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Lactobacillus paracasei HP7 with *Portulaca oleracea* Linn. Alleviates Scopolamine-Induced Cognitive Decline via Regulation of Neurotrophic Factor and Inflammation Signals in Mice

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ABSTRACT: People often experience cognitive deterioration of various degrees, from early-stage mild cognitive impairment to severe cognitive decline. Cognitive deterioration is related to many diseases and studied to alleviated inflammation reaction or oxidative stress. In the present study, the levels of various memory-related proteins: brain-derived neurotrophic factor (BDNF), amyloid beta (Aβ) 42, Aβ40, interleukin-6 and tumor necrosis factor-alpha were measured. Among *Lactobacillus paracasei* HP7 (HP7), *Portulaca oleracea* Linn. (PO) and HP7 together with PO (HP7A), the HP7A group had the best effect on increasing BDNF expression and suppressing Aβ40 expression. Also, we measured the protective effect on scopolamine-induced cognitive decline in mice. In the acquisition test, the HP7A group most reliably relieved cognitive decline from days 2 to 5 of scopolamine injection. When the probe test was performed on the day 6 of scopolamine injection, the HP7A group had the shortest escape latency. Based on the results of the Morris water maze tasks, we suggest that HP7A is most useful for ameliorating cognitive decline. It is suggested that the HP7A ameliorating scopolamine-induced cognitive decline via the increase of BDNF expression and the suppression of Aβ40 expression.

Keywords: cognitive decline, inflammation, *Lactobacillus*, neurotrophic factor, *Portulaca oleracea* Linn.

INTRODUCTION

Cognitive deterioration with human aging is related to many diseases characterized by chronic processes, such as inflammation or oxidative stress (Lu et al., 2014; Paniz et al., 2017). There has been showed a critical role of inflammation in cognitive ability or memory deficits, since cognitive deteriorations like Alzheimer disorders have relation with up-regulated inflammatory cytokine levels (Barrientos et al., 2009). Moreover, according to Lu et al. (2014), deficits of brain-derived neurotrophic factor (BDNF) lead to the deterioration of brain disorders, such as cognitive decline and up-regulated BDNF level can inhibit the amyloid beta (Aβ) accumulation, which lead to memory loss and learning disabilities (Eckert et al., 2003; Arancibia et al., 2008; Lu et al., 2014). Thus, the development of drugs or supplements for cognitive decline is becoming ever more urgent.

Recent evidence has revealed that probiotics can improve cognitive function by alleviating increased inflammatory cytokine level. Namely, *Lactobacillus pentosus* has been found to improve age-related memory impairment and scopolamine-induced cognitive impairment *in vivo* (Jeong et al., 2015). *Lactobacillus paracasei* has been reported to prevent age-related cognitive decline in senescent accelerated prone 8 mice (Corpuz et al., 2018; Huang et al., 2018). According to Yun et al. (2020), probiotics including *Lactobacillus* have alleviative effect against cognitive decline by regulating inflammatory cytokine such as interleukin (IL)-1β, IL-6, or tumor necrosis factor (TNF) expression.

Furthermore, *Portulaca oleracea* Linn. (PO) is well known for its cognition-enhancing properties (Sumathi and Christinal, 2016; Noorbakhshnia and Karimi-Zandi, 2017; Wang et al., 2017). This PO has been termed the 'Global Panacea' and listed as one of the most-used medicinal plants by the World Health Organization (Lim and Quah, 2017). As the nickname 'longevity vegetable' suggests, pharmacological studies have demonstrated that this plant has anti-aging and cognition-improving properties (Hongxing et al., 2007). PO, like prebiotics, also has the effect of increasing the amount of *Lactobacillus* in the broiler caecum (Zhao et al., 2013). However, the synergistic effects of probiotics with PO on cognitive impair-

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ment or anti-inflammatory effects have not been studied. Also, when they mixed or administered together, the BDNF or Aβ level which have critical relationship with cognitive decline was not reported yet.

Therefore, this study aims to investigate the synergistic effect of alleviative effect of inflammation as well as regulatory effects of neurotrophic factor of HP7A [*L. paracasei* HP7 (HP7) together with PO], and to confirm the cognitive improvement effect in mice using Morris water maze tasks.

MATERIALS AND METHODS

Reagents and equipment

Chemicals and assay kits used in this study include scopolamine, corticosterone (Sigma-Aldrich Co., St. Louis, MO, USA), mouse Aβ40, Aβ42, BDNF, TNF-α and IL-6 enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA), human IL-6 and TNF ELISA kit (BD Biosciences, Franklin Lakes, NJ, USA). Cell viability assay kit (Precaregene, Anyang, Korea) reagent was used for assay cell proliferation.

