Original article

Intake of *scrophularia buergeriana* extract (Brainon®) shows memory improvement effects in a randomized, double-blind, placebo-controlled clinical study

Sung-Kwon Lee[1], *Hae-Lim Kim*[1,2], *Seong-Hyeon Park*[1], *Bong-Keun Choi*[1], *Dong-Ryung Lee*[1], *Seung-Hwan Yang*[2]

[1]Research Institute, NUON Co., Ltd., Seongnam, Republic of Korea; [2]Department of Biotechnology, Chonnam National University, Yeosu, Republic of Korea

Abstract. *Background and aim: Scrophularia buergeriana* (SB), also known as Hyun-Sam in Korea, is a traditional medicine used to alleviate fever, swollen skin, constipation, and other health issues. The aim of this study was to evaluate the efficacy and safety of *Scrophularia buergeriana* 70% ethanol extract (Brainon®) supplementation on memory improvement in adults who complained of memory impairment. *Methods:* Eighty volunteers were recruited from Wonkwang University, Korean Medicine Hospital in Iksan, Republic of Korea. Participants were given either a daily dose of 300 mg of Brainon® or a placebo. In this study, the Korean Mini-Mental State Examination (K-MMSE), a Computerized Neurocognitive function Test (CNT), Digit Span Test, serum Brain-Derived Neurotrophic Factor (BDNF), and interleukin-6 (IL-6) were used to check effects of Brainon® on memory function in those with mild memory impairment. *Results:* Consumption of Brainon® resulted in significant improvements in immediate recall total score of the CNT-Verbal learning test, correct response and omission error items of the CNT-Visual C.P.T, backward recall of the Digit span test, and serum levels of BDNF and IL-6. Clinically significant adverse events or physical changes were not observed in this study. *Conclusions*: This clinical trial demonstrated for the first time the efficacy and safety of SB extract (Brainon®) in enhancing memory function. The results of this study indicate that consumption of Brainon® could lead to an improvement in memory without any adverse effects. Based on these research findings, Brainon® is expected to be developed as a health functional food ingredient.

Key words: *Scrophularia buergeriana*, clinical trial; memory improvement, memory impairment, health functional food

Introduction

The prevalence of memory impairment is closely related to the aging trend, which is a worldwide phenomenon due to increasing elderly population and low birth rates (1, 2). Complete prevention of memory impairment is not yet possible. The emphasis is on early intervention to delay its onset by slowing down the condition or improving symptoms (3). Alzheimer's disease (AD) involves the most severe level of memory impairment. Since it is mostly irreversible, preventing deterioration of cognitive function is crucial (3, 4).

Memory involves the acquisition, storage, and retrieval of information, facilitated by the hippocampus, amygdala, and prefrontal cortex. The hippocampus is key in forming new memories and integrating them with existing knowledge, the amygdala processes emotional memories, and the prefrontal cortex handles working memory and executive functions (5).

Brain-Derived Neurotrophic Factor (BDNF) is crucial for neuroplasticity, supporting learning and memory by promoting neuron survival and enhancing synapse strength, primarily active in the hippocampus, cortex, and basal forebrain. Higher levels of BDNF are linked to better memory and cognitive abilities, whereas lower levels are associated with neurodegenerative diseases like Alzheimer's, leading to cognitive impairments (6, 7).

Scrophularia buergeriana Miquel (SB) belonging to the Scrophulariaceae family is indigenous to Korea. It also found abundantly in China and Japan. In traditional medicine, dried SB roots have long been used to alleviate high fever, swollen skin, obstipation, pharyngitis, neuro-inflammation, and throat infection (8). SB is known as Hyun-Sam in Korea. It is traditionally used to treat fever, swelling, constipation, and age-related memory loss in Northern China (9).

Our previous study has revealed that SB extract (Brainon®) possesses a protective effect on SH-SY5Y cells (brain nerve cells) (10). Additionally, long-term administration (4 weeks) of Brainon® has a positive effect on the recovery of impaired memory in a β-amyloid-induced memory loss mouse model (11). Moreover, Brainon® administration has a positive effect by improving cognitive ability in a scopolamine-induced cognitive impairment mouse model (12). These studies have shown that Brainon® exhibits neuroprotective effects against memory loss and cognitive impairments through its antioxidant, anti-inflammatory, and anti-apoptotic activities. Brainon® inhibits the production of key inflammatory mediators such as nitric oxide (NO) and pro-inflammatory cytokines (Interleukin-6 (IL-6), IL-1β, Tumor Necrosis Factor-α (TNF- α)). This inhibition occurs through the blocking of the Toll-Like Receptor 4 (TLR4)/Myeloid Differentiation Primary Response 88 (MyD88) and Nucleotide-binding Oligomerization Domain (NOD)-like receptor family, pyrin domain containing 3 (NLRP3) pathways, which in turn suppresses the activation of Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Activator Protein-1 (AP-1) signaling pathways. Additionally, Brainon® enhances antioxidant defense mechanisms to protect neuronal cells from oxidative stress and promotes autophagy and mitophagy to remove damaged cellular components. These processes are regulated via the Mammalian Target of Rapamycin (mTOR)/AMP-activated Protein Kinase (AMPK) signaling pathways. Furthermore, the extract regulates the expression of apoptosis-related proteins such as Bcl-2-associated X protein (Bax) and B-cell lymphoma-2 (Bcl-2) and inhibits caspase activation, thereby preventing programmed cell death in neurons exposed to harmful stimuli. Through these diverse mechanisms, Brainon® demonstrates potential in managing or preventing cognitive decline associated with neurodegenerative diseases.

However, human clinical trial results regarding the effect of SB in improving memory impairment have not been reported yet.

Considering previous research indicating its memory-enhancing effects without adverse effects, Brainon® is believed to be a potential product for improving memory function. Therefore, the present study aimed to investigate the efficacy and safety of Brainon® in improving memory function in individuals aged between 20 and 65 years with memory impairment. In this study, the Korean Mini-Mental State Examination (K-MMSE), a Computerized Neurocognitive function Test (CNT), Digit Span Test, serum BDNF, and IL-6 were used to check effects of Brainon® on memory function in those with mild memory impairment in accordance with previous studies (13–15). Characteristics of our study were analyzed in terms of sample population, sample size, eligibility criteria, proportion of the population for which data were analyzed, mean age, presence of a control group, timing of assessment, lifestyle, nutritional status, and education level.

Materials and methods

Study design and population

This clinical study was a randomized, double-blind, placebo-controlled clinical trial that was designed and conducted over a duration of 12 weeks to evaluate the memory improvement effect of Brainon® use compared to that of a placebo. The clinical trial required 80 participants with memory impairment, allowing for a possible dropout rate of 20%. All participants provided signed informed consent prior to the experiment. Participants' responses were treated confidentially and anonymously. A total of 80 participants

(age, 20–65 years) were recruited from Wonkwang University, Korean Medicine Hospital in Iksan, Republic of Korea. Considering recent studies demonstrating an increase incidence of memory decline in younger adults (16), the age range for participants in this study was set from 25 to 65 years. This configuration is intended to assess the efficacy of Brainon® in enhancing memory across a broad age spectrum, from young to elderly adults.

Inclusion criteria for participants were as follows: 1) adults, both male and female, aged between 20 and 65 years old who are experiencing memory decline, 2) individuals capable of reading and understanding Korean, 3) participants with a score within the borderline range or higher on the Korean Wechsler Memory Scale - Fourth Edition (K-WMS-IV) brief cognitive status exam, and who have either an auditory memory index or a visual memory index score of 85 or below, or those scoring 1 standard deviation below the mean for their age and educational level on the Seoul Neuropsychological Screening Battery-II (SNSB-II)'s Seoul Verbal Learning Test (SVLT) or Rey Complex Figure Test (RCFT) in immediate recall, delayed recall, or recognition scores, 4) individuals who have given written informed consent to participate in this clinical trial prior to its commencement.

