

SCREENING OF THE CENTRAL NERVOUS SYSTEM ACTION OF AGARWOOD LEAVES EXTRACT IN FEMALE OVARIECTOMIZED RATS

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Abstract

Agarwood leaves tea has been consumed among people in the Southeast Asia and Oceania countries as herbal beverage for sedative, antinociceptive, antihyperglycemic and laxative purposes. Since studies have shown that agarwood leaves had mild depressant and antioxidant actions. We, therefore, interested in screening the actions of *Aquilaria subintegra* (AS) leaves extract on the central nervous system (CNS) function and learning and memory in an animal model of Alzheimer's disease. Sixty aged female rats were used. Fifty rats were subjected to bilateral ovariectomy (OVX) and ten rats were served as control sham. These rats were divided into 5 groups as 1) control (OVX+vehicle), 2) sham (non-ovariectomized), 3) OVX + AS 100 mg/kg, 4) OVX + AS 1000 mg/kg, and 5) OVX + diazepam 2.5 mg/kg. After a single dose administration, rats were subjected to behavioral tests. We found that the AS-treated groups exhibited 1) a reduction in spontaneous locomotion, 2) an increase of anxiolytic index, 3) an increase of nociceptive threshold, and 4) marked potentiation of thiopental induced anesthesia. Additionally, rats received AS leaves extract for 2 months showed a significant improvement in both object recognition and spatial memories when compared to the control group. Altogether, the results suggest that agarwood leaves extract has the potential to be developed as herbal beverage and may be used for reducing stress and anxiety, and for the treatment of mild cognitive deficits. However, further investigation is warranted to uncover the mechanism(s) of action of this herbal extract and its bioactive compound(s).

Keywords: agarwood leaves extract, CNS screening, learning and memory

Introduction :

Agarwood (*Thymelaeaceae* family) is an incense tree of *Aquilaria* species which the wood can be used to make incense sticks (cones) and sachets, and the resinous part of the wood can be distilled to produce fragrant volatile oil. In Thailand, agarwood is commonly known as "Mai Krissana" and there are at least four species of *Aquilaria* trees (*A. malaccensis*, *A. subintegra*, *A. crassna*, and *A. sinensis*) that have been cultivated in several provinces, i.e., Trad, Chanthaburi, Nakhon Nayok, and Narathivas, etc. The extract of agarwood oil or wood has been widely used a sedative, analgesic, and digestive herbal medicine over hundreds of years in the Southeast Asia and Oceania for centuries, especially in China, Vietnam, Indonesia, Japan and Thailand, etc. (Okugawa et al., 1992, Yagura et al., 2003, Hashim et al.,

2016). However, the information regarding the pharmacological effects of volatile oil and crude extracts of agarwood has been shown over the past two decades

In 1996, Okugawa and his colleagues have demonstrated that the benzene crude extract of *A. malaccensis* wood has a sedative effect on spontaneous locomotor activity, prolong barbiturate-induced sleeping, decreased rectal temperature and reduce the number of acetic acid-induced writhing in mice (Okugawa et al., 1996). They also conducted a pharmacological study on active compounds, jinkoh-eremol and agarospirol, extracted from *A. malaccensis* wood and found that these two compounds could reduce methamphetamine- and apomorphine-induced locomotor activity in mice. Single oral administration of *A. sinensis* leaves extract at a dose of 848 mg/kg showed the increasing analgesic activity on hotplate analgesic test and reducing inflammation activity on xylene or carrageenan-induced edema, and carboxymethylcellulose sodium-induced leukocyte migration in mice (Zhou et al., 2008). For the *in vivo* study of agarwood oil, inhalation of agarwood oil vapor could sedate mice by decreasing their spontaneous locomotor activities (Takemoto et al., 2008). Moreover, the active ingredient of *A. malaccensis* leaves, 4'-hydroxyacetanilide, has been used as acetaminophen for treating analgesic and antipyretic (Afiffudden et al., 2015).

