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Essential oils from Apiaceae as valuable resources in neurological disorders: *Foeniculi vulgare aetheroleum*

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ABSTRACT

Used as a spice and to improve the palatability of different meat and vegetable dishes, common fennel, *Foeniculum vulgare* Mill. (Apiaceae), was a traditional remedy for the relief of spasms and colic due to gas accumulation, to stimulate gastrointestinal motility, to alleviate productive coughs as well as for the induction of menstruation and lactation. Fennel essential oil extracted from fennel fruits is used as traditional medicine to improve eyesight, promote courage and mental strength, reduce stress/nervousness and produce calming.

Therefore, we wanted to reinforce some of the folk uses with scientific proves. The present study analyzed the anxiolytic and antidepressant of the fennel essential oil in beta-amyloid (1-42) rat model of Alzheimer's disease (AD). The antioxidant activity of the essential oil was tested *in vitro*. The anxiolytic- and antidepressant-like effects of the fennel essential oil were studied by means of *in vivo* (elevated plus-maze and forced swimming tests) approaches. The beta-amyloid (1-42)-treated rats exhibited the following: decrease of the exploratory activity, the percentage of the time spent and the number of entries in the open arm within elevated plus-maze test and decrease of swimming time and increase of immobility time within forced swimming test. Inhalation of the fennel essential oil significantly exhibited anxiolytic- and antidepressant-like effects.

Our results suggest that the fennel essential oil inhalation ameliorates beta-amyloid (1-42)-induced anxiety and depression in laboratory rats. Thus, the results of the present study indicate that the fennel essential oil may have potential clinical applications in the management of anxiety and depression related to AD conditions.

1. Introduction

Although cognitive symptoms are characteristic of Alzheimer's disease (AD), non-cognitive symptoms are becoming increasingly important because of the prevalence and dysfunctions they generate (Cañete et al. 2015). Non-cognitive symptoms, such as agitation, aggression, depression, anxiety and psychosis are often observed in AD patients. These symptoms known as "behavioral and psychological symptoms of dementia" (BPSD) have been reported to occur in about 20% of AD patients (Takeda et al., 2014). These symptoms increase the caregiver stress and the impairment in daily living activities, worsen patients quality of life and also accelerate cognitive decline (Fernandez et al., 2010). It is known that the olfactory system correlates with limbic system by influencing emotions, memory and learning. Experimental studies have shown that olfactory pathway stimuli reduces amyloid beta deposits in transgenic mice brain (Lazarov et al., 2005).

Worldwide, medicinal plant research has progressed constantly in search for new therapeutic products used in neurological disorders. Therefore, different animal models were used to exemplify the pharmacological effectiveness of many plant species (Mesfin et al., 2014).

Foeniculum vulgare Mill. (Apiaceae) commonly known as fennel, is an aromatic plant widely cultivated in temperate and tropical regions (Aprotosoaie et al., 2010). It has been reported that *F. vulgare* exhibited medicinal effects as evidenced by different animal and clinical studies include, antibacterial and antifungal (Özcan et al., 2006), antioxidant (Barros et al., 2009), anti-inflammatory (Chainy et al., 2000), anti-atherosclerotic (Oulmouden et al., 2011), gastroprotective (Birdane et al., 2007), hepatoprotective (Özbek

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et al., 2003) and diuretic (Wright et al., 2007). Moreover, it has been reported that the crude extract of *F. vulgare* has an anxiolytic profile in mice model (Kishore et al., 2012). One of the most recent studies (Liao et al., 2012) examined the effect of short/long-term enriched odor exposure on the tau phosphorylation at multiple sites in the rat brain. The results suggested that long-term odor exposure can decrease the phosphorylation of tau proteins (with an important role in the dynamic structure and function of the neurons), indicating possible therapeutic strategies in the treatment of AD. However, the data regarding neuroprotective activity of the fennel essential oil is scarce.

Here we hypothesized that exposure to *F. vulgare* essential oil could ameliorate amyloid beta (1-42)-induced anxiety and depression in laboratory rats. Beta-amyloid peptide represents the major constituent of amyloid plaques in the brains of patients with Alzheimer's disease and Down's syndrome. Amongst these, amyloid beta (1-42) is the most common protein sequence used in animal models of AD.

Our main objectives were: 1. the extraction and chemical characterization of *F. vulgare* essential oil from a controlled environment; 2. to establish the antioxidant activity of the essential oil; 3. to evaluate the anxiolytic and antidepressant activity of two concentrations of *F. vulgare* essential oil on a rat model of AD. Environmental enrichment (inhalation via an electronic vaporizer) was chosen to administer the essential oil to the rats.

