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The ameliorating effect of the extract of the flower of *Prunella vulgaris* var. *lilacina* on drug-induced memory impairments in mice

Se Jin Park [a], Dong Hyun Kim [a], Il Kyun Lee [b], Won Yong Jung [a], Dong Hyun Park [a], Jong Min Kim [a], Kang Ro Lee [b], Kyung-Tae Lee [a,c], Chan Young Shin [d], Jae Hoon Cheong [e], Kwang Ho Ko [g], Jong Hoon Ryu [a,f,*]

[a] *Department of Life and Nanopharmaceutical Sciences, College of Pharmacy, Kyung Hee University, Dongdaemoon-Ku, Seoul 130-701, Republic of Korea*
[b] *Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon, Republic of Korea*
[c] *Department of Pharmaceutical, College of Pharmacy, Kyung Hee University, Dongdaemoon-Ku, Seoul 130-701, Republic of Korea*
[d] *Department of Pharmacology, School of Medicine and Center for Geriatric Neuroscience Research, Institute of Biomedical Science and Technology, Konkuk University, Seoul, Republic of Korea*
[e] *Department of Pharmacology, School of Pharmacy, Samyook University, Seoul, Republic of Korea*
[f] *Oriental Pharmaceutical Science, College of Pharmacy, Kyung Hee University, Dongdaemoon-Ku, Seoul 130-701, Republic of Korea*
[g] *Department of Pharmacology, College of Pharmacy, Seoul National University, Seoul, Republic of Korea*

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ABSTRACT

Prunella vulgaris var. *lilacina* is widely distributed in Korea, Japan, China, and Europe, and its flowers are used to treat inflammation in traditional Chinese medicine. In the present study, we studied the effects of the ethanolic extract of the flower of *P. vulgaris* var. *lilacina* (EEPV) on drug-induced learning and memory impairment using the passive avoidance, the Y-maze, and the Morris water maze tasks in mice. EEPV (25 or 50 mg/kg, p.o.) significantly ameliorated scopolamine-induced cognitive impairments in the passive avoidance and Y-maze tasks ($P < 0.05$). In the Morris water maze task, EEPV (25 mg/kg, p.o.) significantly shortened escape latencies in training-trials. Furthermore, swimming times within the target zone during the probe-trial were significantly increased as compared with scopolamine-treated mice ($P < 0.05$). In addition, the reduced latency induced by MK-801 treatment in the passive avoidance task was ameliorated by EEPV (25 mg/kg, p.o.) ($P < 0.05$). Additionally, the ameliorating effect of EEPV on scopolamine-induced memory dysfunction was antagonized by a sub-effective dose of MK-801. These results suggest that EEPV would be useful for treating cognitive impairments induced by cholinergic dysfunction, and that it exerts its effects via NMDA receptor signaling.

1. Introduction

It is well known that learning and memory are closely related to the cholinergic and glutamatergic neurotransmitter systems in brain (Bartus et al., 1982; Durand et al., 1996). Blockade of muscarinic receptors by scopolamine, a muscarinic receptor antagonist, impairs learning and memory in mice (Bartus et al., 1982; Renner et al., 2005). Furthermore, scopolamine-induced amnesic animal models are used to screen for potential treatments for cognitive dysfunction. It has been established that cognitive impairment can be caused by normal aging and stress as well as by specific neurodegenerative and psychiatric disorder such as Alzheimer's disease (AD), vascular dementia, and schizophrenia (Hsiao et al., 1996; McEwen, 1999; Gooding and Tallent, 2004; Haenschel et al., 2009). Muscarinic and nicotinic acetylcholine receptor ligands and acetylcholinesterase (AChE) inhibitors, such as tacrine, donepezil, rivastigmine, and galantamine, are being successfully used for the therapeutic approaches for cognitive loss. However, AChE inhibitors have side effects such as nausea, diarrhea, and vomiting (Terry and Buccafusco, 2003; Cummings et al., 2008). Therefore, the development of new side effect-free drugs to treat cognitive impairments would be highly desirable. Furthermore, because treatment with herbal agents is generally cheap and relatively free of side effects, several research groups have focused on the identification of anti-amnesic agents in herbal materials used as traditional medications (Howes et al., 2003).

Prunella vulgaris var. *lilacina* Nakai (Labiatae) is widely distributed in Korea, Japan, China, and Europe, and it continues to be used

Abbreviations: AChE, acetylcholinesterase; AD, Alzheimer's disease; CREB, cAMP response element-binding protein; EEPV, the ethanolic extract of the flower of *Prunella vulgaris* var. *lilacina*; ERK, extracellular signal-regulated kinase; NMDA, *N*-methyl-D-aspartate; LTP, long-term potentiation.