Agarwood leaves have also been demonstrated to enhance the cognitive function. Ethanolic extract of Vietnamese agarwood leaves, showed significant induction of brain-derived neurotrophic factor (BDNF) mRNA expression in rat's cultured cortical neurons (Ueda et al., 2006). A recent *in vitro* study by Ray and colleagues (2014) reported a high antioxidant activity of *A. subintegra* leaves ethanolic extract when compared to vitamin C (Ray et al., 2014). In addition, *A. subintegra* leaves extract showed an AChE inhibitory activity *in vitro* and could reverse valium-induced cognitive impairment mice (Bahrani et al., 2014). Although several studies have been demonstrated the biological effects of *Aquilaria* species, but the screening of general CNS action as well as cognitive enhancing property of *A. subintegra* leaves extract has yet not been fully investigated. Thus, the objectives of the present study were to screen the pharmacological action of *A. subintegra* leaves extract on general CNS function as well as to test the cognitive enhancing action of this crude extract on ovariectomized aging rats which were animal models of amnesia.

Methodology :

Agarwood leaves extract

A. subintegra leaves extract was prepared at the Bioscreening Unit, Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University. In brief, fresh young leaves of *A. subintegra* were collected from an agarwood garden in Bang-Kra-Thum district, Phitsanulok, Thailand. These leaves were dried in a hot-air oven (60 °C) for 48 hr and then ground to dried powder. The dried powder was macerated in hot water (95-100 °C) at the ratio of 100 g: 1 L for 30 minutes, then sieved through cheesecloth. The filtrate was centrifuged and the supernatant was lyophilized for 72 hr. The percent yield of AS crude extract obtained from the dried leaves was ranging between 9.48-22.21%. By using a HPLC analysis, we found that each lot of *A. subintegra* leaves extracts contained mangiferin between 8.81-13.10 % (w/w).

Experimental animals

An animal protocol presented herein had been approved by the board of Naresuan University Animal Care and Use Committee (NUACUC) (Approval number: 56040030). Sixty retired female Sprague Dawley (SD) rats aging 8 months were ordered from National Laboratory Animal Center (NLAC), Mahidol University, Nakhornprathom, Thailand. The animals were housed, two-three rats per a cage, in an animal room of Naresuan University Center for

Animal Research (NUCAR) which have received AAALAC accreditation. The animal holding room was maintained at constant temperature of 22 ± 1.0 °C, relative humidity of 45-65%, and a reverse dark-light cycle (light on 11:00 p.m.-11:00 a.m. and light off 11:00 a.m.-11:00 p.m.). These animals had free access to food and water (food pellet formula G82, CPF PCL, Thailand) in their home cages.

At the age of 12 months, these female rats were subjected to a bilateral ovariectomy surgery and were allowed to recover from the surgery for at least two weeks before any behavioral testing. The screening of effects of AS crude extract on the CNS were conducted in 2 set of experiments. The first set of experiments was aimed to screen an acute effect of AS crude extract. This set of behavior tests consisted of 1) locomotor activity test, 2) elevated plus maze test, 3) hotplate analgesic test, 4) tail-flick analgesic test, and 5) barbiturate potentiation test, respectively. The second set of experiments was aimed to screen the effect of AS crude extract when given daily for 2 months on learning and memory. This set of experiments consisted of 1) a novel object recognition (NOR) test and 2) Morris water maze (MWM) test.

Locomotor activity test

The open field setup consists of an empty circular tank (150 cm diameter, 45 cm height) and a webcam (model C6150, Logitech, USA) connected to a PC computer equipped with a video tracking software (Anymaze version 4.7, Stoelting Co., USA). On the test day, each animal was gently placed in the middle of the floor and allowed to explore the tank for 10 min. After that, animal was administered with vehicle, diazepam, or AS crude extract. Then, the animal was put back in the tank and allowed to explore for another 60 minutes. After finishing each test session, the animal was removed to its home cage and the tank floor was cleaned using 70% alcohol. The parameters such as total distance and immobile time were obtained from Anymaze software.

