**Angelica keiskei** Ameliorates Scopolamine-Induced Memory Impairments in Mice

Sa Rang Oh, Su-Jin Kim, Dong Hyun Kim, Jong Hoon Ryu, Eun-Mi Ahn, and Ji Wook Jung

*College of Pharmacy, Keimyung University; Sindang-dong, Dalseo-gu, Dae-gu 704–701, Republic of Korea: *Department of Cosmeceutical Science, Daegu Haany University; #Department of Herbal Foodceutical Science, Daegu Haany University; #Department of Herbal Medicinal Pharmacology, College of Herbal Bio-industry, Daegu Haany University; Yugoslavia, Kyungsan 712–715, Republic of Korea; and *Department of Oriental Pharmaceutical Science, College of Pharmacy, Kyung Hee University; J Hoeki-dong, Dongdaemoon-Gu, Seoul 130–701, Republic of Korea.

© 2013 The Pharmaceutical Society of Japan

*To whom correspondence should be addressed. e-mail: jwjung@dhu.ac.kr

Memory impairment is the most common symptom in patients with Alzheimer’s disease (AD). *Angelica keiskei* (AK) has traditionally been used as a diuretic, laxative, analeptic and galactagogue. However, the anti-amnesic effects of AK and its molecular mechanisms have yet to be clearly elucidated. The aim of the present study is to evaluate the effects of AK on scopolamine-induced memory impairments in mice. The regulatory effect of AK on memory impairment was investigated using passive avoidance, Y-maze and the Morris water maze tasks. Acetylcholinesterase (AChE) activity assay was performed to investigate the cholinergic antagonistic effect of AK in the hippocampus. The effect of AK on phosphorylation of cAMP response element-binding protein (CREB) and expression of brain-derived neurotrophic factor (BDNF) were evaluated by Western blot assays and immunohistochemistry. The findings showed that AK significantly attenuated scopolamine-induced cognitive impairment in mice. Increase of AChE activity caused by scopolamine was significantly attenuated by AK. Additionally, AK significantly recovered the phosphorylation of CREB and expression of BDNF reduced by scopolamine in the hippocampus. Taken together, these results provide experimental evidence that AK might be a useful agent in preventing deficit of learning and memory caused by AD and aging.

**Key words** *Angelica keiskei*: memory impairment; scopolamine; acetylcholinesterase; cAMP response element-binding protein; brain-derived neurotrophic factor

Numerous people suffer from Alzheimer’s disease (AD) and aging associated memory impairment, and this problem is growing with increase in the life span. Despite extensive research implicating components as possible factors associated with the development of memory impairment, its pathogenesis is still incompletely understood. Accumulating evidence suggested cholinergic deficit is correlated with the severity of cognitive dysfunction and memory loss in AD patients as well as in aged humans. Acetylcholine (ACh) is known to regulate the learning and memory process. It was reported that ACh activity was increased in the training of spatial memory performance and it was decreased in memory deficit. Acetylcholinesterase (AChE) plays a key role in the rapid hydrolysis of ACh and it is responsible for regulation of ACh levels. For this reason, inhibition of AChE activity has been suggested as a strategy for treatment of AD. Recently, several AChE inhibitors including tacrine, donepezil, and galantamine have been approved for AD therapy and they act by counteracting the ACh deficit. However, these drugs are not so ideal in clinical use due to side effects such as hepatotoxicity. Thus, it is necessary to continue to seek alternative drugs for the treatment of AD and aging associated memory impairment and prevention clinical problems.

The cAMP-responsive element-binding protein (CREB), a transcription factor, is involved in memory consolidation. The dysfunction of CREB disrupts hippocampus-dependant memory formation and it has been suggested that CREB is required for memory stability. It was reported that β-amyloid peptide disrupted the hippocampus-dependant memory by affecting the CREB signaling pathway. Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and plays important roles in many developmentally regulated processes, such as cell survival, differentiation and synaptic plasticity of neurons as well as neurogenesis. Growing evidence suggested that BDNF is associated with neurodegenerative diseases such as AD and Parkinson’s disease. Additionally, BDNF is believed to be responsible for synaptic plasticity and memory performance and is coupled to the activation of CREB. These studies demonstrated that the CREB-BDNF pathway is implicated in long-term memory formation.

