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Research Article

Panaxcerol D from *Panax ginseng* ameliorates the memory impairment induced by cholinergic blockade or $A\beta_{25-35}$ peptide in mice

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ABSTRACT

Background: Alzheimer's disease (AD) has memory impairment associated with aggregation of amyloid plaques and neurofibrillary tangles in the brain. Although anti-amyloid β (Aβ) protein antibody and chemical drugs can be prescribed in the clinic, they show adverse effects or low effectiveness. Therefore, the development of a new drug is necessarily needed. We focused on the cognitive function of *Panax ginseng* and tried to find active ingredient(s). We isolated panaxcerol D, a kind of glycosyl glyceride, from the non-saponin fraction of *P. ginseng* extract.

Methods: We explored effects of acute or sub-chronic administration of panaxcerol D on cognitive function in scopolamine- or $A\beta_{25-35}$ peptide-treated mice measured by several behavioral tests. After behavioral tests, we tried to unveil the underlying mechanism of panaxcerol D on its cognitive function by Western blotting.

Results: We found that pananxcerol D reversed short-term, long-term and object recognition memory impairments. The decreased extracellular signal-regulated kinases (ERK) or Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) in scopolamine-treated mice was normalized by acute administration of panaxcerol D. Glial fibrillary acidic protein (GFAP), caspase 3, NF-kB p65, synaptophysin and brain-derived neurotrophic factor (BDNF) expression levels in $A\beta_{25-35}$ peptide-treated mice were modulated by sub-chronic administration of panaxcerol D.

Conclusion: Pananxcerol D could improve memory impairments caused by cholinergic blockade or Aβ accumulation through increased phosphorylation level of ERK or its anti-inflammatory effect. Thus, panaxcerol D as one of non-saponin compounds could be used as an active ingredient of *P. ginseng* for improving cognitive function.

1. Introduction

Dementia is a common feared health obstacle in the elderly [1]. It could be grouped into Alzheimer's disease (AD), vascular dementia or Lewy body disease, with AD accounting for the most among all dementia patients [2]. Although it is prominent that aggregation of amyloid β protein (Aβ) and/or tangle of tau protein [3] in the brain of an AD patient, the exact etiology of AD remains unclear. Acetylcholinesterase (AChE) inhibitors such as donepezil can be prescribed for treating AD patients to delay cognitive decline through activation of the cholinergic neurotransmitter system in the clinic [4]. Although AChE inhibitors can temporarily alleviate the decline of cognitive function, they have adverse effects such as diarrhea, anorexia, and dizziness [5]. Therefore, it is urgent to invent a new drug that can delay the progression or the onset of dementia with few adverse effects.

Panax ginseng Meyer (Araliaceae) has been applied as a tonic agent in conventional herbal prescriptions in Eastern country, especially, China, Japan, and Korea [6]. It has been well acknowledged that *P. ginseng* can enhance immune function [7], cognitive performances of elderly people [8], or AD patients [9]. Ginsenosides

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are primary working compounds of *P. ginseng*. Some of them can reverse impaired cognitive function [10,11]. For example, ginsenoside Rb1 and Re can reduce Aβ production by activating peroxisome proliferator-activated receptor γ (PPAR-γ) [12]. Ginsenoside Rg3 is able to ameliorate memory deficit induced by lipopolysaccharide through its anti-inflammatory activity [13]. Ginsenoside Rg1 can inhibit γ-secretase activity, resulting in reduction of Aβ accumulation in the brain [14]. As described above, most research studies on *P. ginseng* were focused on ginsenosides. However, studies on other chemical groups of *P. ginseng* are limited except for gintonin with respect to its effect on cognitive function [15]. In a pilot study, we found that EtOAc fraction of *P. ginseng* had ameliorating activity against cognitive disorder caused by cholinergic neurotransmitter blocking in mice. Subsequently, we attempted to separate active component(s) from EtOAc fraction and found panaxcerol D, a kind of glycosyl glyceride. Panaxcerol D has anti-inflammatory effect by inhibiting NO production or skin-whitening activity by inhibiting melanin biosynthesis [16,17]. However, whether panaxcerol D can reverse cognitive impairments has not been explored yet.

In the current study, we inspected whether panaxcerol D could ameliorate cognitive impairment in scopolamine- or $A\beta_{25-35}$ peptide-induced AD mice model. We performed several behavioral tests such as the Y-maze test, the novel object recognition test (NORT), the passive avoidance test (PAT) to probe effects of panaxcerol D on cognitive functions. In addition, we explored the underlying mechanism of panaxcerol D by investigating signaling pathway(s) associated with cognitive functions.

2. Materials and methods

2.1. Animals

Six-week-old CD-1 mice (27-30 g body weight) were procured from Orient Co., Ltd. (Gyeonggi-do, Korea). Mice were housed in cages (5 mice/cage) and supplied food and water freely accessible in a university animal care facility. The animal room was maintained under a 12 h light/dark cycle (light on from 07:30-19:30) at a keeping temperature of 23 ± 1 °C with a relative humidity of 60 ± 10%. Animals were cared in line with the Animal Care and Use Guidelines printed by Kyung Hee University. After acclimation in the animal room for one week, mice were used for experiments. All experimental processes using animals were authorized by the Institutional Animal Care and Use Committee of Kyung Hee University (approval number: KHSASP-21-567).

2.2. Materials

Scopolamine hydrobromide, donepezil hydrochloride and avertin (2,2,2-tribromoethanol) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). *tert*-Amyl alcohol (2-methyl-2-butanol) was bought from Thermo Fisher Scientific Co. (Waltham, MA, USA). Aβ protein fragment 25-35 ($A\beta_{25-35}$) was bought from BACHEM Co. (Bubendorf, Switzerland). Rabbit polyclonal anti-phosphorylated extracellular signal-regulated kinase (pERK), anti-ERK, and anti-phosphorylated p65 antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA). Rabbit polyclonal anti-phosphorylated Ca^{2+}/calmodulin-dependent protein kinase II (pCaMKII), mouse monoclonal anti-CaMKII, anti-p65, and anti-β-actin antibodies were bought from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Rabbit polyclonal anti-brain-derived neurotrophic factor (BDNF), anti-synaptophysin, and mouse monoclonal anti-caspase 3 antibodies were acquired from Abcam (Cambridge, UK). Rabbit polyclonal anti-glial fibrillary acidic protein (GFAP) antibodies were bought from GeneTex, Inc. (Irvine, CA, USA). For column chromatography, Sephadex LH-20 (Amersham Pharmacia Biotech, Amersham), silica gel (70–230 mesh, Merck, Kenilworth, NJ, USA) and LiChroprep RP-18 (40–63 μm, Merck, Kenilworth, NJ, USA) were purchased. All other materials were purchased from normal commercial sources. They were of the highest quality available. Panaxcerol D was used after it was resolved in 10% Tween 80 solution with 3% dimethyl sulfoxide. Scopolamine and donepezil were used after they were resolved in 0.9% saline solution.

2.3. Isolation of panaxcerol D

Dried roots of *P. ginseng* were bought from a local market (Chungcheongnam-do, Korea) and verified by one of the authors, Prof. DS Jang. A voucher specimen (PAGI-2019) of the raw material was retained in the Laboratory of Natural Product Medicine, College of Pharmacy, Kyung Hee University, Seoul, Republic of Korea.

The raw material (40.53 kg) was extracted with 70% acetone twice at room temperature for 3 days. The solvent was evaporated at 42 °C. The extract (857.66 g) was then suspended in H_2O and partitioned with EtOAc to yield EtOAc extract (86 g) and water-soluble extract (772 g), respectively. The EtOAc-soluble fraction (84 g) was separated by column chromatography (CC) using silica gel (70–230 mesh; φ 6.5 × 48.0 cm) as stationary phase with a gradient system of *n*-hexane-EtOAc gradient (95:5 to 80:20, v/v) to give 24 fractions (E1 – E24). Fraction E18 (7.87 g) was fractionated further using Sephadex LH-20 CC (φ 5.5 × 65.3 cm, CH_2Cl_2–MeOH = 50:50) to yield eight subfractions (E18-1 – E18-8). Subfraction E18-3 was separated by LiChroprep RP-18 (ODS-A 12 nm S-150 μm, φ 4.0 × 43.0 cm, acetone-H_2O = 75:25 to 100:0 v/v) to obtain panaxcerol D (714.2 mg). The purity of the panaxcerol D isolated was over 98% by DART-MS and NMR analysis. The structure of panaxcerol D was identified based on spectroscopic data, especially based on 1D NMR and DART-MS studies with the literature [16]. The structure of panaxcerol D is presented in Fig. 1.

