Ameliorative Effect of *Apium graveolens* L. on Scopolamine-Induced Amnesia Mice

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Abstract

Introduction: *Apium graveolens* L. has been claimed to possess therapeutic potential for central nervous system disorders. Thus, the present study aimed to evaluate its effect on scopolamine-induced memory impairment in mice **Methods:** Forty-eight C57BL/6 mice were subjected to Morris water maze and object recognition test in order to determine the spatial and non-spatial memory. Additionally, the brain acetylcholinesterase (AChE) activities were also quantified. Aricept was used as a positive control. **Results:** Oral administration of *Apium graveolens* L. extract (125, 250, 375 and 500 mg/kg BW for consecutive 28 days) significantly decreased the escape latency time but increased the discrimination index when compared to vehicle ($p<0.001$) and aricept-treated groups ($p<0.05$). Furthermore, the AChE activities in cerebral cortex and hippocampus significantly decreased in animals receiving 125, 250, 375 and 500 mg/kg BW of *Apium graveolens* L. extract ($p<0.001$) when compare to vehicle treated group and show a level similar to those observed in aricept treated group. **Conclusion:** These result suggested that *Apium graveolens* L. has beneficial effects against scopolamine-induced amnesia by regulating cholinergic system and promoting memory enhancement via decreased activity of AChE.

Keywords: *Apium graveolens* L., Scopolamine, Morris water maze, Object recognition test, AChE activity

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1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder (Scatena *et al*., 2007) which is clinically characterized by a slowly progressive deterioration of memory as well as other higher cognitive dysfunctions. Previous evidence suggested the loss of cholinergic neurons associated with the severity of memory loss and cognitive impairment in AD patients as well as aging population (Terry and Buccafusco, 2003).

Nowadays, several Acetylcholinesterase (AChE) inhibitors such as aricept, reminyl, cognex have been approved for AD treatment to improve memory, behavior and mood (Lahiri *et al*., 2003). However, the side effects of these drugs including muscular cramps, insomnia, diarrhea, nausea, and bronchitis (Doody *et al*., 2001) have made their limited of usage. Hence, traditional herbal medicine has drawn considerable attentions on drug discovery from botanical sources.

Apium graveolens L. (*A. graveolens* L.) has been observed wide range of biological activities including anti-hypertension (Zhang et al., 2012; Popoviç et al., 2010; Tsi and Tan, 1997; Ko *et al*., 1991), anti-cancer (Lin *et al*., 2008; Patel et al., 2007; Gusman *et al*., 2001), antimi crobial effect (Misic *et al*., 2008; Momin and Nair, 2001), antioxidative activity (Iswantini *et al*., 2012; Yao and Ren, 2011; Jain *et al*., 2009; Wei and Shibamoto, 2007; Popovic *et al*., 2006), anti-inflammatory activity (Feng *et al*., 2012; Ovodora *et al*., 2009; Momin and Nair, 2002; Al-Hindawi *et al*., 1989), and AChE inhibitory activity (Szwajgier and Borowiec, 2012; Gholamhoseinian *et al*., 2009).

Scopolamine is an antagonist at mus carinic acetylcholine receptors causing cognitive and short-term memory impairment. Scopolamine-treated animal is one of a well-known pharmaco logical animal model for the cholinergic hypoth esis of cognitive dysfunction (Wang *et al.,* 2010; Ahmed and Gilani, 2009; Sun *et al*., 2007; Petersen, 1977).

To date, there has been no report on the effect of *A. graveolens* L. can target AChE to prevent memory impairments that explained by this animal model. In the present study, we examined the effect of crude extracts of *A. graveolens* L. on scopolamine induced memory disruption in C57BL/6 mice, and its anti-AChE activity in order to elucidate its therapeutic potential for AD.

