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# Cognitive-enhancing and antioxidant activities of inhaled coriander volatile oil in amyloid β(1–42) rat model of Alzheimer's disease

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## HIGHLIGHTS

- Coriander volatile oil significantly improved memory of the Aβ(1–42)-treated rats.
- Coriander volatile oil restored the oxidative status of the Aβ(1–42)-treated rats.
- Coriander volatile oil confers neuroprotection by alleviation of oxidative damage.

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## ABSTRACT

*Coriandrum sativum* L., commonly known as coriander and belonging to the Apiaceae family is cultivated throughout the world for its nutritional value. In traditional medicine, coriander is recommended for the relief of pain, anxiety, flatulence, loss of appetite and convulsions. In the present study, the effects of inhaled coriander volatile oil (1% and 3%, daily, for 21 days) extracted from *C. sativum* var. *microcarpum* on spatial memory performance were assessed in an Aβ(1–42) rat model of Alzheimer's disease. The Aβ(1–42)-treated rats exhibited the following: decrease of spontaneous alternations percentage within Y-maze task and increase of working memory errors, reference memory errors and time taken to consume all five baits within radial arm maze task. Exposure to coriander volatile oil significantly improved these parameters, suggesting positive effects on spatial memory formation. Assessments of oxidative stress markers in the hippocampal tissue of Aβ(1–42)-treated rats showed a significant increase of superoxide dismutase (SOD), lactate dehydrogenase (LDH) and a decrease of glutathione peroxidase (GPX) specific activities along with an elevation of malondialdehyde (MDA) level. Coriander volatile oil significantly decreased SOD and LDH specific activities, increased GPX specific activity and attenuated the increased MDA level. Also, DNA cleavage patterns were absent in the coriander rats, thus suggesting antiapoptotic activity of the volatile oil. Therefore, our results suggest that exposure to coriander volatile oil ameliorates Aβ(1–42)-induced spatial memory impairment by attenuation of the oxidative stress in the rat hippocampus.



## 1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by elevated levels of amyloid-β (Aβ) in the brain and progressive cognitive impairments [1]. Aβ, a 40- to 42-amino-acid peptide, is derived by proteolytic cleavage of an integral membrane protein known as amyloid precursor protein (APP) by the action of β- and γ-secretases. Aβ(1–40) and Aβ(1–42) form the majority of the Aβ found in human brain and have been considered to play a key role in the development and progression of AD [2]. Aβ(1–42) is the more toxic of these species both in vitro and in vivo. Further, a number of studies have suggested that small oligomers of Aβ are the actual toxic species of this peptide, rather than Aβ fibrils [3–5]. Aβ(1–42) accumulates early in amyloid plaques and aggregates more rapidly in vitro than Aβ(1–40) [6]. Also, Aβ(1–42)-induced toxicity is linked to synaptic aberration and neuronal death [7]. Soluble Aβ oligomers cause rapid detrimental effects on excitatory synaptic function [8] leading to impaired learning and memory [9]. Hippocampal long-term potentiation (LTP) and long-term depression (LTD) are cellular correlates of learning and memory and oligomeric Aβ is reported to inhibit LTP [10] and enhance LTD [11].

Previous studies have demonstrated that intracerebral infusion of Aβ causes brain dysfunctions as evidenced by neurodegeneration and an impairment of learning and memory [12–15]. Central administration of Aβ induced learning and memory deficits in mice [14], cholinergic dysfunction [16], neuronal apoptosis [17], oxidative stress [18] and

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neuroinflammation [19]. Specifically, microinjections of Aβ(1–42) impaired memory in various tasks, including Morris water-maze test [20], passive avoidance test [14], Y-maze test [21] and radial arm water maze test [22].

These findings suggested that Aβ has a central role in the pathogenesis of AD while the mechanism by which Aβ causes neuronal injury and cognitive impairment is not yet clearly understood. In fact, in animal models and humans alike, the association between plaques of Aβ densities and the degree of cognitive deficits is not always clear cut [23].

Increasing evidence highlights the role played by oxidative stress in AD [5,15,24]. It has been shown that free radicals and oxidative stress induce memory deficits and enhance behavioral impairments in AD patients [25]. Several studies have suggested that Aβ induced the oxidative stress observed in the neurodegenerative AD brain [26,27]. Aβ toxicity is associated with increases in reactive oxygen species (ROS), including $H_2O_2$ [28–30]. In addition, other studies suggest that Aβ promotes oxidative stress and lipid peroxidation in neuronal cultures [31,32]. ROS and $H_2O_2$ were generally believed to directly affect mitochondria function, inducing a rise in the release of apoptotic factors into the cytosol [30]. Several mechanisms of Aβ(1–42)-mediated oxidative stress and neurotoxicity have been proposed. Previous studies have shown that methionine residue 35 of Aβ(1–42) may play a critical role in Aβ(1–42)-mediated oxidative stress and neurotoxicity [33–35]. Additionally, the aggregation state of the peptide is believed to play a role in the oxidative stress and neurotoxic properties exhibited by Aβ(1–42) [36,37].

