

COGNITION AND MEMORY FUNCTION OF *TARAXACUM COREANUM* IN AN *IN VIVO* AMYLOID-B-INDUCED MOUSE MODEL OF ALZHEIMER'S DISEASE

AH YOUNG LEE¹, NORIKO YAMABE¹, KI SUNG KANG², HYUN YOUNG KIM³, SANGHYUN LEE^{4*}
and EUN JU CHO^{1*}

¹ Department of Food Science and Nutrition & Kimchi Research Institute, Pusan National University, Busan 609-735, Republic of Korea

² Natural Medicine Center, Korea Institute of Science and Technology, Gangneung 210-340, Republic of Korea

³ Department of Food Science, Gyeongnam National University of Science and Technology, Jinju 660-758, Republic of Korea

⁴ Department of Integrative Plant Science, Chung-Ang University, Anseong 456-756, Republic of Korea

*Corresponding author: slee@cau.ac.kr

Abstract - We investigated whether the ethyl acetate fraction of *Taraxacum coreanum* (ETC) had a protective effect against memory impairment in an amyloid beta (A β)-induced mouse model of Alzheimer's disease (AD). The formation of A β in the brain is a hallmark of AD. We examined whether oxidative stress contributes to learning and memory deficits using the T-maze test, the object recognition test, and the Morris water maze test in mice injected with A β . Cognition and memory function were significantly impaired in mice injected with A β , as compared to the normal group. However, mice that received ETC orally at doses of 50 or 100 mg/kg/day for 2 weeks showed high recognition behavior of tasks. ETC may have prevented oxidative stress to the brain tissue by reducing lipid peroxidation levels and a NO scavenger. ETC could be useful for the prevention and treatment of AD.

Key words: *Taraxacum coreanum*; Alzheimer's disease; cognition, memory

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disorder in the aged population and the number of people with AD is expected to reach 81 million by 2040 (Ferri et al., 2006). AD is a progressive neurodegenerative disorder, which results in memory loss, confusion, and a variety of cognitive disabilities (Khachaturian, 1985). Sites in the AD brain where neurodegeneration occurs and where oxidative stress exists are associated with increased amyloid beta (A β) deposits (Hensley et al., 1995). The extent of A β protein deposition correlates with the

degree of neuronal damage, cognitive impairment and memory loss (Mann et al., 1985). Furthermore, the deposition of soluble A β induces the aggregation of peptide-forming amyloid fibrils, which have been reported to be neurotoxic both *in vitro* and *in vivo* (Inestrosa and Reyes, 1998). The mechanisms of A β -mediated neurotoxicity are unknown, but several studies have suggested that oxidative stress plays a key role in A β -mediated neurotoxicity by triggering or facilitating neurodegeneration (Coyle and Puttfarcken, 1993). Amyloid precursor protein (APP)-derived A β , probably as a small, soluble aggregate, inserts into the neuronal and glial membrane bilayer

and generates free radicals that cause lipid peroxidation and protein oxidation (Varadarajan et al., 2000). Therefore, antioxidants may help treat A β -induced neurotoxicity and improve memory in patients with AD.

Plants in the genus *Taraxacum* have been used since ancient time as medicinal herbs to treat dyspepsia, heartburn, the spleen and hepatitis anorexia. Of the nearly 400 plants in this genus, the Korean dandelion (*Taraxacum coreanum*) is one of most common species in Korea and Japan. *T. coreanum* has been used for its diuretic and anti-inflammatory activities (Koo et al., 2004). In addition, the extracts from *T. coreanum* have been shown to protect low-density lipoprotein from oxidation (Yang and Jeon, 1996). The effects of *T. coreanum* are related to its phytochemical constituents, including phenols and flavonoids, which are important sources of natural antioxidants. These characteristics have led to increased demand for the use of *T. coreanum* as a dietary supplement, as well as in pharmaceutical products. However, no previous study has examined whether *T. coreanum* protects against AD or improves cognition impairment in AD. Therefore, in this study, we examined if *T. coreanum* protected against AD in an *in vivo* A β ₂₅₋₃₅-injected animal model.

MATERIALS AND METHODS

Plant materials

Aerial parts of *T. coreanum* Nakai were collected in 2007 near the Westcoast Express Highway in Korea. A voucher specimen (No. LEE 2007-01) was deposited at the Herbarium of Department of Integrative Plant Science, Chung-Ang University, Korea.

Instruments and reagents

A β ₂₅₋₃₅ and malondialdehyde (MDA) were obtained from Sigma Aldrich (Saint Louis, Missouri, USA). Dimethyl sulfoxide and sodium chloride (NaCl) were purchased from Bio Basics Inc. (Ontario, Canada). Thiobarbituric acid (TBA) was obtained from Lancaster Synthesis (Ward Hill, USA). Phosphoric acid

and 1-butanol were acquired from Samchun Pure Chemical Company (Pyeongtaek, Korea).

