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Anxiolytic and free radical scavenging potential of Chinese celery (*Apium graveolens*) extract in mice

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ABSTRACT

Objective: To elucidate the anxiolytic and free radical scavenging effect of methanolic extract of *Apium graveolens* (*A. graveolens*) in adult C57BL/6 mice.
Methods: Sixty male mice were divided into 6 groups: control, vehicle, positive control and *A. graveolens* (125, 250 and 500 mg/kg). Different behavioral models of elevated plus maze, open field, light/dark, hole-board and pentobarbital-induced sleep were used to assess anxiety-like behavior. Biochemical parameters including monoamine oxidase-A (MAO-A) activity, lipid peroxidation, % inhibition of superoxide anion and glutathione peroxidase activity were measured. Histologic studies were also examined.
Results: Mice receiving various doses of *A. graveolens* (125, 250 and 500 mg/kg) showed an alleviation of anxiety-like behavior as evidenced by the battery of behavioral tests. Likewise, *A. graveolens* treatment was found to significantly decrease MAO-A activity, lipid peroxidation as well as cause a significant increase of % inhibition of superoxide anion and glutathione peroxidase activity in both cortex and striatum. The total number of survival neurons found in the frontal cortex and striatum was significantly higher than that of the vehicle-treated group.
Conclusions: Taken together, we showed that *A. graveolens* improve the behavioral changes which might be related to the inhibition of free radicals and modulation of MAO-A activity resulting in an increased number of survival neurons. Our findings suggest the therapeutic potential of *A. graveolens* in the treatment of anxiety.

1. Introduction

Anxiety is one of the most common neuropsychiatric disorders. It is divided into generalized anxiety disorder, panic disorder, post-traumatic stress disorder, social phobias and agoraphobia which contribute to impairing their quality of life and increasing costs of general medical inpatients [1]. It is common in all age groups and characterized by persistent worry occurring over at least six months associated with mental and somatic symptoms including arousal, irritability, fatigue and sleep disturbance [2,3]. Benzodiazepine anxiolytics are the first line of prescription to patients with anxiety. The adverse effects of benzodiazepine usage have been reported including anterograde amnesia, physical dependence, cognitive dysfunction, risk of cancer and risk of falling especially in the elderly [4–6]. Therefore, alternative medicines have drawn an attention in the search for medicinal plants with potent biological activity.

Apium graveolens Linn. (*A. graveolens*) is an annual herb belonging to family Apiaceae with green blanched leaf stalks. It is found in North and South Americas, Southern Europe, Africa

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and Asia. Its major active constituents are L-3-n-butylphthalide, sedanolide, linoleic acid, flavonoids, phenolic compounds and volatile oil, which are extracted from its various part including roots, leaves and seeds [7–9]. Several studies have reported its pharmacological activity on antimicrobial, anti-inflammatory, anti-arthritis, antiulcerogenic, antihyperlipidemia and anti-hypertension [8–11]. Concerning the beneficial effect on the central nervous system, a pure compound extracted from *A. graveolens* (L-3-n-butylphthalide) was demonstrated to improve cognitive impairment in the Alzheimer's mouse model [7]. Its synthesized compound, DL-3-n-butylphthalide, has shown neuroprotective effects on cerebral ischemia and 1-methyl-4-phenylpyridinium ion-induced Parkinson's disease [12,13].

Thus, the present study aimed to elucidate the potential anxiolytic effect of *A. graveolens* in adult C57BL/6 mice, and to examine its effect on free radical scavenging and histological alterations.

2. Materials and methods

2.1. Preparation of plant extracts

Whole plant materials of *A. graveolens* were purchased from Lampang Herb Conservation, Thailand. *A. graveolens* was identified by a botanist and a voucher specimen (BKF: 188856) was deposited at the Forest Herbarium, Bangkok, Thailand. Dried plants were ground into powder and macerated in 70% methanol at 1:10 (w/v) ratio for 72 h. Thereafter, it was filtered through a satin cloth followed by Whatman No. 1 filter paper to achieve the filtrate methanolic extract. The residue was then re-extracted twice following the same procedure. The combined methanolic extract was evaporated to dryness by using a rotary evaporator under vacuum (Rotavapor R-300, BÜCHI, Switzerland) at 50 °C and finally lyophilized to achieve a powder. The yield of methanolic *A. graveolens* extract was found to be 15.88% (w/w). *A. graveolens* was administered *p.o.* in distilled water once daily.

2.2. Drugs and chemicals

Diazepam (2 mg/tablet) (Government Pharmaceutical Organization, Bangkok, Thailand) was used as a positive control for anxiolytic effect. The drug was dissolved in distilled water and administered orally. Sodium pentobarbital was purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade.

2.3. Laboratory animals

Two-month-old adult C57BL/6 mice weighing between 30 and 35 g (n = 10) were obtained from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. The animals were housed five per cage and maintained under standard environmental conditions (12 h light–dark cycle, 23–27 °C temperature, 50% humidity). Mice were fed with standard food and water *ad libitum*. The study was approved by the Institutional Animal Care and Use Committee and was carried out in accordance with the Principles for the Care and Use of Research Animals outlined by Faculty of Science, Prince of Songkla University, Thailand (MOE0521.11/582).