2. Material and methods

2.1. Plant material and essential oil preparation

Knowing that the chemical composition depends on plant origin, ecological and environmental conditions in which the plant grows, the plant material was obtained from a controlled environment, with no pesticides. For this, our collaborators have first obtained the seedlings in the lab, and then introduced them in the experimental fields for two years. The fruits were harvested on the third year of growth. Mature *F. vulgare* fruits were harvested during August 2011 from biological crops of the Biological Research Center "Stejarul" Piatra Neamt, Romania. The plant material was dried in an oven, controlling permanently the air flow, humidity and temperature (35 °C). The voucher specimens (no. 2011/Fv) were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy "Gr. T. Popa" Iasi, Romania, prior to the experiments.

The essential oil was obtained by steam distillation in a Clevenger type apparatus for 3 h. Then, the isolated samples were dried over anhydrous sulphate and stored at −4 °C until testing.

2.2. Essential oil analysis and identification

The analysis of the essential oil was performed with an Agilent 6890 GC–MS system, equipped with a split/splitless injector. GC–MS conditions were: line/detector temperature 250 °C, carrier gas (helium, 1 mL/min), split ratio 100:1, capillary column DB 5MS (30 m × 0.25 mm; film thickness 0.25 μm; Agilent, Palo Alto, CA, USA). The temperature program was established to grow with 10 °C/min up to 280 °C. The injected volume was 0.30 μL with a total scan time of 32 min. The comparison of the retention indices (RI), retention times (RT) and mass spectra with those obtained from authentic Wiley libraries (available through Hewlett Packard) and published mass spectra (Adams, 2007) were used to identify the compounds. The GC-FID analysis used an Agilent type 6890 GC connected to a FID detector and all method and analysis parameters were the same as describe above.

To record KI values of *n*-alkanes (8–20 and 21–40) for further references, aliquots of the solutions (0.30 μL) were subjected to GC analysis in similar conditions to the samples.

2.3. In vitro *antioxidant activity assays*

The antioxidant capacity of the *F. vulgare aetheroleum* was assessed by two complementary tests: free radical scavenging potential and inhibition of 15-lipoxigenase.

2.4. DPPH assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical is stable and strongly absorbs at 517 nm. By reaction with a scavenger of free radicals the DPPH solution changes color from purple to yellow due to the formation diphenyl-picrylhydrazyne (DPPH-H). Briefly, stock solution (60 mg/mL) of the essential oil was diluted in methanol to obtain concentrations ranging from 25 μL/mL to 100 μL/mL. Diluted solutions (1 mL each) were mixed with 1 mL of a freshly prepared 4% DPPH radical methanol solution. The mixture was then incubated in the dark for 30 min at room temperature. The absorbance was recorded on an ultraviolet–visible (UV–vis) spectrometer (ABL&E JASCO) against the blank. The free radical scavenging activity was calculated as percent inhibition according to the following equation:

$$I\% = 100 \times \text{scavengeractivity}(\text{Acontrol} - \text{Asample})/\text{Acontrol},$$

In parallel, for each sample IC50 value (sample concentration providing 50% inhibition) was calculated, expressed in μL/mL final solution. All values were reported as means ± SD of triplicates.

2.5. Inhibition of 15-lipoxygenase

Essential oil compounds have the ability to inhibit the activity of lipoxygenase which catalyzes the oxidation of linoleic acid, thus reducing the absorbance at 234 nm. 0.05 mL of borate buffer lipoxygenase solution was treated with 0.05 mL of fennel essential oil solution in DMSO and left to stand for 10 min at room temperature, after which 2 mL of linoleic acid in borate buffer were added. The absorbance of each sample at 234 nm was recorded for up to 120 s (Maltreud and Rydland, 2000). Essential oil dilutions in DMSO ranged from 0.78 to 25 μL/mL. Lipoxygenase inhibitory capacity was calculated according to the formula:

