Fermented *Laminaria japonica* improves working memory and antioxidant defense mechanism in healthy adults: a randomized, double-blind, and placebo-controlled clinical study

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Abstract

A randomized, double-blind, and placebo-controlled clinical study was used to determine the cognitive functions related to working memory (WM) and antioxidant properties of fermented *Laminaria japonica* (FLJ) on healthy volunteers. Eighty participants were divided into a placebo group (n = 40) and FLJ group (n = 40) that received FLJ (1.5 g/day) for 6 weeks. Memory-related blood indices (brain-derived neurotrophic factor, BDNF; angiotensin-converting enzyme; human growth hormone, HGH; insulin-like growth factor-1, IGF-1) and antioxidant function-related indices (catalase, CAT; malondialdehyde, MDA; 8-oxo-2′-deoxyguanosine, 8-oxo-dG; thiobarbituric acid reactive substances, TBARS) were determined before and after the trial. In addition, standardized cognitive tests were conducted using the Cambridge Neuropsychological Test Automated Batteries. Furthermore, the Korean Wechsler Adult Intelligence Scale (K-WAIS)-IV, and the Korean version of the Montreal Cognitive Assessment (MoCA-K) were used to assess the pre and post intake changes on WM-related properties. According to the results, FLJ significantly increased the level of CAT, BDNF, HGH, and IGF-1. FLJ reduced the level of TBARS, MDA, and 8-oxo-dG in serum. Furthermore, FLJ improved physical activities related to cognitive functions such as K-WAIS-IV, MoCA-K, Paired Associates Learning, and Spatial Working Memory compared to the placebo group. Our results suggest that FLJ is a potential candidate to develop functional materials reflecting its capability to induce antioxidant mechanisms together with WM-related indices.

Keywords: Antioxidant, Cognitive functions, Functional food, *Laminaria japonica*

Introduction

Laminaria japonica, an edible brown seaweed, is widely consumed in East Asian countries as a food source. A large number of *L. japonica* farms are established in seaside areas of Korea and China to meet the significant demand for this seaweed in local markets (Asanka Sanjeewa & Jeon, 2018; Wang et al., 2008). *L. japonica* has been used as a drug in traditional Korean, Chinese, and Japanese medicine for thousands of years (Zha et al., 2012). Several studies suggested that *L. japonica* is used as a treatment for gall disease, hard lump, edema, tuberculosis, detumescence, beriberi disease, phlegm elimination, and weight loss in East Asian countries (Asanka Sanjeewa & Jeon, 2018; Fang et al., 2015). In addition to its medicinal applications, sliced *L. japonica* (5–6 cm long strips) is popular as a condiment and soup ingredient. Dried *L. japonica* is also popular as a snack and pickled in vinegar (Choi et al., 2012). In addition to the traditional applications, secondary metabolites such as fucoidans, phlorotannins, pigments, and sterols isolated from *L. japonica* have been found to possess interesting bioactive qualities, including antioxidant, anti-inflammatory, and anticancer properties (Choi et al., 2012; Kang et al., 2018; Lu et al., 2013; Zha et al., 2012).

In our previous research, *L. japonica* was fermented with lactic acid bacteria (*Lactobacillus* spp.) to increase palatability, nutritional value, and preservative and medicinal properties. Lee et al. (2010) reported that fermentation of *L. japonica* (FLJ) with *Lactobacillus brevis* BJ20 has greater potential to induce 2,2-diphenyl-1-picrylhydrazyl scavenging, superoxide scavenging, and xanthine oxidase inhibition than the commercial antioxidant (butylated hydroxyanisole) tested in the study. Interestingly, Kang et al. (2012) also noticed FLJ has the potential to stimulate anti-oxidant activities in healthy individuals. The study concluded that FLJ enhanced the antioxidant defense-related enzyme secretion (superoxide dismutase, SOD; and catalase, CAT) in healthy individuals (25–60 years old). Taken together, FLJ is a potential candidate to develop functional materials for enhancing anti-oxidant human health applications.

The use of working memory (WM) is ubiquitous and a number of definitions for WM have been proposed by researchers that reflect the diverging theoretical views of individual scientists (Cowan, 2017). However, in general, WM refers to the ability to maintain short-term information via a system, or set of processes, which can hold information temporarily for processing. WM is an essential factor to perform complex tasks including comprehension, learning, and reasoning (Ma et