* Corresponding author at: Oriental Pharmaceutical Science, College of Pharmacy, Kyung Hee University, Dongdaemoon-Ku, Seoul 130-701, Republic of Korea. Tel.: +82 2 961 9230; fax: +82 2 966 3885.
E-mail address: jhryu63@khu.ac.kr (J.H. Ryu).

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to treat inflammation, eye pain, headache, and dizziness in traditional Chinese medicine (Zhu, 1998). Previous studies have shown that *P. vulgaris* var. *lilacina* contains several active compounds including oleanolic acid, betulinic acid, ursolic acid, flavonoids, and rosmarinic acid (Lamaison et al., 1991; Xu et al., 1999; Ryu et al., 2000). Furthermore, this herb has been shown to have anti-allergic, anti-inflammatory, anti-oxidative, anti-microbial, and anti-viral effects (Ryu et al., 2000; Psotová et al., 2003). However, to the best of our knowledge, there have not been any reports on the anti-amnesic effects of the flower of *P. vulgaris* var. *lilacina* or of its constituents. In the present study, we examined the effects of an ethanolic extract of the flower of *P. vulgaris* var. *lilacina* on drug-induced cognitive impairment in mice using the step-through passive avoidance, the Y-maze, and the Morris water maze tasks.

2. Material and methods

2.1. Animals

Male ICR mice (6 weeks old, 25–30 g) were purchased from the Orient Co. Ltd., a branch of Charles River Laboratories (Seoul, Korea). Mice were housed in groups of five. Animals were provided with food and water ad libitum and kept under a 12 h light/ dark cycle (light on 07:00–19:00) at room temperature. After delivery, animal maintenance and treatment were carried out in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and with the Animal Care and Use Guidelines issued by Kyung Hee University, Republic of Korea.

2.2. Materials

(−)-Scopolamine hydrobromide, dizocilpine (MK-801), and 9-amino-1, 2, 3, 4-tetrahydroacridine hydrochloride hydrate (Tacrine) were purchased from Sigma Chemical Co. (St. Louis, MO). Dried *P. vulgaris* var. *lilacina* flowers were obtained from an herbal supplier in Seoul, Korea and voucher specimens (KHUOPS-08-33) were deposited at the herbarium of the College of Pharmacy, Kyung Hee University. The material was authenticated by Emeritus Professor Chang Soo Yook (Department of Oriental Pharmaceutical Science, College of Pharmacy, Kyung Hee University). All other materials were of the highest grades available and were obtained from normal commercial sources. Tacrine, MK-801, and scopolamine were dissolved in 0.9% saline solution. To obtain an ethanolic extract of the flower of *P. vulgaris* var. *lilacina* (EEPV), flowers were extracted with 70% ethanol twice for two hours in an ultrasonic bath. The obtained extract was then filtered, concentrated in a water bath under vacuum, frozen, lyophilized (model FD-5N; Eyela, Tokyo), and then stored at −20 °C until required (yield: 16.85 ± 1.72%). EEPV concentrations were standardized based on the amount of rosmarinic acid, a major constituent (Lamaison et al., 1991). The mean level of rosmarinic acid in EEPV was 11.86 ± 2.05% (n = 3).

2.3. Passive avoidance task

Assessment of acquisition and retention of the passive avoidance task was carried out using identical illuminated and non-illuminated compartments (20 cm × 20 cm × 20 cm) containing a 50 W bulb, as previously described (Kim et al., 2006). The floor of the non-illuminated compartment was composed of 2 mm stainless steel rods spaced 1 cm apart. These two compartments were separated by a guillotine door (5 cm × 5 cm). The animals underwent two separate trials, namely, an acquisition trial, and 24 h later, a retention trial. For the acquisition trial, a mouse was initially placed in the light compartment and, 10 s later, the door between the two compartments was opened. When a mouse entered the dark compartment, the door automatically closed and an electrical foot shock (0.5 mA, 3 s) was delivered through the grid floor. One hour before the acquisition trial, mice were administered EEPV (12.5, 25, 50, or 100 mg/kg, p.o.) or tacrine (10 mg/kg, p.o.). The control group received 0.9% saline vehicle solution rather than EEPV or tacrine. Thirty minutes after the treatment with EEPV, tacrine, or saline, mice were treated with scopolamine (1 mg/kg, i.p.), MK-801 (0.1 mg/kg, s.c.), or vehicle. In our previous study, MK-801 was founded to impaired cognitive performance at 0.1 mg/kg (s.c.) in mice (Kim et al., 2009). The retention trial was conducted 24 h after the acquisition trial. Mice were again placed in the light compartment and the time required (latency) to enter the dark compartment was recorded for each mouse. If a mouse did not enter the dark compartment within 300 s, we concluded that the mouse remembered the acquisition trial. In a separate antagonism study, EEPV (25 mg/kg, p.o.)-treated mice were co-administered scopolamine (1 mg/kg, i.p.) and a sub-effective dose of MK-801 (0.0125 mg/kg, s.c.) 30 min prior to an acquisition trial. In a previous pilot study, MK-801 at this dose did not impair passive avoidance task performance. To investigate the effect of EEPV on learning and memory in unimpaired naïve animals, EEPV was administered one hour before the acquisition trial. To avoid a ceiling effect in unimpaired animals, the intensity of the electrical foot shock was set at 0.25 mA for 3 s. This lower intensity shock allowed for the study of any potential enhancing effects of EEPV.