2. Materials and Methods

2.1 Animals treatments

Adult male (8 weeks old) C57BL/6 mice, weight approximate 25-30 grams were used as experimental model. They were obtained from National Laboratory Animal Center, Mahidol University, Nakorn Pathom, Thailand and supplied with standard protocol from Southern animal unit, Prince of Songkla University, Songkhla, Thailand. They were randomly housed 5 per cage and maintained in a clean room at a temperature between 23 to 27 °C, with a 12 h light-dark cycle and a relative humidity of 50%. Mice were housed in metabolic cages under the supply of filtered pathogen-free air with access to food and water ad libitum. All injections in this study were performed once daily between 8.00-9.00 a.m. The experimental procedures have been performed in accordance with the principles of animal care outlined by Faculty of Science, Prince of Songkla University, Songkhla, Thailand (MOE0521.11/582).

2.2 Drugs and chemicals

Scopolamine has been purchased from Sigma-Aldrich (St Louis, Mo, USA). Donepezil hydrochloride (Aricept 5 mg/tablet) (Pfizer, NY, USA) was purchased and used as standard drugs.

2.3 Preparation of *A. graveolens* L. crude extract

A. graveolens L. has been purchased from Lampang Herb Conservation, Lampang, Thailand. Whole part of the plant was deposited at the Forest Herbarium, Bangkok, Thailand (BKF number 188856) and prepared as methanolic extract by Dr. Wanida Sukketsiri, Department of Pharmacology, Faculty of Science, Prince of Songkla University, Thailand. The dried powder was extracted with 70% methanol in ratio of 1:10 for 72 h. The methanolic fraction was filtered by filtrated paper no.1, dried under vacuum rotary evaporator and lyophilized with freeze dryer. The *A. graveolens* L. methanolic extract was kept in container protected from light and stored at 4°C until use. *A. graveolens* L. extract was freshly prepared everyday by dissolved in distill water in order to obtain the desired concentration before a once-daily oral administration.

2.4 Experimental protocol

Eight groups of animal were employed in this study.

Group I: Normal control group received no treatment.

Group II: Vehicle control group received distill water once daily via oral route for 4 weeks.

Group III: Mice were treated with aricept (a cholinesterase inhibitor : positive control)

Group IV – VIII: Mice received *A. graveolens* L. at various doses ranging from 65, 125, 250, 375 and 500 mg/kg BW once daily for 4 weeks.

The animals in group II-VIII were intra peritoneal injected by scopolamine, a reversible competitive antagonist at specifically muscarinic acetylcholine receptors type 1 (3 mg/kg BW) within 30 min after the last dose of administration. Then, they were determined the cognitive function using Morris water maze and object recognition test.

2.5 Morris water maze test

In order to determine higher cognitive functions such as spatial memory which is believed to be involved to the hippocampal function, one of the most common behavioral paradigms for evaluating the rodents is Morris water maze test. Animals were examined in a round polyvinyl water pool (120 cm diameter, 50 cm height) filled with water (25 °C). Additional of powder was used to provide the water opaque. The pool was divided equally into four quadrants: labeled N-S-E-W. A platform (10 cm diameter) was placed in one of the four quadrants (the target quadrant) and submerged 1.5 cm below the water surface. For animals, the location of the platform was invisible and it remained there throughout the training. The animals must memorize the environ mental cues to locate the platform. Each animal was placed in the water in the starting quadrant and allowed to freely swim in the pool for 60 sec or until it found and climbed onto the platform. During training session, the mice were gently placed on the platform by experimenter when it could not reach the platform in 60 sec. In either case, the subject was left on the platform for 30 sec and removed from the pool. The time for

animals to climb on the hidden platform was recorded as escape latency or acquisition time. In each trial, the animal was quickly dried with towel before being returned to the cage. Acquisition time in Morris water maze tests were carried out within 30 min after administration of vehicle or *A. graveolens* L. or aricept which served as positive control (Morris, 1984).