Elevated plus maze test

The maze used in this study was made of wood, consisted of 2 open arms and 2 close arms which had three side walls. This maze was elevated 50 cm from the floor and had a webcam above the maze. The animal was allowed to acclimatize to the maze for 5 min and the data obtained from this period was recorded as a pre-treatment. Then the animal was fed with either vehicle, 2.5 mg/kg diazepam or AS crude extract. After the drug administration for 30 min, each animal was placed back to the maze and its locomotive behavior was continuously recorded for 5 min. The number of entries into each arm and the total times that animal spent in open arms and closed arms were analyzed by using Anymaze software. The anxiolytic index was calculated from the ratio of time in open arms/time in all 4 arms.

Hotplate analgesic test

An Eddy's hotplate analgesiometer (Orchid Scientific & Innovative India Pvt. Ltd., India) was employed to monitor pain responses. After the drug treatment for 30 min, the animal was placed on a metal plate which its temperature was set at 55 ± 0.5 °C. The pain responses were classified as 1st pain response (i.e., paw shaking front paw licking and hind-paw licking) and 2nd pain response (i.e., climbing and jumping). The cut-off time was set at 60 s to prevent any tissue damage. Response latency to the thermal stimulus were recorded before administration during pre- and 30, 60, and 90 min post drug administrations.

Tail-flick analgesic test

The equipment used in this test was a tail-flick analgesiometer (model 7360, Ugo Basile, Italy). After the administration for 30 min, the animal's tail was placed on the platform in such a way that the middle portion of the tail remained just above the infrared beam. The temperature was set at 55 ± 0.5 °C. The latency period of response was recorded when the

animal responded with a sudden and characteristic flick its tail out of the heat beam. A cut off time of 10 sec was set to avoid any tissue damage.

Barbiturate potentiation test

After the administration for 30 min, each animal was injected with sodium thiopental (Jagsonpal, India) at dose 25 mg/kg by i.p. The onset that the animal lost its righting reaction was recorded as induction time (onset) and the period between the onset and time when animal could resume its upright position was recorded as duration of anesthesia.

Morris's water maze (MWM) test

The MWM setup consisted of a circular tank (diameter 150 cm) filled with tap water and a webcam connected to a computer equipped with Anymaze software. There were 4 different symbols (visual cues) attached to each wall (or curtain). The pool was divided into 4 quadrant corresponding to these visual cues. On Day1 – Day7, each rat was trained in the circular pool to locate the hidden platform within 90 seconds. On Day8, the platform was removed and the rat was allowed to swim in the circular pool for 90 seconds (so called a “probe trial test”). The retention memory (referred as time of spent in target quadrant) were recorded and analyze by using Anymaze software. Spatial memory index of each animal was calculated by using the following formula:

Spatial memory index = [duration in target quadrant / duration in non-target quadrants] × 100

Novel object recognition (NOR) test

The NOR setup consisted of an empty circular tank and 3 different objects (i.e., object A, B and C). In training phase, the animal was placed in the middle of tank floor and allowed to explore 2 different objects (A and B) for 5 min. After that, the animal was removed and put back to its home cage for 5 minutes. During this delay period, object B was removed and replaced with object C which was considered as a novel object. In test phase, the animal was put back into the tank and allowed to explore these 2 objects (A and C) for another 5 min. The durations that the animal explore on object A (TA) and object C (TC) during the test phase were calculated for a recognition index by using the following formula:

Recognition index = [TC / (TA + TC)] × 100

Statistical analysis

All data were expressed as means ± SD. Student's paired or unpaired t-test was used to determine the difference between before vs. after drug administration or the control vs. test group. Each pair of data were considered statistically significant if P value was less than 0.05.