The water extract preparation of *L. paracasei* HP7, PO, and HP7A

HP7 was cultured for 24 h at 35°C in Man-Rogosa-Sharpe broth (Difco Laboratories, Detroit, MI, USA). After drying, the powdered HP7 was stored at −20°C until further use. For *in vivo* assays, HP7 was resuspended at the concentration of 1×10^9 colony forming unit (CFU)/mL in sterile phosphate-buffered saline. To prepare *P. oleracea* extract, dried aerial part of PO was purchased from Humanherb (Daegu, Korea). To prepare the water extract for the animal experiments, the dried sliced aerial part of PO was refluxed by ten to twenty times the weight (w/w) of water for 6 to 16 h. The extracts were concentrated and dried to yield PO water extracts. For *in vivo* assays, PO extract was resuspended at the concentration of 20 mg/mL in sterile phosphate-buffered saline. In order to prepare HP7A, HP7 was resuspended at the concentration of 1×10^9 CFU/mL in sterile phosphate-buffered saline and PO extract was resuspended altogether at the concentration of 20 mg/mL in sterile phosphate-buffered saline.

Cell cultures

The human neuroblastoma cells (SK-N-SH) were cultured in Eagle's minimum essential medium containing 1% antibiotic solution (Gibco, Carlsbad, CA, USA) and 10% heat-inactivated fetal bovine serum in 5% CO_2 at 37°C. For *in vitro* assay, cells (1×10^5 cells/mL) were incubated in 6 well plates with corticosterone (250 μM) in the presence of samples, HP7 (1×10^6 CFU/well), PO (50 μg/mL), or HP7A (1×10^6 CFU HP7 with 100 μg/mL PO/well) (Table 1). The culture supernatants were recovered for analysis by ELISA.

Animals and experiment design

Five weeks old male-C57BL/6 mice were purchased from Orient Bio (Seongnam, Korea). Animals were maintained under controlled climatic conditions (21.2～23.6°C, 43～65% relative humidity, 12-h light cycle with lights on during 07:00～19:00). The animal protocol in this study was reviewed and approved based on ethical procedures and scientific care by the Ethics Committee at Chaon Corp. (Yongin, Korea; IACUC no. CE21047). The mice were divided into control, scopolamine, donepezil, HP7, PO, and HP7A groups, with six mice in each group. Donepezil is a medicine that treats some types of dementia. It alleviates mental dysfunctions such as memory-deficit. Mice in the donepezil, HP7, PO, and HP7A group orally received 5 mg/kg of donepezil (Su et al., 2011), 1×10^8 CFU/mice *L. paracasei* HP7, 100 mg/kg PO, and 1×10^8 CFU/mice *L. paracasei* HP7 with 100 mg/kg PO, respectively, for 13 days (Table 2). On the day 8, all mice except those in the control group started to be injected intraperitoneally with scopolamine (1 mg/kg) 1 h before behavioral tests. After the final behavioral test, mice were sacrificed and hippocampal tissues were collected. The schedule of animal experiments is shown in Fig. 1.

Morris water maze task

The Morris water maze is commonly used to investigate spatial memory and learning. A circular swimming pool which is 90 cm in diameter and 45 cm in height is filled with opaque water mixed with 500 mL of milk to a depth of 30 cm. The water temperature was maintained at 20± 1°C. There were four sectors marked by white platforms which are 6 cm in diameter and 29 cm in height in the pool. The white platforms were invisible as they were lo-

Table 1. The experimental groups in SK-N-SH cells for *in vitro*

Group	Treatment	Corticosterone (250 μM)
Control	Vehicle	(–)
Corticosterone	Corticosterone (250 μM)	(+)
HP7	*Lactobacillus paracasei* HP7 (1×10^6 CFU/well)	(+)
PO	*Portulaca oleracea* Linn. (50 μg/mL)	(+)
HP7A	*L. paracasei* HP7 (1×10^6 CFU/well) added to *P. oleracea* Linn. (100 μg/mL)	(+)

Table 2. The animal experimental groups for *in vivo*

Group	Treatment	No. of mice	Scopolamine (1 mg/kg)
Control	Vehicle	6	(–)
Scopolamine	Scopolamine (1 mg/kg)	6	(+)
Donepezil	Donepezil (5 mg/kg)	6	(+)
HP7	*Lactobacillus paracasei* HP7 (1×10^8 CFU/mice)	6	(+)
PO	*Portulaca oleracea* Linn. (100 mg/kg)	6	(+)
HP7A	*L. paracasei* HP7 (1×10^8 CFU/mice) added to *P. oleracea* Linn. (100 mg/kg)	6	(+)