Preparation of the ethyl acetate (EtOAc) fraction from T. coreanum (ETC)

Freeze-dried *T. coreanum* was extracted with methanol (MeOH) for 3 h and the MeOH extraction process was repeated eight times. The extract was concentrated using a rotary evaporator and suspended in water. The concentrate was suspended in distilled water and partitioned with *n*-hexane, chloroform (CHCl₃), EtOAc, and *n*-butanol (*n*-BuOH), successively. The various fractions, namely *n*-hexane (99 g), CHCl₃ (12 g), EtOAc (23 g), and *n*-BuOH (25 g) fractions, were collected.

Animals and experimental protocols

Male ICR mice (5-weeks-old; Orient Inc. Seongnam, Republic of Korea) weighing 25-27 g were housed in plastic cages with free access to food and water, and were maintained in a controlled environment (20 \pm 2°C, 50 \pm 10%, 12-h light/dark cycle). The animal protocol used in this study was reviewed and approved by Pusan National University-Institutional Animal Care and Use Committee (PNU-IACUC). Mice were divided into four groups comprising 10 individuals in each of the four cages. The groups were defined as follows: Normal = 0.9% NaCl injection + oral administration of water; Control = A β ₂₅₋₃₅ injection + oral administration of water; ETC 50 = A β ₂₅₋₃₅ injection + oral administration of ETC (50 mg/kg/day); ETC 100 = A β ₂₅₋₃₅ injection + oral administration of ETC (100 mg/kg/day) for 14 days using a sonde. There were no significant differences in initial body weight among the groups.

A β ₂₅₋₃₅-infused mouse model

A β ₂₅₋₃₅ was aggregated according to the procedure outlined by Maurice et al., (1996). In brief, the peptide was dissolved and diluted in sterile distilled water to achieve a concentration of 1 mg/ml, aliquoted into tubes, and then dissolved. A β ₂₅₋₃₅ was incubated at 37°C for 3 days before injection to induce aggre-

gation. Distilled water containing aggregated $A\beta_{25-35}$ was injected into mice according to the procedure established by Laursen and Belknap (1986). Mice were lightly anesthetized with ether, and the solution was injected 0.8 mm posterior to the bregma and 1.5 mm lateral to the sagittal suture. All injections were made with a 10 μ l Hamilton microsyringe fitted with a 26-gauge needle that was inserted 2.2 mm beneath the surface of the brain. Animals were injected with 5 μ l of sterile distilled water or 5 nmol of $A\beta_{25-35}$ aggregate in each cerebral lateral ventricle at a rate of 1 μ l/min. The needle was left in the injection site for 1 min.

T-maze test

The T-maze test was conducted according to the procedure established by Montgomery (1952). The maze apparatus was T-shaped, and the walls were made of black boards (length of start and goal stems = 50 cm, width = 13 cm, height = 20 cm) that were glued to a square blackboard bottom. The maze consisted of a start box, left arm and right arm, with a door to separate the two sides. The mice were placed at the start box, and the number of touches and exploration times of the right arm of the T-maze were recorded during a 10-min period (training session). The mice were then placed back into the same apparatus 24 h after the training session. They were allowed to explore the right and left sides of the maze freely for 10 min, and the number of touches and exploration times were recorded (test session). Space perceptive ability (%) was calculated as the ratio of the number of left or right maze entries to the number of total maze entries multiplied by 100.

Novel object recognition test

The object recognition test (Bevins and Besheer, 2006) was performed in a square black open-field apparatus (40 \times 30 \times 20 cm). Two identical objects (plastic bottles) were placed at fixed distances within the square field. The mice were then placed at the center of the square field, and the number of touches of each object was recorded during a 10 min period (training session). The mice were placed back into

the same field 24 h after the training session, but this time one of the objects used during the training session was replaced with a new object (another plastic bottle). The mice were allowed to explore freely for 10 min, and the number of touches was recorded (test session). Object cognitive ability (%), a ratio of the amount of time spent exploring any one of the two original objects (training session) or the novel object (test session) over the total time spent exploring both objects, was used to measure cognitive function.

