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Cognitive-enhancing Activity of Loganin Isolated from *Cornus officinalis* in Scopolamine-induced Amnesic Mice

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We examined anti-amnesic activity of the methanolic extract of *Cornus officinalis* fruits (COT) and a major constituent, loganin using scopolamine-induced (1 mg/kg body weight, s.c.) amnesic mice with both passive avoidance and the Morris water maze tests. Oral treatment of mice with COT (100 mg/kg body weight) and loganin (1 and 2 mg/kg body weight) significantly mitigated scopolamine-induced memory deficits in passive avoidance test. In the Morris water maze test, oral treatment of loganin significantly ameliorated scopolamine-induced memory deficits showing the formation of long-term and/or short-term spatial memory. Moreover, loganin (2 mg/kg body weight) significantly inhibited acetylcholinesterase activity by as much as 45% of control in the mouse hippocampus. These results indicate that loganin may exert anti-amnesic activity in *in vivo* through acetylcholinesterase inhibition.

Key words: *Cornus officinalis*, Loganin, Scopolamine-induced amnesic mice, Passive avoidance test, Morris water maze test, Acetylcholinesterase

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease and causes memory loss and dementia, which mostly affects the elderly population (Francis et al., 1993). Cognitive impairment in AD is caused mainly by death of cholinergic neurons in basal forebrain area, though other neurotransmitter systems could well be involved (Bartus et al., 1982; Collerton, 1986; Kopelman and Corn, 1988). A deficit of acetylcholine in an AD brain is well known (Perry et al., 1978; Wilcock et al., 1982). These findings lead us to search for agents that increase acetylcholine level in the brain. Impairment of learning and memory, as the most characteristic manifestation of dementia, can be induced chemically in experimental animals by scopolamine, a cholinergic antagonist known to interfere with acetylcholine transmission in the central nervous system (Misane and Ogren, 2003). The experimental animal model of scopolamine-induced amnesia has been extensively used to screen for compounds with potential therapeutic value in dementia (Bejar et al., 1999; Rubaj et al., 2003). Thus, we have tried to search for cognitive-enhancing compounds from natural resources by using scopolamine-induced memory impairment in mice.

In the course of screening, a methanolic extracts of *Cornus officinalis* Sieb. et Zucc. (Cornaceae) fruits found show a significant cognitive-enhancing activity. The fruits of *C. officinalis* are a well-known traditional medicine for its tonic, analgesic and diuretic properties in Korea, Japan and China, and this plant is widely distributed in Korea (Kim and Kwak, 1998). Loganin (Fig. 1), a major iridoid glycoside isolated from *C. officinalis* has been reported to exhibit immune regulating function, protective effect on experimental diabetic nephropathy, anti-inflammatory and anti-shock effects (Guo et al., 2001; Mathad et al., 1998; Wang et al., 1999).

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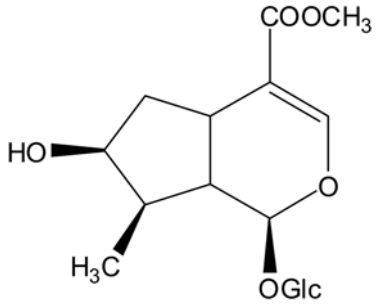

Fig. 1. The chemical structure of loganin

However, there is no report of cognitive-enhancing activity from *C. officinalis* or its constituents.

We tried to examine how the methanolic extract of *C. officinalis* fruits (COT) and a major constituent, loganin mitigated scopolamine-induced memory impairment in mice. To assess the efficacy of COT and loganin, mice were treated with COT or loganin, and then scopolamine was used to induce memory impairment. The restored degree of impairment was gauged using both passive avoidance and the Morris water maze tests with or without treatment.

