Anti-stress effects of *Apium graveolens* on rats subjected to immobilization

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**Abstract**

*Apium graveolens* is a potent antioxidant and effective agent on neurological disease. However, the neuroprotective effect against stress still unclear. The present study investigates the anxiolytic effects of a methanol exact of *A. graveolens* in immobilization stressed rats. The five groups of rats used were control, vehicle, positive controls (diazepam 2 mg/kg) and treated groups with 125 and 250 mg/kg of *A. graveolens* for 21 days. On day 15, the rats were placed in the restrainer (12 h daily for 7 days). Restraint stress-induced anxiety-like behavior were assessed using the open field, hole-board and elevate plus maze test. For biochemical parameters, corticosterone, monoamine oxidase enzyme-A (MAO-A) activity, malondialdehyde (MDA), percentage of inhibition of superoxide anion (O$_2^-$) and glutathione peroxidase (GPx) were measured. Administration of *A. graveolens* showed a significant increase in the frequency of head dips in the hole board, line crossings and rearing in the open field, time spent in open arm in elevate plus maze, the discrimination index in object recognition and an decrease in escape latency time in the Morris water maze test compared with vehicle. Moreover, results of the biochemical parameter were represented by treated versus vehicle group. Corticosterone level (250 mg/kg) was 9.76 ± 1.87 versus 27.75 ± 5.90 ng/mL. In cortex and striatum, MAO-A and MDA were significantly decreased while GPx and % inhibition of O$_2^-$ were significantly increased. These results indicated that *A. graveolens* has anxiolytic potential to prevent stress without cognitive deficit, whereas diazepam can cause cognitive deficit.

**Keywords**

*A. graveolens* Linn.
Stress
Cognitive deficit
Rat

**Article history**

Received: 7 June 2016
Received in revised form: 28 July 2016
Accepted: 3 August 2016

**Introduction**

Stress is defined as a condition in the life of an organism that results from the action of one or more stressors that may be of either external or internal origin (Von Borell, 2001). Stress is one of the basic contributing factors to the development of any disease and has been shown to be associated with altered homeostasis that may lead to an oxidant-antioxidant imbalance (Nadeem et al., 2006). Numerous reports have revealed that immobilization stress can affect the central nervous system functions by producing neurochemical and hormonal abnormalities associated with an imbalance of antioxidant status that results in odd neurological behavior including anxiety-like behavior, motor alteration and changes of biochemical parameters (Liu et al., 1996; Marzatico et al., 1998; Nishimura et al., 1999; Belanoff et al., 2001; Lupien et al., 2001; Şahin and Gümüşlü, 2004; Sevgi et al., 2006; Goyal and Kumar, 2007; Nooshinfar et al., 2011). It is well known that an intensive stress response results in creation of reactive oxygen species (ROS) that causes lipid peroxidation, especially in membranes and can play an important role in tissue injury (Kovacs et al., 1996). It has been suggested that chronic stress and high levels of glucocorticoids, the adrenal steroids secreted during stress, affect diverse processes involving ROS with an increase of ROS by approximate 10% above the base (McIntosh and Sapolsky, 1996). The membrane injury causes disruption of the tissue integrity (Cochrane, 1999). It is reported that a disturbance of the balance of the free radical processes was observed with maximal changes in the brain after immobilization stress...
In order to neutralize ROS, the body uses intracellular antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and also glutathione peroxidase (GPx).

In addition, stress causes several neurobehavioral deficits on a variety of cognitive tasks (Nagata et al., 2009) that are also associated with oxidative damage. On the other hand, studies with short term exposure to stress have been shown to facilitate learning and memory performance both in animals (Oitzl et al., 2001) and humans (Cahill et al., 2003). This increase or decrease in memory performance has been attributed to the hormones and neurotransmitters released under stress conditions. The acute stress may activate the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis causes a surge of arousal that is believed to result in memory consolidation following exposure to stress conditions (Joels et al., 2006). In contrast, chronic stress may activate the hippocampal glucocorticoid receptors (GRs) which to a great extent are responsible for cognitive deficits (Pavlides et al., 1995; McEven, 2000; Lupien et al., 2001; Roozendaal et al., 2003).

