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Original Article

Anti-Alzheimer's disease effect of essential oil from aerial parts of *Salvia miltiorrhiza* Bge

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Abstract: The present study aimed to identify the anti-AD effect of essential oil from aerial parts of *S. Miltiorrhiza.* The essential oil from aerial parts of *S. Miltiorrhiza* was obtained by steam distillation and then was analyzed through GC-MS. The AD mice model was induced by D-gal plus $AlCl_3$, and then the mice in the experimental group were treated with essential oil from aerial parts of *S. Miltiorrhiza*. The protective effects of essential oil on the memory impairment of mice were determined by Morris water maze test. The contents or activity of SOD, CAT, MDA, Ach and AchE in mice brain homogenate and serum were analyzed by Biochemical Determinations (SOD, CAT, MDA, Ach and AchE kits were used). The result showed that terpenoids was the main components of essential oil from aerial parts of *S. Miltiorrhiza*, accounting for about 50.18% of the total essential oil, and β-caryophyllene (8.58%), 6,10,14-trimethyl-2-pentadecanone (7.97%), dihydro-neoproene (7.96%), germacrene D (6.37%) caryop, hyllene (4.22%) were the main and characteristic compositions of the essential oil. The essential oil, given orally, prevented cognitive impairment in AD mice induced by D-gal plus $AlCl_3$. Compared to the model group, SOD activity, CAT activity and Ach content were found to be increased in test group mice, while AchE activity and MDA content were decreased. All the above suggest that essential oil from aerial parts of *S. Miltiorrhiza* improve AD-like symptoms in mice induced by D-gal and $AlCl_3$, and has the potential to develop a new drug for the treatment of AD.

Keywords: Alzheimer's disease, *Salvia miltiorrhiza*, aerial parts, essential oil, anti-AD activity

Introduction

Alzheimer's disease (AD), a commonly chronic progressive neurodegenerative brain disease, is one of the most common dementia diseases, accounting for about 60%-80% of all dementia case [1]. The clinical manifestations of AD are content cognitive and language dysfunction, personality changes and memory decline, etc. It is characterized by the formation of extracellular senile plaques (SPs) by the accumulation of β-amyloid (Aβ), the formation of intracellular neurofibrillary tangles (NFTs) by the abnormal phosphorylation of tau protein, nerve cell necrosis, and vascular amyloidosis of cortical arteries and small arteries [2-6]. With the development of the aging process, the problem of senile dementia on human health is more and more prominent and senile dementia has become the fourth most deadly disease just after cardiovascular disease, cancer and AIDS [7]. With incomplete statistics, the number of AD patients worldwide reached 60 million, of which the number of AD in China is close to 10 million [8, 9]. However, the pathogenesis of AD is not very clear. So to explore the etiology of AD and to find effective treatment for AD drugs is particularly important.

As a traditional Chinese medicine, *Savlia miltiorrhiza Bge.* is mainly distributed in Shandong, Shanxi, Shaanxi, Hebei, Henan, Anhui, Zhejiang, Hubei and Jiangsu province of China and Japan [10]. The main ingredients of *S. Miltiorrhiza* contain tanshinones, phenolic acids, flavonoids, polysaccharides, amino acids and terpenes. This medicinal herb has the efficacy of promoting blood circulation to remove blood stasis, inducing menstruation to relieve menalgia, clearing away heat, relieving vexation, cooling the blood to relieve carbuncles, and so on [11, 12]. Recent pharmacological and phytochemical studies were mainly focused on the fat-soluble quinone abietanes and water-soluble phenolic acids, which have the pharmaco-

logical activity of anti-bacterial, anti-inflammatory, anticoagulant, anti-thrombosis, and cell protection, were the two kinds of major active constituents in the root of *S. Miltiorrhiza* [13-15]. Traditionally, the root and rhizome of *S. Miltiorrhiza* are the main drug parts, the aerial part is not for medicine. With the continuous improvement of people's living standards, the demand for Chinese medicine is increasing. Since *Fufang Danshen Dripping Pills* were listed in US market, the demand of *S. Miltiorrhiza* showed a sharp increase. According to statistics, only in Shandong, China, the annual demand of *Salvia* medicinal herbs reached 10,000 tons and the weight of the aerial parts take up about two thirds of the total weight of *S. Miltiorrhiza* [16]. In the process of search for new drug source, it was found that some of the chemical composition and pharmacological effects of the aerial part of *S. Miltiorrhiza* had some similarity with its root [17-19] which will greatly improve the development and utilization of resources if the aerial parts of *S. Miltiorrhiza* can be full used. This will not only reduce the waste of resources, but also ease the current environmental pollution situation to a certain extent.

Essential oil is one of the important active ingredients of the genus *Salvia*, with antibacterial, anti-inflammatory, anti-oxidation, anxiolytic, anti-tumor and other pharmacological activities [20-25]. The components and pharmacological activities of essential oil from *S. miltiorrhiza* were studied by many researchers [26-30], and the results showed that terpenoids and oxygen compounds were the main components of it. Li [28] analyzed the components of essential oil from aerial parts of *S. Miltiorrhiza.* by GC-MS, and found that β-caryophyllene, α-caryophyllene, germacrene D, bourbonene and other sesquiterpenes were the characteristic components in stems, leaves, flowers of *S. Miltiorrhiza.* Another studies found that terpenoids have significant pharmacological effects, such as antioxidant, anti-inflammatory, antibacterial, anti-tumor and improving the symptoms of Alzheimer's disease [31-35]. These provide a theoretical basis for the study of the anti-AD effect of the essential oil from aerial parts of *S. Miltiorrhiza*.

