Journal of Essential Oil Bearing Plants

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/teop20

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To cite this article: Sükrü Hayta, Özgür Bulmuş, Elif Aksoz, Saliha Uysal & Emel Akbaba (2024) Neuroprotective effects of *Sideritis trojana* essential oil in $AlCl_3$-induced amnesic rats, Journal of Essential Oil Bearing Plants, 27:4, 1035-1048, DOI: 10.1080/0972060X.2024.2373137

To link to this article: https://doi.org/10.1080/0972060X.2024.2373137

HKB PUBLICATIONS

JOURNAL OF ESSENTIAL OIL BEARING PLANTS

Taylor & Francis
Taylor & Francis Group

DOI: 10.1080/0972060X.2024.2373137

Research Article

Neuroprotective effects of *Sideritis trojana* essential oil in $AlCl_3$-induced amnesic rats

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Received 07 May 2024
Revised 05 June 2024
Accepted 10 June 2024

Abstract
In this study, the effects of *Sideritis trojana* essential oil on memory, anxiety, and depression were investigated in rats subjected to $AlCl_3$-induced dementia. The major constituents of *S. trojana* essential oil were analyzed as carvacrol (42.32%), carvone (18.69%), and α-pinene (4.54%). Rats were divided into five groups: Control, positive control (donepezil), $AlCl_3$-only applicated, $AlCl_3$ with 40% *S. trojana* essential oil applicated, and $AlCl_3$ with 80% *S. trojana* essential oil applicated groups. The essential oil was administered for 30 days, after that, the rats were subjected to behavioral tests. In the forced swimming test, *S. trojana* essential oil application demonstrated antidepressant-like effects. In the elevated plus maze and open field tests, *S. trojana* essential oil application exhibited anxiolytic-like effects. Furthermore, the application of *S. trojana* essential oil ameliorated memory impairment induced by $AlCl_3$ by increasing spontaneous alternation in the Y-maze test. Furthermore, *S. trojana* essential oil application reduced the level of malondialdehyde and increased catalase activity, demonstrating antioxidant activity. Molecular docking analyses were performed between the major compounds of *S. trojana* essential oil and the AMPA receptor to evaluate possible mechanisms of action leading to neuroprotection. Carvacrol, carvone, and α-pinene were shown to possess strong binding abilities to the AMPA receptor, which may result in allosteric modulation of the receptor. This study demonstrated the neuroprotective effects of *S. trojana* essential oil in an animal model induced by $AlCl_3$ application, suggesting its potential protective role against central nervous system disorders.

Keywords
Sideritis trojana essential oil, Aluminum chloride, Alzheimer's Disease, Carvacrol, Memory

INTRODUCTION

Aluminum (Al) is a highly prevalent metal in the Earth's crust and has the ability to infiltrate living organisms, including humans, through multiple routes such as ingestion of food, food additives, drinking water, use of cookware, and cosmetic application. There is significant evidence indicating that aluminum can cross the blood-brain barrier and build up in different areas of the brain, such as the cerebral cortex and hippocampus, which are involved in learning and memory formation. The accumulation of aluminum in the brain has been observed to play a role in the development of neurodegenerative conditions like Parkinson's and Alzheimer's disease (AD)[1]. In AD, it is proposed that metal accumulation triggers amyloid-beta aggregation, leading to the accumulation of redox-active biometals inside or in close proximity to neurons. This accumulation is purported to generate reactive oxygen species (ROS), initiating oxidative damage and neurodegeneration[2]. The inevitability of oxidative stress in aerobic organisms has become an accepted fact. The confirmed role of oxidative stress in the etiology of various chronic diseases such as diabetes, cancer, cardiovascular disorders, and AD is clearly acknowledged[3]. The administration of

$AlCl_3$ is a widely employed model for inducing brain damage in experimental animals[4]. AD is a neurodegenerative condition that manifests in association with age, characterized by the accumulation of a protein known as amyloid-beta in the brain. It progressively advances, presenting with gradual memory loss and cognitive decline. It is estimated that AD is responsible for approximately 50-60% of dementia cases among individuals aged 65 and older worldwide[5]. Due to the association of the disease with age, it is anticipated that the number of cases will triple in the next 50 years. In advanced stages, the disease renders individual living unfeasible, imposes numerous financial and emotional responsibilities on the family, and escalates healthcare expenditures. The disease is accompanied by various behavioral symptoms, including anxiety and depression. It is postulated that neurodegeneration begins approximately 20-30 years before the onset of the symptoms of the disease[6].

AMPA receptors (AMPARs) are a type of glutamate receptors responsible for facilitating rapid excitatory communication in the brain. They have a crucial function in the development of the central nervous system[7]. AMPARs facilitate signal transmission between the neuronal circuits of the hippocampus[8]. AMPARs are linked to the augmentation of synaptic excitation and plasticity, which have an impact on memory and learning. The dysregulation of AMPAR occurs prior to the detection of symptomatic plaques and neuronal death. Reducing AMPAR is a critical initial stage that leads to cognitive impairments in AD[9]. Thus, targeting AMPAR signaling may be a promising approach in therapeutic research for AD.

