Study of Ameliorating Effects of Ethanolic Extract of *Centella asiatica* on Learning and Memory Deficit in Animal Models

Saowalak Doknark BSc*,
Salin Mingmalairak PhD**, Anusara Vattanajun PhD***,
Boonyong Tantisira PhD****, Mayuree H. Tantisira PhD****

** Program of Inter-department of Physiology, Graduate School, Chulalongkorn University, Bangkok, Thailand*
*** Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*
**** Department of Physiology, Phramongkutklao College of Medicine, Bangkok, Thailand*
***** Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand*

Objective: *The present study investigated the effect of Centella asiatica ethanolic extract (CE) on learning and memory impairment induced by either transient bilateral common carotid arteries occlusion (T2VO) or an intraperitoneal injection of scopolamine in mice.*

Material and Method: *CE (100, 300, 1,000 or 1,500 mg/kg, p.o.) were administered to learning and memory impaired mice once daily for 8 consecutive days. Learning and memory performance were evaluated by Morris water maze (MWM) and step-down passive avoidance (PA) test. Changes in malondialdehyde (MDA) levels in the brain were determined by lipid peroxidation assay.*

Results: *T2VO mice exhibited learning and memory impairment in the MWM and PA tests. Treatment with CE ameliorated the learning and memory impairment of T2VO mice. Furthermore, CE significantly reduced MDA level in the brain of T2VO mice. On the other hand, administration of CE did not attenuate learning and memory impairment induced by scopolamine in mice.*

Conclusion: *The present study demonstrated ameliorating effect of CE on learning and memory impairment in T2VO mice. Furthermore, it is likely that the positive effect of CE observed could be, at least partly, accounted by its antioxidative property. Thus, CE might be beneficial for memory impairment in which oxidative stress is an underlying cause.*

Keywords: *Centella asiatica, Learning and memory, Transient bilateral common carotid arteries occlusion (T2VO), Scopolamine*

J Med Assoc Thai 2014; 97 (Suppl. 2): S68-S76
Full text. e-Journal: *http://www.jmatonline.com*

Alzheimer's disease is an age-related neurodegenerative disorder which is the most common cause of dementia in the elderly. The pathological features that have been identified in the central nervous system (CNS) in Alzheimer's disease are oxidative and inflammatory processes[1]. Although a number of drugs have been approved for use, they have been shown to produce side effects and yield relative modest benefits. To overcome these limitations of current therapeutics for Alzheimer's disease, extensive research and development are underway to identify drugs that are effective and free of undesirable side effects. Recently, natural medicines have gained considerable attention as alternative candidates for Alzheimer's disease therapy[2].

Centella asiatica is a herb which have been used for centuries in Ayurvedic medicine as one of the main ingredients in Ayurvedic formulation to retard ageing and to prevent dementia. In traditional Chinese medicine, *C. asiatica* has been used for combating physical and mental exhaustion[3]. Preliminary studies on the pro-cognitive effect of *C. asiatica* have demonstrated that extract of this herb enhances memory retention in rodents[4,5]. Phytochemical analyses have shown that *C. asiatica* contains triterpenoid saponins, flavonoids, phytosterols and other components[6]. Asiaticoside, the most abundant triterpene saponin, has been reported as a cognitive enhancer useful in treating dementia[7]. However, there has been no report

Correspondence to:
Tantisira M H, Faculty of Pharmaceutical Sciences, Burapha University, Chonburi 20131, Thailand.
Phone: 0-2218-8325, Fax: 0-2218-8324
E-mail: tmayuree@chula.ac.th, mayureet@buu.ac.th

on the effect of *C. asiatica* on the animal model of transient cerebral ischemia or scopolamine-induced learning memory deficit. Therefore, the present study aimed to explore the effect of ethanolic extract of *C. asiatica* (CE) on transient bilateral common carotid arteries occlusion (T2VO) or scopolamine-induced learning and memory impairment in mice. Since Alzheimer's disease have been associated with increased oxidative stress[8], the effect of CE on marker of brain oxidative stress namely malondialdehyde (MDA) was also conducted.

Material and Method

Plant materials and preparation of the extract

C. asiatica plants were procured from local supplier in Nonthaburee province, Thailand. The leaves were washed thoroughly, sun dried and ground into powder. The dried plant (7.1 kg) was extracted with 95% ethanol for 3 days and filtered. The pooled extract was concentrated under reduced pressure by rotary flash evaporator to obtain syrupy mass which was then evaporated to dryness on water bath. The yield of the crude CE was 12.68 % (W/W).

Chemicals

All chemicals used in the present study were purchased from Sigma-Aldrich (St. Louis, Mo, USA).