In this study, GC/GC-MS was used to analyze the essential oil components of aerial parts of *S. Miltiorrhizae*, and the therapeutic effect on memory impairment induced by D-gal and $AlCl_3$ was tested by mouse pharmacological experiment. Through this experiment, we expected to provide a reference for the comprehensive development and utilization of *S. Miltiorrhiza* resources, and to find a new natural medicine for the treatment of AD.

Materials and methods

Animals

Healthy female adult kunming strain mice (2 months old and weighing 35 ± 5 g) were used in the study. This study was approved by Shandong University of Traditional Chinese Medicine's ethics committee, and all procedures complied with the guidance set out in the Guidelines for Caring for Experimental Animals published by the Ministry of Science and Technology of the People's Republic of China. The mice were taken to minimize discomfort, distress, and pain. They were group housed (10 animals/cage) with a 12:12-hour light/dark cycle and ad libitum access to food and water and allowed to acclimatize to their new conditions for one week before commencing experiments. Before Morris water maze test, the mice were screened by their swimming ability. Then, the mice that can float on the water and do not take the initiative to swim were removed.

Isolation of essential oil

Aerial parts of *S. Miltiorrhiza* were collected in the medicinal botancial garden of Shandong University of Traditional Chinese Medicine, Shandong, Jinan, China in September 2016. It was identified by Pro. Yongqing Zhang and a vouncher specimen were deposited at the Herbarium of Shandong University of Traditional Chinese Medicine. The air-dried aerial parts of *S. Miltiorrhiza* (10 kg) were crushed into a coarse powder, and the essential oil were isolated by hydrodistillation using a Clevenger-type apparatus for 6 h according to the method recommended in the Chinese Pharmacopoeia 2015 edition. The essential oil was dried over anhydrous sodium sulfate and stored at -20°C until analysis.

Identification of components of essential oil

Identification of the components of the essential oil was based on GC retention indices relative to n-alkanes and computer matching with

Table 1. Chemical compositions of essential oil from Salvia miltiorrhiza Bge. aerial parts

NO.	Retention time/min	Compound	Molecular formula	Molecular weight	Relative content/%
1	4.277	O-xylene	C_8H_{10}	106	0.260
2	6.749	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	$C_{10}H_{16}$	136	0.103
3	7.734	1-Octen-3-ol	$C_8H_{16}O$	128	0.826
4	8.332	Camphene	$C_{10}H_{16}$	136	1.633
5	11.370	3-acetoxy-4-(1-hydroxy-1-methylethyl) -1-methyl-cyclohexene	$C_{12}H_{20}O_3$	128	0.466
6	12.422	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{10}H_{18}O$	156	0.650
7	14.467	2-methyl-undecane	$C_{11}H_{24}$	156	6.765
8	15.046	Bicyclo [2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	$C_{10}H_{18}O$	156	0.507
9	15.960	3-Cyclohexene-1-methanol, .alpha., .alpha. 4-trimethyl-	$C_{10}H_{18}O$	156	0.806
10	18.079	2-Cyclohexen-1-one, 5-methyl-2-(1-methylethyl)-	$C_{10}H_{18}O$	156	0.137
11	19.255	N-[4-(Ethyl-methyl-amino)-phenyl]-acetamide	$C_{11}H_{16}N_2O$	192	0.639
12	19.406	Isoborneol	$C_{10}H_{18}O$	154	1.149
13	20.733	β-caryophyllene	$C_{15}H_{24}$	204	8.282
14	22.544	Pinene	$C_{15}H_{24}$	204	0.530
15	22.887	Caryophyllene	$C_{15}H_{24}$	204	3.271
16	24.177	Caryophyllene	$C_{15}H_{24}$	204	0.950
17	25.222	Octahydro-7-methyl-3-methylene-4-(1-methylethyl) -1H-cyclopenta [1, 3] cyclopropane [1, 2]	$C_{15}H_{24}$	204	0.349
18	25.419	α-caryophyllene	$C_{15}H_{24}$	204	1.278
19	25.629	α-caryophyllene	$C_{15}H_{24}$	204	0.849
20	26.670	γ-Vanillin	$C_{15}H_{24}$	204	0.346
21	27.102	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{13}H_{20}O$	192	1.266
22	27.637	Germacrene D	$C_{15}H_{24}$	204	4.366
23	29.102	(1S-cis)-1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl) naphthalene	$C_{15}H_{12}$	192	0.730
24	31.205	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	$C_{15}H_{26}O$	212	0.982
25	32.388	Dihydro-neoproene	$C_{15}H_{26}$	206	5.958
26	32.935	2,6-Lutidine-N-oxide	C_7H_9NO	123	1.103
27	33.768	3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	$C_9H_{14}O$	138	2.040
28	36.190	Cis-2,3,4,4a, 5,6,7,8-octahydro-1,1,4a, 7-tetramethyl-1H-benzocyclohepten-7-ol	$C_{15}H_{26}O$	222	0.824
29	36.947	2,3,4,4a, 5,6,7,8-octahydro-1,1,4a, 7-tetramethyl-1H-benzocyclohepten-7-ol	$C_{15}H_{26}O$	222	2.724
30	45.429	Huma-1,6-dien-3-ol	$C_{15}H_{24}O$	220	1.358
31	46.089	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	7.986
32	51.689	Isophytol	$C_{20}H_{40}O$	296	1.636
33	51.981	β-Caryophyllene oxide	$C_{15}H_{24}O$	220	1.526
34	53.619	7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	$C_{20}H_{30}$	270	0.561
35	55.457	Phytol	$C_{20}H_{40}O$	296	1.574
36	57.182	Dodecane	$C_{22}H_{46}$	310	0.771
37	59.896	Caryophyllene oxide	$C_{15}H_{24}O$	220	1.846
38	60.512	Tricosane	$C_{23}H_{48}$	324	1.329
39	64.865	Tetracosane	$C_{24}H_{50}$	338	1.636
40	66.092	Palmital	$C_{16}H_{32}O$	240	1.871
41	68.726	Pentacosane	$C_{25}H_{52}$	352	1.554
42	76.342	13-Docosenamide, (Z)-	$C_{22}H_{23}NO$	317	1.129
43	76.717	Octadecane, 1-iodo-	$C_{18}H_{37}I$	380	0.881
44	77.818	Squalene	$C_{30}H_{50}$	410	1.823
45	80.575	Methyl, oleate	$C_{19}H_{36}O_2$	296	1.860
46	83.354	Stigmastera-3, 5-diene	$C_{29}H_{48}$	396	1.382
47	86.187	Octacosane	$C_{28}H_{58}$	394	0.486
48	89.614	Ursa-9 (11), 12-dien-3-one	$C_{30}H_{46}O$	424	0.423

