

Heat-Killed *Leuconostoc mesenteroides* H40 Alleviates Cognitive Impairment by Anti-Inflammation and Antioxidant Effects in a Scopolamine-Induced Mouse Model

Na-Kyoung Lee^{1†}, Yunjung Lee^{2†}, Jiyoun Hwang², Eunju Park^{2*}, and Hyun-Dong Paik^{1,3*}

¹Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 05029, Republic of Korea

²Department of Food and Nutrition, Kyungnam University, Changwon 51767, Republic of Korea

³View of Creativity, GHBio Co., Ltd., Konkuk University, Seoul 05029, Republic of Korea

Leuconostoc mesenteroides H40 (H40), originally isolated from kimchi, has been shown to exhibit probiotic characteristics and a neuroprotective effect in SH-SY5Y cells. In this study, we investigated the modulatory effects of heat-killed H40 (H-H40) in a scopolamine-induced (1 mg/kg/day) mouse model of cognitive impairment. H-H40 at either 1×10^8 or 1×10^9 CFU/day alleviated scopolamine-induced cognitive impairment in the novel object recognition and Y-maze tests. Neuroinflammatory cytokines, tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , inducible NO synthase (iNOS), and cyclooxygenase (COX)-2 were all found to be decreased by H-H40 treatment. Moreover, changes in neurotransmitter levels and synaptic plasticity were further confirmed through measurement of acetylcholinesterase, acetylcholine, choline acetyltransferase, and brain-derived neurotrophic factor (BDNF) levels. H-H40 increased β -secretase levels, but decreased amyloid- β levels. In addition, the antioxidant effects of catalase and GPx were demonstrated. Overall, our results showed that H-H40 exerts positive cognitive effects by anti-inflammatory and antioxidant activities in a mouse model of scopolamine-induced cognitive impairment. H-H40 could be used as a prophylactic functional food for improving cognition.

Keywords: Probiotics, *Leuconostoc mesenteroides*, cognition, anti-neuroinflammatory effect, antioxidant effect

Introduction

Cognitive function is influenced by aging, environmental exposure to hazardous contaminants (such as drugs or alcohol), brain injury, and social correlations [1]. Alzheimer's disease (AD) is a neurodegenerative disorder accompanied by both mental and physical weakening [2]. The primary symptoms of AD include cognitive decline, impaired reasoning and judgment, short-term memory loss, and communication difficulties [3]. At the molecular level, AD is characterized by the accumulation of extracellular amyloid- β (A β) peptides in the brain [4]. This accumulation of A β is associated with neuroinflammation, mitochondrial apoptosis, and synaptic dysfunction. Consequently, A β accumulation and oxidative stress are generally acknowledged as major factors affecting AD.

The gut-brain axis is a bidirectional network that links the enteric and central nervous systems (CNS). In addition to anatomical aspects, this network also extends to include humoral, endocrine, immune, and metabolic pathways. Psychobiotics are probiotics that deliver or produce neuroactive substances that can benefit patients with neurodegenerative diseases via the gut-brain axis. The hippocampus is the primary brain region accountable for the formation and consolidation of fresh memories, with crucial influence on adult hippocampal neurogenesis [5].

Oxidative stress and neuroinflammation is associated with neurodegenerative diseases, such as AD, amyotrophic lateral sclerosis (ALS), and Parkinson's disease (PD) [6, 7]. Excessive oxidative stress can also lead to inflammatory conditions, such as neurodegenerative diseases [8]. Neuroinflammation is a defense mechanism that protects the brain by removing or preventing various pathogens [9]. This inflammatory stimulation can persist due to endogenous factors, such as infection, trauma, and drugs, protein aggregation, genetic mutation, or environmental factors [10].

Lactic acid bacteria have previously been reported to exhibit numerous health functions, including anticancer and neurodegenerative effects, through antioxidant and anti-inflammatory activities [11-14]. Probiotics are living organisms that provide health benefits to their hosts. *Leuconostoc mesenteroides* is a heterolactic fermentative bacterium found in the early stages of kimchi fermentation. Some *L. mesenteroides* strains have been used as probiotics [11]. In addition, extracellular vesicles from *L. mesenteroides* have demonstrated anti-inflammatory activities in microglial cells [12]. Recently, paraprobiotics, or heat-killed probiotics, have gained attention because of their novel functions and stability [13]. *L. mesenteroides* H40, a bacterial strain isolated from kimchi, has been

Received: November 9, 2024
Revised: December 13, 2024
Accepted: December 19, 2024

*Corresponding authors
HD Paik
E-mail: hdpaik@konkuk.ac.kr
EJ Park
E-mail: pej@kyungnam.ac.kr

[†]Na-Kyoung Lee and Yunjung Lee contributed equally to this work.

Supplementary data for this paper are available on-line only at <http://jmb.or.kr>.

pISSN 1017-7825
eISSN 1738-8872

Copyright © 2025 by the authors. Licensee KMB. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

shown to exhibit probiotic properties and neuroprotective effects in SH-SY5Y cells (neuroblastoma cell line) [14]. Heat-killed *L. mesenteroides* H40 (H-H40) has not yet been demonstrated to influence cognitive function in scopolamine-treated mouse models. Hence, we aimed in the present study to research the preventive effects of H-H40 against cognitive impairment in scopolamine-treated model mice.

Materials and Methods

Probiotic Samples

L. mesenteroides H40 cells were cultivated as probiotics in lactobacilli MRS media and centrifuged for collection [14]. Two washed *L. mesenteroides* H40 cells were resuspended in phosphate-buffered saline (PBS) (HyClone, USA) or 1% glucose. To obtain H-H40, suspended H40 cells were heated at 80°C for 30 min, and the harvested cells were then freeze-dried. These freeze-dried H-H40 cells were then used in an animal model.

