Acteoside of *Callicarpa dichotoma* Attenuates Scopolamine-Induced Memory Impairments

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We previously reported that ten phenylethanoid glycosides including acteoside isolated from the leaves and twigs of *Callicarpa dichotoma* significantly attenuated glutamate-induced neurotoxicity. In the present study, we examined anti-amnesic activity of acteoside using scopolamine-induced (1 mg/kg body weight, s.c.) amnesic mice with both passive avoidance and Morris water maze tests. Acute oral treatment (single administration prior to scopolamine treatment) of mice with acteoside (1.0, 2.5 mg/kg body weight) significantly mitigated scopolamine-induced memory deficits in the passive avoidance test. It is interesting to note that prolonged oral daily treatment of mice with much lower amount (0.1 mg/kg body weight) of acteoside for 10 d reversed the scopolamine-induced memory deficits. In the Morris water maze, prolonged oral treatment with acteoside (prolonged daily administration of 1.0 mg/kg body weight for 10 d) significantly ameliorated scopolamine-induced memory deficits showing the formation of long-term and/or short-term spatial memory. We suggest, therefore, that acteoside has anti-amnesic activity that may ultimately hold significant therapeutic value in alleviating certain memory impairment observed in Alzheimer’s disease.

Key words *Callicarpa dichotoma*; Verbenaceae; acteoside; scopolamine-induced amnesic mice; passive avoidance test; Morris water maze test

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by irreversible, progressive loss of memory followed by complete dementia. Cognitive impairment in AD is caused mainly by death of cholinergic neurons in basal forebrain area, though other neurotransmitter systems could well be involved. A deficit of acetylcholine (ACh) in an AD brain is well known. At the same time, extent of dementia correlates well with the extent of neuronal death caused by excess of glutamate, the most prevalent excitatory neurotransmitter in the brain.2—4

We previously reported that ten phenylethanoid glycosides including acteoside (Fig. 1) isolated from the leaves and twigs of *Callicarpa dichotoma* (Verbenaceae) significantly attenuated glutamate-induced neurotoxicity.5 In the present study, we tried to examine whether the major constituent, acteoside mitigated scopolamine-induced memory impairment in mice. Acteoside (= Verbascoside, Fig. 1) is a phenylethanoid glycoside distributed in *C. dichotoma* and other medicinal plants. Many studies have reported that acteoside shows anti-oxidative, anti-inflammatory, anti-nephritic and anti-hepatotoxic activities.5—9 It contains a hydroxyphenylethyl and a caffeoyl moiety which are well-known antioxidants. To assess the efficacy of the acteoside, mice were treated with acteoside and then scopolamine was used to induce memory impairment. The degree of impairment was gauged using both the passive avoidance and the Morris water maze tests with or without treatment with acteoside.

MATERIAL AND METHODS

**Extraction and Isolation** The *C. dichotoma* were collected at Baegwoon Mountain, Gwangyang city, Jeollanam-do, Korea and identified by Dr. Jong Hee Park, professor of Pusan National University. The voucher specimen (SNUPH No. 691) has been deposited in the Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University. The air-dried leaves and twigs of *C. dichotoma* (8.5 kg) were extracted three times with 70% MeOH in an ultrasonic apparatus. Upon removal of the solvent in vacuo, the methanolic extracts yield 780 g of material (9.2% by dry weight). The methanolic extract was suspended in H₂O and partitioned successively with n-hexane, CHCl₃, EtOAc, and n-BuOH. Detailed experimental procedures of isolation and identification of phenylethanoid glycosides from n-BuOH fraction (360 g) were shown in our previous work.5 Acteoside was (Fig. 1) demonstrated to be pure (>98% purity) as evidenced by HPLC analysis.

**Animals** Male ICR mice (Experimental Animal Breeding Center of Seoul National University, Seoul, Korea), weighing 25—30 g, were used for passive avoidance and water maze tests following a one-week adaptation period (20 to 23 °C; 12 h light cycle from 9:00 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 San Francisco Inc., U.S.A.) as described in our previous report.10—12 The mice...
were initially placed in the light chamber and 10 s 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 s was delivered through the stainless steel rods (one trial training). Ten mice were used per treatment. For acute treatment experiment, mice received acteoside (0.1, 1.0, 2.5 mg/kg body weight) by oral administration 120 min before the training trial. After 90 min, amnesia was induced in mice with scopolamine (1.0 mg/kg body weight, dissolved in 0.1% DMSO) given subcutaneously. For prolonged treatment experiment, mice were orally treated with acteoside (0.1 mg/kg body weight/d) for 10 d. 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. If the mice did not enter the dark compartment within 180 s, 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 with minor modification described in our previous reports. 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. In the water maze experiments, the first day of the experiment was dedicated to swimming training for 60 s 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 stopwatch, 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 s. If the mouse did not locate the platform within 120 s, it was placed on the platform for 10 s and then removed from the pool. The mouse was given two daily trials for 4 d 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. For prolonged treatment experiment, mice were pretreated with acteoside (1.0 mg/kg body weight/d, p.o.) for 10 d. After 90 min, amnesia was induced in mice with scopolamine (1.0 mg/kg body weight, dissolved in 0.1% DMSO) given subcutaneously. All mice were tested for spatial memory 30 min after the administration of scopolamine.