Animals

Eight-week-old male ICR mice (National Laboratory Animal Center, Mahidol University, Nakornpathom, Thailand) weighing 30-35 g were housed, 8 mice per cage, under standard conditions (ambient temperature 25$\pm$2°C, humidity 60-70%, 12-h light/dark cycle (lights on at 7:00 h)) and allowed free access to both food pellets and water. They were allowed to acclimatize to these housing conditions for 1 week prior to the experiments in which they were divided into experimental groups of 8 animals in each. All behavioral experiments were carried out between 9:00 and 17:00 h. The experimental protocol was approved by the Ethics Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Induction of learning and memory impairment in mice

Learning and memory impairment was induced by T2VO or an administration of scopolamine. T2VO was performed by method described previously by Xu et al[9]. Briefly, mice were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). A ventral midline incision of the neck was made. The common carotid arteries were exposed, carefully separated from surrounding tissue and vagus nerve and then occluded by arterial clips. While the arteries were clamped, blood (0.3 ml) was withdrawn by cutting off the tip of the tail. After 20 min, the arterial clips were removed and the incision was closed. Sham-operated mice were subjected to the same procedure but without carotid clamping and bleeding. Animals were allowed to recovery for 24 h before behavioral studies. For scopolamine-induced learning and memory impairment, mice were intraperitoneally injected with scopolamine (0.5 mg/kg, dissolved in normal saline) 30 min before each behavioral test[10].

Drug administration

CA was dissolved with 15% Tween 20 in distilled water and administered orally by gavage. Vehicle was given to sham operated mice and CE (100, 300, 1,000 or 1,500 mg/kg) was given to T2VO-induced learning and memory deficit mice at 1 h before the onset of behavioral test at every consecutive day (8 days).

In the scopolamine-induced learning and memory impairment study, CE was given 40 min before the onset of behavioral test at every consecutive day (8 days). Vehicle was administered to the control group using the same time schedule.

Morris water maze test

The procedure used was slightly modified from the one that described by Morris[11]. The Morris water maze (MWM) is a black circular pool with a diameter and height of 70 and 23 cm, respectively. The pool was filled to a depth of 13 cm with water (25$\pm$1°C). The pool was divided into four quadrants and placed in a room with various visual cues in fixed positions. A black platform (6 cm in diameter) was placed at the middle of one quadrant of the pool and submerged 1 cm below the water surface. Daily swimming consisted of four trial sessions in which the mice were placed in the water facing the pool wall from four different starting points. Mice were allowed 60 sec to locate the platform in each trial session and 30 sec to rest between the trial sessions. This was conducted for 5 consecutive days. The time taken to find the platform in each trial session was then recorded and averaged as an escape latency of each animal per day. Two days before these days were considered as training days in which the animal that could not locate the platform would be guided by researcher onto the platform and allowed to remain on

it for 10 sec. The mice that failed to locate the platform after four trial sessions were excluded from the experiments.

Step-down passive avoidance test

A step-down passive avoidance (PA) test was examined in a Plexiglas chamber (35x23x20 cm^3) with a stainless steel grid floor (3 mm stainless steel rods spaced 11 mm apart) attached to a shock generator (1 Hz, 1 ms, 36 V DC) as previously described[12]. A wooden platform (5 cm in diameter) was placed in one corner of the chamber serving as a shock free zone. The PA test consisted of two trials; acquisition and retention trial. The acquisition trial was performed on the next day after the last MWM test. In the acquisition trial, the mouse was placed in a chamber without an electric foot shock for 3 min, and then the electric shock was delivered through the stainless steel rods for 5 min. The mouse could escape the electric shock by moving to the platform. Twenty-four hours after the acquisition trial, the mouse was placed on the platform in a chamber for a retention trial, and the electric shock was delivered. The initial step-down latency and the number of errors were recorded for 5 min. Initial step-down latency was the time that elapsed until the mouse stepped down from the platform. If the mice did not step down from the platform within 300 sec, the initial step-down latency was recorded as 300 sec. Upon electric shock, the mice would escape from the grid floor back to the platform and the number of error was counted whenever the animal stepped down from the platform.

Locomotor activity test

Spontaneous locomotor activity was evaluated using an activity cage (model 7430, Ugo Basile, Comerio, Italy). The apparatus consisted of a Plexiglas chamber (35x23x20 cm^3) with a stainless steel grid floor (3 mm stainless steel rods spaced 11 mm apart) connected to the circuit of counting unit. On the day after retention trial of the PA test, the mouse was placed in the activity cage, and locomotion was immediately recorded for 5 min.

Lipid peroxidation assay

Following the locomotor activity test, the animals were sacrificed by decapitation and the brains were quickly removed, cleaned with ice-cold saline and stored at -80°C. Brain tissue samples were thawed and homogenized with ice-cold 0.1 M phosphate buffer (pH 7.4). Aliquots of homogenates were used to determine the MDA content which is a marker of lipid peroxidation by the method previously described by Gupta et al[13]. Briefly, the reagents including 1.5 ml of acetic acid pH 3.5 (20%), 1.5 ml of thiobarbituric acid (0.8%) and 0.2 ml of sodium dodecyl sulphate (8.1%) were added to 0.1 ml of brain tissue samples and then heated at 100°C for 60 min. The mixture was cooled under tap water then 5 ml of n-butanol/pyridine (15:1) and 1 ml of distilled water was added and vortexed vigorously. After centrifugation at 2,500 rpm for 20 min, the organic layer was separated. The supernatant was collected and the absorbance was measured at 532 nm by spectrophotometry. The concentration of MDA was expressed as nmol/g tissue.

