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Anthriscus nemorosa essential oil inhalation prevents memory impairment, anxiety and depression in scopolamine-treated rats



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ABSTRACT

Anthriscus nemorosa (Bieb.) Sprengel is used for medicinal purposes in traditional medicine around the world, including Turkey. Ethnobotanical studies suggest that *Anthriscus* essential oil could improve memory in Alzheimer's disease. The current study was hypothesized to investigate the beneficial effects of inhaled *Anthriscus nemorosa* essential oil on memory, anxiety and depression in scopolamine-treated rats. *Anthriscus nemorosa* essential oil was administered by inhalation in the doses of 1% and 3% for 21 continuous days and scopolamine (0.7 mg/kg) was injected intraperitoneally 30 min before the behavioral testing. Y-maze and radial arm-maze tests were used for assessing memory processes. Also, the anxiety and depressive responses were studied by elevated plus-maze and forced swimming tests. As expected, the scopolamine alone-treated rats exhibited the following: decrease the percentage of the spontaneous alternation in Y-maze test, increase the number of working and reference memory errors in radial arm-maze test, 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. However, dual scopolamine and *Anthriscus nemorosa* essential oil-treated rats showed significant improvement of memory formation and exhibited anxiolytic- and antidepressant-like effects in scopolamine-treated rats.

These results suggest that *Anthriscus nemorosa* essential oil inhalation can prevent scopolamine-induced memory impairment, anxiety and depression.

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1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder, accounting for 70% of all dementia cases [1]. AD affects millions of people worldwide causing massive economic burden and the number of cases is expected to rise dramatically [2].

Several pathological conditions characterize AD including neuronal loss, the accumulation of senile plaques formed by extracellular deposits of amyloid beta (A β) peptides, intracellular neurofibrillary tangles formed by hyperphosphorylated tau proteins, proliferation of astrocytes, and activation of microglia [3].

Cholinergic hypofunction is one of the hallmarks of AD. It was reported to be caused by significant loss of cholinergic neurons, cholinergic neuronal and axonal abnormalities, and reduction in the numbers of postsynaptic neurons accessible to acetylcholine

(ACh). It is evidenced that cholinergic hypofunction is closely related to A β and tau pathologies [4]. According to cholinergic hypothesis, memory deficits in AD are caused by cholinergic hypofunction leading to reduced hippocampal and cortical levels of ACh. In healthy brain, acetylcholinesterase (AChE) is the most important enzyme regulating ACh levels, while butyrylcholinesterase (BChE) has a minor role. Until now, cholinesterase inhibitors are the only approved drugs for treating patients with AD [5].

Epidemiological studies show that neuropsychiatric symptoms (NPS) are common in AD. For example, 51% of new onset AD patients were observed to have at least one NPS. The most common symptoms were depression (25%), apathy (17%), and irritability (17%) [6].

Scopolamine, a muscarinic receptor antagonist, crosses the blood brain barrier and induces dementia and cognitive dysfunction [7]. Amnesic effects of scopolamine have been verified by a variety of learning paradigms such as T-maze alternation task and object recognition test, respectively [8]. This model is frequently employed as a quick way for testing new drugs as cognition enhancers [9]. In healthy adults, scopolamine has been used to

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create impairment in memory and information processing speed and efficiency that resembles impairments in patients with AD [10].

Anthriscus (commonly known as beaked chervil, beaked parsley, rough chervil) is one of the aromatic members of the Apiaceae family [11] used for medicinal purposes in traditional medicine around the world, including Turkey. *Anthriscus nemorosa* (Bieb.) Sprengel is one of the species distributed in Turkey. It has been known as gimigimi in some parts of Turkey [12]. The fruits of this plant are used to treat gastrointestinal ailments [12], for inflammation and rheumatism [13]. *A. nemorosa* is one of the 25 herbs used to make herby cheese in eastern and south-eastern parts of Turkey (especially in Van region), called “Otlı peynir” in Turkish [14]. Furthermore, aerial parts of *A. cerefolium*, called mende, mendu or menda in Turkish, have been eaten as meal after boiled for stomach-ache in eastern parts of Turkey [15]. In a handwritten folk-medicinal document in Switzerland a distilled beverage made of *A. cerefolium* is indicated to improve memory [16]. In addition, the roots of *A. sylvestris* have been used as antitussive, antipyretic, analgesic, diuretic, and cough remedy in Chinese traditional medicine, and the young aerial part of this plant is used for food [17]. Ethnobotanical studies suggest that essential oil of *A. nemorosa* could improve memory in AD [12]. To our knowledge, there is no study indicating the effects of *A. nemorosa* essential oil on scopolamine-induced a rat model of cholinergic amnesia.