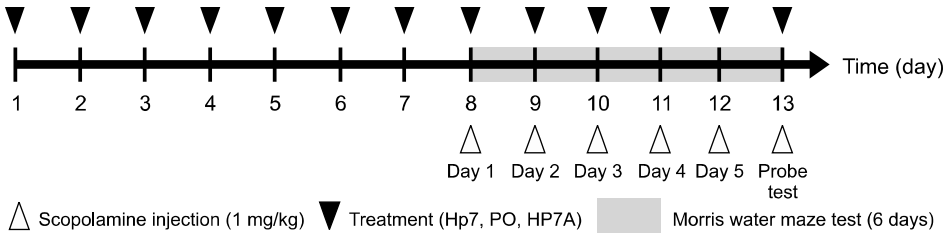

Fig. 1. Experimental design of HP7, PO, and HP7A treatment in scopolamine injected mice. HP7, PO, and HP7A were treated for 7 days before Morris water maze test. Probe test was done after scopolamine was treated for 5 days. HP7, *Lactobacillus paracasei* HP7; PO, *Portulaca oleracea* Linn.; HP7A, HP7 together with PO.

cated 1 cm below the surface of the water. Mice were trained for 60 s on the first day of the experiment. During next 4 days after the training, the learning trials were conducted 4 times per a day; the mouse was allowed to stay on the platform for 10 s either when it found a platform or when it did not find within 60 s. Each trial was performed with inter-trial interval of 30 s. Mice were dried in their cages under an infrared lamp between trials. The latency time was recorded while mice reached to the invisible platform using a Smart 3.0 video tracking system (Panlab, Barcelona, Spain). During 4 trials in a day, mice faced each quadrant of the pool wall. The probe trial session was done after the last training trial period. Mice were placed in the pool without the platform to swim and find a platform for 60 s. Moreover, target crossing time, the motion trail of the mouse and the swimming time in the quadrant were recorded.

Tissue analysis

Hippocampus samples were washed with cold phosphate-buffered saline and homogenized on ice using a bead homogenizer. The samples were then subjected to two freeze-thaw cycles to further degrade cell membranes and the supernatants were collected by centrifugation at 16,000 *g* for min. After all sample concentrations had been equalized, these samples were assayed immediately or stored at −20°C until further use.

Statistical analysis

Datasets are presented as the mean±standard error of the mean. Between-group differences were evaluated using Dunnett's multiple comparison test with one-way or two-way ANOVAs, and were deemed statistically significant at $P<0.05$.

RESULTS

The effect of HP7A on levels of inflammatory cytokines and neuroblastoma cell proliferation in *in vitro* assays

We investigated the inhibitory action of HP7, PO, and HP7A on the levels of IL-6 and TNF production in SK-N-SH cells (Fig. 2). HP7A treatment demonstrated inhibition of IL-6 and TNF production in SK-N-SH cell induced by corticosterone ($P<0.05$). HP7A treatment also showed protective effects on neuroblast cell proliferation in SK-N-SH cells induced by corticosterone ($P<0.05$). Furthermore, the synergistic effect of HP7A on recovery of IL-6 and TNF was observed.

The effect of HP7, PO, and HP7A on the hippocampus

We measured the effects of donepezil, HP7, PO, and HP7A on BDNF, Aβ42, and Aβ40 protein expression in the hippocampus (Fig. 3). The BDNF protein expression level in the hippocampus was reduced from 509.3 pg/mL in the control group to 284.8 pg/mL in the scopolamine group ($P<0.001$). These levels increased to 403.0 pg/mL, 404.7 pg/mL, and 423.3 pg/mL in the donepezil ($P<0.05$), HP7 ($P<0.05$), and HP7A ($P<0.01$; Fig. 3A) group, respectively. We also observed that Aβ42 and Aβ40 protein expression levels in the hippocampus increased from 19.01 pg/mL and 37.72 pg/mL in the control group, respectively, to 35.78 pg/mL and 72.35 pg/mL, respectively, in the scopolamine treated group ($P<0.05$). In terms of the Aβ42 expression level, only the HP7 group exhibited a significant decrease to 22.60 pg/mL ($P<0.05$; Fig. 3B). The Aβ40 expression levels decreased to 39.23 pg/mL, 40.95 pg/mL, and 38.23 pg/mL in the HP7 ($P<0.05$), PO ($P<0.05$), and HP7A ($P<0.01$; Fig. 3C) groups, respectively. We measured the effects of donepezil, HP7,