The central nervous system has traditionally been considered to be a target site for free radical damage because the brain contains abundant amounts of polyunsaturated fatty acids, which are particularly vulnerable to free radical attacks (Gutteridge, 1995) and consumes high amounts of oxygen (Halliwell and Gutteridge, 1985).

The reducing anxiety-like behavior associated with protecting side effect such as amnesia may be alleviated using therapeutic strategies involving medicinal and dietary phyto-antioxidants. Many of nutracelsicals such as Bacopa monniera and Hesperidin have been found to ameliorate the immobilization-stress-induced oxidative-stress, biochemical alterations, and associated neurobehavioral alterations (Rai et al., 2003; Sheikh et al., 2007; Viswanatha et al., 2012). Moreover, one such nutraceutical is Apium graveolens Linn. that possesses very good antioxidant properties (Popovic et al., 2006; Jain et al., 2009; Yao and Ren, 2011; Iswantini et al., 2012) and it has been proved to be very effective in treating various neurological diseases (Chen et al., 2008; Park et al., 2009; Peng et al., 2010; Feng et al., 2012). Moreover, many of nutraceuticals such as Bacopa monniera and Hesperidin has been found to ameliorate the immobilization-stress-induced oxidative-stress, biochemical alterations, and associated neurobehavioral alterations (Rai et al., 2003; Naila et al., 2007; Viswanatha et al., 2012).

With this background, the present study was designed to investigate the possible neuroprotective effect of A. graveolens against immobilization-stress-induced anxiety-like behavior and associated oxidative damage in a rat.

Materials and Methods

Drugs and chemicals

Diazepam (2 mg/tablet) (Government Pharmaceutical Organization) was used as standard drugs. All chemical substances used in this study were analytical grade.

Preparation of A. graveolens crude extract

A. graveolens was collected from Lumpang, Thailand. Identification (BKF No. 188856) was made and deposited at the Forest Herbarium, Royal Forest Department, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. The powdered dried whole plant (2 kg) was extracted with 70% methanol for 3 days at room temperature and filtered. Then, the solvent was evaporated to dryness under reduced pressure (Buchi, Switzerland). A. graveolens methanol extracts were prepared and analyzed as our previous described (Choosri et al., 2016).

Animals

Adult male Wistar rats (250 ± 50 g, 10 weeks old) were obtained from the Southern animal unit, Prince of Songkla University, Songkhla, Thailand. They were randomly housed 6 per cage and maintained in a clean room at a temperature of 26 ± 1°C, with a 12 h light-dark cycle and a relative humidity of 50%. Rats were housed in metabolic cages and supplied with filtered pathogen-free air with access to food and water ad libitum. All administration activities in this study were performed once daily between 8:00-9:00 a.m. The experimental procedures were performed in accordance with the principles of animal care outlined by the Faculty of science, Prince of Songkla University, Songkhla, Thailand (MOE 0521.11/462).

Immobilization stress

On the 15th-21st day all the animals (except naive control) were immobilized for 12 h (6:00 p.m.-6:00 a.m.) by putting them in plexiglass tubes. In the unstressed group, the rats were kept in an animal cage with soft bedding in the experimental room. Following each stress session, animals were returned to their home cages and had free access to food and water for the remainder of the experiment.

Experimental procedures

The A. graveolens extract suspension and diazepam in distilled water was freshly prepared before being administered. All the treatments were...
given once daily for three weeks and the animals were subjected to immobilization stress on the 15th to 21st day. All the animals were randomly divided into five groups with six animals in each group. The following protocol was used: Group I: unstressed animals (control, daily oral administration of 0.5 mL distilled water. Group II: stressed animals, daily oral administration of 0.5 mL distilled water. Group III-IV: stressed animals, with daily oral administration of A. graveolens extract at doses of 125 and 250 mg/kg, respectively. Group V: stressed animals, daily oral administration of diazepam 2 mg/kg.

Behavioral assessments

The rats were acclimatized for 5 min on each behavioral test apparatus consecutively for 3 days before initiating the actual experimental protocol, except for the Morris water maze test, were acclimatized for 1 min (5 times/day) consecutively for 5 days before initiating the actual experimental protocol. It was necessary to acclimatize the animals to the test apparatus in the behavioral studies to avoid any potentially conflicting effects induced by the novelty of the testing apparatus that in turn reduced the variations in the experimental data. Thereafter, the rats were subjected to immobilization stress and the battery of behavioral tests was performed with the sequence of the elevated plus maze, the hole board, the open field, the object recognition and the Morris water maze. All behavioral test equipments were cleaned after each test with alcohol and water. At the end of behavioral tests, blood was withdrawn from the heart for the estimation of corticosterone. The corticosterone levels were at their highest levels in the morning; therefore, all the experiments were performed during 8:00 a.m.-12:00 a.m.