2.6 Object recognition test

The object recognition test was performed to determine non-spatial memory of shape, color and texture. The apparatus consisted of a circular arena with 100 cm in diameter and 40 cm high wall. The open field and the objects were cleaned between each trial using 70% ethanol to avoid odor trails. Before the experiment day, the animals were allowed to acclimatize to the experimental environment. During habituation, the animals were allowed to freely explore the apparatus without objects for 5 min, once a day for three consecutive days before testing. On the experimental day, animals were submitted to two trials spaced. During the first trial (T1), animals were placed in the area containing the same two identical objects for an amount of time necessary to spend 15 sec exploring these two objects in a limit of 3 min. Any mice which did not explore the objects for 15 sec within the 3 min period were excluded from experiments. 1 h after exposing to the first trial, the animal was exposed to the second trial (T2). According to this trial, one of the objects presented in the first trial was replaced by a novel object. Animals were

placed back in the arena for 3 min, the total times which the animals spent to explore or directed the nose within 2 cm of the object while looking at, sniffing, or touching the novel object were recorded and recognized as total exploration time upon novel object or time of approach to both objects were recorded and calculated according to the equation (Antunes and Biala, 2012).

Discrimination index = $\dfrac{\text{(Time of approach to object 2-Time of approach to object 1)}}{\text{(Time of approach to object 2+Time of approach to object 1)}}$

2.7 The AChE assay in hippocampus and cerebral cortex

The activity of AChE was measured according to a method developed by Ellman et al (Ellman et al., 1961). This method employs acetylthiocholine iodide (ATChI) as a synthetic substrate for AChE. ATChI is broken down to thiocholine and acetate by AChE and thiocholine is reacted with dithiobisnitrobenzoate (DTNB) to produce a yellow color. The quantitative measurement of AChE activity has been determined by yellow color development using a spectrophotometer. The data were expressed as μ moles hydrolyzed per min per g of tissue and compared with other groups.

2.8 The statistical analysis

All data were expressed as mean ± SD and were analyzed by ANOVA (Turkey's post hoc test). A probability level less than 0.05 were accepted as significance.

3. Results

3.1 Effect of *A. graveolens* L. on spatial memory

Figure 1 showed the vehicle administration with scopolamine significantly prolong escape latency time than control group suggesting the spatial memory deficit. Co-administration of *A. graveolens* L. with scopolamine from 125, 250, 375 and 500 mg/kg BW could reverse memory deficit condition particularly 250 mg/kg BW. However, our data did not show a dose-dependent effect of *A. graveolens* L.
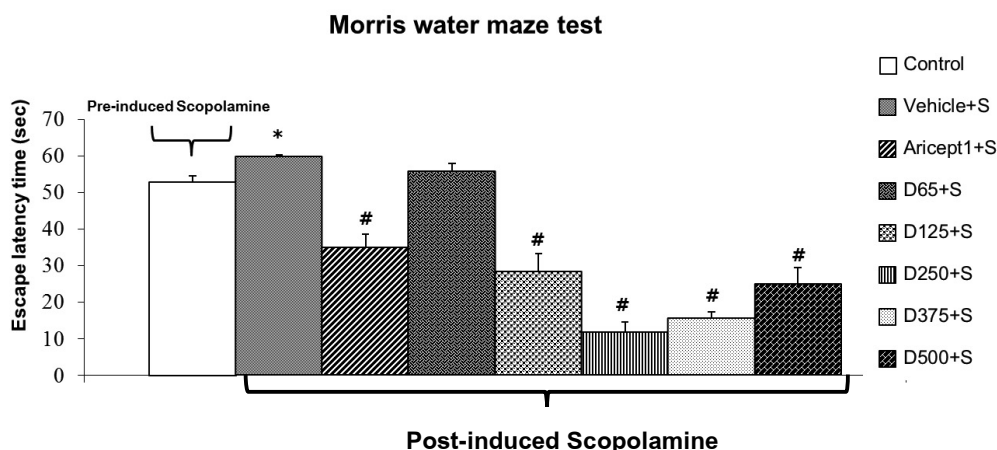
Morris water maze test