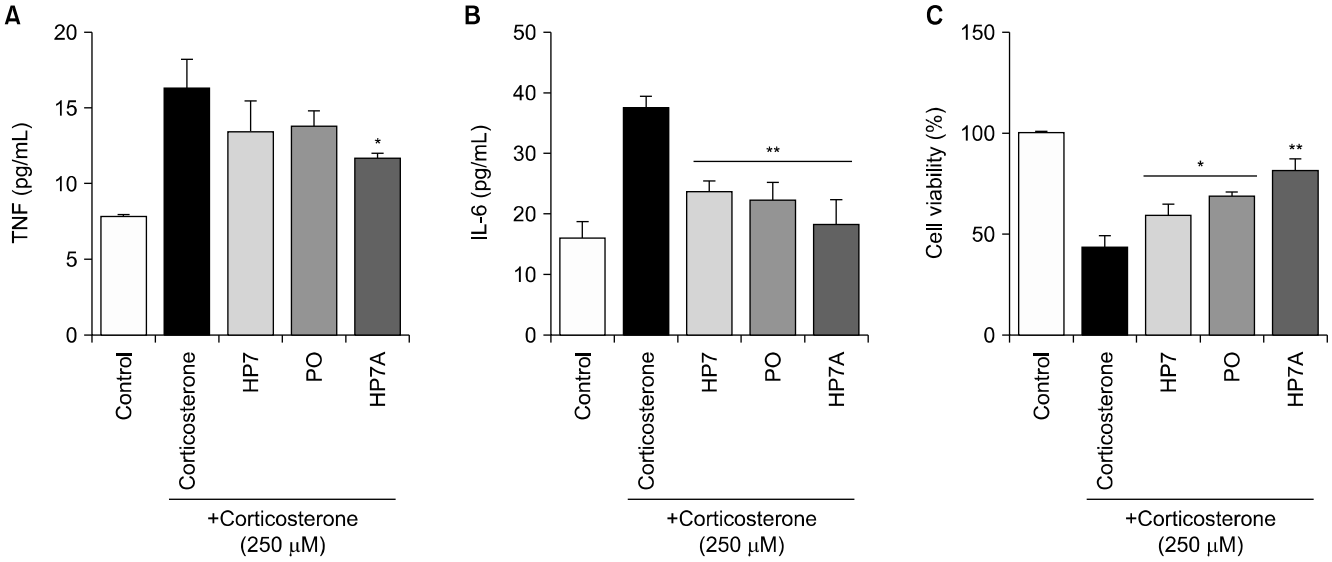

Fig. 2. The effects of HP7, PO, and HP7A on corticosterone-stimulated SK-N-SH assay. Tumor necrosis factor (TNF) (A) and interleukin (IL)-6 (B) concentrations were assessed using ELISA and neuroblastoma cell proliferation (C) was measured by using cell viability assay kit. Neuroblastoma cells stimulated with corticosterone except the control group. $^{*}P<0.05$ and $^{**}P<0.01$ vs. corticosterone-treated group. Control, vehicle treated cell; Corticosterone, corticosterone 250 μM; HP7, corticosterone-treated cell with *Lactobacillus paracasei* HP7 (1×10^{6} CFU/well); PO, corticosterone-treated cell with *Portulaca oleracea* Linn. (50 μg/mL); HP7A, HP7 (1×10^{6} CFU/well) added to PO (100 μg/mL); CFU, colony forming unit.

PO, and HP7A on IL-6 and TNF-α protein expression in the hippocampus (Fig. 4). The levels of inflammatory markers IL-6 and TNF-α in the hippocampus increased from 5.89 pg/mL and 27.79 pg/mL in the control group to 14.23 pg/mL and 340.0 pg/mL, respectively, in the scopolamine group ($P<0.05$ and $P<0.01$, respectively). HP7 was effective in reducing IL-6 and TNF-α levels to 7.06 pg/mL and 96.57 pg/mL, which were similar to those of the control group ($P<0.05$).

The effect of HP7, PO, and HP7A on acquisition test for cognitive performance

As shown in Fig. 5A, the escape latency of the five groups tended to decrease from days 1 to day 4, which indicates that training improved learning capacity. On days 2, 3, and 4, escape latencies in the scopolamine group in-