**Statistical Analysis**  All data were expressed as mean ± S.D. Passive avoidance latencies were analyzed by one-way ANOVA. 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 present study has been performed in an attempt to evaluate the cognitive-enhancing activity of the major constituent, acteoside isolated from the leaves and twigs of *C. dichotoma* for the possibility of preventing and/or treating memory deficits such as those seen in AD.

We examined whether acteoside mitigated the scopolamine-induced memory deficits through the passive avoidance test. The step-through latency of the scopolamine-treated (1.0 mg/kg body weight, s.c.) group was significantly shorter than that of the 0.5% carboxymethylcellulose (CMC)-saline-treated control group. The shorter step-through latency induced by scopolamine was significantly reversed by acteoside (1.0—2.5 mg/kg body weight, p.o.) (Table 1). It was also found that escape latencies of acteoside alone-treated groups of each dose were not significantly different from that of control group. Donepezil, an AChE inhibitor and most widely used treatment for AD, was used as a positive control. Donepezil was reported to exhibit a bell-shaped dose–response curve (0.01—10 mg/kg body weight, p.o.) in passive avoidance tests using scopolamine (2.0 mg/kg body weight i.p.)-induced amnesic rats and restore the step-through latency by about 60.8% at a dose of 0.1 mg/kg body weight as compared to the saline-treated control group. Consistent with this result, donepezil at a dose of 0.1 mg/kg body weight (p.o.) restored the step-through latency by 45.6% as compared to the 0.5% CMC-saline 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 acteoside is comparable to that of donepezil. Next we tested the effect of the prolonged treatment of acteoside on passive avoidance test. Daily oral treatments with acteoside (0.1 mg/kg body weight) for 10 d produced no significant effect on body weight compared to the vehicle group. Animals appeared to behave normally in their home cage and test environments. In contrast with the

<table>
<thead>
<tr>
<th>Experimental treatment</th>
<th>Step through latency (s) ( % of control)</th>
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<tr>
<td>Control ^a^</td>
<td>176.4 ± 7.2 (100%)</td>
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<tr>
<td>Acteoside ^c^</td>
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<tr>
<td>0.1 mg/kg</td>
<td>171.5 ± 15.6 (97.3%)</td>
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<tr>
<td>1.0 mg/kg</td>
<td>179.2 ± 1.7 (101.6%)</td>
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<tr>
<td>2.5 mg/kg</td>
<td>174.4 ± 7.0 (98.9%)</td>
</tr>
<tr>
<td>Scopolamine ^d^</td>
<td>25.2 ± 6.6 (14.3%)</td>
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<tr>
<td>Scopolamine + acteoside</td>
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<tr>
<td>0.1 mg/kg</td>
<td>32.4 ± 21.3 (18.4%)</td>
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<tr>
<td>1.0 mg/kg</td>
<td>78.8 ± 33.6** (44.7%)</td>
</tr>
<tr>
<td>2.5 mg/kg</td>
<td>88.4 ± 33.9** (50.1%)</td>
</tr>
<tr>
<td>Scopolamine + donepezil</td>
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<tr>
<td>0.1 mg/kg</td>
<td>80.4 ± 27.4** (45.6%)</td>
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*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 ± S.D. Results differ significantly from value in scopolamine-treated group: 

**p<0.01.

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

c) Acteoside means acteoside and saline-treated group.

d) Scopolamine means 0.5% CMC and scopolamine-treated group (1.0 mg/kg body weight, s.c.).
result of acute treatment at the dose of 0.1 mg/kg body weight, prolonged treatment of acteoside (0.1 mg/kg body weight) significantly improved the memory deficit in mice with amnesia induced by scopolamine (Table 2).