Results :

Effect on locomotor activity

Spontaneous locomotor activity of the animals during a 60-min period after drug administration are shown in Figure 1A and 1B. The total distance of OVX+vehicle and sham+vehicle groups were not statistically different and measured 26.13 ± 10.73 m and 24.63 ± 13.53 m, respectively. On the other hand, the total distance of OVX+Diazepam, OVX+AS100 and OVX+AS1000 groups measured 7.02 ± 2.29 m, 20.65 ± 10.77 m and 21.37 ± 13.05 m which were significantly shorter than that of the negative control group. In addition, the total immobilization time of OVX+vehicle and sham+vehicle groups measured 3016.39 ± 269.92 s and 2884.21 ± 296.43 s. However, the total immobilization time of OVX+Diazepam, OVX+AS100 and OVX+AS1000 groups measured 3474.70 ± 32.11 s, 3262.82 ± 120.07 s and 3336.57 ± 96.63 s. Altogether, these results indicate that AS leaves extract has a mild CNS depressant action on lab rats.

Anxiolytic effect

In this study, the anxiolytic indices of control group show no significant difference between Pre (0.45 ± 0.12) and post administration (0.49 ± 0.16) ($P > 0.05$, paired t-test). Interestingly, the index of OVX+Diazepam group during pre administration (0.36 ± 0.05) show significantly lower than that of during post administration (0.52 ± 0.04) ($P < 0.05$, paired t-test). However, the index of Sham+vehicle group during pre administration show a slight increase during pre (0.41 ± 0.11) but no significant difference when compared to post administration (0.37 ± 0.11) ($P > 0.05$, paired t-test). Similarly, the indices of OVX+AS100 and OVX+AS1000 groups during pre treatment are 0.35 ± 0.14 and 0.36 ± 0.12 which do not significantly different when compared to those from post treatments which are 0.36 ± 0.07 and 0.41 ± 0.12 , respectively ($P > 0.05$, paired t-test). These results indicate that AS leaves extract does not have anxiolytic action.

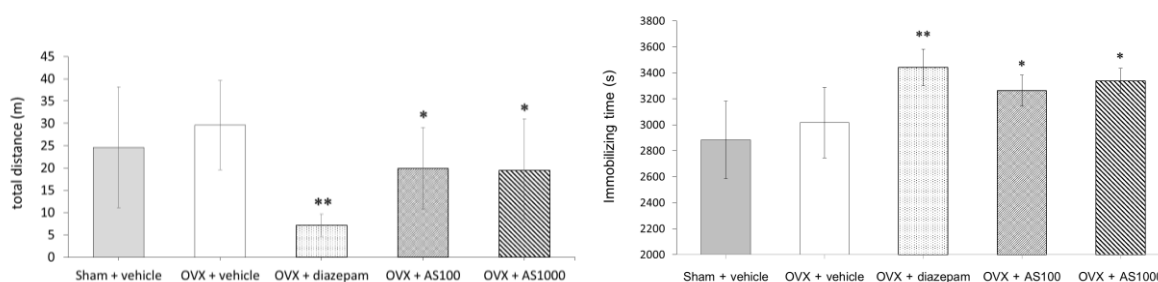


Figure 1 (A) Total distance post administration. (B) Total immobilization time post administration. Each bar represents mean \pm SD. * = $p < 0.05$ when compare to the control, ** = $p < 0.01$ when compare to the control.

Anti-nociceptive effect

1. Tail-flick analgesic test

By using a tail-flick analgesiometer, the latencies of pain response obtained from each group are shown in Figure 2. The pain response latencies of control (OVX+vehicle) and sham groups during post administration for 30, 60 and 90 min measured 1.3 ± 0.5 , 1.2 ± 0.3 , and 1.2 ± 0.3 s, and 1.06 ± 0.3 s, 1.90 ± 0.3 and 1.4 ± 0.7 s, respectively. The pain response latencies of OVX+AS100 group during post administration for 30, 60 and 90 min show no significant difference when compared to the control ($P > 0.05$, unpaired t-test). However, the pain response latencies during post administration for 30, 60 and 90 min of OVX+AS1000 measured 2.0 ± 1.0 s, 1.9 ± 0.8 and 2.0 ± 0.9 s and positive control groups (OVX+D#1) measured 2.2 ± 0.5 s, 2.2 ± 0.9 and 2.1 ± 1.0 s which were significantly longer than the control ($P < 0.05$, unpaired t-test).