MATERIALS AND METHODS

Extraction and isolation

The fruits of *C. officinalis* were purchased from Kyungdong Oriental Medicinal Herb Market, Seoul, Korea and a voucher specimen (SNUPH-060929) has been deposited in the Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University. The fruits of *C. officinalis* (1 kg) were extracted three times with 80% MeOH in an ultrasonic apparatus. Upon removal of the solvent *in vacuo*, the methanolic extract (COT) was yielded 400 g of material. The methanolic extract was suspended in H_2O and partitioned successively with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. The *n*-BuOH (20.5 g) fraction was subjected to column chromatography (CC) over silica gel eluted with *n*-hexane-EtOAc-MeOH mixture (50:1:0, 20:1:0, 10:1:0, 5:1:0, 3:1:0, 2:1:0, 1:1:0, 0:1:0, 0:50:1, 0:20:1, 0:10:1, 0:5:1, 0:3:1, 0:2:1, 0:1:1, 0:0:1, 2 L of each solvent) to afford 13 fractions (B1-B13). Loganin (250 mg) was purified by recrystallization with $CHCl_3$ from B9 and demonstrated to be pure (> 97% purity) as evidenced by HPLC analysis.

Loganin was obtained as white amorphous powder. IR (KBr) ν_{max} : 3431, 1711, 1646, 1074 cm^{-1}; FABMS m/z 413 $[M+Na]^+$; ^1H NMR (400 MHz, CD_3OD) : 1.12 (3H, d, J = 6.8 Hz, H-10), 1.65 (1H, m, H-6_{eq}), 1.90 (1H, m, H-8), 2.06 (1H, m, H-6_{ax}), 2.25 (1H, m, H-9), 3.15 (1H, m, H-5), 3.20-3.42 (4H, m, H-2', 3', 4', 5'), 3.68 (1H, dd, J = 11.0, 5.2 Hz, H-6'$_a$), 3.70 (3H, s, OCH_3), 3.93 (1H, dd, J = 11.0, 5.2 Hz, H-6'$_b$), 4.06 (1H, t, J = 4.8 Hz, H-7), 4.67 (1H, d, J = 8.0 Hz, H-1'), 5.30 (1H, d, J = 4.4 Hz, H-1), 7.42 (1H, s, H-3) ppm; ^{13}C NMR (100 MHz, CD_3OD) : 170.3 (C-11), 152.9 (C-3), 114.8 (C-4), 100.8 (C-1'), 98.5 (C-1), 79.3 (C-5'), 78.8 (C-3'), 75.8 (C-7), 75.5 (C-2'), 72.3 (C-4'), 63.5 (C-6'), 52.4 (OCH3), 47.3 (C-9), 41.6 (C-6), 40.7 (C-8), 32.9 (C-5), 14.2 (C-10) ppm

Animals

Male ICR mice (Experimental Animal Breeding Center of Seoul National University, Seoul, Korea), weighing 25-30 g, were used for passive avoidance and the Morris water maze tests following a one-week adaptation period (20 to 23°C; 12 h light cycle from 09:00 to 21:00; food, Agribrand Purina Korea, and water *ad libitum*). All experiments were conducted according to the guidelines of the Committee on Care and Use of Laboratory Animals of the Seoul National University.

Passive avoidance test

Training for and testing of passive avoidance performance were carried out in two, identical, light and dark square boxes (Gemini avoidance system, San Diego Instrument Inc., USA) as described in our previous report (Kim et al., 1999; Kim et al., 2003; Kang et al., 2003). The mice were initially placed in the light chamber and ten seconds later the door between compartments was opened. When mice entered the dark compartment, the door automatically closed and an electrical foot shock (0.1 mA/10 g body weight) for a time period of 2 sec was delivered through the stainless steel rods (one trial training). Ten mice were used per treatment. Mice received 0.5% carboxymethylcellulose (CMC), COT (100 mg/kg body weight, dissolved in 0.5% CMC) or loganin (1 and 2 mg/kg body weight, dissolved in 0.5% CMC) by oral administration 120 min before the training trial. After 90 min, amnesia was induced in mice with scopolamine (1 mg/kg body weight, dissolved in saline) given subcutaneously. Twenty-four hrs after the training trial, the mice were again placed in the light compartment. The latency time to enter the dark compartment was measured. If the mice did not enter the dark compartment within 180 sec, we concluded that the mice had memorized the passive avoidance training after one training trial.