2.4. $A\beta_{25-35}$ peptide injection

$A\beta_{25-35}$ peptide injection mouse model has been used for memory impaired animal model became $A\beta_{25-35}$ peptide induces multiple disturbances in cellular integrity [18,19]. $A\beta_{25-35}$ peptide was resolved in filtered sterilized 0.05 M PBS (1 μg/μl) and incubated at 38 °C for 72 h for aggregation. After anesthesia with avertin (5 mg/kg, i.p.), mice were injected with aggregated $A\beta_{25-35}$ peptide or vehicle (3 μl/3 min) into the right lateral ventricle at stereotaxic coordinates (AP, −0.2 mm; ML, +1.0 mm; DV, −2.5 mm) taken from the atlas of the mouse brain [20]. Backflow was minimized by holding the needle for 2 min after injection. Mice were then placed in a warm (32-33 °C) incubator and kept until they awoke.

2.5. Drug administration

For acute treatment studies, panaxcerol D (3, 10 or 30 mg/kg), donepezil (5 mg/kg) or vehicle was administered 1 h before Y-maze, the acquisition trial in PAT, or the training session of NORT.

To explore the memory improvement of sub-chronic treatment with panaxcerol D, the mice were administered of panaxcerol D (30 mg/kg), donepezil (5 mg/kg) or vehicle once a day for 7 days from the day after $A\beta_{25-35}$ injection. In addition, the administration of panaxcerol D, donepezil or vehicle was continuously conducted during behavioral tests. The administration was performed 1 h before the Y-maze task, habituation and training session in NORT, acquisition trial in PAT. Also, the administration of panaxcerol D or donepezil was performed after probe session in NORT, and retention trial in PAT, as presented in Fig. 3.

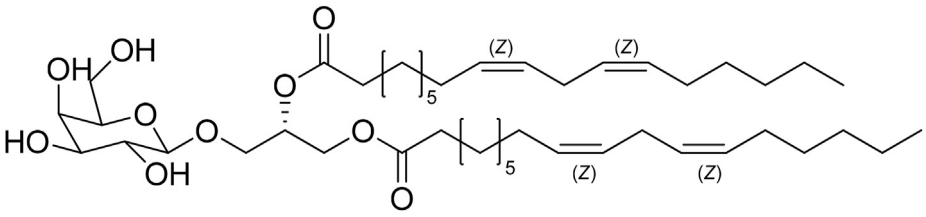


Fig. 1. The chemical structure of panaxcerol D.

2.6. Y-maze test

The Y-maze test has been employed for measuring hippocampus-dependent spatial working and short-term memory of mouse [21]. The Y-maze has Y-shaped arms with equal length (40 cm-long, 4 cm-wide, 11 cm-high walls) and distance, apart from one another by 120° angles as described previously [22]. Mice were positioned in the center of the maze. They were let to move without restraint. They were recorded for 6 min with a video camera. The successive order and number of arm entries were manually counted (i.e. ABCBCBA). An actual alternation was determined as a mouse enter three different arms successively (i.e., ABC, BAC, or CBA). The percentage of spontaneous alternation was defined as the following formula: alternation (%) = [(number of real alternations)/(total arm entries-2)] × 100.

2.7. NORT

NORT has been used for measuring the recognition memory in mouse [23]. NORT was executed carried for 4 days: 2 days for habituation, 1 day for training and the final day for probe test as described previously [24]. In the habituation session, mice were placed to habituate for 10 min in a NORT box (25 × 25 × 25 cm), Mice were let to move without restraint within the NORT box without any object. In the training session, mice were introduced into the test box for habituation. After habituation for 5 min, two indistinguishable objects were placed in slanted corners of the test box. Mice were let to perceive objects for 5 min. The following day after the training session, the probe session was executed. After habituation for 5 min, one of two familiar objects was substituted with a novel object that had a nonidentical shape with comparable material to the familiar object. Mice were let to move freely for 5 min. Their exploring behaviors were recorded with a video camera. They were completely dried before each test. The discrimination ratio (%) was defined as the following formula: $[(T_{novel} - T_{familiar})/(T_{novel} + T_{familiar})] \times 100$.

2.8. PAT

PAT has been used for assessment of the long-term memory of mouse [25]. PAT was executed for two continuous days as acquisition and retention trials in a device configuring two connected same chambers (20 × 20× 20 cm), a light chamber and a dark chamber, divided by a guillotine door (5× 5 cm) as reported previously [26]. The light chamber had a light source (50 W bulb). However, the dark chamber had no light source. A stainless steel grid (diameter: 2 mm) was set in the floor of chambers. At the day of the acquisition trial, each mouse was positioned softly in the light chamber. The guillotine door was opened carefully 30 s after placing the mouse. When mice went inside the dark chamber, the door was get down and mice were given an electric foot shock (0.5 mA, 3 s) through steel rods. The retention trial was performed by placing mice in the light chamber at 24 h after the acquisition trial. The guillotine door was gently lifted after 30 s and the latency that a mouse went in the dark chamber was recorded. The step-through latency was recorded up to 300 s.

2.9. Western blot analysis

The day after PAT, mice were sacrificed by cervical dislocation at 1 h after administration of panaxcerol D (3, 10 or 30 mg/kg, p.o.) or donepezil (5 mg/kg, p.o.) with 30 min after administration of scopolamine (1 mg/kg, i.p.) and their brains were removed. Isolated whole cortex and hippocampus were homogenized in ice-cold Tris-HCl buffer (20 mM, pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride, 1 mM orthovanadate, 1 mM sodium fluoride, 1 mM EDTA, 0.32 M sucrose, complete protease inhibitor (1 tablet / 50 mL of buffer) and a phosphatase inhibitor cocktail (1 tablet / 10 mL). The homogenized tissue was centrifuged twice at 14,000 rpm for 20 min at 4 °C. The supernatant was collected separately. Quantified proteins (15 μg) were loaded onto sodium dodecyl sulfate polyacrylamide gel electrophoresis gels (10% or 12%) under reduced conditions. After transfer, polyvinylidene difluoride (PVDF) membranes were incubated in 5 % skim milk for 2 h at room temperature. They were then incubated with primary antibodies (1:1000 dilution in 3 % skim milk) at 4 °C overnight. After membranes were washed with Tris-buffered saline (20 mM, pH 7.4) containing Tween-20 (TBST) six times for 10 min each, they were incubated with secondary antibodies (1: 5,000 dilution) suitable for each primary antibody at room temperature for 2 h. After membranes were washed with TBST six times for 10 min each, immunoblots were developed with enhanced chemiluminescence (Amersham Life Science, Arlington Heights, IL, USA) and analyzed using a Molecular Image Gel Doc XR + system (Bio-Rad Laboratory, Berkeley, CA, USA).