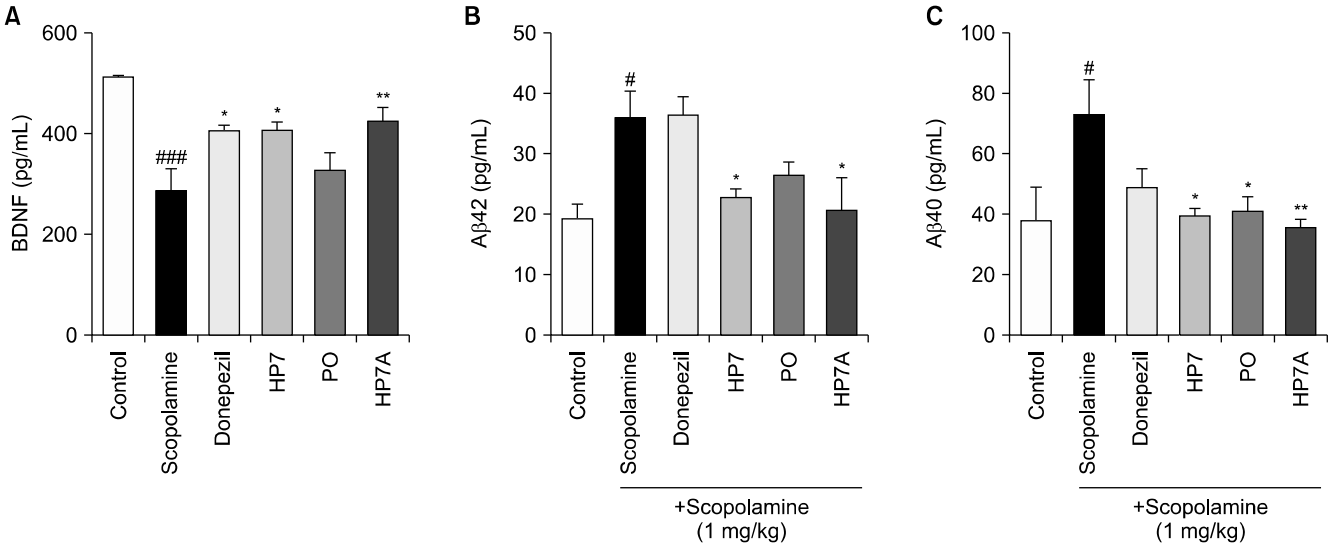

Fig. 3. The effects of HP7, PO, and HP7A on hippocampal brain-derived neurotrophic factor (BDNF), amyloid beta (Aβ) 42, and Aβ40 levels. Mouse hippocampus samples were collected after induced by scopolamine and BDNF (A), Aβ42 (B), and Aβ40 (C) concentrations were assessed using ELISA. Mice were orally administered test agents or an equal volume of vehicle once daily for 13 days. $^{\#}P<0.05$ and $^{\#\#\#}P<0.001$ vs. control group; $^{*}P<0.05$ and $^{**}P<0.01$ vs. scopolamine-treated group. Control, mice treated with vehicle; Scopolamine, scopolamine-injected mice treated with vehicle; Donepezil, scopolamine-injected mice treated with donepezil (5 mg/kg); HP7, scopolamine-injected mice treated with *Lactobacillus paracasei* HP7 (1×10^{8} CFU/mice); PO, scopolamine-injected mice treated with *Portulaca oleracea* Linn. (100 mg/kg); HP7A, HP7 (1×10^{8} CFU/mice) added to PO (100 mg/kg); CFU, colony forming unit.