The genus *Sideritis*, belonging to the Lamiaceae family, are locally known as 'dağ çayı' in Turkish. *Sideritis trojana* Bornm is consumed as tea and used for therapeutical purposes. *S. trojana* is known as an insecticidal, and anti-candidal plant species[10]. The essential oil of *S. trojana* has been reported to possess anti-microbial properties[11]. Previously antioxidant, anti-cholinesterase and anti-butyrylcholinesterase activities of *S. trojana* have been reported suggesting that it could be beneficial against neurodegenerative diseases, especially AD[12,13]. However, there is currently no existing literature on the effects of *S. trojana* essential oil on AD, memory, anxiety or depression. In this study, the effects of *S. trojana* essential oil on memory, anxiety, and depressive-like behavior were evaluated in $AlCl_3$-induced demented rats for the first time. Also, antioxidant effects of *S. trojana* essential oil in the brains of $AlCl_3$-toxicated rats were assessed. Furthermore, molecular docking studies between AMPA receptor and the major components of *S. trojana* essential oil were carried out to determine molecular interactions and evaluate possible mechanisms of action.

Materials and Methods

Harvesting of plants and extraction of essential oil

Plant species were collected from their natural habitats in Turkey (GPS: 39°42'27.95"K; 26°52'3.67"D, 1670 m) (Fig. S1). The plant species was identified by Prof. Dr. Sukru Hayta (Fig. S2). The specimens were stored in Balikesir University Herbarium (collection number: SH 4233). The plant samples were dried naturally in the shade. Dry weights of the plant samples were weighed. Essential oil was obtained from dried plant samples using the Clevenger apparatus through water distillation (hydrodistillation) method for a duration of 3 hours[14]. Essential oil yield was calculated based on dry weight.

Characterization of *S. trojana* essential oil

The essential oil components were identified and characterized using an HP-5MS column (30 m x 0.25 mm i.d., film thickness, 0.25 µm) connected to a GC-MS (Agilent 5973 N) detector and a GC-FID (Agilent 6890 GC). The samples, which were to be injected using the split injection method, were prepared at a ratio of 1:100. The temperature of the GC oven was initially set at 70ºC for 2 minutes. It was then increased at a rate of 10ºC per minute until it reached 150ºC. After reaching 150ºC, it was held at that temperature for 15 minutes. Finally, the temperature was increased at a rate of 5ºC per minute until it reached 240ºC. The carrier gas employed was

helium, flowing at a rate of 1 mL/min. The mass spectrometry analysis was performed using an energy of 70 electron volts (eV) and a mass range of 35 to 425. The retention index (RI) was determined by comparing the retention times of the sample compounds with those of the *n*-alkane series. Component identification was conducted by comparing mass spectra with electronic libraries such as Wiley and NIST laboratories.

Experimental groups

A total of 35 male Sprague Dawley rats, weighing between 180 and 200 grams, were included in the investigation. Each group consisted of seven rats. The animals were housed in a room with a controlled temperature of 22±2ºC and a light-dark cycle of 12 hours. They had unrestricted access to food and drink. The rats were classified into five categories as follows:

Control Group: Administration of 1% Tween 80 (oral route).

Positive Control (ARIC): Aricept tablet (donepezil) at a dose of 10 mg/kg for 30 days and administration of $AlCl_3$ (100 mg/kg).

$AlCl_3$-only Group (ALM): Intraperitoneal (*i.p.*) administration of 100 mg/kg bw $AlCl_3$ for 30 consecutive days.

$AlCl_3$ with 40% S. trojana essential oil group (EO 40%): Essential oil 40 mg/kg: Application of volatile oil at a dose of 40 mg/kg orally and administration of $AlCl_3$ (100 mg/kg) for 30 days.

$AlCl_3$ with 80% S. trojana essential oil group (EO 80%): Application of essential oil at a dose of 80 mg/kg orally and administration of $AlCl_3$ (100 mg/kg) for 30 days.

Animal model and essential oil administration

The essential oil was dissolved in 1% Tween-80 (Sigma Aldrich, Missouri, USA). Essential oil was administered orally in two doses, 40 mg/kg and 80 mg/kg, for a duration of 30 days. The control group received only 1% Tween-80 without essential oil or $AlCl_3$ to rule out confounding effect. Donepezil (10 mg/kg, oral) was administered as a positive control. $AlCl_3$ (Sigma Aldrich, Missouri, USA) was freshly dissolved in 1 mL sterile water each day and was administered by gavage at a dose of 100 mg/kg for 30 consecutive days. This study was approved by Balikesir University Local Ethical Commitee (2023/4). Animal Studies were performed in Balikesir University Experimental Animals Production, Care, Application and Research Center.

Behavioral tests

Y-maze test

The Y-maze is a memory assessment tool including three arms with dimensions of 35 x 25 x 10 cm, together with a central equilateral triangular region. This experiment, conducted during a 24-hour period, comprised placing rats at the midpoint of the labyrinth and giving them the freedom to explore for a duration of 8 minutes. The hind paws were considered to have entered the maze arm when they were completely inside. Spontaneous alternation behavior is characterized by consecutive entries into three distinct arms. Prior to testing each successive animal, the maze was cleansed using a solution containing 10% ethanol (Sigma Aldrich, Missouri, USA) and then dried using paper towels[15].