*Angelica keiskei* (AK) has traditionally been used as a diuretic, laxative, analeptic and galactagogue. Recently, it was reported that AK has an anti-inflammatory effect through the regulation of inflammatory mediators. Additionally, AK induced an elevation of serum high-density lipoprotein levels and a reduction of liver triglyceride levels. Unfortunately, to date, there is no information available as to whether AK regulates memory impairment.

Scopolamine, an anti-cholinergic agent, inhibits central cholinergic neuronal activity and impairs learning and memory. Therefore, scopolamine challenge is very useful for investigating learning and memory studies in which the cholinergic system is involved. The purpose of this study was to determine the beneficial effects of AK on scopolamine-induced memory impairment in mice. The behavioral...
parameters were evaluated using the passive avoidance task, the Y-maze task, and the Morris water maze task in mice. To investigate whether AK possesses central cholinergic activity, we examined activities of cholinergic enzymes such as AChE. Additionally, the effects of AK on memory-related proteins were evaluated in brain tissue.

MATERIALS AND METHODS

Reagents Tacrine (9-amino-1,2,3,4-tetrahydroacridine hydrochloride), (−)-scopolamine hydrobromide, acetylthiocholine iodide and 5,5-dithiobis[2-nitrobenzoic acid] (DTNB) were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Anti-BDNF antibody, anti-CREB antibody and rabbit secondary antibody were purchased from Santa Cruz Biotech (Santa Cruz, CA, U.S.A.). Anti-pCREB antibody was purchased from Cell Signaling Technology (Danvers, MA, U.S.A.).

Animals Male ICR mice at 5 weeks of age, weighing 25–30 g, were purchased from the Orient Co., Ltd., a branch of Charles River Laboratories (Seoul, Korea). Animals were housed 10 per cage, allowed access to water and food ad libitum, and maintained at a constant temperature (24±1°C) and humidity (60±10%) under a 12-h light/dark cycle (light on 08:00–20:00 h). The animals were maintained under laboratory conditions for an acclimatization period of 7 d before performing the experiment. Animal experimental procedures were approved by the ethics committee of Daegu Haany University.

Preparation of AK Angelica keiskei Koidzumi (AK), an herbal plant belonging to the Umbelliferae family, is found primarily in the Korea. The dried AK was purchased from the Human herb company (Kyungsan, Korea) and voucher specimens (HMP-0701) were deposited at the herbarium of the Daegu Haany University. An ethanol extract of the AK leaves was prepared with 70% ethanol solution under room temperature for 24 h and then concentrated under a vacuum. The extract solution obtained was filtered, concentrated on a water bath under vacuo, frozen and lyophilized to yield ethanol extract solution (yield: 2.1%).

Passive Avoidance Task Passive avoidance task was carried out in identical illuminated and non-illuminated boxes (Gemini Avoidance System, San Diego, U.S.A.). The illuminated compartment (20×20×20 cm) contained a 100 W bulb, and the floor of non-illuminated compartment (20×20×20 cm) was composed of 2 mm stainless steel rods spaced 1 cm apart. These compartments were separated by guillotine door (5×5 cm). One hour after the last administration of each drug or vehicle, a mouse was gently placed in the illuminated compartment for the acquisition trial, and the door between the two compartments was opened 10 s later. When mice entered the dark compartment, the door automatically closed and an electrical foot shock (0.5 mA) of 3 s duration was delivered through the stainless steel rods. Twenty-four hours after this acquisition trial, the mouse was again placed in the illuminated compartment for a retention trial. The time taken for a mouse to enter the dark compartment after its door was opened was defined as latency time for both acquisition and retention trials. Latency for entering the dark compartment was recorded up to 180 s. If a mouse did not enter the dark compartment within 180 s, the mouse was removed and assigned a latency score of 180 s. One hour before the acquisition trial, mice were administration of AK (5, 10, 20, 40 mg/kg, per os (p.o.) or tacrine (10 mg/kg, p.o.) as positive control. Memory impairment was induced by scopolamine treatment (1 mg/kg, intraperitoneally (i.p.) 30 min after the administration of AK, tacrine, or 10% Tween 80 solution. Vehicle animals were administered 10% Tween 80 solution only.

