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ARTICLE

Acer okamotoanum improves cognition and memory function in $A\beta_{25–35}$-induced Alzheimer's mice model

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Abstract We studied the effect of ethyl acetate (EA) fraction from *Acer okamotoanum* on cognitive improvement and protective abilities in amyloid beta $(A\beta)_{25–35}$ peptide-injected Alzheimer's disease (AD) mice. EA was oral administration at 100 and 200 mg/kg/day during the 14 days. We studied the protective effect of EA against AD on the basis of behavioral tests including T-maze test, Novel object recognition test, and Morris water maze test. Control group injected with $A\beta_{25–35}$ showed significant impairments in memory function. But the oral administration of EA (EA 100 and EA 200 groups) improved the cognition and memory function. In addition, EA against $A\beta_{25–35}$ peptide has been shown to inhibit lipid peroxidation levels and nitric oxide production in tissues. Acetylcholinesterase (AChE) was elevated in the brain by $A\beta_{25–35}$ peptide, whereas administration of EA (EA 100 and EA 200 groups) significantly decreased AChE level. Our results indicated that EA improves learning and long-term memory against $A\beta_{25–35}$ peptide–caused deficit through attenuation of oxidative stress.

Keywords *Acer okamotoanum* · Amyloid $\beta_{25–35}$ peptide · Alzheimer's disease · Cognition · Learning · Memory deficit · Oxidative stress

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Introduction

Alzheimer disease (AD) is the major familiar chronic neurodegenerative disease that progresses with age. The characteristics of the brain of an AD patient brain include amyloid-β peptide (Aβ) accumulation, mitochondrial deficits, neurofibrillary tangles, synapse depletion, and oxidative stress (Pi et al. 2012), resulting in progressive memory loss and cognitive impairment (Paban et al. 2014). In particular, Aβ accumulation and oxidative stress are determining factors of neurodegeneration in AD. Neurodegeneration caused by Aβ leads to cognitive impairment, neuro-inflammation (Bicca et al. 2015), and neuronal apoptosis (Park et al. 2009). Aβ originates from proteolytic cleavage of amyloid precursor protein (APP) by β- and γ-secretases via amyloidogenic pathway, and it accumulates in AD brains owing to excessive production and impaired clearance. The Aβ accumulation leads to neurodegeneration and apoptosis, which impair synaptic function and long-term potentiation (Esmaeili et al. 2015). The hydrolysis of acetylcholine by acetylcholinesterase (AChE) occurs within and around the amyloid peptides in the brain of AD patients. AChE expression promotes the Aβ fibril formation and leads to Aβ toxicity (Kwon et al. 2011).

The genus *Acer* is divided into 15 types in Korea and includes typical maple trees widely distributed in the mountainous regions (Kim et al. 1998). In particular, *Acer okamotoanum* (*A. okamotoanum*), which is indigenous to Korea and widely distributed in Ulleung Island was reported to have health benefits (Yim et al. 1981). *A. okamotoanum* is rich in flavonoids and phenolic compounds, such as quercetin, kaempferol, and gallic acid (Qadir et al. 2007; Jeong et al. 2009). Several studies demonstrated that the leaf, branch, and root of *A. okamotoanum* have useful biological properties, such as anti-

HIV-1 integrase, anti-cancer, anti-oxidant, skin whitening, and anti-herpetic effects (Jeong et al. 2010; Woo et al. 1997). In addition, *A. okamotoanum* was reported to affect alcohol detoxification (Yoo et al. 2011) through reduction decline of the levels of ethanol and acetaldehyde in the blood. However, the effects of the *A. okamotoanum* on cognitive impairment associated with AD were not yet studied.

Therefore, we investigated the influence of *A. okamotoanum* on cognitive impairment and neuroprotective function in ICR mice model induced by intracerebroventricular injection of $A\beta_{25–35}$ peptides.

Materials and methods

Plant materials

Acer okamotoanum was obtained from Ulleung Island by Korea National Arboretum and deposited at the Herbarium of our department.

Drugs

$A\beta_{25–35}$, malondialdehyde (MDA), acetylcholine iodide, and 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma Aldrich (Saint Louis, Missouri, USA). Dimethyl sulfoxide and sodium chloride (NaCl) were obtained from Bio Basics Inc. (Ontario, Canada). Thiobarbituric acid (TBA) was acquired from Lancaster Synthesis (Ward Hill, USA).

Arrangement of the ethyl acetate (EtOAc) fraction from *A. okamotoanum* (EA)

Freeze-dried *A. okamotoanum* was extracted with methanol under reflux and repeated 5 times at 3-h intervals. The extract was concentrated using a rotary evaporator and suspended in distilled water (DW). Extract was suspended in DW and partitioned with EtOAc.