al., 2017). Furthermore, WM comprises the central executive system, visuospatial sketch pad, and phonological loop (Ma et al., 2017). In general, the central executive, identified as an attentional-controlling system, is required to play games such as chess, and the visuospatial sketch pad manipulates visual images. The phonological loop stores and rehearses speech-based information. The phonological loop plays an important role during the acquisition of vocabularies of the mother tongue and other languages (Baddeley, 1992). Neuropsychological studies have revealed a systematic decline of cognitive processes, such as the ability to inhibit responses to auditory and visual stimuli and the ability to perform WM tasks. However, the reduction of WM can affect an individual's day-to-day tasks, as WM is required in general functions, such as the control of attention and processing involved in a range of regulatory functions, including the recall of information deposited in long-term memory (Alloway & Alloway, 2010). Thus, it is important to maintain a functional WM to retain quality of life and the ability to carry out the activities of daily living. Recently, functional foods prepared from natural bases have gained considerable attention due to their lower likelihood of causing side effects compared to their commercially available chemical analogues. In particular, functional foods and cosmeceuticals based on seaweeds have received increased attention due to the identification of bioactive metabolites with therapeutic potential among consumers (Asanka Sanjeewa et al., 2016). Thus, in the present study, the authors attempted to evaluate the effects of FLJ on WM in human subjects. The basic objective of this study was to identify whether FLJ has stimulatory effects on different factors related to WM in FLJ-administered adults for 6 weeks.

Materials and Methods

Preparation of fermented *Laminaria japonica* (FLJ) by *Lactobacillus brevis* BJ20 and placebo

FLJ was prepared according to our previous report (Reid et al., 2018). Briefly, *L. japonica* was added to water at a ratio of 1:15 (w/ v) with the addition of glucose and yeast based on *L. japonica* dry weight used. Then the mixture was autoclaved at 121℃ for 30 min to obtain *L. japonica* extract. The solution was incubated with 2% (v/v) *L. brevis* BJ20 (Accession No. KCTC 11377BP) at 37℃ for 5 days. The incubation solution was freeze dried to obtain moisture free FLJ. The capsules made for clinical trial contained FLJ (250 mg). A placebo was made from the same ingredients but which contained 0 mg FLT and 250 mg dextrins.

Study protocols

This study was designed as a randomized, double-blind, and placebo-controlled clinical trial. The subjects were divided into a placebo group and an FLJ treatment group by block randomization. After screening for eligibility, the 80 participants were healthy adults from 20 to 50 years old regardless of gender. We excluded people who had any other health problems or took medicine. The random allocation sequence was generated using the SAS version 9.2 program (SAS Institute, Cary, NC, USA) and was supervised by the Korean National Institute of Health. Allocation concealment and double-blinding were carried out in accordance with the International Conference on Harmonization Good Clinical Practice guideline. The study protocol was approved by the Institutional Review Board of Institute for Cognitive Science, Seoul National University (IRB no. 1508/001-005). Participants were randomly assigned to FLJ (n = 36) or

placebo (n = 35) groups and ingested two capsules for three times per day (1,500 mg intake; Fig. 1). The treatment protocol continued for 6 weeks. However, of the initial 80 participants, 71 completed the clinical trial; nine dropped out due to their intention to discontinue. Fasting venous blood samples were taken before and after the 6 weeks. Collected blood samples were clotted for 30 min centrifugation for 15 min at 1,000×g separated into serum and stored at −80℃ and used to analyze tested parameters. The number of participants in the study, for each assay, are noted in Table 1.

Antioxidant assays
Serum thiobarbituric acid reactive substances (TBARS) content determination

The thiobarbituric acid reactive substances (TBARS) level was determined with the OxiSelect™ TBARS Assay Kit, purchased from
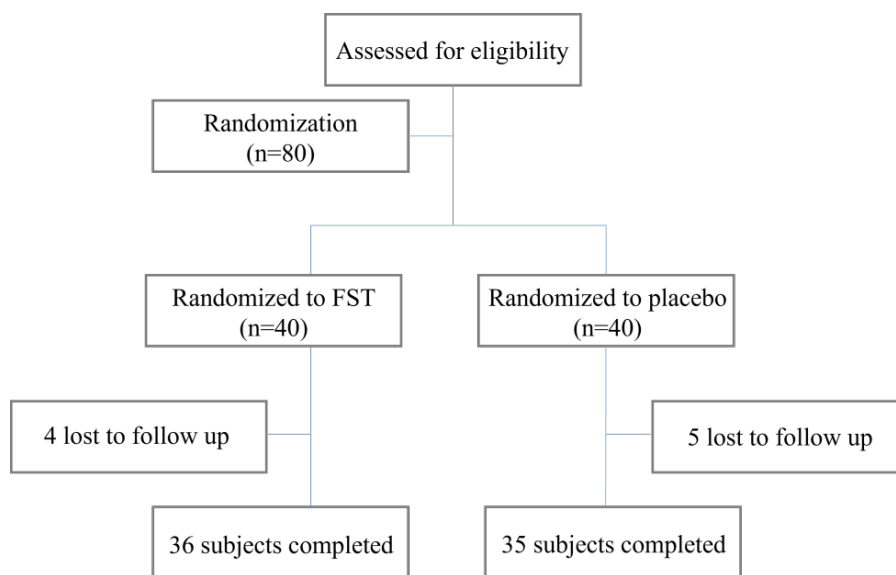

Fig. 1. Participant flow diagram. Subjects recruited for the study were assessed and those eligible were enrolled.