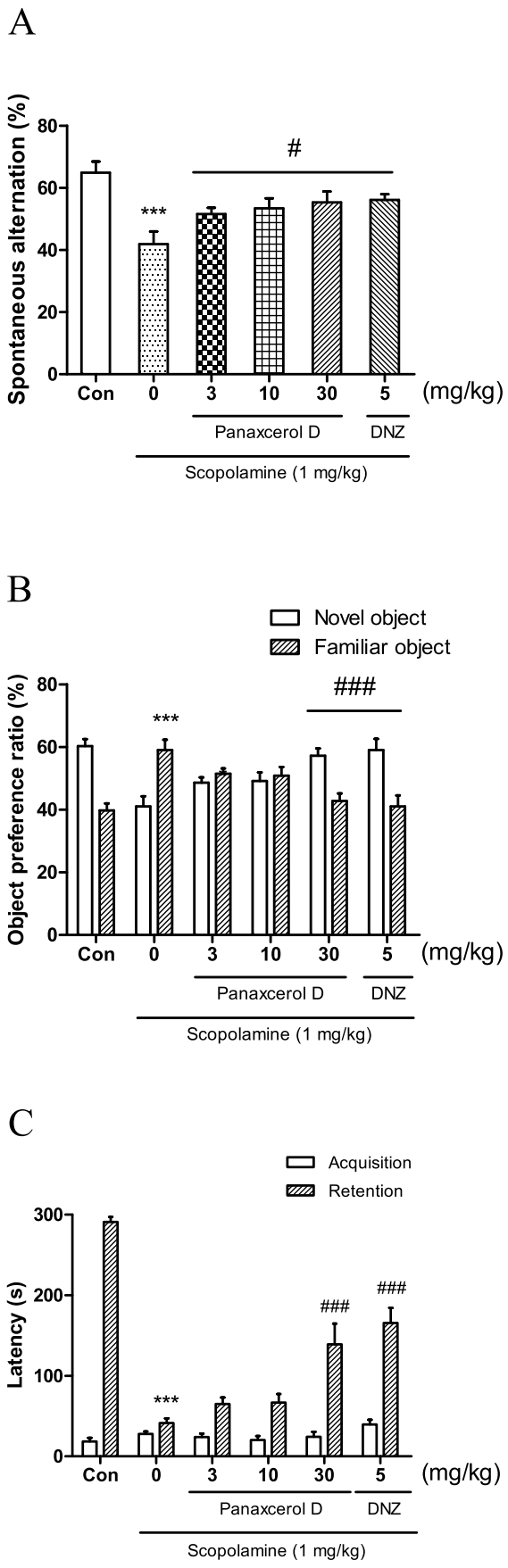
2.10. Statistical analysis

Data are presented as mean ± standard error of the mean (S.E.M.). Results of the Y-maze test, PAT, and Western blot analysis were analyzed by one-way analysis of variance (ANOVA) with the Newman-Keuls multiple comparison test. Two-way ANOVA followed by Bonferroni's post hoc test was used to analyze the object preference ratio in NORT. Differences between groups were considered significant at $P < 0.05$. All statistical analyses were performed with Prism 5.0 software (GraphPad, La Jolla, CA, USA). Researchers who were blinded to each treatment analyzed behavioral data.

3. Results

3.1. Panaxcerol D improves cognitive impairment caused by cholinergic neurotransmitter blocking in behavioral tests

Y-maze test was employed to evaluate effects of panaxcerol D on working memory and short-term memory. There were significant



group differences in spontaneous alternation [F [5,50] = 5.099, P = 0.0007; Fig. 2A]. The spontaneous alternation was decreased by injection of scopolamine. However, it was significantly recovered by panaxcerol D administration ($P < 0.05$), as observed in donepezil-administered group ($P < 0.05$). Total arm entries showed no significant group differences (data not shown), suggesting that panaxcerol D did not affect their spontaneous locomotor behavior [27].

Next, we conducted NORT to investigate the effect of panaxcerol D on object recognition memory in scopolamine-induced memory impaired mice. Two-way ANOVA showed significant group differences in object preference ratio [object, $F(1, 102) = 10.41$, $P = 0.0017$; treatment, $F(5, 102) = 15.40$, $P < 0.0001$; interaction $_{\text{object x treatment}}$ $F(5, 102) = 0$, $P = 1$; Fig. 2B]. The increase of preference ratio to familiar object of scopolamine-treated group was significantly recovered after administration of panaxcerol D ($P < 0.0001$), as observed in donepezil-administered group.

PAT was also executed to measure effects of panaxcerol D on long-term memory in memory impairment induced by cholinergic blockade. There were no significant group differences in step-through latency in the acquisition trial [F [5,54] = 1.937, P = 0.1033, Fig. 2C]. However, there were significant group differences in the latency between groups in the retention trial [F [5,54] = 41.07, $P < 0.0001$; Fig. 2C]. The latency in the scopolamine-administered group was significantly decreased compared to that in the control group ($P < 0.001$). However, treatment with panaxcerol D at 30 mg/kg significantly ($P < 0.001$) reversed such decrease of latency caused by scopolamine treatment. The effect of panaxcerol D was similar to that of donepezil.

3.2. Sub-chronic administration of panaxcerol D ameliorates cognitive dysfunction impaired by $A\beta_{25\text{-}35}$ peptide injection in behavioral tests

In the acute treatment study, panaxcerol D reversed memory impairment induced by cholinergic blockade. Therefore, we next explored whether sub-chronic treatment with panaxcerol D could rescue cognitive dysfunction induced by $A\beta_{25\text{-}35}$ peptide injection. There were no significant difference in spontaneous alternation behavior among groups in Y-maze test [F [3,38] = 2.433, P = 0.0813; Fig. 3A]. Total arm entries were not significantly different across groups either (data not shown).

In NORT, there were significant group differences in object preference ratio based on two-way ANOVA [object, $F(1, 70) = 234.5$, $P < 0.0001$; treatment $F(3, 70) = 14.50$, $P < 0.0001$; interaction $_{\text{object x treatment}}$ $F(3, 70) = 0$, $P = 1$; Fig. 3B]. The decrease of novel object preference ratio by $A\beta_{25\text{-}35}$ peptide treatment was significantly recovered by panaxcerol D administration ($P < 0.001$), as observed in donepezil-administered group.

In PAT, there were no significant group differences in the acquisition trial [F [3,38] = 1.790, P = 0.1670; Fig. 3C]. On the other hands, there were significant group differences in the retention trial [F [3,38] = 29.47, $P < 0.0001$; Fig. 3C]. The latency in the $A\beta_{25\text{-}35}$-treated group was significantly lower than in the sham group. However treatment with panaxcerol D significantly reversed such

Fig. 2. Effects of panaxcerol D on cognitive function measured by Y-maze test, NORT, and PAT in mice
The spontaneous alternation (A) in Y-maze test, the object preference ratio (B) in NORT, and the step-through latencies in the acquisition trial and retention trial (C) in PAT were presented. Data were presented means ± S.E.M. (n = 8-10/group) (***$P < 0.001$ versus the vehicle-administered group; $^{\#}P < 0.05$ versus the scopolamine administered group; $^{\#\#\#}P < 0.001$ versus the scopolamine administered group). Con, control.