In this study we examined the effects of *Anthriscus nemorosa* essential oil on memory processes, anxiety and depressive-like behaviors in scopolamine-treated rats. Correlation between the behavioral scores of scopolamine-treated rats, as a result of inhalation of essential oil was also investigated.

2. Materials and methods

2.1. Plant materials and volatile oil preparation

Aerial parts of *Anthriscus nemorosa* were collected in the flowering stage in Adiyaman, Eastern Anatolia, Turkey, in June 2013 and identified by Prof. dr. Eyup Bagci at the Herbarium of Department of Biology, Firat University where a voucher specimen (no. Hayta 4829) was registered and deposited for ready reference. Air-dried aerial parts of the plant samples were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus to obtain the essential oil. The total essential oil yield was 0.7% (v/w).

2.2. Gas chromatography (GC–MS/GC–FID) analysis

GC–MS analysis of the *Anthriscus nemorosa* essential oil was performed in Plant Products and Biotechnology Research Laboratory (BUBAL), Firat University, using Hewlett Packard–Agilent 5973N GC–MS system with 6890 GC equipped with a flame ionization detector (FID). The procedure was performed as described previously [18]. Individual components were identified by comparison with the retention index from the GC–MS NIST library [19].

2.3. Animals

36 male Wistar rats aged 3–4 months (250 ± 10 g) upon arrival to the laboratory were used in this study. 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 (6 animals per group): (1) the Control group received 0.9% saline with 1% Tween 80 treatment; (2) the Scopolamine (Sco, 0.7 mg/kg b.w., i.p.) – alone-treated group received 0.9% saline with 1% Tween 80 treatment, as

negative control; (3) the Diazepam alone-treated group (DZP, 1.5 mg/kg) received 0.9% saline with 1% Tween 80 treatment, as positive control; (4) the Tramadol alone-treated group (TRM, 10 mg/kg) received 0.9% saline with 1% Tween 80 treatment, as positive control; (5) the Scopolamine-treated group received by inhalation *Anthriscus nemorosa* essential oil 1% (Sco + AEO1%) and (6) the Scopolamine-treated group received by inhalation *Anthriscus nemorosa* essential oil 3% (Sco + AEO3%). Control, DZP, TRM- and scopolamine alone-treated groups were caged in the same conditions but in the absence of the tested essential oil. Rats were treated in accordance with the guidelines of animal bioethics from 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. This study was approved by the Committee on the Ethics of Animal Experiments of the Alexandru Ioan Cuza University of Iasi (permit number: 2192) and also, efforts were made to minimize animal suffering and to reduce the number of animal used.

2.4. Y-maze test

Short-term memory was assessed by spontaneous alternation behavior in the Y-maze task. The Y-maze used in the present study consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. 15 min after the inhalation of *Anthriscus nemorosa* essential oil (AEO1% and AEO3%), rats were placed at the end of one arm and allowed to move freely through the maze for 8 min. An arm entry was counted when the hind paws of the rat were completely within the arm. Spontaneous alternation behavior was defined as entry into all three arms on consecutive choices. The number of maximum spontaneous alternation behaviors was then the total number of arms entered minus 2 and percent spontaneous alternation was calculated as (actual alternations/maximum alternations) $\times$ 100 [20]. Spontaneous alternation behavior is considered to reflect spatial working memory, which is a form of short-term memory. Locomotor activity was assessed by the number of arm entries. The maze was cleaned with a 10% ethanol solution and dried with a cloth before the next animal was tested.

2.5. Radial arm-maze test

The radial arm-maze used in the present study consisted of eight arms, numbered from 1 to 8 (48 cm $\times$ 12 cm), extending radially from a central area (32 cm in diameter). The apparatus was placed 50 cm above the floor, and surrounded by various extra-maze visual cues placed at the same position during the study. At the end of each arm there was a food cup that had a single 50 mg food pellet. Prior to the performance of the maze task, the animals were kept on restricted diet and body weight was maintained at 85% of their free-feeding weight over a week period, with water being available *ad libitum*. Before the actual training began, three or four rats were simultaneously placed in the radial arm-maze and allowed to explore for 5 min and take the food freely. The food was initially available throughout the maze, but was gradually restricted to the food cup. The animals were trained for 4 days to run to the end of the arms and consume the bait. To evaluate the basal activity of rats in radial arm-maze, the rats were given 5 consecutive training trials per day to run to the end of the arms and consume the bait. The training trial continued until all 5 baits have been consumed or until the 5 min have elapsed which have been set as the performance criteria. After adaptation, all rats were trained with 1 trial per day. Briefly, 15 min after the inhalation of *Anthriscus nemorosa* essential oil (AEO1% and AEO3%), each animal