2.4. Experimental design

The adult male C57BL/6 mice were divided into 6 groups of ten mice each to receive the daily treatments: (1) no treatment (naive control), (2) distilled water as vehicle control, (3) 2.0 mg/kg body weight diazepam as positive control, (4) 125 mg/kg body weight *A. graveolens*, (5) 250 mg/kg body weight *A. graveolens* and (6) 500 mg/kg body weight *A. graveolens*. Treatments were administered once daily for 4 weeks. The behavioral tests were determined on Day 1, 7, 14, 21 and 28 after 1 h of *A. graveolens*, distilled water or standard drug administration, whereas biochemical assay and histological examination were performed at the end of the experiment.

2.5. Behavioral tests

2.5.1. Elevated plus maze (EPM) test

EPM apparatus (two open arms and two closed arms elevated 50 cm off the floor) was employed to measure the anxiety behavior of the experimental animals. The mice were placed at the center of the plus maze facing one of the open arms. During the 5-min test period, the time spent in the open arm was recorded. Behavioral testing of all animals was conducted every 7 days by observers blind to the treatment group.

2.5.2. Open field test

Open field test was used to evaluate the anxiety-related behavior of the mice. The apparatus consisted of a plexiglass box (height: 38.0 cm; length: 90.0 cm; width: 90.0 cm) divided into 25 squares. One hour after oral administration of *A. graveolens* (125, 250, 500 mg/kg body weight) or standard drug, each mouse was centrally placed in the arena and the behavioral patterns were investigated for 5 min.

2.5.3. Light/dark test

The test apparatus consisted of two equal size compartments by a dark safe compartment and a large illuminated compartment. The experiment was initiated by placing a mouse into the center of the illuminated box facing the hole and the mouse was allowed to freely explore the apparatus for 5 min. Time spent in light and dark box was then recorded with the aid of a video camera.

2.5.4. Hole-board test

The hole-board apparatus consisted of a plexiglass box (height: 50.0 cm; length: 40.0 cm; width: 40.0 cm) with 16 evenly distributed holes (diameter 2.0 cm) was elevated 25 cm from the floor. Mice were then placed in the center of apparatus and allowed to freely move for 5 min. The number of head dips was evaluated.

2.5.5. Pentobarbital-induced sleep time test

Sixty minutes after administration of *A. graveolens*, diazepam or distilled water, the mice were injected with sodium pentobarbital (50 mg/kg body weight, *i.p.*) to induce sleep. The duration of time between the pentobarbital administration and loss of righting reflex was considered as the sleep latency (min) and the duration of time between loss of righting reflex and voluntary recovery of righting reflex was considered as sleeping time (min).

2.6. Biochemical analysis

2.6.1. Assay for monoamine oxidase (MAO)-A activity

Animals were anesthetized with *i.p.* injection of pentobarbital sodium at a dose of 50 mg/kg body weight after the completion of the behavioral tests. Brains (cortex and striatum) were dissected

and homogenized in ice-cold 100 mmol/L potassium phosphate buffer, pH 7.4 with glass Potter-Elvehjem homogenizer. The homogenates were incubated with 500 μmol/L tyramine plus 500 nmol/L pargyline in order to inhibit MAO-B activity. The chromogenic solution prepared in the assay contained vanillic acid (1 mmol/L), 4-aminoantipyrine (500 mmol/L) and horse radish peroxidase (4 IU/mL) in potassium phosphate buffer (0.2 mol/L, pH 7.6). The absorbance was measured spectrophotometrically at 490 nm. The MAO-A activity was then determined and expressed as μmol/min g tissue.

2.6.2. Assay for lipid peroxidation

Malondialdehyde (MDA) which is the most abundant product from lipid peroxidation was evaluated by the reaction of thiobarbituric acid and homogenized brain samples (cortex and striatum). Afterwards, the mixtures were heated at 100 °C for 60 min and cooled under tap water. After centrifugation at 4 000 r/min for 10 min, the organic layer was separated and the optical density (OD) was measured at 532 nm using a spectrophotometer. The concentration of MDA was expressed as nmol/mg protein.

2.6.3. Assay for % inhibition of superoxide anion (O_2^-)

The O_2^- assay was conducted according to xanthine/xanthine oxidase system which converted nitro blue tetrazolium to formazan. The reagent mixture (ethylenediaminetetraacetic acid, nitro blue tetrazolium, xanthine and xanthine oxidase) was incubated with homogenized brain samples (cortex and striatum) and the OD was measured at 560 nm in comparison to standard curve of tempol. The data were expressed as % inhibition calculated based on the following formula:

$$\%\text{Inhibition} = [(\text{OD of reagent} - \text{OD of sample})/\text{OD of reagent}] \times 100$$

2.6.4. Assay for glutathione peroxidase (GPx) activity

Supernatant from the brain tissue (cortex and striatum) was incubated with the reaction mixture containing 48 mmol/L sodium phosphate, 0.38 mmol/L ethylenediaminetetraacetic acid, 0.12 mmol/L β-nicotinamide adenine dinucleotide phosphate, 0.95 mmol/L sodium azide, 3.2 units of glutathione reductase, 1 mmol/L glutathione, 0.02 mmol/L DL-dithiothreitol and 0.000 7% H_2O_2 in comparison to the standard enzyme GPx solution. The disappearance of nicotinamide adenine dinucleotide phosphate was measured at 340 nm, 25 °C, and the data were expressed as units/g protein.