$$I\% = (\text{AEFI} - \text{AECI}) \times 100/\text{AEFI}.$$

2.6. Animals

The study used 60 male Wistar rats (3 months old) weighing 250 ± 50 g at the start of the experiment. The animals were housed in a temperature and light-controlled room (22 °C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water *ad libitum*. The rats were divided into 6 groups (10 animals per group): (1) Control group received 0.9% saline with 1% Tween 80 treatment; (2) Aβ (1-42) alone-treated group received 0.9% saline with 1% Tween 80 treatment, as negative control; (3) Diazepam alone-treated group (DZP, 1.5 mg/kg) received 0.9% saline with 1% Tween 80 treatment, as positive control; (4) Tramadol alone-treated group (TRM, 10 mg/kg) received 0.9% saline with 1% Tween 80 treatment, as positive control; (5) Aβ (1-42)-treated group received by inhalation *F. vulgare* essential oil 1% (Aβ (1-42) + FVO1%) and (6) Aβ (1-42)-treated group received by inhalation *F. vulgare* essential oil 3% (Aβ (1-42) + FVO3%). Control, DZP, TRM- and Aβ (1-42) alone-treated groups were caged in the same conditions but in the

absence of the tested essential oil. They were subjected to inhale 0.9% saline with 1% Tween 80 solution.

Animals used in this study were treated in accordance with the guidelines of the animal bioethics of the Act on Animal Experimentation and Animal Health and Welfare from Romania and all procedures were in compliance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Alexandru Ioan Cuza University of Iasi. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize animal suffering and to reduce the number of animal used.

2.7. Neurosurgery

All surgical procedures were conducted under aseptic conditions, under sodium pentobarbital (50 mg/kg b.w., i.p., Sigma-Aldrich, Germany) anesthesia. Rats were mounted in the stereotaxic apparatus with the nose oriented 11° below horizontal zero plane. Animal model of AD was established by intracerebroventricular (i.c.v.) injection of 400 pmol Aβ (1-42) (Sigma-Aldrich, Germany), 20 days prior to inhalation of fennel essential oil (1% and 3%) according to the procedure established by Laursen and Belknap (1986). Aβ (1-42) was administered right-unilaterally through Hamilton syringe over 4 min, and the syringe was left in place for 5 min after injection before being slowly removed. The injection volume (4 μL) was delivered gradually (1 μL/min) using the following coordinates: 1.5 mm lateral to the midline; 7.4 mm ventral to the surface of the cortex (Paxinos and Watson, 2005). The sham-operated rats were injected with saline.

2.8. Inhalation apparatus

The inhalation apparatus consisted of a Plexiglas chamber (50 cm × 40 cm × 28 cm). Two chambers were used, one for the control, DZP, TRM and Aβ(1-42) alone-treated animals, which were exposed to 0.9% saline with 1% Tween 80 solution, and the other one for the experimental animals, which were exposed to fennel essential oil. Fennel essential oil were diluted with 1% Tween 80 (v/v). Fennel essential oil exposure (200 μL, either 1% or 3%) was via an electronic vaporizer placed at the bottom of chamber, but out of reach of the animals. Rats in the fennel groups were exposed to oil vapors for controlled 60 min period, daily, for 21 continuous. 60 min is a suitable inhalation period for the expected effects (Linck et al., 2010).

2.9. Behavioral tests

2.9.1. Elevated plus-maze test

Behavior in the elevated plus-maze (EPM) is also utilized to assess exploration, anxiety, and motor behavior. The EPM consists of four arms, 49 cm long and 10 cm wide, elevated 50 cm above the ground. Two arms were enclosed by walls 30 cm high and the other two arms were exposed. 60 min after the inhalation of *F. vulgare* essential oil (1% and 3%), each rat was placed in the center of the maze facing one closed arm. Behavior was observed for 5 min, and the time spent and number of entries into the open and enclosed arms was counted (Hayashi et al., 2012). The percentages of time spent in the open arms (time spent in the open arms/time spent in all arms × 100) were calculated. An entry was defined as an animal placing all four paws into an arm, and no time was recorded when the animal was in the central area. The maze floor was cleaned with cotton and 10% ethanol solution between subjects.

Table 1
Chemical composition (selective) of the essential oil from *Foeniculum vulgare* seeds.

No.	Compound	RT (min)	RI[a]	%
1	α-thujene	6.1	930	tr
2	β-pinene	6.4	939	2.6
3	camphene	6.5	954	tr
4	sabinene	6.6	975	0.2
5	myrcene	6.8	991	0.3
6	*p*-cymene	7.0	1025	0.1
7	L-limonene	7.2	1029	0.9
8	γ-terpinene	7.4	1060	0.2
9	*trans*-sabinene hydrate	7.8	1071	3.1
10	α-fenchone	8.2	1094	0.5
11	α-thujone	8.5	1116	0.1
12	camphor	8.9	1146	21.3
13	estragole	9.6	1195	0.6
14	(+)-carvone	10.4	1243	7.8
15	*p*-anisaldehyde	11.3	1251	1.5
16	*trans*-anethole	11.8	1289	58.1
17	germacrene D	12.7	1561	0.3
Total identified				97.6
Other compounds[b]				2.4

[a] RI provided for HP-5MS column.
[b] this category includes monoterpenes and oxidized terpenes with amounts less than 0.1% that are not included in the table.