Mice and Experimental Design

All animal protocols performed in this study followed the guidelines of the Institutional Animal Care Committee of Kyungnam University (KUICA-22-02). ICR mice (7-weeks-old, male) were acquired from Koatech (Republic of Korea). Mice were contained in groups of three to four per cage at a constant temperature of $23 \pm 2^\circ\text{C}$ and relative humidity of $53 \pm 2\%$, with a 12-h light/dark cycle. After one week of adaptation, the mice were divided into five groups ($n = 10$ per group), as follows: normal control (NC, no treatment), positive control (PC, scopolamine-injected control, 1 mg/kg/day), H-H40L (treated with scopolamine 1 mg/kg/day, and 1×10^8 CFU/day H-H40), H-H40H (treated with scopolamine 1 mg/kg/day, and 1×10^9 CFU/day H-H40), and DO (treated with scopolamine and donepezil, 2 mg/kg; medicinal control). The samples or donepezil were administered for 2 weeks, and scopolamine was injected intraperitoneally for 7 to 14 days to induce memory impairment starting one week before the experiment.

The animals were starved for 12 h and then sacrificed with 4 ml/kg isoflurane. Blood samples were gathered and centrifuged ($2,000 \times g$ for 30 min) to split the serum. Each organ was separated on ice and frozen at -80°C for further study.

Behavioral Tests

To conduct the behavioral tests, we used novel object recognition and Y-maze tests. For the novel object recognition test, mice were set in a box plus two similar objects (O1+O2), and permitted to adapt for 3 min. The consumed time with each object and touched number over 5 min were documented. The next day, object O1 was switched with object O2. The mice were allowed to explore the objects for 5 min, and the time consumed on the new object was used to estimate the recognition index. The touched numbers on a new object represents the cognitive recognition of that object.

The Y-maze test was performed following the method of Salahuddin *et al.* [15], with some modifications. A device with three arms (50 cm (length) \times 20 cm (height) \times 10 cm (width)) was used. The arms were tapered 10 cm away from the free ends. All mice experienced two training trials per day over two serial days. Each mouse was set at the end of one arm and admitted to move freely through the maze for 8 min. During the trial assemblies, the mice were set at the center of the apparatus and allowed to move generously for 8 min to explore the arms at random. The experimental assemblies were conducted over 4 days. The total number of entries into each arm was recorded during each session. The spontaneous alternation behavior of the mice was calculated using the following formula:

$$\text{Spontaneous alternation (\%)} = \frac{\text{Number of alternations}}{\text{Total number of arm entries} - 2} \times 100$$

Neuroinflammatory Cytokines, iNOS, and COX-2

Hippocampal tissues were standardized and these cells were then centrifuged ($10,000 \times g$, 5 min) to obtain supernatants. TNF- α and IL-1 β were analyzed using a commercial enzyme-linked immunosorbent assay (ELISA) kit (BD, USA). The absorbances of TNF- α and IL-1 β were measured at 450 nm using a microplate reader.

Total RNA was extracted from the brain tissue using TRIzol reagent (Invitrogen, USA), and cDNA was synthesized from 1 μg of RNA using M-MLV reverse transcriptase (Promega, USA). After cDNA synthesis, quantitative real-time PCR was performed using 25 μl of SYBR Green master mix (PhileKorea, QuantiSpeed SYBR No-ROX Kit, Republic of Korea) with a real-time DNA thermal cycler (CFX Duet Real-Time PCR System, Bio-RadUSA). The reaction mixtures were finally incubated for an initial denaturation at 95°C for 10 min, followed by 50 cycles of PCR. Each cycle was performed as per the following parameters: 95°C for 10 s, 55°C for 20 s, and 72°C for 20 s. The sequences of the sense and antisense primers used for amplification are listed as follows: COX-2, sense, 5'-acctggtagactacgactgc-3' and antisense, 5'-ctaggaggaggactgctcat-3'; iNOS, sense, 5'-ctatggccgtttgatgtgc-3' and antisense, 5'-tggggatgctcatggtcac-3'. The β -actin-encoding gene was used as a reference gene. Normalized target gene expression levels in each sample were calculated using $2^{-\Delta\Delta\text{CT}}$. Values are expressed as the fold change compared to control, and are presented as the mean \pm SE ($n = 7$).

β -Secretase and Amyloid- β Contents

The hippocampal tissues were standardized and these cells were then centrifuged ($10,000 \times g$, 5 min) to harvest the supernatants. The β -secretase levels in supernatants were measured using the Mouse Bace1 ELISA Kit (Express Biotech International, USA).

To obtain A β 1-40 and A β 1-42 levels, the hippocampal tissue was homogenized in 5 M guanidine-HCl/50 mM Tris buffer (8 \times , pH 8.0) on ice. This hippocampal homogenate was incubated at room temperature for 3–4 h, mixed with cold BSAT-PBS (10 \times), and centrifuged (16,000 \times g, 20 min) to acquire the supernatant. A β 1-40 and A β 1-42 levels in the supernatant were determined using the Mouse Amyloid β 40 and β 42 ELISA kits from BioVendor R&D (Czech Republic), respectively.

Acetylcholine, Acetylcholinesterase, and Choline Acetyltransferase

Hippocampal tissues were standardized and centrifuged (10,000 \times g, 5 min) to get the supernatant. Obtained supernatants were measured to analyze the AChE, ACh, and choline acetyltransferase (ChAT) activities. These ELISA kits were obtained from Bioassay Systems (USA) and MyBiosource, Inc. (USA).

BDNF Level

BDNF levels were investigated in the hippocampus tissue. The rt-PCR was conducted similarly to cytokine expression. The sequences of the sense and antisense primers used for amplification are listed in Lee *et al.* [14].

Antioxidant Activity

Hippocampal tissues were standardized and centrifuged (10,000 \times g, 5 min) to get a supernatant. Then, the catalase and glutathione peroxidase (GSH-Px) activities were measured. Catalase activity was calculated following the method of Ostovan *et al.* [16], with some modifications. First, 100 μ l of supernatant was dissolved in 50 ml of 50 mM phosphate buffer (pH 7), and 2 ml of the mixture was added to a cuvette. The reaction was then initiated through the addition of 1 ml of 30 nM H₂O₂ at 20°C. The H₂O₂ decomposition rate was measured at 240 nm for 30 s using a spectrophotometer.