The Morris water maze test

The Morris water maze test was conducted according to the procedure established by Morris (1984) with slight modifications. The apparatus used in the Morris water maze test consisted of a dark plastic circular pool, 80 cm in diameter, surrounded by a 40-cm high wall, randomly divided into quadrants. White poster color was added to the pool water to make it opaque, and the water temperature was maintained at $22 \pm 1^\circ\text{C}$. A platform 8 cm in diameter was placed 1 cm below the water surface in the middle of one quadrant. The position of the platform was unchanged during the training session. Four posters on the walls of the apparatus provided visual cues for navigation. Three training trials per day were conducted for 3 days. In the training trials, the mice were placed randomly in the water facing the pool wall and allowed to swim for a maximum of 60 s. The latency time required to find the platform was recorded. Mice that found the platform were allowed to rest there for 15 s. A probe trial of the Morris water maze test was performed 1 day after the 3 days of training were completed. In the primary test, the experiment was performed as before. However, in the secondary test, the trial was performed without the platform. The mice were placed in the pool and swam for 60 s looking for the platform, and the latency time that the mice spent in the position previously occupied by the platform was recorded. In the tertiary test, the water was transparent and the number of times it took the mouse to reach the platform, which was visible 1 cm above the surface of the water, was counted. Occupancy of the target quadrant (%) was calculated as the per-

centage of time spent in the target quadrant during a 60 s trial.

Measurement of lipid peroxidation

MDA levels were measured by the method described by Ohkawa et al., (1979). After completion of the behavioral observations, mice were anesthetized with ether. Mice brains, livers, and kidneys were removed immediately and placed on ice. The dissected tissue was homogenized in saline solution, and mixed with 1% phosphoric acid and 0.67% TBA solution. After boiling for 45 min, the solution mixture was cooled in an ice bath, and 2 mL of 1-butanol was added followed by centrifugation at 3 000 rpm for 10 min. The absorbance values of the supernatant were measured at 535 and 520 nm. The level of lipid peroxidation was calculated using a MDA standard curve.

Nitric oxide (NO) scavenging activity

The NO concentration in tissues was determined by the method described by Schmidt et al., (1992). One hundred fifty microliters of supernatant from the lipid peroxidation procedure was mixed with 130 μ L of distilled water, and 20 μ L of dilution was added to the same amount of phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamide dihydrochloride. The absorbance value of the mixture was measured at 540 nm. The NO yield was calculated using a sodium nitrite (NaNO_2) standard curve.

Statistical analysis

Results are expressed as means \pm SD. Statistical significance was determined using one-way ANOVA, followed by Duncan's post-hoc tests. Significance was set at $P < 0.05$.

RESULTS

T-maze test

To investigate short-term memory, a T-maze test was carried out after oral administration of ETC for 1 week to mice injected with $\text{A}\beta_{25-35}$. The effect of ETC

on preventing impaired spatial cognition is shown in Fig. 1. Mice in the normal group had a shorter latency to find the platform using new routes than the control group, which means that injection of $\text{A}\beta_{25-35}$ resulted in functional impairment of short-term memory function. However, the cognitive ability of mice treated with ETC (50 and 100 mg/kg/day) to find a new route improved dose-dependently compared with the control group.

Novel object recognition test

The novel object recognition test was conducted on the day following the T-maze test. The same two objects were explored during the acquisition phase. After 24 h of training, mice in the normal group touched the new object frequently, while mice injected with $\text{A}\beta_{25-35}$ showed less ability to recognize the new object than mice in the normal group. In contrast, groups that received ETC (50 and 100 mg/kg/day) touched the new object significantly more than control mice and spent longer exploring the novel object than the control group mice. In particular, mice that received 100 mg/kg/day ETC had an object cognition ability of 61.43% versus an object cognition ability of 48.42% in the control group. These results demonstrated that oral administration of ETC protected against the $\text{A}\beta_{25-35}$ injection-induced impairment in spatial cognition ability (Fig. 2).

The Morris water maze test

The Morris water maze test was conducted on days 16-19 after the injections. Results are shown in Figs. 3 and 4. During the training session, latency to reach the platform decreased in all groups over a period of 3 days. However, the control group took a relatively longer time to reach the platform than the two groups that received ETC. These results indicated that oral administration of ETC significantly improved $\text{A}\beta_{25-35}$ -induced cognitive deficits. The exercise and visual capacity of mice as assessed by the hidden and exposed platforms are shown in Fig. 4. The distance swum to the platform is the most reliable indicator of learning and memory in the water maze, because latency to reach the platform can be affected by mo-

Table 1. Protective activity of ETC against lipid peroxidation in mice injected with A β_{25-35} .

Group	MDA (nmol/mg protein)		
	Brain	Liver	Kidney
Normal	22.69 \pm 0.91 ^c	59.61 \pm 0.95 ^b	30.77 \pm 2.95 ^d
Control	27.69 \pm 1.55 ^a	59.04 \pm 3.05 ^a	51.92 \pm 5.02 ^a
ETC 50	26.73 \pm 0.98 ^{ab}	62.88 \pm 0.53 ^b	46.15 \pm 3.27 ^b
ETC 100	25.19 \pm 1.26 ^b	62.50 \pm 3.89 ^b	40.38 \pm 5.58 ^c

ETC 50: Oral administration of ETC (50 mg/kg/day)

ETC 100: Oral administration of ETC (100 mg/kg/day)

Values are means \pm SD.Different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.**Table 2.** Effects of oral administration of ETC on A β_{25-35} -induced nitric oxide formation.

