



Evaluation of anti-amnesic activity of *Salvia multicaulis* essential oil on scopolamine-induced amnesia in rats: *in vivo* and *in silico* approaches



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ABSTRACT

Plants of genus *Salvia* have been used in folk medicine for wound healing, in the alleviation of stomach, liver, and rheumatism pains, as antioxidant, cognitive-enhancer, sedative and antiseptic, stimulant and tonic agents. The present study aimed to evaluate whether *Salvia multicaulis* essential oil (1% and 3%) administered for 21 days attenuates cognitive deficits and exhibits anxiolytic and antidepressant-profile in the scopolamine-induced amnesia in rats. Rats were randomly divided into six groups (n = 6): (1) control, (2) scopolamine (Sco, 0.7 mg/kg) (3, 4) *S. multicaulis* essential oil treatment groups (SEO, 1% and 3%), (5) diazepam (DIAZ, 1.5 mg/kg) and (6) tramadol (TRM, 10 mg/kg). To establish an animal model of amnesia, Sco (0.7 mg/kg), a muscarinic acetylcholine receptor antagonist, was injected into male Wistar rats. Y-maze (memory), radial arm-maze (memory), elevated plus-maze (anxiety), and forced swimming (depression) tests were employed. Molecular interactions of chemical compounds from the essential oil with the GABA_A receptor was explored via molecular docking experiments. Using behavioral tests, we demonstrated that inhalation of *S. multicaulis* essential oil exerts significant anti-amnesic activity as well as anxiolytic-antidepressant-like effects in the Sco-treated rats.

Our data revealed that *S. multicaulis* oil could act as a promising phytopharmaceutical agent for improving dementia-related abnormalities.

1. Introduction

Alzheimer's disease (AD) is one of the most common causes of dementia. Until 2060, the percentage of people over 65 years with this disease will increase as reported by the European Statistical Institute. Moreover, the incidence of dementia increases dramatically with age (Tromp et al., 2015). Also, non-cognitive symptoms such as agitation, apathy, depression, and psychosis are associated by patients with the AD (Rosenberg et al., 2015), and it has been reported that the prevalence of these symptoms oscillates between 60% to 90% of cases (Baquero and Martín, 2015).

Scopolamine (Sco) is a standard drug, centrally acting as a cholinergic receptor antagonist, for experimentally inducing memory impairment in both humans and animals (Jawaid et al., 2015). This effect has been proposed to mimic the cognitive and behavioral deficits seen during aging or in the AD. Sco produces a reversible impairment in maintaining attention, processing of information, and the acquisition of new knowledge in both rodents and humans (Deb et al., 2015).

Cognitive deficits that present with many of neuropsychiatric

conditions demand the use of nootropics to boost cognitive abilities. Recently, there is a tremendous urge to explore medicinal plants globally for improving cognitive function owing to their less adverse effects (Kulkarni et al., 2012).

Among 900 of *Salvia* L. species (Lamiaceae) in the world, 95 species of them are naturally distributed in Turkey (Orhan et al., 2013). Species of *Salvia* are known as “adaçayı” in Turkey, and their leaves are used as the herbal tea (Koşar et al., 2011). *Salvia* species have been used in traditional medicine for wound healing, in alleviation of stomach, liver, and rheumatism pains, as analgesic, antioxidant, sedative and antiseptic (Porres-Martínez et al., 2015b), stimulant, tonic (Eissa et al., 2014), and for treating the common cold in the form of infusion and decoction in various parts of the world. Moreover, the ethanolic extract of *S. officinalis* potentiated memory retention, as previously described by Eidi et al. (2006). These plants and their essential oils are also used in food flavoring, pharmaceuticals, and in perfumery (Ozkan et al., 2010). Despite extensive studies about the uses of *Salvia* species extracts, there is no study clarifying the possible neurological effects. Therefore, we searched for the possible effects of inhaled *S. multicaulis* essential oil in

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terms of improving the cholinergic dysfunction and characterize its underlying mechanism of action.

2. Materials and methods

2.1. Plant material and essential oil preparation

Air-dried aerial parts of *S. multicaulis* were collected from Elazig, Turkey on June 2014 and identified by Prof. Dr. Eyup Bagci at the Herbarium of Department of Biology, Firat University where a voucher specimen (No. 2014HFU) was deposited. The essential oil was extracted by hydrodistillation for 3 h using a Clevenger-type apparatus. The yield of the essential oil was 0.7 % (v/w) and was stored at 4 °C until utilized.