GSH-Px levels were determined according the method of Martens *et al.* [17]. First, 10 μ l of supernatant was added to 100 μ l of 1 M Tris-HCl-5 mM EDTA buffer (pH 8.0), 100 μ l of 10 U/ml glutathione reductase, 20 μ l of 0.1 M glutathione, and 100 μ l of 2 mM NADPH, and filled with distilled water to 1 ml. After incubation at 37°C for 10 min, the reaction was begun by the addition of 10 μ l of t-butyl hydroperoxide, and the absorbance was observed at 340 nm. The reaction was run for 90 s, and NADPH loss was monitored based on the change in the A₃₄₀/min.

Statistical Analyses

All data were analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range test for multiple comparisons. SPSS software (IBM Corp., USA) was used for statistical analyses and the results were considered statistically significant on the basis of $p < 0.05$.

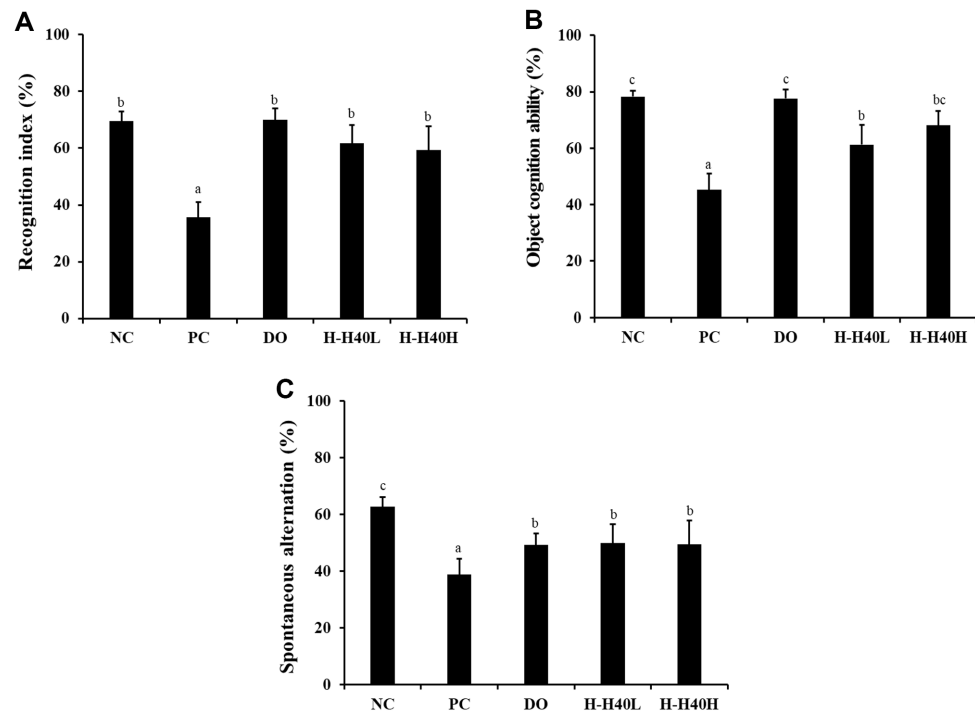


Fig. 1. Behavioral effects of heat-killed *Leuconostoc mesenteroides* H40 using the novel object exploration and Y-maze test in mice with scopolamine-induced memory impairment ($n = 10$). (A) Recognition index (%), (B) object cognition ability (%), and (C) spontaneous alternation (%). NC, normal control without scopolamine; PC, positive control with scopolamine; DO, administration of 2 mg/kg donepezil with scopolamine; H-H40-L, administration of 1×10^8 CFU/day H-H40 with scopolamine; H-H40-H, administration of 1×10^9 CFU/day H-H40 with scopolamine. Data are presented as the mean \pm standard deviation, and alphabetic letters on the error bars indicate significant differences ($p < 0.05$).

Results

Effect of H-H40 on Behavioral Tests

The recognition index and object cognitive ability were investigated using new objects to reveal changes in scopolamine-treated mice. Specifically, the scopolamine-treated control (PC) group (35.6%) represented a significant reduction in the recognition index over time for new objects compared to the NC group (69.5%) (Fig. 1A). The H-H40-L and H-H40-H groups revealed 61.8% and 59.3%, respectively. These values indicated a significant increase in contrast with the PC group. With regard to object cognitive ability, the PC group (45.3%) had a significantly decreased cognition ability as the touched number of new objects increased, compared to the NC group (78.3%) (Fig. 1B). The H-H40-L and H-H40-H groups showed 61.3% and 68.1%, respectively. A slight increase showed contrast with the PC group. The Y-maze test exposed evident patterns of spontaneous alternations in the tested groups (Fig. 1C). The PC group (38.9%) showed significantly lowered alternation behavior compared with the NC group (62.7%), indicating the induction of cognitive impairment. Furthermore, all H-H40 or DO groups showed a significant increase in spontaneous alternation compared to the PC group, thus demonstrating the effect of improved cognitive ability. Therefore, in all behavior tests, the H-H40 group showed similar results to that in the DO group, with no significant differences between groups.

Anti-Neuroinflammatory Effect of H-H40 in Hippocampal Protein

TNF- α and IL-1 β protein production levels were assessed (Fig. 2A and 2B). The PC group (2,756.5 pg/ml) showed increased TNF- α production compared to the NC group (1,134.5 pg/ml), while the values in the H-H40-H and DO groups were 1,314.7 and 917.1 pg/ml, respectively. The PC group (1,466.2 pg/ml) also showed an increase in IL-1 β production compared to the NC group (818.8 pg/ml). The corresponding values in the H-H40-H and DO groups were 818.8 and 714.4 pg/ml, respectively. Notably, the H-H40 groups exhibited concentration-dependent results.

The mRNA expression levels of iNOS and COX-2 were also determined (Fig. 2C and 2D). Specifically, the PC group (10.2-fold) showed a significant increase in iNOS in brain tissue compared to the NC group (1.0-fold), indicating damage to brain tissue cells following scopolamine treatment. However, the H-H40-L and H-H40-H groups showed 6.39- and 5.58-fold lower expression levels, respectively. Although the DO group showed a higher expression value (10.64-fold), these values were similar to those observed in the PC group. For COX-2, the PC group (3.02-fold) showed an increased value compared to the NC group (1.00-fold). Groups H-H40-L, H-H40-H, and DO further exhibited slightly lower expression levels (2.88-, 2.80-, and 2.78-fold, respectively).