was placed individually in the center of the maze and subjected to working and reference memory tasks, in which same 5 arms (nos. 1, 2, 4, 5 and 7), were baited for each daily training trial. The other 3 arms (nos. 3, 6 and 8) were never baited. The selection of the baited arms is based on the fact that animals prefer to solve the maze using an adjacent arm selection strategy. In this case, we altered adjacent arm patterning behavior by only baiting 5 arms (nos. 1, 2, 4, 5, and 7) subjecting animals to change their strategy and avoid the unbaited arms. An arm entry was counted when all four limbs of the rat were within an arm. Measures were made of the number of working memory errors (entering an arm containing food, but previously entered) and reference memory errors (entering an arm that was not baited) [20]. Reference memory is regarded as a long-term memory for information that remains constant over repeated trials (memory for the positions of baited arms), whereas working memory is considered a short-term memory in which the information to be remembered changes in every trial (memory for the positions of arms that had already been visited in each trial). The maze was cleaned with a 10% ethanol solution and dried with a cloth before the next animal was tested.

2.6. Elevated plus-maze test (EPM)

Behavior in EPM is utilized to assess exploration, anxiety, and motor behavior. 15 min after the inhalation of *Anthriscus nemorosa* essential oil (AEO1% and AEO3%), 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 [21]. The percentages of time spent in the open arms (time spent in the open arms/time spent in all arms $\times$ 100) were calculated. In addition, the total number of open- and enclosed-arm entries (number of crossing), which indicates the exploratory activity of animals [22], was measured. In the EPM, diazepam alone-treated group was used as positive control.

2.7. Forced swimming test (FST)

The FST is used for assessing depressive-like response [23]. The depressive-like response was assessed, basically using the same method described by Campos et al. [24], 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 $26 \pm 1^\circ\text{C}$. 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), 15 min after the inhalation of *Anthriscus nemorosa* essential oil (AEO1% and AEO3%). 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). In the FST, tramadol alone-treated group was used as positive control.

2.8. Statistical analysis

Behavioral scores within Y-maze, radial arm-maze, elevated plus-maze and forced swimming tests were analyzed by two-way analysis of variance (ANOVA) followed by Tukey *post hoc* test using GraphPad Prism 6 software for Windows. In order to evaluate differences between groups in the radial arm-maze task, separate repeated-measures ANOVA were calculated on the number of working memory errors and the number of reference memory errors with group (Control, Sco, Sco + AEO1% and Sco + EO3%) as between-subject factor and days (1–7) as within-subjects factors. All results are expressed as mean $\pm$ standard error of mean (S.E.M). *F* values for which $p < 0.05$ were regarded as statistically

significant. Pearson's correlation coefficient and regression analysis were used in order to evaluate the connection between behavioral measures.

3. Results

3.1. Chemical composition of the *Anthriscus nemorosa* essential oil

The GC–MS/GC–FID analysis of the volatile profiles in *Anthriscus nemorosa* essential oil is listed in Table 1. A total of 18 different compounds were isolated which constituted 85.9% (w/w) of the total essential oil. As a result of GC–MC analysis, caryophyllene (23.6%) was the major compound for the sample studied. Additionally, other major compounds were *trans*-pinocarveol (9.8%), germacrene D (5.6%), β -elemene (4.2%) and α -terpineol (2.7%). Sesquiterpenes represented 66.50% of the essential oil and consisted of caryophyllene (23.6%), β -elemene (4.2%), α -farnesene (1%), germacrene D (5.6%), bergamotene (0.9%), valencene (0.6%), δ -cadinene (12.1%), caryophyllene oxide (12.3%) and carotol (6.2%). Monoterpenes represented 19.40% of the essential oil and consisted of α -pinene (0.5%), sabinene (1.7%), β -pinene (0.5%), β -phellandrene (0.4%), β -ocimene (1%), *trans*-pinocarveol (9.8%), 3-cyclohexen-1-ol (0.8%), α -terpineol (2.7%) and pregeijerene (2%).

3.2. Effect of the *Anthriscus nemorosa* essential oil on spatial memory in Y-maze test

Analyses of the spontaneous alternation percentage within Y-maze task showed significant overall differences between all groups ($F(3,20) = 9.73$, $p < 0.0001$ (Fig. 1b). Both doses of inhaled *Anthriscus nemorosa* essential oil, significantly improved memory formation in scopolamine-treated rats as compared to scopolamine alone-treated rats. The changes in the spontaneous alternation percentages of scopolamine-treated rats exposed to *Anthriscus nemorosa* essential oil are not related to the changes in motor activity, as evidenced by the number of arm entries as compared to control rats (Fig. 1a).

3.3. Effect of the *Anthriscus nemorosa* essential oil on spatial memory in radial arm-maze task

To investigate whether exposure to *Anthriscus nemorosa* essential oil of scopolamine-treated rats affects spatial memory,

Table 1
Chemical composition (%) of identified compounds in the essential oil of *Anthriscus nemorosa* aerial parts.