Statistical analysis

Results are expressed as mean $\pm$ SEM. Data obtained from behavioral and biochemical study were evaluated using Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value <0.05 was considered statistically significant.

Results

Effects of CE on memory impairment induced by T2VO

Morris water maze test

The effect of CE (100, 300, 1,000 or 1,500 mg/kg, p.o.) on spatial learning and memory impairment was evaluated using the MWM test. The MWM test is a behavioral procedure widely used in behavioral neuroscience to study spatial learning and memory[14]. In this test, mice are required to learn to locate the hidden platform, and the latency to locate the platform serves as a measure to spatial learning and memory performance. As shown in Fig. 1, mice subjected to T2VO exhibited longer latencies (44.88$\pm$0.38 sec on day 1) to locate the platform than sham-operated mice (23.91$\pm$3.17 sec on day 1) throughout the testing trial indicating impairment of learning and memory. On day 5, the escape latency of the T2VO group was 36.38$\pm$2.08 sec while it was 9.66$\pm$1.81 sec in sham-operated group. Orally given CE, 100, 300, 1,000 or 1,500 mg/kg, significantly attenuated the T2VO-induced deficit in learning and memory. The escape latency on day 5 was found to be 14.44$\pm$0.97, 11.88$\pm$1.77, 10.44$\pm$0.76, and 8.09$\pm$0.61 sec for respective groups of CE.

Step-down passive avoidance test

As shown in Fig. 2A, the initial step-down latency of T2VO mice (46.13$\pm$1.56 sec) was significantly

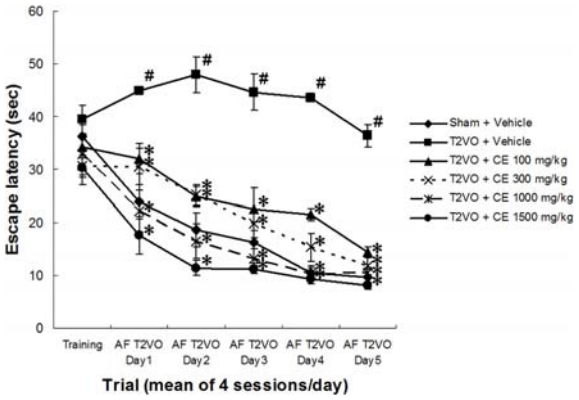

Fig. 1 Effects of CE on the impairment of Morris water maze performance in transient bilateral common carotid artery occlusion (T2VO)-induced memory deficit mice. CE (100, 300, 1,000 or 1,500 mg/kg, p.o.) or vehicle (15% Tween 20) was administered to mice 60 minutes before the testing trial of each day. The Morris water maze task was conducted as described in Material and Method. Each datum presents the mean ± SEM for n = 8. $^{\#}p<0.001$ versus vehicle-treated sham group (Student's t-test), $^{*}p<0.001$ versus vehicle-treated T2VO group (one-way ANOVA followed by Tukey's post hoc test).

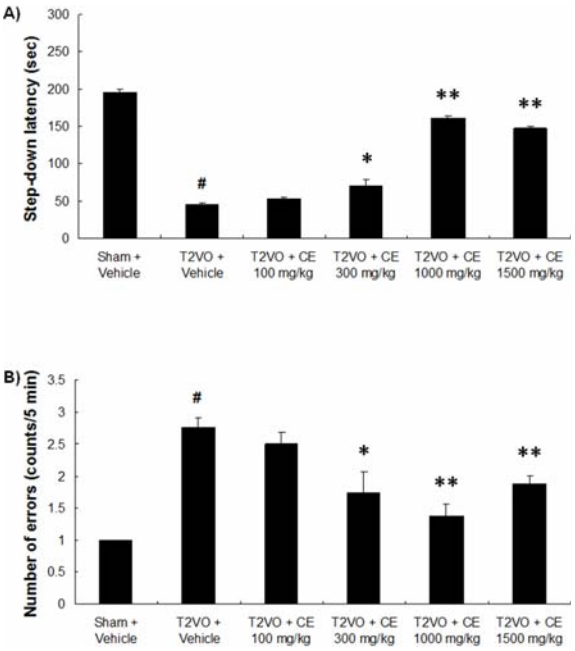

Fig. 2 Effects of CE on transient bilateral common carotid artery occlusion (T2VO)-induced memory deficit as determined by the step-down passive avoidance test. Mice were orally treated with CE (100, 300, 1,000 and 1,500 mg/kg, p.o.) or vehicle (15% Tween 20) for 7 days. Final administration was performed 60 minutes before the test. Step-down latency (A) and number of error (B) were measured for 5 min. Each datum presents the mean ± SEM for n = 8. $^{\#}p<0.001$ versus vehicle-treated sham group (Student's t-test), $^{*}p<0.01$; $^{**}p< 0.001$ versus vehicle-treated T2VO group (one-way ANOVA followed by Tukey's post-hoc test).

shorter than that of the sham-operated mice (195.38±3.78 sec). Administration of CE, 300, 1,000 or 1,500 mg/kg, significantly increased the initial step-down latency in T2VO mice to 70.75±8.22, 160.38±3.78 and 147.88±2.68 sec, respectively. Similar results were accordingly observed in terms of the number of error in which CE significantly decreased an increment of number of error observed in T2VO mice (Fig. 2B).