Mice model and experimental design

The male 5-week-old ICR mice (weighing 24.0–26.0 g) were gained from the Orient Inc. (Seongnam, Korea). The experimental models were housed 4 per plastic cages fed with a standard diet and free drinking edible water. Also, they were sustained under the constant temperature (20 ± 2 °C), 12-h light–dark illumination period. The mice management was conducted in accordance with the animal care and use guidelines (approval No. PNU-2014-0056) approved by the Pusan National University-Institutional Animal Care and Use Committee (PNU-IACUC). The experimental models were separated into 4 groups, consisting of six individuals each: normal group = 0.9% NaCl-lesioned + intraorally water; control group = $A\beta_{25–35}$-lesioned + intraorally water; EA 100 group = $A\beta_{25–35}$-lesioned + intraorally EA (100 mg/kg/day); EA 200 group = $A\beta_{25–35}$-lesioned + intraorally EA (200 mg/kg/day) during the 14 days using a zonde. As shown in Fig. 1, the behavioral experiments design.

Induction of AD by $A\beta_{25–35}$-injection to brain of ICR mice

$A\beta_{25–35}$ peptide was solubilized in saline solution to a final concentration of 1 mg/ml. The solution was incubated for 3 days (37 °C) to obtain the aggregated form (Maurice et al. 1996). The aggregated $A\beta_{25–35}$ peptide was injected into the experimental models hippocampus in accordance with the process constituted by Laursen and Belknap (1986). Experimental models in the control, EA 100 and 200 groups were anesthetized with CO_2 and injected with aggregated 5 nmol of $A\beta_{25–35}$ peptide (5 µl) into the left lateral ventricle at a rate of 1 µl/min. Normal group was injected with saline solution (0.9% NaCl) in the same area. All the injections were performed using a 10-µl Hamilton syringe (26-gauge needle, 2.2 mm depth).

Morris water maze test

Classical water maze test procedure was based on a method described by Morris (1984). Water maze apparatus that was composed of circular water tank pool (diameter, 80 cm; height, 50 cm) was randomly divided into quadrants. An escape platform (8 cm in diameter) was submerged 1 cm underneath the water surface in the middle of 1 quadrant. The water temperature was maintained at 22 ± 1 °C, and then white poster color added to water in order that make hidden the platform. The platform position was not changed during the training period. 4 stickers on the walls of the apparatus provided visual cues for navigation. The experimental models were instructed to locate the obscure platform in three times per day for consecutive 3 days. In every trial, the experimental models were released in the water pool facing a different sticker and permitted to swim within 60 s. After successfully reaching the platform, they were allowed to remain on the platform for 15 s. If the mice failed to find the platform, the experimenter released the mice on the platform and permitted to stay on it for 15 s. The memory retention session (probe trial test) was conducted after 3 days of training. In the primary test on day 4, the experiment was carried out in a manner as described based on Morris (1984). In the secondary test, the platform was removed and then the mice were placed in the water and allowed to swim within 60 s to look for the

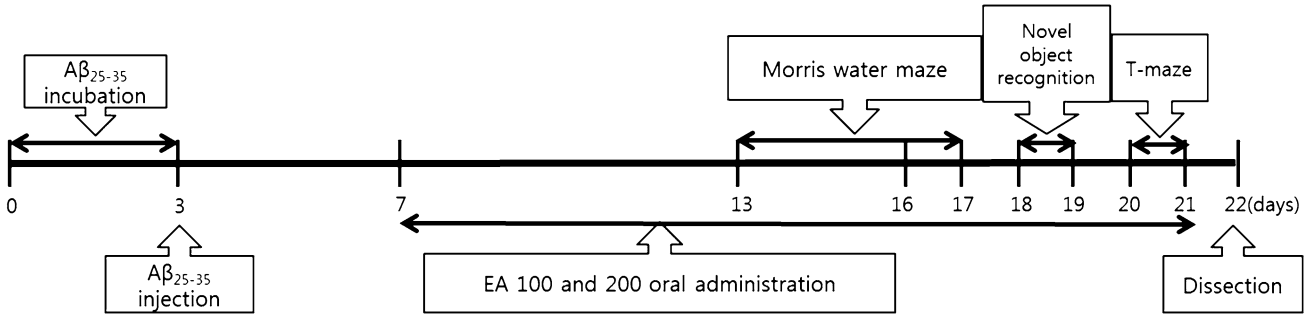

Fig. 1 Experimental schedule of behavioral tests for mice injected with $A\beta_{25–35}$

platform. The time spent in the target quadrant was set down. In the tertiary test, time the mice spent to reach the platform in transparent water was counted. The percentage of occupancy of the target quadrant was calculated as the percentage of time spent in the target quadrant during a 60 s trial.