2.9.2. Forced swimming test (FST)

The FST is the most widely used model for assessing depressive-like response (Cryan et al., 2002). The depressive-like response was assessed, basically using the same method described by Campos et al. (2005), but with modification. On the first day of the experiments (pretest session), rats were individually placed into cylindrical recipients (diameter 30 cm, height 59 cm) containing 25 cm of water at 26 ± 1 °C. The animals were left to swim for 15 min before being removed, dried and returned to their cages. The procedure was repeated 24 h later, in a 6 min swim session (test session), 60 min after the inhalation of *F. vulgare* essential oil (1% and 3%). During the test session, the following behavioral responses were recorded: (1) immobility (time spent floating with the minimal movements to keep the head above the water); and (2) swimming (time spent with active swimming movements).

3. Statistical analysis

The animal's behavioral activities within elevated plus-maze and forced swimming tests were statistically analyzed with two-way analysis of variance (ANOVA). All results are expressed as mean ± standard error of mean (S.E.M.). *F* values for which p\0.05 were regarded as statistically significant. Significant differences were determined by Tukey's *post hoc* test.

4. Results

4.1. Chemical composition

The fennel essential oil was prepared by hydrodistillation of the dried plant material and its chemical composition were analyzed by GC–MS/GC-FID. In total, 71 components were identified, but among them only 11 representing 96.8% (Table 1, Fig. 1) from the total amount. *trans*-Anethole (58.1%), a phenylpropanoid, was found as the main component. Camphor (21.3%), a terpene ketone, was the second major compound detected in fennel essential oil, followed by carvone (7.8%), *trans*-sabinene hydrate (3.1%), β-pinene (2.6%), and others were found to be the minor components in the essential oil of fennel seeds. According to this profile of the components, our fennel essential oil has anxiolytic activity and works mainly as tonic agent for the central nervous system (CNS).