No.	Compounds	RI	Concentration (%)
1	α -pinene	1021	0.5
2	sabinene	1051	1.7
3	β -pinene	1056	0.5
4	β -phellandrene	1077	0.4
5	β -ocimene	1107	1
6	<i>trans</i> -pinocarveol	1176	9.8
7	3-cyclohexan-1-ol	1203	0.8
8	α -terpineol	1214	2.7
9	pregeijerene	1290	2
10	β -elemene	1369	4.2
11	caryophyllene	1390	23.6
12	α -farnesene	1399	1
13	germacrene D	1432	5.6
14	bergamotene	1438	0.9
15	valencene	1442	0.6
16	δ -cadinene	1458	12.1
17	caryophyllene oxide	1496	12.3
18	carotol	1508	6.2
Total			85.9

RI: experimental retention indices relative to *n*-alkanes on the HP-5 MS column.

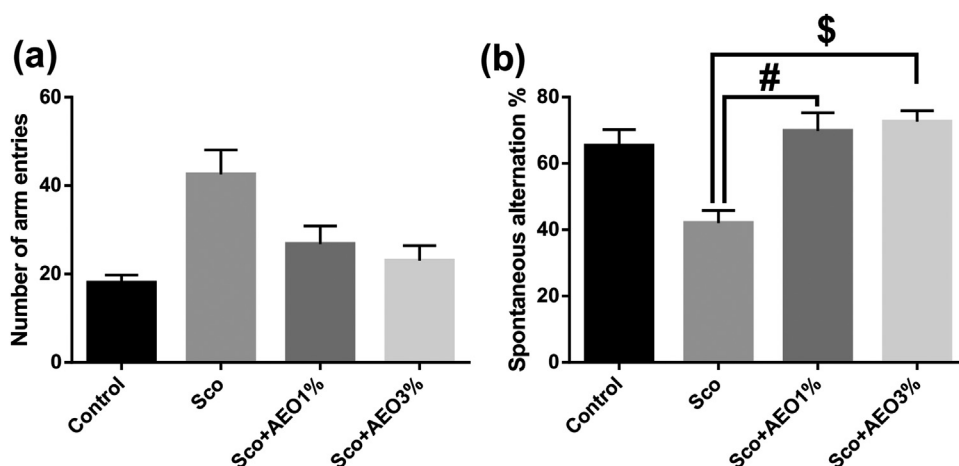


Fig. 1. Effects of the inhaled *Anthriscus nemorosa* essential oil (AEO1% and AEO3%) in the Y-maze on the number of arm entries (a) and spontaneous alternation% (b) in the scopolamine (Sco)-treated rats. Values are means $\pm$ S.E.M. (n = 6 animals per group). For Turkey's post hoc analyses – #Sco vs. Sco + AEO1%: $p < 0.001$ and \$Sco vs. Sco + AEO3%: $p < 0.0001$.

the rats were further evaluated in the radial arm-maze task. For working memory errors, repeated-measures ANOVA revealed a significant time difference ($F(6140)=2.30$, $p < 0.01$) and a significant group difference ($F(3140)=3.11$, $p < 0.01$) (Fig. 2a). For reference memory errors, repeated-measures ANOVA revealed a significant time difference ($F(6140)=3.06$, $p < 0.001$) and group difference ($F(3140)=7.60$, $p < 0.0001$) (Fig. 2b).

3.4. Effect of the *Anthriscus nemorosa* essential oil on elevated plus-maze behavior

As can be seen in Fig. 3a, in the elevated plus-maze task ANOVA revealed a significant overall differences between all groups ($F(4,25)=10.80$, $p < 0.0001$) on the percentage of the time spent in the open arms. Both doses of the *Anthriscus nemorosa* essential oil, but especially 3%, significantly increased the percentage of the time spent in the open arms in scopolamine-treated rats as compared to scopolamine alone-treated rats. As can be seen in Fig. 3b, in the elevated plus-maze task ANOVA revealed a significant overall differences between all groups ($F(4,25)=4.87$, $p < 0.01$) on the number of open-arm entries. Inhalation of the *Anthriscus nemorosa* essential oil, but especially 3%, significantly increased on the number of open-arm entries of scopolamine-treated rats as compared to scopolamine alone-treated group. As can be seen in Fig. 3c, in the elevated plus-maze task ANOVA revealed a significant overall differences between all groups (F

(4,25)=11.30, $p < 0.0001$) on the number of crossing (exploratory activity). Inhalation of the *Anthriscus nemorosa* essential oil, but especially 3%, significantly increased the number of crossing of scopolamine-treated rats as compared to scopolamine alone-treated rats. The diazepam treatment, as positive control, significantly increased the percentage of the time spent in the open arms, the number of open-arm entries and the number of crossing in the elevated plus-maze, acting as anxiolytic agent.