2.2. Gas chromatography-mass spectrometry (GC-MS/GC-FID) analysis

GC-MS was performed according to the method described by Aydin et al. (2016) using an Agilent 5973 N GC-MS system (Agilent Technologies, USA) with 6890 GC equipped with a flame ionization detector (FID). Compounds were separated on HP-5 MS column (30 m × 0.25 mm i. d., the film thickness, 0.25 µm) and the helium was used as the carrier gas. The injector temperature was set at 250 °C; the split flow was 1 ml/min. The GC oven temperature was kept at 70 °C for 2 min and programmed to 150 °C at a rate of 10 °C/min and then kept constant at 150 °C for 15 min to 240 °C at a rate of 5 °C/min. Retention indices (RI) were calculated using alkanes as reference points. The ionization energy was 70 eV and a mass range of 35–425. The chemical compounds of the essential oil were identified by comparing their RI to those of *n*-alkanes (C₈–C₂₂) as external references, their retention times (RT) and their mass spectra with that reported in MS libraries (Wiley) and the literature (Adams, 2007).

2.3. Animals and drug treatment

Thirty-six male Wistar rats (250 ± 10 g) were used during the experiment. The animals had access to food and water *ad libitum* and were housed in a room maintained at 22 °C with a 12-h light-dark cycle starting at 08:00. After acclimatization, the rats were randomly divided into six groups (n = 6 per group): control, scopolamine (Sco, 0.7 mg/kg), *S. multicaulis* essential oil treatment groups (SEO, 1% and 3%, daily for 21 consecutive days). Additionally, we used two groups (n = 6 per group) as referred to diazepam (DIAZ, 1.5 mg/kg, b. w., i. p., as the positive drug control in the elevated plus-maze test) and tramadol (TRM, 10 mg/kg, b. w., i. p., as the positive drug control in the forced swimming test). Except for SEO groups, the Control, DIAZ, TRM- and Sco-treated groups inhaled 0.9% saline with 1% Tween 80 solution. DIAZ and TRM were administered intraperitoneally 1h before the elevated plus-maze and forced swimming tests while Sco (0.7 mg/kg, b. w., i. p.), a muscarinic acetylcholine receptor antagonist, was delivered intraperitoneally once 30 min before the Y-maze test for 7 consecutive days, and also 30 min before the radial arm maze task. The protocol was approved by the Committee on the Ethics of Laboratory Animal Experiments of the Firat University, Elazig, Turkey (permit number: 236) and was conducted by the 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.

2.4. Inhalation equipment

For the inhalation session, two Plexiglas chambers (50 × 40 × 28 cm) were used: one for the Control, DIAZ, TRM- and Sco-treated groups which were exposed to 0.9% saline with 1% Tween 80 solution, and the second one for the groups exposed to SEO (1% and 3%). SEO was diluted with 1% Tween 80 (v/v) solution and was delivered (200 µL) by an electronic vaporizer (KBAYBO) for a controlled 15 min session, daily for 21 consecutive days.

2.5. In silico docking experiments

For the molecular docking simulation, VEGA ZZ software was used in conjunction with GriDock/AutoDock 4 to assess the interaction between the chemical compounds from essential oil with GABA_A receptor as previously used by Bagci et al. (2016). Structure of the bioactive compounds from the essential oil were retrieved from PubChem Compound Database (Bolton et al., 2008) and the selected compounds: α-pinene (CID 6654), α-eudesmol (CID 92762), valeranone (CID 171455), camphene (CID 6616), 1,8-cineole (CID 2758), β-caryophyllene (CID 5281515), calarene (CID 6432176) and β-selinene (CID 442393). Also, GABA (CID 119), diazepam (CID 3016) and benzamidine (CID 2332) were tested.

2.6. Y-maze test

The Y-maze test was performed as previously reported by Postu et al. (2018). The apparatus had three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. 15 minutes after the inhalation of SEO (1% and 3%), rats were individually placed at the end of one arm and allowed to move freely inside the maze for 8 min session. The percent of spontaneous alternation was calculated as (number of alternations/total entries - 2) X 100.

2.7. Radial arm-maze test (RAM)

A RAM was performed as described by Postu et al. (2018). The equipment was placed 50 cm above the floor and consisted of 8 arms (48 × 12 cm) which surrounded a central arena (32 cm in diameter). A food cup ended each arm with a single 50 mg food pellet. Before the training period, the animals were subjected to one-week restricted diet until the body weight was maintained at 85% of their weight. For 4 days pre-training period, the animals were subjected to explore the maze and to consume the bait. In the training period (7 days), the animals were trained with one trial per day. Briefly, 15 min after the inhalation of SEO (1% and 3%), rats were subjected to working and reference memory tasks for 5 min session, and the number of working memory errors and the number of reference memory errors were recorded for 7 days period of training.

2.8. Elevated plus-maze test (EPM)

An EPM was utilized as previously described by Aydin et al. (2016). The device showed 4 arms (49 × 10 cm), elevated 50 cm above the floor. Two arms were enclosed by walls 30 cm high, and the other two arms were opened. 15 minutes after the inhalation of SEO (1% and 3%), each rat was individually tested for 5 min session, and the examined parameters were: (1) the percentages of time spent in the open arms, (2) total number of open arm entries and (3) total number of open- and enclosed-arm entries (number of crossing). DIAZ was used as a positive reference drug in EPM.