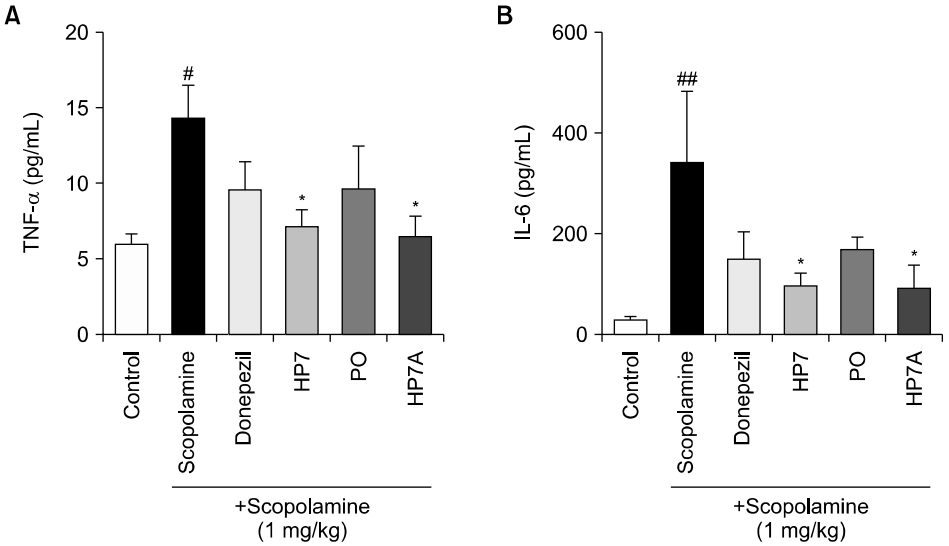

Fig. 4. The effects of HP7, PO, and HP7A on hippocampal interleukin (IL)-6 and tumor necrosis factor (TNF)-α levels. Mouse hippocampus samples were collected after induced by scopolamine, and TNF-α (A) and IL-6 (B) were assessed by ELISA. Mice were orally administered test agents or an equal volume of vehicle once daily for 13 days. $^{\#}P<0.05$ and $^{\#\#}P<0.01$ vs. control group; $^{*}P<0.05$ vs. scopolamine-treated group. Control, mice treated with vehicle; Scopolamine, scopolamine-injected mice treated with vehicle; Donepezil, scopolamine-injected mice treated with donepezil (5 mg/kg); HP7, scopolamine-injected mice treated with *Lactobacillus paracasei* HP7 (1×10^8 CFU/mice); PO, scopolamine-injected mice treated with *Portulaca oleracea* Linn. (100 mg/kg); HP7A, HP7 (1×10^8 CFU/mice) added to PO (100 mg/kg); CFU, colony forming unit.

creased to 1.95, 2.09, and 4.44 times of those of the control group ($P<0.01$, $P<0.001$, and $P<0.001$, respectively), which indicated that chronic treatment of scopolamine impaired the learning capacity of mice. The average escape latency of the donepezil, HP7, and HP7A groups on day 2 reduced to 86%, 87%, and 72% of that of the scopolamine group; however, only the PO group reached significance, at 70% ($P<0.05$). Compared to the scopolamine group, the average escape latency of the donepezil and PO groups on day 3 reduced to 70% and 69%; however, only the HP7 and HP7A groups reached the significance levels of 57% and 60% ($P<0.01$). Compared to the scopolamine group, the average escape latency of the PO group on day 4 reduced to 73%; however, only the donepezil, HP7, and HP7A groups reached the significance levels of 52%, 51%, and 50% ($P<0.01$), which indicates that HP7 and HP7A can enhance the learning capacity of mice.

The effect of HP7, PO, and HP7A on probe test for cognitive performance

We measured the effects of donepezil, HP7, PO, and HP7A on the escape latency in the probe test of the Morris water maze task (Fig. 5B). Scopolamine increased the escape latencies to 41.09 s, which indicates that repetitive treatment of scopolamine impairs the spatial memory capacity of mice. Donepezil, HP7, PO, and HP7A reduced the escape latency to 14.69 s, 18.40 s, 13.80 s, and 10.99 s on the probe test, respectively, which indicates that HP7, PO, and HP7A alleviate spatial memory impairment, similar to donepezil ($P<0.01$, $P<0.05$, $P<0.01$, and $P<0.01$, respectively). Scopolamine decreased target crossings to 23% in the probe test. Donepezil, HP7, PO, and HP7A restored the target crossings to 91%, 73%, 77%, and 82%, but these were not significant (Fig. 5C). As shown in Fig. 5D and 5E, the time spent in the target platform position decreased non-significantly to 31.01% in scopolamine-treated group, compared to 52.88% in the control group. Compared to the scopolamine-treated mice, the time donepezil, HP7, PO, and HP7A groups spent in the target platform position quadrant non-significantly increased to 48.06%, 36.4%, 39.65%, and 39.57%.

DISCUSSION

Cholinergic disorder is an important feature of Alzheimer's disease pathology (Sarter and Bruno, 2004; Ishrat et al., 2009), since it can lead to memory loss and cognitive impairment over the years (Terry and Buccafusco, 2003). Previous reported that scopolamine is an anti-cholinergic drug, but it can cause memory loss in healthy young subjects mimicking the memory impairment observed in elderly people not under treatment with anti-dementia drugs (Tariot et al., 1996). Thus, we investigated the anti-inflammatory effects of HP7, PO, and HP7A on scopolamine-induced cognitive decline in mice as well as the protective effects of neurotrophic factor, such as BDNF.

Numerous studies have explored that BDNF plays a critical role in memory and learning (Hall et al., 2000; Mizuno et al., 2000; Alonso et al., 2002; Broad et al.,