Morris water maze test

A spatial memory test was performed by the method of Morris (1984) with minor modification described in our previous reports (Kim et al., 2003; Kang et al., 2003). The Morris water maze is a white circular pool (90 cm in diameter and 45 cm in height) with a featureless inner surface. The circular pool was filled to a height of 30 cm with water (20± 1°C), in which 500 mL of milk was mixed. The pool was divided into four quadrants of equal area. A white platform (6 cm in diameter and 29 cm in height) was centered in one of the four quadrants of the pool and submerged 1 cm below the water surface so that it was invisible at water level. The continuous location of each swimming mouse from the start position to the platform was monitored by a video tracking system (Smart 2.5). In the water maze experiments, the first day of the experiment was dedicated to swimming training for 60 sec in the absence of the platform. In the following days, the mice were given two trial sessions each day for four consecutive days. During each trial, the escape latencies of mice, as measured with a stop-watch, were recorded by the same experimenter. This parameter was averaged for each session of trials and for each mouse. Once the mouse located the platform, it was permitted to remain on it for 10 sec. If the mouse did not locate the platform within 120 sec, it was placed on the platform for 10 sec and then removed from the pool. The mouse was given two daily trials for 4 days with an inter-trial interval of 20 min. The point of entry of the mouse into the pool and the location of the platform for escape remained unchanged between trials 1 and 2 but was changed on each day. The decrease in escape latency from day to day in trial 1 represents long-term memory or reference memory while that from trial 1 to trial 2, represents short-term memory or working memory. Ten mice were used per treatment. Mice were pretreated with 0.5% CMC, COT (100 mg/kg body weight, dissolved in 0.5% CMC) or loganin (1 and 2 mg/kg body weight, dissolved in 0.5% CMC) by oral administration 120 min before the training trial. After 90 min, amnesia was induced in mice with scopolamine (1 mg/kg body weight, dissolved in saline) given subcutaneous. All mice were tested for spatial memory 30 min after the administration of scopolamine.

Acetylcholinesterase assay

Mice were administered with 0.5% CMC, COT (100 mg/kg body weight, p.o.) or loganin (1 and 2 mg/kg body weight, p.o.). Ten mice were used per treatment. The mice were decapitated 40min after treatment and the brains were removed. Acetylcholinesterase activity was measured by the method of Ellman et al. (1961), with slight modification (Kang et al., 2003). The cerebral cortex and hippocampus were each dissected out. Each part of the brain tissue was rapidly homogenized, respectively, with sodium phosphate buffer (0.1 mM, pH 7.4). Each homogenate was preincubated for 5min at 37°C with 0.1 mM tetraisopropyl pyrophosphoramide (TPPA), a selective inhibitor of butyrylcholinesterase. For the assay of acetylcholinesterase activity, a reaction mixture that contained sodium phosphate (0.1 mM, pH 8.0) 470 μL, 4% 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) 167 μL and 33 μL of homogenate was incubated for 5 min at 37°C. The acetylcholine iodide (1 mM) 280 μL was added to the reaction mixture. After incubation for 3 min at 37°C, the reaction was terminated by adding 50 μL neostigmine (2 mM). Acetylcholinesterase activity was calculated as the optical density (OD) value per mg protein. Protein concentrations were determined using BCA Kit with bovine serum albumin as a standard (Simith et al., 1985).

Statistical analysis

Data for the Morris water maze and passive avoidance tests were expressed as mean±S.E.M. The activities of acetylcholinesterase were expressed as mean±S.D. Passive avoidance latencies were analyzed by one-way ANOVA. The Morris water maze latencies were analyzed by two-way ANOVA with the day as one variable and the treatment as a second. The data were considered to be significant statistically if the probability had a value of 0.05 or less.

RESULTS AND DISCUSSION

The effects of COT and loganin on the scopolamine-induced memory deficit were evaluated using passive avoidance test. The step-through latency of the scopolamine-treated (1 mg/kg body weight s.c.) group was significantly shorter than that of the 0.5% CMC-treated control group. The shorter step-through latency induced by scopolamine was significantly reversed by COT (100 mg/kg body weight p.o.) and loganin (1 and 2 mg/kg body weight p.o.) (Table I). It was also found that step-through latencies of the only COT or loganin treated group of each dose were not significantly different from that of control group (data not shown). Donepezil, an acetylcholinesterase inhibitor and the most widely used tre-

Table I. Effects of acute oral treatment of loganin and COT on the scopolamine-induced amnesic mice in the passive avoidance test[a]