2.7. Cresyl violet staining

Frontal cortex and striatum from various groups of treatment were sectioned and stained with 0.5% cresyl violet in order to determine neuronal density. The sections were observed and photographed under light microscopy (E600, Nikon, Japan).

2.8. Statistical analysis

Experimental data were expressed as mean ± SD. Significant differences were compared by ANOVA (Tukey's *post hoc* test). The statistical difference was regarded as $P < 0.05$.

3. Results

3.1. EPM test

We observed that 2 mg/kg diazepam and *A. graveolens* at all doses (125, 250, 500 mg/kg) significantly increased the time spent in the open arms; the 125 mg/kg dose demonstrated the highest effect ($P < 0.05$) (Figure 1).

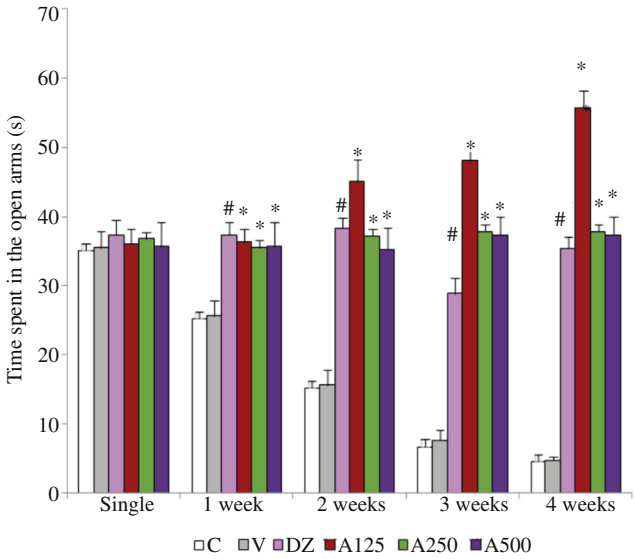

Figure 1. Effects of *A. graveolens* (A125, 250 and 500 mg/kg, *i.p.*), diazepam (DZ 2 mg/kg, *i.p.*), vehicle (V) or control (C) on the time spent in the open arms during EPM test in mice.
Data are presented as mean ± SD, $n = 10$ each group. #: $P < 0.05$ positive control versus vehicle, *: $P < 0.05$ *A. graveolens* versus vehicle.

3.2. Open field test

Diazepam (2 mg/kg) and *A. graveolens* (125, 250, 500 mg/kg) significantly increased the ambulation activity at the central area ($P < 0.05$) with a peak effect at 125 mg/kg (Figure 2).

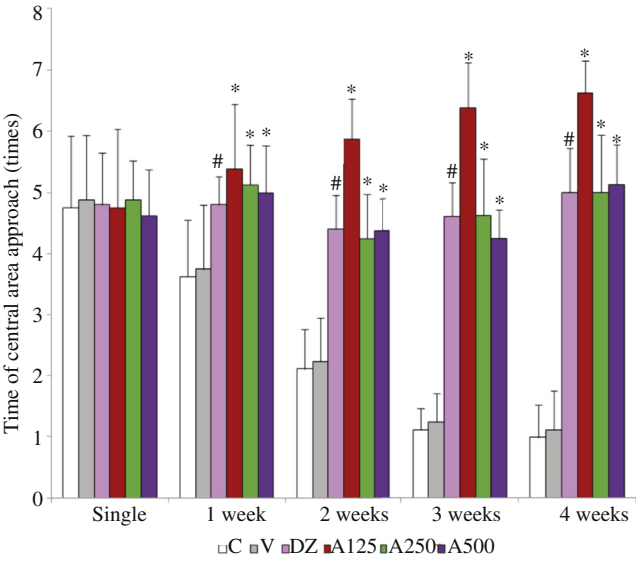

Figure 2. Effects of *A. graveolens* (A125, 250 and 500 mg/kg, *i.p.*), diazepam (DZ 2 mg/kg, *i.p.*), vehicle (V) or control (C) on the time of central area approach in the open field test in mice.
Data are presented as mean ± SD, $n = 10$ each group; #: $P < 0.05$ positive control versus vehicle, *: $P < 0.05$ *A. graveolens* versus vehicle.

3.3. Light/dark test

Figure 3 demonstrates a significant increase of time spent in the light chamber in the diazepam and *A. graveolens* (125, 250, 500 mg/kg) treated groups ($P < 0.05$).