3.5. Effect of the *Anthriscus nemorosa* 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 ($F(4,25)=46.18$, $p < 0.0001$) (Fig. 4a) and on the immobility time ($F(4,25)=43.01$, $p < 0.0001$) (Fig. 4b). Both doses of *Anthriscus nemorosa* essential oil, but especially 3%, significantly increased swimming time and decreased immobility time of scopolamine-treated rats as compared to scopolamine alone-treated rats. The tramadol treatment, as positive control, increased the swimming time and decreased the immobility time in the forced swimming test, acting as antidepressant agent.

More importantly, when linear regression was determined, significant correlations between the percentage of the spontaneous alternation vs. the percentage of the open arms time (n = 24, $r=0.529$, $p < 0.001$) (Fig. 5a), the percentage of the spontaneous

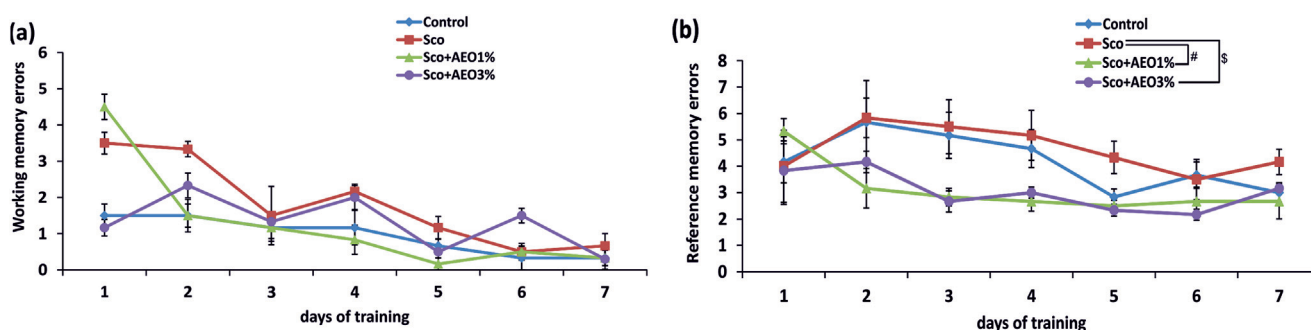


Fig. 2. Effects of the inhaled *Anthriscus nemorosa* essential oil (AEO1% and AEO3%) on the working memory errors (a) and the reference memory errors (b) during 7 days training in the radial arm-maze in the scopolamine (Sco)-treated rats. Values are means $\pm$ S.E.M. (n = 6 animals per group). For Turkey's post hoc analyses – #Sco vs. Sco + AEO1%: $p < 0.01$ and \$Sco vs. Sco + AEO3%: $p < 0.001$.