2.4. Y-maze task

The Y-maze test was conducted in a three-arm maze with angles of 120° between the arms, which were 40 cm long and 3 cm wide with walls that were 12 cm high each. The maze floor and walls were constructed from dark opaque polyvinyl plastic as previously described (Kim et al., 2006). Mice were initially placed within one arm, and the sequence and number of arm entries were recorded manually for each mouse over an 8 min period. The percentage of triads in which all three arms were represented, i.e., ABC, CAB, or BCA but not BAB, was recorded as an 'alternation' to estimate short-term memory (Sarter et al., 1988). One hour before the test, mice were administered EEPV (12.5, 25, 50, or 100 mg/kg, p.o.) or tacrine (10 mg/kg, p.o.). Control group animals received 0.9% saline solution rather than EEPV or tacrine. Scopolamine (1 mg/kg, i.p.) or vehicle was introduced to induce memory impairment 30 min before the test. Arms were cleaned with water spray between tests to remove odors and residues. The alternation score (%) for each mouse was defined as the ratio of the actual number of alternations to the possible number (defined as the total number of arm entries minus two) multiplied by 100 as shown by the following equation: % Alternation = [(Number of alternations)/(Total arm entries − 2)] × 100. The number of arm entries was used as an indicator of locomotor activity.

2.5. Morris water maze task

The Morris water maze is a circular pool (90 cm in diameter and 45 cm in height) with a featureless inner surface. The pool was filled to a depth of 30 cm with water containing 500 ml of milk (20 ± 1 °C). The tank was placed in a dimly lit, soundproof test room with various visual cues. The pool was conceptually divided into quadrants. A white platform (6 cm in diameter and 29 cm high) was then placed in one of the pool quadrants and submerged 1 cm below the water surface so that it was not visible. The test was conducted as previously described (Kim et al., 2006; Kim and Ryu, 2008), with slight modifications. The first experimental day was dedicated to swimming training for 60 s in the absence of the platform. During the four subsequent days, the mice were given four training-trials per session per day with the platform in place. When a mouse located the platform, it was permitted to remain on it for 10 s. If a mouse did not locate the platform within 60 s, then it was placed on the platform for 10 s. The animals were returned to home cages and allowed to dry under an infrared lamp after each trial. The time between training-trials was 30 s. During each training session, the time taken to find the hidden platform (latency) was recorded using a video camera-based Ethovision System (Nodulus, Wageningen, The Netherlands). For each training-trial, mice were placed in the water facing the pool wall in a randomly selected pool quadrant. The day after the last training-trial session, mice were subjected to a probe-trial session, in which the platform was removed from the pool, and mice were allowed to search for it for 60 s. A record was kept of the swimming time in the pool quadrant where the platform had been located previously. EEPV (25 mg/kg, p.o.) or tacrine (10 mg/kg, p.o.) were administered daily one hour before the first training-trial of each session. Memory impairment was induced by scopolamine (1 mg/kg, i.p.) 30 min after EEPV treatment. The control group received 0.9% saline solution only.

2.6. Statistics

The results of the behavioral studies are expressed as means ± S.E.M. Passive avoidance task latencies, Y-maze task spontaneous alternation (%), and Morris water maze test probe-trial swimming times were analyzed by one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test for multiple comparisons. The interactions between the agonist and the antagonist that were determined by the passive avoidance task were analyzed by two-way ANOVA, and Tukey's *post hoc* test was used to perform pairwise comparisons to determine antagonist or agonist effects. The Morris water maze test training-trial latencies were analyzed by two-way ANOVA followed by Tukey's *post hoc* analysis using the day as one variable and treatment as a second. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Effects of EEPV on scopolamine-induced memory impairment in the step-through passive avoidance task

We tested the effect of EEPV on scopolamine-induced memory deficit using the step-through passive avoidance task which is largely dependent on long-term memory (Myhrer, 2003). A significant group effect was observed in step-through latency in the retention trial [F (6, 109) = 18.603, $P < 0.001$] (Fig. 1A). The mean