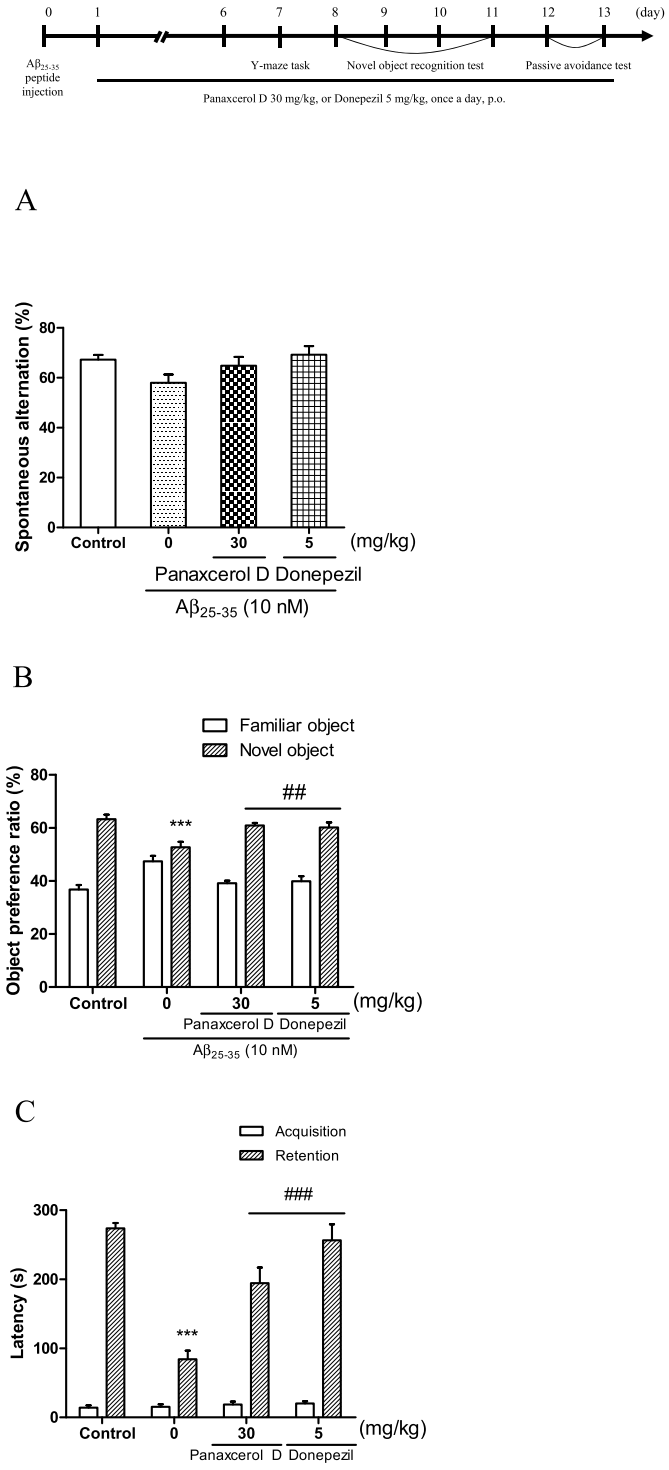


Fig. 3. Effects of sub-chronic panaxcerol D treatment on $A\beta_{25\text{-}35}$ peptide-induced memory impairment in Y-maze test, NORT, and PAT in mice
The spontaneous alternation (A) in Y-maze test, the object preference ratio (B) in NORT, and the step-through latencies in the acquisition trial and retention trial (C) in PAT were presented. Data were presented means ± S.E.M. (n = 9-10/group) (***P < 0.001 versus the vehicle-administered group; ##P < 0.01 versus the scopolamine administered group; ###P < 0.001 versus the scopolamine administered group). Con, control.

decrease in latency by $A\beta_{25\text{-}35}$ peptide treatment. The effect of panaxcerol D on latency was similar to that of donepezil.

3.3. Effects of panaxcerol D on phosphorylation levels of ERK and CaMKII in memory impairment mice model induced by cholinergic blockade

We examined phosphorylation levels of ERK and CaMKII in the hippocampus after panaxcerol D administration. Expression levels of each signaling molecule in the hippocampus were analyzed by one-way ANOVA [p-ERK, $F[5,30] = 18.25$, $P < 0.0001$; p-CaMKII, $F[5,30] = 9.804$, $P < 0.0001$]. Panaxcerol D administration significantly increased levels of p-ERK and p-CaMKII compared to scopolamine administration ($P < 0.05$, Fig. 4), as shown in donepezil-treated group.

3.4. Effects of panaxcerol D sub-chronic administration on expression levels of ERK, synaptophysin, GFAP, caspase 3, NF-kB, and BDNF in $A\beta_{25\text{-}35}$ peptide-injected AD mice model

We inspected expression levels of GFAP, caspase 3, synaptophysin, and BDNF, and phosphorylation levels of ERK and NF-kB in the cortex after panaxcerol D administration. Expression levels of each signaling molecule were analyzed by one-way ANOVA in the cortex [GFAP, $F[3,20] = 6.137$, $P = 0.0039$; caspase 3, $F[3,18] = 4.800$, $P = 0.0125$; p-ERK, $F[3,20] = 3.620$, $P = 0.0310$; p-p65, $F[3,20] = 5.068$, $P = 0.009$; BDNF, $F[3,20] = 8.628$, $P = 0.0007$]. In the cortex, panaxcerol D administration (30 mg/kg) significantly decreased GFAP and caspase 3 expression levels ($P < 0.05$, Fig. 5). Panaxcerol D administration (30 mg/kg) significantly decreased phosphorylation levels of NF-kB p65, but increased BDNF expression levels ($P < 0.05$, Fig. 5). Panaxcerol D administration (30 mg/kg) also significantly increased synaptophysin expression levels and phosphorylation levels of ERK compared to Aβ25-35 peptide treatment ($P < 0.05$, Fig. 5). However, expression levels of in GFAP, caspase 3, ERK, NF-kB p65, and BDNF in the hippocampus showed no significant changes (Supplementary Fig. S1).

4. Discussion

In the current study, we discovered that panaxcerol D alleviated scopolamine- or $A\beta_{25\text{-}35}$ peptide -induced cognitive dysfunction through several behavioral tests as well as the Y-maze test, PAT and NORT. Additionally, administration of panaxcerol D enhanced ERK or CaMKII signaling pathway in the hippocampus under a hypocholinergic state and normalized GFAP, caspase 3, NF-kB p65, synaptophysin, and BDNF levels in the cortex after injection with $A\beta_{25\text{-}35}$ peptide.

It has been reported that *P. ginseng* has an improving effect on AD in animal models [28,29] and patients [30–32]. In addition, ginsenosides are known to be main active components of *P. ginseng* in terms of cognitive function [33]. Recently, in the pilot study, we found that the non-saponin fraction of *P. ginseng* extract could improve cognitive dysfunction in mouse model induced by scopolamine using a PAT (Supplementary Fig. S2), suggesting that non-saponin compound(s) as well as ginsenosides might play important roles in cognitive function of *P. ginseng*. Thereafter, we explored which compound(s) could have memory improving effect in the non-saponin fraction of *P. ginseng* and isolated panaxcerol D. It has been reported that panaxcerol D has anti-inflammatory activity *in vitro* [16]. However, any pharmacological effect of panaxcerol D on cognitive function has not been inspected to the best of our knowledge.

In behavioral tests, acute or sub-chronic administration of panaxcerol D improved long-term memory and recognition memory measured by PAT and NORT in both scopolamine and $A\beta_{25\text{-}35}$ peptide-treated cognitive impairment mouse models. In the Y-

maze test, acute administration of panaxcerol D significantly recovered the memory dysfuntion induced by scopolamine. However, sub-chronic administration of panaxcerol D did not show a significant effect on memory impairment of $A\beta_{25-35}$ peptide-injected mouse. It is unclear why panaxcerol D did not attenuate short-term memory in the $A\beta_{25-35}$ peptide-treated mouse model. The Y-maze test has been employed for measuring hippocampus-dependent spatial working and short-term memory of mouse [21]. It has been reported that hippocampus dependent behavioral changes can be observed at 3 - 4 weeks after injection of Aβ peptide [34,35]. However, in our studies, we injected $A\beta_{25-35}$ peptide into cerebral ventricles of mice and performed experiments at one week after injection. Therefore, no changes in the Y-maze test after administration of panaxcerol D in Aβ peptide-treated mice might not have enough time to affect the hippocampal function associated with short-term memory of mice. No changes of signaling molecules in the hippocampus could also support results of Y-maze test using Aβ peptide-treated mice (*vide infra*).

It has been reported that ERK and CaMKII contribute to neuronal or synaptic plasticity [36]. Recently, we observed that phosphorylation levels of ERK and CaMKII were decreased in a scopolamine-induced cognitive impairment mouse model [37]. In the current study, we found that phosphorylation levels of ERK and CaMKII in the hippocampus were decreased by injection of scopolamine, but increased by administration of panaxcerol D or donepezil. These results suggest that panaxcerol D could ameliorate cognitive function through activation of ERK-CaMKII signaling pathways in a hypocholinergic state. In addition, in the brain of Aβ-induced memory impairment animal model, the level of GFAP expression was increased, consistent with our and other's report [38,39]. It is known that upregulation of GFAP controls damage of CNS diseases [40]. For example, GFAP levels are increased in brains of patients with AD [41] or multiple sclerosis [42]. In addition, caspase 3-mediated GFAP proteolysis could induce degeneration of astrocytes in brains of AD patients [43]. To investigate changes of molecules associated with AD pathology, we explored GFAP and caspase 3 expression levels. GFAP and caspase 3 expression levels were increased in $A\beta_{25-35}$ peptide-injected group, but decreased by panaxcerol D administration. These results mean that administration of panaxcerol D could normalize impaired expression levels of GFAP and caspase 3 observed in AD patients [43]. In the present study, anti-inflammatory effect of panaxcerol D was observed via the Western blot analysis of GFAP, however, specific brain areas associated with such effect were hard to clarify. Further researches should be investigated to know the expression of GFAP and microglia activation associated with neuroinflammation and AD with immunohistochemistry analysis. In addition, it has been reported that Aβ peptides can stimulate ROS production, resulting in activation of NF-kB with phosphorylation of p65 and dysregulated expression of various genes including BDNF [44]. In the current study, the BDNF expression level was increased and the phosphorylation level of p65 was decreased in panaxcerol D administration group. Additionally, we inspected that the phosphorylation level of ERK was normalized in panaxcerol D administrated group, as in scopolamine-induced animal model, suggesting that panaxcerol D treatment could restore synaptic plasticity. These increased phosphorylation levels of ERK and CaMKII might be associated with the activation of TrkB. BDNF bound with TrkB activated downstream signaling pathways including ERK and CaMKII, thereafter, promote the expression of BDNF. Although the administration of panaxcerol D did not increase the activation of TrkB significantly, it showed a tendency to increase the phosphorylation level of TrkB (Supplementary figure S3) and significantly increased the phosphorylation level of ERK and CaMKII. Therefore, panaxcerol D might exert its pharmacological activities after binding with TrkB. Further study is needed to clarify the receptor of panaxcerol D. Synaptophysin plays a critical role in synaptic plasticity [45]. Expression levels of synaptophysin are decreased in AD