Figure 1 Effect of *A. graveolens* L. on escape latency time of mice subjected to Morris water maze test. Control: pre-induced scopolamine control group, Vehicle+S: a scopolamine-treated vehicle group, Aricept+S: 1 mg/kg BW aricept plus scopolamine-treated group, D65+S: 65 mg/kg BW *A. graveolens* L. plus scopolamine-treated group, D125+S: 125 mg/kg BW *A. graveolens* L. plus scopolamine-treated group, D250+S: 250 mg/kg BW *A. graveolens* L. plus scopolamine-treated group, D375+S: 375 mg/kg BW *A. graveolens* L. plus scopolamine-treated group, D500+S: 500 mg/kg BW *A. graveolens* L. plus scopolamine-treated group. (n=8 each, $*p<0.05$ compared with control group; $\#p<0.001$ compared with Vehicle+S treated group).

3.2 Effect of *A. graveolens* L. on non-spatial memory

During the experimental period, vehicle treated group plus scopolamine revealed prominent decrease in the discrimination index indicating memory deficit. Co-administration of *A. graveolens* L. (125, 250, 375 and 500 mg/kg BW) with scopolamine significantly increased the discrimination index especially 250 mg/kg BW. Interestingly, *A. graveolens* L. show significant difference higher than aricept-treated group as depicted in Figure 2.
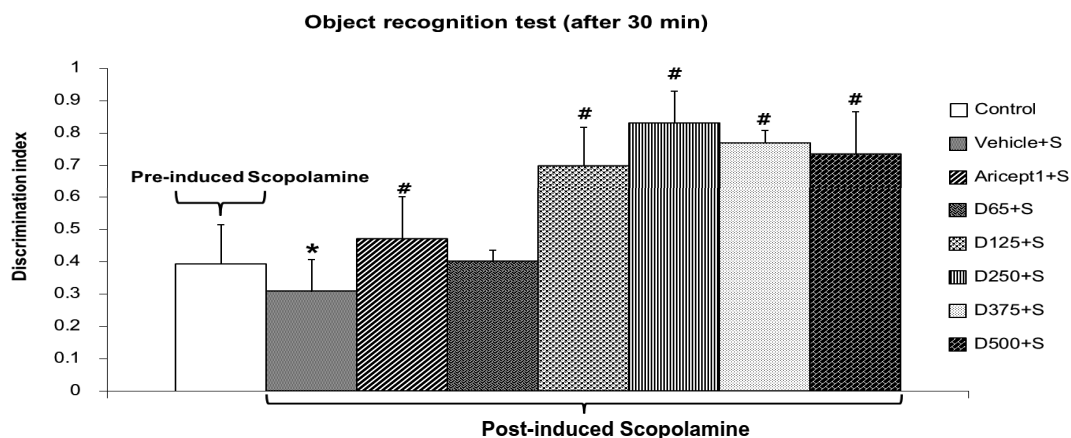
Object recognition test (after 30 min)

Figure 2   Effect of *A. graveolens* L. on discrimination index of mice subjected to object recognition test. Control: pre-induced scopolamine control group, Vehicle+S: a sccopolamine-treated vehicle group, Aricept+S: 1 mg/kg BW aricept plus scopolamine-treated group, D65+S: 65 mg/kg BW *A. graveolens* L. plus scopolamine-treated group, D125+S: 125 mg/kg BW *A. graveolens* L. plus scopolamine-treated group, D250+S: 250 mg/kg BW *A. graveolens* L. plus scopolamine-treated group, D375+S: 375 mg/kg BW *A. graveolens* L. plus scopolamine-treated group, D500+S: 500 mg/kg BW *A. graveolens* L. plus scopolamine-treated group. (n=8 each, *$p<0.05$ compared with control group; #$p<0.001$ compared with Vehicle+S treated group).