Open field test

The open field has been considered to be a non-conditioned anxiety test based on the creation of a conflict between the exploratory drive of the rat and its innate fear to exposure in an open area (Angrini et al., 1998). The open field test has been employed to assess the spontaneous activity, general exploration and ambulation of the rodents. Each rat was placed individually in the center of the apparatus and observed for 5 min to record its locomotor activity (the number of line crossings), exploratory activity (indicated by frequency of rearing) and time spent in the center (Manchanda et al., 2011).

Elevated plus maze test

Anxiety was evaluated in an elevated plus maze. The experimental procedure was similar to that described by Pellow et al. (1985). Immediately after the pretest exposure rats were placed in the center of the elevated plus maze facing one of the open arms. During the 5 min test period the number of entries into the open arms and the time spent there were measured manually. An entry was defined as all four feet into one arm. An increase in open arms entries and increase in time spent in open arms were accepted as the measures of potential anxiolytic activity.

Hole board test

The hole board apparatus was used to determine a high-anxiety-like state in rats. Each animal was placed in the hole board and allowed to freely explore the apparatus for 5 min. The numbers of head dips by the rats were counted (Takeda et al., 1998).

Morris water maze test

In order to determine higher cognitive functions such as spatial memory which is believed to be involved with hippocampal functions, one of the most common behavioral paradigms for evaluating the rodents is the Morris water maze test. Animals were watched in a round polyvinyl water pool (120 cm diameter, 50 cm height) filled with water (25°C). Additional powder was used to provide the opaque water. 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 environmental 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 s or until it found and climbed onto the platform. During the training session, the mice were gently placed on the platform by the experimenter when it could not reach the platform in 60 s. In either case, the subject was left on the platform for 30 s and removed from the pool. The time for animals to climb onto 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 (Morris et al., 1984).

Object recognition test

The object recognition test was performed to determine the non-spatial memory of shape, color and texture. The apparatus consisted of a circular area 100 cm in diameter with a 40 cm high wall. The open field and the objects were cleaned between each trial using 70% ethanol to avoid odor trails. Before the experimental 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 spaced trials. 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 s exploring these two objects in a limit of 3 min. Any mice which did not explore the objects for 15 s within the 3 min period were excluded from the 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 present in the first trial was replaced by a novel object. Animals were placed back in the area for 3 min, the total times which the animals spent to explore or directed their nose within 2 cm of the object while looking at, sniffing, or touching the novel object were recorded and calculated according to the equation (Antunes and Biala, 2012).

\[
\text{Discrimination index} = \frac{(A-B)}{(A+B)}
\]

A = Time of approach to object 2
B = Time of approach to object 1

**Biochemical parameters**

At the end of the experimental periods, each rat was deeply anesthetized with sodium thiopental 50 mg/kg BW intraperitonelly. Whole blood was immediately withdrawn from the heart and collected in heparin-coated test tubes and centrifuged at 1500 g for 10 min to separate plasma from the erythrocytes. All the animals were then perfused by intracardiac perfusion with 500 ml 0.9% normal saline solution for 30 minutes and immediately separated from the brain area. The protein content from brain tissue was measured according to the method of Lowry et al. (1951) using bovine serum albumin as a standard.

**Plasma corticosterone measurement**

Post-stress corticosterone was measured in the plasma by an enzyme-linked immunosorbant assay using the corticosterone ELISA kits in accordance with the manufacturer’s protocol (Enzo life sciences Inc, USA.). The resulting concentration of plasma corticosterone was expressed as ng/mL using prepared corticosterone standards.