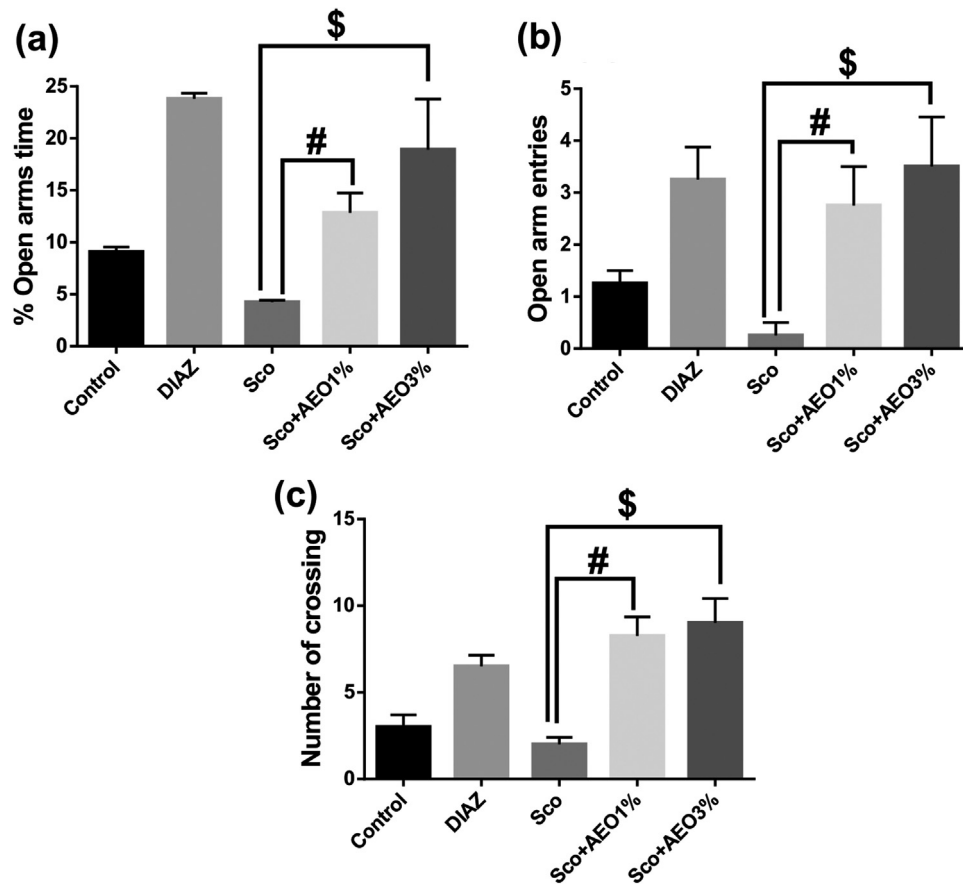


Fig. 3. Effects of the inhaled *Anthriscus nemorosa* essential oil (AEO1% and AEO3%) in the elevated plus-maze test on the percentage of the time spent in the open arms (a), the number of open-arm entries (b) and number of crossing (c) in the scopolamine (Sco)-treated rats. Values are means $\pm$ S.E.M. (n = 6 animals per group). For Turkey's post hoc analyses – [#]Sco vs. Sco + AEO1%: $p < 0.01$ and ^{\$}Sco vs. Sco + AEO3%: $p < 0.001$ (a), [#]Sco vs. Sco + AEO1%: $p < 0.001$ and ^{\$}Sco vs. Sco + AEO3%: $p < 0.0001$ (b) and [#]Sco vs. Sco + AEO1%: $p < 0.001$ and ^{\$}Sco vs. Sco + AEO3%: $p < 0.0001$ (c).

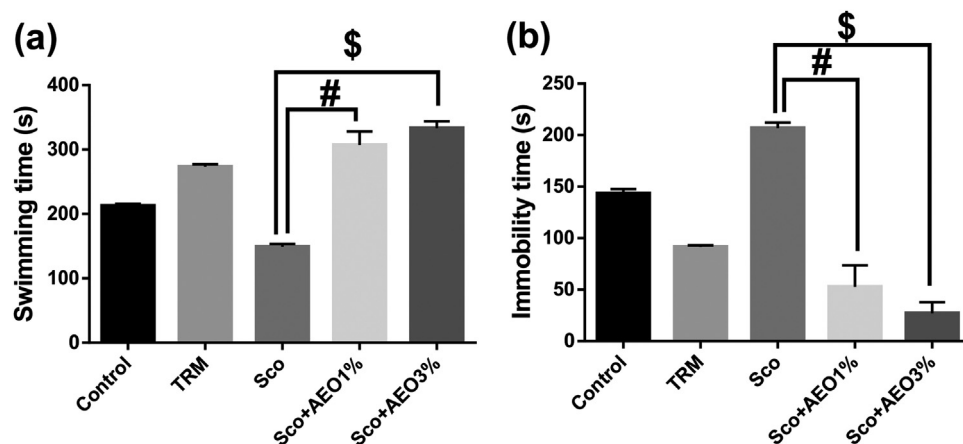


Fig. 4. Effects of the inhaled *Anthriscus nemorosa* essential oil (AEO1% and AEO3%) on swimming time (a) and immobility time (b) in the scopolamine (Sco)-treated rats during the 6 min period in the forced swimming test. Values are means $\pm$ S.E.M. (n = 6 animals per group). For Turkey's post hoc analyses – [#]Sco vs. Sco + AEO1%: $p < 0.0001$ and ^{\$}Sco vs. Sco + AEO3%: $p < 0.001$ (a) and [#]Sco vs. Sco + AEO1%: $p < 0.0001$ and ^{\$}Sco vs. Sco + AEO3%: $p < 0.0001$ (b).

alternation vs. swimming time (n = 24, $r = 0.562$, $p < 0.001$) (Fig. 5b), the percentage of the spontaneous alternation vs. immobility time (n = 24, $r = -0.556$, $p < 0.001$) (Fig. 5c), the reference memory errors vs. swimming time (n = 24, $r = -0.595$, $p < 0.001$) (Fig. 5d) and the reference memory errors vs. immobility time (n = 24, $r = 0.585$, $p < 0.001$) (Fig. 5e) were evidenced.