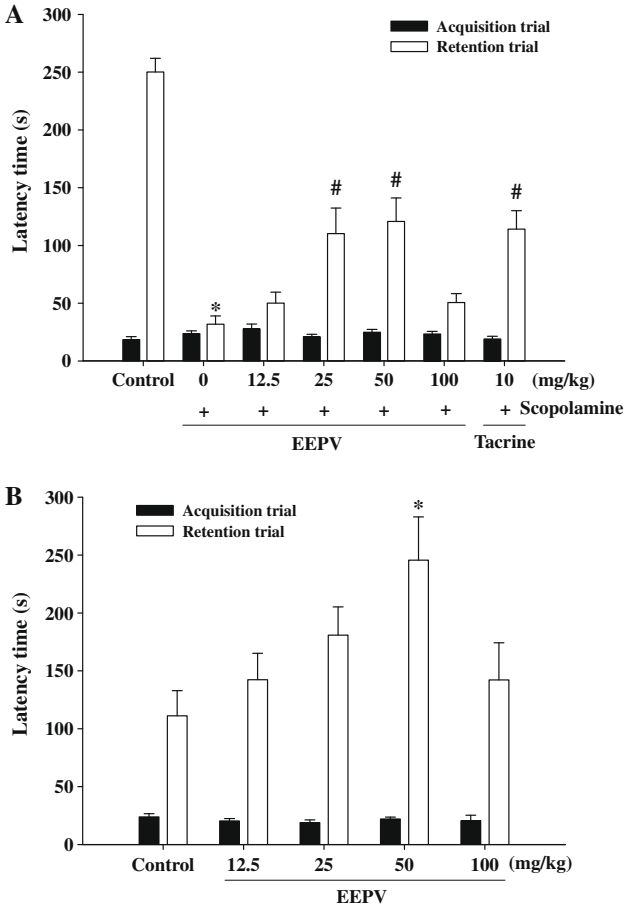

Fig. 1. The effects of the ethanolic extract of the flower of *P. vulgaris* var. *lilacina* (EEPV) on scopolamine-induced memory impairment mouse model (A) and on unimpaired naïve mice (B) in the passive avoidance task. (A) EEPV (12.5, 25, 50, or 100 mg/kg, p.o.), tacrine (10 mg/kg, p.o.), or the same volume of vehicle (0.9% saline solution) were administered to mice 60 min before an acquisition trial. Memory impairment was induced by scopolamine (1 mg/kg, i.p.) 30 min before the acquisition trial. (B) To investigate the effect of EEPV alone in unimpaired naïve mouse, EEPV (12.5, 25, 50, or 100 mg/kg, p.o.) was administered 1 h before acquisition trial, and 30 min before trial vehicle was administered instead of scopolamine. At 24 h after the acquisition trial, a retention trial was conducted for 300 s. Data represent means ± S.E.M (A, $n = 15–16$; B, $n = 9–10$ per group) ($^{*}P < 0.05$ versus the vehicle-treated controls, $^{\#}P < 0.05$ versus the scopolamine-treated group).