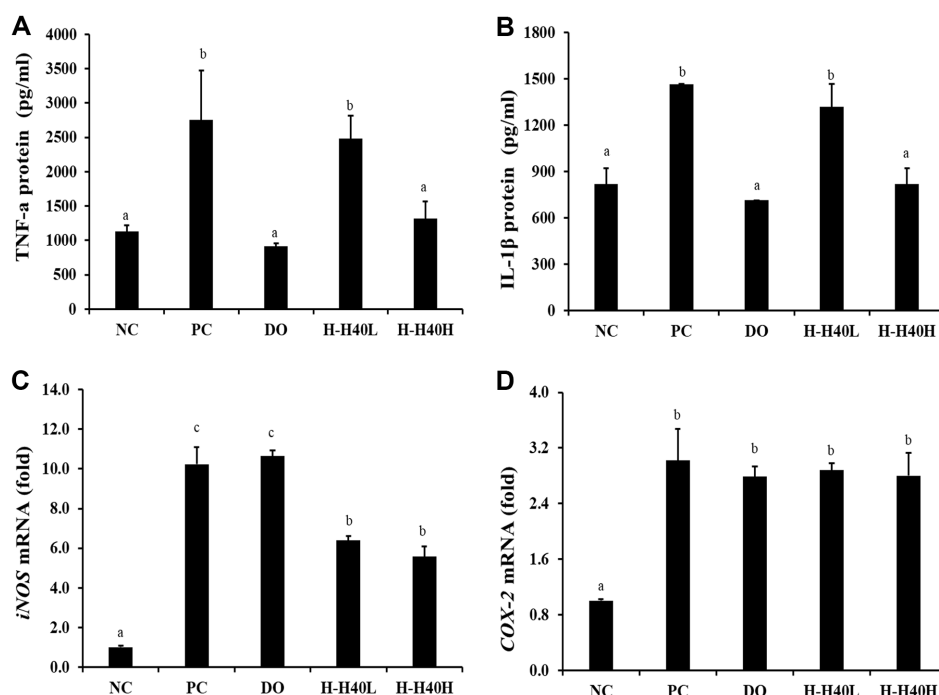


Fig. 2. Anti-neuroinflammatory effect of heat-killed *L. mesenteroides* H40 on hippocampal proteins. Protein expression of (A) TNF- α and (B) IL-1 β . mRNA expression of (C) iNOS and (D) COX-2. NC, normal control without scopolamine; PC, positive control with scopolamine; DO, administration of 2 mg/kg donepezil with scopolamine; H-H40-L, administration of 1×10^8 CFU/day H-H40 with scopolamine; H-H40-H, administration of 1×10^9 CFU/day H-H40 with scopolamine. Data are presented as the mean \pm standard deviation, and alphabetic letters on the error bars indicate significant differences ($p < 0.05$).

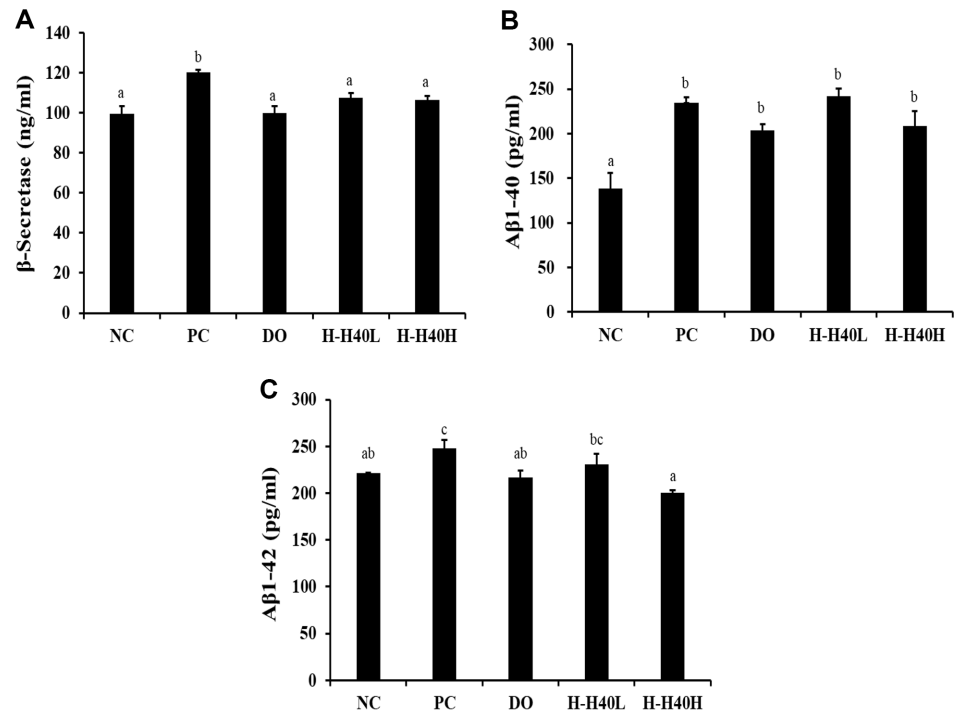


Fig. 3. Effects of heat-killed *L. mesenteroides* H40 on amyloid β accumulation in the brain tissue, as assessed by ELISA. (A) β -Secretase, (B) amyloid β 1-40 (A β 1-40), and (C) amyloid β 1-42 (A β 1-42) activities. NC, Normal control without scopolamine; PC, positive control with scopolamine; DO, administration of 2 mg/kg donepezil with scopolamine; H-H40-L, administration of 1×10^8 CFU/day H-H40 with scopolamine; H-H40-H, administration of 1×10^9 CFU/day H-H40 with scopolamine. Data are presented as the mean \pm standard deviation, and alphabetic letters on error bars indicate significant differences ($p < 0.05$).