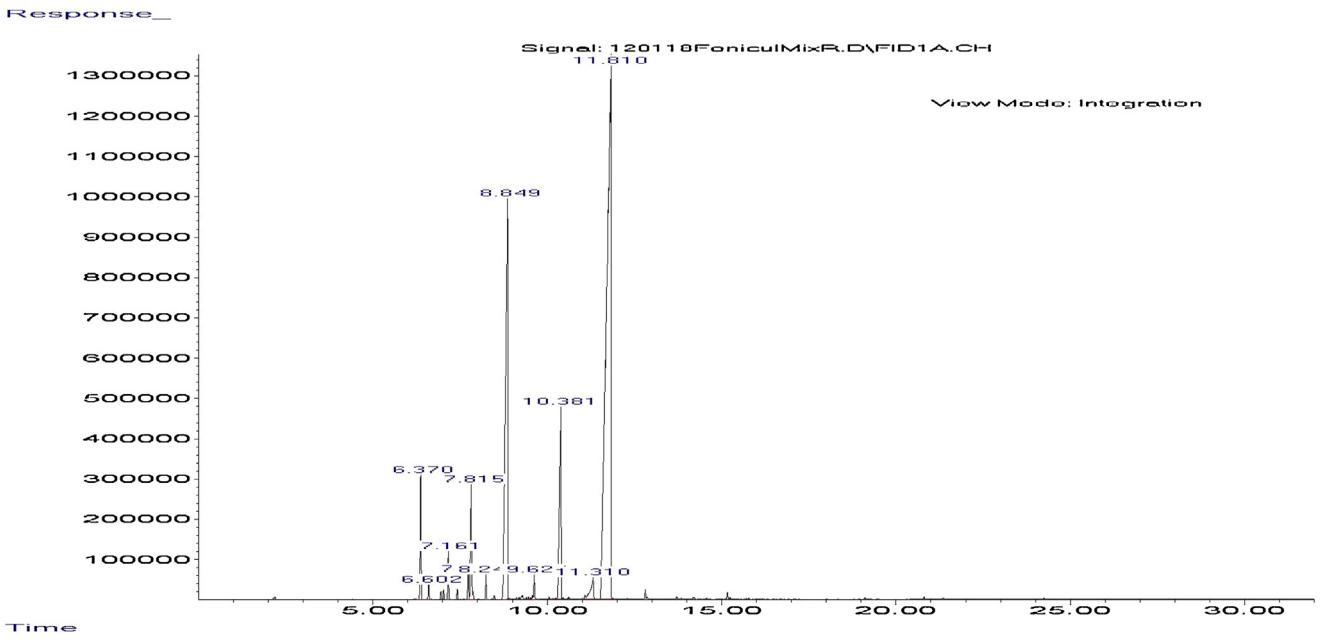

Fig. 1. The GC profile of the *Foeniculum vulgare* essential oil.

4.2. Antioxidant capacity

For *in vitro* testing we chose linalool and *trans*-anethole as controls, but in most of the assays their activity was lower than that of the diluted essential oil. Thus, we believe that the actual biologic activity belongs to the phytocomplex possibly by synergistic effects and is less given by a single compound. Similar results were obtained by Misharina and Polshkov (2005), when *trans*-anethole as a single compound had a lower antioxidant activity than coriander essential oil.

In DPPH assay our data showed that the intensity of the scavenger activity is directly proportional to and depends on the concentration of the sample. IC50 value for fennel essential oil was 27.5 ± 0.3 μL/mL. The *F. vulgare* essential oil tested in this research has protective action against lipoxygenase because concentrations of 25 μL/mL inhibit the enzyme by over 75%. Moreover, low IC50 values (0.071 ± 0.02 μL/mL) are an indicator of promising opportunities to use the essential oil in therapy, such low doses being safe and non-toxic.

4.3. Effect of fennel essential oil on elevated plus-maze behavior

In the elevated plus-maze task (Fig. 2a) ANOVA revealed a significant overall differences between all groups ($F(4,45) = 11.01$, $p < 0.0001$) on the percentage of the time spent in the open arms. Both doses of the fennel essential oil (1% and 3%), but especially 3%, significantly increased the percentage of the time spent in the open arms in Aβ (1-42)-treated groups as compared to Aβ (1-42) alone-treated group.

In Fig. 2b ANOVA revealed a significant overall difference between all groups ($F(4,45) = 10.06$, $p < 0.0001$) on the number of open-arm entries. The inhalation of the fennel essential oil (1% and 3%), but especially 3%, significantly increased on the number of open-arm entries of Aβ (1-42)-treated groups as compared to Aβ (1-42) alone-treated group.

Significant overall differences between all groups ($F(4,45) = 9.23$, $p < 0.0001$) on the number of crossing (exploratory activity) are shown in Fig. 2c. The inhalation of the fennel essential oil (1% and 3%), but especially 3%, significantly increased the exploratory activity of Aβ (1-42)-treated groups as compared to Aβ(1-42) alone-treated group. This behavior indicates the lack of anxiety of exploring open spaces (Caspani et al., 2014).

The diazepam treatment significantly increased the percentage of the time spent in the open arms, the number of open-arm entries and the number of crossing as compared to Aβ (1-42)-alone-treated group, acting as an anxiolytic agent.

4.4. Effect of fennel essential oil in the rat forced swimming test

In the forced swimming test, ANOVA revealed a significant overall differences between all groups on the swimming time ($F(4,45) = 10.26$, $p < 0.0001$) (Fig. 3a) and on the immobility time ($F(4,45) = 8.90$, $p < 0.0001$) (Fig. 3b). Both doses of the fennel essential oil (1% and 3%), but especially 3%, significantly increased swimming time and decreased immobility time of Aβ (1-42)-treated groups as compared to Aβ (1-42) alone-treated group.

The tramadol treatment increased the swimming time and decreased the immobility time as compared to Aβ (1-42)-alone-treated group, acting as an antidepressant agent.

5. Discussion

The present study was aimed to examine the anxiety and depressive-like response following inhalation of the *F. vulgare* essential oil (200 μL, 1% and 3%) in rats subjected to injection of Aβ (1-42). Consequently, injection of Aβ (1-42) causes anxiety-like behavior and depressive-like response, in accordance with our previous investigation (Colaianna et al., 2010).