Exclusion criteria for participants were as follows: 1) individuals currently hospitalized or discharged within the past three months due to malignant tumors, severe cerebrovascular diseases, or severe cardiac conditions, 2) individuals with a current or past diagnosis of schizophrenia, 3) individuals currently meeting the criteria for major depressive disorder according to Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V), 4) individuals with a Center for Epidemiologic Studies Depression Scale (CES-D) score of 16 or above, indicating depression, 5) individuals with diseases associated with cognitive impairment, such as dementia, Parkinson's disease, or stroke (Dementia diagnosis based on DSM-V criteria), 6) individuals who have undergone estrogen replacement therapy (excluding topical applications) within two months prior to Visit 1, 7) individuals who have taken medications that could affect cognitive function or memory (such as antipsychotics, degenerative disease agents, cognitive enhancers, tricyclic antidepressants) within four weeks prior to Visit 1, 8) individuals who have consumed health supplements related to cognitive function or memory within two weeks prior to Visit 1, 9) individuals taking daily doses of vitamin E supplements exceeding 400 IU or where a dose reduction is anticipated, 10) individuals with high alcohol consumption or dependency, 11) individuals with thyroid disorders with TSH levels below 0.1 μU/mL or above 10 μU/mL, 12) individuals with creatinine levels more than twice the upper normal limit of the testing institution, 13) individuals with AST (GOT) or ALT (GPT) levels more than three times the upper normal limit of the testing institution, 14) individuals with uncontrolled hypertension (systolic blood pressure above 160 mmHg or diastolic blood pressure above 100 mmHg, measured after a 10-minute rest), 15) individuals with uncontrolled diabetes (fasting blood glucose levels above 180 mg/dL), 16) individuals who are pregnant, breastfeeding, or planning to become pregnant within three months, 17) individuals who have participated in any other interventional clinical trials (including human trials) within three months prior to the start of this trial or who plan to participate in other interventional clinical trials during this trial, 18) individuals deemed inappropriate for participation in this trial by the investigator.

During the intervention period, participants were advised to maintain their usual dietary patterns, physical activity levels, and dietary intake. They were asked to avoid consumption of foods containing SB. The food consumption was assessed between Visit 1 and Visit 2 (maximum interval: three weeks). In addition, participants recorded the frequency of SB-containing food intake once a week during the intervention.

This interventional study was registered with Protocol no. CNU_Brainon (Version 1.4). It was approved by the medical ethics committee of the institutional review board (IRB) of Wonkwang University, Korean Medicine Hospital (IRB No. WKUIOMH-IRB-2019-07) in accordance with the International Conference on Harmonisation of Good Clinical Practice (ICH GCP).

Randomization and blinding

Eighty eligible participants were randomly assigned to a test group or a control group. The study was double-blinded, with investigators and participants

unaware of the treatment intervention. Unique codes related to blinding were managed and sealed by the study director. The production, packaging, and labeling of the food used in the study were also controlled. Disclosure of these codes was prohibited until the end of the study except in cases of serious adverse drug reactions or other important clinical situations.

Trial nutritional supplement and placebo

Trial nutritional supplement was supplied by Nuon Co., Ltd., (Seongnam, Republic of Korea). Instructions were given to take the trial nutritional supplement 1 tablet once a day, Brainon® or control, regardless of the time of consumption or meal times. The supplement consisted of either 300 mg of standardized Brainon® (experimental group) or 300 mg of maltodextrin (placebo). In our previous *in-vivo* studies that evaluated the brain function enhancement activity of Brainon®, tests were conducted at two human equivalent daily dosages of approximately 150 mg and 500 mg (11, 12). Significant efficacy was confirmed at both concentrations. The dosage for the clinical trials was set at 300 mg, an intermediate level between the minimal effective dosage of 150 mg and the maximal effective dosage of 500 mg. This decision was based on the efficacy results from prior studies and the feasibility of developing the extract as a health supplement product.

Efficacy measurements

This study compared effects of Brainon® and a placebo on the following factors. First, it compared the ability of Brainon® to improve memory function with that of the placebo using auditory learning, visual working memory, verbal learning, and digit span tests. Second, it evaluated the efficacy and safety of Brainon® in improving memory function compared to the placebo by assessing K-MMSE results and serum levels of BDNF and IL-6.

Inclusion criteria for participants were as follows: 1) age 20–65 at the time of screening, 2) borderline or worse cognitive assessment on the Korean Wechsler Memory Scale-Fourth Edition (K-WMS-IV), with an auditory or visual memory index score of 85 or less, 3) a score on Seoul Verbal Learning Test (SVLT) or Rey Complex Figure Test (RCFT) of the Seoul Neuropsychological Screening Battery (SNSB)-II that was one standard deviation (SD) or less than the mean for age and educational level, 4) Center for Epidemiologic Studies Depression Scale (CES-D) score of less than 16, and 5) voluntary participation and compliance with study requirements.

After group assignment, participants took either Brainon® or placebo samples once a day for 12 weeks (test group: 300 mg/day of Brainon®, placebo group: 300 mg/day of maltodextrin). Subsequently, participants visited Wonkwang University, Korean Medicine Hospital every three weeks to undergo evaluations for memory improvement, vitality signs, drug administration, changes in health status, adverse reactions, and tests specified in the clinical trial protocol (Figure 1).

All participants had a blood test at baseline prior to the start of the trial. Blood samples were collected at Visits 1 and 4 to investigate effects of Brainon® on serum BDNF and IL-6 levels. BDNF and IL-6 levels were measured using a Human BDNF ELISA kit and an IL-6 Human ELISA kit, respectively.

Statistical analysis

Changes in CNT, Digit Span Test, K-MMSE, serum BDNF, and IL-6 levels before and after intake of the trial nutritional supplement were analyzed using paired t-test. The degree of change between the test group and the placebo group at each time point was evaluated for statistical significance using a two-sample *t*-test or Wilcoxon rank sum test depending on the normality. Additional statistical analyses were conducted using Generalized Linear Model (GLM) by adding age, alcohol consumption, and stress as covariates. These are potential variables that could affect memory (17–19).

Data from this clinical study were analyzed in three different forms: Safety Set, Full Analysis Set (FAS), and Per Protocol Set (PPS). Safety set analysis was conducted for participants who took the trial nutritional supplement at least once for safety evaluation. The FAS followed the intention-to-treat (ITT) principle and included all randomly assigned participants.

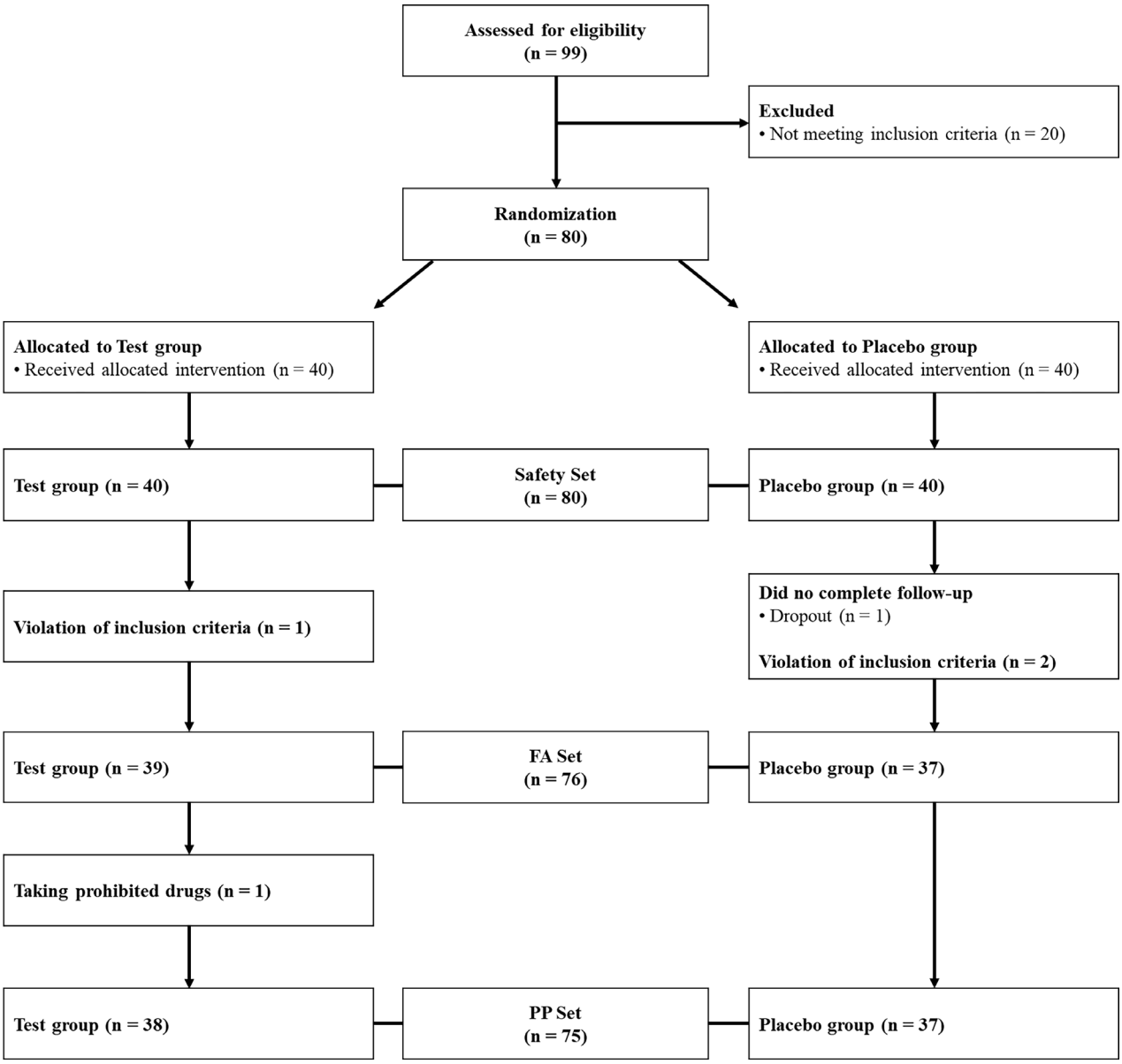

Figure 1. Demographic characteristics of participants.