Table 1. Composition of the experimental diets (% of dry matter)

	FLJ group			Placebo group		
	Recruitment	Suspension	Completion	Recruitment	Suspension	Completion
Behavioral experiments	40	4	36	40	5	35
Blood samples	40	17	23	40	21	19
Working memory capacity	40	36	4	40	35	5
Biochemical indicators	40	36	4	40	35	5

FLJ, fermented *Laminaria japonica*.

Cell Biolabs (San Diego, CA, USA). The samples were prepared according to the instructions provided by Cell Biolabs. Spectrophotometric measurement was used to calculate TBARS at 532 nm absorbance. Serum TBARS levels were expressed as μM.

8-Oxo-2'-deoxyguanosine (8-oxo-dG) level determination

8-Oxo-2'-deoxyguanosine (8-oxo-dG) levels were determined using a competitive ELISA kit from JaICA (Japan Institute for the Control of Aging, Shizuoka, Japan). The vendor's instructions were followed to prepare samples. Absorbance levels were measured at 450 nm using an ELISA plate reader.

Malondialdehyde (MDA) level determination

The malondialdehyde (MDA)-containing samples were first reacted with TBA at 95℃. After a brief incubation, the MDA-protein adduct content in the serum was determined by comparison with a predetermined MDA standard curve. Spectrophotometric measurement was used to calculate TBARS at 532 nm absorbance. TBARS content in the serum samples were expressed as μM.

Catalase (CAT) level determination

CAT level in the serum was determined using a commercial ELISA kit (BioVision, Milpitas, CA, USA). One unit of CAT was defined as the amount required to decompose 1 μM of H_2O_2 within 60 seconds. The decomposition rate of H_2O_2 was determined spectrophotometrically at 570 nm. Serum CAT level was reported as mU/mL.

Cognitive function-related assays

Brain derived neurotrophic factor (BDNF) determination

Brain derived neurotrophic factor (BDNF) level in the serum samples were measured using a commercial kit purchased from R&D Systems (Minneapolis, MN, USA). The optical density of diluted serum samples was measured within 30 min at 450 nm.

Human growth hormone (HGH) determination

Human growth hormone (HGH) level was determined using Immulite 2000, a commercial kit purchased from Siemend AG (Muenchen, Germany). A growth hormone releasing hormone + arginine stimulation test was calibrated with both IS 80/505 and IS 98/574 (GRH Growth Hormone-Recombinant 98/574-kit).

Insulin-like growth factor-1 (IGF-1) determination

Insulin-like growth factor-1 (IGF-1) level were measured using

a solid-phase enzyme-labelled chemiluminescent immunometric assay on the Immunlite 2000 automated Immunoanalyzer (Siemens AG).

Angiotensin converting enzyme (ACE) determination

Angiotensin converting enzyme (ACE) intermediates the breakdown of the synthetic substrate N-[3-(2-furyl)acryloyl]-L-phenylalanyl-L-glycyl-L-glycine (furanacryloyl-L-phenylalanylglycylglycine, FAPGG) into dipeptides and amino acid derivatives. The kinetic of this angiotensin peptide cleavage reaction was calculated by recording the reduction of absorbance at 340 nm using a commercial kit purchased from BÜHLMANN Laboratories (Basel, Switzerland).

Working memory related assays

Iconic Memory Test (pre, simultaneous, post)

Participants were required to remember and report the sensory stimuli presented on a screen for a short time in order to assess sensory memory capacity. Three experimental conditions were used in this study: pre-cue condition, indicator signal appears for 200 ms before the stimulus array; simultaneous condition, instructional signal appears simultaneously with the stimulus array; post-cue condition, stimulation signal appears 200 ms after the stimulus array.

Korean Wechsler Adult Intelligence Scale (K-WAIS)

Korean Wechsler Adult Intelligence Scale (K-WAIS) is a standardized cognitive test, a Korean version of the Wechsler Adult Intelligence Scale-Revised (WAIS-R), which is used to evaluate verbal and performance intelligence. The three-subtests from the K-WAIS, comprising digit span, digit symbol coding, and block design, were administered to estimate cognitive function (Yeom et al., 1992). Digit span measures WM, short-term memory, and attention. Digit symbol coding measures speed of information processing, visual short-term memory, and set-shifting ability. Block design measures visual-motor coordination and perceptual organization.

The Korean version of the Montreal Cognitive Assessment (MoCA-K)

The Montreal Cognitive Assessment (MoCA) is a screening instrument to detect mild cognitive impairment developed by Nasreddine et al. (2005). To complete the Korean version of the Montreal Cognitive Assessment (MoCA-K) takes about 10–15 min and lower scores indicate poor cognition. The maximum score for the test is 30 (Nasreddine et al., 2005).