Effect of H-H40 on Amyloid- β Accumulation in Brain Tissue

The PC group (120.2 ng/ml) displayed significant increase of β -secretase levels compared with the NC group (99.4 ng/ml) (Fig. 3A). All H-H40 and DO groups (49.3–50.0 ng/ml) represented a significant decrease of β -secretase levels contrasted with the PC group.

The A β 1-40 content showed significant increase in the PC group (234.9 pg/ml) compared with the NC group (138.3 pg/ml) (Fig. 3B), while the corresponding values in the H-H40-H and DO groups were 208.3 pg/ml and 203.3 pg/ml, respectively. In addition, the A β 1-42 content was found to be increased in the PC group (247.9 pg/ml) compared with the NC group (221.5 pg/ml) (Fig. 3C), with values of 200.3 pg/ml and 216.7 pg/ml in the H-H40-H and DO groups, respectively. These results showed a slight decrease in A β accumulation.

Effect of H-H40 on Neurotransmitters

Fig. 4A shows that the PC group (125.9%) exhibited significant increase of AChE activity compared to the NC group (100%). The H-H40-L (116.6%), H-H40-H (89.2%), and DO (106.6%) groups also displayed significantly lower AChE activity than the PC group, demonstrating enhanced cognitive ability.

The ACh content was reduced in the PC group (64.91 μ M) compared with that in the NC group (90.06 μ M) (Fig. 4B). The H-H40-L (69.28 μ M), H-H40-H (67.33 μ M), and DO (68.91 μ M) groups all showed slight increases compared to the PC group. However, this difference was not statistically significant.

In the ChAT activity (Fig. 4C), the PC group (4.48 U/g fresh weight) showed significant decrease of ChAT activity compared with the NC group (11.44 U/g fresh weight), while the H-H40-L (7.94 U/g fresh weight), H-H40-H (8.66 U/g fresh weight), and DO (9.56 U/g fresh weight) groups displayed significant increase of ChAT activity compared to the PC group.

Effect of H-H40 on Synaptic Plasticity

In Fig. 5, the *BDNF* mRNA expression levels showed the variation in the hippocampi of mice (Fig. 5). The PC group (0.79-fold) displayed significant decrease of *BDNF* expression compared with the NC group (1.02-fold), while the H-H40-L (1.28-fold), H-H40-H (1.69-fold), and DO group (1.77-fold) showed significant increases in *BDNF* expression compared with the PC group, representing an enhancement in cognitive ability.

Antioxidant Effect of H-H40 in Hippocampal Protein

The antioxidant effects of H-H40 were confirmed by catalase and GSH-Px production. In terms of catalase production, the PC group (14.7 K/mg protein) showed a significant decrease in the brain tissue compared with the

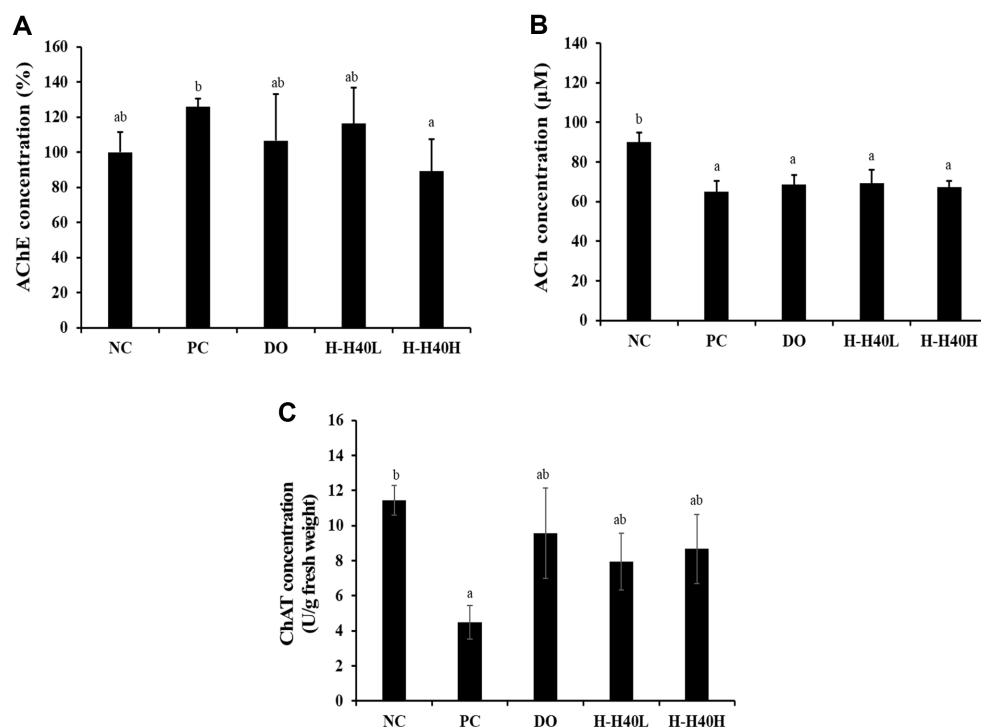


Fig. 4. Effects of heat-killed *L. mesenteroides* H40 on choline-based substances in brain tissue, assessed by ELISA. Production of (A) acetylcholinesterase (AChE), (B) acetylcholine (ACh), and (C) choline acetyltransferase (ChAT). NC, Normal control without scopolamine; PC, positive control with scopolamine; DO, administration of 2 mg/kg donepezil with scopolamine; H-H40-L, administration of 1×10^8 CFU/day H-H40 with scopolamine; H-H40-H, administration of 1×10^9 CFU/day H-H40 with scopolamine. Data are presented as the mean \pm standard deviation, and alphabetic letters on the error bars indicate significant differences ($p < 0.05$).

NC group (29.8 K/mg protein), while brain tissue cells were oxidized by scopolamine treatment (Fig. 6A). H-H40 groups (18.0–22.0 K/mg protein) showed lower catalase activity, while the DO group showed the lowest value, at 15.4 K/mg protein.