the Wiley 275 L mass spectra library. In addition, the analysis included comparisons of the fragmentation patterns of the mass spectra to those reported in the literature.

Animal experiments

An appropriate amount of essential oil was diluted with normal saline containing 3% Tween 20 to produce the final working concentration for pharmacological tests. In one set of experiments, the mice were orally treated with 100 mg/kg·d of $AlCl_3$ by gavage and treated with 100 mg/kg·d of D-gal by i.p., once a day for 8 weeks, with normal saline as a control. Then, the vehicle group mice were treated orally with normal saline, and other mice were divided into four groups (10 mice per group): with 3% Tween 20, essential oil (50 mg/kg·d), essential oil (150 mg/kg·d) and essential oil (450 mg/kg·d) 60 min after being given the $AlCl_3$ and D-gal, once a day for 8 weeks.

Morris water maze test

After 8 weeks of treatment with essential oil, the mice were subjected to Morris water maze test. The Morris water maze test was minor modifications according to previously described. A circular pool (diameter: 100 cm; height: 30 cm) with a white inner surface was applied to perform the Morris water maze test, and was located in a laboratory with a clear immovable clue. The pool was divided into 4 quadrants with an equal area, and a transparent platform (diameter: 8 cm; height: 12 cm) was fixed in one of the quadrants of the pool. The pool was filled with nontoxic white-dyed water being kept at 20-25°C, and the water surface higher than the transparent platform 0.5-1.0 cm so that the platform was invisible. The mice were released into the water at water-level (not dropped it), facing the tank wall. The released place was 1 of 3 randomly quadrant and the mice were allowed to find the hidden platform. The time spent from being released to finding and climbing onto the platform was defined as the escape latency, and it was recorded by a recorder. The mice was in a consecutive trial for 5 days (3 times/day), and the test time was 90 s (after climbing the platform, each mouse allowed to stay about 30 s, 10-15 min interval every two trainings). If the mouse failed to find the platform within 90 s, the experimenter should guide the mouse to the platform, and the escape latency was recorded as 90 s.

A probe test

The mice were subjected to a probe test to detect the spatial memory after 24 h of the last study exercise. In this experiment, the mice were placed in the pool (the position and way were the same as the Morris water maze test) after the platform was removed. The time spent in the target quadrant area and the time on the location of platform was recorded in 60 s, and was used to estimate the spatial memory retention of the mice.

Biochemical determinations

After a probe test, the mice were killed by cervical dislocation, the blood was collected, and the brains were removed. The blood was allowed to stand still at room temperature for 2 hours, then was centrifuged at 3000 g for 10 min. The serum of blood was used to determine the activity of AchE and the content of Ach according to the AchE, Ach kit instructions. The brains of mice were weighed and calculated brain body weight ratio, then homogenized in normal saline (all the operation under 4°C). The homogenized of the brains was centrifuged at 3000 g for 10 min, and the supernatant was diluted 10 times with the normal saline. Then the diluent was used to determine the activity of SOD, CAT and content of MDA according to the SOD, CAT, MDA kit instructions.

Statistical analysis

All data were analyzed by a one-way analysis of variance, and the differences between means were established by Duncan's multiple-range test. All data were expressed as mean ± S.E.M. The values of $P<0.05$ were considered significant.

Results

GC-MS analysis

The results of composition analysis of essential oil from *S. Miltiorrhiza* aerial parts and their percentages were given in **Table 1**, and the components are listed in order of their elution from the HP-5MS column. The total ion current of essential oil from *S. Miltiorrhiza* aerial parts are given in **Figure 1**.