Experimental treatment	Step through latency (s) (% of control)
Control[b]	180.0 ± 0.0 (100%)
Scopolamine[c]	40.1 ± 9.3 (22.3%)
Scopolamine + COT 100 mg/kg	102.9 ± 16.8** (57.2%)
Scopolamine + Loganin 1 mg/kg	93.2 ± 27.0* (51.8%)
2 mg/kg	134.6 ± 23.4** (74.8%)
Scopolamine + Donepezil 2 mg/kg	130.0 ± 17.4** (72.7%)

[a] 120 min before the training trial, mice received test sample (p.o.). After 90 min, amnesia was induced in mice with scopolamine (1 mg/kg body weight, s.c.). Twenty-four hours after the training trial, the mice were again placed in the light compartment. The latency time to enter the dark compartment was measured. The values shown are the mean latency ± S.E.M. Results differ significantly from value in scopolamine-treated group: *P<0.01; **P<0.001.

[b] Control means 0.5% CMC and saline-treated group (10 mL/kg body weight, p.o.).

[c] Scopolamine means 0.5% CMC and scopolamine-treated group (1 mg/kg body weight, s.c.)

atment for AD, was used as a positive control (Lahiri et al., 2002). Donepezil at a dose of 2mg/kg body weight (p.o.) restored the step-through latency by 72.7% when compared to the 0.5% CMC treated control group in the passive avoidance test in our present study. Considering the cognition-enhancing activity of donepezil, it seems that the potency of loganin (2 mg/kg body weight p.o.) is comparable to that of donepezil.

We examined whether COT and loganin affected working or reference memory using the Morris water maze, a test that can evaluate spatial memory. Animals in the saline-treated control group rapidly learned the location of the platform. This was demonstrated by exhibiting a reduction in swimming times from the first to the second trial on day 1 and by reaching stable latencies at day 2 (Fig. 2A). Furthermore, we found the swimming pathway required to find the submerged platform was simplified in groups given saline (data not shown). The shortened swimming distance was well correlated with the decrease of swimming time. The only COT or loganin treated group also showed similar behavioral result to that of control group (Fig. 2B-2D). By contrast, in the scopolamine-treated group (1 mg/kg body weight, s.c.), a characteristic swimming behavior, consisting of circling around the pool, was observed; the swimming times in trials 1 and 2 remained unchanged throughout the entire 4-day testing period (Fig. 2E). Treatment of COT (100 mg/kg body weight, p.o.) did not antagonize the effect of scopolamine on swimming time in the testing period (Fig. 2F). Treatment of loganin (1 and 2 mg/kg body weight, p.o.) significantly antagonized the effect of scopolamine on swimming time in the testing period (Fig. 2G, 2H). In the loganin-treated mice, the latencies for trial 2 were lower than that in trial 1. Furthermore, loganin treatment tended to decrease the swimming time in each of the 1st trials over the four days (Fig. 2G, 2H) and also simplified the swimming pathway, which was well correlated with the degree of the shortened swimming time. The swimming times in control or scopolamine-treated group with or without loganin showed highly significant effects with respect to the day ([trial 1]: $F(3, 144) = 14.4$, $P<0.0001$; [trial 2]: $F(3, 144) = 6.77$, $P<0.0005$), with respect to treatment ([trial 1]: $F(3, 144) = 30.9$, $P<0.0001$; [trial 2]: $F(3, 144) = 59.3$, $P<0.0001$) and with respect to the day and treatment interaction ([trial 1]: $F(9, 144) = 1.9$, $P<0.05$; [trial 2]: $F(9, 144) = 2.7$, $P<0.01$).

Scopolamine interferes with memory and cognitive function and subsequently causes similar degrees of impairment in both reference and working memory in the Morris water maze test (Bejar et al., 1999). The paradigm used in the Morris water maze test enables the simultaneous analysis of distinctions between working and reference memory processes (Bejar et al., 1999; Muir et al., 1993). In this study, the 0.5% CMC-treated group exhibited that both working and reference memories were being formed. In contrast, in the scopolamine-treated group, neither working nor reference memory was exhibited. Treatment of loganin (1 and 2 mg/kg body weight p.o.) significantly reduced the deficits in working and reference memories induced by scopolamine (Fig. 2G, 2H). Especially, loganin (2 mg/kg body weight p.o.)-treated group exhibited significant gradual improvement in reference memory.