**Assay for monoamine oxidase (MAO-A) activity**

The MAO-A activity from the supernatant of the brain tissues were determined by spectrophotometric measurement using the method of Elliott et al. (1991) with a slight modification (Holt et al., 1991). Brain tissue homogenates were incubated with the mixed substrate and 500 μM tyramine plus 500 nM pargyline to inhibit MAO-B. The chromogenic solution contained vanillic acid (1 mM), 4-aminoantipyrine (500 mM) and peroxidase (4 U/mL) in potassium phosphate buffer (0.2 M, pH 7.6). The assay mixture contains 50 μL of supernatant, 50 μL chromogenic solution and 100 μL of 100 mM amine substrate plus 1% H₂O₂ and the optical density (OD) was measured at 490 nm. The MAO-A activity was designated as μmol/min/ g tissue.

**Lipid peroxidation assay**

The quantitative measurement of lipid peroxidation in the brain tissues were assessed as per the modified method of Wills (1966). The amount of malondialdehyde (MDA) formed was measured by the reaction with thiobarbituric acid at 532 nm using a microplate reader. The results were expressed as micromole of malondialdehyde per mg protein.

**Superoxide anion (O₂⁻) assay**

The O₂⁻ level was determined by a spectrophotometric measurement. The method was based on a xanthine/xanthine oxidase (XO) system that converted nitro blue tetrazolium (NBT)-yellow color to a formazan-blue color. The reagent mix was prepared with EDTA, NBT, xanthine and XO, mixed with a sample and measured at 560 nm compared with the standard curve of TEMPOL. The data was expressed as % inhibition which was calculated following the equation (Toda et al., 1991).

\[
\% \text{ inhibition} = \frac{(A-B)}{A} \times 100
\]

A= OD of reagent only
B = OD of sample

**Assay for GPx activity**

The GPx activity was determined by the method of Wendel (1980), which measures the activity indirectly by a coupled reaction with glutathione reductase. The oxidation of NADPH to NADP⁺ was accompanied by a decrease in absorbance at 340 nm. The rate of decrease in the A₃₄₀nm level was directly proportional to the GPx activity. The final 1 mL of the system mixture contained 48 mM sodium phosphate, 0.38 mM EDTA, 0.12 mM β-NADPH, 0.95 mM sodium azide, 3.2 units of glutathione reductase, 1 mM glutathione (GSH), 0.02 mM DL-dithiothreitol,
0.0007% H$_2$O$_2$, and the standard enzyme GPx solution or supernatant from the brain tissue sample. The GPx solution was used to represent the standard enzyme activity. The standard curve was plotted as the rate of A$_{340}$nm per min against the GPx activity. One unit of activity was defined as the amount of enzyme necessary to catalyze the oxidation by H$_2$O$_2$ of 1 µmol of GSH to GSSG per min at pH 7 and 25°C. The data were reported in units of GPx per g protein.

**Statistical analysis**

All data are presented as mean values ± standard error of the mean (SEM). Statistical analysis using Statistical Package for the Social Sciences (SPSS) was performed by one-way analysis of variance (ANOVA), followed by the Least Significant Difference (LSD’s post-hoc test). A probability value of less than 0.05 was considered significant.

**Results**

**Behavioral estimations**

The control animals showed consistent and stable locomotor activity, exploratory activity and anxiety-like behavior. The immobilization stress had significantly reduced the locomotor activity and exploratory activity (as indicated by the decreased number of line crossings and frequency of rearing in the open field test) and induced anxiety-like behavior (decreased time spent in central area in the open field test, decreased frequency of head dips in the hole board test and decreased time spent in an open arm in the elevated plus maze test) as compared to the unstressed control group (P<0.05) (Figure 1).

Twenty-one days of *A. graveolens* extract (both 125 and 250 mg/kg) treatment had significantly inhibited the anxiety-like behavior (increased time spent in the open arm (P<0.05 and P<0.05), frequency of head dips) (P<0.05 and P<0.05), improved locomotor...
activity (increased number of line crossings) (P<0.05 and P<0.05) and exploratory activity (increased frequency of rearing (data not shown)) as compared to the vehicle (restraint stress) group (P<0.05 and P<0.05). Diazepam also showed the same pattern with A. graveolens extract but was more potent in increasing the time spent in the opened arm of the elevated plus maze test than both concentration of A. graveolens extract (Figure 1). In the Morris water maze test, stress led to a prolongation of the average escape latency, which indicated a spatial learning deficit. Treatment with A. graveolens extract prior to stress prevented this spatial deficit but diazepam was better at reducing the novel exploration time (Figure 2).