Elevated plus maze test

The plus-maze is comprised of four arms, each measuring 49 cm in length and 10 cm in breadth. The maze is elevated 50 cm from the ground by metal legs. Two arms were surrounded by walls of 30 cm in height, while the other two arms were left uncovered. Every rat was positioned at the midpoint of the maze, with its front directed towards an unobstructed arm. Observations were conducted for a duration of 5 minutes, during which the amount of time spent in and the number of entries made into both open and closed arms were documented. After each test, the floor was sanitized using a solution consisting of 10% ethanol[16].

Forced swimming test

During the pre-test session, the rats were individually placed in a tank filled with water. The tank had a diameter of 30 cm and a height of 59 cm. The water temperature was maintained at 26±1°C. The animals were permitted to engage in a 15-minute swimming session, after which

they were dried and placed back in their cages. The aforementioned method was replicated after a span of 24 hours, namely during a 6-minute swimming session referred to as the test session. The test session involved recording two specific behaviors: (1) immobility, which refers to the time spent making efforts to keep the head above water, and (2) high mobility, which refers to the time spent in active swimming and climbing movements[17].

Open field test

A box of 100 x 100 x 40 cm was utilized for the open field test. The middle area was partitioned into 16 identical squares, with a central area measuring 50 x 50 cm and outlying parts measuring 25 cm a piece. Every rat was positioned at the midpoint of the arena and monitored for a duration of 5 minutes. The recorded behaviors encompassed the duration of time spent in, as well as the count of entries and departures from, both the central and periphery areas[18,19]. The behavioral tests were observed with a camera setup and the software of EthoVision XT (version 10), Noldus Information Technology, Wageningen, The Netherlands.

Biochemical analyses

After all behavioral experiments were finished, rats were fasted overnight; the next day they were euthanized under anesthesia, dissected and whole brains were removed. Hippocampus samples were separated, washed with cold (+4ºC) potassium phosphate buffer (Sigma Aldrich, Missouri, USA), dried with blotting paper, and rapidly frozen in liquid nitrogen for preservation until analysis. Tissue dry weights were measured before analysis, and tissues were homogenized in a cold 0.1 M potassium phosphate buffer (pH 7.4) at a ratio of 1:9 (w:v) using a homogenizer. The prepared homogenates were centrifuged at 16000 rpm for 2 minutes at +4°C, and the supernatants were collected.

Molecular docking analyses

Molecular interactions between AMPA receptor and the major components of *S. trojana* essential oil were evaluated via molecular docking using Autodock 4. 3D structure of AMPA receptor was obtained from RCSB Protein Data Bank. The structures of carvacrol, carvone and α-pinene were received from PubChem. Openbabel was used to convert the structures from sdf to pdb format. The receptor and the ligands were prepared via AutoDock 4. Water molecules and the ligand were removed, polar hydrogens and charges were added to the receptor. The grid box was generated covering the ligand binding domain. Binding free energies were also calculated by Autodock 4. The interactions were saved as pdbqt format. Discovery Studio 2024 was used to illustrate the ligand/receptor interactions.

Results

Essential oil components of *Sideritis trojana*

In the study, the essential oil yield of *S. trojana* was determined as 0.2% (v/w). GC and GC-MS analyses identified a total of 28 components, constituting 95.83% of the total oil. The main components were identified as carvacrol (42.32%), carvone (18.69%), and α-pinene (4.54%), as shown in Table 1.

Behavioral tests

Forced swimming test

In the forced swimming test, two parameters, namely, high mobility time and immobility time, were considered. Generally, significant differences were observed in the high mobility time (Fig. 1a) (p<0.0001). In the Tukey's *post hoc* analysis, the inter-group differences were found as follows: Control vs. ARIC (p=0.0002), control vs. EO40% (p=0.0104), control vs. EO80% (p<0.0001), ARIC vs. ALM (p<0.0001), EO40% vs. ALM (p<0.0001), EO80% vs. ALM (p<0.0001). For the immobility time (Fig. 1b), ANOVA yielded overall significant differences (p<0.0001). According to the Tukey's *post hoc* analysis, inter-group differences were as follows: Control vs. ARIC (p=0.0061), ARIC vs. ALM (p<0.0001), EO40% and ALM (p<0.0061), EO80% and ALM (p<0.0004).

Elevated plus maze test

In the elevated plus maze test, the time spent in the open arms and the number of entrances