Novel object recognition test

This test was conducted according to the process constituted by Bevins and Besheer (2006), in a rectangular black open box (40 cm × 30 cm × 20 cm). During first session (training session), two identical objects (plastic models) were placed at the opposite sides in the box. The mice were released at the center of the box and permitted to explore liberally during the 10 min. In this session, the number of touch times for each object was set down. The experimental models were released again into the same field after 24 h. However, in this session (test session), one of the original objects was changed with a novel (another plastic model). In test session, mice were permitted to search and explore freely during the 10 min, and all trials in the object recognition test were recorded.

T-maze test

This test was based on the process described by Montgomery (1952). T-maze apparatus was composed of a stem and two arms made of black boards (length of start and goal stems, 50 cm; width, 13 cm; height of walls, 20 cm). The apparatus composed of a start position, left route, and right route with a black door to divide the routes from one another. Experimental models were released in the start position, and the number of total entry times in the right route of the T-maze was measured for each mouse for 10 min. Experimental models were released back into the same apparatus for 24 h after the training session. The mice were permitted to explore freely through the maze within 10 min, and the number of total entry times was measured (test session). The percentage of space perceptive alternations was calculated as the ratio (%) of the number of entries into the left or right route to the number of total route entries, multiplied by 100.

Determination of lipid peroxidation

The lipid peroxidation levels in organs were conducted by the method of Ohkawa et al. (1979). After the finish of behavioral experiments, experimental models were anesthetized using CO_2 gas. The brains, livers, and kidneys of the mice were directly isolated and placed on ice. Followed by dissection, these tissues were homogenized. The obtained homogenate was blended with 1% phosphoric acid and 0.67% TBA solution. After boiling for 45 min, the mixture was cooled on the ice and 2 ml of 1-butanol was mixed prior to centrifugation at 13,000×*g* for 10 min (4 °C), absorbance was measured at 540 nm. The lipid peroxidation levels were determined and calculated using the standard curve of MDA content.

Estimation of reduced nitric oxide (NO)

Reduced NO level in brain, liver and kidney were assayed by Schmidt et al. (1992). The 150 µl of supernatant from the lipid peroxidation process was blended with 130 µl of DW, and then mixed solvent 20 µl was blended to the identical amount of phosphoric acid and 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride solvent and the absorbance of the mixture was assayed at 540 nm. The manufacture of NO level was calculated with the standard curve of sodium nitrite content.

AChE inhibitory activity assay

AChE activity assay was performed as described by Ellman et al. (1961). The reaction solution included 4 ml of (0.1 M) phosphate buffer saline, 100 µl of DTNB, and 200 µl of acetylthiocholine iodide mixture, which were blended and incubated during the 30 min (25 °C). After

5 min, samples (400 µl) were added to the reaction mixtures and then the absorbance was measured at 412 nm.

Statistical analysis

Our data are expressed as mean ± SD ($n = 6$). All statistical significances were performed using a one-way ANOVA, followed by Duncan's post hoc tests. Comparisons with $P < 0.05$ were considered statistical significance.

Results

Body weight change

This study did not show any dramatic difference in the body weights among these four groups, normal, control, EA 100, and EA 200, during experimental periods (data not shown).

Morris water maze test

The influence to EA on spatial memory was examined in the water maze. In the training session, normal group immediately learned the hidden platform location by swimming across the water tank pool and promptly reached the hidden platform. EA 100 and EA 200 groups reached the platform on a period of 3 days with less time. In the final test, the groups administered with EA 100 and EA 200 significantly reduced the time to reach platform. The EA 200 group dramatically reduced time to reach the platform from 58.5 to 18.0 s (Fig. 2). When the platform was removed, the staying time in the target quadrant was shorter in $A\beta_{25-35}$-injected control group than normal group (Fig. 3). However, of the EA administration increased the staying time in the target quadrant. It indicates that the EA administration protected against the deficit of memory role induced by $A\beta_{25-35}$. In addition, Fig. 4(A) and (B) indicated that EA administration did not affect the visual and exercise ability. There were no significance differences among all of groups in latency to reach the exposed platform (Fig. 4(A)). On the other hand, EA-treated groups took shorter to find the platform than the control groups, when the platform was hidden (Fig. 4(B)).