Essential oil from aerial parts of S. Miltiorrhiza treatment rescues the memory impairment of mice

The results showed that the essential oil from aerial parts of *S. miltiorrhiza* could significantly

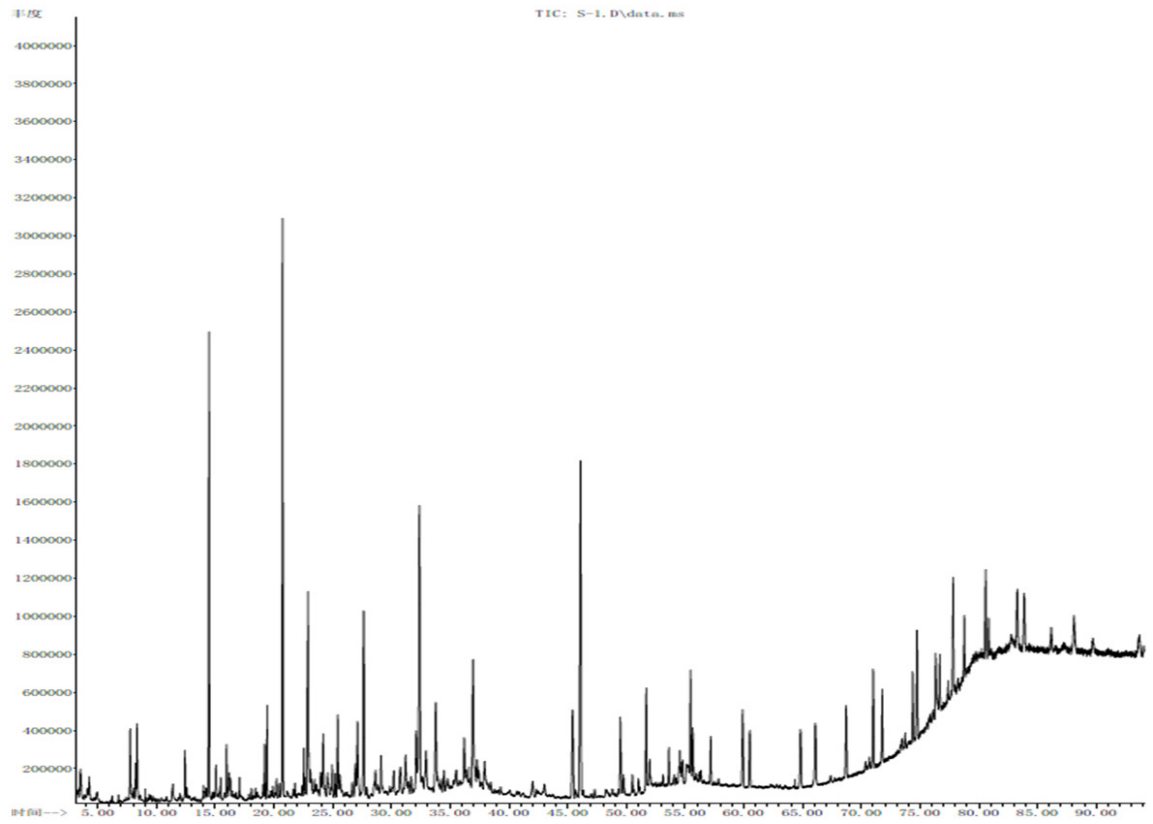

Figure 1. The total ion current of essential oil from Salvia miltiorrhiza Bge. aerial parts by GC-MS analysis.

improve the learning ability and spatial memory ability of mice induced by D-gal and $AlCl_3$, and slow down the process of AD. In previous experiments, the observations showed that at the lowest dose (50 mg/kg·d), the medium dose (150 mg/kg·d) and the highest dose (450 mg/kg·d) of treatment with essential oil performed different effects on rescuing the memory impairment of nice, and the effects showed a roughly dose-dependent manner. Among them, the lowest dose and the medium dose had significant difference (P<0.05), but no significant difference was found between the medium dose and the highest dose (P>0.05). So the medium dose was employed in the subsequent experiments, and the times on this dose were given in the **Figure 2**.

The effects of essential oil on the learning ability of mice were showed in **Figure 2A**. Through the learning trial, the escape latency of the mice reached the platform was gradually shortened. Compared with control group, model group mice developed an evident retardation in obtaining spatial memory, as the escape latency was not shortened in the training sessions (P>0.05). Compared with the model group, the escape latency of the treatment with essential oil group (150 mg/kg·d) was significantly shortened from the 4th day, and with significant difference (P<0.05).

Effects of essential oil on the retention of spatial memory of mice were showed in **Figure 2C**. The retention of spatial memory was detected through a probe test. Compared with the model group, the results showed that the test group mice treated with essential oil spent more time in the target quadrant, and with significant difference (P<0.05). The time spent in the target quadrant of the model group mice was significant less than the control group (P<0.05). There were no significant differences in times of passing through the location of platform by each group mice (P>0.05; **Figure 2D**). The effect of essential oil on the brain weight ratio of mice was showed in **Figure 2B**. The brain body weight ratio of the mice by treatment with essential oil was not significantly higher than that in the model group.