2.9. Forced swimming test (FST)

The FST was performed according to the method described previously by Aydin et al. (2016). The equipment consisted of a cylindrical recipient (30 cm diameter, 59 cm high) with tap water (25 cm) at 26 ± 1 °C. In the pretest session (first day), rats were independently placed into the cylinder and left to swim for 15 min before being removed. Additionally, in the second day was performed the test session (6 min). 15 minutes before the test session, the rats were subjected to the inhalation of SEO (1% and 3%). The following 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). TRM was used as a positive reference drug in FST.

2.10. Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test for multiple comparisons of behavior. Also, for the RAM, behavioral data were supplementary analyzed using repeated-measures ANOVA. Correlation analysis was done using Pearson's correlation analysis. Results are expressed as the mean $\pm$ standard error of the mean (S.E.M). For all statistical tests, $p < 0.05$ were regarded as significant. All statistical analyses were performed using GraphPad Prism 7 software.

3. Results

3.1. The identification of *S. multicaulis* essential oil constituents

The constituents of the essential oil were analyzed by GC-MS/GC-FID as listed in Table 1. 31 different compounds were isolated which constituted 97.10 % (w/w) of the essential oil as: α -pinene (28.10 %), camphene (6.40 %), β -pinene (2.80 %), DL-limonene (2.70 %), 1,8-cineole (4.10 %), β -caryophyllene (3.50 %), calarene (3.50 %), α -eudesmol (13.10 %), β -selinene (3.70 %) and valeranone (8.50 %).

3.2. Molecular modeling

In silico studies revealed new insights about the possible binding mode of different compounds with receptors (Zengin et al., 2018). Assay performed on the essential oil indicated that α -pinene, α -eudesmol, valeranone, camphene, 1,8-cineole, β -caryophyllene, calarene and β -selinene ligands with the receptor protein, GABA_A exhibit the lower value of the inhibition constant (K_i) and the lowest amount of free energy of binding as compared with diazepam (Table 2).

Table 1

Chemical composition (%) of identified compounds in the essential oil of *Salvia multicaulis* aerial parts.

Compound No.	Compound name	RI	Concentration (%)
1.	Tricyclene	919	0.30
2.	α -Pinene	937	28.10
3.	Camphene	950	6.40
4.	β -Pinene	977	2.80
5.	β -Myrcene	989	1.40
6.	<i>p</i> -Cymene	1018	1.00
7.	DL-Limonene	1094	2.70
8.	β -Phellandrene	1095	0.90
9.	1,8-Cineole	1096	4.10
10.	Camphor	1145	0.70
11.	Borneol	1167	0.50
12.	Myrtenol	1214	1.20
13.	Copaene	1358	0.40
14.	Zingiberene	1378	1.40
15.	β -Caryophyllene	1391	3.50
16.	Aromadendrene	1404	0.70
17.	α -Curcumene	1433	1.00
18.	Germacrene B	1443	0.60
19.	[$\pm$]-epibicyclosesquiphellandrene	1456	0.50
20.	α -Copaene-11-ol	1471	0.90
21.	α -Bergamotene	1479	1.20
22.	Caryophyllene oxide	1496	2.20
23.	α -Himachalene	1500	0.50
24.	Azulene	1503	1.00
25.	Calarene	1524	3.50
26.	Diepi- α -Cedrene epoxide	1531	0.70
27.	β -Eudesmol	1534	1.50
28.	α -Eudesmol	1538	13.50
29.	β -Selinene	1545	3.70
30.	Valeranone	1548	8.50
31.	Pentacosane	1654	1.70
	Total		97.10

RI- Retention index in the HP-5 MS column relative to *n*-alkanes.

3.3. Effects on anti-amnesia in Y-maze and RAM tests

The effects of *S. multicaulis* essential oil on the Sco-induced memory impairment were evaluated using Y-maze and RAM tests. In the Y-maze test, the rats in the Sco treated-group displayed decreased spontaneous alternation ($p < 0.01$) as compared to the control group, whereas inhalation of essential oil significantly elevated the spontaneous alternation ($p < 0.01$ for SEO1%) compared with Sco, indicating a memory-enhancing effect (Fig. 1a).

In the RAM, Sco treatment increased the number of working memory errors ($p < 0.001$) and the number of reference memory errors ($p < 0.01$) as compared to the control group. Repeated-measures ANOVA showed a time difference ($F(6,84) = 6.66$, $p < 0.0001$) and a group difference ($F(3,84) = 8.15$, $p < 0.0001$) (Fig. 1b) for working memory errors while for reference memory errors indicated a significant time difference ($F(6,84) = 4.58$, $p < 0.0001$) and a significant group difference ($F(3,84) = 6.23$, $p < 0.0001$) (Fig. 1c).