Y-Maze Task Y-Maze is used as a measure of immediate spatial working memory which is form of short-term memory.27 The Y-maze is a three arm horizontal maze (40 cm long and 3 cm wide with walls 12 cm high) in which the arms are symmetrically disposed at 120° angles from each other. The maze floor and walls were constructed from dark opaque polyvinyl plastic as has been described previously.28 Mice were initially placed within one arm, and the sequence (i.e., ABCCAB, etc.) and number of arm entries were recorded manually for each mouse over an 8 min period. An actual alternation was defined as entries into all three arms on consecutive choices (i.e., ABC, CAB, or BCA but not ABA). Maze arms were thoroughly cleaned between tasks to remove residual odors. One hour after the last administration of each drug or vehicle, mice were gently placed in the maze. The percentage of alternations was defined according to the following equation: % alternation=([number of alternations]/(total arm entries−2))×100. The number of arm entries serves as an indicator of locomotor activity.

Morris Water Maze Task The Morris water maze is a circular pool with a featureless inner surface and a diameter and height 90 and 45 cm, respectively. 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 containing various visual cues. A white platform (6 cm in diameter, 1 cm below the surface of the water) was then placed in one of the pool quadrants. 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 trial sessions per day with the platform in place. The time interval between each trials session was 1 min. For four trials sessions, mice were placed in the water facing the pool wall in one of the pool quadrants. The entry point was changed in a different order each day. Drug or vehicle was administered 1 h before the first training trial. When a mouse located the platform, it was permitted to remain on it for 10 s. If the mouse did not locate on the platform within 60 s, it was placed on the platform for 10 s. The animal was returned to its home cage and was allowed to dry up under an infrared lamp after each trial. During each trial session, the time taken to find the hidden platform (latency) was recorded using a video camera-based Ethovision System (Noldus, Wageningen, the Netherlands). One day after the last training trial sessions, mice were subjected to a probe trial session in which the platform was removed from the pool, mice were allowed to swim for 120 s to search for it. A record was kept of the swimming time in the pool quadrant where the platform had been previously placed.

AChE Activity Assay AChE activity assay were carried out using an acetylthiocholine iodide substrate by a colorimetric analysis. Whole brains of male ICR mice (25–30 g) were homogenized in a glass Teflon homogenizer (Eyela, Japan) containing 10 volumes of homogenizer buffer (12.5 mM sodium phosphate buffer pH 7.0, 400 mM NaCl), and then centrifuged at 1000×g for 20 min at 4°C. The supernatant so obtained
was used as a source of enzyme for the assay. AK or tacrine was initially dissolved in 1% dimethyl sulfoxide (DMSO) and diluted to various concentrations in Buffer A (100 mM sodium phosphate buffer, pH 8.0) immediately before use. An aliquot of diluted drug solution in Buffer A (10 μL) was then mixed with 640 μL of Buffer A, 100 μL of enzyme source and 25 μL of buffered Ellman’s reagent (10 mM 5,5′-dithio-bis[2-nitrobenzoic acid] and 15 mM sodium bicarbonate) and reacted at room temperature for 30 min. Absorbance was measured at 410 nm immediately after adding the 5 μL acetylthiocholine iodide solution (75 mM), Neostigmine (10 μL) to the reaction mixtures (Tecan Infinite 200, North Carolina, U.S.A.). The concentration of durg required to inhibit acetylcholine esterase activity by 50% (IC50) was calculated using an enzyme inhibition dose response curve. Tacrine was used as a positive control.

