	0.0791
		Placebo group	30	6.0	±	3.6	
VA	0W	Seabuckthorn juice group	30	6.3	±	4.0	0.1407
		Placebo group	30	7.9	±	4.5	
	4W	Seabuckthorn juice group	30	8.0	±	3.8	0.3262
		Placebo group	30	9.1	±	5.0	
	8W	Seabuckthorn juice group	30	11.5	±	4.7	0.0067
		Placebo group	30	8.1	±	4.7	
F	0W	Seabuckthorn juice group	30	9.6	±	4.6	0.3125
		Placebo group	30	10.7	±	3.7	
	4W	Seabuckthorn juice group	30	10.5	±	3.6	0.7648
		Placebo group	30	10.2	±	4.1	
	8W	Seabuckthorn juice group	30	12.3	±	4.0	0.0141
		Placebo group	30	9.7	±	3.9	

Table 3 shows the results of POMS2, which is indicated by TMD scores and subscales of Anger-Hostility (A-H), Confusion-Bewilderment (CB), Depression-Dejection (DD), Fatigue-Inertia (FI), Tension-Anxiety (TA), Vigor-Ativity (VA), and Friendliness (F). Compared to the placebo group, the seabuckthorn juice group showed significantly lower TMD and FI scores and significantly higher VA and F scores after 8 weeks of consumption.

Secondary Endpoints

Blood Test

Table 4. Result of anemia test and EPO in blood.

Item	Group	Week	N	Average	±	SD	p-value
Fe (μg／dL)	Seabuckthorn juice group	0W	30	88.4	±	36.7	0.2936
	Placebo group		30	98.3	±	35.9	
	Seabuckthorn juice group	4W	30	98.4	±	38.6	0.2959
	Placebo group		30	110.2	±	47.3	
	Seabuckthorn juice group	8W	29	92.1	±	34.5	0.7462
	Placebo group		30	95.6	±	46.9	
	Seabuckthorn juice group	Δ4W	30	10.0	±	45.3	0.8813
	Placebo group		30	11.9	±	49.3	

Item	Group	Week	N	Average	±	SD	p-value
	Seabuckthorn juice group	Δ8W	29	1.3	±	39.7	0.7128
	Placebo group		30	-2.7	±	44.1	
Ferritin (ng/mL)	Seabuckthorn juice group	0W	30	28.3	±	18.7	0.8921
	Placebo group		30	29.1	±	24.0	
	Seabuckthorn juice group	4W	30	33.3	±	20.3	0.3177
	Placebo group		30	27.9	±	21.0	
	Seabuckthorn juice group	8W	29	35.3	±	23.4	0.1558
	Placebo group		30	27.1	±	20.3	
	Seabuckthorn juice group	Δ4W	30	5.0	±	13.8	0.0478
	Placebo group		30	-1.2	±	9.2	
	Seabuckthorn juice group	Δ8W	29	6.2	±	14.5	0.0105
	Placebo group		30	-2.0	±	8.6	
Hb (g/dL)	Seabuckthorn juice group	0W	30	12.31	±	0.78	0.9034
	Placebo group		30	12.34	±	0.70	
	Seabuckthorn juice group	4W	30	12.78	±	0.96	0.2605
	Placebo group		30	12.47	±	1.18	
	Seabuckthorn juice group	8W	29	12.78	±	1.08	0.1575
	Placebo group		30	12.38	±	1.08	
	Seabuckthorn juice group	Δ4W	30	0.47	±	0.49	0.0408
	Placebo group		30	0.13	±	0.74	
	Seabuckthorn juice group	Δ8W	29	0.42	±	0.64	0.0312
	Placebo group		30	0.04	±	0.67	
EPO (erythropoietin) (mIU/mL)	Seabuckthorn juice group	0W	30	11.49	±	2.52	0.5842
	Placebo group		30	11.10	±	2.87	
	Seabuckthorn juice group	4W	30	12.15	±	2.06	0.0429
	Placebo group		30	10.88	±	2.66	
	Seabuckthorn juice group	8W	29	12.63	±	2.36	0.0208
	Placebo group		30	11.04	±	2.74	
	Seabuckthorn juice group	Δ4W	30	0.66	±	0.87	<0.0001
	Placebo group		30	-0.23	±	0.50	
	Seabuckthorn juice group	Δ8W	29	1.17	±	0.81	<0.0001
	Placebo group		30	-0.06	±	0.35	