We examined whether acteoside affected working or reference memory using the Morris water maze, a test that can evaluate spatial memory. However, acute treatment of acteoside 1.0 mg/kg body weight did not significantly antagonize the effect of scopolamine on the escape latency in the test period (data not shown). The once daily oral treatment with acteoside (1.0 mg/kg body weight, p.o.) for ten days on spatial memory was also investigated in the Morris water maze test. 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. Acteoside alone-treated group also showed similar behavioral result to that of control group. By contrast, in the scopolamine-treated group (1.0 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-d testing period (Fig. 2B). Prolonged treatment of acteoside (1.0 mg/kg body weight, p.o.) significantly antagonized the effect of scopolamine on swimming time in the testing period (Fig. 2C). In the acteoside-treated mice, the latencies for trial 2 were significantly lower than that in trial 1. Furthermore, acteoside treatment tended to decrease the swimming time in each of the 1st trials over the 4 d (Fig. 2C) and also simplified the swimming pathway, which was well correlated with the degree of the shortened swimming time. The swimming times in groups control or scopolamine with or without

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<tr>
<td>Control**</td>
<td>174.8±14.7 (100%)</td>
<td></td>
</tr>
<tr>
<td>Scopolamine**</td>
<td>34.7±18.2 (19.9%)</td>
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<tr>
<td>Scopolamine + acteoside 0.1 mg/kg</td>
<td>91.6±28.7** (52.4%)</td>
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a) After daily oral treatment of acteoside for 10 d, mice were given the passive avoidance test. The values shown are the mean latency±S.D. Results differ significantly from value in scopolamine-treated group: **p<0.01.

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 (1.0 mg/kg body weight, s.c.)

Fig. 2. The Effect of Prolonged Oral Treatment of Acteoside (1.0 mg/kg Body Weight) on the Scopolamine-Induced Amnesic Mice in the Morris Water Maze Test

After daily oral treatment of acteoside (1.0 mg/kg body weight) for 10 d, mice were given two sessions of trials each day for four consecutive days. Trial 2 was carried out 20 min after trial 1. The swimming time required for the mouse to escape was recorded in each trial. Each day, the mice were treated with acteoside (1.0 mg/kg body weight, p.o.). After 90 min, amnesia was induced in mice with scopolamine (1.0 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.D. 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) Acteoside (1.0 mg/kg body weight, p.o.) alone-treated group. (C) Scopolamine (1.0 mg/kg body weight, s.c.)-treated group. (D) Acteoside (1.0 mg/kg body weight, p.o.)-treated group before 90 min of scopolamine (1.0 mg/kg body weight, s.c.) treatment.
acteoside showed highly significant effects with respect to the day ([trial 1]: $F(3,72)=50.4$, $p<0.0001$; [trial 2]: $F(3,72)=26.9$, $p<0.0001$), with respect to treatment ([trial 1]: $F(2,72)=178.7$, $p<0.0001$; [trial 2]: $F(2,72)=253.0$, $p<0.0001$) and with respect to the day and treatment interaction ([trial 1]: $F(6,72)=21.9$, $p<0.0001$; [trial 2]: $F(6,72)=16.7$, $p<0.0001$).

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.\(^{16}\) The paradigm used in the Morris water maze test enables the simultaneous analysis of distinctions between working and reference memory processes.\(^{16,17}\) In this study, the 0.5% CMC-saline-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. Prolonged treatment of acteoside (1.0 mg/kg body weight, $p\alpha$) significantly reduced the deficits in working and reference memories induced by scopolamine (Fig. 2C). From the second day of testing, acteoside-treated group remarkably improved the deficit in working memory induced by scopolamine. In addition, acteoside-treated group exhibited significant gradual improvement in reference memory.

Although acteoside exerted its remarkable cognitive-enhancing action on memory deficits induced by a muscarinic antagonist, scopolamine, in experiment mice, acteoside did not show a significant effect on AChE activity (data not shown). Therefore, we think that acteoside could have a direct/indirect action as cholinergic agonist or a modulatory action on cholinergic transmission, because acteoside attenuated muscarinic antagonist-induced memory impairments. Recently, scopolamine is additionally known to trigger the action on cholinergic transmission, because acteoside attenuated muscarinic antagonist-induced memory impairments.

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 acteoside had remarkable cognitive-enhancing activity. Recently, natural products and/or such synthetically-developed active components as galanthamine and huperzine A have received approval for the treatment of AD or have been under clinical study.\(^{24,25}\) Natural compounds such as acteoside that exert anti-amnesic activity in vivo might offer a useful therapeutic choice in the treatment of AD.

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REFERENCES