Although many studies have been directed to AD treatment, there is still yet promising intervention for curing the disease. Neuroprotection is the attempt to preserve normal cellular interaction in the brain and minimize loss of neuronal functions in pathological conditions. Currently, much attention has been focused on the potential of using natural herbs as neuroprotective agents [12].

In traditional medicine, *Coriandrum sativum* L. (Apiaceae) has been indicated for a number of medical problems such as dyspeptic complaints, loss of appetite, convulsion, insomnia and anxiety [38]. The fresh leaves of *C. sativum* are routinely added for their delicious taste and flavor which they impart to various dishes in Asian countries. The leaves contain volatile oil, proteins, flavonoid glycosides (quercetin, isoquercitrin and rutin), caffeic acid and traces of fats, minerals (like calcium, phosphorus, and iron), carotene, fibers and carbohydrates. Coriander fruit is also reputed to be tonic, diuretic and aphrodisiac, while the oil is considered useful in flatulent colic, rheumatism, neuralgia etc. [39]. There are two varieties of *C. sativum*: *vulgare* Alef. and *microcarpum* DC. These varieties differ in the fruit size and oil yield: *vulgare* has fruits of 3–5 mm diameter and yields 0.1–0.35% essential oil, while *microcarpum* fruits are 1.5–3 mm and yield 0.8–1.8% essential oil [40,41]. A recent review suggested that the volatile oil of coriander seeds possesses antibacterial, antioxidant and memory improving proprieties [42]. The main constituents of coriander oil are linalool, α-pinene, γ-terpinene, geranyl acetate, camphor and geraniol (corresponding to GC chromatogram of coriander oil, European Pharmacopoeia (Ph. Eur.) 6th edition, 2008). Ph. Eur. 6th edition describes a capillary gas-chromatographic method and imposes limits for linalool (65.0%–78.0%), α-pinene (3.0%–7.0%), γ-terpinene (1.5%–8.0%), geranyl acetate (0.5%–4.0%), camphor (3.0%–6.0%) and geraniol (0.5%–3.0%), respectively.

Currently, aromatherapy is mostly used in the management of chronic pain, depression, cognitive disorders, anxiety, insomnia and stress-related disorders [43]. Furthermore, the essential oils chosen for use in aromatherapy are those that are known to be least harmful, with fewest potential risks for users. Lavender is considered to be the safest, along with others such as basil, chamomile, coriander, lemon, lemon balm and neroli are also generally safe. Widespread current use of aromatherapies, together with contemporary clinical data, indicate that if these oils are carefully used within the directions suggested, they can provide treatment for AD, dementia and other psychiatric disorders, without any of the adverse effects associated with some of the conventional drugs already in use. Aromatherapy may therefore be a much safer option than conventional drugs such as antipsychotics, which are often used to treat agitation or other non-cognitive symptoms that accompany dementia [44].

The data available on the toxicity of coriander oil are limited. However, coriander and its oil have a long history of dietary use, with no record of harm caused by consumption of these ingredients. Therefore, the use of coriander oil is considered safe [41].

In the present study, we investigated whether chronic inhalation of the coriander volatile oil prevents memory impairment and oxidative damage in Aβ(1–42)-induced a rat model of AD.

## 2. Materials and methods

### 2.1. Essential oil and chemical analyses

Mature fruits of coriander were collected from the experimental fields of Agricultural Research and Development Center, Secuieni, Neamt (Eastern Romania) in June 2012 and identified. Voucher specimen is preserved at the Department of Pharmacognosy, Faculty of Pharmacy (University of Medicine and Pharmacy "Gr T. Popa", Iasi, Romania) for ready reference. Air dried fruits of *C. sativum* var. *microcarpum* sample were subjected to hydro-distillation for 3 h using a Clevenger type apparatus to obtain the volatile oil. The essential oil was dried over anhydrous sodium sulfate and kept at −4 °C until analysis.

#### 2.1.1. GC/MS

Analysis of the coriander volatile oil was performed using an Agilent 6890 GC–MS system, equipped with a split/splitless injector (200 °C). The transfer line temperature was 250 °C. Helium was used as carrier gas (1 ml/min) and the capillary column used was DB 5MS (30 m × 0.25 mm; film thickness 0.25 μm; Agilent, Palo Alto, CA, USA). The temperature program started with 40 °C, growing with 10 °C/min up to 280 °C; split ratio 100:1. The injected volume was 0.30 μl. Total scan time 32 min. Acquisition mass range 40–400 amu. The identification of the compounds was based on comparison of their retention indices (RI), their retention times (RT) and mass spectra with those obtained from authentic Wiley libraries (available through Hewlett Packard) and the literature [45].