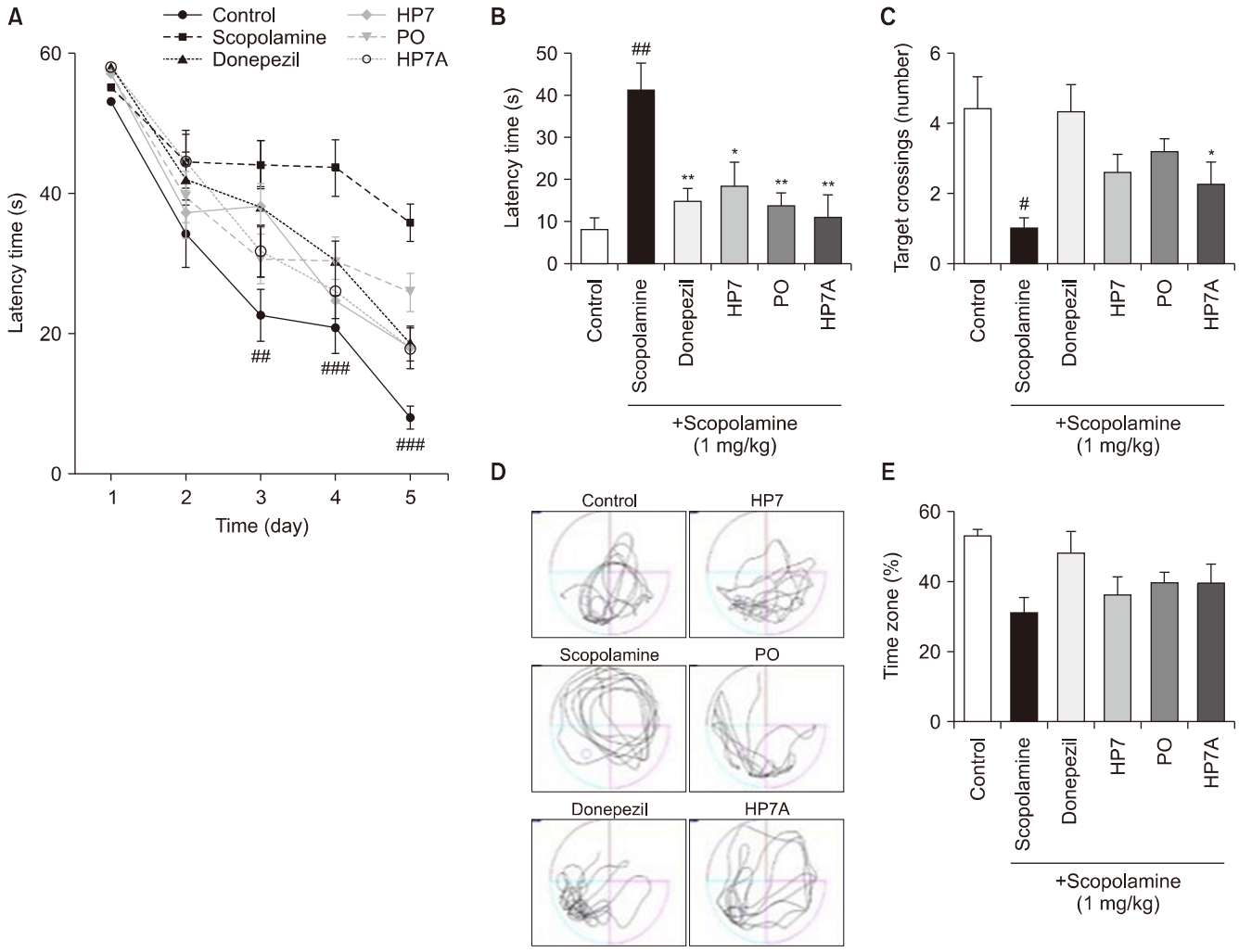

Fig. 5. The effect of HP7, PO, and HP7A on cognitive performance in Morris water maze tests. (A) Latency time (s) in the acquisition test. (B) Latency time (s), (C) target crossing, (D) motion trails of mice, and (E) time in zone (%) in the probe test. All tests were carried out 1 h after the administration of test agents or vehicle. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ vs. control group; $^{*}P<0.05$ and $^{**}P<0.01$ vs. scopolamine-treated group. Control (●), mice treated with vehicle; Scopolamine (■), scopolamine-injected mice treated with vehicle; Donepezil (▲), scopolamine-injected mice treated with donepezil (5 mg/kg); HP7 (◆), scopolamine-injected mice treated with *Lactobacillus paracasei* HP7 (1×10^8 CFU/mice); PO (▼), scopolamine-injected mice treated with *Portulaca oleracea* Linn. (100 mg/kg); HP7A (○), HP7 (1×10^8 CFU/mice) added to PO (100 mg/kg); CFU, colony forming unit.

2002; Ma et al., 2011). However, this is the first study to examine BDNF expression levels in the hippocampus after HP7 or PO administration in mice. BDNF promotes synaptic transmission and plasticity, which play a key role in memory formation and storage (Park et al., 2012). BDNF participates in the formation of long-term potentiation by increasing N-methyl-D-aspartate receptor sensitivity (Figurov et al., 1996; Madara and Levine, 2008). Our results showed that the HP7A group almost restored hippocampal BDNF levels in scopolamine-injected mice, which suggests that HP7A ameliorates scopolamine-induced cognitive decline by the increase of BDNF expression.