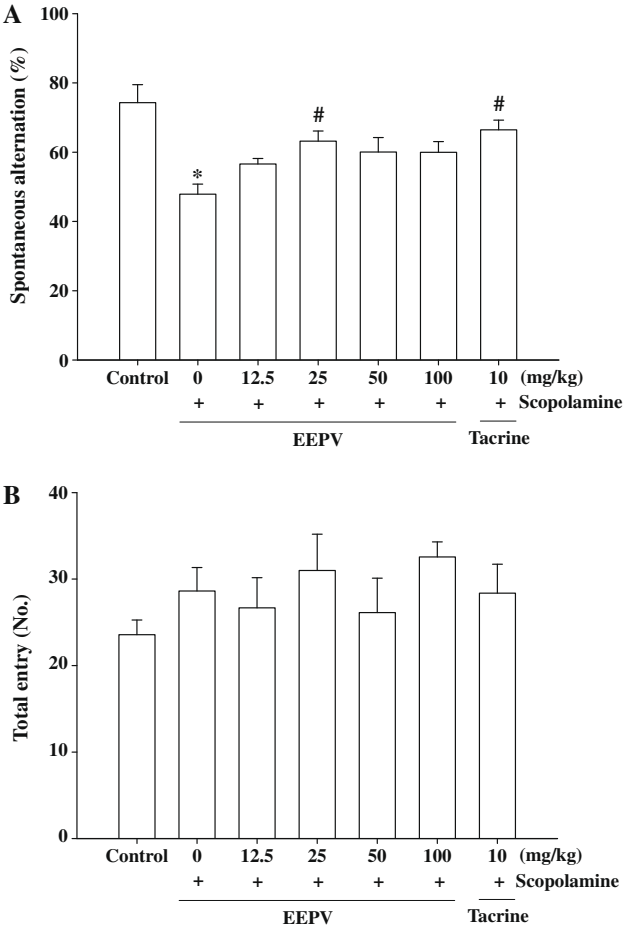

Fig. 2. The effects of the ethanolic extract of the flower of *P. vulgaris* var. *lilacina* (EEPV) on scopolamine-induced memory impairment in mice in the Y-maze task. EEPV (12.5, 25, 50, or 100 mg/kg, p.o.), tacrine (10 mg/kg, p.o.), or the same volume of vehicle (0.9% saline solution) were administered to mice 60 min before Y-maze tests. Memory impairment was induced by scopolamine (1 mg/kg, i.p.) 30 min before the Y-maze tests. Spontaneous alternation behavior (A) and numbers of arm entries (B) during an 8 min session were recorded. Data represent means ± S.E.M ($n = 8–9$ per group) ($^{*}P < 0.05$ versus the vehicle-treated controls, $^{\#}P < 0.05$ versus the scopolamine-treated group).

step-through latency of scopolamine-treated mice was significantly shorter than that of vehicle-treated control mice (Fig. 1A, $P < 0.05$). Furthermore, the step-through latency reductions induced by scopolamine were significantly ameliorated by EEPV (25 and 50 mg/kg, p.o.) and by tacrine (Fig. 1A, $P < 0.05$). Moreover, step-through latency in mice administered EEPV only (50 mg/kg, p.o.) was also significantly increased when compared to vehicle controls [F (4, 41) = 3.451, $P = 0.016$] (Fig. 1B, $P < 0.05$). During the acquisition trials, no significant inter-group differences in step-through latency were observed.

3.2. Effects of EEPV on scopolamine-induced memory deficit in the Y-maze task

We tested the effect of EEPV on scopolamine-induced memory deficit using the Y-maze task. A significant group effect was observed on spontaneous alternation behavior [F (6, 47) = 6.179, $P < 0.001$]. Spontaneous alternation (%) in the scopolamine-treated group was significantly lower than in the vehicle-treated control group (Fig. 2A, $P < 0.05$), and this reduced spontaneous alternation was significantly ameliorated by EEPV (25 mg/kg, p.o.) and by tacrine (10 mg/kg, p.o.) (Fig. 2A, $P < 0.05$). However, the mean numbers of the arm entries were similar in all experimental groups (Fig. 2B), which demonstrated that locomotor activity was not affected by EEPV.

3.3. Effects of EEPV on scopolamine-induced learning and memory dysfunction in the Morris water maze task

The effect of EEPV (25 mg/kg, p.o.) on spatial learning was evaluated using the Morris water maze task. As shown in Fig. 3A, the scopolamine-treated group (1 mg/kg, i.p.) exhibited longer escape latencies than vehicle-treated controls and showed similar escape latencies during the four training days (Fig. 3A). However, the EEPV (25 mg/kg, p.o.) or tacrine (10 mg/kg, p.o.) plus scopolamine-treated groups showed significantly shorter mean escape latencies than the scopolamine-treated group during trial sessions 3 and 4 [trial session 3, F (3, 32) = 8.572, $P < 0.001$; trial session 4, F (3, 32) = 18.864, $P < 0.001$] (Fig. 3A). On the probe-trial session, groups were found to be significantly different in terms of swimming times (%) within the quadrant that normally contained the platform (target quadrant) [F (3, 32) = 6.412, $P = 0.002$] (Fig. 3B). Mean