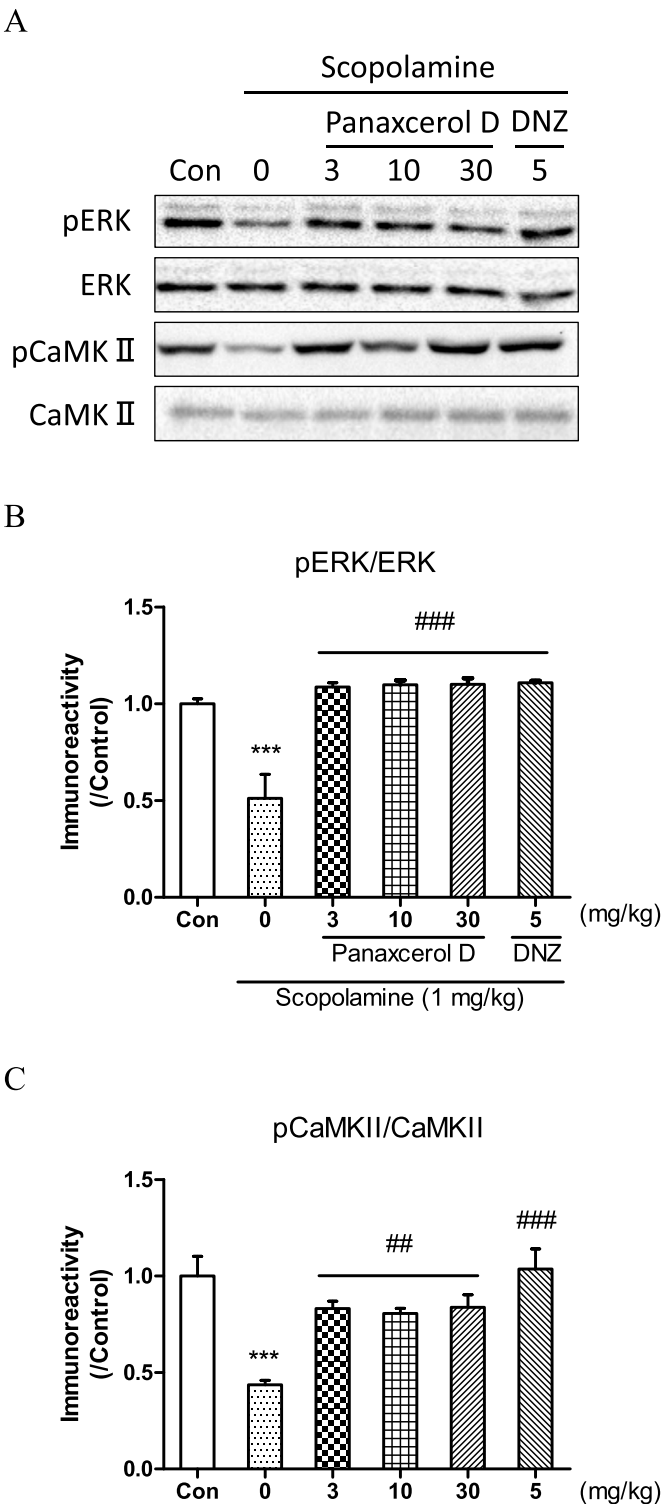


Fig. 4. Effects of panaxcerol D on the scopolamine-induced phosphorylation levels of ERK and CaMKII in the hippocampus
The immunoreactivity (A) and quantitative analysis of expression levels of ERK (B) and CaMKII (C) were presented. Data were presented means ± S.E.M. (n = 5-6/group) (***P < 0.001 versus the vehicle-administered group; ##P < 0.01 versus the scopolamine administered group; ###P < 0.001 versus the scopolamine administered group). Con, control.

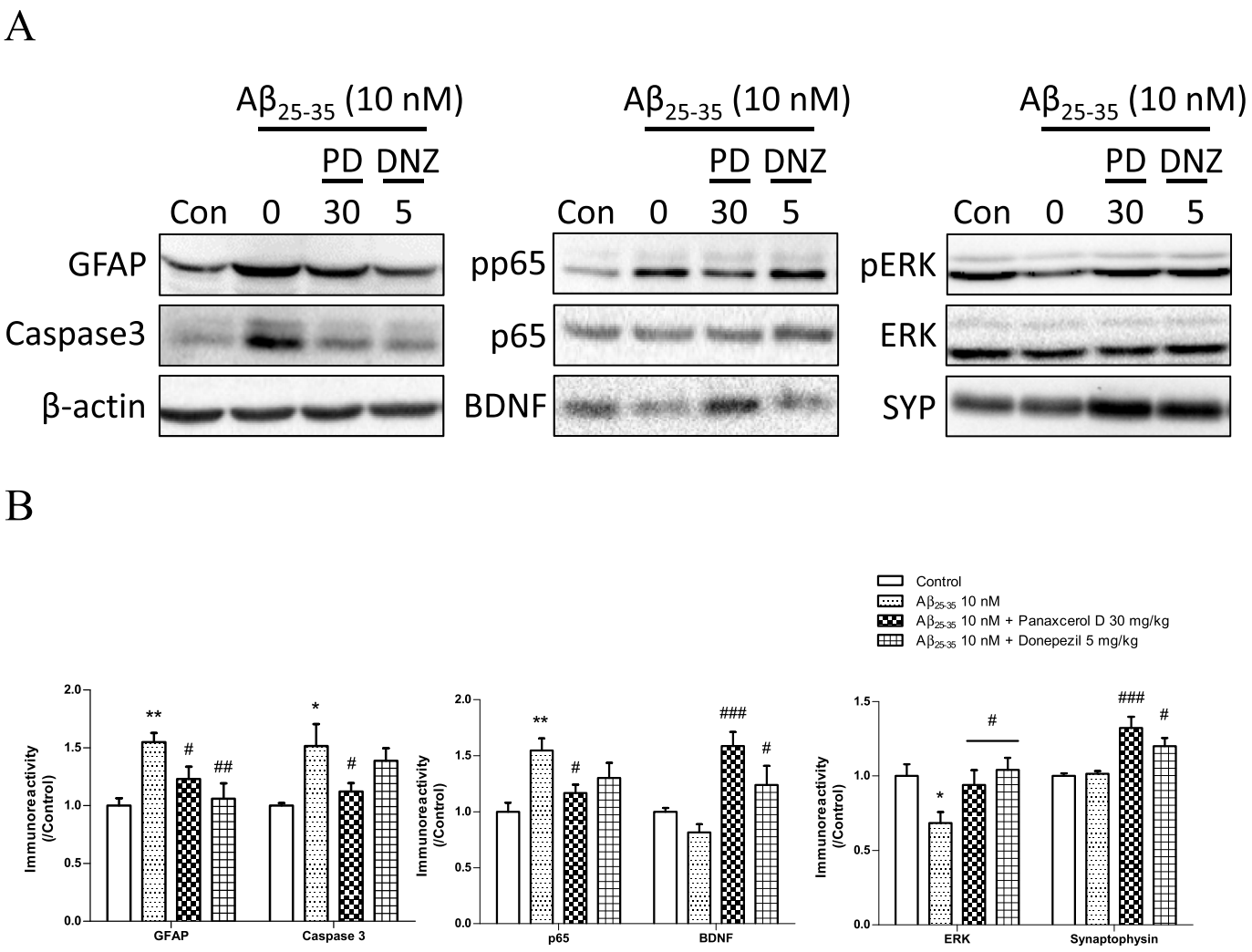


Fig. 5. Effects of sub-chronic treatment of panaxcerol D on $A\beta_{25\text{-}35}$ peptide-induced the expression level of GFAP and caspase 3 and phosphorylation level of ERK and p65 and expression level of synaptophysin and BDNF in the cortex