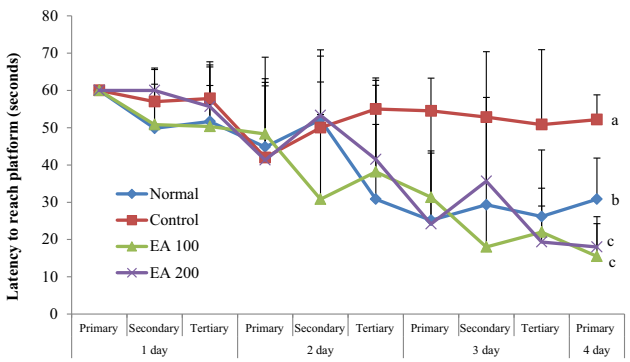

Fig. 2 Effect of EA on spatial learning in the Morris water maze test. The groups are defined as follows: normal = 0.9% NaCl injection + oral administration of water; control = $A\beta_{25-35}$ injection + oral administration of water; EA100 = $A\beta_{25-35}$ injection + oral administration of EA (100 mg/kg/day); EA200 = $A\beta_{25-35}$ injection + oral administration of EA (200 mg/kg/day). Values are represented as mean ± SD. [a–c]The *different letters* represent significant differences ($p < 0.05$) by Duncan's multiple range test

Novel object recognition test

The effect of EA on novel object recognition impairment was shown in Fig. 5. There was no significant difference in exploratory preference among the groups in the training session. After 24 h of training session, cognitive ability of the normal group toward the novel object was significantly higher than familiar object recognition, whereas the $A\beta_{25-35}$-injected control group did not show significant difference on object recognition ability between familiar and novel object. However, the EA 100 and EA 200 groups increased novel object cognitive ability to 60.7 and 65.1%, respectively. It indicated that administration of EA from *A. okamotoanum* evidently improved object recognition ability.

T-maze test

Influence of EA on short-term memory was examined using the T-maze test (Fig. 6). $A\beta_{25-35}$-injected control group did not show the spatial perception toward the novel route. However, EA 100- and EA 200-treated groups showed a higher preference for the novel route to 61.4 and

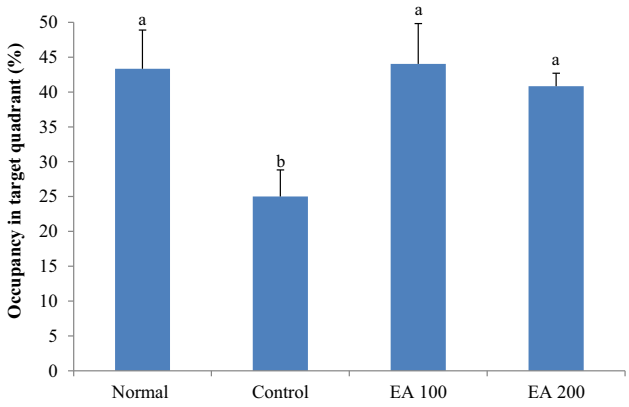

Fig. 3 Effect of EA on memory impairment induced by $A\beta_{25-35}$ injection in the Morris water maze test. The groups are defined as follows: normal = 0.9% NaCl injection + oral administration of water; control = $A\beta_{25-35}$ injection + oral administration of water; EA100 = $A\beta_{25-35}$ injection + oral administration of EA (100 mg/kg/day); EA200 = $A\beta_{25-35}$ injection + oral administration of EA (200 mg/kg/day). Values are represented as mean ± SD. [a–b]The *different letters* represent significant differences ($p < 0.05$) by Duncan's multiple range test

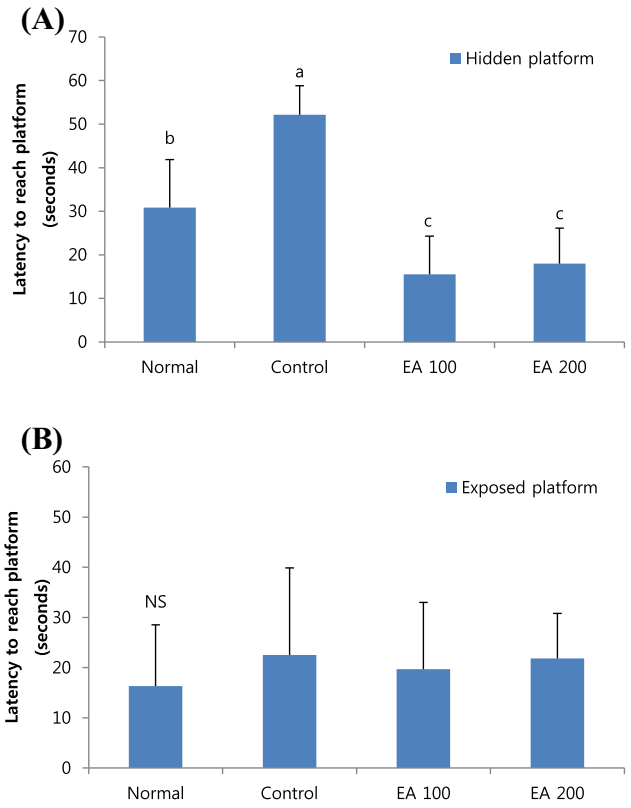

Fig. 4 Effects of EA on the performance of $A\beta_{25-35}$-treated mice in finding the hidden (**A**) and exposed (**B**) platforms in the Morris water maze test. The groups are defined as follows: normal = 0.9% NaCl injection + oral administration of water; control = $A\beta_{25-35}$ injection + oral administration of water; EA100 = $A\beta_{25-35}$ injection + oral administration of EA (100 mg/kg/day); EA200 = $A\beta_{25-35}$ injection + oral administration of EA (200 mg/kg/day). Values are represented as mean ± SD. [a–c]The *different letters* represent significant differences ($p < 0.05$) by Duncan's multiple range test. *NS* No significant

61.1%, respectively. It indicated that oral administration of EA from *A. okamotoanum* enhanced the ability to spatial perception toward novel route.