4. Discussion

In the present study, we investigated whether inhalation of the *Anthriscus nemorosa* essential oil (1% and 3% for 21 continuous days) causes behavioral effects as memory-enhancing, anxiolytic and antidepressant-like effects in scopolamine-treated rats based

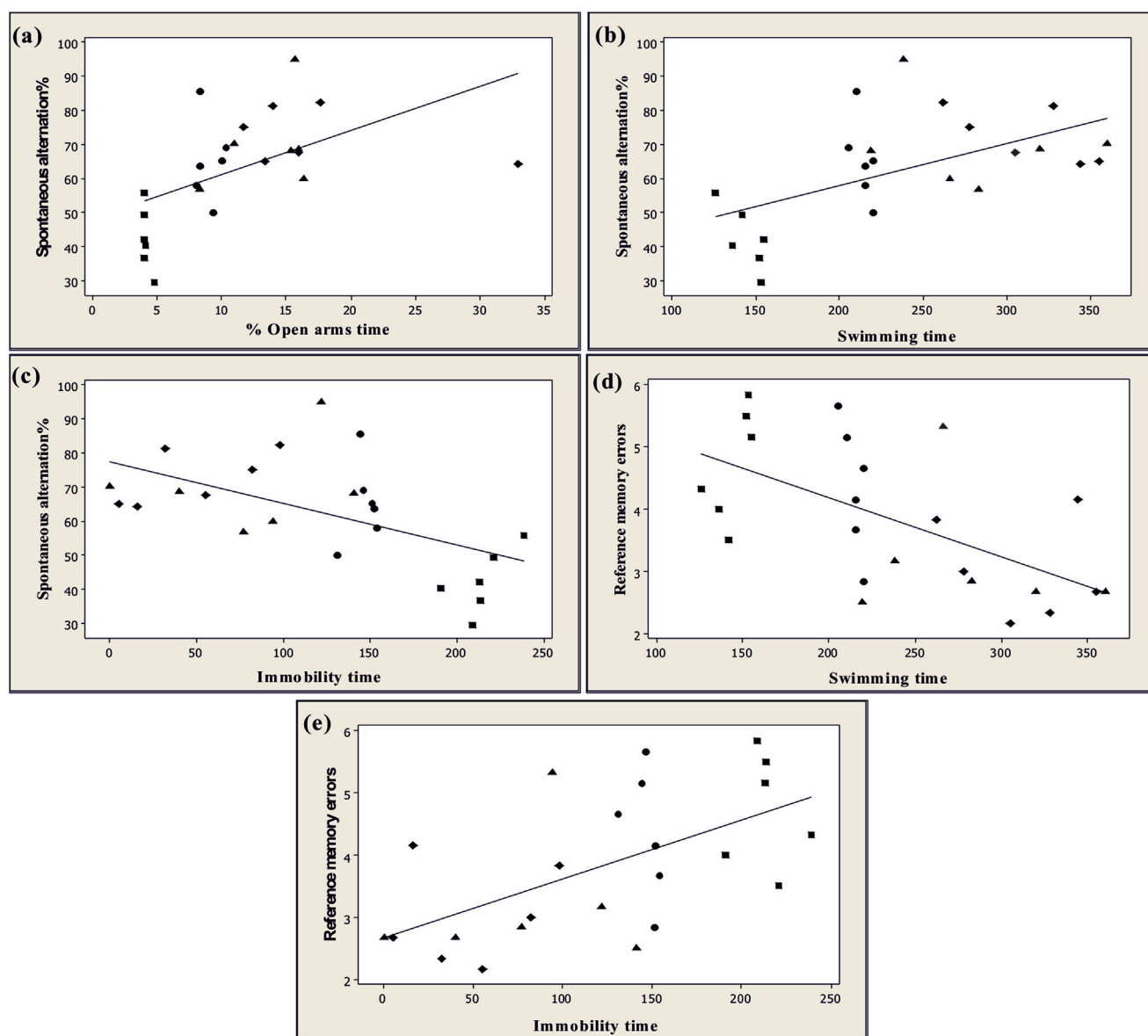


Fig. 5. Pearson's correlation between spontaneous alternation% vs.% open arms time (a), spontaneous alternation% vs. swimming time (b), spontaneous alternation% vs. immobility time (c), reference memory errors vs. swimming time (d) and reference memory errors vs. immobility time (e) in control group (●), scopolamine (Sco) alone-treated group (■), Sco + AEO1% group (▲) and Sco + AEO3% group (◆).

on specific behavioral tests (Y-maze, radial arm-maze, EPM and FST).

The GC-MS/GC-FID analyses indicated caryophyllene (23.6%), followed by *trans*-pinocarveol (9.8%), germacrene D (5.6%), β -elemene (4.2%) and α -terpineol (2.7%), as the main components of our *Anthriscus nemorosa* essential oil suggesting that these constituents could be responsible for the observed behavioral effects in scopolamine-treated rats. It has been reported that β -caryophyllene prevented cognitive impairment in APP/PS1 mice, and this positive cognitive effect was associated with reduced β -amyloid burden in both the hippocampus and the cerebral cortex. Also, it has been suggested that β -caryophyllene is an attractive molecule for the development of new drugs with therapeutic potential for the treatment of AD [25]. In the light of this study, we presume that our high-caryophyllene (23.6%) containing essential oil sustains spatial memory formation in

scopolamine-induced amnesia in the specific behavioral tests (Y-maze, radial arm-maze).