Table 1. Chemical composition of *Sideritis trojana* essential oil

Components	KI*	KI¥	%
Monoterpenes			
α-Pinene	936	935-939 [a-c]	4.54
β-Pinene	977	974-982 [a-c]	2.94
α-Phellandrene	1004	1002c	1.30
α-Terpinene	1017	1015–1019 [d]	1.05
Limonene	1028	1024-1032 [a,b]	0.95
Oxygenated Monoterpenes			
1,8-Cineol	1031	1031 [b]	1.23
Verbenol	1144	1125–1144 [d]	0.53
p-Mentha-1,5-dien-8-ol	1165	1131–1165 [d]	0.81
α-Terpineol	1189	1186-1188 [a]	1.07
cis-Carveol	1226	1196–1224 [d]	0.55
Carvone	1242	1242 [c]	18.69
Sesquiterpenes			
cis-α-bergamotene	1414	1406–1416 [d]	0.32
trans-α-bergamotene	1434	1429–1435 [d]	0.80
trans-(β)-Caryophyllene	1421	1411-1419 [a,b]	1.27
Germacrene-D	1480	1473-1485 [a,b]	0.69
Bicyclogermacrene	1497	1486–1497 [d]	0.87
Oxygenated Sesquiterpenes			
Spathulenol	1576	1577-1578 [a]	3.54
Caryophyllene oxide	1581	1572-1583 [a]	3.31
Cubenol	1635	1632–1644 [d]	1.88
Valeranone	1670	1674 [a]	2.11
α-Bisabolol	1680	1685-1688 [a]	1.49
Phenolic Components			
Thymol	1290	1290 [b]	1.76
Carvacrol	1301	1298-1299 [a-c]	42.32
Hydrocarbons			
Benzenemethanol, 4-(1-methylethyl)-	1245	1240-1245 [d]	0.51
Cuminaldehyde	1247	1196–1226 [d]	0.39
α-Ionone	1425	1426 [d]	0.35
2-Pentadecanone, 6,10,14-trimethyl-	1844	1832-1844 [d]	0.76
Total			95.83

*KI Kovats indices of the sample, ¥KI of literature a[55] b[56] c[57] d[58]

and exits (frequency) to the open arms were taken into account. ANOVA revealed significant overall differences in the time spent in the open arms (Fig. 2a) (p=0.0022). Tukey's *post hoc* analysis revealed significant differences between control vs. ALM (p=0.0095), ARIC vs. EO80% (p=0.0482), ARIC vs. ALM (p=0.0034), and EO40% vs. ALM (p=0.0407). Furthermore, general overall differences were obtained in the open arm frequency (Fig. 2b) (p<0.0001). Significant differences between control vs. ARIC (p=0.0023), control vs. ALM (p=0.0241),

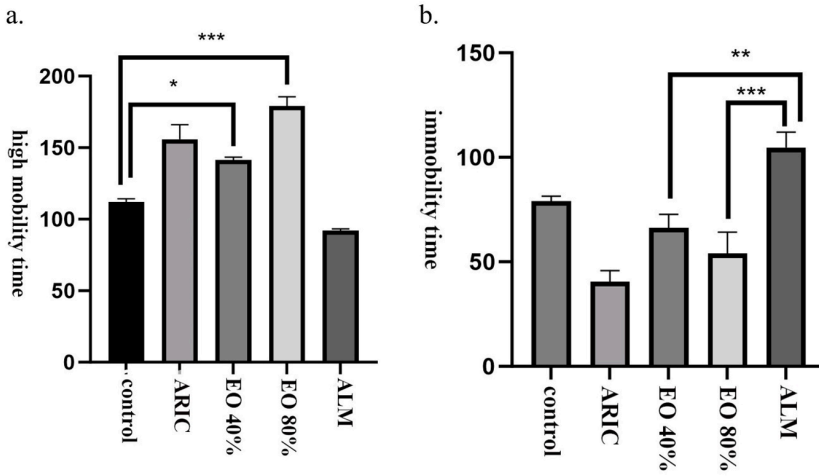

Figure 1. High mobility time (a) and immobility time (b) in the forced swimming test. ARIC stands for Aricept tablet that includes donepezil; ALM stands for $AlCl_3$

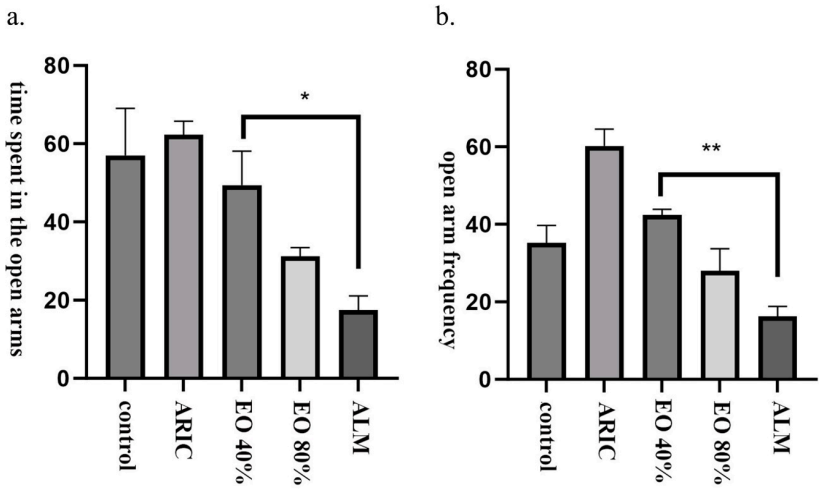

Figure 2. Time spent in the open arms (a) and open arm frequency (b) in the elevated plus maze. ARIC stands for Aricept tablet that includes donepezil; ALM stands for $AlCl_3$

ARIC vs. EO40% (p=0.0377), ARIC vs. EO80% (p<0.0001), ARIC vs. ALM (p<0.0001), EO40% vs. ALM (p=0.0014) were obtained through Tukey's *post hoc* analysis.