Spontaneous locomotor activity

An assessment of locomotor activity revealed no significant difference between T2VO and sham-operated mice (Fig. 3). Moreover, CE (100, 300, 1,000 or 1,500 mg/kg, p.o.) treatment had no effect on the spontaneous locomotor activity of T2VO mice (Fig. 3).

Lipid peroxidation

The brain MDA levels, an indicator of lipid peroxidation, of different experimental groups were shown in Fig. 4. The brain MDA levels of the T2VO mice (634.63±19.09 nmol/g tissue) were significantly higher than that of the sham-operated mice (201.88±7.08 nmol/g tissue). The brain MDA levels of T2VO mice were significantly attenuated by CE (100, 300, 1,000 or 1,500 mg/kg, p.o.) treatment (Fig. 4).

Effects of CE on memory impairment induced by scopolamine

Morris water maze test

The effect of CE (100, 300, 1,000 or 1,500 mg/kg, p.o.) on memory impairment induced by scopolamine was shown in Fig. 5. In comparison to normal saline-treated mice, significant increase of the escape latency in MWM test was observed in mice receiving scopolamine, an anticholinergic drug used to induce experimental dementia[15]. This effect was seen on the first day of testing trial and persisted throughout the testing period (Fig. 5). The escape latency on day 5 of normal saline-treated mice was 7.69±1.03 sec whereas it was 39.53±2.83 sec in scopolamine-treated mice. Administration of CE (100, 300, 1,000 or 1,500 mg/kg, p.o.) to scopolamine treated-mice 40 min before the first testing trial of each day did not affect the impaired

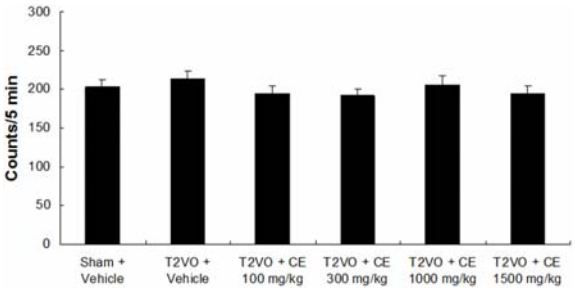

Fig. 3 Effects of CE on spontaneous locomotor activity of transient bilateral common carotid artery occlusion (T2VO)-induced memory deficit mice. Mice were orally treated with CE (100, 300, 1,000 and 1,500 mg/kg, p.o.) or vehicle (15% Tween 20) for 8 days. Final administration was performed 60 minutes before the test. Locomotor activity was measured for 5 min. Each datum presents the mean ± SEM for n = 8. No significant differences were detected.

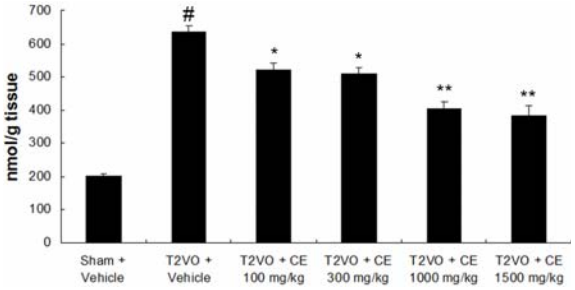

Fig. 4 Effects of CE on brain MDA levels of transient bilateral common carotid artery occlusion (T2VO)-induced memory deficit mice. Following the locomotor activity test, the mice were sacrificed and the brains were collected. MDA level was determined using lipid peroxidation assay. Each datum presents the mean ± SEM for n = 8. $^{\#}p<0.001$ versus vehicle-treated sham group (Student's t-test), $^{*}p<0.01$; $^{**}p<0.001$ versus vehicle-treated T2VO group (one-way ANOVA followed by Tukey's post-hoc test).

spatial learning performance in MWM test of scopolamine-treated mice (Fig. 5).

Step-down passive avoidance test

In the PA test, scopolamine significantly shortened the initial step-down latency and increased the number of error (Fig. 6A and B). CE (100, 300, 1,000 or 1,500 mg/kg, p.o.) treatment had no effect on the step-down latency and the number of error of scopolamine-treated mice (Fig. 6A and B).