In regard to GSH-Px, the PC group (0.29 U/mg protein) showed a significant decrease in the brain tissue compared with the NC group (0.56 U/mg protein), while brain tissue cells showed increased oxidation following scopolamine treatment (Fig. 6B). H-H40 groups (0.33–0.36 U/mg protein) showed relatively high GSH-Px activity, while the DO group showed the lowest value, at 0.27 U/mg protein.

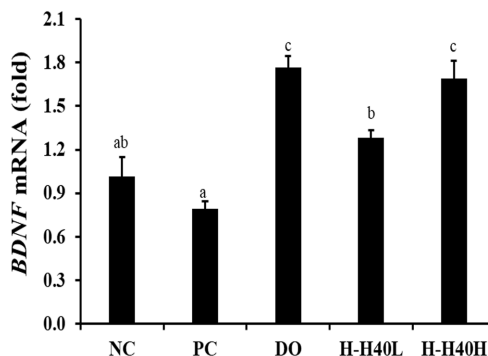


Fig. 5. Effects of heat-killed *L. mesenteroides* H40 on BDNF mRNA expression. BDNF, brain-derived neurotrophic factor; NC, normal control without scopolamine; PC, positive control with scopolamine; DO, administration of 2 mg/kg donepezil with scopolamine; H-H40-L, administration of 1×10^8 CFU/day H-H40 with scopolamine; H-H40-H, administration of 1×10^9 CFU/day H-H40 with scopolamine. Data are presented as the mean \pm standard deviation of triplicate experiments. Different letters on the error bars indicate significant differences ($p < 0.05$).

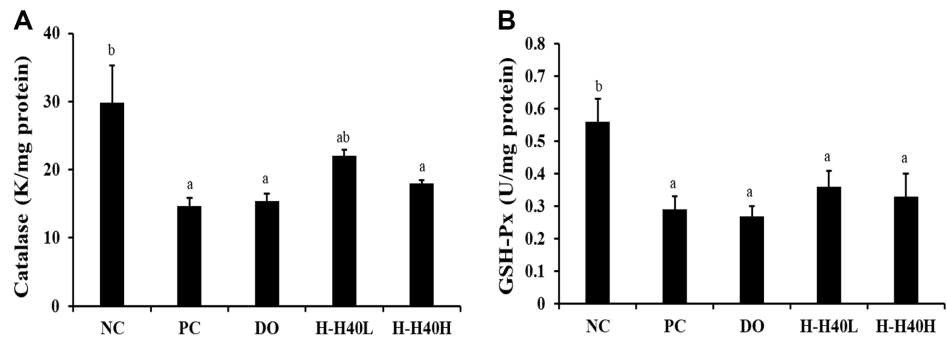


Fig. 6. Antioxidant effects of heat-killed *L. mesenteroides* H40. Activity of (A) catalase and (B) GSH-Px. NC, normal control without scopolamine; PC, positive control with scopolamine; DO, administration of 2 mg/kg donepezil with scopolamine; H-H40-L, administration of 1×10^8 CFU/day H-H40 with scopolamine; H-H40-H, administration of 1×10^9 CFU/day H-H40 with scopolamine. Data are presented as the mean \pm standard deviation and alphabetical letters on the error bars indicate significant differences ($p < 0.05$).

Discussion

Overall, in the present study, we investigated the cognitive-alleviating effects of H-H40 in a mouse model of scopolamine-induced cognitive impairment. H-H40 treatment further exhibited improved learning and memory capacities, and suppressed neuroinflammation and A β accumulation. Furthermore, H-H40 treatment increased the neurotransmitter and antioxidant effects.

Scopolamine, aging, and LPS have all been used in mouse models of cognitive impairment [6, 18]. Among these conditions, scopolamine has been shown to cause deficits in learning acquisition and consolidation, in addition to promoting neuroinflammation and oxidative stress [19]. Cholinergic neurons in the CNS degenerate in a manner that associates with functional loss of cognition in patients with AD and senile dementia. Fig. 1 shows the cognitive variations induced by scopolamine treatment. The PC group treated with scopolamine exhibited reduced cognitive function and spontaneous alternation, while treatment with H-H40 or donepezil alleviated the effects of scopolamine. These behavioral benefits could be confirmed by administration of H-H40. However, to explain the mechanisms of the neuroprotective effects of H-H40, additional research, including on the intestinal microbiome, appears to be necessary.

Neuroinflammation is activated by the conjugation of immune cells in the CNS connecting microglia and astrocytes. This neuroinflammation can accelerate neurological deficits, whereas inhibition of the inflammatory responses can be neuroprotective [20]. IL-1 β and TNF- α are directly related to brain function and can promote inflammatory response inside the brain [21, 22]. IL-1 β is associated with the inflammatory activity, including the accumulation of A β peptides, and has further been linked to stroke, AD, and multiple sclerosis [22]. Increased TNF- α has also been shown to be associated with lower hippocampal volume and cognitive impairment [21]. iNOS and COX-2 are neurotoxic mediators modulated by the NF- κ B signaling pathway [22]. Therefore, these pro-inflammatory cytokines and mediators are also associated with brain diseases. NF- κ B is an inducible regulator of inflammation in the brain. *Lactobacillus acidophilus* has shown neuroprotective effects by lowering IL-1 β [22]. Folic acid ameliorated memory impairment by antioxidant effect [23]. *Levilactobacillus brevis* KU15147 also demonstrated anti-inflammatory effect in macrophage cells through action on the NF- κ B, AP-1, and MAPK signaling pathways [24]. TNF- α further induces iNOS and COX-2 transcription. As shown in Fig. 2, H-H40 reduced IL-1 β , TNF- α , iNOS, and COX-2 levels in a concentration-dependent manner, indicating a protective effect against neuroinflammation.

A β is an important initiator of AD pathology. The production of A β is related with mitochondria, the major source of energy for the brain. β -Secretase levels have been shown to be upregulated in dementia [25]. β -Secretase regulates ordinarily amyloid precursor protein, A β , and A β plaques [26]. The inhibition of β -secretase can prevent A β aggregation. In our study, we found that H-H40 can lower β -secretase and A β production in mice treated with scopolamine, in a manner similar to treatment with donepezil (Fig. 3). Similarly, one prior study showed that *Bifidobacterium breve* MCC1274 reduced A β production in APP knock-in mouse by exerting anti-inflammatory effects through the modulation of the ERK/HIF-1 α signaling pathway [27]. Similarly, another study showed that *Lactococcus lactis* KC24 inhibited β -secretase and A β production [28].