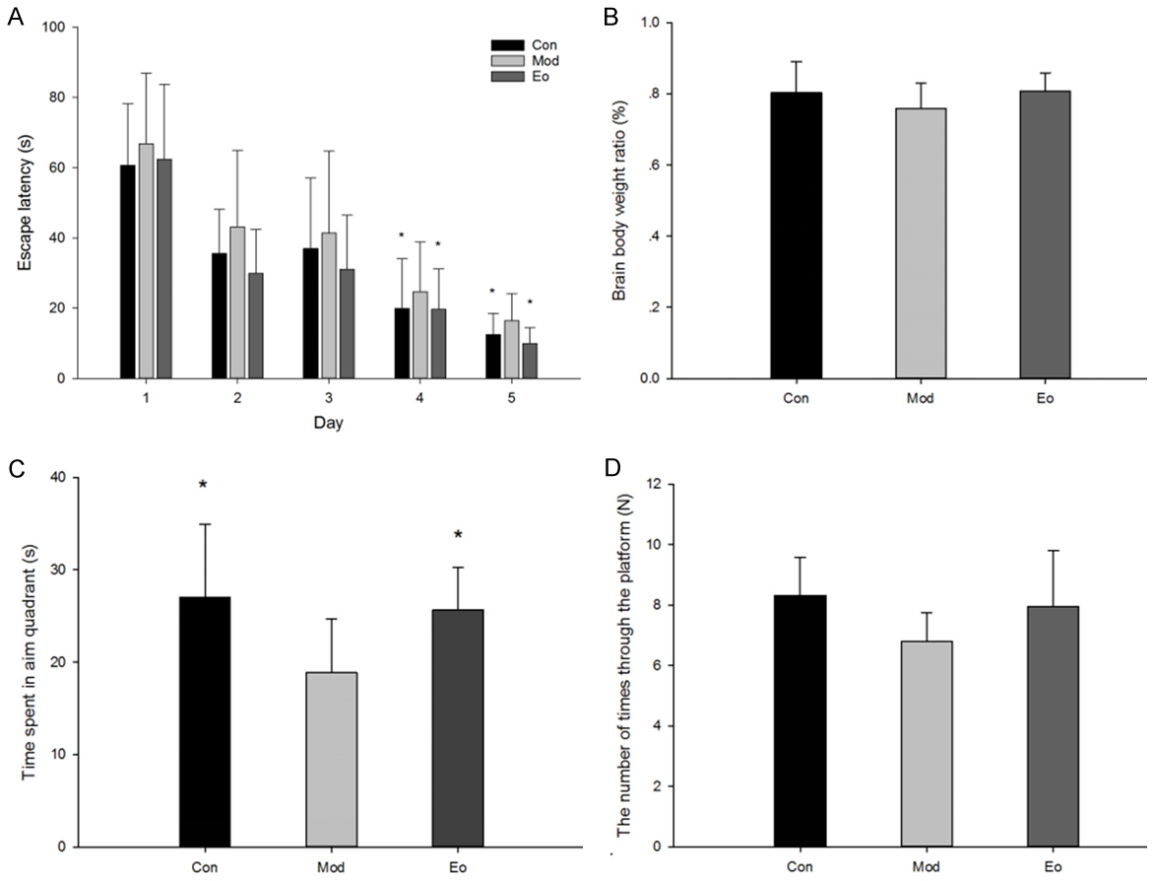

Figure 2. Essential oil, given orally, reduced memory impairment of AD mice induced by D-gal plus $AlCl_3$. 16 weeks later, the learning ability of mice was tested by Morris water maze test, and the escape latency (A) was recorded from the first day to the fifth day. Capacity of retained spatial memory was tested by a probe test, and the time spent in aim quadrant (C) and the number of times through the platform (D) was recorded. Then, the mice were killed by cervical dislocation, and the brain body weight ratio (B) was measured. All data were expressed as mean ± S.E.M. Con = control group mice; Mod = model group mice; Eo = test group mice with essential oil treatment (150 mg/kg·d). **P*<0.05, ***P*<0.01 *vs.* Mod.

Effect of essential oil from S. Miltiorrhiza aerial parts on brains oxidative stress levels

The superoxide radicals, harmful to health, generated by the brain metabolism, can be catalyzed by SOD transformed into H_2O_2, and then catalytic decomposed into completely non-toxic water by CAT, then was excreted. It has been suggested that the accumulation of superoxide free radicals can lead to the destruction of cell structure and function in vivo, and thus promote human aging. MDA, as the body's aging indicators, is a product of lipid peroxidation, and its content is gradually increased with the body's aging. In this experiment, the relative activities of SOD and CAT and the content of MDA in the brain homogenate were determined, then the accumulation of superoxide and the degree of oxidative stress injury in the brain of mice were measured and the aging level of the mice was determined. Compared to the model group, the results showed that the activities of SOD and CAT of the test group mice treated with essential oil were significantly stronger (*P*<0.05; **Figure 3A**, **3B**). The content of MDA in the model group was significantly more than the control group and the test group (*P*<0.05; **Figure 3C**).

Effect of essential oil from S. Miltiorrhiza aerial parts on serum AchE and Ach levels

Ach is an important neurotransmitter in the central cholinergic nervous system, which is closely related to the learning and retention of spatial memory ability of the organism. Throu-