Fisheries and Aquatic Sciences

Paired Associates Learning (PAL) and Spatial Working Memory (SWM)

A well-trained staff member and specialist administered all neuropsychological tests and the Cambridge Neuropsychological Test Automated Battery (CANTAB®) was used to examine some components of cognition. The CANTAB® computerized platform is well known and widely used for assessing cognitive functions (Lee et al., 2013). Based on the study purpose a battery of two tests was selected to evaluate alertness and cognitive functions. Paired Associates Learning (PAL) and Spatial Working Memory (SWM) tests were used to evaluate WM of placebo and FLJ groups. A high score represents poor WM and low scores equates to good performance for WM (Lee et al., 2013).

Liver function related assays

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) level determination

In general, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are capable of catalyzing the interconversion of amino acids and α-keto acids by transfer of amino groups. A standardized protocol obtained from the manufacturer (COBAS; Roche Diagnostics, Basel, Switzerland) was used to determine the AST and ALT levels. ALT determination included optimization of substrate levels, employment of tris buffers, pre-incubation of a combined buffer and serum to allow side reactions with nicotinamide adenine dinucleotide hydride (NADH), substrate start, and pyridoxal phosphate activation. AST measurement included optimization of substrate levels, employment of tris buffers, pre-incubation of a combined buffer and serum to allow side reactions with NADH to occur, substrate start, and optional pyridoxal phosphate activation.

Statistical analysis

Data were analyzed by intention-to-treat analysis and expressed as mean ± SD. An independent *t*-test was used to identify between-group differences. A paired *t*-test was used to compare the mean changes from baseline within each group. All statistical analyses were conducted with Statistical Package for Social Sciences (SPSS for Windows version. 21.0; IBM, Armonk, NY, USA). A *p*-value < 0.05 was considered statistically significant.

Results

Fermented *Laminaria japonica* (FLJ) enriched anti-oxidant related factors in serum

As shown in Table 2, antioxidant function-related indices, such as TBARS, MDA, and CAT, as well as oxidative DNA damage-related factors including 8-oxo-dG, were determined before and after the oral administration of FLJ and placebo for a 6-week period. According to the results, significant differences of TBARS were observed in the pre and post variation between the FLJ and placebo groups. Specifically, the levels of TBARS and MDA were sufficiently suppressed in the FLJ-administered group compared to the placebo group. Furthermore, the CAT index, before and after the functional food digestion for 6 weeks, was measured. The results demonstrated a statistically significant difference in the pre and post variation between the FLJ and placebo groups, and the level of improvement was higher in the FLJ group compared to the placebo group (Table 2). In addition, the oxidative DNA damage index-related factor 8-oxo-dG was also measured using ELISA assay. A statistically significant difference was observed in the pre and post variation between the FLJ-administered and placebo groups, and the de-

Table 2. Pre and post test comparison results of anti-oxidant parameters tested in the study

		FLJ (n = 23)		Placebo (n = 19)			
		M (SD)	Difference M (SD)	M (SD)	Difference M (SD)	*t*-value	*p*-value
TBARS (µM)	Pre	1.09 (0.62)	−0.27 (0.43)	1.06 (0.77)	0.07 (0.41)	−2.581	0.014
	Post	0.82 (0.56)		1.13 (0.84)			
MDA (µM)	Pre	58.91 (16.41)	−6.67 (16.03)	57.25 (16.51)	2.90 (13.85)	−2.046	0.047
	Post	52.24 (14.11)		60.14 (17.67)			
CAT (mU/mL)	Pre	9.86 (3.80)	2.77 (4.08)	9.65 (1.71)	0.67 (1.85)	2.08	0.044
	Post	12.63 (3.79)		10.31 (1.40)			
8-Oxo-dG (ng/mL)	Pre	1.48 (0.31)	−0.45 (0.38)	1.55 (0.47)	0.02 (0.22)	−4.884	0
	Post	1.02 (0.45)		1.57 (0.55)			

FLJ, fermented *Laminaria japonica*; M, means of each parameter; TBARS, thiobarbituric acid reactive substances; MDA, malonialdehyde; CAT, catalase; 8-oxo-dG, 8-oxo-2′-deoxyguanosine.

gree of level decrease was larger in the FLJ group.