The PPS analysis was conducted for a subset of the FAS who complete the trial without any major violations. Efficacy evaluation was conducted using the PPS as the main analysis group. Demographic, and nutritional data were analyzed primarily using the PPS. Safety data were analyzed using Safety Set analysis as the primary analysis. SAS® version 9.4 (SAS Institute, Cary, North Carolina, USA) was used for analysis, and statistical significance level was set at $p < 0.05$.

Results

Demographic information and compliance assessment

A total of 75 participants (38 in the Brainon® group and 37 in the placebo group) were included in the PP set. Results of the demographic information analysis are shown in Table 1. Average age of participants was 50.97 years (the Brainon® group: 53.32 years, the

Table 1. Demographic characteristics of participants.

Variables		Brainon® (n = 38)	Placebo (n = 37)	Total (n = 75)	*p*-Value
Sex n (%)	male	8 (21.05)	12 (32.43)	20 (26.67)	0.2652†
	female	30 (78.95)	25 (67.57)	55 (73.33)	
Age (years)	Mean ± SD	53.32 ± 12.22	48.57 ± 10.13	50.97 ± 11.42	0.0182&
	Min, Max	20.00, 65.00	28.00, 65.00	20.00, 65.00	
Education n (%)	• No education or elementary school dropout	0 (0.00)	0 (0.00)	0 (0.00)	0.0115‡
	• Elementary school graduation or middle school dropout	4 (10.53)	1 (2.70)	5 (6.67)	
	• Middle school graduation or high school dropout	6 (15.79)	1 (2.70)	7 (9.33)	
	• High school graduation	9 (23.68)	9 (24.32)	18 (24.00)	
	• Junior college graduation or attending in university or higher	4 (10.53)	0 (0.00)	4 (5.33)	
	• University graduation or attending in graduate school or higher	15 (39.47)	26 (70.27)	41 (54.67)	
Smoking history n (%)	Non-Smoker	33 (86.84)	9 (24.32)	18 (24.00)	1.0000‡
	Ex-Smoker (smoking cessation ≥ 1year)	4 (10.53)	0 (0.00)	4 (5.33)	
	Smoker	15 (39.47)	26 (70.27)	41 (54.67)	
Cigarette consumption (Cigarette / day)	Mean ± SD	15.00 ± 5.00	13.40 ± 4.22	14.20 ± 4.44	0.5994*
	Min, Max	10.00, 20.00	10.00, 20.00	10.00, 20.00	
Smoking duration (year)	Mean ± SD	24.00 ± 8.94	20.00 ± 11.73	22.00 ± 10.06	0.5762&
	Min, Max	10.00, 30.00	5.00, 30.00	5.00, 30.00	
Alcohol use n (%)	No	25 (65.79)	19 (51.35)	44 (58.67)	0.0820‡
	Stop	0 (0.00)	0 (0.00)	0 (0.00)	
	< 1 bottle / week	10 (26.32)	7 (18.92)	17 (22.67)	
	1 ~ 3 bottles / week	3 (7.89)	10 (27.03)	13 (17.33)	
	≥ 4 bottles / week	0 (0.00)	1 (2.70)	1 (1.33)	
Physical activity	No	13 (34.21)	12 (32.43)	25 (33.33)	0.8921†
	1 ~ 2 times / week	7 (18.42)	8 (21.62)	15 (20.00)	
	3 ~ 4 times / week	10 (26.32)	9 (24.32)	19 (25.33)	
	5 ~ 6 times / week	6 (15.79)	4 (10.81)	10 (13.33)	
	Everyday	2 (5.26)	4 (10.81)	6 (8.00)	
Perceived stress assessment	No	4 (10.53)	1 (2.70)	5 (6.67)	0.7455‡
	Low	27 (71.05)	28 (75.68)	55 (73.33)	
	High	6 (15.79)	7 (18.92)	13 (17.33)	
	Very high	1 (2.63)	1 (2.70)	2 (2.67)	
Height (cm)	Mean ± SD	159.62 ± 8.32	162.75 ± 8.72	161.17 ± 8.61	0.1159*
	Min, Max	147.30, 178.10	147.70, 183.60	147.30, 183.60	

Values are presented as mean ± standard deviation (SD). *: *p*-value for Two sample *t*-test. &: *p*-value for Wilcoxon rank sum test. †: *p*-value for Chi-square test. ‡: *p*-value for Fisher's exact test.

placebo group: 48.57 years), showing statistically significant difference between the two groups.

In the Brainon® group and the control group, education levels were distributed as follows: elementary school dropout or no education (0.00% vs 0.00%), elementary school complete or middle school dropout (10.53% vs 2.70%), middle school complete or high school dropout (15.79% vs 24.32%), high school graduate (23.68% vs 24.32%), junior college graduate or currently attending university (10.53% vs 0.00%), and university graduate or currently attending graduate school (39.47% vs 70.27%).

Variables showing significant differences between the two groups based on demographic information and other pre-intake characteristics were analyzed by adjusting for validity evaluation. There was no statistically significant difference in gender, smoking status, alcohol consumption, physical activity, perceived stress evaluation, or height between the two groups. At Visit 4, a survey was conducted regarding smoking status, alcohol consumption, physical activity, and perceived stress evaluation. Results showed no statistically significant difference between the two groups. In the analysis of compliance with intake, the overall product compliance was 94.53% in the Brainon® group and 97.62% in the placebo group, showing no statistically significant difference between the two groups (Table 2).

CNT-verbal learning test

Table 3 presents results of CNT-Verbal Learning Test. After 12 weeks of intervention, in the analysis of total score changes of immediate recall (A1~A5), the Brainon® group and the placebo group showed statistically significant increases of 7.13 points and 3.78 points compared to the baseline, respectively (Figure 2). There was a statistically significant difference between the two groups. In the immediate recall of A1 score, the Brainon® group and control group had a significant increase, showing no statistically significant difference between the two groups. In the analysis of the immediate recall of A5, the Brainon® group showed a significant increase of 1.63 points, while the placebo group showed an increase of 1.05 points (no statistically significant difference between the two groups). In the analysis of delayed recall changes, the Brainon® group showed an increase of 2.18 points, while the placebo group showed an increase of 1.30 points (no statistically significant difference between the two groups). In the analysis of delayed recognition, the Brainon® group showed an increase of 1.21 points and the placebo group showed an increase of 0.62 points (no statistically significant difference observed between the two groups).

CNT-auditory C.P.T

Table 4 presents results of the analysis of changes in CNT-Auditory C.P.T measured at Visit 1 and Visit 4. After 12 weeks of intervention, correct responses increased by 10.84 times in the Brainon® group and by 4.97 times in the placebo group (Fig. 2). Omission errors decreased by 10.84 times in the Brainon® group and by 4.97 times in the placebo group. Commission errors decreased by 7.97 times in the Brainon® group and by 3.43 times in the placebo group. Response time for correct responses decreased by 0.02 seconds in the

Table 2. Assessment of participants compliance.

	Brainon® group (n = 38)		**Placebo group (n = 37)**		
	n	**Mean ± SD**	**n**	**Mean ± SD**	***p*-Value**
Visit 3	38	92.24 ± 9.85	37	93.45 ± 10.69	0.9492[&]
Visit 4	38	96.82 ± 12.37	36	102.30 ± 15.64	0.1694[&]
Total	38	94.53 ± 8.53	36	97.62 ± 8.66	0.1267[*]

Values are presented as mean ± standard deviation (SD). [*]: Compared between groups; *p*-value for Two sample *t*-test. [&]: Compared between groups; *p*-value for Wilcoxon rank sum test. [a]: Participant who lost the investigational product was excluded from the total analysis for the 12-week human trial.

Table 3. Changes in CNT-Verbal Learning Test by visit.