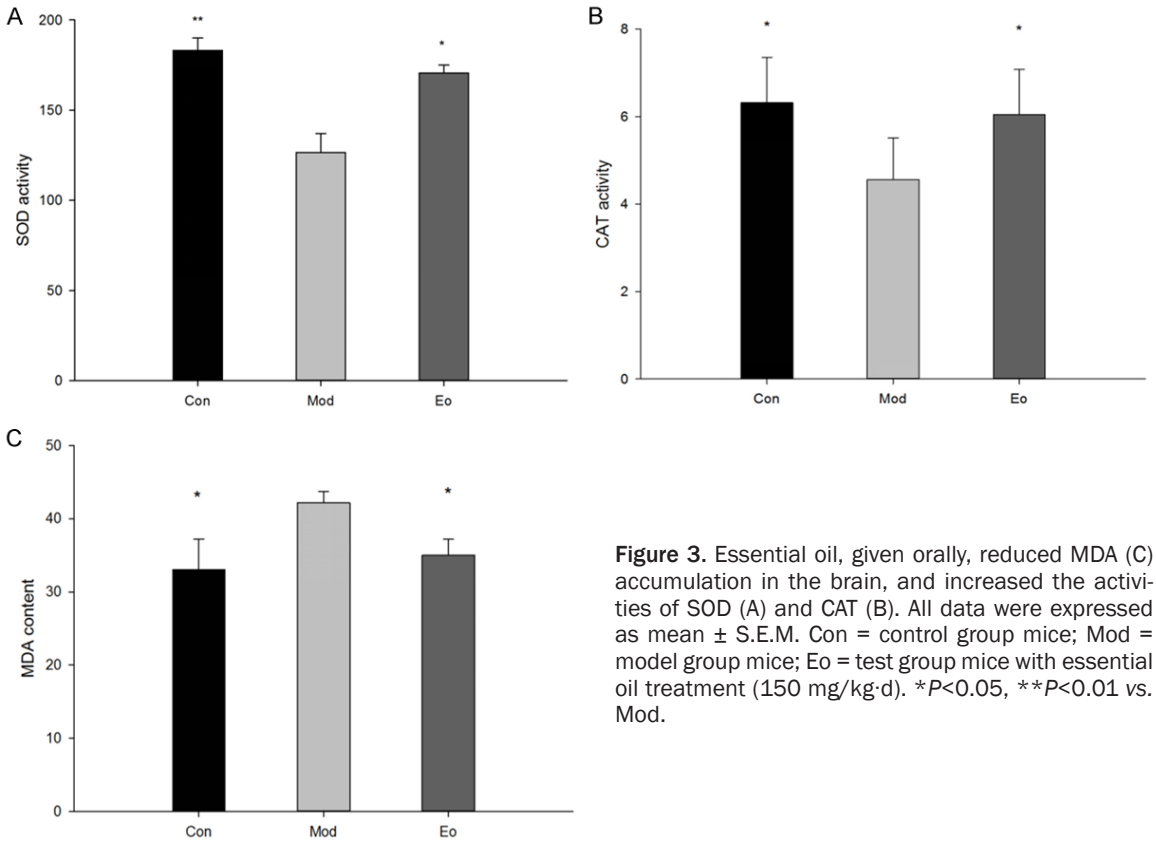

Figure 3. Essential oil, given orally, reduced MDA (C) accumulation in the brain, and increased the activities of SOD (A) and CAT (B). All data were expressed as mean ± S.E.M. Con = control group mice; Mod = model group mice; Eo = test group mice with essential oil treatment (150 mg/kg·d). **P*<0.05, ***P*<0.01 *vs.* Mod.

ghout the literature there have been numerous reports that the serum and the brain have a lot of Ach, and the contents will gradually decrease with aging. AchE is a kind of degrading enzyme of Ach, and its activity is negatively correlated with Ach content. Compared to the model group, the experiment results showed that the activities of AchE of test group was significantly lower (*P*<0.01; **Figure 4A**), and the contents of Ach were significantly higher (*P*<0.05; **Figure 4B**).

Discussion

In this experiment, GC-MS was used to analyze the components of the essential oil from *S. Miltiorrhiza*e aerial parts, and the total ion current map and the peak mass spectrum were obtained. The components were identified by computer search (NIST05a.L libraries date of the GC-MS system), artificial interpretation and literature proofing. The results were given in the **Table 1**. A total of 79 species were detected from the essential oil of aerial parts of *S. Miltiorrhiza*, and 48 species were identified, accounting for 81.42% of the total essential oil. The most of which is terpenoids, accounting for about 50.18% of the essential oil. Among terpenoids, β-caryophyllene, dihydro-neoproene, germacrene D, caryophyllene and α-caryophyllene were the main constituents of sesquiterpenes, representing 8.58%, 7.96%, 6.37%, 4.22% and 2.13%, respectively. Phytol and isophytol were the main constituents of diterpenes, representing 1.64% and 1.55%, respectively. Camphene (1.63%) and isoborneol (1.15%) were the main constituents of monoterpenes. Other compounds constituted 31.24% of the essential oil with 6,10,14-trimethyl-2-pentadecanone (7.97%) of ketone compounds, 3,4-dimethyl-3-cyclohexene-1-carbaldehyde (2.04%) of aldehydes and methyl oleate (1.86%) of esters. The essential oil from *S. Miltiorrhiza* aerial parts was characterized by a high content of β-caryophyllene (8.58%) as the principal compound, followed by 6,10,14-trimethyl-2-pentadecanone (7.97%), dihydro- neoproene (7.96%), germacrene D (6.37%) and caryophyllene (4.22%).