Since acetylcholinesterase inhibitors are known to antagonize scopolamine-induced amnesia, the effect of loganin on acetylcholinesterase activities in cerebral cortex and hippocampus of the brain of mice was tested (Table II). At a dose of 1 and 2 mg/kg, loganin significantly inhibited acetylcholinesterase activity in hippocampus (25%, 45%, respectively, $P<0.05$) as compared to control mice injected with 0.5% CMC. However, in the cortex, loganin did not inhibit acetylcholinesterase activity. Treatment of

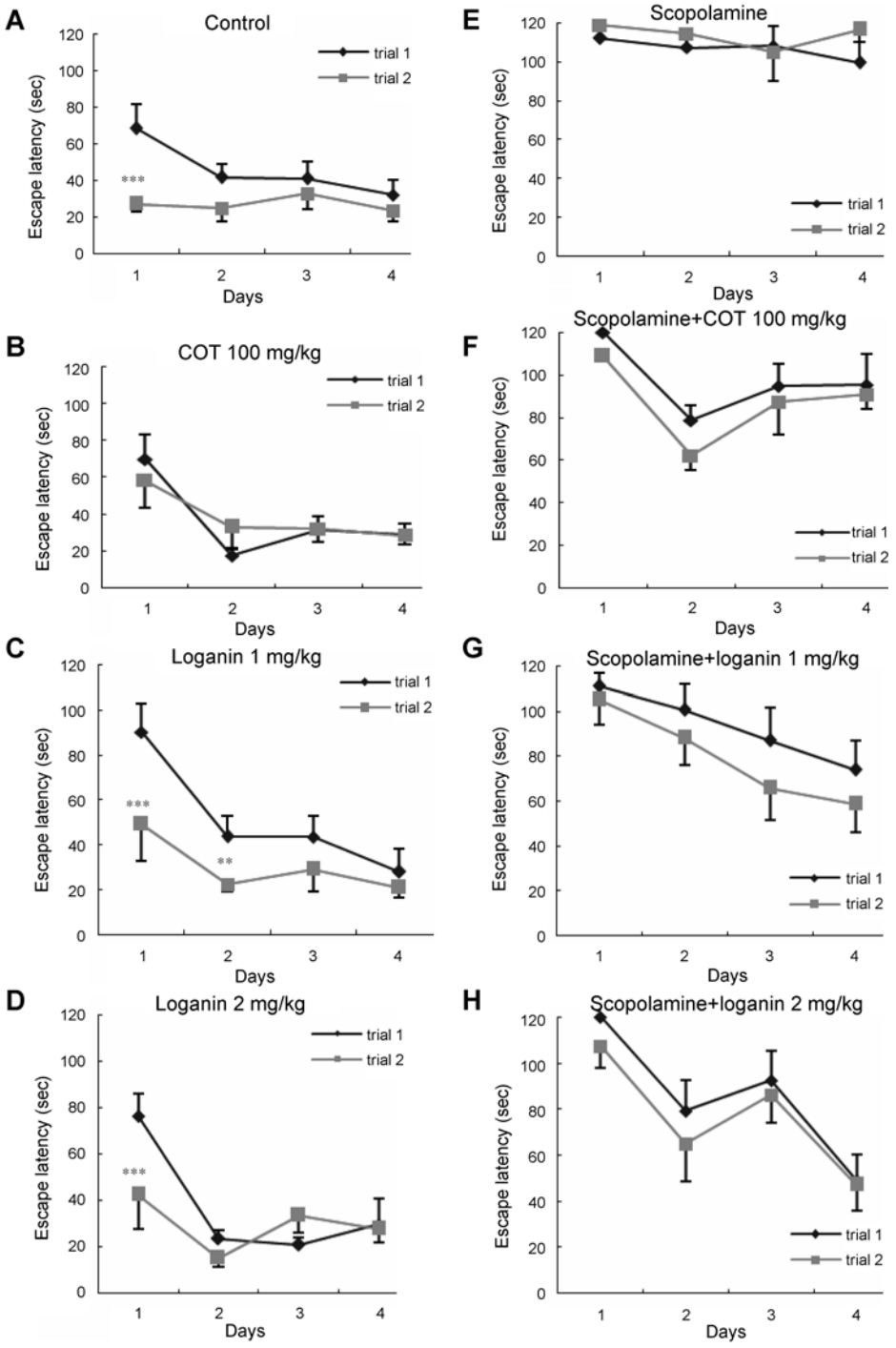

Fig. 2. The effect of oral treatment of COT and loganin on the scopolamine-induced amnesic mice in the Morris water maze test. After oral treatment of COT (100 mg/kg body weight) and loganin (1 and 2 mg/kg body weight), mice were given two sessions of trails each day for four consecutive days. Trial 2 was carried out 20 min after trail 1. The swimming time required for the mouse to escape was recorded in each trial. Each day, the mice were treated with COT and loganin. After 90 min, amnesia was induced in mice with scopolamine (1 mg/kg body weight, s.c.). All mice were tested for spatial memory 30 min after the injection of scopolamine. The values shown are the mean latency ± S.E.M. Results significantly differ from value in trial 1: $p < 0.01^{**}$; $p < 0.001^{***}$. **A**. 0.5 % CMC – saline (10 mL/kg body weight, p.o.) - treated control group. **B**. COT (100 mg/ kg body weight, p.o.) - saline - treated group. **C**. loganin (1 mg/ kg body weight, p.o.) - saline - treated group. **D**. loganin (2 mg/ kg body weight, p.o.) - saline - treated group. **E**. Scopolamine (1 mg/kg body weight, s.c.) - treated group. **F**. COT (100 mg/ kg body weight, p.o.) - treated group before 90 min of scopolamine (1 mg/kg body weight, s. c.) treatment. **G**. loganin (1 mg/ kg body weight, p.o.)- treated group before 90 min of scopolamine (1 mg/kg body weight, s. c.) treatment. **H**. loganin (2 mg/ kg body weight, p.o.) - treated group before 90 min of scopolamine (1 mg/kg body weight, s. c.) treatment.

Table II. Effects of COT and loganin on AChE activity in cortex and hippocampus of the brain[a]

Treatment		OD value/mg protein	
		Cortex	Hippocampus
Control		0.1203 ± 0.007	0.1081 ± 0.010