CNT – Verbal Learning Test		Brainon® group (n = 38)		Placebo group (n = 37)		*p*-Value	*p*-Value[$]
		n	Mean ± SD	n	Mean ± SD		
Immediate recall A1~A5 Total score (point)	Baseline (Visit 2)	38	43.97 ± 8.00	37	48.00 ± 7.81	0.0306[*]	
	Visit 4	38	51.11 ± 8.65	37	51.78 ± 8.52		
	Change from baseline	38	7.13 ± 5.87	37	3.78 ± 7.33	0.0320[*]	0.0195
	p-value[**]		< 0.0001		0.0034		
Immediate recall A1 (point)	Baseline (Visit 2)	38	6.03 ± 1.50	37	6.22 ± 1.67	0.7868[&]	
	Visit 4	38	7.18 ± 1.57	37	7.00 ± 1.78		
	Change from baseline	38	1.16 ± 1.81	37	0.78 ± 2.11	0.4120[*]	0.3214
	p-value[**]		0.0003		0.0300		
Immediate recall A5 (point)	Baseline (Visit 2)	38	10.58 ± 2.24	37	11.78 ± 1.69	0.0104[*]	
	Visit 4	38	12.21 ± 2.32	37	12.84 ± 1.95		
	Change from baseline	38	1.63 ± 1.73	37	1.05 ± 1.91	0.1745[*]	0.2219
	p-value[**]		< 0.0001		0.0019		
Delayed recall (point)	Baseline (Visit 2)	38	8.89 ± 2.64	37	10.88 ± 2.49	0.0776[*]	
	Visit 4	38	11.08 ± 2.66	37	11.57 ± 2.68		
	Change from baseline	38	2.18 ± 2.04	37	1.30 ± 2.07	0.0653[*]	0.3261
	p-value[**]		< 0.0001		0.0005		
Delayed recognition (point)	Baseline (Visit 2)	38	12.50 ± 1.98	37	13.27 ± 1.57	0.7589[&]	
	Visit 4	38	13.71 ± 1.16	37	13.89 ± 1.58		
	Change from baseline	38	1.21 ± 1.79	37	0.62 ± 1.26	0.2622[&]	0.3198
	p-value[**]		< 0.0001		0.0050		

Values are presented as mean ± standard deviation (SD). [*]: Compared between groups; *p*-value for Two sample *t*-test. [&]: Compared between groups; *p*-value for Wilcoxon rank sum test. [$]: Compared between groups; *p*-value for GLM adjusted by baseline, age, drink, stress, education, plant protein, dietary, ash, Ca, plant Ca, animal Ca, P, Na, potassium, plant Fe, smoke, exercise. [**]: Compared within group; *p*-value for Paired *t*-test. [#]: Compared within group; *p*-value for Wilcoxon Signed rank test.

Brainon® group and by 0.02 seconds in the placebo group. Additionally, standard deviation of response time decreased by 0.01 seconds in the Brainon® group and by 0.01 seconds in the placebo group. There was no statistically significant difference between Brainon® and placebo groups in any of these CNT-Auditory C.P.T measures.

CNT-visual C.P.T

Changes in CNT-Visual C.P.T measured at Visit 1 and Visit 4 are shown in Table 5. After 12 weeks of intervention, correct responses increased by 1.47 times in the Brainon® group, which were statistically significant compared to those at baseline, but not significantly different from those in the placebo group. Omission errors decreased by 1.47 times in the Brainon® group, which were statistically significant compared to those at baseline, but not significantly different from those in the placebo group (Figure 2). Commission errors decreased by 0.42 times in the Brainon® group and by 0.19 times in the placebo group. Response time for correct responses decreased by 0.01 seconds in the Brainon® group and by 0.00 seconds in the placebo group. Similarly, standard deviation of response time decreased by 0.01 seconds in the Brainon® group and by 0.00 seconds in the placebo group. However, there was no statistically significant difference between

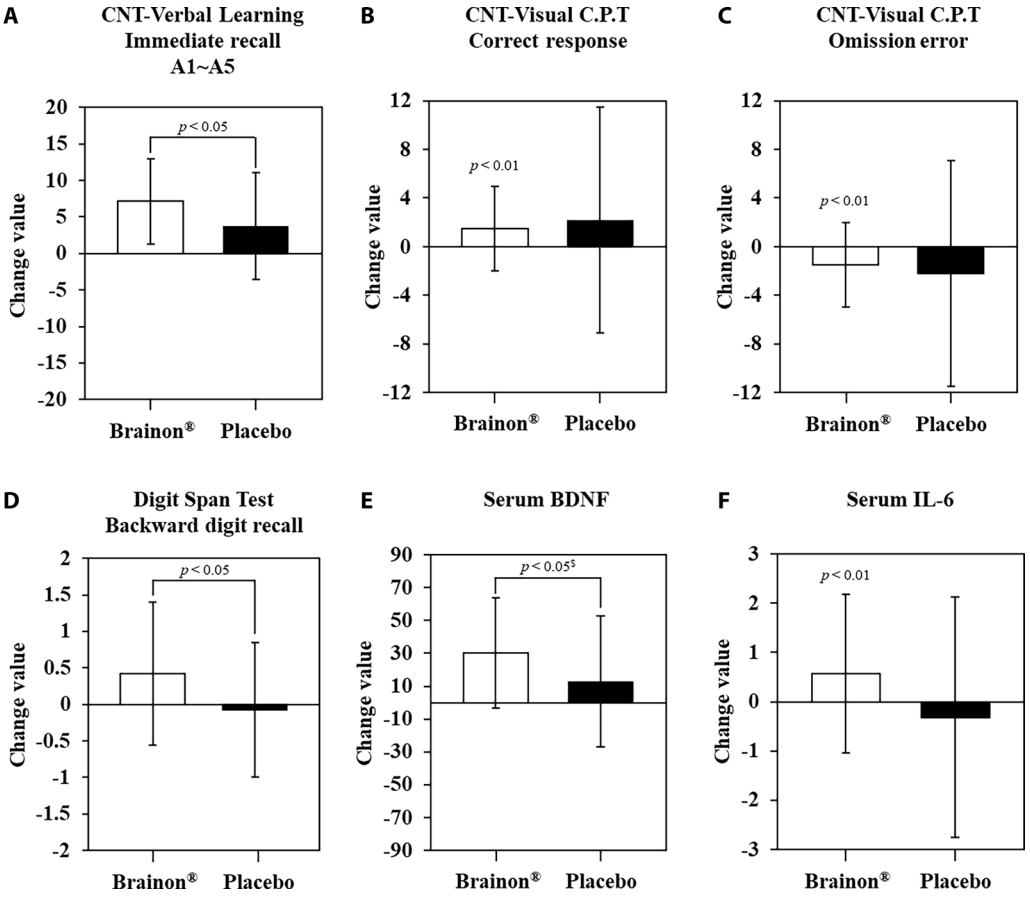

Figure 2. Changes of values in (A) CNT-Verbal learning test immediate recall A1~A5, (B) CNT-Visual C.P.T correct response, (C) CNT-Visual C.P.T omission error, (D) Digit span test backward digit recall, (E) serum BDNF levels, and (F) serum IL-6 levels of participants. There were significant differences in changes for the verbal learning test and digit span test between Brainon® and placebo groups at Visit 4 ($p < 0.05$). Changes in serum BDNF levels showed statistically significant differences between the experimental group and the placebo group based on covariate-adjusted analysis results ($p < 0.05^{\$}$). The Brainon® group showed significant changes in CNT-visual C.P.T correct responses, omission errors, and serum IL-6 levels at Visit 4 compared to baseline (all $p < 0.01$).

the two groups in any of these CNT-Visual C.P.T measures.

Digit span test

Immediate digit recall score increased by 0.26 points in the Brainon® group and by 0.11 points in the placebo group at Visit 4 (Table 6). However, there was no statistically significant difference between the two groups. Backward digit recall score increased by 0.42 points in the Brainon® group and decreased by 0.08 points in the placebo group, showing a statistically significant difference between the two groups after 12 weeks of intervention (Figure 2).

K-MMSE

After 12 weeks of intervention, the total K-MMSE score increased by 0.13 points in the Brainon® group and by 0.65 points in the placebo group (Table 7). However, there was no statistically significant difference between the two groups. The score for time

Table 4. Changes in CNT-Auditory C.P.T by visit.