**Western Blot Analysis** To investigate the effects of AK on pCREB and BDNF expression in the hippocampus, acquisition trial-treated mice were sacrificed 1 h after single administration of AK (10 mg/kg, p.o.). Isolated hippocampal tissues were homogenized in an ice-chilled Tris–HCl buffer (20 mM, pH 7.4) containing 0.32 M sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ethylene glycol bis(2-aminoethyl ether)-N,N′,N″,N‴-tetraacetic acid (EGTA), 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM sodium orthovanadate and one tablet of protease inhibitor (Roche, Seoul, Korea) per 50 mL of buffer. Samples of homogenates (30 μg of protein) were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. Proteins were transferred to polyvinylidene fluoride (PVDF) membranes in the transfer buffer. After transferring, the membranes were blocked in phosphate buffered saline (PBS) with 0.01% Tween 20 (PBST) containing 5% non-fat dried milk for 1 h at room temperature. Western blots were then incubated for 1 h with a blocking solution (5% skim milk) at room temperature and incubated 1 h with primary antibodies. After three washes in PBST for 30 min, the membranes were incubated for 30 min with secondary antibodies (Abs). After three washes, the protein bands were visualized by an enhanced chemiluminescence detection system according to the recommended producere (Amersham Corp., Newark). The membrane was analyzed with the bio-imaging program of the LAS-4000 mini (FUJIFILM Lifescience U.S.A., Stanford, CT, U.S.A.).

**Immunohistochemistry** Animals were deeply anesthetized by zoletil and transcardially perfused with cold saline followed by 4% paraformaldehyde (PFA). Their brains were removed and post-fixed for 24 h in PFA before further processing. Serial coronal sections (40 μm thickness) were cut for the entire hippocampus using a vibration microtome (Leica, CM 1850, Germany). Free floating sections were incubated for 24 h in PBS (4°C) containing anti-pCREB (1:1000 dilution) or BDNF (1:1000 dilution) antibody, 0.3% Triton X-100, 0.5 mg/mL bovine serum albumin, and 1.5% normal horse serum. The sections were then incubated for 90 min with biotinylated secondary antibody (1:200 dilution), treated with avidin–biotin temperature, and reacted with 0.02% 3,3′-diaminobenzidine and 0.01% H2O2 for approximately 3 min. After each incubation step, sections were washed three times with PBS. Finally, they were mounted on gelatin-coated slides, dehydrated in an ascending alcohol series, and cleared in xylene.

**HPLC Apparatus and Measurements** The extracts used for HPLC analysis were filtered through a 0.45 mm syringe filter (Milipore, MSL, Westboro, MA, U.S.A.) and 20 μL of the filtrate was injected to the HPLC system. The HPLC was Shimadzu LC-20AD instrument equipped with a YMC-PackODS-AM column (5 μm, 4.6×250 mm). HPLC condition were as follows: elution A, water; elution B, MeOH; gradient, 0 min (10% B), and 60 min (100% MeOH). The flow rate of the mobile phase was 1.0 mL/min, and the peak was monitored at a wavelength of 254 nm.

**Statistics** All data was expressed as means±S.E.M. In the passive avoidance task, Y-maze task, Morris water maze task, and in vitro AChE inhibition assay, data were analyzed by one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test for multiple comparisons. A value of p<0.05 was considered statistically significant.