Synaptic plasticity is associated with AChE, ACh, and ChAT activities. In our study, scopolamine treatment reduced AChE, ACh, and ChAT activities (Fig. 4). ACh plays a crucial role in the peripheral and CNS, while AChE hydrolyzes ACh at the synaptic cleft. ChAT is responsible for acetylcholine synthesis, and is the most important indicator of the functional state of cholinergic neurons. Cholinergic neurons are located in the basal forebrain, including those that form the nucleus basalis of Meynert cells in AD [29]. H-H40 and donepezil treatment increased choline-related enzyme activity, with ChAT exhibiting the greatest influence. Especially, cognitive and plasticity-related factors were affected by lower doses of H-H40 than by donepezil.

Neurotransmitters include ACh, 3,4-dihydroxy-phenylacetic acid, dopamine, and homovanillic acid [30]. BDNF is related to neural plasticity and neuron survival in the release of mBDNF and pro-BDNF in cellular

membrane [31]. In this study, BDNF levels were found to be reduced in the brain tissue by scopolamine treatment, and these results were comparative to memory deficits. BDNF levels have further been reported to be decreased by oxidative stress [32]. H-H40 treatment alleviated the decrease in BDNF levels (Fig. 5). Lab4b and *L. lactis* KC24 increased BDNF mRNA expression [28]. H-H40 treatment alleviated the decrease in BDNF levels (Fig. 5). Lab4b and *L. lactis* KC24 increased BDNF mRNA expression.

Lactobacillus gasseri NK109, *Lactiplantibacillus plantarum* MWFLp-182, *Lactobacillus johnsonii* CJLJ103, and *L. lactis* KC24 alleviated LPS, D-galactose, or scopolamine-induced cognitive impairment by upregulation of inflammation-mediated BDNF expression [28, 33–35]. *L. plantarum* MWFLp-182 further induces memory impairment and inflammation triggered by D-galactose in an aging mouse model [35]. H-KC24 and H-H40 demonstrate neuroprotective effects against oxidative stress on the apoptotic factors, *Bax* and *Bcl2* [14, 28]. *L. brevis* KU15151 has further been shown to inhibit inflammation in RAW 264.7 macrophages [36]. Together, these results confirmed the hypothesis that H-H40 alleviates cognition impairment through the modulation of neuroinflammation, neurotransmitters, synaptic plasticity, A β accumulation, and antioxidant effects.

Conclusion

H-H40 treatment alleviated cognitive damage in a scopolamine-induced mouse model with cognitive impairment. Behavioral deficits were further found to be alleviated by H-H40 treatment. The effects of H-H40 were demonstrated by the control of neuroinflammation, neurotransmitters, synaptic plasticity, A β accumulation, and antioxidant effect in brain tissue. Although our results suggest that *L. mesenteroides* H40 is a potent, natural neuroprotective agent, for H-H40 to be employed as a preventive functional food, additional clinical research will need to be conducted.

Acknowledgments

This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry (IPET) through (Technology Commercialization Support Program), funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (RS-2024-00402311).

Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

- World Health Organization. 2020. Risk Reduction of Cognitive Decline and Dementia: WHO Guidelines, pp. 1–96. <https://www.who.int/publications/i/item/9789241550543> (accessed on 23 November 2023).
- Brejijeh Z, Karaman R. 2020. Comprehensive review on Alzheimer's disease: causes and treatment. *Molecules* **25**: 5789.
- D'Argenio V, Sarnataro D. 2021. Probiotics, prebiotics and their role in Alzheimer's disease. *Neural Regen. Res.* **16**: 1768–1769.
- Rahman MM, Lendel C. 2021. Extracellular protein components of amyloid plaques and their roles in Alzheimer's disease pathology. *Mol. Neurodegener.* **16**: 59.
- Toda T, Gage FH. 2018. Review: adult neurogenesis contributes to hippocampal plasticity. *Cell Tissue Res.* **373**: 693–709.
- Gul S, Attallah S, Alsugoor MH, Bawazeer S, Shah SA, Khan S, et al. 2023. Folicitin abrogates scopolamine induced oxidative stress, hyperlipidemia mediated neuronal synapse and memory dysfunction in mice. *Heliyon* **9**: e16930.
- Olufumilayo EO, Gerke-Duncan MB, Holsinger RMD. 2023. Oxidative stress and antioxidants in neurodegenerative disorders. *Antioxidants* **12**: 517.
- Scarian E, Viola C, Dragoni F, Di Gerlando R, Rizzo B, Diamanti L, et al. 2024. New insights into oxidative stress and inflammatory response in neurodegenerative diseases. *Int. J. Mol. Sci.* **25**: 2698.
- Kwon HS, Koh SH. 2023. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. *Transl. Neurodegener.* **14**: 42.
- Zhang W, Xiao D, Mao Q, Xia H. 2023. Role of neuroinflammation in neurodegeneration development. *Signal Transduct. Target. Ther.* **8**: 267.
- Park J, Heo S, Lee G, Hong SW, Jeong DW. 2024. Bacterial diversity of baechu-kimchi with seafood based on culture-independent investigation. *Food Sci. Biotechnol.* **33**: 1661–1670.
- Kim SH, Lee JH, Kim EH, Reaney MJT, Shim TT, Chung MJ. 2022. Immunomodulatory activity of extracellular vesicles of kimchi-derived lactic acid bacteria (*Leuconostoc mesenteroides*, *Lactilactobacillus curvatus*, and *Lactiplantibacillus plantarum*). *Foods* **11**: 313.
- Lee NK, Park YS, Kang DK, Paik HD. 2023. Paraprobiotics: definition, manufacturing methods, and functionality. *Food Sci. Biotechnol.* **32**: 1981–1991.
- Lee NK, Lim SM, Cheon MJ, Paik HD. 2021. Physicochemical analysis of yogurt produced by *Leuconostoc mesenteroides* H40 and its effects on oxidative stress in neuronal cells. *Food Sci. Anim. Resour.* **41**: 261–273.
- Salahuddin HS, Attallah S, Shah SA, Khan S, Zahid M, Ullah M, et al. 2023. Alsugoor. Ranuncoside's attenuation of scopolamine-induced memory impairment in mice via Nrf2 and NF- κ B signaling. *Saudi Pharm. J.* **31**: 101702.
- Ostovan F, Gol A, Javadi A. 2022. Protective properties of *Rydingia persica* in reproductive complications induced by diabetes in male rats: An experimental study. *Int. J. Reprod. BioMed.* **20**: 123–136.
- Martens GA, Folkow LP, Burmester T, Geßner C. 2022. Elevated antioxidant defense in the brain of deep-diving pinnipeds. *Front. Physiol.* **13**: 064476.
- Anand A, Khurana N, Ali N, AlAsmari AF, Alharbi M, Waseem M, et al. 2022. Ameliorative effect of vanillin on scopolamine-induced dementia-like cognitive impairment in a mouse model. *Front. Neurosci.* **16**: 1005972.
- Cheon SY, Koo BN, Kim SY, Kam EH, Nam J, Kim EJ. 2021. Scopolamine promotes neuroinflammation and delirium-like neuropsychiatric disorder in mice. *Sci. Rep.* **11**: 8376.
- Ma Y, Liu T, Fu J, Fu S, Hu C, Sun B, et al. 2019. *Lactobacillus acidophilus* exerts neuroprotective effects in mice with traumatic brain injury. *J. Nutr.* **149**: 1543–1552.
- Pamies D, Sartori C, Schwartz D, González-Ruiz V, Pellerin L, Nunes C, et al. 2021. Neuroinflammatory response to TNF α and IL1 β cytokines is accompanied by an increase in glycolysis in human astrocytes in vitro. *Int. J. Mol. Sci.* **22**: 4065.