Memory-related blood indices

For the analysis, levels of memory-related blood indices, including BDNF, HGH, IGF-1, and ACE, were measured before and after the intake of FLJ and placebo for 6 weeks. According to the results, the differences between before and after food intakes were significantly higher in the FLJ group compared to the placebo group (Fig. 2). Specifically, the differences between before and after meal of BDNF, IGF-1, and HGH were recorded as 879.67, 16.89, and 16.89 in the FLJ group, respectively. Meanwhile, the levels of BDNF, IGF-1, and HGH were recorded as −624.19, 0.84, and −0.07 in the placebo group. These results suggest FLJ has the potential to improve memory-related blood indices. In addition, the ACE index before and after the intake of FLJ for 6 weeks showed a statistically significant difference in pre and post variation between the FLJ group and the placebo group, and the degree of level decrease was larger in the FLJ

group. This suggests that the intake of functional food induced the improvement of memory-related blood indices.

Liver function-related blood indices

As part of the study, the levels of AST and ALT in the blood samples were measured using commercial ELISA assays. As shown in Fig. 3, the AST index before and after the intake of FLJ for 6 weeks demonstrated no statistically significant difference in the pre and post variation between the FLJ group and the placebo group. Furthermore, pre and post variation of the ALT index between the FLJ and placebo group showed no statistically significant difference. Additionally, the FLJ treated group had low levels of AST (−0.61 ± 4.24) and ALT (0.22 ± 1.62) in their blood samples compared to the placebo group (Fig. 3).

Pre and post test comparison results of memory-related behaviors

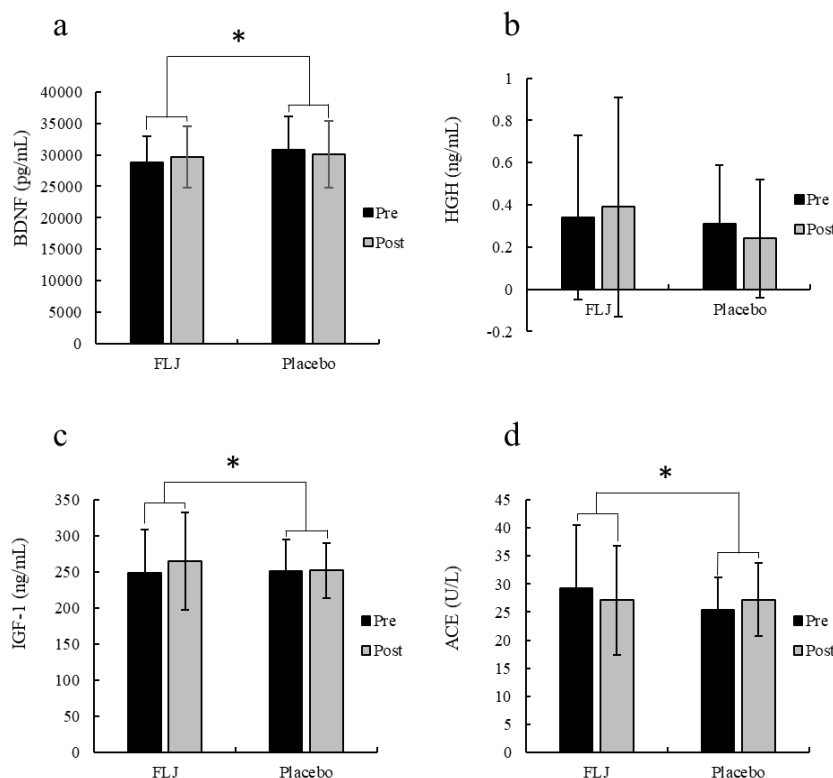The factors related to iconic memory, such as MoCA-K, K-WAIS-

Fig. 2. Changes in serum growth-related markers. BDNF (a), HGH (b), IGF-1 (c), and ACE (d) levels were determined in the FLJ and placebo group, pre and post experimental trial. Each bar represents the mean ± SD. Student's paired *t*-tests were used to determine the statistical significances (* *p* < 0.05) between the FLJ and placebo group. BDNF, brain derived neurotrophic factor; HGH, human growth hormone; IGF-1, insulin-like growth factor-1; ACE, angiotensin converting enzyme; FLJ, fermented *Laminaria japonica*.
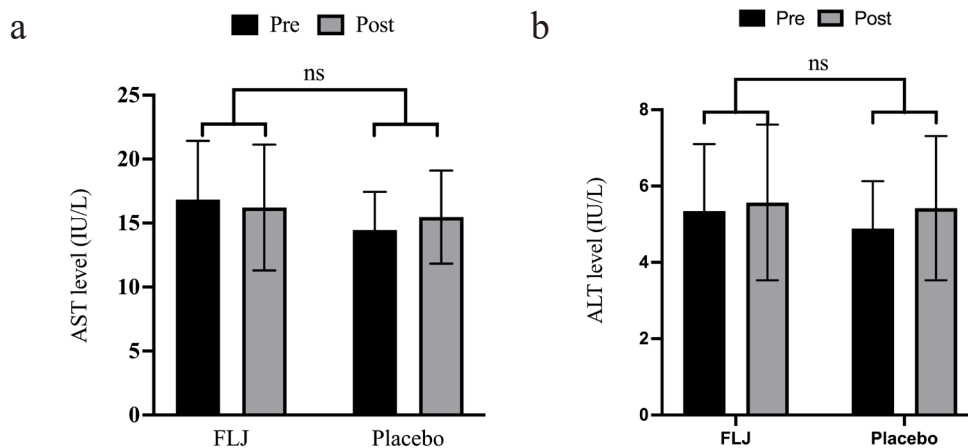
Fig. 3. Serum AST and ALT levels. Serum AST (a) and ALT (b) levels were determined in the FLJ group (2 capsules/3 times/day for 6 weeks) and the placebo group, before and after completing the trial. Each bar represents the mean ± SD. Student's paired *t*-tests were conducted to identify significant differences. * $p < 0.05$ between before and after in each group. [†] $p < 0.05$ between the FLJ and placebo groups. AST, aspartate aminotransferase; ALT, alanine aminotransferase; FLJ, fermented *Laminaria japonica*.