Open field test

Central area frequency, time spent in the central area, periphery area frequency and time spent in the periphery area were used in this test. Significant overall differences were obtained through one-way ANOVA (p=0.0172) in central area frequency (Fig. 3a). Furthermore, group differences were obtained between control vs. EO40% (p=0.0187) and EO40% vs. ALM (p=0.0297). For the time spent in the central area (Fig. 3b), ANOVA showed a lower *p* value (*p*=0.0038) and therefore significant differences were obtained in general. Group differences between ARIC vs. ALM (p=0.0124) and EO40% vs. ALM (p=0.0040) were determined. In addition, overall differences in the periphery area frequency (Fig. 3c) (p=0.0384), and significant group difference between EO80% vs. ALM (p=0.0273) were determined. ANOVA revealed overall differences in the time spent in the peripheral area (Fig. 3d) (p=0.0168). Tukey's

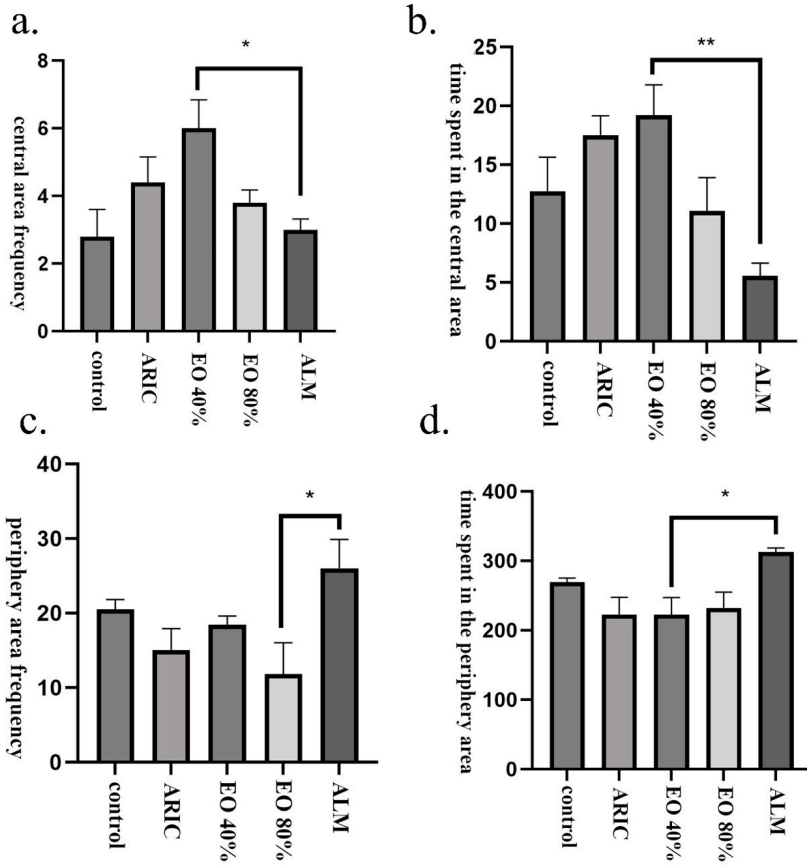

Figure 3. Central area frequency (a), time spent in the central area (b), periphery area frequency (c), and time spent in the periphery area (d) in the open field test. ARIC stands for Aricept tablet that includes donepezil; ALM stands for $AlCl_3$

post hoc test revealed significant differences between ARIC vs. ALM (p=0.0299), and EO40% vs. ALM (p=0.0295).

Y-maze test

In the Y-maze test, the spontaneous alternation was generally found to be significant (p<0.0001). Differences between groups were obtained with the Tukey *post hoc* test and significant differences were found between Control vs. ARIC (p=0.0037), ARIC vs. EO80% (p=0210), ARIC vs. ALM (p<0.0001), EO40% vs. ALM (p=0.0026), EO80% vs. ALM (p=0.0150) (Fig. 4).

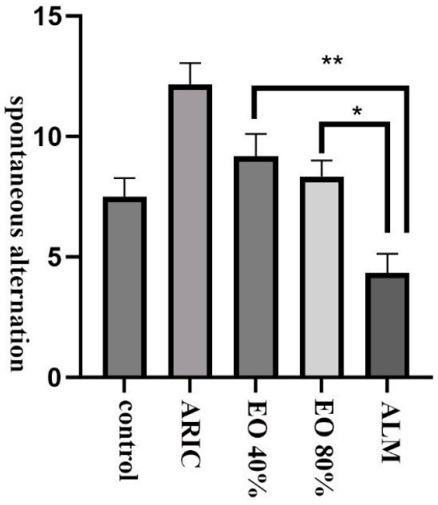

Figure 4. Spontaneous alternation in the Y-maze test. ARIC stands for Aricept tablet that includes donepezil; ALM stands for $AlCl_3$

Catalase and MDA levels

An overall difference in catalase level in the hippocampus was determined as p=0.0046 through ANOVA (Fig. 5a). Differences between

groups were examined with the Tukey *post hoc* test. Accordingly, significant differences were obtained between ARIC vs. ALM (p=0.0119), EO40% vs. ALM (p=0.0226), and EO80% vs. ALM (p=0064). Data show that aluminum application significantly reduced the level of catalase in the hippocampus, while essential oil application led to a relative increase.