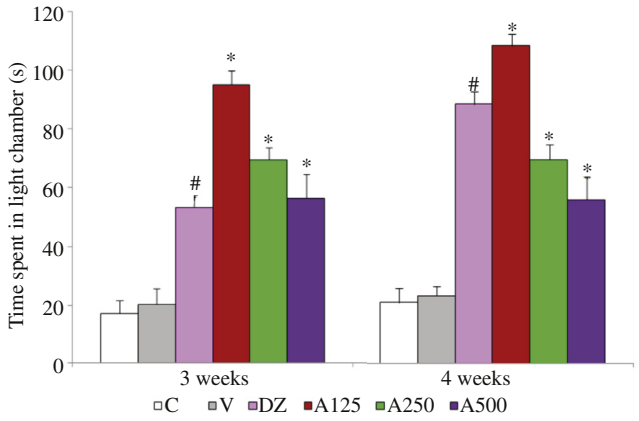

Figure 3. Effects of *A. graveolens* (A125, 250 and 500 mg/kg, *i.p.*), diazepam (DZ 2 mg/kg, *i.p.*), vehicle (V) or control (C) on the time spent in the light chamber during light/dark test in mice.
Data are presented as mean ± SD, n = 10 each group; #: $P < 0.05$ positive control versus vehicle, *: $P < 0.05$ *A. graveolens* versus vehicle.

3.4. Hole-board test

The administration of *A. graveolens* (125, 250, 500 mg/kg) significantly increased the number of head dips compared to the vehicle control. Peak effect was demonstrated at dose of 125 mg/kg. Mice treated with standard drug (2 mg/kg diazepam) demonstrated a significant increase on head dips compared to those observed in the vehicle control group ($P < 0.05$) (Figure 4).

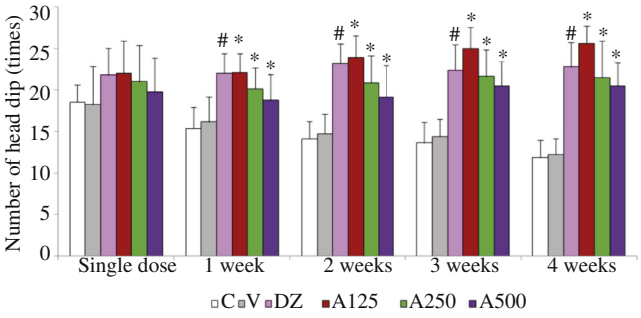

Figure 4. Effects of *A. graveolens* (A125, 250 and 500 mg/kg, *i.p.*), diazepam (DZ 2 mg/kg, *i.p.*), vehicle (V) or control (C) on number of head dips during hole-board test in mice.
Data are presented as mean ± SD, n = 10 each group; #: $P < 0.05$ positive control versus vehicle, *: $P < 0.05$ *A. graveolens* versus vehicle.

3.5. Pentobarbital-induced sleeping time test

In vehicle-treated animals, the righting reflex was lost after pentobarbital injection for 5.47 min (Figure 5A). However, the animals who received 125, 250 and 500 mg/kg *A. graveolens* and diazepam showed a significantly decreased sleep latency compared to the vehicle-treated group ($P < 0.05$). The sleeping time (Figure 5B) was significantly increased by *A. graveolens* (125, 250 and 500 mg/kg) ($P < 0.05$) and 2 mg/kg diazepam treatment ($P < 0.05$).

3.6. MAO-A activity

The effect of *A. graveolens* on brain MAO-A is shown in Figure 6. The administration of *A. graveolens* (125, 250 and 500 mg/kg) markedly decreased MAO-A activity when compared to the vehicle ($P < 0.05$) in the mouse specific brain regions including cortex and striatum. Peak effect was found in the 125 mg/kg *A. graveolens* treatment group.

3.7. Brain lipid peroxidation and antioxidant enzyme activity

The cortical MDA level (Table 1) in *A. graveolens*-treated group (125, 250 and 500 mg/kg) was significantly decreased compared with the vehicle group ($P < 0.05$). This effect was parallel with that in the striatum (Table 2), which was also characterized by a significant decrease of MDA level in *A. graveolens* (125 and 250 mg/kg) treated group compared to the vehicle ($P < 0.05$). In addition, the administration of *A. graveolens* extract (125 and 250 mg/kg) significantly increased % inhibition of O_2^- both in the cortex and striatum ($P < 0.05$) (Tables 1 and 2). The most important antioxidant enzyme GPx was found to be significantly increased at all doses of *A. graveolens* treatment in the cortex and striatum ($P < 0.05$) (Tables 1 and 2).

3.8. Cresyl violet staining

The administration of 250 mg/kg *A. graveolens* significantly increased neuron density in frontal cortex and 125 mg/kg *A. graveolens* significantly increased neuron density in striatum (Figure 7).