3.4. Effects on anxiety-like behavior in the EPM test

Rats treated with Sco spent less time in the open arms ($p < 0.01$) (Fig. 2a), scored significantly low number of open arm entries ($p < 0.01$) (Fig. 2b) and low number of crossing ($p < 0.01$) (Fig. 2c) as compared to control group. Furthermore, it was also observed that the administration of DIAZ or inhalation of the essential oil significantly reversed these parameters, suggesting anxiolytic effects.

3.5. Effects on depressive-like behavior in the FST

Administration of Sco significantly increased anhedonia in animals, as evidenced by decreased swimming time ($p < 0.01$) (Fig. 3a) and increased immobility time ($p < 0.01$) (Fig. 3b) as compared to control group. Moreover, a significant improvement of anhedonia was observed following TRM treatment or inhalation of the essential oil.

Pearson's correlations between the percentage of time spent in the open-arm vs. spontaneous alternation percentage ($n = 24$, $r = 0.667$, $p < 0.001$) (Fig. 4a), the number of crossings vs. spontaneous alternation percentage ($n = 24$, $r = 0.531$, $p < 0.01$) (Fig. 4b), immobility time vs. spontaneous alternation percentage ($n = 24$, $r = -0.718$, $p < 0.001$) (Fig. 4c), the percentage of time spent in the open-arm vs. number of crossings ($n = 24$, $r = 0.563$, $p < 0.01$) (Fig. 4d), the percentage of time spent in the open-arm vs. the number of open arm entries ($n = 24$, $r = 0.508$, $p < 0.05$) (Fig. 4e), the number of crossings vs. the number of open arm entries ($n = 24$, $r = 0.815$, $p < 0.0001$) (Fig. 4f), the percentage of time spent in the open-arm vs. the swimming time ($n = 24$, $r = 0.666$, $p < 0.001$) (Fig. 4g), the percentage of time spent in the open-arm vs. the immobility time ($n = 24$, $r = -0.666$, $p < 0.001$) (Fig. 4h), the number of open arm entries vs. immobility time ($n = 24$, $r = -0.617$, $p < 0.01$) (Fig. 4i) and the number of crossings vs. immobility time ($n = 24$, $r = -0.658$, $p < 0.001$) (Fig. 4j) were evidenced.

4. Discussion

The purpose of this study was to investigate if there was a behavioral improvement following inhalation of the *S. multicaulis* essential oil (1% and 3% for 21 consecutive days) in Sco-treated rats based on specific *in vivo* behavioral approaches such as Y-maze, RAM, EPM, and FST. Sco is a pharmacological tool used to induce transient memory deficits via increasing acetylcholinesterase activity in the hippocampus (Lim et al., 2018). On the other hand, as compared to humans, rats given Sco displayed high levels of anxiogenic behavioral responses in specific tests such as open field test and light/dark test as previously reported (Hamilton et al., 2017; Hughes and Otto, 2013).

The main phytochemicals of the essential oil identified by GC-MS/GC-FID analyses were: α -pinene (28.10 %), camphene (6.40 %), β -pinene (2.80 %), DL-limonene (2.70 %), 1,8-cineole (4.10 %), β -caryophyllene

Table 2
Ligand-protein interaction parameters.

Molecule	Inhibition constant K_i (μ M)	Lowest free energy of binding (kcal/mol)	Mean free energy of binding (kcal/mol)	Total inter-molecular energy (kcal/mol)	vdW + Hbond + desolv energy (E_{VHD}) (kcal/mol)	Electrostatic energy (kcal/mol)	Final total internal energy (kcal/mol)	Torsional free energy (kcal/mol)	Unbound system's energy (kcal/mol)	PubChem CID
α -pinene	39.30	-6.01	-6.00	-6.01	-6.00	-0.01	0.00	0.00	0.00	6654
α -eudesmol	3.99	-7.37	-7.32	-7.37	-7.36	-0.01	0.00	0.00	0.00	92762
valeranone	1.49	-7.95	-7.88	-7.95	-7.98	0.03	0.00	0.00	0.00	171455
camphene	40.80	-5.99	-5.94	-5.99	-5.99	0.00	0.00	0.00	0.00	6616
1,8-cineole	37.70	-6.04	-6.00	-6.04	-6.00	-0.04	0.00	0.00	0.00	2758
β -caryophyllene	2.27	-7.70	-7.60	-7.70	-7.70	0.00	0.00	0.00	0.00	5281515
calarene	4.56	-7.29	-7.18	-7.29	-7.29	0.00	0.00	0.00	0.00	6432176
β -selinene	5.62	-7.16	-6.92	-7.46	-7.47	0.01	-0.19	0.30	-0.18	442393
diazepam	3.25	-7.49	-6.95	-7.75	-7.83	0.07	-0.43	0.30	-0.40	3016
GABA	2640.00	-3.52	-3.24	-4.47	-4.12	-0.36	0.00	0.89	-0.06	119
benzamidine	241.00	-4.94	-4.80	-5.53	-5.45	-0.08	-0.04	0.60	-0.04	2332
benzamidine (4COF)	1520.00	-3.85	-	-4.78	-4.72	-0.06	0.04	0.89	0.00	-