**RESULTS**

**Effect of AK on Scopolamine-Induced Memory Impairment in the Passive Avoidance Task** We tested the effect of AK on scopolamine-induced memory deficit using the step-through passive avoidance task which is largely dependent on long-term memory. As shown in Fig. 1, the step-through latency of scopolamine-treated (1 mg/kg, i.p.) mice was significantly shorter than that of vehicle-treated control mice. In addition, we observed that the reduced step-through latency induced by scopolamine was significantly ameliorated by AK (10 mg/kg, p.o.) in the retention trial. In this study, tacrine, an anti-amnestic drug, was used as a positive control. The step-through latency of tacrine -treated group, step-through latency was significantly shorter than that of vehicle-treated control mice (p<0.05). However, during the acquisition trials, latency times were not changed by treatment of AK in the scopolamine-induced amnestic model.

**Effect of AK on Memory Impairment Induced by Scopolamine in the Y-Maze Task** The effects of AK on
showed similar escape latencies during the four training days in Fig. 3A, the scopolamine-treated groups exhibited longer impairments using the Morris water maze task. As shown the effect of AK on scopolamine-induced spatial and memory polamine in the Morris Water Maze Task.

We investigated experimental groups, showing that general locomotor activity was not affected by AK treatment (Fig. 2B).

However, the number of arm entries was similar across all p<0.05 as compared with the control group. spontaneous alternation behavior was examined using the Y-maze task. As shown in Fig. 2A, spontaneous alternation of the scopolamine-treated (1 mg/kg, i.p.) mice was significantly lower than that of the vehicle-treated control mice (p<0.05), and the lowered spontaneous alternation induced by scopolamine was significantly reversed by AK treatment (p<0.05).

However, the number of arm entries was similar across all experimental groups, showing that general locomotor activity was not affected by AK treatment (Fig. 2B).

Effect of AK on Memory Impairment Induced by Scopolamine in the Morris Water Maze Task

We investigated the effect of AK on scopolamine-induced spatial and memory impairments using the Morris water maze task. As shown in Fig. 3A, the scopolamine-treated groups exhibited longer escape latencies than the vehicle-treated control group and showed similar escape latencies during the four training days (p<0.05). However, the AK treatment showed significantly

shorter mean escape latencies than the scopolamine treated group during trial sessions 3 and 4 (p<0.05). Tacrine also significantly decreased escape latencies compared with the scopolamine-treated group (p<0.05). On the day following the final day of training trial sessions, swimming times within the target quadrant in the scopolamine-treated groups were significantly lower than those in the vehicle-treated control group (Fig. 3B, p<0.05). Moreover, the shortened swimming time within the platform quadrant induced by scopolamine was significantly increased by AK treatment (p<0.05).

Effect of AK on Scopolamine-Induced AChE Activity

To investigate the cholinergic antagonistic effect of AK, AChE inhibition assay was performed in the hippocampus. The result showed that AK inhibited scopolamine-induced AChE activity in a dose dependent manner with an IC_{50} value of 230.7 µg/mL. The IC_{50} value of tacrine, a positive control, was 18.51 µg/mL (Table 1).

Effect of AK on Scopolamine-Attenuated pCREB and BDNF Expression in the Hippocampus

To investigate the effects of AK on the expression of pCREB and BDNF which are crucial molecules in memory formation, we conducted immunohistochemical and Western blot analyses using the brain tissues. AK administration increased the number of pCREB and BDNF positive cells in the hippocampal CA1 and dentate gyrus regions (Figs. 4A, C). Moreover, the results from the western blot analysis also revealed that the hippocampal pCREB and BDNF expressions in the mice with the AK administration were significantly higher than those in the scopolamine-treated control mice (Figs. 4B, D).

Qualitative Analysis of the AK by HPLC

To confirm the constituents of AK, we performed the HPLC analysis. As a result, hyperin (quercetin 3-O-glucoside) was detected (Fig. 5).

DISCUSSION

Herbal medicine has been a subject of increased interest for its potential in the treatment of AD. Although AK has a variety of pharmaceutical properties, little information is available on the effects of AK in improving memory and in treatment of neurodegenerative diseases including AD. In this study, we first demonstrated the ameliorative effect of AK on scopolamine-induced memory impairments in mice. Additionally, this study suggested an important molecular mechanism by which AK regulated the activity of AChE and expression of CREB and BDNF in brain tissue.