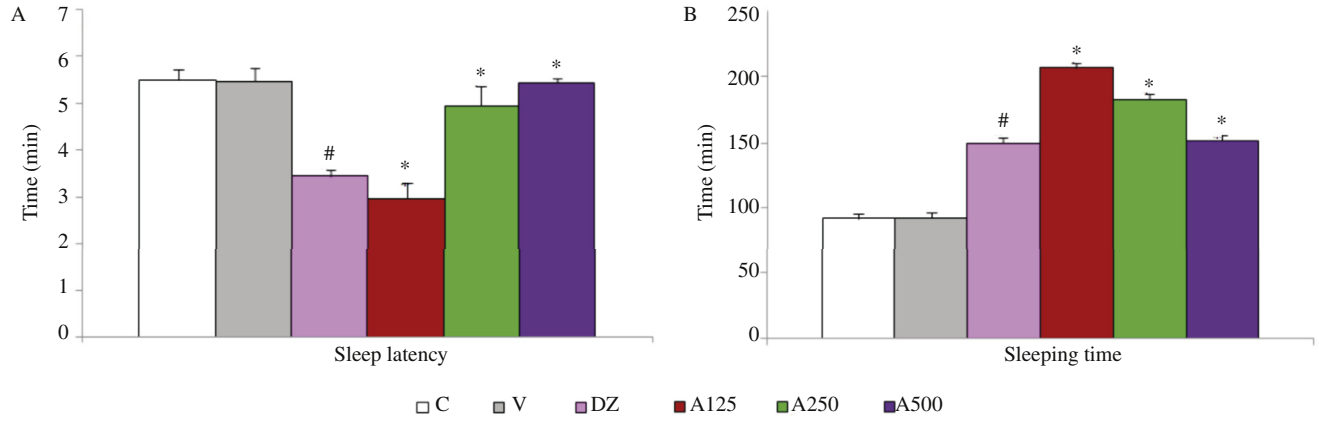

Figure 5. Effects of *A. graveolens* (A125, 250 and 500 mg/kg, *i.p.*), diazepam (DZ 2 mg/kg, *i.p.*), vehicle (V) or control (C) on the sleep latency (A) and sleeping time (B) after pentobarbital-induced sleeping time test in mice.
Data are presented as means ± SD, n = 10 each group; #: $P < 0.05$ positive control versus vehicle, *: $P < 0.05$ *A. graveolens* versus vehicle.

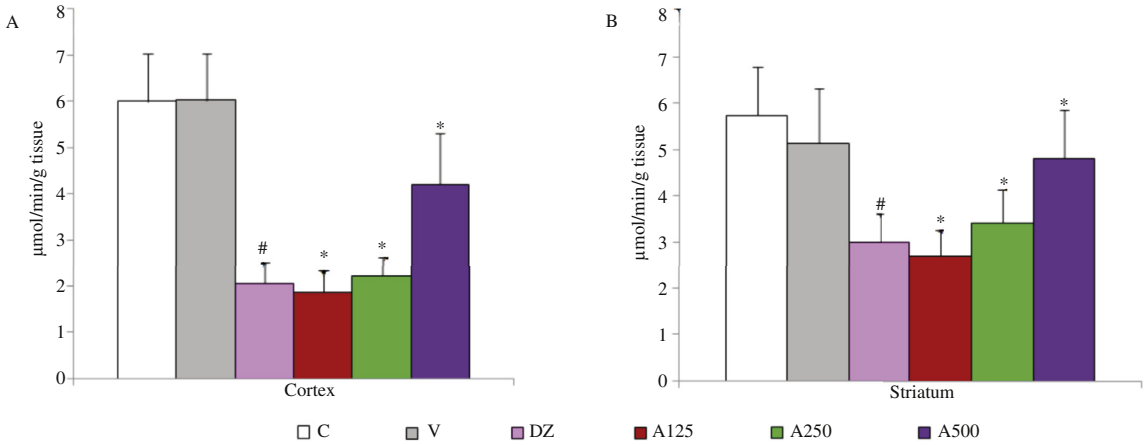

Figure 6. Effects of *A. graveolens* (A125, 250 and 500 mg/kg, *i.p.*), diazepam (DZ 2 mg/kg, *i.p.*), vehicle (V) or control (C) on MAO-A activity in the cortex (A) and striatum (B).
Data are presented as means ± SD, $n = 5$ each group. #: $P < 0.05$ positive control versus vehicle, *: $P < 0.05$ *A. graveolens* versus vehicle.

Table 1
Effects of oral administration of *A. graveolens* on lipid peroxidation and antioxidant status in the cerebral cortex.

Cortex	MDA (nmol/mg protein)	O_2^- (% inhibition)	GPx (IU/g protein)
Control	5.06 ± 0.62	10.93 ± 1.01	8.45 ± 0.71
Vehicle	4.82 ± 0.44	10.85 ± 1.72	9.09 ± 0.27
Diazepam	3.10 ± 0.51[a]	15.19 ± 0.95[a]	15.73 ± 0.90[a]
A. graveolens 125 mg/kg	3.13 ± 0.11[a]	20.68 ± 1.71[a]	16.55 ± 1.18[a]
A. graveolens 250 mg/kg	2.95 ± 0.41[a]	27.60 ± 1.29[a]	19.55 ± 1.18[a]
A. graveolens 500 mg/kg	3.35 ± 0.64[a]	14.77 ± 0.85	14.43 ± 1.04[a]

Data are presented as mean ± SD, $n = 5$ each group. [a]: $P < 0.05$ positive control or *A. graveolens* versus vehicle.