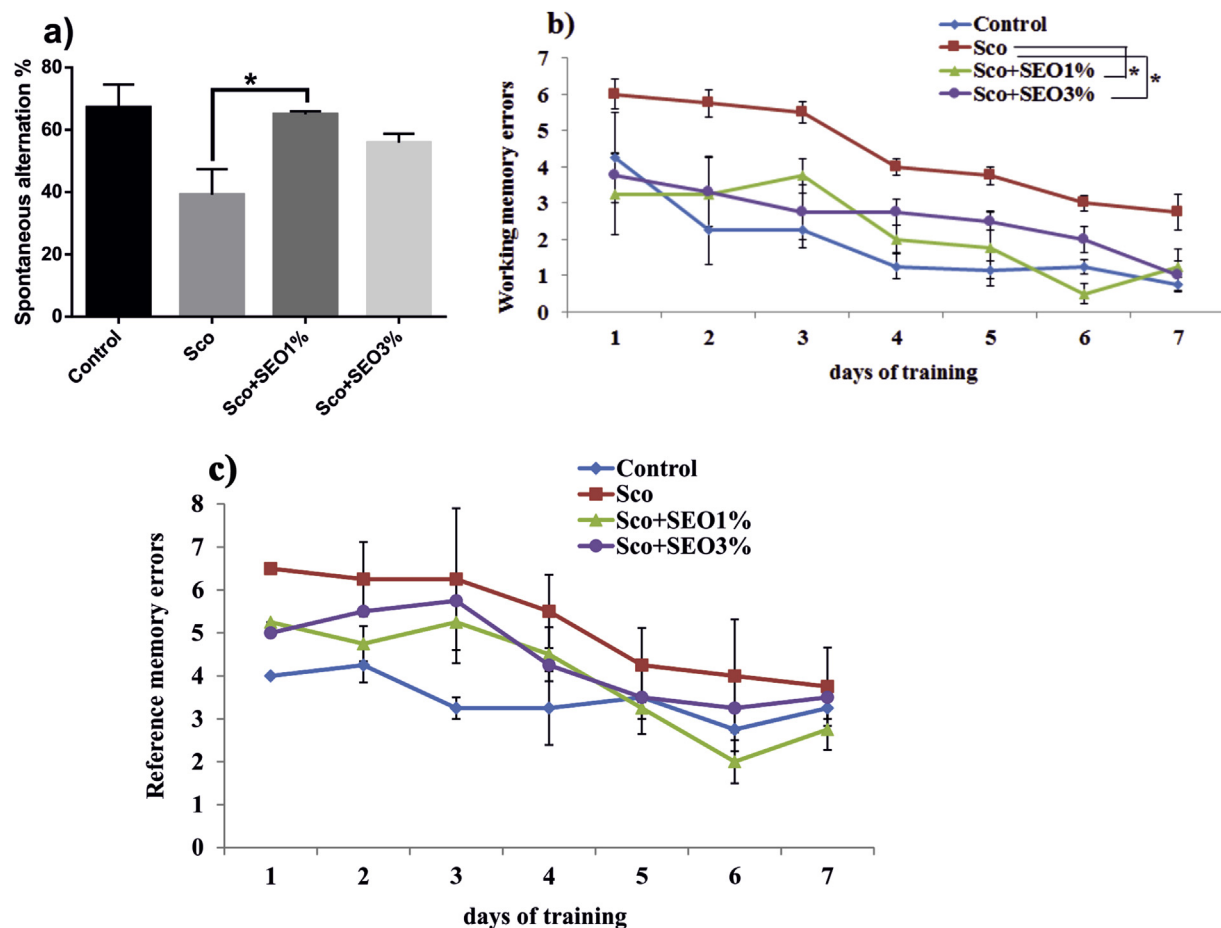


Fig. 1. Effects of the inhaled *S. multicaulis* essential oil (SEO1% and SEO3%) on the spontaneous alternation percentage (a) in the Y-maze task and on the working memory errors (b) and the reference memory errors (c) during 7 days training in the RAM task in the Sco-treated rats. Values are means $\pm$ S.E.M. ($n = 6$ animals per group). For Tukey's *post hoc* analyses – *Sco vs. Sco + SEO1%: $p < 0.01$ (a) and *Sco vs. Sco + SEO1%: $p < 0.01$, *Sco vs. Sco + SEO3%: $p < 0.01$ (b).