Group	NaNO ₂ (μ mol/mg protein)		
	Brain	Liver	Kidney
Normal	8.88 \pm 0.54 ^b	15.66 \pm 0.04 ^c	21.94 \pm 1.87 ^b
Control	11.06 \pm 0.61 ^a	28.37 \pm 0.09 ^a	29.59 \pm 0.30 ^a
ETC 50	10.50 \pm 0.56 ^a	23.42 \pm 0.01 ^{ab}	23.98 \pm 1.94 ^b
ETC 100	10.04 \pm 1.08 ^a	20.71 \pm 0.07 ^{bc}	22.45 \pm 2.90 ^b

ETC 50: Oral administration of ETC (50 mg/kg/day)

ETC 100: Oral administration of ETC (100 mg/kg/day)

Values are means \pm SD.Different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

tor function. There were no significant differences in latency to reach the exposed platform among groups. However, when the platform was hidden, the control group took longer to find the platform than the normal and ETC-treated groups. These data indicated that damage induced by A β_{25-35} did not affect swimming or visual ability, but did affect recognition ability. These results indicate that ETC not only protects against cognitive impairment, but also has a strong positive influence on learning and memory abilities.

Measurement of lipid peroxidation

Lipid peroxide levels in the brain, liver, and kidney of mice injected with A β_{25-35} are shown in Table 1. Injection of A β_{25-35} into the cerebral ventricle increased lipid peroxide levels in the brain. The MDA levels in the brain of normal and control groups were 22.69 and 27.69 nmol/mg protein, respectively. The MDA values in the ETC (50 and 100 mg/kg/day) groups

were 26.13, and 25.19 nmol/mg protein, respectively, demonstrating that oral administration of ETC inhibited the formation of MDA in the brain. In addition, when we examined lipid peroxidation levels in the kidney, we found that the MDA value of the control group was 51.92 nmol/mg protein, while that of the 50 and 100 mg/kg/day ETC groups was 46.15 and 40.38 nmol/mg protein, respectively. Furthermore, the MDA concentrations in the liver of the groups that received ETC were significantly lower than that of the control group. These results indicate that administration of ETC protected the brain, kidney, and liver from lipid peroxidation.

NO scavenging activity

As shown in Table 2, ETC significantly inhibited NO production in a dose-dependent manner. The NO level in the normal group was 8.88 μ mol/mg protein, while in the control group it was significantly high-

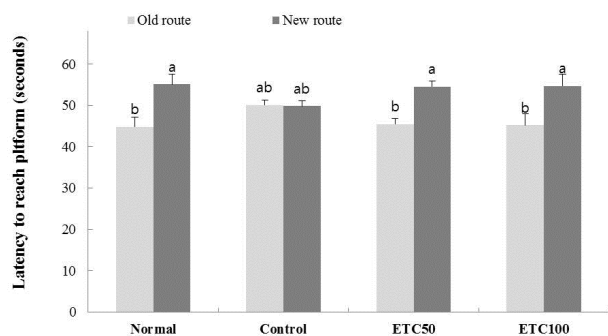


Fig. 1. Spatial perceptive ability scores by group as assessed by the T-maze test.

After training to explore the right arm of the T-maze for 10 min, the number of touches and exploration times of the right and left sides of the maze were calculated. The groups were defined as follows: Normal = 0.9% NaCl injection + oral administration of water; $A\beta_{25-35}$ = $A\beta_{25-35}$ injection + oral administration of water; ETC 50 = $A\beta_{25-35}$ injection + oral administration of ETC (50 mg/kg/day); ETC 100 = $A\beta_{25-35}$ injection + oral administration of ETC (100 mg/kg/day). Values are reported as means \pm SD. Spatial perception of the old route was not significantly different among experimental groups. ^{a-b}Means of the space perceptive ability to find new routes indicated with different letters are significantly different ($P < 0.05$) between groups.