AD is the most common form of age-related neurodegenerative disorder that is characterized with an insidious loss of
At 60 min before training trial sessions, AK or tacrine (10 mg/kg, p.o.) was administered 60 min prior to the first training trial of each training day. Memory impairment was induced by scopolamine treatment (1 mg/kg, i.p.). The training trial and probe trial sessions were performed as described in Materials and Methods. Data represent means ± S.E.M., (n = 10). *p < 0.05 as compared with the control group, #p < 0.05 as compared with the scopolamine-treated group.

Fig. 4. Effect of AK on BDNF and pCREB Expressions in the Hippocampus

(A) Photomicrographs of immunohistochemical data of pCREB. (B) Western blot data of pCREB. (C) The relative levels of pCREB were represented. (D) Photomicrographs of immunohistochemical data of BDNF. (E) Western blots data of BDNF. (F) The relative levels of BDNF were represented in the hippocampus. Values are expressed as means ± S.E.M. (n = 10). *p < 0.05 as compared with the control group, #p < 0.05 as compared with the scopolamine-treated group.
memory, associated functional decline, and behavioral disturbances.25,26 The cholinergic system plays an important role in learning and memory.3) Cholinergic deficit is a major feature that is associated with memory loss and cognitive dysfunction in AD. Cholinergic agonists can facilitate memory, whereas cholinergic antagonists can impair memory. Animal models of memory impairment have been used to understand its molecular basis and search its therapeutic targets. Scopolamine is an anti-cholinergic agent and induces memory deficits. It is well known that scopolamine-induced memory deficits are similar to those found in age-related senile central nervous system dysfunction.27) Therefore, scopolamine challenge could serve as a useful tool for investigating learning and memory systems that involve the cholinergic system. In this study, we investigated whether AK has memory-enhancing effects in the scopolamine-induced memory impairment animal model. The results show that step-through latency reduced by scopolamine was recovered by treatments of AK. However, during the training trial, no differences in latencies were observed between any groups, demonstrating no effect of AK on general behavior changes. In this study, we observed that the inhibitory effect of AK on step-through latency reduced by scopolamine is not dependent of its doses. We presuppose that it may be related the sedative effect of AK. With preliminary examination, we observed that AK has the sedative effect (data not shown). Further studies will be necessary in order to clarify precisely the role of AK on the sedative effect. Like the study of the passive avoidance task, AK had an ameliorative effect in other behavioral studies including the Y-maze and the Morris water maze tasks, in which the behavior is regulated by hippocampus. These results suggest that AK ameliorates memory dysfunction induced by cholinergic dysfunction.

In order to elucidate the underlying mechanism of action of AK, we assessed the effect of AK on AChE activity in scopolamine-induced learning and memory deficient mouse brains. It is well known that the anti-amnestic effect of tacrine is due to the inhibition of AChE in the brain. Tacrine, as a positive control, inhibited the activity of AChE with an IC50 value of 18.51 µg/mL, which is consistent with previously published data.28) In the present study, we found that AK also inhibited AChE activity in a dose dependent manner (IC50=230.7 µg/mL). This result demonstrated that the anti-amnestic effect of AK is mediated through the suppression of AChE in brain.