(3.50 %), calarene (3.50 %), α -eudesmol (13.10 %), β -selinene (3.70 %) and valeranone (8.50 %). The main identified components of the oil are mostly similar with those identified in another study by [Ghorbani and Esmailizadeh \(2017\)](#), suggesting that our essential oil could be considered a good source of bioactive compounds responsible for the observed

effects in the Sco-treated rats. Supporting evidence suggests that the main selected monoterpenes α -pinene and 1,8-cineole found in *S. lavandulifolia* essential oil act as regulators of cellular redox balance in astrocytes ([Porres-Martínez et al., 2015a](#)). Also, some authors demonstrated that oral administration of essential oil of *S. miltiorrhiza* presents the anxiolytic-like

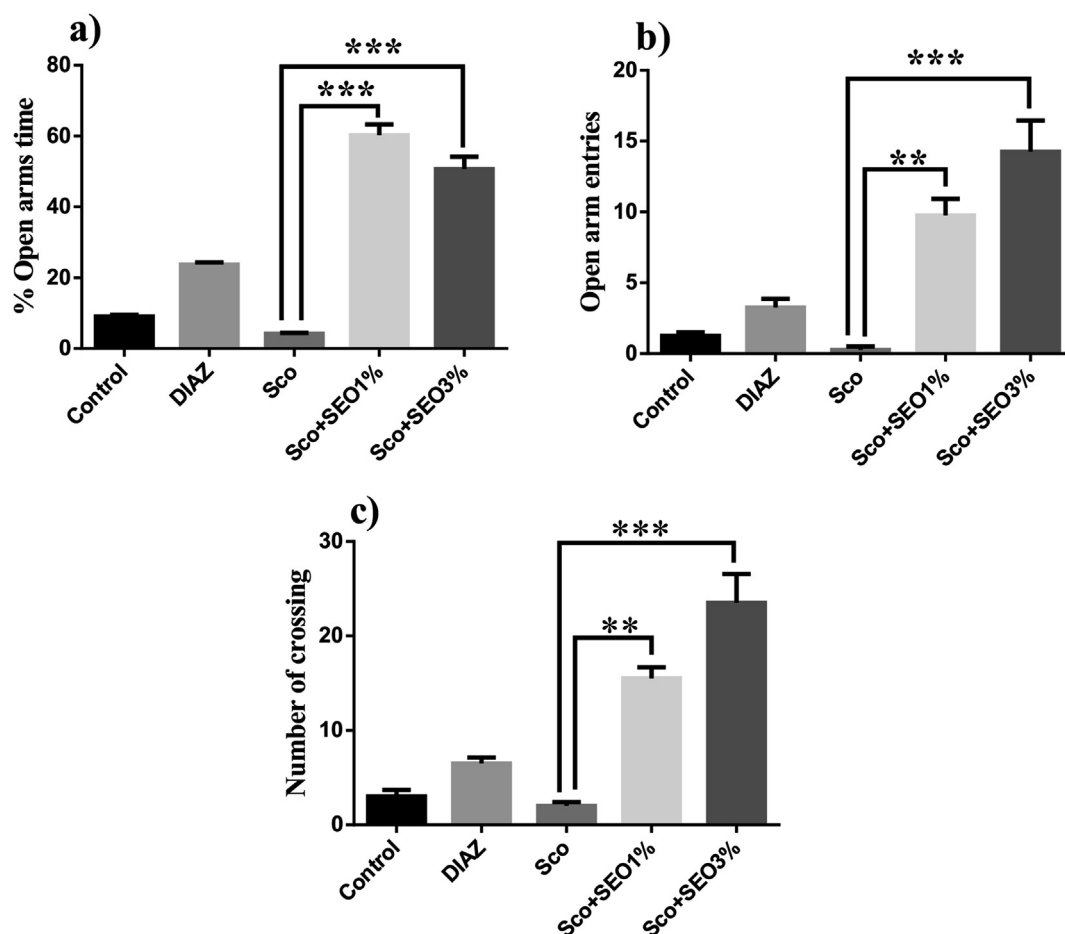


Fig. 2. Effects of the inhaled *S. multicaulis* essential oil (SEO1% and SEO3%) in the EPM test on the percentage of the time spent in the open arms (a), the number of open-arm entries (b) and the number of crossing (c) in the Sco-treated rats. Values are means $\pm$ S.E.M. ($n = 6$ animals per group). For Tukey's *post hoc* analyses – ***Sco vs. Sco + SEO1%: $p < 0.0001$, ***Sco vs. Sco + SEO3%: $p < 0.0001$ (a), **Sco vs. Sco + SEO1%: $p < 0.001$, ***Sco vs. Sco + SEO3%: $p < 0.0001$ (b), and **Sco vs. Sco + SEO1%: $p < 0.001$, ***Sco vs. Sco + SEO3%: $p < 0.0001$ (c).