22. Versele R, Sevin E, Gosselet F, Fenart L, Candela P. 2022. TNF- α and IL-1 β modulate blood-brain barrier permeability and decrease amyloid- β peptide efflux in a human blood-brain barrier model. *Int. J. Mol. Sci.* **23**: 10235.
23. Xu X, Yang J, Lu Z, Ding J, Lin S. 2024. Folic acid ameliorated the scopolamine-induced memory impairment in mice and the neuroprotective mechanisms. *Food Biosci.* **60**: 104351.
24. Hyun JH, Yu HS, Woo IK, Lee NK, Paik HD. 2023. Anti-inflammatory activities of *Levilactobacillus brevis* KU15147 in RAW 264.7 cells stimulated with lipopolysaccharide on attenuating NF- κ B, AP-1, and MAPK signaling pathways. *Food Sci. Biotechnol.* **32**: 2105-2115.
25. Zuliani G, Trentini A, Brombo G, Rosta V, Guasti P, Romagnoli T, et al. 2021. Serum beta-secretase 1 (BACE1) activity increases in patients with mild cognitive impairment. *J. Neurochem.* **159**: 629-637.
26. Hampel H, Hardy J, Blennow K, Chen C, Perry G, Kim SH, et al. 2021. The amyloid- β pathway in Alzheimer's disease. *Mol. Psychiatry* **26**: 5481-5503.
27. Abdelhamid M, Zhou C, Ohno K, Kuhara T, Taslima F, Abdullah M, et al. 2022. Probiotic *Bifidobacterium breve* prevents memory impairment through the reduction of both amyloid- β production and microglia activation in app knock-in mouse. *J. Alzheimer's Dis.* **85**: 1555-1571.
28. Lee NK, Lee Y, Park JY, Park E, Paik HD. 2024. Heat-killed *Lactococcus lactis* KC24 ameliorates scopolamine-induced memory impairment in ICR mice. *Probiotics Antimicrob. Proteins* <https://doi.org/10.1007/s12602-024-10268-6>
29. Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM. 2016. Alzheimer's disease: targeting the cholinergic system. *Curr. Neuropharmacol.* **14**: 101-115.
30. Rahimzadegan M, Soodi M. 2018. Comparison of memory impairment and oxidative stress following single or repeated doses administration of scopolamine in rat hippocampus. *Basic Clin. Neurosci.* **9**: 5-14.
31. Pisani A, Paciello F, Del Vecchio V, Malesci R, De Corso E, Cantone E, et al. 2023. The role of BDNF as a biomarker in cognitive and sensory neurodegeneration. *J. Pers. Med.* **13**: 652.
32. Lim SM, Lee NK, Paik HD. 2020. Potential neuroprotective effects of heat-killed *Lactococcus lactis* KC24 using SH-SY5Y cells against oxidative stress induced by hydrogen peroxide. *Food Sci. Biotechnol.* **29**: 1735-1740.
33. Yun SW, Park HS, Han MJ, Kim DH. 2023. *Lactobacillus gasseri* NK109 alleviates lipopolysaccharide-induced cognitive impairment in mice by up-regulating inflammation-mediated BDNF expression. *Curr. Top. Lact. Acid Bact. Probiotics* **9**: 1-8.
34. Lee HJ, Lim SM, Kim DH. 2018. *Lactobacillus johnsonii* C/JL103 attenuates scopolamine-induced memory impairment in mice by increasing BDNF expression and inhibiting NF- κ B activation. *J. Microbiol. Biotechnol.* **28**: 1443-1446.
35. Nie H, Wang X, Luo Y, Kong F, Mu G, Wu X. 2024. Mechanism explanation on improved cognitive ability of D-gal inducing aged mice model by *Lactiplantibacillus plantarum* MWFLp-182 via the microbiota-gut-brain axis. *J. Agric. Food Chem.* **72**: 9523-10162.
36. Kim WJ, Yu HS, Lee NK, Paik HD. 2022. *Levilactobacillus brevis* KU15151 inhibits *Staphylococcus aureus* lipoteichoic acid-induced inflammation in RAW 264.7 macrophages. *Probiotics Antimicrob. Proteins* **14**: 767-777.