#### 2.1.2. GC/FID

GC analysis of the coriander volatile oil was carried out using an Agilent 6890 GC-FID system, equipped with DB-5 capillary column (30 m × 0.32 mm; film thickness 0.25 μm; J & W, CA, USA) and connected to a FID detector. The injector and detector temperature was 280 °C. The carrier gas was helium, at flow rate of 1.0 ml/min. The thermal program was 40 °C–280 °C at a rate of 10 °C/min; split ratio 100:1. Two replicates of coriander volatile oil were processed in the same way. The injected volume was 0.3 μl.

From the total of 64 separated peaks, monoterpenes was the predominant class of compounds, with linalool (69.358%) being the major component. Other important substances were: γ-terpinene (7.729%), α-pinene (6.509%), pinocarvone (4.388%), carvone (2.314%), β-ocimene (E + Z) (3.105%) and geranyl acetate (1.580%). The results were in accordance with literature, where monoterpenes have been reported to be the main class in *C. sativum* fruit oils [45–51] with linalool being the predominant compound, while camphor and limonene were absent from the oil, but pinocarvone and carvone were present in significant amounts. Nevertheless, linalool represents almost 70% of the volatile oil, therefore an important pharmacological potential will belong to this compound.

### 2.2. Animals

40 male Wistar rats (3 months old) weighing 250 ± 50 g at the start of the experiment were used. The animals were housed in a temperature and light-controlled room (22 °C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water ad libitum. The rats were divided into 4 groups (10 animals per group): (1) Control group (sham-operated) received saline treatment (0.9% NaCl); (2) Aβ(1–42) alone-treated group; (3) Aβ(1–42)-treated group received coriander volatile oil 1% (CO1% + Aβ(1–42)); and (4) Aβ(1–42)-treated group received coriander volatile oil 3% (CO3% + Aβ(1–42)). Control and Aβ(1–42) alone-treated groups were caged in the same conditions but in the absence of the tested oil. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare from Romania and all procedures were in compliance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. This study was approved by the local Ethic Committee and also, efforts were made to minimize animal suffering and to reduce the number of animal used.

### 2.3. Neurosurgery

All surgical procedures were conducted under aseptic conditions, under sodium pentobarbital (50 mg/kg b.w., i.p., Sigma-Aldrich, Germany) anesthesia. Rats were mounted in the stereotaxic apparatus with the nose oriented 11° below horizontal zero plane. Animal model of AD was established by intracerebroventricular (i.c.v.) injection of 400 pmol Aβ(1–42) (beta-amyloid peptide 1–42, Rat, Sigma-Aldrich, Germany), 20 days prior to inhalation of coriander volatile oil (CO1% and CO3%) according to the procedure established by Laursen and Belknap [52]. Aβ(1–42) was administered right-unilaterally through Hamilton syringe over 4 min, and the syringe was left in place for 5 min after injection before being slowly removed. The injection volume (4 μl) was delivered gradually (1 μl/min) using the following coordinates: 1.5 mm lateral to the midline; 7.4 mm ventral to the surface of the cortex [53]. The sham-operated rats were injected with saline.

### 2.4. Inhalation apparatus

The inhalation apparatus consisted of a Plexiglas chamber (50 × 40 × 28 cm). Two chambers were used, one for the control and Aβ(1–42) alone-treated animals, which were not exposed to any substance, and the other one for the experimental animals, which were exposed to coriander volatile oil. Coriander volatile oil was diluted with 1% Tween 80 (v/v). Coriander volatile oil exposure (200 μl, either 1% or 3%) was via an electronic vaporizer placed at the bottom of chamber, but out of reach of the animals. Rats in the coriander groups were exposed to oil vapors for controlled 60 min period, daily, for 21 continuous days. 60 min is a suitable inhalation period for the expected effects [43]. Chambers were always cleaned up (10% ethanol solution).

### 2.5. Y-maze task

Short-term memory was assessed by spontaneous alternation behavior in the Y-maze task. The Y-maze used in the present study consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. 60 min after the inhalation of coriander volatile oil (CO1% and CO3%), rats were placed at the end of one arm and allowed to move freely through the maze for 8 min. An arm entry was counted when the hind paws of the rat were completely within the arm. Spontaneous alternation behavior was defined as entry into all three arms on consecutive choices. The number of maximum spontaneous alternation behaviors was then the total number of arms entered minus 2 and percent spontaneous alternation was calculated as (actual alternations / maximum alternations) × 100 [54,55]. The maze was cleaned with a 10% ethanol solution and dried with a cloth before the next animal was tested. Spontaneous alternation behavior is considered to reflect spatial working memory, which is a form of short-term memory.