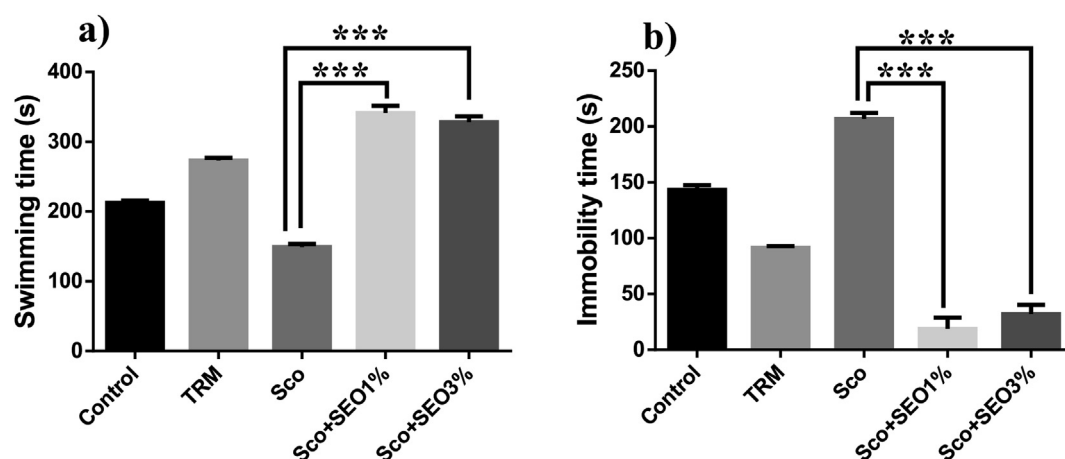


Fig. 3. Effects of the inhaled *S. multicaulis* essential oil (SEO1% and SEO3%) on swimming time (a) and on immobility time (b) in the scopolamine (Sco)-treated rats during the 6 min in the FST. Values are means $\pm$ S.E.M. ($n = 6$ animals per group). For Tukey's *post hoc* analyses – ***Sco vs. Sco + SEO1%: $p < 0.0001$, ***Sco vs. Sco + SEO3%: $p < 0.0001$ (a) and ***Sco vs. Sco + SEO1%: $p < 0.0001$, ***Sco vs. Sco + SEO3%: $p < 0.0001$ (b).

effects in rats. The authors attributed this effect to the interaction of essential oil compounds with GABA_A receptor for induction of the anxiolytic effect (Liu et al., 2015). Also, docking calculations have shown that the GABA_A receptor interacts with α -pinene (binding energy -6.01), α -eudesmol (binding energy -7.37), valeranone (binding energy

-7.95), camphene (binding energy -5.94), 1,8-cineole (binding energy -6.00), β -caryophyllene (binding energy -7.60), calarene (binding energy -7.18) and β -selinene (binding energy -6.92) from *S. multicaulis* essential oil effectively and among which diazepam (binding energy -6.95) exhibit a close interaction with GABA_A receptor.