The immunoreactivity (A) and the quantitative analysis (B) of expression levels of GFAP, caspase 3, synaptophysin and BDNF or phosphorylation levels of ERK and p65 were presented. Data were shown as the means ± S.E.M. (n = 5-6/group) (*P < 0.05 versus the vehicle-administered group; **P < 0.01 versus the vehicle-administered group; #P < 0.05 versus the $A\beta_{25\text{-}35}$ administered group; ##P < 0.01 versus the $A\beta_{25\text{-}35}$ administered group). PD, panaxcerol D; SYP, synaptophysin; Con, control.

patients [46] and AD animal models [47,48] with decreased synaptic plasticity [49]. In the present study, panaxcerol D administration enhanced expression levels of synaptophysin in $A\beta_{25\text{-}35}$ peptide-induced AD mouse model, meaning that administration of panaxcerol D could improve synaptic plasticity in $A\beta_{25\text{-}35}$ peptide-induced AD mouse model. Taken together, these results suggest that panaxcerol D could normalize impaired GFAP-caspase 3 and NF-kB-BDNF signaling pathways and enhance synaptic plasticity by increasing phosphorylation levels of ERK and synaptophysin expression levels in an AD mouse model.

Regarding the effect of *P. ginseng* on cognitive function, most studies have mainly focused on saponin components [50,51]. Besides ginsenosides, non-saponin components of *P. ginseng* such as panaxynol as a polyacetylene component [52] and gintonin as a glycolipoprotein component [53] could improve cognitive function [54,55]. In this study, we also observed that panaxcerol D would be one of the active components of the non-saponin fraction of *P. ginseng*. This is the first study to show that panaxcerol D is effective in improving memory in scopolamine- and Aβ-induced cognitive impairment mouse models. However, the proportion of panaxcerol D in the non-saponin fraction of *P. ginseng* is only about 1 %, suggesting that other components including panaxcerol D and gintonin might also play roles in effects of *P. ginseng* on cognitive function. Also, it is likely that panaxcerol D directly exerts its effects in the brain because of its polarity and anti-inflammatory effect. However, we did not have any data on the pharmacokinetic parameters of panaxcerol D, such as blood-brain barrier penetration. Therefore, further researches on synergistic effects of panaxcerol D with other non-saponin component(s) and pharmacokinetic research of panaxcerol D are needed to explain the contribution of panaxcerol D on cognitive function. Further research on synergistic effects of panaxcerol D with other non-saponin component(s) is needed to explain the contribution of panaxcerol D to effects of *P. ginseng* on cognitive function.

In summary, we found that panaxcerol D, a glycosyl glyceride isolated from *P. ginseng*, could enhance memory dysfunction induced by scopolamine treatment or $A\beta_{25\text{-}35}$ peptide injection in mice measured by several behavioral experiments. These memory improvements were due to increased levels of phosphorylation of ERK and CaMKII in a cholinergic blockade state. Decreases of expression levels of GFAP and caspase 3 and phosphorylation levels of p65 with increased phosphorylation of ERK and expression level

of synaptophysin and BDNF might have ameliorated memory impairment in $A\beta_{25-35}$ peptide-induced AD mice model. Taken together, these results suggest that panaxcerol D could be a candidate for treating cognitive dysfunction observed in a hypocholinergic or Aβ deposition state such as in AD.

Authors contributions

Jong Hoon Ryu designed the studies and wrote the paper with Keontae Park. Keontae Park and Seo Yun Jung performed and analyzed the molecular studies. Mijin Jeon and Woo Chang Kang analyzed the data of the behavioral studies. Kyungnam Cho and Chang Hyoen Kong performed the gavages and behavioral tests. Ranhee Kim and Dae Sik Jang isolated panaxcerol D and analyzed the chemical data.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2023.08.002.