Table 4 shows the serum iron, ferritin, and Hb levels and blood erythropoietin levels. Regarding ferritin and Hb levels, the seabuckthorn juice group showed a significant difference from the placebo group in the amount of change from baseline at 4 and 8 weeks after intake, with the seabuckthorn juice group exhibiting an increase. Additionally, blood erythropoietin levels were significantly higher in the seabuckthorn juice group than in the placebo group in the measurements taken after 4 and 8 weeks of intake, as well as in the amount of change from before intake.

Fatigue by VAS due to Kraepelin Test Load

Table 5. Fatigue VAS questionnaire results before and after Kraepelin test.

Item	Group	N	Average		SD	p-value
0W Before Kraepelin test	Seabuckthorn juice group	30	3.83	±	2.30	0.5989
	Placebo group	30	4.18	±	2.77	
0W After Kraepelin test	Seabuckthorn juice group	30	5.28	±	1.99	0.4722
	Placebo group	30	5.67	±	2.19	
0W Change in fatigue before and after Kraepelin test	Seabuckthorn juice group	30	1.45	±	1.92	0.9440
	Placebo group	30	1.50	±	2.73	
4W Before Kraepelin test	Seabuckthorn juice group	30	3.42	±	2.68	0.1285
	Placebo group	30	2.47	±	2.08	
4W After Kraepelin test	Seabuckthorn juice group	30	5.92	±	1.99	0.0297
	Placebo group	30	7.04	±	1.90	
4W Change in fatigue before and after Kraepelin test	Seabuckthorn juice group	30	2.50	±	3.06	0.0050
	Placebo group	30	4.57	±	2.40	
8W Before Kraepelin test	Seabuckthorn juice group	30	3.42	±	2.68	0.1326
	Placebo group	30	2.47	±	2.08	
8W After Kraepelin test	Seabuckthorn juice group	30	4.87	±	2.56	0.1861
	Placebo group	30	5.75	±	2.53	
8W Change in fatigue before and after Kraepelin test	Seabuckthorn juice group	30	1.45	±	2.96	0.0111
	Placebo group	30	3.27	±	2.40	

Table 5 shows the amount of change in fatigue levels from before to after the Kraepelin test using the VAS method. The seabuckthorn juice group showed significantly less fatigue than the placebo group, with significant differences in the measurements post-Kraepelin test after 4 weeks of intake and in the amount of change from pre-Kraepelin test. The seabuckthorn juice group was also significantly less fatigued than the placebo group after 8 weeks of intake in the amount of change from before the Kraepelin test.

Table 6. Psychological Index Testing with SRS-18.

Item	Week	Group	N	Average	±	SD	p-value
Total score	0W	Seabuckthorn juice group	30	16.67	±	12.63	0.6725
		Placebo group	30	15.30	±	12.28	
	4W	Seabuckthorn juice group	30	10.30	±	8.49	0.1852
		Placebo group	30	13.27	±	8.64	
	8W	Seabuckthorn juice group	30	8.47	±	6.57	0.0542
		Placebo group	30	11.80	±	6.57	
Depression/anxiety	0W	Seabuckthorn juice group	30	5.03	±	4.90	0.7526
		Placebo group	30	4.67	±	4.03	
	4W	Seabuckthorn juice group	30	2.80	±	2.82	0.1058
		Placebo group	30	4.20	±	3.72	
	8W	Seabuckthorn juice group	30	2.40	±	2.34	0.0673
		Placebo group	30	3.60	±	2.63	
Displeasure/anger	0W	Seabuckthorn juice group	30	5.03	±	3.89	0.5425
		Placebo group	30	4.43	±	3.69	
	4W	Seabuckthorn juice group	30	3.57	±	3.05	0.7420
		Placebo group	30	3.83	±	3.20	
	8W	Seabuckthorn juice group	30	2.90	±	2.64	0.3521
		Placebo group	30	3.63	±	3.37	

Item	Week	Group	N	Average	±	SD	p-value
Apathy	0W	Seabuckthorn juice group	30	6.60	±	5.18	0.7706
		Placebo group	30	6.20	±	5.40	
	4W	Seabuckthorn juice group	30	3.93	±	3.79	0.1945
		Placebo group	30	5.23	±	3.88	
	8W	Seabuckthorn juice group	30	3.17	±	2.84	0.0774
		Placebo group	30	4.57	±	3.18	

Table 6 shows the changes in psychological indices using the SRS-18, which is assessed by the total score and subscales of "depression and anxiety," "mood and anger," and "lethargy." Although there was no significant difference between the seabuckthorn juice group and the placebo group on any of the scales, a trend toward improvement was observed in the total, depression/anxiety, and apathy scores ($p<0.10$) after 8 weeks of intake.