2. Hotplate analgesic test

By using a constant (55 ± 0.5 C^o) temperature hotplate as a pain stimulus, the mean latencies of 1st and 2nd pain responses calculated from each group are shown in Figure 2A. For the results of control (OVX+vehicle) and sham groups, 1st and 2nd pain response latencies measured 6.02 ± 5.38 s and 20.07 ± 8.38 s, and 9.81 ± 5.06 s and 23.41 ± 6.19 s, respectively. For the results of AS-treated groups, OVX+AS100 group measured 13.08 ± 5.14 s and 27.24 ± 5.99 s, whereas those of OVX+AS1000 group measured 27.27 ± 9.01 s and 30 ± 1.63 s. When compared to the control, the 1st and 2nd pain response latencies of OVX+AS100 and OVX+AS1000 groups were significantly longer ($P < 0.05$, unpaired t-test). Altogether, from the results obtained from these 2 analgesic tests, we can conclude that AS leaves extract has an acute antinociceptive effect in rats.

Barbiturate potentiation

After administration of drinking water for 30 min, the onset time (induction time) and duration of thiopental induced anesthesia for control (OVX+vehicle) and sham groups

measured 2.97 ± 0.72 min and 15.10 ± 3.46 min, 1.94 ± 0.31 min and 17.26 ± 2.34 min, as shown in Figure 3. For the test groups, the onset times of OVX+AS100 and OVX+AS1000 groups (measured 2.82 ± 0.62 min and 3.37 ± 0.68 min, respectively) did not differ from the control ($P > 0.05$, unpaired t-test). However, the duration of anesthesia of OVX+AS100 and OVX+AS1000 groups (measured 26.88 ± 6.70 min and 20.98 ± 6.24 min, respectively) were significantly longer than the control ($P < 0.05$, unpaired t-test). The result indicates that AS leaves extract has mild efficacy on potentiating barbiturate effect.

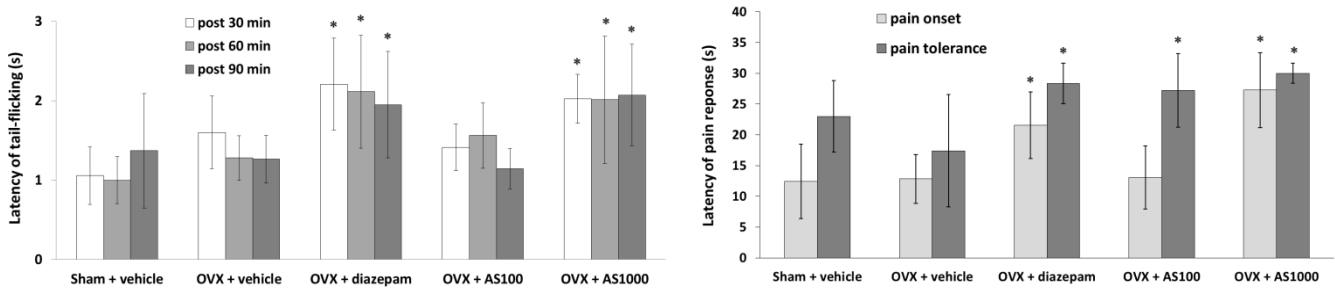


Figure 2 Screening of the acute antinociceptive action of AS leaves extract using a tail-flick (A) and hotplate (B) analgesic tests. A: pain response latencies were recorded at 30, 60, and 90 min after drug administration. B: response latencies were determined as pain onset and tolerance to the thermal stimulus. Each bar represents mean \pm SD, * $p < 0.05$ when compared to the control (unpaired t-test).