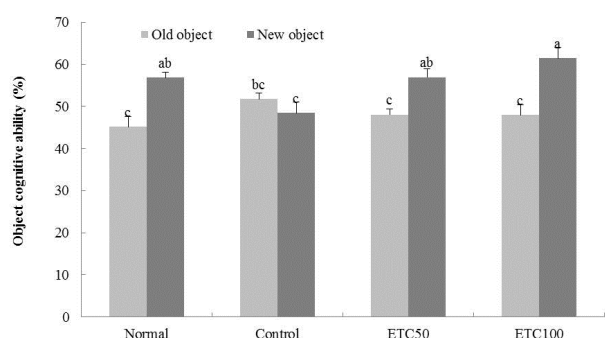


Fig. 2. Percentage change in object cognitive ability test scores. After training with two identical objects, the mice were allowed to explore one familiar object from training and one novel object. The time that the mice spent with the novel object was recorded: Normal = 0.9% NaCl injection + oral administration of water; $A\beta_{25-35}$ = $A\beta_{25-35}$ injection + oral administration of water; ETC 50 = $A\beta_{25-35}$ injection + oral administration of ETC (50 mg/kg/day); ETC 100 = $A\beta_{25-35}$ injection + oral administration of ETC (100 mg/kg/day). Values are reported as means \pm SD. The ability to recognize the old object was not significantly different among experimental groups. However, there were significant differences in ability to recognize a novel object among groups; ^{a-c} means with different letters are significantly different ($P < 0.05$) from each other.

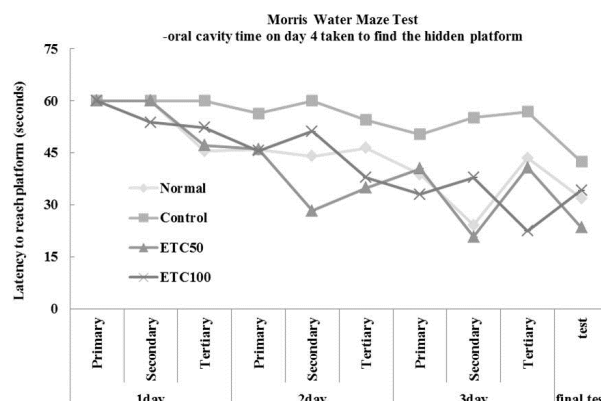


Fig. 3. Effect of ETC on spatial learning in the Morris water maze test.

Mice were trained to swim and find the platform for 3 days. The latency time to reach the platform during training and on the final test day was calculated: Normal = 0.9% NaCl injection + oral administration of water; $A\beta_{25-35}$ = $A\beta_{25-35}$ injection + oral administration of water; ETC 50 = $A\beta_{25-35}$ injection + oral administration of ETC (50 mg/kg/day); ETC 100 = $A\beta_{25-35}$ injection + oral administration of ETC (100 mg/kg/day). Values are reported as means \pm SD.

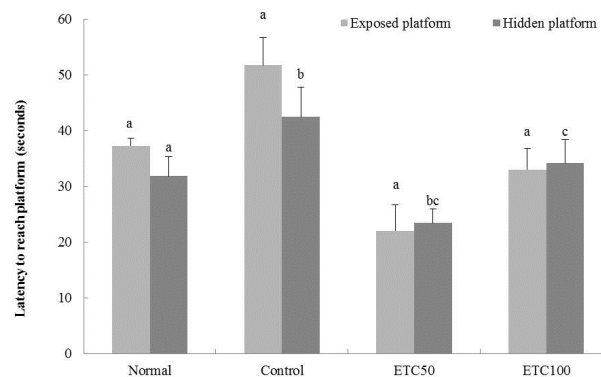


Fig. 4. Latency to reach hidden and exposed platform in the Morris water maze test.

The time to find hidden and exposed platform was recorded on the final test day of the water maze test: Normal = 0.9% NaCl injection + oral administration of water; $A\beta_{25-35}$ = $A\beta_{25-35}$ injection + oral administration of water; ETC 50 = $A\beta_{25-35}$ injection + oral administration of ETC (50 mg/kg/day); ETC 100 = $A\beta_{25-35}$ injection + oral administration of ETC (100 mg/kg/day). Values are reported as means \pm SD. The mean latency to find the exposed platform was not show significantly different among experimental groups. ^{a-c}Means with different letters indicate significant differences between groups in the time taken to reach the hidden platform ($P < 0.05$).

er at 11.06 $\mu\text{mol}/\text{mg}$ protein. Interestingly, the NO levels in the ETC groups administered ETC (50 and 100 mg/kg/day) were 10.50 and 10.04 $\mu\text{mol}/\text{mg}$ protein, revealing its inhibitory activity that was higher than in the control group. Moreover, NO levels in the kidney were also elevated by $\text{A}\beta_{25-35}$ injection. Mice that received ETC at oral doses of 50 and 100 mg/kg/day had a NO level of 23.98 and 22.45 $\mu\text{mol}/\text{mg}$ protein, respectively, in comparison with the NO concentration in the control group: 29.59 $\mu\text{mol}/\text{mg}$ protein ($P < 0.05$). In addition, the level of NO in the liver of the normal group was 15.66 $\mu\text{mol}/\text{mg}$ protein, whereas that of the control group was higher at 28.37 $\mu\text{mol}/\text{mg}$ protein. The liver NO levels of the ETC groups were lower than in the control group, exhibiting a significant dose-dependent relationship. These results indicate that administration of ETC had a strong inhibitory effect on NO generation in the brain, kidney, and liver.