The components of the essential oil from *S. Miltiorrhizae* aerial parts have been analyzed

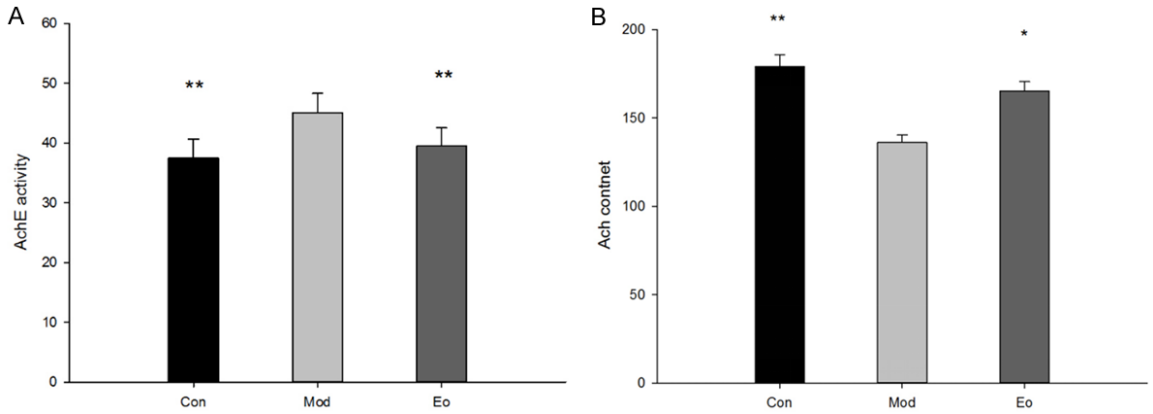

Figure 4. Essential oil, given orally, reduced the activity of AchE (A) in serum, and restored the content of Ach (B). All data were expressed as mean ± S.E.M. Con = control group mice; Mod = model group mice; Eo = test group mice with essential oil treatment (150 mg/kg·d). **P*<0.05, ***P*<0.01 *vs.* Mod.

by many researchers. According to the study carried out by Li et al. [28], hexadecanoic acid (17.0%), germacrene D (9.1%), phytol (8.9%), β-caryophyllene (7.1%) and methyl linolenate (5.3%) were characterized as the major constituents in the leaves of *S. Miltiorrhizae.* The components of essential oil in stem, leaf, and flower of *S. Miltiorrhizae* were identified by Chen [29] through the headspace solid-phase microextraction coupled by gas chromatography-mass spectrometry (HS-SPME/GC-MS). The result showed that β-caryophyllene (22.22%, 5.41%, 36.16%), germacrene D (9.69%, 16.76%, 8.00%) and bourbonene (4.61%, 13.05%, 13.05%) were the main compositions of the essential oil in stem, leave, flower of *S. Miltiorrhizae,* respectively. Ji [36] used the method of steam distillation extracted the essential oil from stem, leave and flower of *S. Miltiorrhizae,* and the components of essential oil were identified by GC-MS. The result showed that germacrene D (15.47%, 36.68%, 23.42%), caryophyllene (15.37%, 15.32%, 22.77%) and α-caryophyllene (5.97%, 6.06%, 10.37%) were the main compositions of the essential oil in stem, leave and flower of *S. Miltiorrhizae,* respectively. Throughout the literature there have been numerous reports on the chemical compositions of the essential oils from the aerial parts of *S. Miltiorrhizae.* The main components of essential oils have significantly different according to different researchers, and most of the reports indicated that β-caryophyllene, α-caryophyllene, caryophyllene, germacrene D, hexadecanoic acid, phytol and bourbonene were the main and/or characteristic constituents of the essential oil of the aerial parts of *S. Miltiorrhizae.* The present study showed that β-caryophyllene, dihydroneoproene, 6,10,14-trimethyl-2-pentadecanone, germacrene D, caryophyllene and α-caryophyllene were detected in the aerial parts essential of *S. Miltiorrhizae,* among which dihydro-neoproene and 6,10,14-trimethyl-2-pentadecanone as the main compositions of aerial parts essential oil of *S. Miltiorrhizae.* were rare reported in previously researches. The extraction method, the variety of *S. Miltiorrhiza*, the growth period, the time of harvesting and the drying method may be the cause of the difference in the composition of the essential oil.

AD is one of the most common in the elderly dementia group, accounting for about 60%-80% of all dementia case [1]. Now, the etiology of AD is not yet clear, the hypotheses on the pathogenesis of AD contains hypothesis of Aβ toxic, hypothesis of Al poisoning, hypothesis of free radical damage, hypothesis of inflammatory immune, hypothesis of genetic, hypothesis of Tau protein abnormal phosphorylation and hypothesis of cholinergic deficiency [37-41]. The establishment of AD animal model plays an important role in the prevention and cure of AD. The ideal AD animal model exhibits not only in the behavior of animal aging, but also in biochemistry, pathology and neurotransmitters and other aspects of change. The establishment of AD animal model has a history from a simple single factor induction to the multi-fac-

tor joint induction, and transgenic animal model, in which the model based on D-gal induced, is the most commonly used one.

In this study, the AD mouse model was induced by D-gal (D-galactose) combined with $AlCl_3$. The AD animal model of D-gal induction was put forward by Pro. XU of China Pharmaceutical University in the first time [42], and the mechanism of aging in mice is that the excess D-gal is converted to D-gal alcohol under the action of aldose reductase, then converted to D-galaldehyde and H_2O_2 catalyzed by galactose oxidase (GAO). GAO can also catalyze O_2 to convert to O_2^- in vivo, and then H_2O_2 and O_2^- produce HO· under the action of Fe^{2+}. H_2O_2, O_2^- and HO· are all referred to as reactive oxygen species (ROS), which accumulate *in vivo* to reduce intracellular mitochondrial dysfunction to reduce ATP synthesis and further increase ROS synthesis. ROS can induced neuronal apoptosis and body aging through effect on the membrane of nerve cell, membrane of mitochondrial, nucleic acid and enzymes, etc. In *vivo*, accumulation of Al can induce body aging through three major ways. Firstly, Al induced neuroinflammation through activated astrocytes, then leading to accumulation of Aβ in nerve cells. Accumulation of Aβ induced membrane potential imbalance of neuronal cells, resulting in ROS accumulation and the induced apoptosis neuronal cells. Secondly, Al interferes the metabolism of zinc, resulting in GSH, CAT, SOD and other antioxidant enzyme activity decrease, then causes ROS and MAD accumulation in *vivo*. MAD promotes the synthesis of APP, the precursor protein of Aβ, by neurotoxicity and further increases the accumulation of Aβ in neuronal cells. ROS and Aβ can induce neuronal apoptosis. Thirdly, Al activates the protein kinase C (PKC) through affecting the Ca steady state, causes the tau protein abnormal phosphorylation, resulting in neurofibrillary tangles (NFTs) in neuronal cell, and finally leading to neuronal cell apoptosis.