Evaluation of lipid peroxidation

The results of the preventive activity of EA against lipid peroxidation caused by $A\beta_{25-35}$ are shown in Fig. 7. $A\beta_{25-35}$-injected control group raised lipid peroxide values in the brain. Compared with the normal group, the MDA value of the control group was raised dramatically by 75.1 nmol/mg protein in the brain. However, the administration of EA 100 and EA 200 groups was 40.0 and 40.2 nmol/mg protein, respectively. Additionally, the MDA values in the kidney of normal and control groups were 63.5 and 87.3 nmol/mg protein, respectively. On the other hand, the levels were reduced 53.0 and 53.1 nmol/mg protein in EA 100 and EA 200 groups, respectively. Moreover, the MDA concentration in the liver increased in control group to 47.7 nmol/mg protein, compared with that

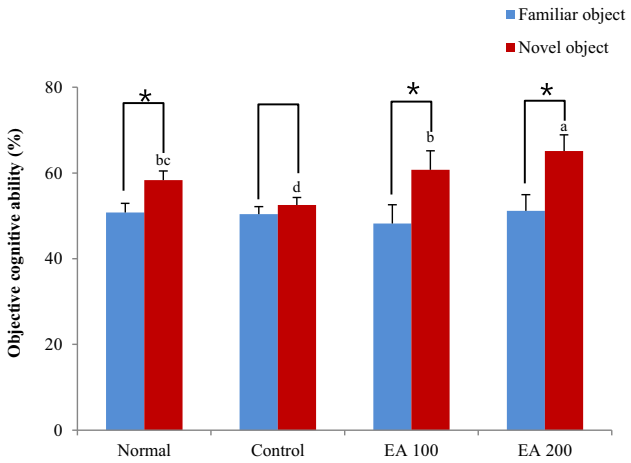

Fig. 5 Effect of EA on objective recognition test. The groups are defined as follows: normal = 0.9% NaCl injection + oral administration of water; control = $A\beta_{25-35}$ injection + oral administration of water; EA100 = $A\beta_{25-35}$ injection + oral administration of EA (100 mg/kg/day); EA200 = $A\beta_{25-35}$ injection + oral administration of EA (200 mg/kg/day). Values are represented as mean ± SD. [a–d]The *different letters* represent significant differences ($p < 0.05$) by Duncan's multiple range test. * The object cognitive abilities for familiar and novel objects are significantly different as determined by Student' s *t* test ($p < 0.05$)

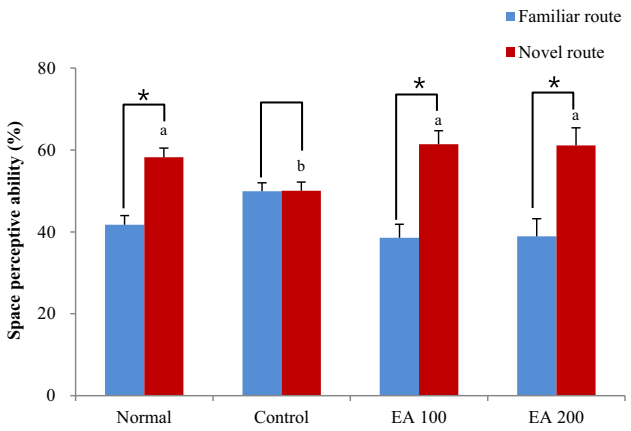

Fig. 6 Spatial alternation test in the T-maze test. The groups are defined as follows: normal = 0.9% NaCl injection + oral administration of water; control = $A\beta_{25-35}$ injection + oral administration of water; EA100 = $A\beta_{25-35}$ injection + oral administration of EA (100 mg/kg/day); EA200 = $A\beta_{25-35}$ injection + oral administration of EA (200 mg/kg/day). Values are represented as mean ± SD. [a–b]The *different letters* represent significant differences ($p < 0.05$) by Duncan's multiple range test. * The object cognitive abilities for familiar and novel routes are significantly different as determined by Student' s *t* test ($p < 0.05$)

of normal group, 19.6 nmol/mg protein. The hepatic MDA levels in the EA 100 and EA 200 groups decreased to 13.9 and 10.9 nmol/mg protein, respectively. These results demonstrated that EA from *A. okamotoanum* significantly restored the level of MDA in the brains, kidneys, and livers of $A\beta_{25-35}$-injected experimental models.