CNT – Auditory C.P.T		Brainon® group (n = 38)		Placebo group (n = 37)		*p*-Value[&]	*p*-Value[$]
		n	Mean ± SD	n	Mean ± SD		
Correct response (count)	Baseline (Visit 2)	38	119.18 ± 21.97	37	123.86 ± 11.90	0.6556	
	Visit 4	38	130.03 ± 4.32	37	128.84 ± 7.13	0.3341	0.2046
	Change from baseline	38	10.84 ± 20.63	37	4.97 ± 12.72		
	p-value[**]		< 0.0001		0.0114		
Omission error (count)	Baseline (Visit 2)	38	15.82 ± 21.97	37	11.14 ± 11.90	0.6556	
	Visit 4	38	4.97 ± 4.32	37	6.16 ± 7.13	0.3341	0.2046
	Change from baseline	38	-10.84 ± 20.63	37	-4.97 ± 12.72		
	p-value[**]		< 0.0001		0.0114		
Commission error (count)	Baseline (Visit 2)	38	13.53 ± 16.79	37	9.38 ± 8.75	0.3898	
	Visit 4	38	5.55 ± 3.70	37	5.95 ± 5.52	0.2881	0.1634
	Change from baseline	38	-7.97 ± 15.99	37	-3.43 ± 9.12		
	p-value[**]		< 0.0001		0.0110		
Response time for correct response (second)	Baseline (Visit 2)	38	0.69 ± 0.05	37	0.68 ± 0.04	0.4057	
	Visit 4	38	0.67 ± 0.05	37	0.66 ± 0.04	0.9365	0.2383
	Change from baseline	38	-0.02 ± 0.05	37	-0.02 ± 0.04		
	p-value[**]		0.0125[#]		0.0211[**]		
Standard deviation of response time (second)	Baseline (Visit 2)	38	0.09 ± 0.02	37	0.09 ± 0.02	0.8716	
	Visit 4	38	0.08 ± 0.02	37	0.08 ± 0.02	0.4988	0.5066
	Change from baseline	38	-0.01 ± 0.02	37	-0.01 ± 0.02		
	p-value[**]		< 0.0001		0.0043		

Values are presented as mean ± standard deviation (SD). [&]: Compared between groups; *p*-value for Wilcoxon rank sum test. [$]: Compared between groups; *p*-value for GLM adjusted by baseline, age, drink, stress, education, plant protein, dietary, ash, Ca, plant Ca, animal Ca, P, Na, potassium, plant Fe, smoke, exercise. [**]: Compared within group; *p*-value for Paired *t*-test. [#]: Compared within group; *p*-value for Wilcoxon Signed rank test.

orientation increased by 0.00 points in the Brainon® group and by 0.05 points in the placebo group. The score for attention and calculation increased by 0.00 points in the Brainon® group and by 0.38 points in the placebo group. The score for recall increased by 0.13 points in the Brainon® group and by 0.22 points in the placebo group. For Place orientation, Registration, Language and Visual construction components of the K-MMSE test, no significant change was observed in the Brainon® group or the placebo group.

Serum BDNF and IL-6 levels

After 12 weeks of intervention, serum BDNF levels increased by 30.22 pg/mL in the Brainon® group and by 13.07 pg/mL in the placebo group (Table 8). After adjusting for age, alcohol consumption, stress level, education level, nutritional analysis, smoking status, and physical activity status as covariates, a GLM analysis revealed a statistically significant difference between the two groups in terms of the change in BDNF (Figure 2). Serum IL-6 levels increased by 0.57 pg/mL

Table 5. Changes in CNT-Visual C.P.T by visit.

CNT – Visual C.P.T		Brainon® group (n = 38)		Placebo group (n = 37)		*p*-Value[&]	*p*-Value[$]
		n	Mean ± SD	n	Mean ± SD		
Correct response (count)	Baseline (Visit 2)	38	132.34 ± 4.72	37	131.89 ± 9.06	0.7648	
	Visit 4	38	133.82 ± 2.79	37	134.08 ± 1.71	0.4950	0.5879
	Change from baseline	38	1.47 ± 3.47	37	2.19 ± 9.26		
	p-value[**]		0.0081		0.1985		
Omission error (count)	Baseline (Visit 2)	38	2.66 ± 4.72	37	3.11 ± 9.06	0.7648	
	Visit 4	38	1.18 ± 2.79	37	0.92 ± 1.71	0.4950	0.5879
	Change from baseline	38	-1.47 ± 3.47	37	-2.19 ± 9.26		
	p-value[**]		0.0081		0.1985		
Commission error (count)	Baseline (Visit 2)	38	1.47 ± 2.38	37	1.54 ± 1.56	0.2042	
	Visit 4	38	1.05 ± 1.43	37	1.35 ± 1.51	0.9956	0.7585
	Change from baseline	38	-0.42 ± 2.33	37	-0.19 ± 1.98		
	p-value[**]		0.5955		0.4790		
Response time for correct response (second)	Baseline (Visit 2)	38	0.46 ± 0.07	37	0.44 ± 0.05	0.6248	
	Visit 4	38	0.45 ± 0.05	37	0.44 ± 0.03	0.9746	0.9029
	Change from baseline	38	-0.01 ± 0.07	37	-0.00 ± 0.04		
	p-value[**]		0.8157		0.9923		
Standard deviation of response time (second)	Baseline (Visit 2)	38	0.06 ± 0.03	37	0.06 ± 0.02	0.8928	
	Visit 4	38	0.05 ± 0.01	37	0.05 ± 0.02	0.6675	0.4713
	Change from baseline	38	-0.01 ± 0.02	37	-0.00 ± 0.02		
	p-value[**]		0.1546		0.4566		

Values are presented as mean ± standard deviation (SD). [&]: Compared between groups; *p*-value for Wilcoxon rank sum test. [$]: Compared between groups; *p*-value for GLM adjusted by baseline, age, drink, stress, education, plant protein, dietary, ash, Ca, plant Ca, animal Ca, P, Na, potassium, plant Fe, smoke, exercise. [#]: Compared within group; *p*-value for Wilcoxon Signed rank test.

Table 6. Changes in Digit Span Test by visit.

Digit Span Test		Brainon® group (n = 38)		Placebo group (n = 37)		*p*-Value[&]	*p*-Value[$]
		n	Mean ± SD	n	Mean ± SD		
Immediate digit recall (point)	Baseline (Visit 2)	38	6.34 ± 1.19	37	7.00 ± 1.13	0.0152	
	Visit 4	38	6.61 ± 1.31	37	7.11 ± 0.99	0.4588	0.7317
	Change from baseline	38	0.26 ± 0.79	37	0.11 ± 0.81		
	p-value[**]		0.0579		0.5065		
Backward digit recall (point)	Baseline (Visit 2)	38	4.66 ± 1.21	37	5.46 ± 1.17	0.0066	
	Visit 4	38	5.08 ± 1.24	37	5.38 ± 1.11	0.0139	0.4904
	Change from baseline	38	0.42 ± 0.98	37	-0.08 ± 0.92		
	p-value[**]		0.0109		0.7232		

Values are presented as mean ± standard deviation (SD). [&]: Compared between groups; *p*-value for Wilcoxon rank sum test. [$]: Compared between groups; *p*-value for GLM adjusted by baseline, age, drink, stress, education, plant protein, dietary, ash, Ca, plant Ca, animal Ca, P, Na, potassium, plant Fe, smoke, exercise. [#]: Compared within group; *p*-value for Wilcoxon Signed rank test.

Table 7. Changes in K-MMSE by visit.