Table 2
Effects of oral administration of *A. graveolens* on lipid peroxidation and antioxidant status in the striatum.

Striatum	MDA (nmol/mg protein)	O_2^- (% inhibition)	GPx (IU/g protein)
Control	3.73 ± 0.36	12.43 ± 0.94	8.87 ± 0.68
Vehicle	3.62 ± 0.59	12.82 ± 0.71	8.54 ± 2.04
Diazepam	2.84 ± 0.58[a]	19.16 ± 1.06[a]	15.81 ± 1.22[a]
A. graveolens 125 mg/kg	2.93 ± 0.33[a]	29.68 ± 1.08[a]	18.37 ± 1.15[a]
A. graveolens 250 mg/kg	3.16 ± 0.47[a]	21.90 ± 0.73[a]	15.71 ± 1.26[a]
A. graveolens 500 mg/kg	3.56 ± 0.45	18.00 ± 0.83	12.10 ± 1.22[a]

Data are presented as mean ± SD, $n = 5$ each group. [a]: $P < 0.05$ positive control or *A. graveolens* versus vehicle.

4. Discussion

Anxiety is a psychophysiological and behavioral condition characterized by an increased arousal, expectancy, neurochemical activation and changes in behavioral patterns [1,2,14]. The pathophysiology of anxiety disorder is not yet fully elucidated; however, an involvement of neurotransmitter including γ-aminobutyric acid, serotonin and noradrenergic system in several brain regions is evidenced [15]. In the present work, we examined the anxiolytic effect of *A. graveolens* using a battery of behavioral tests including EPM, open field, light/dark, hole-board, pentobarbital-induced sleeping tests comparable to diazepam as a standard drug. Furthermore, biochemical alterations and histological study were also evaluated.

The validity of the EPM behavioral test as an anxiety model of a neuropsychiatric phenotype has been evaluated [16]. The EPM paradigm relies on response of rodents to the open spaces or the maze height suggesting anxiogenic stimuli. Diazepam, a classical anxiolytic drug commonly prescribed to target anxiety disorder, has been used in this study as a positive control. We found that diazepam treatment had a positive anxiolytic effect on EPM, similar to the previous observation [17,18]. Following various doses of *A. graveolens* administration (highest effect was found at dose of 125 mg/kg), the mice demonstrated a significantly increased time spent in the open arms compared to the control group. Hereafter, light/dark transition test has also been measured based on their aversion to the bright areas. The present study demonstrated that all doses of *A. graveolens* used in this study increased time spent in light chamber particularly at the

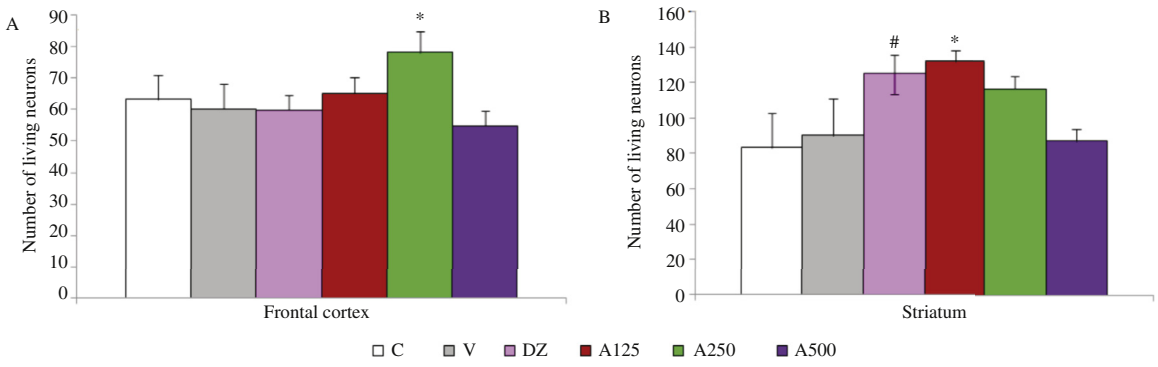

Figure 7. Effects of *A. graveolens* (A125, 250 and 500 mg/kg, *i.p.*), diazepam (DZ 2 mg/kg, *i.p.*), vehicle (V) or control (C) on neuronal density of cresyl violet staining in the frontal cortex (A) and striatum (B).
Data are presented as mean ± SD, $n = 5$ each group. #: $P < 0.05$ positive control versus vehicle, *: $P < 0.05$ *A. graveolens* versus vehicle.