The GC–MS/FID analysis of our *F. vulgare* essential oil indicated *trans*-anethole (58.135%), a phenylpropanoid, followed by camphor (21.297%), a terpene ketone, as the main components of the essential oil suggesting that these constituents could be responsible for the observed anxiolytic-antidepressant like-behavior in Aβ (1-42)-treated rats. The major component of the essential oil, *trans*-anethole, has been reported to display a potent anxiolytic activity in mice (Miyagawa et al., 2014).

The antioxidant assays used for this research are commonly used, nevertheless the lipophilic character of the essential oil imposes careful amendments to the methods. Although the DPPH scavenging activity is the primary test used to indicate the

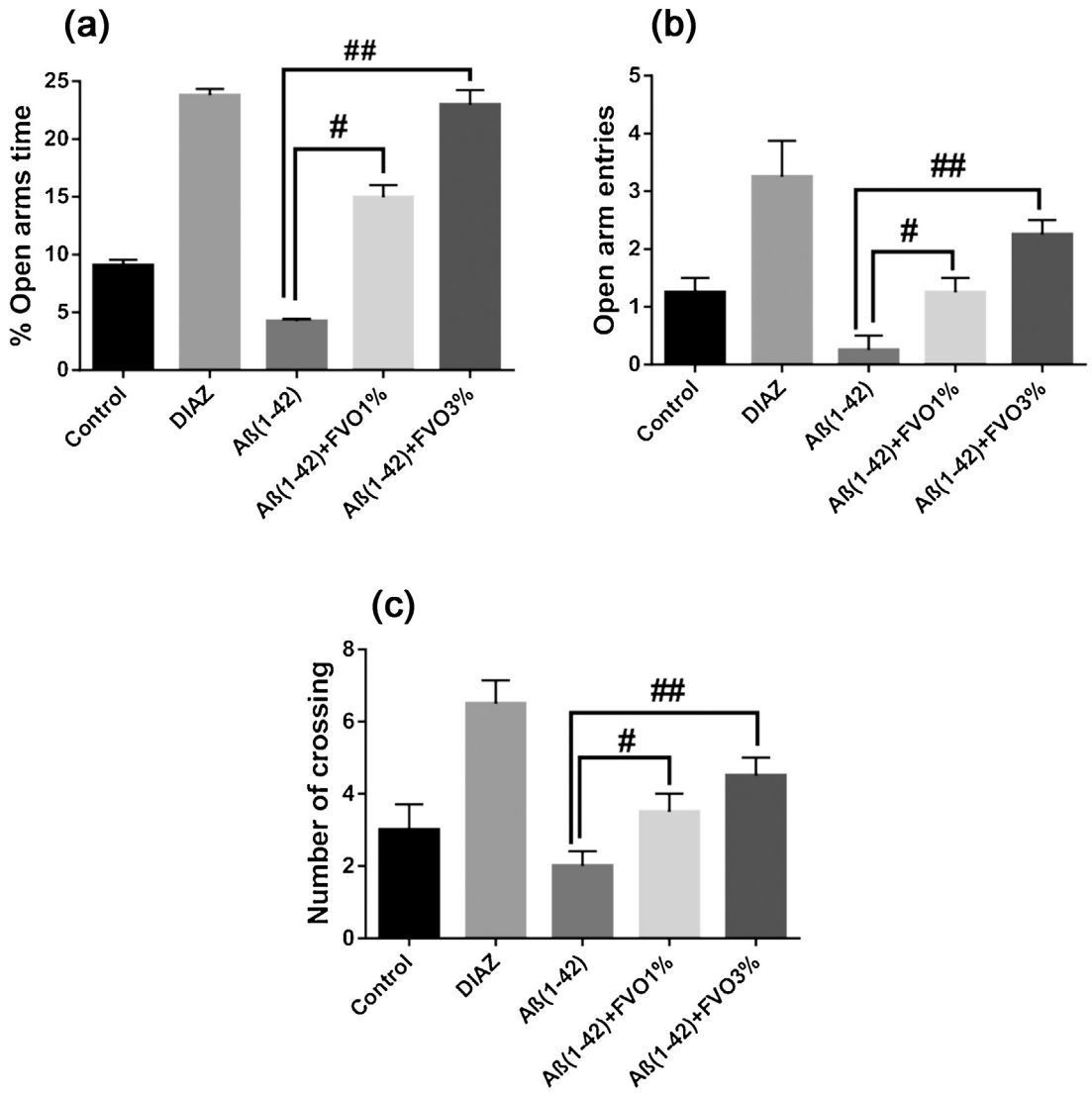

Fig. 2. Effects of inhaled *Foeniculum vulgare* essential oil (1% and 3%) in the elevated plus-maze test on the percentage of the time spent in the open arms (a), the number of open-arm entries (b) and the number of crossing (c) in the Aβ (1-42)-treated rats. Values are means ± S.E.M. (n = 10 animals per group). For Turkey's *post hoc* analysis — $^{\#}$Aβ (1-42) *vs.* Aβ (1-42) + FOV1%: $p < 0.0001$ and $^{\#\#}$Aβ (1-42) *vs.* Aβ (1-42)+ FOV3%: $p < 0.0001$ (a), $^{\#}$Aβ (1-42) *vs.* Aβ (1-42)+ FOV1%: $p < 0.001$ and $^{\#\#}$Aβ (1-42) *vs.* Aβ (1-42)+ FOV3%: $p < 0.0001$ (b) and $^{\#}$Aβ (1-42) *vs.* Aβ (1-42)+ FOV1%: $p < 0.001$ and $^{\#\#}$Aβ (1-42) *vs.* Aβ (1-42)+ FOV3%: $p < 0.0001$ (c).

antioxidant capacity of a plant extract, the inhibition of 15-lipoxigenase was chosen because this oxide-reductase enzyme is widely distributed in the brain. Also, literature data showed that the overexpression of this enzyme could cause Alzheimer's disease and various anxiety disorders. Therefore, the inhibition of 15-lipoxigenase decreases the oxidation processes of proteins post-ischemia and impaired blood-brain barrier (Maltreud and Rydland, 2000; Schneider and Bucar, 2005). However, these results should be correlated with *in vivo* data for future research perspectives.