K-MMSE		Brainon® group (n = 38)		Placebo group (n = 37)		*p*-Value[&]	*p*-Value[$]
		n	Mean ± SD	n	Mean ± SD		
Total score (point)	Baseline (Visit 2)	38	28.61 ± 1.26	37	28.32 ± 1.47	0.4103	
	Visit 4	38	28.74 ± 1.18	37	28.97 ± 0.76		
	Change from baseline	38	0.13 ± 1.36	37	0.65 ± 1.69	0.1991	0.9193
	p-value[**]		0.5497		0.0298		
Time orientation (point)	Baseline (Visit 2)	38	4.95 ± 0.23	37	4.95 ± 0.23	0.9891	
	Visit 4	38	4.95 ± 0.23	37	5.00 ± 0.00		
	Change from baseline	38	0.00 ± 0.33	37	0.05 ± 0.23	0.4238	0.8360
	p-value[**]		1.0000		0.5000		
Place orientation (point)	Baseline (Visit 2)	38	5.00 ± 0.00	37	5.00 ± 0.00	1.0000	
	Visit 4	38	5.00 ± 0.00	37	5.00 ± 0.00		
	Change from baseline	38	0.00 ± 0.00	37	0.00 ± 0.00	1.0000	-
	p-value[**]		-		-		
Registration (point)	Baseline (Visit 2)	38	3.00 ± 0.00	37	3.00 ± 0.00	1.0000	
	Visit 4	38	3.00 ± 0.00	37	3.00 ± 0.00		
	Change from baseline	38	0.00 ± 0.00	37	0.00 ± 0.00	1.0000	-
	p-value[**]		-		-		
Attention and calculation (point)	Baseline (Visit 2)	38	4.74 ± 0.60	37	4.43 ± 0.99	0.1892	
	Visit 4	38	4.74 ± 0.50	37	4.81 ± 0.52		
	Change from baseline	38	0.00 ± 0.66	37	0.38 ± 1.14	0.1623	0.9065
	p-value[**]		1.0000		0.0620		
Recall (point)	Baseline (Visit 2)	38	1.92 ± 0.88	37	1.95 ± 0.88	0.8975	
	Visit 4	38	2.05 ± 1.04	37	2.16 ± 0.69		
	Change from baseline	38	0.13 ± 1.09	37	0.22 ± 1.18	0.8520	0.9472
	p-value[**]		0.4842		0.2884		
Language and visual construction (point)	Baseline (Visit 2)	38	9.00 ± 0.00	37	9.00 ± 0.00	1.0000	
	Visit 4	38	9.00 ± 0.00	37	9.00 ± 0.00		
	Change from baseline	38	0.00 ± 0.00	37	0.00 ± 0.00	1.0000	-
	p-value[**]		-		-		

Values are presented as mean ± standard deviation (SD). [&]: Compared between groups; *p*-value for Wilcoxon rank sum test. [$]: Compared between groups; *p*-value for GLM adjusted by baseline, age, drink, stress, education, plant protein, dietary, ash, Ca, plant Ca, animal Ca, P, Na, potassium, plant Fe, smoke, exercise. [#]: Compared within group; *p*-value for Wilcoxon Signed rank test.

in the Brainon® group but decreased by -0.32 pg/mL in the placebo group (Figure 2). There was a statistically significant difference between the two groups.

Safety issues and adverse events

In terms of the severity of adverse events that occurred during this clinical study, there were 5 mild and 7 moderate events in the Brainon® group. Regarding the relation to the investigational food, 11 cases were judged to be 'not considered relevant' and one case was judged to be 'possibly relevant'. Participants with adverse events that could not be excluded as related to the investigational food were confirmed to have completely recovered the next day without corrective treatment. No serious adverse events occurred during the

Table 8. Changes in serum BDNF and IL-6 by visit.

		Brainon® group (n = 38)		Placebo group (n = 37)			
		n	Mean ± SD	n	Mean ± SD	*p*-Value	*p*-Value[$]
BDNF	Baseline (Visit 2)	38	1019.18 ± 373.89	37	960.28 ± 368.42	0.4942[*]	
	Visit 4	38	1049.41 ± 374.35	37	973.35 ± 378.07		
	Change from baseline	38	30.22 ± 33.78	37	13.07 ± 39.88	0.1407[&]	0.0410
	p-value[**]		< 0.0001[**]		0.0448[#]		
IL-6	Baseline (Visit 2)	38	1.97 ± 2.18	37	2.82 ± 3.95	0.2727[&]	
	Visit 4	38	2.53 ± 3.08	37	2.50 ± 3.76		
	Change from baseline	38	0.57 ± 1.61	37	-0.32 ± 2.44	0.0644[&]	0.1231
	p-value[**]		0.0090		0.9941		

Values are presented as mean ± standard deviation (SD). [*]: Compared between groups; *p*-value for Two sample *t*-test. [&]: Compared between groups; *p*-value for Wilcoxon rank sum test. [$]: Compared between groups; *p*-value for GLM adjusted by baseline, age, drink, stress, education, plant protein, dietary, ash, Ca, plant Ca, animal Ca, P, Na, potassium, plant Fe, smoke, exercise. [**]: Compared within group; *p*-value for Paired *t*-test. [#]: Compared within group; *p*-value for Wilcoxon Signed rank test.

Table 9. Adverse events among the participants.

		Brainon® group (n = 40)		Placebo group (n =40)		Total (n = 80)		
		n	Ratio (%)	n	Ratio (%)	n	Ratio (%)	*p*-value[‡]
Symptom severity	Mild	5	41.67	0	0.00	5	41.67	-
	Moderate	7	58.33	0	0.00	7	58.33	
	Severe	0	0.00	0	0.00	0	0.00	
Relevance to Investigational Foods	Clearly relevant	0	0.00	0	0.00	0	0.00	-
	Considered relevant	0	0.00	0	0.00	0	0.00	
	Possibly relevant	1	8.33	0	0.00	1	8.33	
	Not considered relevant	11	91.67	0	0.00	11	91.67	
	Not clearly relevant	0	0.00	0	0.00	0	0.00	
	Unknown	0	0.00	0	0.00	0	0.00	

Values are presented as mean ± standard deviation (SD). [‡]: *p*-value for Fisher's exact test.

study period (Table 9). There were no dropouts due to adverse events.

For safety evaluation in this human clinical trial, clinical laboratory tests including hematology and blood chemistry were conducted at Visit 1 and Visit 4. At visit 4, the two groups showed no statistically significant differences in any items of hematology and blood chemistry (Table 10). Additionally, there were no statistically significant differences in vital signs (pulse, blood pressure) or body weight at Visit 3 or Visit 4 between the two groups (Table 11).

Discussion

Previous efficacy studies using cell and animal models have reported that SB has protective effects on brain nerve cells, ability to inhibit memory loss, and capability of recovering impaired memory (10–12). In this human clinical trial, we evaluated the efficacy and safety of 12 weeks of Brainon® (SB extract) supplementation on memory improvement in adult men and women who complained of memory impairment.

Table 10. Changes in the diagnostic medical test before and after 12 weeks of Brainon® product consumption.

		Brainon® group (n = 40)		Placebo group (n = 40)		
		n	Mean ± SD	n	Mean ± SD	*p*-Value
WBC (10^3/μL)	Baseline (Visit 1)	40	5.57 ± 1.75	40	5.42 ± 1.41	0.9309[&]
	Visit 4	40	5.63 ± 1.68	39	5.46 ± 1.44	
	Change from baseline	40	0.06 ± 1.31	39	0.08 ± 1.42	0.9432[*]
	p-value[**]		0.7832		0.7290	
RBC (10^3/μL)	Baseline (Visit 1)	40	4.49 ± 0.42	40	4.40 ± 0.40	0.6100[&]
	Visit 4	40	4.49 ± 0.36	39	4.40 ± 0.43	
	Change from baseline	40	0.01 ± 0.23	39	0.01 ± 0.22	0.9905[*]
	p-value[**]		0.8659		0.8735	
Hb (g/dL)	Baseline (Visit 1)	40	13.73±1.26	40	13.57±1.52	0.6031[&]
	Visit 4	40	13.51 ± 1.23	39	13.49 ± 1.36	
	Change from baseline	40	-0.22 ± 0.86	39	-0.05 ± 0.96	0.7948[&]
	p-value[**]		0.1647		0.2663	
Hct (%)	Baseline (Visit 1)	40	40.87 ± 3.32	40	40.30 ± 4.04	0.8398[&]
	Visit 4	40	40.75 ± 3.43	39	40.49 ± 3.72	
	Change from baseline	40	-0.12 ± 2.42	39	0.26 ± 2.58	0.6840[&]
	p-value[**]		0.9237		0.6559	
Platelet (10^3/μL)	Baseline (Visit 1)	40	240.55 ± 61.65	40	249.55 ± 55.46	0.4945[*]
	Visit 4	40	239.65 ± 54.15	39	241.26 ± 56.74	
	Change from baseline	40	-0.90 ± 31.18	39	-7.92 ± 26.15	0.2821[*]
	p-value[**]		0.8561		0.0662	
Neutrophol (%)	Baseline (Visit 1)	40	52.00 ± 8.29	40	54.27 ± 8.60	0.2330[*]
	Visit 4	40	52.95 ± 9.54	39	53.60 ± 9.25	
	Change from baseline	40	0.96 ± 7.28	39	-0.48 ± 10.33	0.4775[*]
	p-value[**]		0.4117		0.7712	
Lymphocyte (%)	Baseline (Visit 1)	40	38.44 ± 8.19	40	35.62 ± 8.86	0.1430[*]
	Visit 4	40	37.32 ± 8.92	39	36.56 ± 8.22	
	Change from baseline	40	-1.12 ± 5.93	39	0.69 ± 9.93	0.3300[*]
	p-value[**]		0.2381		0.6669	
Monocyte (%)	Baseline (Visit 1)	40	6.46 ± 1.34	40	6.23 ± 1.74	0.5143[*]
	Visit 4	40	6.85 ± 2.71	39	6.25 ± 1.76	
	Change from baseline	40	0.39 ± 2.18	39	0.05 ± 1.63	0.8599[&]
	p-value[**]		0.6250[#]		0.8526[**]	
Eosinophil (%)	Baseline (Visit 1)	40	2.40 ± 2.32	40	3.27 ± 4.28	0.3029[&]
	Visit 4	40	2.16 ± 2.18	39	2.88 ± 2.88	
	Change from baseline	40	-0.24 ± 1.26	39	-0.36 ± 2.70	0.7240[&]
	p-value[**]		0.1777		0.5484	
Basophil (%)	Baseline (Visit 1)	40	0.71 ± 0.30	40	0.62 ± 0.30	0.2140[&]
	Visit 4	40	0.72 ± 0.36	39	0.72 ± 0.46	
	Change from baseline	40	0.02 ± 0.38	39	0.10 ± 0.47	0.4188[&]
	p-value[**]		0.9599		0.1945	