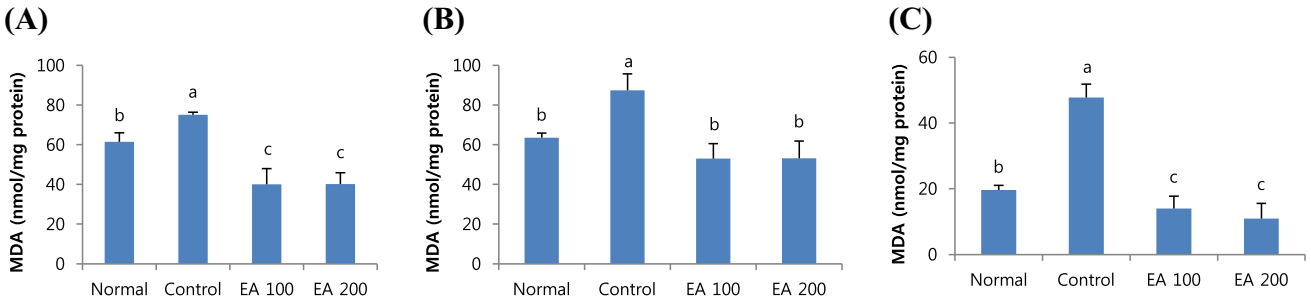

Fig. 7 Effect of EA administration on lipid peroxidation in mice brain (**A**), kidney (**B**), and liver (**C**). The groups are defined as follows: normal = 0.9% NaCl injection + oral administration of water; control = $A\beta_{25–35}$ injection + oral administration of water; EA100 = $A\beta_{25–35}$ injection + oral administration of EA (100 mg/kg/day); EA200 = $A\beta_{25–35}$ injection + oral administration of EA (200 mg/kg/day). Values are represented as mean ± SD. [a–c]The *different letters* represent significant differences ($p < 0.05$) by Duncan's multiple range test

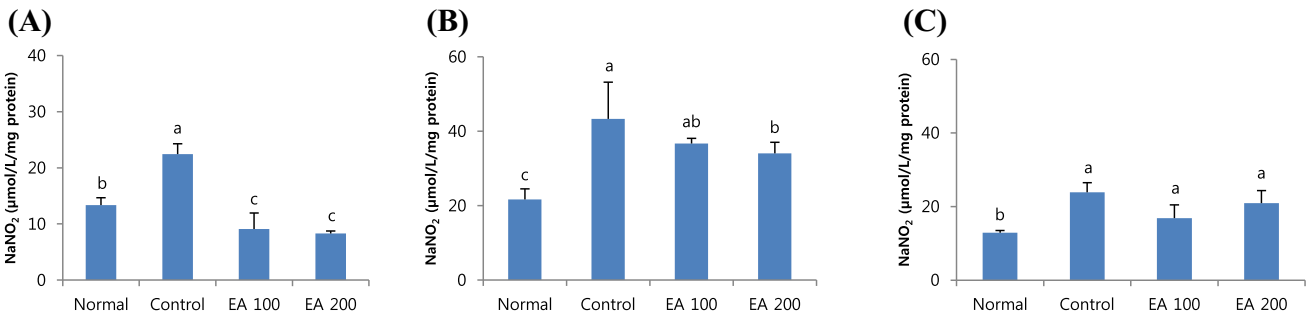

Fig. 8 Effect of EA administration on $A\beta_{25–35}$ induced NO production in mice brain (**A**), kidney (**B**), and liver (**C**). The groups are defined as follows: normal = 0.9% NaCl injection + oral administration of water; control = $A\beta_{25–35}$ injection + oral administration of water; EA100 = $A\beta_{25–35}$ injection + oral administration of EA (100 mg/kg/day); EA200 = $A\beta_{25–35}$ injection + oral administration of EA (200 mg/kg/day). Values are represented as mean ± SD. [a–c]The *different letters* represent significant differences ($p < 0.05$) by Duncan's multiple range test

NO scavenging activity

Figure 8 shows the effects of EA toward NO formation in the brains, kidneys, and livers of mice-injected $A\beta_{25–35}$. The value of NO in the brain of the normal group was 13.3 μmol/L/mg protein, while that of the $A\beta_{25–35}$-injected control group was 22.4 μmol/L/mg protein. However, the brain NO values of the EA 100 and EA 200 groups showed 9.1 and 5.3 μmol/L/mg protein, respectively. NO formation in the kidney of the control group was 43.2 μmol/L/mg protein, but NO levels were decreased to 36.6 and 34.0 μmol/L/mg protein by oral administration of EA 100 and EA 200, respectively. The liver NO formation of the control group was also improved compared to the normal group showing 23.8 and 12.9 μmol/L/mg protein, respectively. However, the EA 100 and EA 200 groups led to the significant decrease in NO levels from 23.8 to 16.8 and 20.9 μmol/L/mg protein, respectively. It was indicated that oral administration of EA 100 and EA 200 significantly inhibited NO formation in the brain, kidney, and liver.