Adverse Events: The common cold was observed in both groups; however, both were transient, and a causal relationship between the consumption of seabuckthorn juice and the placebo juice was ruled out. Consequently, based on these results, no safety concerns were raised.

EPO Production Test

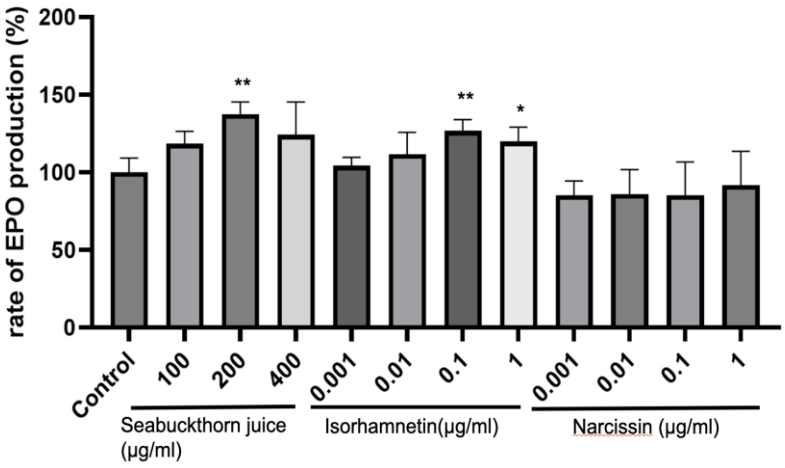


Figure 2. The amount of EPO produced per cell by the addition of the lyophilized product of seabuckthorn juice, isorhamnetin, or narcissin (isorhamnetin glycoside), mean±SD, n=3, **p<0.01, *p<0.05 compared to Control by t-test

Figure 2 shows the amount (%) of EPO produced per cell by the addition of the lyophilized product of seabuckthorn juice, isorhamnetin, or narcissin (isorhamnetin glycoside). The results showed that the addition of lyophilized seabuckthorn juice (200 μg/mL) significantly increased EPO production compared to the control. On the other hand, the addition of narcissin at any of the concentrations examined did not significantly affect EPO production in the cell supernatant compared to the control. In contrast, the addition of isorhamnetin (0.1, 1 μg/mL) significantly enhanced EPO production compared with the control.

DISCUSSION

This randomized, double-blind, placebo-controlled, parallel-group study was conducted to investigate the anti-fatigue and QOL-improving effects of seabuckthorn juice in women over an 8-week intake period. The results showed that TMD scores on the POMS2 were significantly improved, FI was significantly decreased, and VA and F were significantly increased in the juice group compared to the control group. POMS2 is a measure of mood state over the past week, including today, and is widely used as a measure of temporary mood changes in daily life. The results of