3.3 Effect of *A. graveolens* L. on the alteration of AChE activity

In the present study, we examined the AChE activity in hippocampus and cerebral cortex isolated from mice brains to ensure whether the efficacy of *A. graveolens* L. in behavioral test (Morris water maze and object recognition test) is related to AChE alteration. The AChE activity in hippocampus (Figure 3) and cerebral cortex (Figure 4) was remarkably decrease in aricept (1 mg/kg BW) and/or *A. graveolens* L. (125, 250, 375, 500 mg/kg BW) treated animals ($p<0.001$). Interestingly, *A. graveolens* L. at dose of 250 mg/kg BW treatment showed significant decreased AChE activity than positive control drug in both brain regions ($p<0.001$).
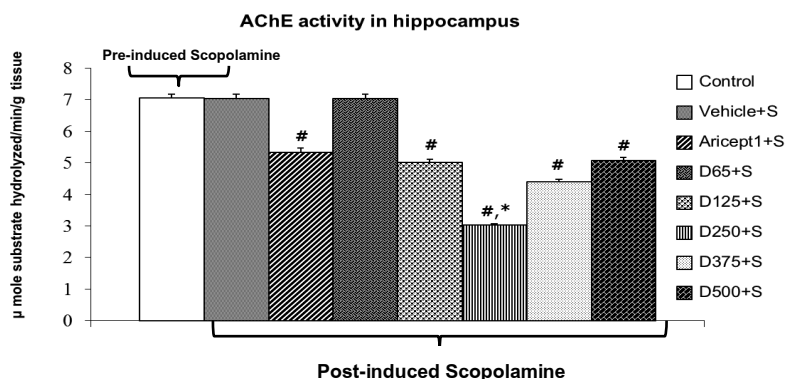
Figure 3 Effect of *A. graveolens* L. on AChE enzyme activity in hippocampus treated with scopo-
lamine: Control: a pre-induced scopolamine control group, Vehicle+S: a scopolamine-
treated vehicle group, Aricept+S: 1 mg/kg BW aricept plus scopolamine-treated group,
D65+S: 65 mg/kg BW *A. graveolens* L. plus scopolamine-treated group, D125+S: 125 mg/
kg BW *A. graveolens* L. plus scopolamine-treated group, D250+S: 250 mg/kg BW A. gra-
veolens L. plus scopolamine-treated group, D375+S: 375 mg/kg BW *A. graveolens* L. plus
scopolamine-treated group, D500+S: 500 mg/kg BW *A. graveolens* L. plus scopolamine-
treated group. (n=8 each, *$p<0.001$ compared with Aricept+S treated group; #$p<0.001$
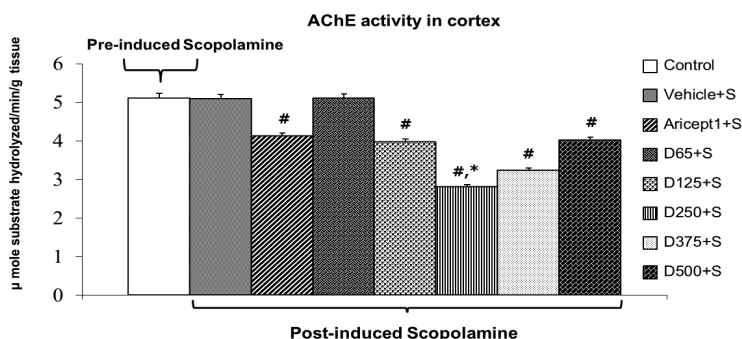compared with Vehicle+S treated group).

Figure 4. Effect of *A. graveolens* L. on AChE enzyme activity in cerebral cortex treated with scopo-
lamine: Control: pre-induced sccopolamine control group, Vehicle+S: a scopolamine-
treated vehicle group, Aricept+S: 1 mg/kg BW aricept plus scopolamine-treated group,
D65+S: 65 mg/kg BW *A. graveolens* L. plus scopolamine-treated group, D125+S: 125 mg/
kg BW *A. graveolens* L. plus scopolamine-treated group, D250+S: 250 mg/kg BW *A. gra-
veolens* L. plus scopolamine-treated group, D375+S: 375 mg/kg BW *A. graveolens* L. plus
scopolamine-treated group, D500+S: 500 mg/kg BW *A. graveolens* L. plus scopolamine-
treated group. (n=8 each, *$p<0.001$ compared with Aricept+S treated group; #$p<0.001$
compared with Vehicle+S treated group).