Corticosterone level

Figure 3 shows the plasma corticosterone level in all the experimental groups and indicated the significant differences among the groups. Comparison of the control group and vehicle group indicated that the immobilization stress significantly increased plasma corticosterone (P<0.05). In contrast, the corticosterone level in the A. graveolens extract groups (125 and 250 mg/kg) were significantly reduced after treatment with A. graveolens extract (P<0.05). These finding indicated that A. graveolens extract did significantly prevent stress-induced elevation of corticosterone levels.

Monoamine oxidase-A (MAO-A) activity

The vehicle (immobilization stress) group exhibited a significant higher level of brain MAO-A activity as compared to the control (unstressed rats) in both the cortex and striatum of the brain (P<0.05 and P<0.05). A. graveolens extract (125 and 250 mg/kg) and diazepam (2 mg/kg) administered for 21 days significantly reduced brain MAO-A activity as compared to the vehicle group (P<0.05 and P<0.05) (Figure 4).

Brain lipid peroxidation and antioxidant enzyme activity

Effects of A. graveolens extract following immobilization stress on the MDA levels, % inhibition of O₂ and antioxidant enzyme activity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/mg tissue)</th>
<th>O₂ (nmol/mg tissue)</th>
<th>GPx (nmol/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>striatum</td>
<td>cortex</td>
<td>striatum</td>
</tr>
<tr>
<td>Control</td>
<td>0.08±0.02</td>
<td>0.08±0.03</td>
<td>49.99±0.03</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.13±0.03</td>
<td>0.12±0.04</td>
<td>13.36±0.13</td>
</tr>
<tr>
<td>A. graveolens</td>
<td>0.12±0.02</td>
<td>0.08±0.02</td>
<td>42.56±0.63</td>
</tr>
<tr>
<td>A. graveolens</td>
<td>0.07±0.02</td>
<td>0.06±0.07</td>
<td>49.16±10.94</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± standard error of the mean. "P<0.05 compared with the control; #P<0.05 compared with the vehicle;

Table 1. Effects of A. graveolens extract following immobilization stress on the MDA levels, % inhibition of O₂ and antioxidant enzyme activity.

Figure 3. Post-stress corticosterone level in the plasma. Data are presented as the mean ± standard error of the mean. *P<0.05 compared with the control; #P<0.05 compared with the vehicle.

Figure 4. The MAO-A activity in striatum and cortex after stress. Data are presented as the mean ± standard error of the mean. *P<0.05 compared with the control; #P<0.05 compared with the vehicle; ** compared with diazepam.
compared to the unstressed group (P<0.05), that indicated induction of stress in the rats. *A. graveolens* extract (125 and 250 mg/kg) produced a significant rise in the levels of the % inhibition of the O$_2^-$, GPx activity and markedly decrease MDA as compared to the vehicle group (P<0.05).

**Discussion**

Stress is able to activate the HPA axis and change the catecholamine, GABA and serotonin levels. Stress thereby influences several neurological functions at both the central and peripheral levels (Von Borell, 2001), and exposure to chronic immobilization stress in animals and psychological stress in humans has implicated the pathophysiology of anxiety and mood disorder (Walesiuk et al., 2006). Immobilization stress, both acute and chronic, has affected motor activity, anxiety-like behavior, and depression-like behavior in animals (Sevgi et al., 2006). Furthermore chronic immobilization stress has been reported to influence cognitive impairment (Nishimura et al., 1999; Sevgi et al., 2006). In the present study, immobilization stress for 12 h daily for 7 days indicated similar results to previous reports along with neurobehavioral alterations due to acute and chronic stress. In this study the behavioral changes observed in the experiments might be due to alterations in the brain regions that control motor activity and anxiety-like behavior. *A. graveolens* extract (125, 250 mg/kg, p.o.) provided significant protection against immobilization stress. The neuroprotective effect against stressful conditions occurred via locomotor activity and anxiety-like behavior, oxidative damage control, monoaminergic system changes and elevation of corticosterone levels.