IV-number memorization task, K-WAIS-IV-arithmetic task, K-WAIS-IV-ordering task, Cambridge Computerized Neuropsychological Test-PAL test, and Cambridge Computerized Neuropsychological Test-SWM test were used to determine the effect of functional food intake. The results from each assay are reported Table 2.

Korean version of the Montreal Cognition Assessment (MoCA-K)

MoCA-K scores of tested subjects were measured before and after the intake of FLJ and placebo. According to the results, no statistically significant difference was observed in the pre and post variation between the FLJ group and placebo group. However, the level of improvement was higher in the FLJ group (Table 2).

Korean Wechsler Adult Intelligence Scale (K-WAIS)-IV-number memorization task

K-WAIS-IV-number memorization task scores were recorded before and after the intakes of FLJ and placebo for 6 weeks. A statistically significant difference was observed in the pre and post variation between the FLJ and placebo groups, and the level of improvement was higher in the FLJ group. This suggests that the intake of functional FLJ has a positive effect on the improvement of WM ability associated with number memorization.

Korean Wechsler Adult Intelligence Scale (K-WAIS)-IV-arithmetic task

From the analysis of the arithmetic task scores before and after

the intake of functional food for 6 weeks, a statistically significant difference was shown in the pre and post variation between the FLJ and placebo groups. Furthermore, the level of improvement was higher in the FLJ group. These results suggest FLJ has a positive effect on the improvement of WM ability associated with arithmetic task performance.

Korean Wechsler Adult Intelligence Scale (K-WAIS)-IV-ordering task

Ordering task scores before and after the intake of FLJ and placebo for 6 weeks were evaluated to determine the effects of FLJ as a functional food. According to the results, improvements of the ordering task in the FLJ group demonstrated significant improvements compared to the placebo group. This suggests that FLJ had positive effects on the improvement of WM ability associated with ordering task performance.

Cambridge Computerized Neuropsychological Test-Paired Associates Learning (PAL) test

PAL scores were recorded in tested individuals before and after the intake of FLJ and placebo for 6 weeks. The results demonstrated a significant difference in the pre and post variation between FLJ and placebo groups, and the level of improvement (reduction of search errors) was increased with the intake of FLJ. These observations support the fact that FLJ has a stimulatory effect on PAL task performance.

Table 3. Pre and post test comparison results of memory-related behaviours of subjects using a *t*-test

		FLJ (n = 23)		Placebo (n = 19)			
		M (SD)	Difference M (SD)	M (SD)	Difference M (SD)	*t*-value	*p*-value
MoCA-K	Pre	27.50 (1.68)	0.14 (1.40)	27.74 (1.36)	−0.23 (2.57)	0.752	0.455
	Post	27.64 (2.18)		27.51 (2.13)			
K-WAIS-IV-number memorization task	Pre	29.19 (4.56)	2.47 (3.76)	30.34 (4.87)	0.51 (2.29)	2.64	0.01
	Post	31.67 (4.40)		30.86 (5.27)			
K-WAIS-IV-arithmetic task	Pre	14.72 (3.12)	2.69 (2.05)	14.17 (4.00)	1.51 (2.69)	2.08	0.041
	Post	17.42 (2.67)		15.69 (4.21)			
K-WAIS-IV-ordering task	Pre	19.17 (3.21)	2.08 (2.10)	19.63 (2.91)	1.14 (1.72)	2.061	0.043
	Post	21.25 (3.02)		20.77 (3.25)			
PAL	Pre	10.22 (8.60)	−3.61 (5.69)	10.06 (8.65)	−1.26 (3.64)	−2.07	0.042
	Post	6.61 (7.89)		8.80 (8.06)			
SWM	Pre	14.89 (11.01)	−3.92 (9.83)	10.23 (12.14)	0.97 (7.54)	−2.347	0.022
	Post	10.97 (9.76)		11.20 (11.34)			

FLJ, fermented *Laminaria japonica*; M, means of each parameter; MoCA-K, Korean version of Montreal Cognitive Assessment; K-WAIS, Korean Wechsler Adult Intelligence Scale; PAL, Paired Associates Learning; SWM, Spatial Working Memory.