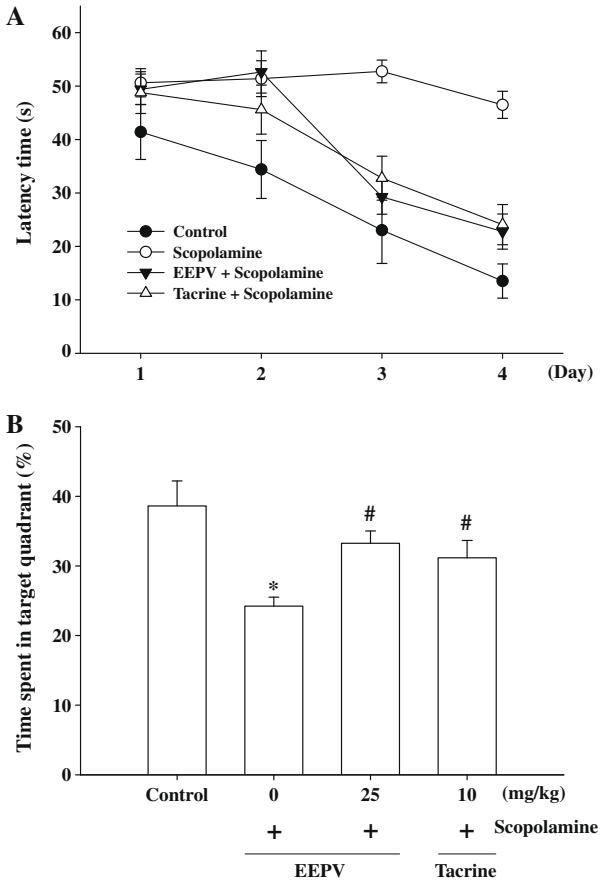

Fig. 3. The effects of the ethanolic extract of the flower of *P. vulgaris* var. *lilacina* (EEPV) on escape latencies during the training sessions (A), and on swimming times during the probe-trial session (B) in the Morris water maze task on scopolamine-induced memory dysfunction in mice. At 60 min before the first training-trial of each session, EEPV (25 mg/kg, p.o.), tacrine (10 mg/kg, p.o.), or the same volume of vehicle (0.9% saline solution) were administered to the mice. Memory impairment was induced by administering scopolamine (1 mg/kg, i.p.) 30 min before the first training-trial. Training-trial and probe-trial sessions were performed over 60 s as described in Section 2. Data represent means ± S.E.M (n = 8–9 per group) ($^{*}P < 0.05$ versus the vehicle-treated controls, $^{\#}P < 0.05$ versus the scopolamine-treated group).

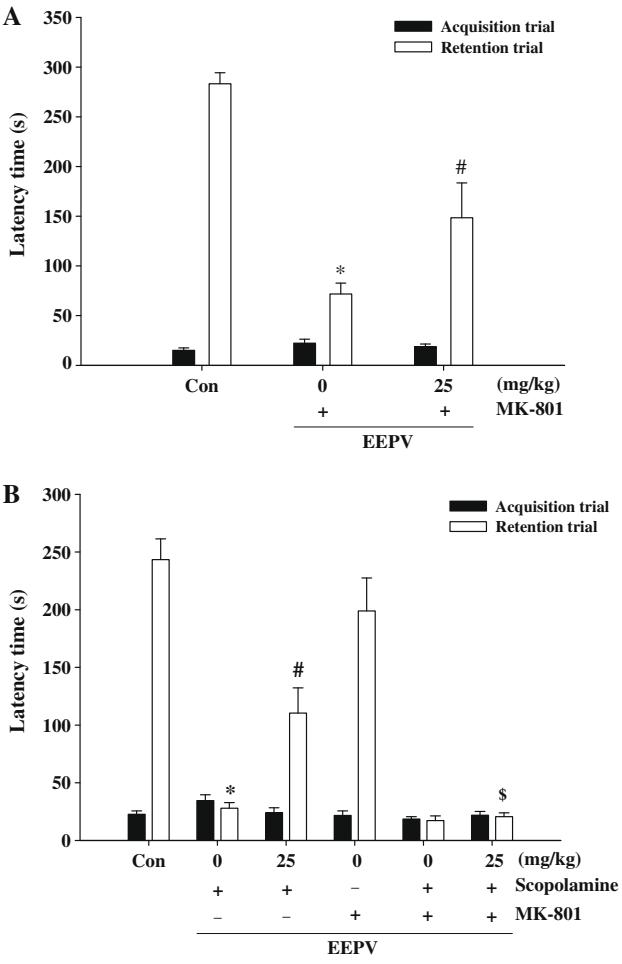

Fig. 4. The role of NMDA receptor signaling in the effects of the ethanolic extract of the flower of *P. vulgaris* var. *lilacina* (EEPV) on drug-induced memory impairments, as assessed by measuring latencies in the acquisition trial and retention trials in the passive avoidance task. (A) To investigate the effects of EEPV on MK-801-induced memory impairment, EEPV (25 mg/kg, p.o.) was administered 1 h before an acquisition trial. Memory impairment was induced by administering MK-801 (0.1 mg/kg, s.c.) 30 min before the acquisition trial. The retention trial was conducted 24 h after the acquisition trial. (B) To test the antagonistic effect of a low dose of MK-801 on the effect of EEPV on scopolamine-induced memory impairment, EEPV (25 mg/kg, p.o.) or the same volume of vehicle (0.9% saline solution) were administered 60 min before the acquisition trial, and 30 min later, mice were injected with a sub-effective dose of MK-801 (0.0125 mg/kg, s.c.). Scopolamine (1 mg/kg, i.p.) was administered 5 min after the MK-801 treatment. The acquisition trial was carried out 25 min later. The retention trial was conducted 24 h after the acquisition trial. Data represent means ± S.E.M (A, n = 8; B, n = 9–10 per group) ($^{*}P < 0.05$ versus the vehicle-treated controls, $^{\#}P < 0.05$ versus the MK-801 (A) or scopolamine (B)-treated groups, $^{\$}P < 0.05$ versus EEPV plus scopolamine-treated group).

swimming time (%) within the platform quadrant in scopolamine-treated mice was significantly reduced compared to vehicle-treated controls (Fig. 3B, $P < 0.05$). Furthermore, the shorter swimming time (%) within the platform quadrant induced by scopolamine was significantly ameliorated by EEPV (25 mg/kg, p.o.) and by tacrine (Fig. 3B, $P < 0.05$). However, no significant differences were observed between groups in terms of swimming speeds within the platform zone (data not shown).