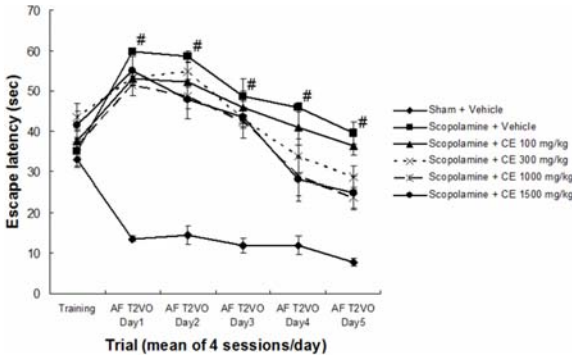

Fig. 5 Effects of CE on the impairment of Morris water maze performance in scopolamine-induced memory deficit mice. CE (100, 300, 1,000 or 1,500 mg/kg, p.o.) or vehicle (15% Tween 20) was administered to mice 40 minutes before the testing trial of each day. Memory impairment was induced by scopolamine treatment (0.5 mg/kg, i.p.) 30 min before the test. The Morris water maze task was conducted as described in Material and Method. Each datum presents the mean ± SEM for n = 8. $^{\#}p<0.001$ versus vehicle-treated control group (Student's t-test).

Spontaneous locomotor activity

The spontaneous locomotor activity of all experimental groups was similar (Fig. 7).

Lipid peroxidation

As shown in Fig. 8, no significant differences in the brain MDA levels were found between scopolamine- and saline-treated mice. Treatment with CE (100, 300, 1,000 or 1,500 mg/kg, p.o.) showed no significant effect on the brain MDA levels of scopolamine-treated mice (Fig. 8).

Discussion and Conclusion

In the present study, CE was evaluated in mice for its effect on learning and memory impairment induced by T2VO or an intraperitoneal injection of scopolamine. In line with previous report[16] that animal models of T2VO exhibited progressive spatial cognitive deficits, T2VO adversely affected learning and memory performances assessed by MWM and PA tests, the tests commonly used to screen for changes in learning and memory due to damage of the hippocampus[17]. The finding that T2VO produced no effect on spontaneous locomotor activity but significantly increased escape latencies in the MWM test and produced a decrease in initial step-down latency of PA test agrees well with the fact that hippocampus is extremely vulnerable to cerebral ischemia. Additionally,

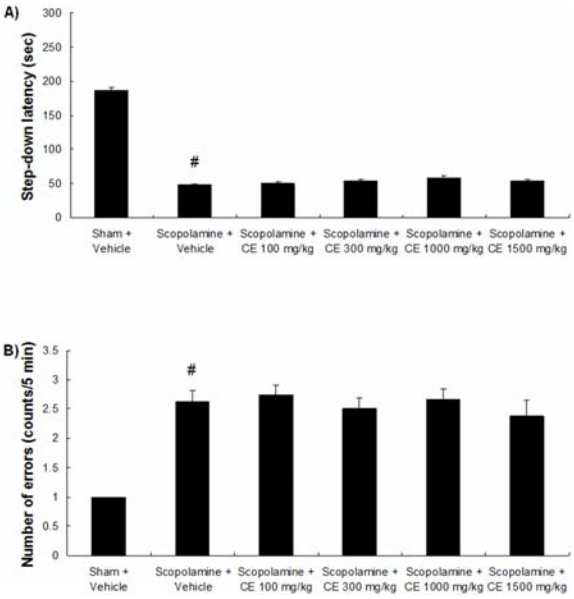

Fig. 6 Effects of CE on scopolamine-induced memory deficit as determined by the step-down passive avoidance test. Mice were orally treated with CE (100, 300, 1,000 and 1,500 mg/kg, p.o.) or vehicle (15% Tween 20) for 7 days. Final administration was performed 40 minutes before the test. Memory impairment was induced by scopolamine treatment (0.5 mg/kg, i.p.) 30 min before the test. Step-down latency (A) and number of error (B) were measured for 5 min. Each datum presents the mean ± SEM for n = 8. $^{\#}p<0.001$ versus vehicle-treated sham group (Student's t-test).

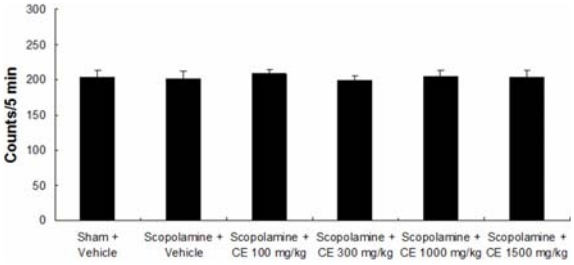

Fig. 7 Effects of CE on spontaneous locomotor activity of scopolamine-induced memory deficit mice. Mice were orally treated with CE (100, 300, 1,000 and 1,500 mg/kg, p.o.) or vehicle (15% Tween 20) for 8 days. Final administration was performed 40 minutes before the test. Memory impairment was induced by scopolamine treatment (0.5 mg/kg, i.p.) 30 min before the test. Locomotor activity was measured for 5 min. Each datum presents the mean ± SEM for n = 8. No significant differences were detected.