Cambridge Computerized Neuropsychological Test-Spatial Working Memory (SWM) test

The changes in the SWM scores are presented in Table 3. According to the results, the pre vs. post comparison of the SWM scores within each group showed a significant difference in all variables by paired sample *t*-test ($p < 0.05$).

The safety of fermented *Laminaria japonica* (FLJ)

No adverse events or reactions to FLJ or placebo treatments occurred in any subject after 6 weeks of treatment. No clinically significant differences were observed in the medical check-up, hematologic examinations, vital signs, or electrolyte analysis.

Discussion

Seaweeds have been well documented to contain essential nutrients such as proteins, polysaccharides, fatty acids, amino acids, minerals, carotenoids, bioactive peptides, and vitamins (Kadam et al., 2013; Mahadevan, 2015). In general, Asians pioneered the utilization of seaweeds for food and medicinal applications. On the other hand, Europeans used seaweeds to extract phycocolloids (Kadam et al., 2013). Among the edible seaweeds, *L. japonica* has long been used as an ingredient in traditional medicine to treat vomiting and hemorrhoids in Korea. In general, dried *Laminaria* contains 8%–30% mannitol, 17%–32% alginic acid, 6%–19% crude protein, and 19%–45% ash (mainly iodine and potassium) (Kim & Bhatnagar, 2011). Furthermore, *L. japonica* is also rich in alginic acids, polyunsaturated fatty acids, amino acids, and minerals required to maintain human health. Recently, a number of studies highlighted that compounds isolated from *L. japonica* using organic solvent-assisted extraction had numerous bioactive properties (Chen et al., 2018; Yang et al., 2010). However, it has been reported that extensive use of organic solvents for food grade extracts is hazardous and toxic for long-term consumption. In addition, organic solvent-assisted extraction is generally not considered an eco-friendly extract, at a time when many consumers and sustainability programs are looking to develop and consume functional products developed from eco-friendly extraction methods (Martins et al., 2011). Therefore, in the present study, the authors evaluated the health effects of . extract prepared using *L. brevis* BJ20 to develop functional products to reduce oxidative stress and increase WM in humans.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are end products of several endogenous and exogenous processes. The effects of ROS and RNS are neutralized by antioxidant defense mechanisms. However, failure to neutralize ROS and RNS to optimal levels by antioxidant defense systems is called oxidative stress (Asanka Sanjeewa et al., 2017; Wojtunik-Kulesza et al., 2016). A close relationship between oxidative stress and the pathogenesis of chronic diseases (cardiovascular diseases, kidney disease, sarcopenia, obstructive pulmonary disease, neurodegenerative diseases, and cancer) are well documented (Liguori et al., 2018). Specifically, increased

levels of TBARS, 8-oxo-dG, and MDA, together with lower activity of antioxidant enzymes (SOD and CAT) are characteristic features of oxidative stress-related disease conditions (Khan & Ali, 2018). A number of studies have reported that oxidative stress could led to development of diseases such as Alzheimer's disease and mild cognitive impairment (Mecocci & Polidori, 2012). Head (2009) reported that the administration of synthetic drugs to treat Alzheimer's disease and mild cognitive impairment showed both positive and negative effects of the use of antioxidant supplements. Thus, natural products, which are capable of monitoring oxidative stress-related markers, have great potential for development as functional products. Previously, Kang et al. (2013) reported the TBARS inhibitory effect of dieckol isolated from Ecklonia cava using a mouse model. According to the authors, dieckol further stimulated the activities of antioxidant enzymes, which caused a reduction of oxidative stress in mouse tissues (Kang et al., 2013). Similarly, FLJ-administered adults showed lower TBARS, 8-oxo-dG, and MDA serum levels, together with upregulated anti-oxidant enzymes.

Age-related decline in cognitive functions has been extensively studied in recent decades. Studies have reported that cognitive functions decline with the ageing process (Lommatzsch et al., 2005). Proteins such as BDNF, IGF-I, and HGH have been found to decline with age. Specifically, reduction of BDNF causes an increase in the vulnerability to pathogenesis of neurodegenerative diseases such as Parkinson's and Alzheimer's. In addition, BDNF, IGF-1, and HGH are thought to play a key role in learning, long-term and short-term memory, executive functioning, and behavior, and therefore warrant attention (Lommatzsch et al., 2005; Sytze van Dam & Aleman, 2004). Thus, supplements with BDNF, IGF-I, and HGH stimulatory properties serve as functional foods for adults to maintain their daily functions without disturbance. Previously, several studies have demonstrated the effect of IGF-1 and BDNF on cognitive function and neuronal regeneration using mice models and rodents (Cirulli et al., 2004; Markowska & Mooney, 1998). According to the authors, performance of cognitive function-related tasks, such as the Morris water-maze task, has been significantly increased following injection with either IGF-I or BDNF. On the other hand, Um et al. (2018) reported the potential of seaweed extracts to increase BDNF levels using male ICR mice. In addition, studies have revealed the relationship between high levels of ACE in blood circulation. Specifically, elevated ACE levels can affect cognitive impairment and the progression of pathogenesis of diseases such as Alzheimer's disease (Zhang