MDA levels in serum generally showed significant differences (Fig. 5b) (p=0.0011). Also, significant group differences were obtained between EO40% vs. ALM (p=0.0027), and EO80% vs. ALM (p=0.0018). Compared to rats treated with aluminum alone, MDA levels were significantly reduced in rats treated with essential oil. However, no significant difference was observed between EO40% and EO80% applications.

Molecular docking analyses

Table 2 shows the binding free energies, inhibition constants, H bonds and other interactions between AMPA receptor and the major components of *S. trojana* essential oil which are carvacrol, carvone and α-pinene. Carvacrol was found to possess a strong binding affinity to AMPAR (Fig. 6a). The binding free energy between AMPAR and carvacrol was found to be -7.76 kcal/mol. H bond, Van der Waals and alkyl interactions were obtained in AMPAR/carvacrol complex. On the other hand, AMPAR/carvone complex also revealed a strong binding interaction (-7.90 kcal/mol) with several Van der Waals and alkyl interactions but without any H bonds (Fig. 6b). Lastly, α-pinene was found to possess strong binding affinity to AMPAR with a -7.27 kcal/mol binding free energy and many Van der Waals and alkyl interactions (Fig. 6c). Free binding energy of -6.00 kcal/mol and lower values are generally considered as strong binding affinities. Furthermore, low inhibition constant values suggest that the compounds are effective in low concentrations. Therefore, the three compounds in this research possibly interact with AMPAR in low concentrations.

Discussion

Turkey's endemic plant, *Sideritis trojana* Bornm., is used both in traditional medicine and as a popular herbal tea[13]. *S. trojana* is a plant that is grown in gardens for its ability to treat kidney diseases, stomach issues, sore throats, and pain in the abdomen[20].

The major constituents of *S. trojana* in this study were determined to be carvacrol (42.32%), carvone (18.69%) and α-pinene (4.54%). A previous study reported the major constituents of *S. trojana* as β-pinene (18.4%), α-pinene (13.2%), and germacrene (5.3%)[21]. As a result of our analyses, the volatile oil of *S. trojana* was found

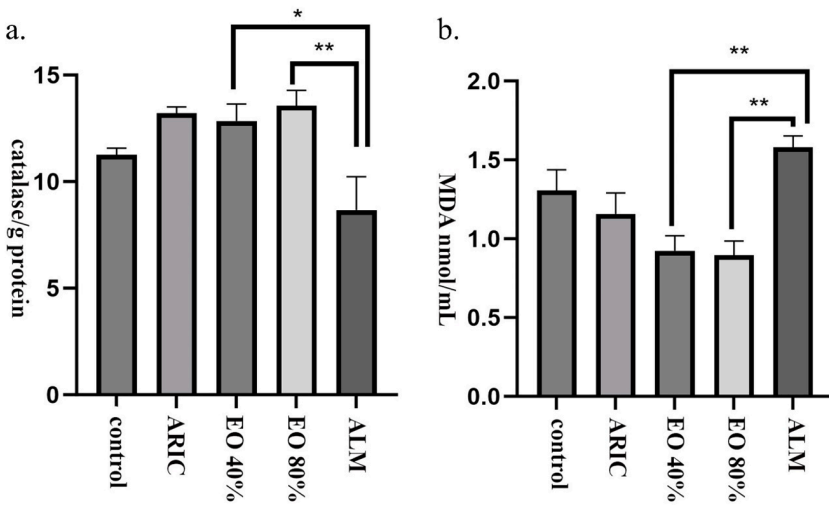

Figure 5. Catalase gram protein (a), and MDA nmol /mL (b) in the hippocampus samples of the rats. ARIC stands for Aricept tablet that includes donepezil; ALM stands for $AlCl_3$

Table 2. RMSD results of the major compounds in *Sideritis trojana* essential oil

Compound	Binding free energy (kcal/mol)	Inhibition constant (μM)	H bonds	Van der Waals interactions	Alkyl interactions
Carvacrol	-7.76	2.05	Leu 751 B	Glu 755B Ile 477C	Lys 726C
Carvone	-7.90	1.62	-	Ile 477C Glu 755B Lys 726C Asp 724C	Leu 751B Phe 654C
α-Pinene	-7.27	4.72	-	Gly 727C	Ile 477C Lys 493B Lys 726C Pro 494B Leu 751B Pro 490C