3.4. The effects of EEPV on MK-801-induced memory deficit and the antagonistic effect of MK-801 on the effects of EEPV in the passive avoidance task

We tested the effect of EEPV on MK-801-induced memory deficit using the step-through passive avoidance task. A significant group effect was observed for step-through latency during the retention trial [F (2, 22) = 19.818, $P < 0.001$] (Fig. 4A). The MK-801-treated group (0.1 mg/kg, s.c.) showed a significant reduction in step-through latency as compared with the vehicle-treated controls (Fig. 4A, $P < 0.05$). Furthermore, the reduced latency induced by MK-801 was ameliorated by EEPV (25 mg/kg, p.o.) (Fig. 4A, $P < 0.05$).

In order to determine whether the ameliorating effect of EEPV on scopolamine-induced memory dysfunction was exerted via *N*-methyl-D-aspartate (NMDA) receptor signaling, an additional group of EEPV (25 mg/kg, p.o.)-treated mice were co-treated with scopolamine and a sub-effective dose of MK-801 (0.0125 mg/kg, s.c.) to block NMDA receptors. A significant group effect was observed in terms of step-through latency during the retention trial [F (5, 52) = 35.435, $P < 0.001$] (Fig. 4B). The reduced latency induced by scopolamine (1 mg/kg, i.p.) was ameliorated by EEPV, but this effect was eliminated to the level of the scopolamine-treated group by administration of MK-801. Moreover, two-way ANOVA analysis revealed that the interaction between the EEPV and MK-801 treatments showed a significant group effect [F (1, 34) = 12.985, $P < 0.001$]. However, in the acquisition trial, no

significant differences in step-through latency were observed between groups.

4. Discussion

In the present study, we confirmed that scopolamine- and MK-801-induced learning and memory dysfunction could be ameliorated by treatment with EEPV. This effect was observed in the passive avoidance, the Y-maze, and the Morris water maze tasks in mice. We also found that the ameliorating effects of EEPV on scopolamine-induced memory dysfunction were antagonized by MK-801 in the passive avoidance task. Furthermore, in unimpaired naïve mice, a single-treatment with EEPV resulted in enhanced memory in this same task.

Retention latency in the step-through passive avoidance task is known to reflect long-term memory formation in rodents (Myhrer, 2003). In addition, spontaneous alternation behavior in the Y-maze task is considered to be an indicator of short-term memory (Sarter et al., 1988; Myhrer, 2003). In the present study, the step-through latency reduction by scopolamine was ameliorated by administration of EEPV (25 or 50 mg/kg, p.o.). In addition, EEPV also ameliorated scopolamine-induced spontaneous alternation impairment in the Y-maze task. These effects of EEPV were not attributable to a change in locomotor activity, because EEPV administration did not affect total entries in the Y-maze task nor the latencies during acquisition trials in the passive avoidance task. Interestingly, the 100 mg/kg dose of EEPV did not any significant effects in the passive avoidance task. Previously, several reports suggested that the increasing of acetylcholine in synaptic clefts by cholinesterase inhibitors or cholinomimetics would activate presynaptic autoreceptors, which resulted in an inverted U-shaped dose–response curve (Braida et al., 1996; Calabrese, 2008). Although we did not measure the level of acetylcholine in synaptic clefts, we supposed that the inverted U-shaped dose–response curve of EEPV is due to its cholinomimetic properties. However, the exact mechanisms underlying the inverted U-shaped dose–response curve in the passive avoidance task by EEPV are not clear at present.