DISCUSSION

AD is the most common cause of progressive cognitive decline and dementia in aged humans (Meziane et al., 1998). Although the causes of AD are not well known, one widely discussed hypothesis is that deposits of $\text{A}\beta$ are the causative agents of AD (Hardy and Allsop 1991). The “amyloid theory” is based on the close correlation between $\text{A}\beta$ production and the neurodegenerative process of AD. Neurofibrillary tangles and $\text{A}\beta$ deposits have been found primarily in regions of the brain associated with memory and cognition in both AD patients and AD transgenic mice (Manczak et al., 2006). In the brain, neuropathological hallmarks of AD lesions include diffuse and neuritic extracellular $\text{A}\beta$ peptides generated by endoproteolytic cleavage of APP, reactive microglial cells, dystrophic neuritis, and bundles of astrocytic processes and intracellular neurofibrillary tangles (Tran et al., 2002; Kar et al., 2004). Furthermore, excessive reactive oxygen species (ROS) production can lead to neuronal apoptosis in neurodegenerative disorders, observed as $\text{A}\beta$ -induced neuronal apoptosis (Butterfield et al., 2001; Fukui et al., 2005). According to the oxidative stress hypothesis of AD, $\text{A}\beta$ inserts into the neuronal membrane

bilayer and generates oxygen-dependent free radicals that cause lipid peroxidation, protein oxidation and ROS formation (Fu et al., 2006). Furthermore, antioxidants have been shown to have a beneficial effect in neurodegenerative disorders (Calabrese et al., 2007) and $\text{A}\beta$ -induced neurotoxicity (Sultana et al., 2004).

Plants of the genus *Taraxacum* are members of the plant family Asteraceae. Several studies have demonstrated that *T. coreanum* had beneficial biological effects, including antioxidant, free-radical scavenging and anti-inflammatory properties. According to Choi et al., (2012), ETC showed very strong radical scavenging activity and protective activity against oxidative stress in a cellular system. In a previous chromatographic study of ETC, two flavonoids were identified as active compounds: luteolin and luteolin 7-O-glucose. They both exhibit strong aldose reductase inhibitory activity (IC_{50} values, 0.15 and 1.05 μM , respectively) (Mok et al., 2011). The total content of luteolin and luteolin 7-O-glucose in dandelions (*T. coreanum*, 15.8 mg/g; *T. officinale*, 12.6 mg/g; *T. ohwianum*, 8.5 mg/g) was determined by high-performance liquid chromatography (Lee et al., 2011). This finding provides a logical basis for the use of *T. coreanum* as a functional food. However, no studies have investigated whether ETC protects against oxidative stress-related deficits in cognition using an animal model. To determine whether ETC can inhibit neurodegenerative disorders such as AD and improve hippocampal-dependent learning and memory, we used a mouse model of AD.

Injection of $\text{A}\beta$ has been shown to effectively impair learning and memory behavior in mice. However, we found that ETC significantly improved the cognitive ability of $\text{A}\beta$ -injected mice. We subjected mice to the T-maze test, object recognition test, and Morris water maze test. We tested spatial learning and memory skill in the T-maze test and found that mice injected with $\text{A}\beta$ displayed significantly impaired spatial working memory, whereas mice that received ETC extract showed an improvement in memory function. In addition, in the novel object recognition test, which measures time spent explor-

ing a novel object versus a previously seen object novel, the percentage of novel objects recognized by the groups that received ETC was higher than that of the control group, suggesting that ETC improved cognition ability. Furthermore, ETC improved short-term memory in the object recognition task when administered after the first trial. These experiments demonstrate that ETC protects against A β -induced impairments in learning and memory function in mice.

The Morris water maze is a well-known experimental method to study long-term memory. In the training session, the latency times of mice administered ETC decreased remarkably after training for 3 days, but the latency time of the control group did not decline with training. Furthermore, the time it took the mice to reach the exposed platform was not significantly different among groups, while the time was shorter in the ETC group than in the control group when the platform was hidden. These results indicate that mice that received ETC retained memories significantly longer and therefore had shorter latencies in the water maze test than the control group, suggesting that ETC improves long-term memory ability.