Alterations in the level or function of transcription factors have been associated with memory impairment due to the requirement for proteins-synthesis in long-term memory formation.29,30) Previously, other studies reported that dysfunction of CREB impairs long-term memory, suggesting that CREB-mediated gene expression is involved in memory formation.12,13) Also, it was reported that up-regulation of CREB transcriptional activity regulates memory consolidation and affects memory performance by regulating BDNF expression. These indicated that the CREB signaling pathway is involved in memory enhancement. To find other mechanisms of AK in improving memory, we tested the effect of AK on pCREB and BDNF expression, which are believed to be key molecules for formation of memory.31) In this study, we observed that scopolamine reduced BDNF expression and CREB activation in the hippocampus, and their reductions were found to be proportional to the memory deficits. Treatment with AK significantly prevented scopolamine-induced reduction of BDNF expression and CREB activation. These results demonstrate that AK exerts an anti-amnestic effect via the regulation of CREB and BDNF expression. Although AK reversed the reduction of CREB and BDNF expression in hippocampus to some degree, the effect of AK on the other pathways involving CREB and BDNF upstream/downstream was not determined. It was reported that the changes of CREB and BDNF in memory impairment were accompanied by increases in the phospholylation state of extracellular signal-related kinase.31) Therefore, further studies will be necessary in order to clarify more precisely the role of AK on the CREB-mediated signaling pathway.

AK was effective in improving or ameliorating spatial long-term memory and short-term memory, showed by the performance of mice in the Morris water maze and Y-maze tasks, respectively. In addition, the cognitive-enhancing activities of AK might result from the regulation of AChE activity and pCREB and BDNF expression in the hippocampus. These results provide pharmacological evidence that AK could have a significant therapeutic value in alleviating memory impairments.

Acknowledgement This research was supported by Research Center for Biochemical Resources of Oriental Medicine (B0009008).

REFERENCES

11) Kida S, Josselyn SA, Peña de Ortiz S, Kogan JH, Chevere I, Ma-

7) Ahmed T, Gilani AH. Inhibitory effect of curcuminoids on acetyl-

8) Alahiri DK, Farlow MR, Sambamurti K, Greig NH, Giacobini E,

5) Fadda F, Cocco S, Stancampiano R. Hippocampal acetylcholine re-


Ballard CG, Greig NH, Guillozet-Bongaarts A l, Enz A, Darvesh S.

12) Pittenger C, Huang YY, Paletzki RF, Bourtchouladze R, Scanlin

10) Yang JH, Han SJ, Ryu JH, Jang IS, Kim DH. Ginsenoside Rh2

14) Saura CA, Valero J. The role of CREB signaling in Alzheimer’s


17) Bekinschtein P, Cammarata M, Katec C, Slipczuk L, Rossato JJ,


18) Inamori Y, Baba K, Tsujibo H, Taniguchi M, Nakata K, Kozawa
M. Antibacterial activity of two chalcones, xanthoangelol and 4-hy-
droxyderricin, isolated from the root of Angelica keiskei Koidzumi.

19) Okuyama T, Takata M, Takayasu J, Hasegawa T, Tokuda H, Nishi-

20) Ogawa H, Nakashima S, Baba K. Effects of dietary Angelica keiskei

21) Sun XL, Ito H, Masuoka T, Kamei C, Hatano T. Effect of Polygala
tenuifolia root extract on scopalamine-induced impairment of rat
spatial cognition in an eight-arm radial maze task. Biol. Pharm.

1748–1754 (2010).

exhibits a novel anti-cancer effect on human adenocarcinoma. Bio-

24) Kim DH, Hung TM, Bae KH, Jung JW, Lee S, Yoon BH, Cheong


(2004).

YC. Decursin from Angelica gigas mitigates amnesia induced by

28) Nielsen JA, Mena EE, Williams IH, Nocerini MR, liston D. Cor-
relation of brain levels of 9-amino-1,2,3,4-tetrahydroacridine (THA)
with neurochemical and behavioral changes. Eur. J. Pharmacol.,

29) Jung WY, Park SJ, Park DH, Kim JM, Kim DH, Ryu JH. Quercetin

30) Kim JM, Kim DH, Park SJ, Park DH, Jung SY, Kim HJ, Lee YS,
Jin C, Ryu JH. The n-butanol extract of Opuntia ficus-indica
var. saboten enhances long-term memory in the passive avoidance task in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry, 34,
1011–1017 (2010).

31) Williams CM, El Mohsen MA, Vauzour D, Rendeiro C, Butler LT,