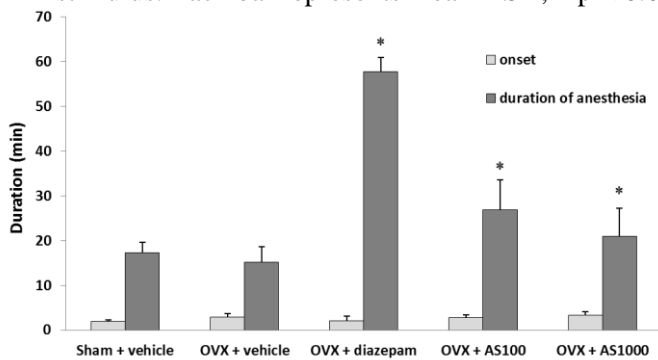


Figure 3 Screening of barbiturate potentiation on induction time and duration of anesthesia after administration for 30 minutes. OVX+diazepam, OVX+AS100 and OVX+AS1000 groups showed significantly longer duration of anesthesia than the control. Each bar represents mean \pm SD, * = $p < 0.05$ when compared to the control (OVX+vehicle) using unpaired t-test.

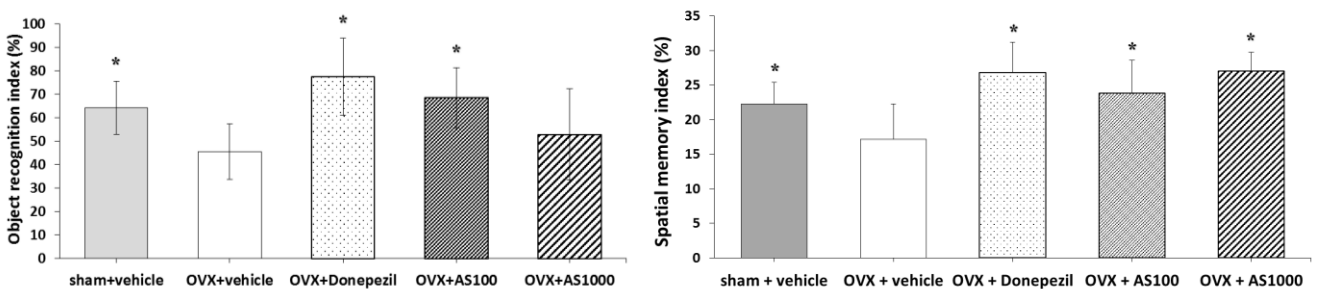


Figure 4 Comparison of object recognition index (A) and spatial memory index (B) of each group following a 2 month period of drug administration. Each bar represent the mean \pm SD, * = $p < 0.05$ when compare to the control (OVX+vehicle) using unpaired t-test.

Effect on cognitive impairment

1. Novel object recognition test

The recognition memory of each group was determined by using a novel object recognition test after a 2 month period of administration. As shown in Figure 4A, the recognition index of control group (OVX) and sham (FOS) 42.63 ± 8.41 and 64.13 ± 11.32 , respectively. When compared to the control group, the indices of both OVX+AS100 and donepezil treated group (OVX+donepezil) were significantly higher (69.27 ± 12.07 and 77.61 ± 16.44 , respectively) ($P > 0.05$, unpaired t-test). However, the index of OVX+AS1000 group (52.86 ± 19.42) did

not significantly differ when compared to the control. This result may imply that AS leaves extract at the dose of 100 mg/kg can help to improve recognition memory in ovariectomized aging rats as well.

2. Morris's water maze test

On Day8, the spatial memory indices of control group and sham group measured 17.2 ± 5.1 and 22.3 ± 3.10 , respectively (Figure 4B). However, the spatial memory index of OVX+AS100 and OVX+AS1000 measured 25.99 ± 5.59 and 25.53 ± 3.76 , which were significantly higher compared to the control ($P < 0.05$, unpaired t-test). The values of these test groups were close to that of the donepezil treated group (OVX+donepezil) which received 5 mg/kg donepezil for 2 months and the index was 26.79 ± 4.43 . This indicates that AS leaves extract can help to improve spatial memory in ovariectomized aging rats.