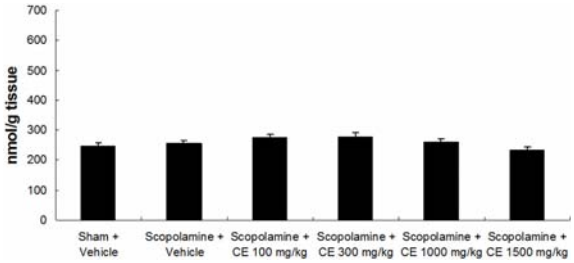

Fig. 8 Effects of CE on brain MDA levels of scopolamine-induced memory deficit mice. Following the locomotor activity test, the mice were sacrificed and the brains were collected. MDA level was determined using lipid peroxidation assay. Each datum presents the mean ± SEM for n = 8. No significant differences were detected.

the findings observed clearly indicated that poor performances in MWM and PA tests of T2VO group could not be explained by deficit or impairment of spontaneous locomotor activity. Furthermore, lipid peroxidation, one of the pathways underlying neuronal damage and cell death in Alzheimer's disease[18] was found to be significantly increased exclusively in T2VO model but not in scopolamine treated mice. It is therefore suggestive that impairment of learning and memory in T2VO model is likely liked to an increase of MDA.

As the level of brain lipid peroxide in Alzheimer's disease patient is higher than that of normal[19], MDA, marker of lipid peroxidation has been used as an index of oxidative stress in Alzheimer's disease. A growing number of studies demonstrate that natural extracts and phytochemicals have a positive impact on brain aging[2,20]. Moreover, clinical data suggest that nutritional antioxidants might exert some protective effect against Alzheimer's disease[21]. Oxidative stress is important in pathogenesis of Alzheimer's disease, in clinical trials, a potential antioxidant, idebenone, showed a clear dose-related anti-dementia activity in Alzheimer's disease[22]. With regards to natural antioxidant enzymes or agents which are capable of augmenting the functions of these enzymes, several reports have shown that the natural remedies possessing antioxidant property, for example *Ginkgo biloba*, *Celastrus paniculatus*, *Clitoria ternates*, *Lycoris radiate*, *Polygala tenuifoloa* and *Salvia miltiorrhiza* could be relevant to the treatment of Alzheimer's disease[2,20]. Accordingly, oral administration of CE at the doses of 100, 300, 1,000 and 1,500 mg/kg showed an improvement in learning and memory deficits as evidenced by decreased escape

latency in the MWM and increased initial step-down latency and decreased number of errors in the PA test. Furthermore, an increase of cerebral MDA induced by T2VO was significantly reduced but not totally abolished by the administration of CE suggesting that ameliorating effect of CE on impairment of learning and memory observed in behavioral study could be partly accounted by antioxidant property of CE. Though antioxidant properties of *C. asiatica* have been similarly proposed to underlie positive effect on learning and memory in normal rats[5,23] some other mechanisms possibly be involved and warranted further investigation.

Scopolamine is a tropane alkaloid drug with anticholinergic property, acting as a competitive antagonist at muscarinic cholinergic receptors. It has been shown to impair memory in humans and experimental animals to mimic the cognitive deficits found in Alzheimer's dementia[15]. Although pathogenesis of Alzheimer's disease remains to be fully defined, several pharmacological strategies for treatment of Alzheimer's disease are under active investigation. Cholinergic therapy that is designed to increase cholinergic functions and antioxidant are most attractive approach for treatment of Alzheimer's disease[24]. Tacrine is the first acetylcholine esterase inhibitor that has been approved for treatment of Alzheimer's disease[25]. In animal model, tacrine improved impairment of performance in the PA and the MWM tests[9,26]. Furthermore, tacrine produced an ameliorative effect on eight-arm radial maze test deficit caused by T2VO in rats, and raised the levels of extracellular acetylcholine in the brain[27].

In the present study, an intraperitoneal administration of scopolamine induced a deficit of learning and memory performances in MWM as well as in the PA tests. However, unlike T2VO model, impairment of learning and memory induced by scopolamine was not associated with an increase of cerebral MDA. Interestingly, CE (100, 300, 1,000 or 1,500 mg/kg, p.o.) which has been found to improve learning and memory deficit in T2VO showed no effect on scopolamine-induced learning and memory deficit. Cerebral MDA level was neither affected by scopolamine or CE. Rivastigmine, an anti-cholinestease drug currently used for Alzheimer's disease, has been reported that in parallel with the ability to inhibit cholinesterase in cortex and hippocampus, it could improve scopolamine-induced deficit in the MWM and the PA tests[28]. Therefore, the negative effect of CE on learning and memory function in mice receiving scopolamine suggests the lack of its effect on cholinergic system.