References

[1] Gale SA, Acar D, Daffner KR. Dementia. Epub 2018/02/10 Am J Med 2018;131(10):1161–9. https://doi.org/10.1016/j.amjmed.2018.01.022. PubMed PMID: 29425707.
[2] Ljubenkov PA, Geschwind MD. Dementia. Epub 2016/09/20 Semin Neurol 2016;36(4):397–404. https://doi.org/10.1055/s-0036-1585096. PubMed PMID: 27643909.
[3] Gallardo G, Holtzman DM. Amyloid-β and tau at the crossroads of alzheimer's disease. Epub 2020/02/26 Adv Exp Med Biol 2019;1184:187–203. https://doi.org/10.1007/978-981-32-9358-8_16. PubMed PMID: 32096039.
[4] Sharma K. Cholinesterase inhibitors as Alzheimer's therapeutics (Review). Epub 2019/07/02 Mol Med Rep 2019;20(2):1479–87. https://doi.org/10.3892/mmr.2019.10374. PubMed PMID: 31257471; PubMed Central PMCID: PMCPMC6625431.
[5] Tayeb HO, Yang HD, Price BH, Tarazi FI. Pharmacotherapies for Alzheimer's disease: beyond cholinesterase inhibitors. Epub 2011/12/27 Pharmacol Ther 2012;134(1):8–25. https://doi.org/10.1016/j.pharmthera.2011.12.002. PubMed PMID: 22198801.
[6] Park HJ, Kim DH, Park SJ, Kim JM, Ryu JH. Ginseng in traditional herbal prescriptions. Epub 2013/05/30 J Ginseng Res 2012;36(3):225–41. https://doi.org/10.5142/jgr.2012.36.3.225. PubMed PMID: 23717123; PubMed Central PMCID: PMCPMC3659587.
[7] Kang S, Min H. Ginseng, the 'immunity boost': the effects of panax ginseng on immune system. Epub 2013/05/30 J Ginseng Res 2012;36(4):354–68. https://doi.org/10.5142/jgr.2012.36.4.354. PubMed PMID: 23717137; PubMed Central PMCID: PMCPMC3659612.
[8] Kiefer D, Pantuso T. Panax ginseng. Am Fam Physician 2003;68(8):1539–42. Epub 2003/11/05. PubMed PMID: 14596440.
[9] Razgonova MP, Veselov VV, Zakharenko AM, Golokhvast KS, Nosyrev AE, Cravotto G, Tsatsakis A, Spandidos DA. Panax ginseng components and the pathogenesis of Alzheimer's disease (Review). Epub 2019/03/01 Mol Med Rep 2019;19(4):2975–98. https://doi.org/10.3892/mmr.2019.9972. PubMed PMID: 30816465; PubMed Central PMCID: PMCPMC6423617.
[10] Wang H, Lv J, Jiang N, Huang H, Wang Q, Liu X. Ginsenoside Re protects against chronic restraint stress-induced cognitive deficits through regulation of NLRP3 and Nrf2 pathways in mice. Epub 2021/03/31 Phytother Res: PTR 2021. https://doi.org/10.1002/ptr.6947. PubMed PMID: 33783035.
[11] Kim J, Shim J, Lee S, Cho WH, Hong E, Lee JH, Han JS, Lee HJ, Lee KW. Rg3-enriched ginseng extract ameliorates scopolamine-induced learning deficits in mice. Epub 2016/02/19 BMC Compl Alternative Med 2016;16:66. https://doi.org/10.1186/s12906-016-1050-z. PubMed PMID: 26887326; PubMed Central PMCID: PMCPMC4758096.
[12] Changhong K, Peng Y, Yuan Z, Cai J. Ginsenoside Rb1 protected PC12 cells from Aβ(25-35)-induced cytotoxicity via PPARγ activation and cholesterol reduction. Epub 2020/12/29 Eur J Pharmacol 2021;893:173835. https://doi.org/10.1016/j.ejphar.2020.173835. PubMed PMID: 33359145.
[13] Lee B, Sur B, Park J, Kim SH, Kwon S, Yeom M, Shim I, Lee H, Hahm DH. Ginsenoside rg3 alleviates lipopolysaccharide-induced learning and memory impairments by anti-inflammatory activity in rats. Epub 2013/11/19 Biomol Therapeut 2013;21(5):381–90. https://doi.org/10.4062/biomolther.2013.053. PubMed PMID: 24244826; PubMed Central PMCID: PMCPMC3825202.
[14] Fang F, Chen X, Huang T, Lue L-F, Luddy JS, Yan SS. Multi-faced neuroprotective effects of Ginsenoside Rg1 in an Alzheimer mouse model. Biochim Biophys Acta (BBA) - Mol Basis Dis 2012;1822(2):286–92. https://doi.org/10.1016/j.bbadis.2011.10.004.
[15] Hwang SH, Shin EJ, Shin TJ, Lee BH, Choi SH, Kang J, Kim HJ, Kwon SH, Jang CG, Lee JH, et al. Gintonin, a ginseng-derived lysophosphatidic acid receptor ligand, attenuates Alzheimer's disease-related neuropathies: involvement of non-amyloidogenic processing. Epub 2012/05/01 J Alzheim Dis: JAD 2012;31(1):207–23. https://doi.org/10.3233/jad-2012-120439. PubMed PMID: 22543851.
[16] Cha BJ, Park JH, Shrestha S, Baek NI, Lee SM, Lee TH, Kim J, Kim GS, Kim SY, Lee DY. Glycosyl glycerides from hydroponic Panax ginseng inhibited NO production in lipopolysaccharide-stimulated RAW264.7 cells. Epub 2015/06/06 J Ginseng Res 2015;39(2):162–8. https://doi.org/10.1016/j.jgr.2014.10.005. PubMed PMID: 26045690; PubMed Central PMCID: PMCPMC4452534.
[17] Kim G-s. Development of functional foods and beauty cosmetic materials using ginseng and medicinal plants. In: 한국약용작물학회 학술대회논문집 2018; 2018. p. 1–16. 2.
[18] Kaminsky YG, Marlatt MW, Smith MA, Kosenko EA. Subcellular and metabolic examination of amyloid-beta peptides in Alzheimer disease pathogenesis: evidence for Abeta(25-35). Epub 2009/09/16 Exp Neurol 2010;221(1):26–37. https://doi.org/10.1016/j.expneurol.2009.09.005. PubMed PMID: 19751725.
[19] Kim MJ, Jung JE, Lee S, Cho EJ, Kim HY. Effects of the fermented Zizyphus jujuba in the amyloid β(25-35)-induced Alzheimer's disease mouse model. Epub 2021/04/13 Nutr Res Pract 2021;15(2):173–86. https://doi.org/10.4162/nrp.2021.15.2.173. PubMed PMID: 33841722; PubMed Central PMCID: PMCPMC8007403.
[20] Paxinos G, Franklin KB. The mouse brain in stereotaxic coordinates. Third. Academic Press; 2008.
[21] Kraeuter AK, Guest PC, Sarnyai Z. The Y-maze for assessment of spatial working and reference memory in mice. Epub 2018/12/12 Methods Mol Biol 2019;1916:105–11. https://doi.org/10.1007/978-1-4939-8994-2_10. PubMed PMID: 30535688.
[22] Bae HJ, Kim J, Jeon SJ, Kim J, Goo N, Jeong Y, Cho K, Cai M, Jung SY, Kwon KJ, et al. Green tea extract containing enhanced levels of epimerized catechins attenuates scopolamine-induced memory impairment in mice. Epub 2020/05/04 J Ethnopharmacol 2020;258:112923. https://doi.org/10.1016/j.jep.2020.112923. PubMed PMID: 32360798.
[23] Lueptow LM. Novel object recognition test for the investigation of learning and memory in mice. Epub 2017/09/12 J Vis Exp 2017;126. https://doi.org/10.3791/55718. PubMed PMID: 28892027; PubMed Central PMCID: PMCPMC5614391.
[24] Koo B, Bae HJ, Goo N, Kim J, Kim J, Cai M, Jung IH, Cho K, Jung SY, Chang SW, et al. A botanical drug composed of three herbal materials attenuates the sensorimotor gating deficit and cognitive impairment induced by MK-801 in mice. Epub 2019/11/13 J Pharm Pharmacol 2020;72(1):149–60. https://doi.org/10.1111/jphp.13199. PubMed PMID: 31713882.
[25] Kim JY, Lee HK, Jang JY, Yoo JK, Seong YH. Ilex latifolia prevents amyloid β protein (25-35)-induced memory impairment by inhibiting apoptosis and tau phosphorylation in mice. Epub 2015/08/21 J Med Food 2015;18(12):1317–26. https://doi.org/10.1089/jmf.2015.3443. PubMed PMID: 26291170; PubMed Central PMCID: PMCPMC4685495.
[26] Kim J, Seo YH, Kim J, Goo N, Jeong Y, Bae HJ, Jung SY, Lee J, Ryu JH. Casticin ameliorates scopolamine-induced cognitive dysfunction in mice. Epub 2020/05/08 J Ethnopharmacol 2020;259:112843. https://doi.org/10.1016/j.jep.2020.112843. PubMed PMID: 32380246.
[27] Sarter M, Bodewitz G, Stephens DN. Attenuation of scopolamine-induced impairment of spontaneous alteration behaviour by antagonist but not inverse agonist and agonist beta-carbolines. Epub 1988/01/01 Psychopharmacology 1988;94(4):491–5. https://doi.org/10.1007/bf00212843. PubMed PMID: 2836875.
[28] Kim J, Kim SH, Lee DS, Lee DJ, Kim SH, Chung S, Yang HO. Effects of fermented ginseng on memory impairment and β-amyloid reduction in Alzheimer's disease experimental models. Epub 2013/05/30 J Ginseng Res 2013;37(1):100–7. https://doi.org/10.5142/jgr.2013.37.100. PubMed PMID: 23717163; PubMed Central PMCID: PMCPMC3659620.
[29] Choi JG, Kim N, Huh E, Lee H, Oh MH, Park JD, Pyo MK, Oh MS. White ginseng protects mouse hippocampal cells against amyloid-beta oligomer toxicity. Epub 2017/01/24 Phytother Res 2017;31(3):497–506. https://doi.org/10.1002/ptr.5776. PubMed PMID: 28112442.
[30] Lee ST, Chu K, Sim JY, Heo JH, Kim M. Panax ginseng enhances cognitive performance in Alzheimer disease. Epub 2008/06/27 Alzheimer Dis Assoc Disord 2008;22(3):222–6. https://doi.org/10.1097/WAD.0b013e31816c92e6. PubMed PMID: 18580589.
[31] Heo JH, Lee ST, Oh MJ, Park HJ, Shim JY, Chu K, Kim M. Improvement of cognitive deficit in Alzheimer's disease patients by long term treatment with Korean red ginseng. Epub 2011/11/01 J Ginseng Res 2011;35(4):457–61.