Discussion and Conclusion :

In the present study, the effects of agarwood leaves extract on general CNS function were evaluated in ovariectomized aging rats. The results of behavioral tests indicate that *A. subintegra* leaves extract has mild sedative, anxiolytic, mild antinociceptive, and memory enhancing properties. We also demonstrated that the CNS action of agarwood leaves extract was similar to those of agarwood oil extracts (Okugawa et al., 1996; Takemoto et al., 2008). Thus, the sedative effect of *A. subintegra* leaves extract did not likely due to estrogen depletion in ovariectomized rats (Zhang et al.1999; Chaves et al. 2009). It is possible that *A. subintegra* leaves extract may contain similar bioactive compounds as found in agarwood oil that can produce mild depressant on animal's spontaneous locomotor activity. Another possibility is that the reduction of locomotor activity in AS leaves extract treated group may be caused by the effect on lower dopamine levels in the brain (Carlsson 1974, Dolphin et al., 1975).

Some of bioactive compounds including flavonoids, terpenoids, and alkaloids contained in *A. subintegra* leaves extract and agarwood oil are known to have ability to cross the blood-brain barrier (Bahrani et al., 2014). These bioactive compounds can bind to benzodiazepine site of GABA-A receptors producing membrane hyperpolarization (Verma et al., 2010, Hanrahan et al., 2011). Since, carvacrol, one of monoterpenes in subclass of terpenoids found in agarwood leaves, can bind to GABA-A receptors and produce an anxiolytic-like property (Pires et al., 2013, Guimarães et al., 2014, Manayi et al., 2016). Our result from elevated plus maze test indicated that the rats treated with AS leaves extract at the dose 100 mg/kg could significantly explore the open arms more than the closed arms. The anxiolytic property of AS leaves extract may be resulting from its bioactive compounds that act through benzodiazepine binding sites of GABA-A receptors (Guimarães et al., 2014). Thus, both sedative and anxiolytic effects of *A. subintegra* leaves extract may be produced by mild depressive action of the bioactive compounds.

In the present study, the results from tail-flick and hotplate analgesic tests in rats similar to that of Zhou et al. who reported the analgesic action of *A. sinensis* leaves extract in mice by using a hotplate analgesic test (Zhou et al., 2008). In addition, Zhou et al. (2008) also reported that a single oral administration of *A. sinensis* leaves extract at the dose of 848 mg/kg could reduce the inflammation induced by xylene- or carrageenan-injection to a mouse's paw. One of the possible mechanisms is that *A. subintegra* extract may act directly at peripheral nociceptors to become desensitizing to pain stimuli. Another possibility is that the crude extract may activate the descending pain control pathway in the brainstem such as periaqueductal gray and nucleus raphe magnus causing a reduction of pain signal transmission to the higher center (Heinricher et al., 2009).

For the results of tail-flick and hotplate analgesic tests, researchers found that *A. sinensis* leaves extract also has antinociceptive action in rats. Zhou and his group used an *in vitro* study to demonstrate the decreases of inducible nitric oxide synthase (iNOS) and lipopolysaccharide-induced nitric oxide release from mouse peritoneal macrophages after the exposure of AS leaves extract (Zhou et al., 2008). In addition, Afiffudden and his co-workers have recently reported that *A. malaccensis* leaves contain 4'-hydroxyacetanilide which is an acetaminophen compound generally used in the synthesis of non-steroid anti-inflammatory drugs (Afiffudden et al., 2015). Since the results from control and sham groups showed no difference in 1st and 2nd pain respond latencies, thus the bioactive compounds in AS leaves extract might exert its action at peripheral nociceptors or through iNOS mechanism, and/or at the central (pain descending pathway) through GABA-A receptors but did not cause by estrogen depletion (Karami et al. 2011; Li et al. 2014).