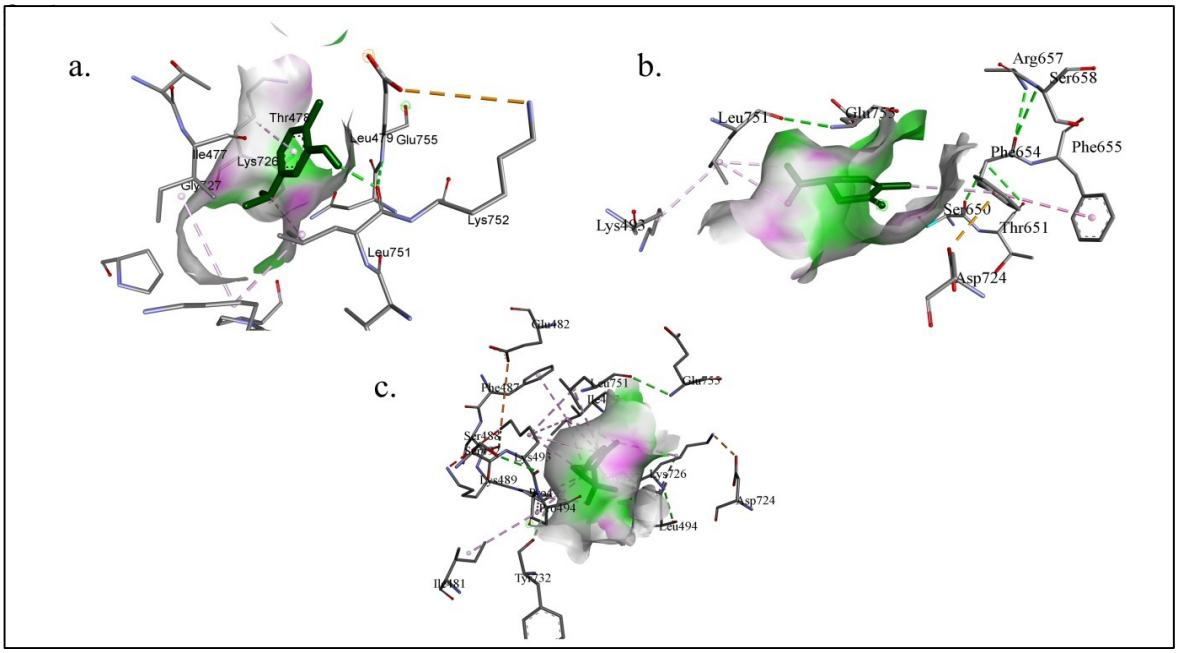

Figure 6. Molecular interactions between AMPA receptor and a. Carvacrol, b. Carvone, c. α-pinene. Green lines show H-bonds. Light pink lines show alkyl interactions

to be rich in monoterpenes, consistent with the existing literature. However, the variation in the major constituents are thought due to harvesting periods[22]. The essential oil yield of *S. trojana* was found to be 0.2% in this study. Similarly, Pasa *et al*.[23] reported a 0.25-0.30% essential oil yield of *S. trojana.*

The forced swimming test was developed more than 40 years ago. Since that time, forced swimming test has been used as a depression animal model[24]. Immobility time shows depressive-like behavior and despair. Therefore, anti-depressant activity can be detected with a decline in the immobility time[25]. The application of *S. trojana* essential oil in the forced swimming test demonstrated significant differences compared to the control group and the group treated with $AlCl_3$. Both doses of *S. trojana*

essential oil, but especially 80% increased high mobility time while decreasing immobility time in forced swimming test, as compared to control group. Therefore, *S. trojana* essential oil exhibited an antidepressant effect in rats treated with $AlCl_3$.

The elevated plus maze test is a commonly employed method for studying anxiety-like behavior in rodents. The presence of unconditioned aversive behavior in response to open and elevated areas can serve as an indicator of anxiety and can be evaluated using the plus maze test. Number of entries and time spent in the open arms are used as indicators of anxiety[26]. *S. trojana* essential oil, especially 80% group decreased the time spent in the open arms. Open arm frequency was also decreased in the *S. trojana* essential oil-received groups, especially in the 80% group. On the other hand, the open-field test is another widely used test to assess exploratory behavior and anxiety-related behavior. Animals that spent most of the time of the experiment near central part are considered as less-fearful or less-anxious rather than those that prefer perimeter area[27]. In this study, *S. trojana* essential oil decreased the central area frequency and time spent in the central area, while increasing the periphery area frequency and time spent in the periphery area, as compared to control and $AlCl_3$-alone treated rats. *S. trojana* essential oil, thus, showed anxiolytic effect in $AlCl_3$-induced rats.

Previously, carvacrol was reported to induce anxiolytic and anti-depressant behaviors in lipopolysaccharide-induced rats[28]. Anti-depressant-like effect of carvacrol was also reported in mice in another study[29]. In addition, Vilmosh *et al.*[30] showed that carvacrol significantly increased the time spent with a new congener in the social interaction test, suggesting anxiolytic effect. In a previous study, carvone was suggested to have anti-convulsant activity[31]. On the other hand, α-pinene was reported to mitigate oxidative damage, cognitive deficits, and depressive and anxiogenic-like behaviors in ketamine-treated mice[32].

The Y-maze spontaneous alternation test is extensively employed in several animal models to evaluate short-term memory. An elevated level of spontaneous alternation has been regarded as a reliable indicator of good spatial memory[33]. *S. trojana* essential oil increased spontanous alternation as compared to control and $AlCl_3$-only induced rats, suggesting memory-enhancing properties. Carvacrol was reported to improve memory and cognition by reducing oxidative stress, inflammation, and amyloid beta-induced neurotoxicity in AD[34]. In another study, carvacrol was suggested to improve memory impairment induced by neuroinflammation[35]. Carvacrol was also reported to improve learning and memory in aged rats with antioxidative effect[36]. A study found that carvacrol can significantly protect neurons from damage caused by persistent cerebral hypoperfusion, which is frequent in neurological illnesses like AD[37]. The neuroprotective effects of carvacrol was also shown previously against cerebral ischemia/reperfusion injury[38]. In this study, carvacrol was found to be the most abundant component of *S. trojana* essential oil. Therefore, carvacrol possibly enhanced memory in aluminum-induced rats. On the other hand, carvone was reported to successfully improve the cerebral ischemia/reperfusion induced neuroinflammation in rats[39]. In a study, carvone inhalation affected memory capacity in a fear-conditioning test[40].