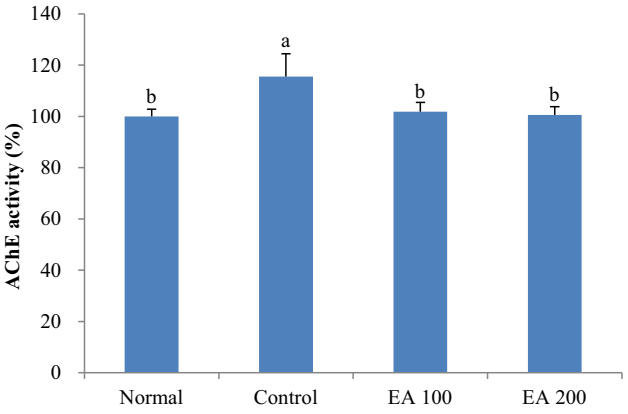

Fig. 9 Effect of EA administration on AChE inhibitory activity in mice brain. The groups are defined as follows: normal = 0.9% NaCl injection + oral administration of water; control = $A\beta_{25–35}$ injection + oral administration of water; EA100 = $A\beta_{25–35}$ injection + oral administration of EA (100 mg/kg/day); EA200 = $A\beta_{25–35}$ injection + oral administration of EA (200 mg/kg/day). Values are represented as mean ± SD. [a–b]The *different letters* represent significant differences ($p < 0.05$) by Duncan's multiple range test

AChE activity

As shown in Fig. 9, we investigated the effect of EA from *A. okamotoanum* on AChE activity in the left brain. $A\beta_{25-35}$-injected control group demonstrated that dramatic increase in the AChE activity in the left brain compared with normal group. On the other hand, EA 100- and EA 200-oral administered groups significantly inhibited AChE activity compared with the control group in the brain. It indicated that EA from *A. okamotoanum* has a excellent prevention influence on AChE activity in brain.

Discussion

AD is the most familiar form of dementia and it is an ascending worldwide public health issue (Brookmeyer and Gray 2000). AD patients were characterized by continuing memory deficit and cognitive impairment, including personality changes, aphasia, and apraxia (Paban et al. 2014). AD neuropathological features include deposits of Aβ peptides, neurofibrillary tangles, neuronal and synaptic degeneration, and neuronal inflammation (Rozemuller et al. 1989). Aβ is created by sequential endoproteolytic cleavage of APP by β- and γ-secretase, and penetrates a dynamic equilibrium among soluble and deposited forms (De Mattos et al. 2002). The most prominent Aβ type in the AD patient brain is $A\beta_{1-40}$, which can be divided into a more toxic fragment, $A\beta_{25-35}$ (Kubo et al. 2002). $A\beta_{25-35}$ has related to induce the hydrogen peroxide production and lipid peroxide in the brain (Yatin et al. 2000). Therefore, $A\beta_{25-35}$ injection into the intracerebroventricular region of mice causes numerous adverse effects, such as impairment of learning, memory, and cognition. In the current study, $A\beta_{25-35}$ was injected into the brain of mice to induce AD, and then the neuroprotective activities of EA from *A. okamotoanum* on learning, memory, and cognitive impairment were evaluated using the Morris water maze, novel object recognition test, and T-maze.

Morris water maze test is a well-established method for the assessment of standard learning and long-term memory (Morris 1984). In the current study, the normal group showed rapid decrease in time to reach the hidden platform by training. However, the control group injected with $A\beta_{25-35}$ did not decrease the latency time to reach the platform. It indicated that the injection of $A\beta_{25-35}$ led to long-term memory impairment. However, EA 100 and EA 200 administration significantly shortened the latency time to reach the hidden platform, opposed to that of control group. On the contrary, the time to reach the exposed platform did not show any significant difference among groups. It indicated that the improvement of long-term spatial cognition impaired by $A\beta_{25-35}$ was not involved in swimming and visual ability, but it was not related to the memory function.

$A\beta_{25-35}$-injection led to the impairment of object recognition ability. Mori et al. (2011) reported that injection of $A\beta_{25-35}$ into mice led to an impairment of object recognition ability. Our finding also demonstrated that $A\beta_{25-35}$-injected control group did not show significant difference on recognition between familiar and novel object. In contrast, EA 100 and EA 200 groups showed higher novel objective recognition. It indicated that injection of $A\beta_{25-35}$-induced deficits in recognition of novel object. However, EA from *A. okamotoanum* improved the $A\beta_{25-35}$-induced recognition impairment.