In this study, we found that AS leaves extract could potentiate the anesthetic action of thiopental as indicated by significantly longer durations of anesthesia than the control group. Previously, Okugawa and his group have reported that two active compounds extracted from AS leaves, such as jinkoh-eremol and agarospirol, show potentiating action on hexobarbital-induced sleeping time in mice (Okugawa et al., 1996). Subsequently, several *in vivo* studies have demonstrated that some bioactive compounds in agarwood leaves such as flavonoids, terpenoids, and alkaloids also have barbiturate potentiating action (Kang et al., 2000, Sousa et al., 2007, Bahrani et al., 2014). From this study, *A. subintegra* leaves extract potentiated barbiturate action did not caused by the effect of ovariectomy (Selye, 1971) or aging (Stevenson and Bull, 1974) as the result showed that there was no different in duration of anesthesia between the control and sham groups.

Finally, *A. subintegra* leaves extract at the doses of 100 and 1000 mg/kg, has been demonstrated to improve learning and memory in ovariectomized rats by using MWM and NOR tests. However, the mechanism(s) of this herbal extract is (are) still unknown. It is possible that this cognitive enhancing property of *A. subintegra* leaves extract may act through these mechanisms: 1) antioxidant activity, 2) increasing some nerve growth factors (NGF), and/or 3) inhibition of acetylcholinesterase (AChE) activity. Previously, Ingkaninan and her colleagues conducted an *in vitro* screening of an antioxidant property of mangiferin extracted from AS leaves using thiobarbituric acid reactive substances (TBAR) and 2,2-diphenylpicrylhydrazyl (DPPH) assays. They found that extract mangiferin could decrease lipid peroxidation in both TBAR and DPPH assays when compared to Trolox which was a derivative of vitamin E (Ingkaninan, et al., unpublished data). Recently, Ray and colleagues using thin-layer chromatography (TLC) and DPPH assay to determine bioactive compounds and antioxidant activity from the ethanolic extract from *A. crassna* leaves. They found 2 main compounds which were mangiferin and genkwanin in the crude extract and these 2 compounds had IC₅₀ values on DPPH free radical scavenging activity approximately 15 and 70 %, respectively (Ray et al., 2014). Thus, it is likely that the cognitive enhancing effect of *A. subintegra* leaves extract may cause by antioxidant property of its bioactive compounds such as mangiferin and genkwanin.

There is evidence showing that agarwood extract can induce a production of brain derived nerve growth factor (BDNF) which is a putative nerve growth factor (NGF). In 2006, Ueda et al. reported that ethanol extract of Vietnamese agarwood could significantly induce BDNF mRNA expression in rat cultured neurons (Ueda et al., 2006). BDNF is known to effect the growth of neurons in the hippocampus, cortex, and basal forebrain areas which are essential for learning, memory, and higher thinking process (Benraiss et al., 2001, Yamada and Nabeshima, 2003, Bekinschtein et al., 2008). In 2014, Bahrani and his co-researchers used a TLC technique and could indicate 2 main compounds such as kaempferol and

dimethoxyflavone from chloroform extracts of the stem and leaves of *A. subintegra*. Then, they employed an *in vitro* screening of AChE activity and found that *A. subintegra* leaves extract and AS stem extract had IC₅₀ values of 80% and 93%, respectively. Subsequently, they used a RAM test to demonstrate that adult male and female mice treated with these chloroform extracts could improve their working memory deficits caused by an administration of valium (Bahrani et al., 2014). These results imply that *A. subintegra* leaves extract is a natural AChE inhibitor for the treatment of some chronic memory impairments such as Alzheimer's disease.

In conclusion, the administration of agarwood leaves extract in aged OVX rats produces mild sedative effect, reduces anxiety, relieves pain, potentiates barbiturate sleeping time, and restores learning and memory. Altogether, the results from the present study can be summarized that *A. subintegra* leaves extract has mild CNS depressive and cognitive restoration effects in ovariectomized aging rats. This herbal extract has a potential to be developed as the food supplement such as herbal tea that has a mild CNS depressant and help to restore the memory impairment. However, the underlying mechanism(s) of action of this herbal extract is still inconclusive. Therefore, further investigations are needed to elucidate this (these) underlying mechanism(s).

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