dose of 125 mg/kg. Behavioral response in hole-board test was then further confirmed to measure head dip activity. The decrease in number of head dip reflects anxiety-like behavior. Our finding showed that *A. graveolens* (125–500 mg/kg, peak effect at 125 mg/kg) increase the number of head dips. Another test to confirm anxiety-related behavior, open field test, has been suggested as a model of normal anxiety sensitive to the anxiolytic effect in rodent which provides the stress created by the brightly lit, unprotected center and novel environment exploration [16]. The anxiogenic-like behavior is indicated by the avoidance of the central area approach while increased time spent in central area indicates anxiolytic effect. The *A. graveolens* extract, at all doses (peak effect at 125 mg/kg), similar to diazepam, increased the time spent in the central area. This is in good agreement with previous studies which reported the low level of anxiety-related behaviors obtained from EPM, light/dark, hole-board and open field test in response to the benzodiazepine diazepam [16,17,19]. According to our result, *A. graveolens* administration provided consistent results in the various experimental models of anxiety reinforcing its anxiolytic effect at all doses (125–500 mg/kg); however, peak effect was obtained at 125 mg/kg.

It has been shown that anxiety is one of the major causes of sleep disturbance [3]. Hence, we addressed the question of whether *A. graveolens* treatment attenuated sleep deprivation. The monoaminergic system (serotonin, 5-hydroxytryptamine) plays an important role in anxiety and control of sleep-wake behavior [20,21]. Our study demonstrated that *A. graveolens* prolonged sleep time and decreased sleep latency as shown in pentobarbital-induced sleeping time test, consistent with the reduction in the activity of MAO-A, which is the major enzyme oxidizing serotonin and may potentiate anxiety treatment. We found that treatment with *A. graveolens* preferentially inhibited MAO-A and speculated that a consequent increase in synaptic serotonin concentration reduced the anxiety-like behavior.

Recent evidence has indicated a close interrelation between anxiety disorder and oxidative stress in both human and animal which may play a major role in anxiety-related behavior [22,23]. The brain tissue is high susceptible to oxidative damage for several reasons, including (i) its high oxygen consumption, thus producing large amounts of oxygen-free radical and reactive-oxygen substrates, (ii) its rich content of radical sensitive poly-unsaturated fatty acids constituting the neuronal membranes which are ready for oxidation, and (iii) its neurotoxic metabolites effects from auto-oxidation of neurotransmitters [24]. The imbalance between reactive-oxygen species (*e.g.* O_2^-, hydrogen peroxide) overproduction and reduction of intracellular antioxidative protection (*e.g.* GPx, superoxide dismutase, catalase) results in damage to cell structures (lipids, proteins, and DNA) which are oxidized, hence causing cellular dysfunction and ultimately neuronal cell death. Our study indicated that *A. graveolens* treatment can significantly decrease the end product of lipid peroxidation MDA, increase the level of enzymatic GPx activity and % inhibition of O_2^- in the cortex and striatum of homogenated brain tissue. Ultimately, neuronal survival was shown to be increased in the brain.

Flavonoids, major bioactive compounds present in celery, have been reported on the antioxidation function via radical scavenging capacity on MDA and enhanced the activities of superoxide dismutase and GPx [25–27]. Therefore, it could be suggested the anxiolytic potential of *A. graveolens* possibly acting via the antioxidant defense mechanism and preferentially modulating the brain serotoninergic system specifically in the cortex and striatum.