4. Discussion

The present study further examined the effects of *A. graveolens* L. on memory deficit of AD model using scopolamine. The scopolamine -treated mice were examined for cognitive function using Morris water maze and object recognition test. The vehicle plus scopolamine (3 mg/kg BW, i.p.) group displayed significant increase of the escape latency time ($p<0.001$) and decrease discrimination index ($p<0.001$) indicating successful scopolamine-induced memory impairment. Aricept, a member of AChE inhibitors, has been used as a standard drug for AD treatment to reverse cognitive deficit and used as a positive control in this study (Čolović *et al*., 2013; Martin and Jeffrey, 2007). *A. graveolens* L. at dose of 125, 250, 375 and 500 mg/kg BW demonstrated the ameliorative effects against scopolamine-induced memory impairment in C57BL/6 mouse model. The *A. graveolens* L. treated mice spent shorter escape latency time but higher discrimination index than vehicle plus scopolamine groups. Apparently, the mice received 250 mg/kg BW *A. graveolens* L. exhibited maximal effect when compared to the others whereas the mice received 375 and 500 mg/kg BW *A. graveolens* L. decreased neuroprotective effect than 250 mg/ kg BW. This finding is probably due to the reduction of gastrointestinal absorption which is consistent with Al-Howiriny and Baananou found that high concentration of celery extract can decrease the function of gastrointertinal tract (Baananou *et al*., 2012; Al-Howiriny *et al*., 2010). In addition, previous study reported that the ethanolic extract of *A. graveolens* L. exerted gastroprotective effect. Its

stem and leaves extract at concentration of 500 mg/kg BW displayed gastric ulcer prevention by the inhibition of gastric acid secretion and ultimately protect gastric mucosa (Al-Howiriny *et al*., 2010). Furthermore, the volatile oil of celery seeds at concentration of 300 mg/kg BW can also prevent peptic ulcer in rat by decreased gastric secretion and acidic condition in stomach (Baananou *et al*., 2012).

In the present work, *A. graveolens* L. significantly reversed memory impairment in scopolamine-treated mice implying that it may improve cognitive function. Previous research showed that *A. graveolens* L. seeds, stems and leaves containing the pure compound of L-3-n-butylphthalide (L-NBP) ameliorated cognitive impairment, promoted long-term spatial memory, decreased amyloid-β and regulated amyloid precursor protein (APP) production in transgenic AD mice (Peng *et al.*, 2010).

We determined the effect of *A. graveolens* L. on brain AChE activities. In the *A. graveolens* L. at dose of 125, 250, 375 and 500 mg/kg BW treated group demonstrated a significant decreased AChE activity. Concurrently the 250 mg/kg BW *A. graveolens* L. administration denoted maximal responsiveness in accordance with the behavioral tests. The brain AChE activities were directly associated with duration of escape latency time implies that a higher AChE activity causing an increased in escape latency time. From these results, we found that the improvement of memory deficit by *A. graveolens* L. is presumably due to its inhibiting effect on AChE activity in the cerebral cortex and hippocampus. This finding

agrees well with Dhingra and Kumar (2012) reported the relationship between AChE activity and escape latency of Morris water maze test (Dhingra and Kumar, 2012). In addition, The water extract of celery roots can inhibit AChE enzyme *in vitro* study (Swajgier and Borowiec, 2012). Attempt has been made on the methanolic extract of celery stems reported to prevent amyloid-β induced neuronal PC12 cells death (Park *et al*., 2009).

5. Conclusion

In conclusion, based on the results of Morris water maze, object recognition tests and the AChE activities in hippocampus and cerebral cortex play a key role in memory and learning function using amnesia mice induced by scop olamine. We found that *A. graveolens* L. at dose of 250 mg/kg BW had remarkable cognitive-enhancing effect. The natural product as *A. graveolens* L. crude extract exerted anti-amnesic function *in vivo* might offer a useful alternative treatment for AD.

Acknowledgement

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