According to a recent study, chronic administration of scopolamine results in an increase of Aβ levels in brain (Hernández-Rodríguez et al., 2020). Amyloid plaques are one of the main remarks of Alzheimer's disease. The soluble Aβ molecules consisted of 40- or 42-residue peptide transforms into plaque-associated fibers (Walsh et al., 1997). The Aβ42 variant is more hydrophobic and tends to aggregate easily to develop fibrillogenesis than Aβ40. Aβ40 takes more various conformational structures than Aβ42, which attribute to the oligomerization pathway and detection time during aggregation (Vestergaard et al., 2005). Aβ accumulation results in cell death, which in turn leads to memory loss and learning disabilities (McGleenon et al., 1999; Eckert et al., 2003). However, it has been reported that increased BDNF signaling protects cells from Aβ deposition and improves cognitive decline (Arancibia et al., 2008; Moghbelinejad et al., 2014). We measured Aβ concentrations in hippocampal tissue. The HP7A group most markedly reduced Aβ40 levels, compared to the scopolamine group. These results demonstrate that HP7A can suppress Aβ accumulation by in-

creasing BDNF expression, as previously reported (Koh et al., 2018).

In the acquisition test, the HP7A group most reliably relieved cognitive decline from day 2 to 5. Compared to the scopolamine group on the day 6 for probe test, the HP7A group had the shortest escape latency. Although the results of target crossing and time in zone were not significant, our results suggest that HP7A can most alleviate spatial memory impairments and excellently increase the learning capacity of mice. Next, we measured the levels of various memory-related proteins: BDNF, Aβ 42, Aβ40, TNF-α, and IL-6.

We confirmed the recovery effects of HP7, PO and HP7A on inflammatory cytokine such as TNF and IL-6 in corticosterone stimulated neuroblastoma cell, SK-N-SH (Kim et al., 2018). HP7 and PO also showed protective effects of neuroblastoma cell proliferation that stimulated by corticosterone, respectively. Interestingly, HP7A showed synergic effect on recovery of inflammatory cytokine as well as neuroblastoma cell protective effects on corticosterone stimulation. There is growing evidence that neuritis can interfere with cognition (Li et al., 2012; Allison and Ditor, 2014; Moon et al., 2014). Neuritis also plays an important role in neurodegenerative diseases such as Alzheimer's disease and ischemic stroke (DeLegge and Smoke, 2008; Hovens et al., 2014). TNF-α is a key pro-inflammatory cytokine that induces the secretion of other cytokines (Qin et al., 2008). Previous studies have revealed that inflammatory mediators stimulate apoptosis pathways and neuronal cell death (Semmler et al., 2007; Belarbi et al., 2012). It has been found that cytokines such as TNF-α interfere with long-term potentiation and neurogenesis in the hippocampus (Kim and Diamond, 2002; Monje et al., 2003). It has also been demonstrated that increased production of IL-6 leads to the dysfunction of neuronal stem cells and the inhibition of neurogenesis, resulting in impaired cognition and learning abilities (Monje et al., 2003).

Collectively, our findings suggest HP7A, a mixture of *L. paracasei* HP7 and PO, showed synergies of alleviative effects on inflammatory cytokines or protective effect in neuroblastoma cell. In addition, HP7A administration improved cognitive decline in scopolamine-induced mice via regulation of neurotrophic factor, BDNF and suppression of inflammatory signals, such as TNF, IL-6 and Aβ40 or 42 level in hippocampus. Also, HP7A alleviated cognitive performance on Morris water maze test.

These health-promoting effect of the administration of HP7A can be related not only to the viability of probiotics but also to gut microbiota. According to the previous study, the combination of probiotics and plant extracts can improve the viability of probiotics during storage (Michael et al., 2015). It is possible that PO enhanced the viability of HP7, resulting in HP7A showed greater efficacy than either PO or HP7. The combination of probiotics and prebiotics is called synbiotics when there is a proven health-benefit in co-administration (Bilal et al., 2022). The other possible mechanism of the synbiotics of HP7A may be relevant to gut microbiota. Supplementation with synbiotics can affect gut microbiota and regulate microbial metabolites including short chain fatty acids and secondary metabolites of plant extracts which are small organic molecules beneficial to gut health (Russell and Duthie, 2011; Bilal et al., 2022). However, further study for underlying mechanism of synergistic effect of HP7A remains the subject of investigation.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: JHK, JHR, SDP, JJS, JLL. Methodology: JHK, JHR. Software: JHK, JHR. Validation: JHK, HK. Formal analysis: JHK. Investigation: JHK, JHR. Writing—original draft preparation: JHK. Writing review and editing: SDP. Visualization: JHK. All authors have read and agreed to the published version of the manuscript.

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