The lipid bilayer of the brain is rich in polyunsaturated fatty acids (PUFA) and oxygen, and the interaction of polyunsaturated fatty acids with free radicals results in lipid peroxidation. Lipid peroxidation occurs in several neurodegenerative diseases (Reed 2011). To determine the level of brain lipid peroxidation in AD, we measured MDA levels, as MDA is widely used as an index of oxidative stress. MDA is produced during the oxidative degradation of some macromolecules. Because peroxidation of PUFA is its major source, MDA can be considered a marker of lipid peroxidation. Increased peroxynitrite formation and membrane lipid peroxidation are directly associated with degenerating neurons in AD patients (Behl et al., 1994), suggesting that peroxynitrite-induced lipid peroxidation may play a key role in the cell death process induced by A β in AD. In our A β mice, MDA increased and so did NO levels. When we measured lipid peroxidation, we found that injec-

tion of A β induced free radical damage in the neurons. However, the groups that received ETC had a lower concentration of free radicals, which in turn decreased lipid peroxidation, leading to a significant reduction in MDA in tissues.

NO is linked to many neuropathological conditions as it plays many roles in the central nervous system as a messenger molecule. Either it can have a neuroprotective or a neurotoxic function, depending on the concentration; when generated in excess, NO can be neurotoxic (Mark et al., 1996). A β can activate NO synthase, stimulating excessive NO release (Hu et al., 1998). We found that treatment of AD mice with ETC attenuated oxidative stress through inhibition of lipid peroxidation and NO production. In comparison with the control group, the groups that received ETC showed a significant decrease in NO levels. These results suggest that the administration of ETC protected mice against A β -induced memory deficits and attenuated oxidative stress. Thus, neurodegenerative diseases, including those that involve learning and cognitive impairment, may be inhibited by increased dietary intake of ETC.

We found that ETC protected against A β -induced memory deficits in mice and significantly reduced oxidative stress. Moreover, administration of 100 mg/kg/day of ETC was more effective than administration of 50 mg/kg/day. These results suggest that ETC could protect against progressive neurological damage associated with AD.

Acknowledgments - This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0026053).

REFERENCES

- Behl, C., Davis, J.B., Lesley, R. and D. Schubert (1994). Hydrogen peroxide mediates amyloid beta protein toxicity. *Cell* 77, 817-827.
- Bevins, R.A. and J. Besheer (2006). Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. *Nature Protocols* 1, 1306-1311.