COT	100 mg/kg	0.1293 ± 0.007	0.1154 ± 0.028
Loganin	1 mg/kg	0.1436 ± 0.021	0.0814 ± 0.012*
	2 mg/kg	0.1192 ± 0.013	0.0598 ± 0.027*

[a] The values shown are the mean optical density/mg protein ± S.D. Results differ significantly from values in same area of control: *P<0.05.

COT (100 mg/kg body weight, p.o.) did not inhibit acetylcholinesterase activity in hippocampus and cortex. These results could explain that COT did not antagonize the effect of scopolamine on swimming time in Morris water maze test.

In recent study, on the pharmacokinetics and tissues distribution of loganin in rats showed that loganin is absorbed and excreted quickly and distributed widely in many tissues or organs after being taken orally. There was no long-term accumulation of loganin in rat tissues and it was difficult for loganin cross the blood-brain barrier. However, the absolute bioavailability of loganin was calculated to be 13.2%; only 5% of administered prototype medicine was detected in urine, very little was detected in bile, and it was undetected in feces (Li et al., 2006, 2007). The result indicated that loganin is probably metabolized by liver microsomes or by intestinal bacteria. Therefore, we speculate that this conversion will play a role in the absorption, bioavailability and activities of loganin and metabolites of loganin will exert acetylcholinesterase inhibitory activity in hippocampus of the brain. Li et al. (2008) reported that by using microbial transformation methods, two metabolites of loganin (log-1 and log-2) were purified and identified. To assess a more relevant relationship between the metabolite and activity, metabolites should be assessed for their cognitive enhancing activity.

In conclusion, based on the results of passive avoidance and the Morris water maze tests using mice with amnesia induced by scopolamine, we found that loganin had cognitive-enhancing activity. Recently, natural products such as galanthamine and huperzine A have received approval for the treatment of AD or have been under clinical study (Mantle et al., 2000; Ma and Gang, 2008). Natural compounds such as loganin that exert anti-amnesic activity in *in vivo* might offer a useful therapeutic choice in the treatment of AD.

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