Oxidative stresses progressively elevate in intensity throughout the course of an individual's life, leading to a decline in mitochondrial function and causing harm to various bodily systems, with a particular emphasis on the central nervous system. Therefore, oxidative stress plays a significant role in the advancement of aging and AD[41]. Oxidative stress is a primary cause of cognitive decline throughout aging or age-related neurodegenerative disorders. The hippocampus and frontal lobe of the brain are particularly susceptible to damage caused by oxidative stress[41]. In this study, *S. trojana* essential oil increased catalase activity, while reducing MDA levels suggesting reduced oxidative stress in the hypocampus of $AlCl_3$-induced rats. Catalase plays a key role in maintaining oxidative balance in cells. Deficiencies in catalase activity is associated with oxidative stress, aging and

various diseases. Catalase has been also shown to protect cells from amyloid-beta toxicity which is a key factor of AD[42]. MDA is a marker of lipid peroxidation, which reflects the production of oxygen free radicals, causing neurodegenerative diseases. Oxidative damage in neurons disrupts micro-environment in the brain causing memory decline and other AD associated symptoms[43]. This study shows a reduction of MDA with increased catalase activity in *S. trojana* essential oil-administered rat brain. Therefore, in this study, reduced oxidative stress possibly resulted in neuroprotective effects of *S. trojana* essential oil. Anxiety disorders are characterized by reduced antioxidant defenses and elevated oxidative damage to proteins, lipids, and nucleic acids[44]. Oxidative stress has been linked to the development of depression and anxiety disorders[45]. In this study, *S. trojana* induced anxiolitic and antidepressant effects could be related to its antioxidant profile.

Carvacrol has been reported to possess antioxidant properties and *in vitro* DNA protective effects[46]. *In vivo* antioxidant effects of carvacrol have also been shown in the brain samples[47]. Furthermore, carvacrol-induced rats were reported to exhibit decreased oxidative stress and inflammation[48]. Carvacrol also decreased antioxidant parameters such as total glutathione and increased MDA levels in hepatotoxic rats[49]. Carvone was reported to possess antioxidant and anti-inflammatory properties[50]. Carvone administration was shown to increase catalase activity in doxorubicin-induced toxic mice[51]. Furthermore, α-pinene was reported to enhance antioxidant capacity in Huntington's disease animal model[52]. In addition, α-pinene was shown to alleviate amyloid β-induced oxidative/nitrosative stress, neuro-inflammation, and behavioral deficits[53].

The modification of glutamatergic signaling through positive allosteric modulation of AMPA receptors could be an alternative AD treatment that may restore synaptic integrity and memory[54]. In this study, strong binding affinities of carvacrol, carvone and α-pinene to AMPAR in low concentrations were obtained. These results show strong interactions between AMPAR and the three major components of *S. trojana* essential oil. Neuroprotective effects of *S. trojana* essential oil could occur through positive allosteric modulation of AMPA receptors. However, *in vitro* studies are necessary to validate the results. The major compound within the *S. trojana* essential oil in this study is carvacrol. Therefore, the effects of the essential oil could be attributed to carvacrol. Furthermore, carvone and α-pinene which are also abundant compounds in this study could contribute the neuroprotective effects of *S. trojana* essential oil. It is also possible that the components of *S. trojana* essential oil acted synergistically.

The limitations of the study include the duration (30 days) of the essential oil application. Further studies of longer duration could provide long-term effects of *S. trojana* essential oil on $AlCl_3$-induced dementia. In addition, this study only covers male rats. Further studies including female rats or both genders could provide more comprehensive understanding of the effects of *S. trojana* essential oil. Also, the effects of carvacrol, carvone and α-pinene should be further studied in $AlCl_3$-induced rats as well as in other animal models of neurodegenerative diseases. Further pre-clinical studies may include the validation of molecular mechanisms of *S. trojana* essential oil and the major components leading to neuroprotective properties. Allosteric modulation of AMPA receptor by *S. trojana* essential oil should be validated. The main step of drug development is dosage optimization. After these studies, clinical trials could be performed.

Conclusions

Sideritis trojana essential oil has been found to enhance memory exhibiting, anxiolytic and antidepressant-like effects in rats treated with $AlCl_3$. Additionally, it has increased catalase activity and reduced MDA level. Due to its neuroprotective and antioxidant effects, *Sideritis trojana* essential oil may offer protection against central nervous system disorders including AD. The neuroprotective effects of *Sideritis trojana* essential oil are likely attributed to the high concentrations of carvacrol, carvone and α-pinene present in its composition.

Conflict of interest statement

Authors declare no conflict of interest.

Acknowledgement

This study was funded by Balikesir University, Scientific Research Projects Unit (2022/082).

Supplementary Data

Figures S1 and S2 are provided in supplementary file.

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