		Brainon® group (n = 40)		Placebo group (n = 40)		
		n	Mean ± SD	n	Mean ± SD	*p*-Value
Glucose (mg/dL)	Baseline (Visit 1)	40	88.98 ± 13.16	40	93.20 ± 15.56	0.0910[&]
	Visit 4	40	87.48 ± 13.90	39	91.18 ± 14.64	
	Change from baseline	40	-1.50 ± 8.64	39	-1.79 ± 10.34	0.8908[*]
	p-value[**]		0.2788		0.2852	
Uric acid (mg/dL)	Baseline (Visit 1)	40	4.90 ± 1.41	40	4.77 ± 1.37	0.8928[&]
	Visit 4	40	4.72 ± 1.30	39	4.72 ± 1.49	
	Change from baseline	40	-0.18 ± 0.69	39	-0.03 ± 0.65	0.7020[&]
	p-value[**]		0.2390[#]		0.7517[**]	
Total protein (g/dL)	Baseline (Visit 1)	40	6.94 ± 0.40	40	7.08±0.34	0.1023[&]
	Visit 4	40	6.99 ± 0.37	39	7.01 ± 0.38	
	Change from baseline	40	0.04 ± 0.39	39	-0.07 ± 0.40	0.1961[*]
	p-value[**]		0.4746		0.2716	
Albumin (g/dL)	Baseline (Visit 1)	40	4.28 ± 0.22	40	4.27 ± 0.22	0.9961[&]
	Visit 4	40	4.38 ± 0.27	39	4.33 ± 0.19	
	Change from baseline	40	0.10 ± 0.24	39	0.06 ± 0.23	0.4971[*]
	p-value[**]		0.0126		0.0837	
Total bilirubin (mg/dL)	Baseline (Visit 1)	40	0.73 ± 0.22	40	0.75 ± 0.31	0.9149[&]
	Visit 4	40	0.70 ± 0.18	39	0.79 ± 0.31	
	Change from baseline	40	-0.02 ± 0.24	39	0.06 ± 0.25	0.1503[*]
	p-value[**]		0.5498		0.1617	
AST (GOT) (IU/L)	Baseline (Visit 1)	40	29.58 ± 13.42	40	24.93 ± 6.74	0.2360[&]
	Visit 4	40	27.00 ± 10.48	39	25.08 ± 8.70	
	Change from baseline	40	-2.58 ± 11.93	39	0.28 ± 10.44	0.4174[&]
	p-value[**]		0.1000		0.5782	
ALT (GPT) (IU/L)	Baseline (Visit 1)	40	27.83 ± 21.19	40	21.38 ± 11.92	0.3141[&]
	Visit 4	40	23.90 ± 14.62	39	21.00 ± 10.14	
	Change from baseline	40	-3.93 ± 13.82	39	-0.15 ± 10.72	0.3634[&]
	p-value[**]		0.2071		0.8896	
BUN (mg/dL)	Baseline (Visit 1)	40	13.61 ± 3.67	40	13.09 ± 3.41	0.5114[*]
	Visit 4	40	14.25 ± 3.41	39	14.12 ± 3.48	
	Change from baseline	40	0.64 ± 3.80	39	1.03 ± 3.90	0.7686[&]
	p-value[**]		0.2931[**]		0.0856[#]	
Creatinine (mg/dL)	Baseline (Visit 1)	40	0.81 ± 0.15	40	0.87 ± 0.15	0.0545[&]
	Visit 4	40	0.84 ± 0.15	39	0.87 ± 0.16	
	Change from baseline	40	0.03 ± 0.07	39	0.01 ± 0.08	0.4445[&]
	p-value[**]		0.1222		0.5862	
Total cholesterol (mg/dL)	Baseline (Visit 1)	40	200.40 ± 43.13	40	192.08 ± 37.51	0.3598[*]
	Visit 4	40	208.15 ± 41.37	39	196.56 ± 32.51	
	Change from baseline	40	7.75 ± 29.59	39	4.41 ± 23.83	0.5828[*]
	p-value[**]		0.1057		0.2550	

Values are presented as mean ± standard deviation (SD). [*]: Compared between groups; *p*-value for Two sample *t*-test. [&]: Compared between groups; *p*-value for Wilcoxon rank sum test. [**]: Compared within group; *p*-value for Paired *t*-test. [#]: Compared within group; *p*-value for Wilcoxon Signed rank test.

Table 11. Changes in test results concerning vital signs and body weight before and after 12 weeks of Brainon® product consumption.

		Brainon® group (n = 40)		Placebo group (n = 40)		
		n	Mean ± SD	n	Mean ± SD	*p*-Value
Systolic blood pressure (mmHg)	Baseline (Visit 1)	40	127.30 ± 12.94	40	125.28 ± 14.17	0.5064*
	Visit 3	40	130.28 ± 15.89	40	123.90 ± 13.79	
	Change from baseline	40	2.98 ± 13.45	40	-1.38 ± 11.78	0.1279*
	p-value**		0.1697		0.4647	
	Visit 4	40	127.10 ± 12.77	39	124.31 ± 15.51	
	Change from baseline	40	-0.20 ± 11.85	39	-1.18 ± 10.98	0.9452&
	p-value**		0.5540#		0.5063**	
Diastolic blood pressure (mmHg)	Baseline (Visit 1)	40	77.33 ± 9.31	40	74.28 ± 9.59	0.1531*
	Visit 3	40	76.95 ± 9.91	40	73.53 ± 7.81	
	Change from baseline	40	-0.38 ± 10.47	40	-0.75 ± 7.94	0.8572*
	p-value**		0.8219		0.5539	
	Visit 4	40	75.35 ± 8.34	39	75.15 ± 10.23	
	Change from baseline	40	-1.98 ± 8.25	39	0.97 ± 7.97	0.1102*
	p-value**		0.1381		0.4496	
Heart rate (beats/min)	Baseline (Visit 1)	40	72.80 ± 8.30	40	73.88 ± 9.52	0.5919*
	Visit 3	40	77.33 ± 10.68	40	75.80 ± 9.20	
	Change from baseline	40	4.53 ± 9.33	40	1.93 ± 9.86	0.5061&
	p-value**		0.0034#		0.2243**	
	Visit 4	40	75.05 ± 11.48	39	74.90 ± 10.92	
	Change from baseline	40	2.25 ± 9.97	39	1.03 ± 12.17	0.6258*
	p-value**		0.1614		0.6018	
Body weight (kg)	Baseline (Visit 1)	40	62.32 ± 11.87	40	63.36 ± 11.12	0.3971&
	Visit 3	40	62.46 ± 11.83	40	63.37 ± 11.08	
	Change from baseline	40	0.15 ± 1.16	40	0.02 ± 1.14	0.6141*
	p-value**		0.4345		0.9338	
	Visit 4	40	62.37 ± 11.96	39	62.89 ± 11.24	
	Change from baseline	40	0.05 ± 1.12	39	-0.38 ± 1.23	0.1025*
	p-value**		0.7675		0.0591	

Values are presented as mean ± standard deviation (SD). *: Compared between groups; *p*-value for Two sample *t*-test. &: Compared between groups; *p*-value for Wilcoxon rank sum test. **: Compared within group; *p*-value for Paired *t*-test. #: Compared within group; *p*-value for Wilcoxon Signed rank test.

Previous studies have confirmed that the MMSE alone cannot detect mild cognitive impairment perfectly and that more detailed cognitive evaluation tools are needed. Additionally, it has been revealed that decreased concentration is related to functional prognosis (20, 21). Furthermore, it has been shown that 6.3% of patients who have normal results in the MMSE test demonstrate impairment in specific cognitive functions when evaluated using CNT (22, 23). To address these limitations, in this clinical study, we conducted K-MMSE and CNT tests together with objective and detailed evaluation provided by CNT being set as the main efficacy evaluation variable.