The Morris water maze is commonly used to assess hippocampal-dependent spatial memory in rodents (Morris, 1984). In this task, scopolamine increased escape latencies during the training-trial sessions, which suggested that long-term and working memory were impaired by scopolamine (Morris, 1984; Barnes et al., 1996). In the present study, EEPV (25 mg/kg, p.o.) ameliorated scopolamine-induced memory impairment, and recovered escape latencies to the vehicle-treated control group level. In addition, during the probe-trial session, the scopolamine-induced reduction in swimming times within the platform quadrant was significantly ameliorated by EEPV and by tacrine, indicating a positive effect on spatial learning and memory. Collectively, these behavioral results suggest that EEPV ameliorates both short-term and long-term memory in the scopolamine-induced amnesic mouse model. As mentioned in the introduction, previous studies have not provided any clues regarding the mechanism whereby EEPV enhances learning and memory. Initially, since EEPV attenuated scopolamine-induced cognitive dysfunction in mice, we examined whether EEPV inhibits AChE activity. However, we found that AChE activity was not inhibited by EEPV in vitro (IC_{50} > 1000 μg/ml) or ex vivo (data not shown). These results suggested that the memory ameliorating effects of EEPV were not derived from AChE inhibition, but rather from an indirect effect on cholinergic signaling.

It is well known that the glutamatergic excitatory neurotransmitter system plays a crucial role in learning and memory (Durand et al., 1996; Dingledine et al., 1999; Rao and Finkbeiner, 2007). The activation of NMDA receptors increases intracellular calcium levels and triggers a cascade of events leading to enhanced synaptic activity in the hippocampus; a phenomenon known as long-term potentiation (LTP) that is believed to be important for learning and memory (Lynch et al., 1990; Tsien et al., 1996). Moreover, it has been reported that 4'-demethylnobiletin, a metabolite of nobiletin, enhanced extracellular signal-regulated kinase (ERK)-cAMP response element-binding protein (CREB) signaling and ameliorated cognitive dysfunction through NMDA receptor activation (Al Rahim et al., 2009). In contrast, Greenamyre et al. (1987) reported that a loss of glutamatergic neurotransmission in the hippocampus in AD patients is likely to cause cognitive decline and memory deficit. In addition, NMDA receptor antagonists such as MK-801 have been shown to induce dose-dependent learning and memory dysfunctions (Venable and Kelly, 1990; Mandillo et al., 2003; de Lima et al., 2005; McDonald et al., 2005). Previously, we employed the water finding test to measure latent learning and spatial attention in animals (Noda et al., 2001; Mouri et al., 2007). We observed that EEPV markedly improved MK-801-induced spatial attention dysfunction (unpublished data). In addition, we found that EEPV enhanced ERK-CREB phosphorylation in normal naïve mice (data not shown). These results persuaded us to investigate whether EEPV attenuates MK-801-induced cognitive dysfunction. In the present study, we observed that EEPV (25 mg/kg) ameliorated MK-801-induced memory impairment in the passive avoidance task. Furthermore, it has been reported that direct and/or indirect interactions between central cholinergic and NMDA receptor systems are involved in learning and memory processes (Abe et al., 2004; Monteiro Moreira et al., 2005). The administration of acetylcholine has been reported to increase excitatory postsynaptic potentials via NMDA receptor activation, which facilitates NMDA receptor-dependent LTP (Markram and Segal, 1990; Calabresi et al., 1998). Furthermore, hippocampal acetylcholine levels were found to be increased by the intra-septal administration of NMDA or by an NMDA receptor-selective agonist (Moor et al., 1994). In the present study, the ameliorating effects of EEPV on scopolamine-induced memory dysfunction were antagonized by a sub-effective dose of MK-801, and interactions were also observed between the MK-801- and EEPV-treated groups in the passive avoidance task. These results suggest that the ameliorating effects of EEPV on learning and memory may be due to an enhancement of glutamatergic and subsequent cholinergic signaling or vice versa. Recently, galantamine (an AChE inhibitor) was also found to affect NMDA and nicotinic acetylcholine receptor activation (Narahashi et al., 2004; Moriguchi et al., 2004), which are thought to contribute to its positive effects on learning and memory in AD. Moreover, cognitive enhancers, such as D-cycloserine (a partial agonist of the NMDA receptor-associated glycine site), are known to improve learning and memory formation and to ameliorate MK-801-induced memory impairment in various behavioral tasks (Baxter et al., 1994; Schwartz et al., 1996; Gabriele and Packard, 2007). In the present study, we also observed that EEPV enhanced cognitive performances in normal naïve mice, which may be related to the enhancement of glutamatergic neurotransmission.

In summary, we found that EEPV ameliorated scopolamine-induced memory impairment in the passive avoidance, the Y-maze, and the Morris water maze tasks in mice. Moreover, MK-801-induced memory dysfunction was also ameliorated by EEPV in the passive avoidance task. These results suggest that the beneficial effects of EEPV are mediated by enhancement of cholinergic neurotransmitter system via NMDA receptor signaling. Although the findings of this study may not provide clinically useful outcomes in patients or in normal humans, they do suggest that EEPV would be useful for treating diseases that cause cognitive impairments such as AD and schizophrenia.

Conflict of interest

The authors declare that there are no conflicts of interest.

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