Taken together, we have demonstrated the action of *A. graveolens* extract with maximal effect at 125 mg/kg body weight on anxiety and might be beneficial for the establishment in the clinical use of *A. graveolens* in patients with anxiety disorder. However, its active constituents and precise mode of actions need to be further investigated.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Koen N, Stein DJ. Pharmacotherapy of anxiety disorders: a critical review. *Dialogues Clin Neurosci* 2011; **13**: 423-37.
[2] Kessler RC, Ruscio AM, Shear K, Wittchen HU. Epidemiology of anxiety disorders. *Curr Top Behav Neurosci* 2010; **2**: 21-35.
[3] Cox RC, Olatunji BO. A systematic review of sleep disturbance in anxiety and related disorders. *J Anxiety Disord* 2016; **37**: 104-29.
[4] Ripoll LH. Psychopharmacologic treatment of borderline personality disorder. *Dialogues Clin Neurosci* 2013; **15**: 213-24.
[5] Ravindran LN, Stein MB. The pharmacologic treatment of anxiety disorders: a review of progress. *J Clin Psychiatry* 2010; **71**: 839-54.
[6] Farach FJ, Pruitt LD, Jun JJ, Jerud AB, Zoellner LA, Roy-Byrne PP. Pharmacological treatment of anxiety disorders: current treatments and future directions. *J Anxiety Disord* 2012; **26**: 833-43.
[7] Peng Y, Sun J, Hon S, Nylander AN, Xia W, Feng Y, et al. L-3-*n*-butylphthalide improves cognitive impairment and reduces amyloid-beta in a transgenic model of Alzheimer's disease. *J Neurosci* 2010; **30**: 8180-9.
[8] Sowbhagya HB. Chemistry, technology, and nutraceutical functions of celery (*Apium graveolens* L.): an overview. *Crit Rev Food Sci Nutr* 2014; **54**(3): 389-98.
[9] Uddin Z, Shad AA, Bakht J, Ullah I, Jan S. *In vitro* antimicrobial, antioxidant activity and phytochemical screening of *Apium graveolens*. *Pak J Pharm Sci* 2015; **28**: 1699-704.
[10] Powanda MC, Whitehouse MW, Rainsford KD. Celery seed and related extracts with antiarthritic, antiulcer, and antimicrobial activities. *Prog Drug Res* 2015; **70**: 133-53.
[11] Dianat M, Veisi A, Ahangarpour A, Fathi Moghaddam H. The effect of hydro-alcoholic celery (*Apium graveolens*) leaf extract on cardiovascular parameters and lipid profile in animal model of hypertension induced by fructose. *Avicenna J Phytomed* 2015; **5**: 203-9.
[12] Li J, Li Y, Ogle M, Zhou X, Song M, Yu SP, et al. DL-3-n-butylphthalide prevents neuronal cell death after focal cerebral ischemia in mice via the JNK pathway. *Brain Res* 2010; **1359**: 216-26.
[13] Huang JZ, Chen YZ, Su M, Zheng HF, Yang YP, Chen J, et al. DL-3-n-butylphthalide prevents oxidative damage and reduces mitochondrial dysfunction in an $MPP(^+)$-induced cellular model of Parkinson's disease. *Neurosci Lett* 2010; **475**: 89-94.
[14] Gerez M, Suarez E, Serrano C, Castanedo L, Tello A. The crossroads of anxiety: distinct neurophysiological maps for different symptomatic groups. *Neuropsychiatr Dis Treat* 2016; **12**: 159-75.

[15] Nuss P. Anxiety disorders and GABA neurotransmission: a disturbance of modulation. *Neuropsychiatr Dis Treat* 2015; **11**: 165-75.
[16] Sestakova N, Puzserova A, Kluknavsky M, Bernatova I. Determination of motor activity and anxiety-related behaviour in rodents: methodologic aspects and role of nitric oxide. *Interdiscip Toxicol* 2013; **6**: 126-35.
[17] Kurhe YV, Radhakrishnan M, Thangaraj D, Gupta D. Anti-anxiety effect of a novel 5-HT_3 receptor antagonist N-(benzo[*d*]thiazol-2-yl)-3-ethoxyquinoxalin-2-carboxamide (6k) using battery tests for anxiety in mice. *Indian J Pharmacol* 2014; **46**: 100-4.
[18] Mesfin M, Asres K, Shibeshi W. Evaluation of anxiolytic activity of the essential oil of the aerial part of *Foeniculum vulgare* Miller in mice. *BMC Complement Altern Med* 2014; **14**: 310.
[19] Nirwane AM, Gupta PV, Shet JH, Patil SB. Anxiolytic and nootropic activity of *Vetiveria zizanioides* roots in mice. *J Ayurveda Integr Med* 2015; **6**: 158-64.
[20] Jindal A, Mahesh R, Kumar B. Anxiolytic-like effect of linezolid in experimental mouse models of anxiety. *Prog Neuropsychopharmacol Biol Psychiatry* 2013; **40**: 47-53.
[21] Fajemiroye JO, Galdino PM, Alves SF, de Paula JA, de Paula JR, Ghedini PC, et al. Involvement of 5-HT_{1A} in the anxiolytic-like effect of dichloromethane fraction of *Pimenta pseudocaryophyllus*. *J Ethnopharmacol* 2012; **141**: 872-7.
[22] Salim S, Sarraj N, Taneja M, Saha K, Tejada-Simon MV, Chugh G. Moderate treadmill exercise prevents oxidative stress-induced anxiety-like behavior in rats. *Behav Brain Res* 2010; **208**(2): 545-52.
[23] Patki G, Solanki N, Atrooz F, Allam F, Salim S. Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rcat model of social stress. *Brain Res* 2013; **1539**: 73-86.
[24] Barros MP, Poppe SC, Bondan EF. Neuroprotective properties of the marine carotenoid astaxanthin and omega-3 fatty acids, and perspectives for the natural combination of both in krill oil. *Nutrients* 2014; **6**(3): 1293-317.
[25] Jung WS, Chung IM, Kim SH, Kim MY, Ahmad A, Praveen N. *In vitro* antioxidant activity, total phenolics and flavonoids from celery (*Apium graveolens*) leaves. *J Med Plant Res* 2011; **5**: 7022-30.
[26] Li P, Jia J, Zhang D, Xie J, Xu X, Wei D. *In vitro* and *in vivo* antioxidant activities of a flavonoid isolated from celery (*Apium graveolens* L. var. *dulce*). *Food Funct* 2014; **5**: 50-6.
[27] Yao Y, Sang W, Zhou M, Ren G. Phenolic composition and antioxidant activities of 11 celery cultivars. *J Food Sci* 2010; **75**: C9-13.