The elevated plus-maze is recognized as a valuable model able to predict anxiolytic- or anxiogenic-like effects of drugs in rodents (Blainski et al., 2010). The percentage of the time spent in the open arms and the number of open-arm entries of Aβ (1-42)-treated rats was significantly decreased (Fig. 2a and b). This indicates that the Aβ (1-42)-treated rats experienced high levels of anxiety and were suitable for evaluating the presumed anxiolytic substances as our essential oil (Hayashi et al., 2012). Furthermore, after the Aβ (1-42)-treated rats being exposed to *F. vulgare* essential oil, the percentage of time spent in the open arms significantly increased in a dose-dependent manner as compared to Aβ (1-42)-alone treated rats. Additionally, the number of open arms entries and number of crossing (exploratory activity) (Fig. 2c) increased in the Aβ (1-42)-treated rats exposed to *F. vulgare* essential oil (3%).

As expected, diazepam (DZP) as a benzodiazepine drug used as positive control produced significant increase in the percentage of time spent in the open arms, the number of open-arm entries and the number of crossing as compared to Aβ (1-42)-alone treated rats. These data are consistent with the results of numerous previous studies, which have shown that DZP and other benzodiazepines produce significant anxiolytic effects in a variety of anxiolytic screening procedures, including elevated plus-maze test procedures (Adebesin et al., 2015; Leggio et al., 2015). The pharmacological action of diazepam enhances the effect of the neurotransmitter GABA by binding to the benzodiazepine site on the GABA-A receptor (via the constituent chlorine atom) leading to central nervous system (CNS) depression (Riss et al., 2008). The anxiety indicators in the elevated plus-maze (the percentage of the time spent in the open arms and the number of open-arm entries) showed up being sensitive to the agents which were thought to act via the GABA-A receptor complex (Emamghoreishi et al., 2005). Moreover, it has been reported that *trans*-anethole display a potent anxiolytic activity in mice (Miyagawa et al., 2014). In light with