The HPA axis activity can be regulated by monoamines (Garabadu et al., 2011; Krishnamurthy et al., 2013). Generally the monoaminergic level varies among brain regions such as the hippocampus, hypothalamus, prefrontal cortex and amygdale in stress conditions (Greenwood et al., 2001; Garabadu et al., 2011; Krishnamurthy et al., 2013). In this study *A. graveolens* did cause changes in the monoaminergic system in both the cortex and striatum regions as shown by the decrease of the MAO-A enzyme. Moreover, a corticotrophin-releasing factor (CRF) was considered to be part of the extra-hypothalamic stress system. Due to the abundance of CRF terminals and receptors, Amygdala becomes an important neuroanatomical area for the extra-hypothalamic stress system (Jankord and Herman, 2008). Prior to *A. graveolens* treatment, changes in the monoaminergic system induced by immobilization stress in rats could be due to decreased corticosterone levels. A similar result for corticosterone was also observed in our diazepam treated group, however the mechanism seems to be different from the case of the *A. graveolens* treated group (Kalman et al., 1997).

Hippocampal areas are heavily engaged in cognitive processes and are very sensitive to corticosteroid damage (McEwen, 2000, 2001; Belanoff et al., 2001), partly because of their high density of mineralcorticoids (MRs) and GRs. The activation of the hippocampal GRs may, to a great extent, be responsible for the behavioral deficits in animal and humans with hypercortisolemia (Pavlidis et al., 1995; McEwen, 2000; Lupien et al., 2001; Roozendaal et al., 2003). The result from our study was consistent with other reported studies that immobilization stress in rats increased corticosterone secretion and decreased memory (Nooshinifar et al., 2011) whereas the ability of *A. graveolens* extracts provided an effect that was in contrast to the corticosterone level and memory. In another way, gamma-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter of the brain and its main actions are mediated by fast-acting GABA-A receptors. Binding of benzodiazepines at the benzodiazepine site of GABA-A receptors resulted in a potentiation of inhibitory neurotransmission with consequent behavioral changes. Their capacity to elicit sedative, anxiolytic, myorelaxant, hypnotic and antiepileptic effects has made benzodiazepines the drugs widely used in clinical practice. However, they may also induce some effects, such as amnesia (McNamara and Skelton, 1991; Nakamura and Roelke, 1997; Stewart, 2005; Sasaki et al., 2013). Our result correspond to previous findings that showed that the escape latency of spatial memory in the Morris water maze test and the discrimination index in the object recognition test were impaired in the diazepam-treated group but not in the *A. graveolens*-treated rats. We thus provide further evidence for the potential usefulness of *A. graveolens* as an anxiolytic without causing amnesia. Furthermore, *A. graveolens* (125, 250 mg/kg) displayed a similar efficacy to diazepam in the suppression anxiety in immobilization stressed rats.

Apart from the mentioned immobilization stress that contributed to a drastic increase in the reactive oxygen species and consequent oxidative damage with a proportionate decrease in the intracellular antioxidant defense (Şahin and Gümüşlü, 2004). Oxidative stress leads to cellular damage and accelerates neuronal death by inducing ROS via oxidizing vital cellular components such as lipids, proteins and DNA (Voronych and Lemel, 1994;
Marzatico et al., 1998; Şahin and Gümüşlü, 2004). In our study, immobilized stress for 12 h daily for 7 days caused significant oxidative damage as indicated by the rise in MDA, GPx activity and a decrease of the % inhibition of the O₂−. Pretreatment with A. graveolens extracts significantly attenuated the MDA concentration, decreased the GPx activity and increased the % inhibition of the superoxide anion and this indicated its antioxidant-like effect. In support of our study, a clinical trial also indicated a raised level of MDA in patients with affective disorders (Ozcan et al., 2004). In accordance with the above findings, in the present study, A. graveolens elevated the antioxidant enzyme defense system against immobilization stress-induced oxidative damage. The possible mechanism behind the protective effect of A. graveolens is inhibition of synthesis of the O₂− in the brain.

**Conclusion**

Our finding has proposed that an A. graveolens extract can ameliorate the immobilization-stress-induced oxidative stress, biochemical alterations and associated anxiety-like behavior in the rat, as well as its involvement of the neurotransmitter pathway in the protective effect of the A. graveolens extract against immobilization-stress-induced anxiety-like behavior without memory loss. In summary A. graveolens could serve as a novel approach for anxiety treatment.

**Acknowledgements**

This project received financial support from Development and Promotion of Science and Technology Talents Project, General project and invention of Prince of Songkla University (SCI570371S), Thailand and BRAND’S Brain Research Centre, Thailand. Thanks to Dr Brian Hodgson for assistance with the English.

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