et al., 2011). Similarly, the present study results demonstrate a significant increase in serum IGF-1, HGH, and BDNF, as well as reduced ACE levels in serums, compared to the placebo. Taken together, our results suggest that FLJ improves cognitive functions via upregulating BDNF, IGF-I, and HGH levels and decreasing ACE in blood circulation.

The level of ALT in serum can be used to determine damage to hepatocytes and is considered a sensitive and accurate preclinical and clinical biomarker of hepatotoxicity. Furthermore, increased serum ALT activity levels have also been associated with other organ toxicities (Ozer et al., 2008). Thus, determination of pre and post serum AST and ALT levels can be used to determine the safety of functional products. As a part of the study the authors also evaluated the serum levels of AST and ALT. According to the results, serum AST and ALT levels were decreased in the FLJ group. Nevertheless, we could not observe significant changes of AST and ALT compared to the placebo group. These results indicate that long-term consumption of FLJ has no side effects such as hepatotoxicity.

Studies have reported that WM begins to decline in middle age. Therefore, enhancing cognitive function in middle age is becoming a major scientific challenge aimed at preventing age-related cognitive decline and sustaining normal cognitive performance in response to cognitively demanding environments (Socci et al., 2017). Specifically, with the global increase of the aged population, health issues related to WM are considered a major health threat (Ossoukhova et al., 2015). One of the possible approaches to prevent age-related cognitive decline is a daily intake of functional foods capable of enhancing cognitive functions. Previously, a number of studies have reported that intake of functional foods has the potential to improve WM-related factors such as SWM, PAL, MoCA, and WAIS-IV using natural plant extracts (Spellman et al., 2015). In general, SWM is required for goal-directed action, such as locating a resource, a threat, or even oneself within a dynamic or unfamiliar environment. Such a task requires a cached representation of relevant spatial features that must be continuously updated, preserved, and applied as needed for the execution of adaptive behaviours (Barnett et al., 2016). PAL is generally used as an instrument for detecting dementia and mainly used for the testing of drugs for Alzheimer's and other central nervous system disorders (Kaser et al., 2017). With disorders such as Alzheimer's and the ageing process, PAL and SWM scores increase, reflecting an increasing number of errors during the test process. Thus, reduction of pre and post PAL and SWM values reflects the improvement

of cognitive functions (Cha et al., 2011). As shown in Table 3, FLJ-administered subjects showed improved PAL and SWM scores, which, in addition, were significantly higher than those of the placebo group. Additionally, pre and post scores of MoCA-K and K-WAIS-IV showed significant improvement in the FLJ group compared to the placebo group (with the exception of MoCA-K). On the other hand, in Figs. 1 and 2 we reported the levels of antioxidant enzymes and cognitive function-related protein levels in the serum samples collected from FLJ and placebo groups. The administration of FLJ for a period of 6 weeks (2 tablets/3 times per day) clearly demonstrated the upregulation of those tested factors compared to the placebo group. Similarly, physical activities related to WM also demonstrated improvements in the FLJ group compared to the placebo group. In addition to the present research, a number of other studies reported the functional properties of FLJ using different *in vivo* and *in vitro* assays (Asanka Sanjeewa & Jeon, 2018; Cha et al., 2011; Choi et al., 2012; Fang et al., 2015; Kang et al., 2012; Lee et al., 2010; Lin et al., 2016; Park & Han, 2006; Zha et al., 2012). However, to the best of our knowledge, this is the first randomized, double-blind, and placebo-controlled clinical study presenting novel results of WM-related blood indices, as well as activity improvement, of human subjects after the administration of FLJ for a six-week period.

Conclusion

Results reported in the present study showed that oral administration of FLJ for 6 weeks, of 2 capsules/3 times per day after meals, has a significant effect on WM-related properties. Specifically, the results highlighted that FLJ has the potential to induce antioxidants and cognitive function-related protein levels in the blood stream. Increased levels of BDNF, HGH, IGF-1, and ACE could be the reason for the increased performance observed during the neuropsychological activities related to WM in the FLJ group after 6 weeks. The results in the present study might be useful in the development of commercial grade functional products to increase neuropsychological activities.

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