- Butterfield, D.A., Drake, J., Pocernich, C. and A. Castegna (2001). Evidence of oxidative damage in Alzheimer's disease brain: central role of amyloid beta-peptide. *Trends. Mol. Medi.* **7**, 548-554.
- Calabrese, V., Guagliano, E., Sapienza, M., Panebianco, M., Calafato, S., Puleo, E., Pennisi, G., Mancuso, C., Allan, B.D. and A.G. Stella (2007). Redox regulation of cellular stress response in aging and neurodegenerative disorders: role of vitagenes. *Neurochemi. Res.* **32**, 757-773.
- Choi, J.M., Choi, M.J., Lee, S., Yamabe, N., Lee, S. and E.J. Cho (2012). Protective effects of ethylacetate fraction from *Taraxacum coreanum* against peroxynitrite-induced oxidative damage under cellular system. *Can. Prev. Res.* **17**, 251-256.
- Coyle, J.T. and P. Puttfarcken (1993). Oxidative stress, glutamate, and neurodegenerative disorders. *Science* **262**, 689-695.
- Ferri, C.P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., Hall, K., Hasegawa, K., Hendrie, H., Huang, Y., Jorm, A., Mathers, C., Menezes, P.R., Rimmer, E. and M. Scazufca (2006). Global prevalence of dementia: a Delphi consensus study. *The Lancet* **366**, 2112-2117.
- Fu, A.L., Dong, Z.H. and M.J. Sun (2006). Protective effect of N-acetyl-L-cysteine on amyloid β -peptide-induced learning and memory deficits in mice. *Brain Res.* **1109**, 201-206.
- Fukui, K., Takatsu, H., Shinkai, T., Suzuki, S., Abe, K. and S. Ura-no (2005). Appearance of amyloid beta-like substances and delayed-type apoptosis in rat hippocampus CA₁ region through aging and oxidative stress. *J. Alzheimer's Dis.* **8**, 299-309.
- Hardy, J. and D. Allsop (1991). Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacolo. Sci.* **12**, 383-388.
- Hensley, K., Hall, N., Subramaniam, R., Cole, P., Harris, M., Aksenov, M., Aksenova, M., Gabbita, P., Wu, J.F., Crney, J.M., Lovell, M., Markesbery, W.R. and D.A. Butterfield (1995). Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. *J. Neurochem.* **65**, 2146-2156.
- Hu, J., Akama, K.T., Krafft, G.A., Chromy, B.A. and L.J. Van Eldik (1998). Amyloid-beta peptide activates cultured astrocyte: morphological alterations, cytokine induction and nitric oxide release. *Brain Res.* **785**, 195-206.
- Inestrosa, N.C. and A.E. Reyes (1998). Acetylcholinesterase induces amyloid formation and increases neurotoxicity of Alzheimer's fibrils. *Neurobiol. Aging* **19**, S44.
- Kar, S., Slowikowski, S.P., Westaway, D. and H.T. Mount (2004). Interactions between beta-amyloid and central cholinergic neurons: Implications for Alzheimer's disease. *J. Psy. and Neurosci.* **29**, 427-441.
- Khachaturian, Z.S. (1985). Diagnosis of Alzheimer's disease. *Archives of Neurology* **42**, 1097-1105.
- Koo, H.N., Hong, S.H., Song, B.K., Kim, C.H., Yoo, Y.H. and H.M. Kim (2004). *Taraxacum officinale* induces cytotoxicity through TNF- α and IL-1 α secretion in Hep G2 cells. *Life Sci.* **74**, 1149-1157.
- Laursen, S.E. and J.K. Belknap (1986). Intracerebroventricular injections in mice. Some methodological refinements. *J. Pharmacol. Met.* **16**, 355-357.
- Lee, S., Han, S., Kim, H.M., Lee, J.M., Kim, J., Park, C.G. and S. Lee (2011). Simultaneous determination of luteolin and luteoloside in dandelions using HPLC. *Horticult., Environ., and Biotechnol.* **52**, 536-540.
- Manczak, M., Anekonda, T.S., Henson, E., Park, B.S., Quinn, J. and P.H. Reddy (2006). Mitochondria are a direct site of A β accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. *Hum. Mol. Gen.* **15**, 1437-1449.
- Mann, D.M., Yates, P.O. and B. Marcyniuk (1985). Correlation between senile plaque and neurofibrillary tangle counts in cerebral cortex and neuronal counts in cortex and subcortical structures in Alzheimer's disease. *Neurosci. Let.* **56**, 51-55.
- Mark, R.J., Blanc, E.M. and M.P. Mattson (1996). Amyloid β -peptide and oxidative cellular injury in Alzheimer's disease. *Mol. Neurobi.* **12**, 211-224.
- Maurice, T., Lockhart, B.P. and A. Privat (1996). Amnesia induced in mice by centrally administered β -amyloid peptides involves cholinergic dysfunction. *Brain Res.* **706**, 181-193.
- Meziane, H., Dodart, J.C., Mathis, C., Little, S., Clements, J., Paul, S.M. and A. Ungerer (1998). Memory-enhancing effects of secreted forms of the of β -amyloid precursor protein in normal and amnesic mice. *Pro. Nati. Acad. Sci.* **95**, 12683-12688.
- Mok, S.Y., Lee, S., Kim, H.M., Lee, J.M., Lee, D.G., Ahn, Y.H., Park, C.G., Cho, E.J. and S. Lee (2011). Inhibition of rat lens aldose reductase by flavonoids from dandelions. *Nat. Prod. Sci.* **17**, 130-134.
- Montgomery, K.C. (1952). A test of two explanations of spontaneous alternation. *J. Comparat. Physiol. Psycholo.* **45**, 287-293.
- Morris, R. (1984). Developments of a water-maze procedure for studying a spatial learning in the rat. *J. Neurosci. Meth.* **11**, 47-60.
- Ohkawa, H., Ohishi, N. and K. Yagi (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**, 351-358.

- Reed, T.T. (2011). Lipid peroxidation and neurodegenerative disease. *Free Radical Biol. Med.* **51**,1302-1319.
- Schmidt, H.H., Warner, T.D., Nakane, M., Forstermann, U. and F. Murad (1992). Regulation and sub cellular location of nitrogen oxide synthases in RAW264.7 macrophages. *Mole. Pharmacol.* **41**, 615-624.
- Sultana, R., Newman, S., Mohammad-Abdul, H., Keller, J.N. and D.A. Butterfield (2004). Protective effect of the xanthate, D609, on Alzheimer's amyloid beta-peptide (1-42)-induced oxidative stress in primary neuronal cells. *Free Radical Res.* **38**, 449-458.
- Tran, M.H., Yamada, K. and T. Nabeshima (2002). Amyloid beta-peptide induces cholinergic dysfunction and cognitive deficits: A minireview. *Peptides* **23**, 1271-1283.
- Varadarajan, S., Yatin, S., Aksenova, M. and D.A. Butterfield (2000). Review: Alzheimer's amyloid β -peptide-associated free radical oxidative stress, and neurotoxicity. *Journal of Structural Biolo.* **130**, 184-208.
- Yang, K.S. and C.M. Jeon (1996). Effect of *Taraxacum coreanum* Nakai on low density lipoprotein oxidation. *Korean J. Pharmacog.* **27**, 267-273.