In conclusion, by means of MWM and the PA tests, the present study demonstrated the beneficial effects of CE on learning and memory impairment exclusively in T2VO mice but not that induced by scopolamine. Based on the finding that T2VO but not scopolamine significantly increased the lipid peroxidation which could be partly ameliorated by CE, therefore, it is suggestive that anti-oxidative property of CE could, at least partly, contributed to its positive effect on memory deficit in T2VO mice. This may explain also the lack of effect of CE on memory impairment induced by scopolamine in which oxidative stress was not its feature. Thus, CE might be beneficial for memory impairment in Alzheimer's disease which oxidative stress is an underlying cause. Further study is needed to identify the active components underlying positive effect of CE on learning and memory deficit associated with cerebral ischemia.

Acknowledgements

The authors wish to thank the Graduate School, Chulalongkorn University for financial support and Faculty of Pharmaceutical Sciences, Chulalongkorn University for providing facilities for this project.

Potential conflicts of interest

None.

References

1. Niranjan R. Molecular basis of etiological implications in Alzheimer's disease: focus on neuroinflammation. Mol Neurobiol 2013; 48: 412-28.
2. Wollen KA. Alzheimer's disease: the pros and cons of pharmaceutical, nutritional, botanical, and stimulatory therapies, with a discussion of treatment strategies from the perspective of patients and practitioners. Altern Med Rev 2010; 15: 223-44.
3. Shinomol GK, Muralidhara, Bharath MM. Exploring the role of "Brahmi" (Bocopa monnieri and Centella asiatica) in brain function and therapy. Recent Pat Endocr Metab Immune Drug Discov 2011; 5: 33-49.
4. Veerendra Kumar MH, Gupta YK. Effect of different extracts of Centella asiatica on cognition and markers of oxidative stress in rats. J Ethnopharmacol 2002; 79: 253-60.
5. Rao MK, Rao MS, Rao GS. Treatment with Centalla

asiatica (Linn) fresh leaf extract enhances learning ability and memory retention power in rats. Neurosciences (Riyadh) 2007; 12: 236-41.
6. Orhan IE. Centella asiatica (L.) Urban: From Traditional Medicine to Modern Medicine with Neuroprotective Potential. Evid Based Complement Alternat Med 2012; 2012: 946259.
7. Inamdar PK, Yeole RD, Ghogare AB, de Souza NJ. Determination of biologically active constituents in Centella asiatica. J Chromatogr A 1996; 742: 127-30.
8. Tang CT, Belani LK, Das S, Jaafar MZ. Treatment of dementia with herbs: a short review. Clin Ter 2013; 164: 43-6.
9. Xu J, Murakami Y, Matsumoto K, Tohda M, Watanabe H, Zhang S, et al. Protective effect of Oren-gedoku-to (Huang-Lian-Jie-Du-Tang) against impairment of learning and memory induced by transient cerebral ischemia in mice. J Ethnopharmacol 2000; 73: 405-13.
10. Bejar C, Wang RH, Weinstock M. Effect of rivastigmine on scopolamine-induced memory impairment in rats. Eur J Pharmacol 1999; 383: 231-40.
11. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984; 11: 47-60.
12. Andersen JM, Lindberg V, Myhrer T. Effects of scopolamine and D-cycloserine on non-spatial reference memory in rats. Behav Brain Res 2002; 129: 211-6.
13. Gupta YK, Veerendra Kumar MH, Srivastava AK. Effect of Centella asiatica on pentylenetetrazole-induced kindling, cognition and oxidative stress in rats. Pharmacol Biochem Behav 2003; 74: 579-85.
14. Morris RGM. Spatial localization does not require the presence of local cues. Learn Motiv 1981; 12: 239-60.
15. Molchan SE, Martinez RA, Hill JL, Weingartner HJ, Thompson K, Vitiello B, et al. Increased cognitive sensitivity to scopolamine with age and a perspective on the scopolamine model. Brain Res Brain Res Rev 1992; 17: 215-26.
16. Ji HJ, Hu JF, Wang YH, Chen XY, Zhou R, Chen NH. Osthole improves chronic cerebral hypo-perfusion induced cognitive deficits and neuronal damage in hippocampus. Eur J Pharmacol 2010; 636: 96-101.
17. Sharma S, Rakoczy S, Brown-Borg H. Assessment of spatial memory in mice. Life Sci 2010; 87: 521-36.
18. Aliev G, Palacios HH, Walrafen B, Lipsitt AE, Obrenovich ME, Morales L. Brain mitochondria as a primary target in the development of treatment strategies for Alzheimer disease. Int J Biochem Cell Biol 2009; 41: 1989-2004.
19. Dumont M, Lin MT, Beal MF. Mitochondria and antioxidant targeted therapeutic strategies for Alzheimer's disease. J Alzheimers Dis 2010; 20 (Suppl 2): S633-43.
20. Kim J, Lee HJ, Lee KW. Naturally occurring phytochemicals for the prevention of Alzheimer's disease. J Neurochem 2010; 112: 1415-30.
21. Pratico D. Oxidative stress hypothesis in Alzheimer's disease: a reappraisal. Trends Pharmacol Sci 2008; 29: 609-15.
22. Yamazaki N, Kiyota Y, Take Y, Miyamoto M, Nagawa Y, Nagaoka A. Effects of idebenone on memory impairment induced in ischemic and embolization models of cerebrovascular disturbance in rats. Arch Gerontol Geriatr 1989; 8: 213-24.
23. Hsieh MT, Peng WH, Wu CR, Ng KY, Cheng CL, Xu HX. Review on experimental research of herbal medicines with anti-amnesic activity. Planta Med 2010; 76: 203-17.
24. Hansen RA, Gartlehner G, Webb AP, Morgan LC, Moore CG, Jonas DE. Efficacy and safety of donepezil, galantamine, and rivastigmine for the treatment of Alzheimer's disease: a systematic review and meta-analysis. Clin Interv Aging 2008; 3: 211-25.
25. Summers WK. Tacrine, and Alzheimer's treatments. J Alzheimers Dis 2006; 9: 439-45.
26. Kim DH, Yoon BH, Kim YW, Lee S, Shin BY, Jung JW, et al. The seed extract of Cassia obtusifolia ameliorates learning and memory impairments induced by scopolamine or transient cerebral hypoperfusion in mice. J Pharmacol Sci 2007; 105: 82-93.
27. Murakami Y, Ikenoya M, Matsumoto K, Li H, Watanabe H. Ameliorative effect of tacrine on spatial memory deficit in chronic two-vessel occluded rats is reversible and mediated by muscarinic M1 receptor stimulation. Behav Brain Res 2000; 109: 83-90.
28. Buccafusco JJ. The revival of scopolamine reversal for the assessment of cognition-enhancing drugs. In: Buccafusco JJ, editor. Methods of behavior analysis in neuroscience. 2nd ed. Boca Raton, FL: CRC Press; 2009: Chapter 17 [1-13].