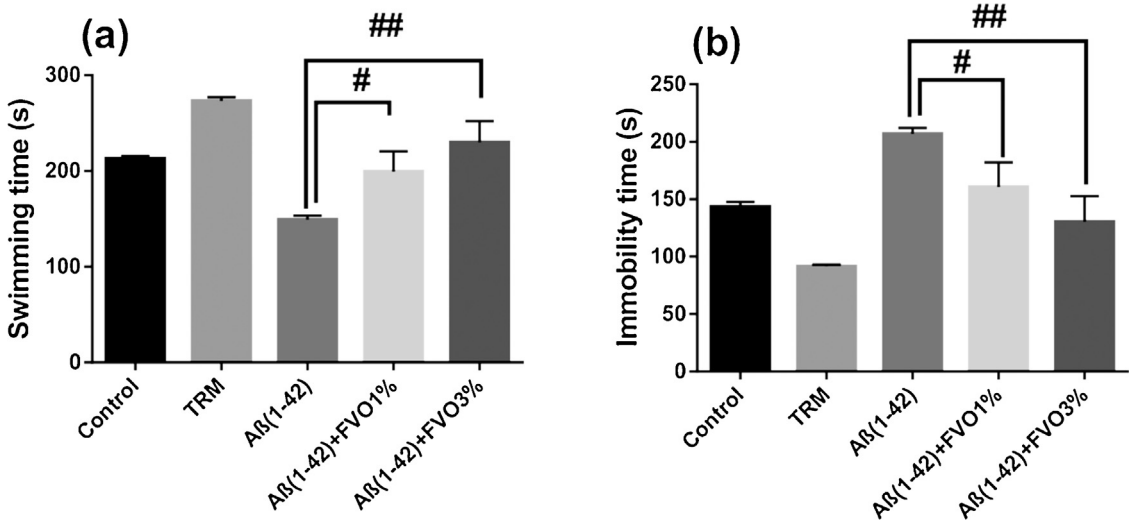

Fig. 3. Effects of inhaled *Foeniculum vulgare* essential oil (1% and 3%) on swimming time (a) and immobility time (b) in the Aβ (1-42)-treated rats during the 6 min period in the forced swimming test. Values are means ± S.E.M. (n = 10 animals per group). For Turkey's *post hoc* analysis – #Aβ (1-42) *vs*. Aβ (1-42) + FOV1%: $p < 0.001$ and ##Aβ (1-42) *vs*. Aβ (1-42)+ FOV3%: $p < 0.001$ (a) and #Aβ (1-42) *vs*. Aβ (1-42) + FOV1%: $p < 0.001$ and ##Aβ (1-42) *vs*. Aβ (1-42)+ FOV3%: $p < 0.0001$ (b).

these reports, our high- *trans*-anethole (58.135%) containing *F. vulgare* essential oil have increased anxiolytic-like behavior and anti-depressive-like response in Aβ (1-42)-treated rats.

The forced swimming test has been validated as a suitable tool for predicting the antidepressant properties of drugs (Cioanca et al., 2014). When rodents are forced to swim in a confined space, after an initial period of struggling, they would become immobile, resembling a state of despair and mental depression. This inescapable stressful situation can be evaluated by assessing different behavioral strategies (Porsolt et al., 1977). As shown in Fig. 3a and b, the swimming time decreased and the immobility time increased in Aβ (1-42) alone-treated rats as compared to control rats. This indicates that the Aβ (1-42) alone-treated rats exhibited depression. After being exposed to both doses of *F. vulgare* essential oil (1% and 3%), the swimming time significantly increased in a dose-dependent manner. Moreover, the decrease of the immobility time in a dose-dependent manner was also observed.

These results suggested that *F. vulgare* essential oil possesses a strong antidepressant-like response to an inescapable stress. In our study, tramadol (TRM), as positive control, produced significant increases in the swimming time and decreases the immobility time as compared to Aβ (1-42)-alone treated rats. Tramadol is a unique drug with multiple modes of action. It is a weak agonist of the μ-opioid receptor but it also inhibits the reuptake of serotonin as well as norepinephrine. It is an analgesic and it is also considered as an antidepressant (Caspani et al., 2014).

6. Conclusions

Our data provide evidence for the link of enriched fennel odor exposure with anxiolytic and antidepressant effects in an AD rat model. These biologic activities are dose dependent and should be correlated to the chemical composition of the essential oil. Moreover, literature indicates a direct connection between the cognitive decline and anxiety in patients with dementia. Often, depression and anxiety increase the severity of cognitive impairment for these patients (Wuwongse et al., 2010). Therefore, inhalation of *F. vulgare* essential oil might offer a useful alternative or complementary choice in either the prevention or the treatment of psychiatric condition close related to AD conditions.

A very important aspect is related to the source of the vegetal material. Our choice was based on our previous experience regarding the chemical and microbial quality of a vegetal product that is to be used for therapy. The organic crops that are the best controlled environment represent the best choices in such cases, the repeatability being assured. For pharmaceutical industry high qualitative crops ensure a good productivity and lower costs for herbal extracts production. Furthermore, the use of phytopharmaceuticals as complementary or alternative medicine, either to prevent or to ameliorate many diseases, still remains a trend for the future generations to come. On the other hand, there are no potentially adverse impacts on the environment and human health.

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