T-maze test results showed that EA 100 and EA 200 groups display significantly higher preference for the novel route, compared to $A\beta_{25-35}$-injected control group. In addition, EA could prevent memory and cognitive deficits in AD mice model.

Aβ causes oxidative stress by inducing lipid peroxidation, reactive oxygen species, and reactive nitrogen species, such as NO (Markesbery and Carney 1999; Avdulov et al. 1997; Butterfield and Lauderback 2002). It induced the formation of senile plaques, mitochondrial DNA damage, and thus might cause neuronal dysfunction and cell death (Reddy 2006). Eventually, oxidative stress induces the pathogenesis of the neurodegenerative disorders, such as AD (Christen 2000; Yin et al. 2013).

NO contributes to various neurodegenerative disorders, including AD (Stepanichev et al. 2008). Torreilles et al. (1999) reported that the oxidative damage due to the overproduction of NO contributes to Aβ-caused neurotoxicity and memory impairment. In addition, NO in the brain contributes to the pathologies of AD (Aliev et al. 2009). Lipid peroxidation products of several aldehydes, including MDA and 4-hydroxy-2,3-nonenal levels were elevated by oxidative stress. Among them, MDA was considered as a key marker of the lipid peroxidation in the AD model (Esterbauer et al. 1991). Thus, NO and MDA represent important markers of oxidative stress in the AD model. Several studies reported increased MDA (Cotman and Su 1996), NO (Butterfield 1997), carbonyls, and heme oxygenase-1 (Smith et al. 1994, 1996) in the AD model. The present study showed that the level of MDA and NO in mice brain, kidney, and liver were elevated by $A\beta_{25-35}$ injection. Choi et al. (2013) demonstrated that $A\beta_{25-35}$ injection in mice resulted in a dramatic improvement in MDA and NO level in mice brain, kidney, and liver, suggesting that $A\beta_{25-35}$-induced oxidative stress and neurodegeneration. However, oral administration of EA had an prevention influence on MDA and NO production in brain, kidney, and liver of AD mice. It indicated that EA alleviated the oxidative stress and neurodegeneration induced by $A\beta_{25-35}$.

AChE was found to be involved in the Aβ of senile plaques, pre-Aβ dispersed sediment, and cerebral blood vessels (Geula and Mesulam 1989). AChE is accelerate the lump of Aβ peptide into fibrils deposited in the AD patients' brain and forms an AChE complex with Aβ. This Aβ–AChE complex has higher toxic effect than Aβ peptide (Melo et al. 2003). Moreover, the Aβ protein also regulates AChE expression and glycosylation in cell, transgenic mice, and AD patient brain. It indicated that AChE creates a vicious cycle, leading to an increased Aβ accumulation (Parnetti et al. 2002). Several reports have shown that AChE activity raised within and encompassing amyloid plaques in AD brain (Ulrich et al. 1990; Chiapinotto Spiazzi et al. 2015). Nakdook et al. (2010) similarly showed that injection of $A\beta_{25-35}$ caused the increased AChE activity in the cortical and hippocampal regions. The present study also demonstrated that AChE activity in the brain was raised in $A\beta_{25-35}$-induced mice compared with that of the normal group. Furthermore, in the groups administered with EA, the AChE activity in brain was dramatically lower than that of the control group. It was demonstrated that EA from *A. okamotoanum* significantly inhibited $A\beta_{25-35}$-induced AChE activity.

Acer okamotoanum has several biological active compounds. Kim et al. (1998) demonstrated that *A. okamotoanum* contains quercetin 3-*O*-(2″,6″-*O*-digalloyl)-β-D-galactoside, flavonol glycoside gallate ester, six kinds of flavonol glycosides, and three kinds of phenolic compounds. These active compounds have the beneficial function against HIV-1 and oxidative stress. In addition, Yun et al. (2001) reported that *A. okamotoanum* was important functional plant that contains antioxidants, several coumarinolignans, and cleomiscosins A and C. These compounds exerted strong antioxidant effects against lipid peroxidation and LDL oxidation (Thuong et al. 2005). However, no previous report has demonstrated that whether *A. okamotoanum* protects against improves $A\beta_{25-35}$-induced cognition impairment. This study is the initial report on the effects of *A. okamotoanum* against AD. In conclusion, our results indicate that EA improved learning and long-term memory ability against $A\beta_{25-35}$–induced impairment. Moreover, the present study suggests the potential of EA from *A. okamotoanum* as a promising dietary agent with protective effect against AD.

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