Both doses of *Anthriscus nemorosa* essential oil in scopolamine-treated rats significantly improved spatial working memory, as evidenced by the increase of spontaneous alternation percentage as compared to scopolamine alone-treated rats. This result suggests that both doses of *Anthriscus nemorosa* essential oil used in this study displays an improved effect on acquisition of the short-term memory of scopolamine-treated rats within the Y-maze task. However, no differences were observed between both doses of *Anthriscus nemorosa* essential oil on spatial working memory within the Y-maze task. The improvement of spatial working memory within Y-maze task cannot be attributed to locomotor activity, because significant changes in the number of entries of the groups treated with the *Anthriscus nemorosa* essential oil as compared with control rats were observed.

The scopolamine-treated rats exposed to dose of 1% *Anthriscus nemorosa* essential oil exhibited an improvement of working memory, along with an improvement of long-term memory, explored by reference memory when exposed to both doses of *Anthriscus nemorosa* essential oil (1% and 3%) as compared with scopolamine alone-treated rats within the radial arm-maze task. These findings suggest that inhalation of the *Anthriscus nemorosa* essential oil plays an important role in spatial memory formation, especially on both working memory and reference memory. However, non-significant differences were observed between both doses of the *Anthriscus nemorosa* essential oil on working memory and reference memory in the radial arm-maze task.

The present study also evaluated whether memory impairment induced by scopolamine is related with anxiety and depressive behaviors as assessed in specific behavioral tests (elevated plus-maze and forced swimming test). Scopolamine alone-treated rats have decreased the percentage of the time spent in the open arms, the number of open-arm entries and the number of crossing (exploratory activity) in the elevated plus-maze test. This indicates that the scopolamine-alone treated rats experienced high levels of anxiety and were suitable for evaluating the presumed anxiolytic substances as our essential oil [21].

In the present study we demonstrated that inhalation of *Anthriscus nemorosa* essential oil in scopolamine-treated rats produces anxiolytic-like activity. Furthermore, after the scopolamine-treated rats being exposed to *Anthriscus nemorosa* essential oil, the percentage of time spent in the open arms significantly increased, especially in the group exposed to 3% essential oil as compared to scopolamine-alone treated rats. Additionally, the number of open arms entries and the number of crossing (exploratory activity) increased, especially in the group of scopolamine-treated rats exposed to *Anthriscus nemorosa* essential oil 3% as compared to scopolamine-alone treated rats.

As expected, diazepam (DZP) as a benzodiazepine drug used as positive control produced significant increases in the percentage of time spent in the open arms, the number of open-arm entries and the number of crossing (exploratory activity) as compared to scopolamine-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 [26]. 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 [27]. 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 [28].

It has been reported that β -caryophyllene produced multiple behavioral changes relevant to anxiety and depression in C57BL/6 mice by modulating the GABA_A receptors activity [29]. In light with this report, our high-caryophyllene (23.6%) containing *Anthriscus nemorosa* essential oil has increased anxiolytic-like behavior and anti-depressive-like response in scopolamine-treated rats.

Within the forced swimming test, the swimming time decreased and the immobility time increased in scopolamine alone-treated rats as compared to control rats. This indicates that the scopolamine alone-treated rats exhibited depression. After being exposed to both doses of *Anthriscus nemorosa* essential oil (1% and 3%), the swimming time significantly increased and the immobility time significantly decreased, especially in group exposed to 3% essential oil as compared to scopolamine alone-treated rats.

These results suggested that *Anthriscus nemorosa* 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 scopolamine-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 [30].

Moreover, we found a significant correlation between the percentage of the spontaneous alternation vs. the percentage of the open arms time, the percentage of the spontaneous alternation vs. swimming time, the percentage of the spontaneous alternation vs. immobility time, reference memory errors vs. swimming time and reference memory errors vs. immobility time when linear regression was determined.

These data suggest that memory-enhancement scores within Y-maze and radial arm-maze tests along with decrease of anxiety and depressive-like behaviors within elevated plus-maze and forced swimming tests could be related with the involvement of the inhaled *Anthriscus nemorosa* essential oil against scopolamine-induced memory impairment, anxiety and depression in laboratory rats.

5. Conclusion

Considering the need for novel compounds that could improve conventional therapies as well as provide new agents targeting psychiatric disorders, the present study has clearly demonstrated the memory-enhancer, anxiolytic and antidepressant effects of the inhaled *Anthriscus nemorosa* essential oil in scopolamine-treated rats.

Conflict of interest

The authors declare no conflicts of interest.

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