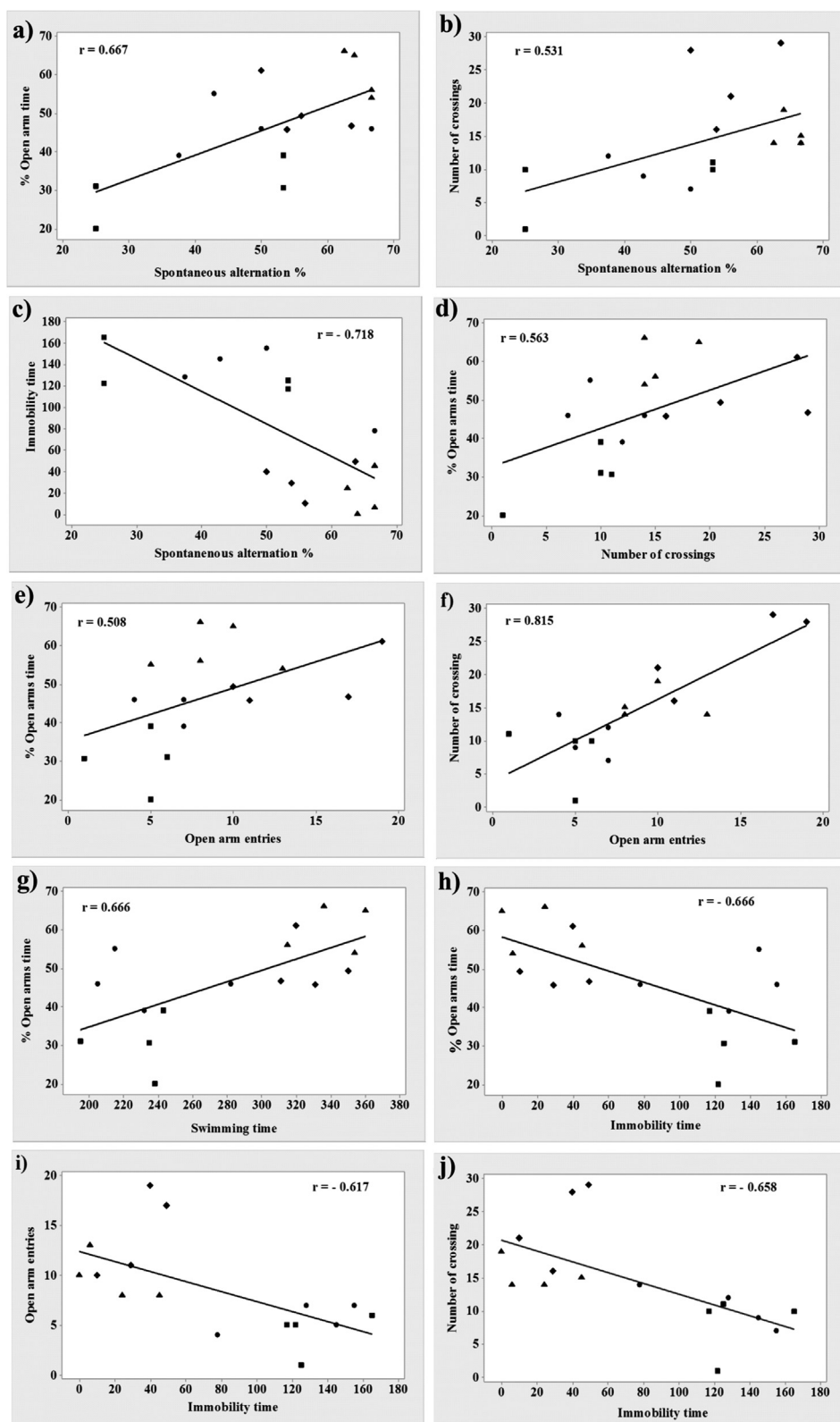


Fig. 4. Pearson's correlation between the percentage of time spent in the open-arm vs. spontaneous alternation percentage (a), the number of crossings vs. spontaneous alternation percentage (b), immobility time vs. spontaneous alternation percentage (c), the percentage of time spent in the open-arm vs. number of crossings (d), the percentage of time spent in the open-arm vs. the number of open arm entries (e), the number of crossings vs. the number of open arm entries (f), the percentage of time spent in the open-arm vs. the swimming time (g), the percentage of time spent in the open-arm vs. the immobility time (h), the number of open arm entries vs. immobility time (i) and the number of crossings vs. immobility time (j) in control group (●), Sco alone-treated group (■), Sco + SEO1% group (▲) and Sco + SEO3% group (◆).

In the Y-maze test, spontaneous alternation behavior is related to short-term memory loss (Hong et al., 2018). Rats given Sco subjected to SEO inhalation (especially 1%) had significantly increased spontaneous alternation behavior, which prevented Sco-induced memory impairment. Also, the RAM is widely used for the investigation of spatial memory impairment (Egashira et al., 2018). Our results showed that SEO inhalation (particularly 1%) significantly attenuated the amnesic behavior, especially on working memory. Our results were consistent with a previous observation about positive effects of acute doses of *S. lavandulaefolia* essential oil on cognitive performance of healthy young volunteers (David et al., 2010; Tildesley et al., 2005).

Also, the present study evaluated if *S. multicaulis* essential oil could have beneficial effects on anxiety and depression as previously demonstrated to have positive effects on cognitive performance. EPM test is commonly used for screening anxiety-modulating drugs (Reddy et al., 2017). The results of the present study suggest that rats with cholinergic deficits subjected to SEO inhalation displayed an anxiolytic behavioral response in the EPM test. As expected, DIAZ, used as a positive control, produced an anxiolytic effect, consistent with previous studies that described anxiolytic-like effects of DIAZ in the EPM method (Fraga et al., 2018). Our study complies with another study where is reported that essential oil of *S. miltiorrhiza* produces anxiolytic-like effects in rats. Furthermore, the essential oil of *S. miltiorrhiza* induced the increased of intracellular Cl^- concentration in cultured human neuroblastoma cells, suggesting that its anxiolytic effects are probably mediated through a GABAergic mechanism of action (Liu et al., 2015).

For assessing the antidepressant effect of *S. multicaulis* essential oil, the FST was used (Lin et al., 2015). Results showed that the inhalation of the essential oil produced a diminution of the immobility time of rats exposed to the FST, suggesting an intense antidepressant activity. These behavioral effects were very similar to that demonstrated by the other authors after treating rats with antidepressant drugs such as TRM (Ostadhadi et al., 2017). Also, our study is in line with other studies reported antidepressant and anxiolytic effects of hydroalcoholic extract from *S. elegans* (Herrera-Ruiz et al., 2006).

5. Conclusion

In conclusion, data here obtained allow us to propose the *S. multicaulis* essential oil as an excellent pharmacological agent with a memory-enhancer profile, potential anxiolytic, and antidepressant activity.

Declarations

Author contribution statement

Eyup Bagci, Lucian Hritcu: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Emel Akbaba, Eugen Ungureanu: Performed the experiments; Analyzed and interpreted the data.

Calin Maniu: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

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