https://doi.org/10.5142/jgr.2011.35.4.457. PubMed PMID: 23717092; PubMed Central PMCID: PMCPMC3659550.
[32] Heo JH, Park MH, Lee JH. Effect of Korean red ginseng on cognitive function and quantitative EEG in patients with alzheimer's disease: a preliminary study. Epub 2016/03/15 J Altern Complement Med 2016;22(4):280–5. https://doi.org/10.1089/acm.2015.0265. PubMed PMID: 26974484.
[33] Lu J, Wang X, Wu A, Cao Y, Dai X, Liang Y, Li X. Ginsenosides in central nervous system diseases: pharmacological actions, mechanisms, and therapeutics. Epub 2022/01/28 Phytother Res 2022;36(4):1523–44. https://doi.org/10.1002/ptr.7395. PubMed PMID: 35084783.
[34] Dhamodharan J, Sekhar G, Muthuraman A. Epidermal growth factor receptor kinase inhibitor ameliorates β-amyloid oligomer-induced alzheimer disease in Swiss albino mice. Epub 2022/08/27 Molecules 2022;27(16). https://doi.org/10.3390/molecules27165182. PubMed PMID: 36014421; PubMed Central PMCID: PMCPMC9412386.
[35] Liu M, Zeng M, Wang S, Cao B, Guo P, Zhang Y, Jia J, Zhang Q, Zhang B, Wang R, et al. Thymidine and 2'-deoxyuridine reduce microglial activation and improve oxidative stress damage by modulating glycolytic metabolism on the Aβ(25-35)-induced brain injury. Epub 2022/08/24 Arch Biochem Biophys 2022;729:109377. https://doi.org/10.1016/j.abb.2022.109377. PubMed PMID: 35998686.
[36] Khan R, Kulasiri D, Samarasinghe S. A multifarious exploration of synaptic tagging and capture hypothesis in synaptic plasticity: development of an integrated mathematical model and computational experiments. Epub 2022/10/25 J Theor Biol 2023;556:111326. https://doi.org/10.1016/j.jtbi.2022.111326. PubMed PMID: 36279957.
[37] Jeon SJ, Kim B, Park HJ, Zhang J, Kwon Y, Kim DH, Ryu JH. The ameliorating effect of 1-palmitoyl-2-linoleoyl-3-acetylglycerol on scopolamine-induced memory impairment via acetylcholinesterase inhibition and LTP activation. Epub 2017/02/01 Behav Brain Res 2017;324:58–65. https://doi.org/10.1016/j.bbr.2017.01.040. PubMed PMID: 28137622.
[38] Kim DH, Kim S, Jeon SJ, Son KH, Lee S, Yoon BH, Cheong JH, Ko KH, Ryu JH. The effects of acute and repeated oroxylin A treatments on Abeta(25-35)-induced memory impairment in mice. Epub 2008/07/16 Neuropharmacology 2008;55(5):639–47. https://doi.org/10.1016/j.neuropharm.2008.05.019. PubMed PMID: 18620712.
[39] Luo SM, Li LY, Guo LZ, Wang L, Wang YF, Chen N, Wang E. Dexmedetomidine exerts an anti-inflammatory effect via α2 adrenoceptors to alleviate cognitive dysfunction in 5xFAD mice. Epub 2022/10/08 Front Aging Neurosci 2022;14:978768. https://doi.org/10.3389/fnagi.2022.978768. PubMed PMID: 36204551; PubMed Central PMCID: PMCPMC9531904.
[40] Brenner M. Role of GFAP in CNS injuries. Epub 2014/02/11 Neurosci Lett 2014;565:7–13. https://doi.org/10.1016/j.neulet.2014.01.055. PubMed PMID: 24508671; PubMed Central PMCID: PMCPMC4049287.
[41] Xu K, Malouf AT, Messing A, Silver J. Glial fibrillary acidic protein is necessary for mature astrocytes to react to beta-amyloid. Epub 1999/02/24 Glia 1999;25(4):390–403. https://doi.org/10.1002/(sici)1098-1136(19990215)25:4<390::aid-glia8>3.0.co;2-7. PubMed PMID: 10028921.
[42] Liedtke W, Edelmann W, Chiu FC, Kucherlapati R, Raine CS. Experimental autoimmune encephalomyelitis in mice lacking glial fibrillary acidic protein is characterized by a more severe clinical course and an infiltrative central nervous system lesion. Epub 1998/01/09. PubMed PMID: 9422542; PubMed Central PMCID: PMCPMC1858102 Am J Pathol 1998;152(1):251–9.
[43] Mouser PE, Head E, Ha KH, Rohn TT. Caspase-mediated cleavage of glial fibrillary acidic protein within degenerating astrocytes of the Alzheimer's disease brain. Epub 2006/03/02 Am J Pathol 2006;168(3):936–46. https://doi.org/10.2353/ajpath.2006.050798. PubMed PMID: 16507909; PubMed Central PMCID: PMCPMC1606516.
[44] Snow WM, Albensi BC. Neuronal gene targets of NF-κB and their dysregulation in alzheimer's disease. Epub 2016/11/25 Front Mol Neurosci 2016;9:118. https://doi.org/10.3389/fnmol.2016.00118. PubMed PMID: 27881951; PubMed Central PMCID: PMCPMC5101203.
[45] Janz R, Südhof TC, Hammer RE, Unni V, Siegelbaum SA, Bolshakov VY. Essential roles in synaptic plasticity for synaptogyrin I and synaptophysin I. Epub 1999/12/14 Neuron 1999;24(3):687–700. https://doi.org/10.1016/s0896-6273(00)81122-8. PubMed PMID: 10595519.
[46] Sze CI, Troncoso JC, Kawas C, Mouton P, Price DL, Martin LJ. Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. Epub 1997/08/01 J Neuropathol Exp Neurol 1997;56(8):933–44. https://doi.org/10.1097/00005072-199708000-00011. PubMed PMID: 9258263.
[47] Hong S, Beja-Glasser VF, Nfonoyim BM, Frouin A, Li S, Ramakrishnan S, Merry KM, Shi Q, Rosenthal A, Barres BA, et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. Epub 2016/04/02 Science (New York, NY) 2016;352(6286):712–6. https://doi.org/10.1126/science.aad8373. PubMed PMID: 27033548; PubMed Central PMCID: PMCPMC5094372.
[48] Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, Guillozet-Bongaarts A, Ohno M, Disterhoft J, Van Eldik L, et al. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. Epub 2006/10/06 J Neurosci: Off J Soc Neurosci 2006;26(40):10129–40. https://doi.org/10.1523/jneurosci.1202-06.2006. PubMed PMID: 17021169; PubMed Central PMCID: PMCPMC6674618.
[49] Chang CH, Lin CH, Lane HY. Machine learning and novel biomarkers for the diagnosis of alzheimer's disease. Epub 2021/04/04 Int J Mol Sci 2021;22(5). https://doi.org/10.3390/ijms22052761. PubMed PMID: 33803217; PubMed Central PMCID: PMCPMC7963160.
[50] Park SJ, Lee D, Kim D, Lee M, In G, Han ST, Kim SW, Lee MH, Kim OK, Lee J. The non-saponin fraction of Korean Red Ginseng (KGC05P0) decreases glucose uptake and transport in vitro and modulates glucose production via down-regulation of the PI3K/AKT pathway in vivo. Epub 2020/03/10 J Ginseng Res 2020;44(2):362–72. https://doi.org/10.1016/j.jgr.2019.12.004. PubMed PMID: 32148419; PubMed Central PMCID: PMCPMC7031776.
[51] Cho DE, Choi GM, Lee YS, Hong JP, Yeom M, Lee B, Hahm DH. Long-term administration of red ginseng non-saponin fraction rescues the loss of skeletal muscle mass and strength associated with aging in mice. Epub 2022/09/13 J Ginseng Res 2022;46(5):657–65. https://doi.org/10.1016/j.jgr.2021.12.001. PubMed PMID: 36090680; PubMed Central PMCID: PMCPMC9459129.
[52] Yamazaki M, Hirakura K, Miyaichi Y, Imakura K, Kita M, Chiba K, Mohri T. Effect of polyacetylenes on the neurite outgrowth of neuronal culture cells and scopolamine-induced memory impairment in mice. Epub 2002/01/05 Biol Pharm Bull 2001;24(12):1434–6. https://doi.org/10.1248/bpb.24.1434. PubMed PMID: 11767118.
[53] Jakaria M, Azam S, Go EA, Uddin MS, Jo SH, Choi DK. Biological evidence of gintonin efficacy in memory disorders. Epub 2020/10/03 Pharmacol Res 2021;163:105221. https://doi.org/10.1016/j.phrs.2020.105221. PubMed PMID: 33007419.
[54] Kim S, Kim MS, Park K, Kim HJ, Jung SW, Nah SY, Han JS, Chung C. Hippocampus-dependent cognitive enhancement induced by systemic gintonin administration. Epub 2016/02/05 J Ginseng Res 2016;40(1):55–61. https://doi.org/10.1016/j.jgr.2015.05.001. PubMed PMID: 26843822; PubMed Central PMCID: PMCPMC4703807.
[55] Nam SM, Choi JH, Choi SH, Cho HJ, Cho YJ, Rhim H, Kim HC, Cho IH, Kim DG, Nah SY. Ginseng gintonin alleviates neurological symptoms in the G93A-SOD1 transgenic mouse model of amyotrophic lateral sclerosis through lysophosphatidic acid 1 receptor. Epub 2021/05/25 J Ginseng Res 2021;45(3):390–400. https://doi.org/10.1016/j.jgr.2020.04.002. PubMed PMID: 34025132; PubMed Central PMCID: PMCPMC8134849.