In this study, the components of the essential oil from *S. Miltiorrhizae* aerial parts were analyzed. The results showed that the compound of terpenoids was the mainly compositions of the essential, in which β-caryophyllene, dihydro-neoproene, germacrene D and caryophyllene were the higher contents of terpenoids. By reference to the extensive literature, it is found that the terpenoids of the essential oil in the genus *Salvia* have strong anti-inflammatory and anti-oxidative ability [31, 32]. In our previous study, the result showed that the essential oil of *S. Miltiorrhizae* aerial parts had a strong ability to remove free radicals through the experiment of clear DPPH and HO· free radical. Cheng [33] relieves AD-like symptoms in transgenic APP/PS1 AD model mice treated with CB2 antagonist AM630 or PPARγ antagonist GW9662 in the investigation of the mechanism of β-caryophyllene. The results showed that β-caryophyllene could reduce the accumulation of Aβ in the hippocampus and cerebral cortex, alleviate the glialization of neurons in the cerebral cortex, inhibit the activity of microglia and Neuroinflammatory of APP/PS1 mice by direct activation of CB2 and PPARγ receptors, then prevent cognitive impairment in APP/PS1 mice. The previous study showed that the mice treated with compounds of sesquiterpene ARD, HDS, PDA can significantly reversal the memory impairment induced by $Aβ_{1-42}$, extend the escape latency in the inhibitory avoidance test, reduce the level of TBARS and restore the activity of GHS in the brain [34].

In the present study, the mice ability of learning was tested by the Morris water maze test. The result showed that the escape latency of test group mice (150 mg/kg·d) was significantly shortened from the 4th day (**Figure 2A**) compared with the model group. The retention of spatial memory was tested by a probe trail and the result showed that the time spent in the target quadrant of test group (150 mg/kg·d) was significantly longer than the model group, but the times of passing through the platform location had no significant differences (**Figure 2C**, **2D**). And the brain body weight ratio of the mice treated with essential oil was not significantly higher than that in the model group (**Figure 2B**). From **Figure 3**, it was found that the activities of SOD and CAT antioxidant enzymes in the brain homogenate of the test group were significantly higher than those in the model group, and the MAD content was lower. According to the mechanism of D-gal plus $AlCl_3$ induced mouse model of AD can be speculated that the alleviation of the symptoms of AD by the essential oil of *S. miltiorrhiza* aerial parts may have the following reasons. First of all, the terpenoids, linoleic acid and other unsaturated compounds of essential oil can

reduce the brain nerve cell necrosis by antioxidant ability to remove the ROS in *vivo*, and slow down the process of AD. Secondly, the terpenoids in the essential oil antagonize the interference of Al on zinc metabolism, restore the activity of antioxidant enzymes such as GSH, CAT and SOD, reduce the accumulation of MAD in the brain, reduce the formation of Aβ precursor protein APP, and reduce the accumulation of Aβ in the brain. Thirdly, these sesquiterpene β-caryophyllene in essential oil through the pathways of CB2 and PPARγ inhibits the activity of astrocytes, reduces the inflammatory response of neurons in the brain, inhibits the accumulation of Aβ in the brain, reduces the apoptosis of neurons induced by Al, and slows down the process of AD. From **Figure 4**, it was found that the activities of AchE in serum of the test group mice were significantly lower than the model group, and the Ach content was higher. The reasons may be that the essential oil removes ROS by its ability of antioxidant activity, reduces damage on the membrane of mitochondrial and increases the synthesis of ATP, then promotes the production of Ach; or one component of essential oil inhibits the interference of Al on Ca-modulating protein, prevents abnormal phosphorylation of tau protein, reduces the formation of NFTs, reduces cholinergic neuronal necrosis, and slows the symptoms of dementia in AD mice.

This study firstly analyzed the composition of the essential oil of the aerial parts of the traditional Chinese medicine *Salvia miltiorrhize* and studied its anti-AD capacity. The result showed that β-caryophyllene, dihydro-neoproene, 6,10,14-trimethyl-2-pentadecanone, germacrene D and caryophyllene were the characteristic constituents of the essential oil. And the animal experiment results demonstrated that the essential oil, given orally, exhibits anti-AD effects on the AD mice model induced by D-gal plus $AlCl_3$. The possible mechanism of aerial parts essential oil of *S. Miltiorrhize* anti-AD has been described in this article, but the specific mechanism is unclear, and this will be the next study focus of our research group. Taken together, the present study indicated that the essential oil of the aerial parts of *S. Miltiorrhize* can prevented cognitive impairment in AD mice and may be potential candidates for AD therapy.

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Disclosure of conflict of interest

None.

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