The CNT-Verbal Learning Test is a computerized version of the Rey Auditory Verbal Learning Test (RAVLT). A verification study has been conducted to confirm the discriminant validity of the Korean language auditory learning test. Results showed that patients performed significantly lower than the normal control group in total scores of A1 to A5 among all basic and combined items (24).

In another previous study that conducted a retest of RAVLT with an interval of 6–14 days, the most reliable items in terms of stability were found to be total score of the 1st to 5th trials of the word list recall and the 6th and 7th trials of the word list recall, while other composite items that included the 1st trial of the word list recall were found to have lower reliability (25). This is because the first and second trials of the word list recall can be influenced by attentional or affective factors as well as degree of adaptation to the test in early stages of testing (24). Therefore, the evaluation result of the total score of A1 to A5 is considered to be more reliable than the result of each individual item.

In this study, total score of A1-A5 immediate recall of the CNT-Verbal Learning Test showed significantly more increase in the Brainon® group than in the placebo group, indicating an improvement effect of Brainon® on memory. Additionally, in the experimental group, there was a statistically significant increase in the number of correct responses in the CNT-Visual C.P.T as well as scores of the Digit Span Test backward recall, while the number of omission errors in the CNT-Visual C.P.T was significantly decreased, confirming a memory improvement effect of Brainon® consumption. Serum levels of BDNF increased by 30.22 pg/mL in the experimental group and by 13.07 pg/mL in the control group. After adjusting for test values as covariates and performing GLM analysis, a statistically significant improvement effect was observed in the Brainon® group compared to the placebo group. IL-6 levels increased by 0.57 pg/mL in the Brainon® group but decreased by 0.32 pg/mL in the placebo group. Although there was no statistical significance compared to the placebo group, a statistically significant increase in IL-6 level was observed in the Brainon® group after 12 weeks of supplementation compared to baseline.

Adverse events occurred in a total of 12 cases in the Brainon® group. Among them, 11 cases were evaluated as unrelated to the investigational food and one case was assessed as potentially related. However, their symptoms were completely recovered the next day without any corrective treatment and participants continued the trial without any further adverse issues. In addition, there were no statistically significant differences between the Brainon® group and the placebo group in clinical laboratory tests, vital signs, or weight measurements, confirming the safety of Brainon® as a health functional food material.

In the experimental group of this human clinical trial, significant improvements were observed in CNT-Verbal Learning Test Immediate recall total scores for A1~A5, CNT-Visual C.P.T correct response and omission errors, Digit Span Test Backward recall scores, and serum BDNF and IL-6 levels (Figure 2). Based on these findings, Brainon® supplementation is expected to have beneficial effects on memory improvement in adult men and women who experience memory impairment.

Conclusion

This study was the first clinical trial to evaluate the efficacy and safety of SB extract for improving memory function. This clinical trial was conducted for 12 weeks using a randomized, double-blind, and placebo-controlled test. The consumption of Brainon® showed a statistically significant effect on immediate recall total score, a major validation indicator of the CNT-Verbal learning test. In addition, significant improvements were observed in correct response and omission error items of the CNT-Visual C.P.T, backward recall of the digit span test, and serum levels of BDNF and IL-6. Clinically significant adverse events or physical changes were not observed, indicating that Brainon® consumption was safe for humans. Therefore, the improvement of memory function by Brainon® ingestion was confirmed in this clinical test for the first time. It was found that symptoms of patients with memory impairment could be improved through continuous intake of Brainon®.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

References

1. Wilson RS, Wang T, Yu L, Bennett DA, Boyle PA. Normative cognitive decline in old age. Ann Neurol 2020;87:816–829.
2. Sengoku R. Aging and Alzheimer's disease pathology. Neuropathology 2020;40:22–29.
3. Lissek V, Suchan B. Preventing dementia? Interventional approaches in mild cognitive impairment. Neurosci Biobehav Rev 2021;122:143–164.
4. Zvěřová M. Clinical aspects of Alzheimer's disease. Clin Biochem 2019;72:3–6.
5. McEwen BS, Nasca C, Gray JD. Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex. Neuropsychopharmacology 2016;41:3–23.
6. Zuccato C, Cattaneo E. Brain-derived neurotrophic factor in neurodegenerative diseases. Nat Rev Neurol 2009;5:311–322.
7. Lu B, Nagappan G, Lu Y. BDNF and synaptic plasticity, cognitive function, and dysfunction. Handb Exp Pharmacol 2014;220:223–250.
8. Kim JK, Kim YH, Lee HH, Lim SS, Park KW. Effect of Scrophularia buergeriana extract on the degranulation of mast cells and ear swelling induced by dinitrofluorobenzene in mice. Inflammation 2011;35:183–191.
9. Jeong EJ, Ma CJ, Lee KY, Kim SH, Sung SH, Kim YC. KD-501, a standardized extract of Scrophularia buergeriana has both cognitive-enhancing and antioxidant activities in mice given scopolamine. J Ethnopharmacol 2009;121:98–105.
10. Lee HJ, Spandidos DA, Tsatsakis A, Margina D, Izotov BN, Yang SH. Neuroprotective effects of Scrophularia buergeriana extract against glutamate-induced toxicity in SH-SY5Y cells. Int J Mol Med 2019;43:2144–2152.
11. Lee HJ, Lee DY, Kim HL, Yang SH. Scrophularia buergeriana extract improves memory impairment via Inhibition of the apoptosis pathway in the mouse hippocampus. Appl Sci 2020;10:7987.
12. Lee HJ, Kim HL, Lee DY, Lee DR, Choi BK, Yang SH. Scrophularia buergeriana extract (Brainon) improves scopolamine-induced neuronal impairment and cholinergic dysfunction in mice through CREB-BDNF Signaling Pathway. Appl Sci 2021;11:4286.
13. Kang Y, Jin JH, Na DL. A Normative Study of the Digit Span Test for the Elderly. Kor J Clin Psychol 2002;21:911–922.
14. Hong YJ, Yang DW, Yoon B, et al. One-year follow-up pilot study for dementia diagnosis in elderly with recall score of 0 in Korean-version of Mini-Mental State Examination. J Korean Geriatr Soc 2014;12:16–23.
15. Shin JH. Diagnosis of Dementia: Neuropsychological Test. Korean J Fam Med 2010;31:253–266.
16. Lukasik KM, Waris O, Soveri A, Lehtonen M, Laine M. The Relationship of Anxiety and Stress With Working Memory Performance in a Large Non-depressed Sample. Front Psychol 2019;10:4.
17. Kim YH. Differences of the visuospatial ability, memory and executive function according to age groups of normal adults. Kor J Health Psychol 2008;13:253–265.
18. Lee CH, Song JK, Kim JH, Choi MJ, Namkoong K. Impairment of Neurocognitive Functions in Young Alcohol Dependent Patients: Problem Solving and Executive Functions. J Korean Neuropsychiatr Assoc 2002;41:1049–1058.
19. Chu SK, Yoo JH. The Effects of Perceived Stress on Cognition in the Community Elderly. J Korean Acad Community Health Nurs 2008;19:368–377.
20. Stephens S, Kenny RA, Rowan E and et al. Association between mild vascular cognitive impairment and impaired activities of daily living in older stroke survivors without dementia. J Am Geriat Soc 2005;53:103–107.
21. Naugle RI, Kawczak K. Limitations of the Mini-Mental State Examination. Cleve Clin J Med 1989;56:277–281.
22. Grigoletto F, Zappala G, Anderson DW, Lebowitz BD. Norms for the Mini-Mental State Examination in a healthy population. Neurology 1999;53:315–320.
23. Lee YN, Kwon HK, Kang YK, Pyun SB. Impact of Cognitive Function on Functional Recovery during Rehabilitation in Patients with Stroke. Brain NeuroRehabil 2011;4:103–109.
24. Cheong SS, Woo JM, Kim E, Yeon BK, Hong KS. Development of Korean Auditory Verbal Learning Test. J Korean Neuropsychiatr Assoc 1999;38:1016–1025.
25. Geffen GM, Butterworth P, Forrester GM, Geffen LB. Auditory verbal learning test components as measures of the severity of closed-head injury. Brain Inj 1994;8:405–411.

Correspondence:
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Seung-Hwan Yang, PhD
Department of Biotechnology,
Chonnam National University, Yeosu 59626, Republic of Korea
Phone: +82-61-659-7306
E-mail: ymichigan@jnu.ac.kr