this study suggest that consumption of seabuckthorn juice may reduce temporary fatigue and (temporary) mental stress in daily life. In addition, in the assessment of fatigue by VAS after the Kraepelin test load, the seabuckthorn juice intake group was significantly less fatigued than the control group. This effect of reducing temporary fatigue due to mental workload can be transferred to work and study in daily life. The anti-fatigue effects of seabuckthorn juice have been reported in several studies. In a study, 30 Sprague-Dawley (SD) rats were randomly divided into three groups (n = 10): sedentary, training-only, and seabuckthorn juice-fed/training groups. The results showed that, compared to the training-only group, the seabuckthorn juice-fed group clearly had a longer time before fatigue, a significant increase in antioxidant enzymes in skeletal muscle, a significant decrease in malondialdehyde content in skeletal muscle, and a clear increase in testosterone and hemoglobin levels in the blood. Hemoglobin levels were clearly increased, and creatine kinase was significantly decreased, reporting that the anti-fatigue effect was associated with an increase in Hb [20]. Although it is difficult to apply the previous results directly to this study, as it measured fatigue experienced temporarily due to daily life rather than fatigue from exercise, it is thought that the effect of seabuckthorn juice on increasing Hb concentration in the blood reported so far is related to its anti-fatigue effect, especially in menstruating women [21]. Women who have menstruation, in particular, cannot avoid a decrease in Hb levels and experience various types of fatigue before and during menstruation. An umbrella review synthesizes that increasing hemoglobin (Hb) tends to improve patient-reported outcomes, notably fatigue, with larger effects at lower baseline Hb levels [22]. In postpartum anemia, a contemporary systematic review shows that intravenous iron reduces fatigue more effectively than oral iron or transfusion, and that improvements in fatigue frequently parallel recovery in Hb and ferritin, supporting a clinical coupling between hematologic correction and fatigue relief [23].In this study, the relief of the temporary fatigue effect of seabuckthorn juice was observed, with significant increases in Hb and ferritin levels compared with placebo. These effects were thought to be influenced by flavonoids, such as isorhamnetin, contained in seabuckthorn juice. In this study, we confirmed that EPO production was promoted by the addition of seabuckthorn juice in cell tests using HepG2 cells. Furthermore, it was shown that the stimulatory effect on EPO production was due to isorhamnetin. EPO is a hormone that promotes erythropoiesis in the body and is produced mainly in the kidneys, although small amounts of EPO are also made in the liver [21]. EPO binds to hematopoietic stem and progenitor cells in the bone marrow and promotes their differentiation and proliferation into red blood cells, increasing the number of red blood cells [24]. Although isorhamnetin is present in seabuckthorn juice as a glycoside, isorhamnetin glycosides are thought to be transferred into the blood as isorhamnetin through metabolic processes [25,26]. Therefore, it is thought that isorhamnetin glycosides in seabuckthorn juice are metabolized to isorhamnetin in the body, which promotes EPO production and results in an increase in Hb. Another possible mechanism of the anti-fatigue effect and temporary reduction of fatigue is that women tend to experience a decrease in Hb due to menstruation; however, Hb is produced by the promotion of EPO production, and isorhamnetin and isorhamnetin glycosides also have antioxidant and anti-inflammatory effects. From the above results, it can be inferred that the anti-fatigue effect is due to the isorhamnetin contained in seabuckthorn juice.

CONCLUSION

This placebo-controlled, randomized, double-blind, parallel-group study investigated the effects of seabuckthorn juice on the quality of life (QOL) of women. The results of the POMS2 analysis after 8 weeks of consumption of the test food showed a significant decrease in TMD scores and FI and a significant increase in VA and F. In the VAS of fatigue after 8 weeks of consumption of the test food, the seabuckthorn juice intake group showed a significant difference in the amount of change from the pre-Kraepelin test load compared to the placebo intake group, indicating a reduction in fatigue. Thus, a

temporary reduction of fatigue effect by seabuckthorn juice in women is evident. In addition, a significant difference in ferritin, Hb, and EPO levels was observed in the juice group compared to the placebo group at 4 and 8 weeks post-ingestion, with an increase in the juice group. In addition, in an EPO production promotion study using HepG2 cells, the addition of seabuckthorn juice and isorhamnetin significantly promoted EPO production compared with the control. The EPO production-promoting effect observed in this clinical study may be due to isorhamnetin and isorhamnetin glycosides. We confirmed that isorhamnetin glycosides are converted to the aglycone isorhamnetin in the body by metabolic studies of isorhamnetin glycosides (data not shown). These results suggest that consumption of seabuckthorn juice may improve blood status and increase Hb, which could be particularly beneficial for women's health.

Abbreviations: A-H, Anger-Hostility subscale; CB, Confusion-Bewilderment subscale; DD, Depression-Dejection subscale; EPO, erythropoietin; F, Friendliness subscale;FI, Fatigue-Inertia subscale; GABA, γ-aminobutyric acid; PBS, phosphate-buffered saline; QOL, quality of life; SD, Sprague-Dawley; SRS-18, psychological stress response scale; TA, Tension-Anxiety subscale; VA, Vigor-Activity subscale

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