การศึกษาผลลดความบกพร่องในการเรียนรู้และความจำของสารสกัดเอทานอลจากบัวบกในโมเดลสัตว์ทดลอง

เสาวลักษณ์ ดอกนาค, สลิล มิ่งมาลัยรักษ์, อนุสรา วัฒนจันทร์, บุญยงค์ ตันติสิระ, มยุรี ตันติสิระ

วัตถุประสงค์: งานวิจัยนี้ศึกษาผลของสารสกัดเอทานอลจากบัวบก ต่อภาวะบกพร่องในการเรียนรู้และความจำในหนูเมาส์ที่ถูกปิดกั้นหลอดเลือดคอมมอนคาโรติดทั้ง 2 ข้างแบบชั่วคราวหรือทีทูวีโอหรือได้รับสารสะโคโพลามีนโดยการฉีดเข้าทางช่องท้อง

วัสดุและวิธีการ: สารสกัดเอทานอลจากบัวบกขนาด 100, 300, 1,000 หรือ 1,500 มิลลิกรัมต่อน้ำหนักตัวสัตว์ทดลอง 1 กิโลกรัมถูกป้อนทางปากให้สัตว์ทดลองที่มีภาวะบกพร่องในการเรียนรู้และความจำ วันละครั้งติดต่อกันทุกวันเป็นเวลา 8 วัน ภาวะบกพร่องในการเรียนรู้และความจำของสัตว์ทดลองถูกทดสอบด้วยวิธีมอรีสวอเตอร์เมสและสะเต็บดาวน์ การเปลี่ยนแปลงระดับมาลอนไดอัลดีไฮในสมองของสัตว์ทดลองจะถูกทดสอบด้วยวิธีลิปิดเปอร์ออกซิเดชัน

ผลการศึกษา: สารสกัดเอทานอลจากบัวบกมีผลลดภาวะบกพร่องในการเรียนรู้และความจำของหนูเมาส์ในโมเดลทีทูวีโอ เมื่อทดสอบด้วยวิธีมอรีสวอเตอร์เมสและสะเต็บดาวน์ และลดระดับมาลอนไดอัลดีไฮด์ที่ถูกเหนี่ยวนำให้เพิ่มขึ้นในสมองหนูเมาส์ที่หลอดเลือดถูกปิดกั้นชั่วคราว ในทางตรงกันข้ามพบว่า สารทดสอบไม่สามารถลดภาวะบกพร่องในการเรียนรู้และความจำที่ถูกเหนี่ยวนำให้เกิดขึ้นโดยสารสะโคโพลามีน

สรุป: การศึกษานี้แสดงให้เห็นถึงผลลดภาวะบกพร่องในการเรียนรู้และความจำของสารสกัดเอทานอลจากบัวบก ในหนูเมาส์ที่หลอดเลือดถูกปิดกั้นชั่วคราว และมีความเป็นไปได้ว่าผลดังกล่าวนั้น อย่างน้อยที่สุดน่าจะมีส่วนหนึ่ง ซึ่งเป็นผลจากคุณสมบัติต้านออกซิเดชัน ดังนั้นจึงอาจนำเอาบัวบกมาใช้ประโยชน์สำหรับภาวะบกพร่องในการเรียนรู้และความจำที่เกี่ยวข้องกับออกซิเดทีพสเตรท