### 2.6. Radial arm-maze task

The radial 8 arm-maze used in the present study consisted of 8 arms, numbered from 1 to 8 (48 × 12 cm), extending radially from a central area (32 cm in diameter). The apparatus was placed 50 cm above the floor, and surrounded by various extra maze visual cues placed at the same position during the study. At the end of each arm there was a food cup that had a single 50 mg food pellet. Prior to the performance of the maze task, the animals were kept on restricted diet and body weight was maintained at 85% of their free-feeding weight over a week period, with water being available ad libitum. Before the actual training began, three or four rats were simultaneously placed in the radial maze and allowed to explore for 5 min and take the food freely. The food was initially available throughout the maze, but was gradually restricted to the food cup. The animals were trained for 4 days to run to the end of the arms and consume the bait. To evaluate the basal activity of rats in radial 8 arm-maze, the rats were given 5 consecutive training trials per day to run to the end of the arms and consume the bait. The training trial continued until all 5 baits have been consumed or until the 5 min have elapsed which have been set as the performance criteria. After adaptation, all rats were trained with 1 trial per day. Briefly, 60 min after the inhalation of coriander volatile oil (CO1% and CO3%), each animal was placed individually in the center of the maze and subjected to working and reference memory tasks, in which same 5 arms (nos. 1, 2, 4, 5 and 7), were baited for each daily training trial. The other 3 arms (nos. 3, 6 and 8) were never baited. The selection of the baited arms is based on the fact that animals prefer to solve the maze using an adjacent arm selection strategy. In this case, we altered adjacent arm patterning behavior by only baiting 5 arms (nos. 1, 2, 4, 5, and 7) subjecting animals to change their strategy and avoid the unbaited arms. An arm entry was counted when all four limbs of the rat were within an arm. Measures were made of the number of working memory errors (entering an arm containing food, but previously entered) and reference memory errors (entering an arm that was not baited). The time taken to consume all five baits was also recorded. The maze was cleaned with a 10% ethanol solution and dried with a cloth before the next animal was tested. Reference memory is regarded as a long-term memory for information that remains constant over repeated trials (memory for the positions of baited arms), whereas working memory is considered a short-term memory in which the information to be remembered changes in every trial (memory for the positions of arms that had already been visited in each trial) [54,55].

### 2.7. Biochemical parameter assay

After behavioral tests, all rats were deeply anesthetized (using sodium pentobarbital, 100 mg/kg b.w., i.p., Sigma-Aldrich, Germany), decapitated and whole brains were removed. The hippocampi were carefully excised. Each of the hippocampal samples were weighted and homogenized (1:10) with Potter Homogenizer coupled with Cole-Parmer Servodyne Mixer in ice-cold 0.1 M potassium phosphate buffer (pH 7.4), 1.15% KCl. The homogenate was centrifuged (15 min at 960 ×*g*) and the supernatant was used for assays of SOD, GPX and LDH specific activities and MDA level.

#### 2.7.1. Determination of hippocampal SOD activity

The activity of superoxide dismutase (SOD, EC 1.15.1.1) was assayed by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). Each 1.5 ml reaction mixture contained

100 mM Tris/HCl (pH 7.8), 75 mM NBT, 2 μM riboflavin, 6 mM EDTA, and 200 μl of supernatant. Monitoring the increase in absorbance at 560 nm followed the production of blue formazan. One unit of SOD is defined as the quantity required to inhibit the rate of NBT reduction by 50% as previously described [56]. The enzyme activity is expressed as units/mg protein.

*2.7.2. Determination of hippocampal GPX activity*

Glutathione peroxidase (GPX, E.C. 1.11.1.9) activity was analyzed by a spectrophotometric assay. A reaction mixture consisting of 1 ml of 0.4 M phosphate buffer (pH 7.0) containing 0.4 mM EDTA, 1 ml of 5 mM $NaN_3$, 1 ml of 4 mM glutathione (GSH), and 0.2 ml of supernatant was pre-incubated at 37 °C for 5 min. Then 1 ml of 4 mM $H_2O_2$ was added and incubated at 37 °C for further 5 min. The excess amount of GSH was quantified by the DTNB method as previously described [57]. One unit of GPX is defined as the amount of enzyme required to oxidize 1 nmol GSH/min. The enzyme activity is expressed as units/mg protein.

*2.7.3. Determination of hippocampal LDH activity*

The specific activity of lactate dehydrogenase (LDH, EC 1.1.1.27) was determined by measuring the rate of oxidation of NADH at 340 nm according to the method of Bergmeyer and Bernt [58]. Briefly, the reaction mixture contained 50 mM potassium phosphate buffer (pH 7.5), 0.5 mM sodium pyruvate, 0.1 mM NADH and the required amount of cytosolic fraction to make the final volume of 1 ml. The reaction was started at 25 °C by addition of NADH and the rate of oxidation of NADH was measured at 340 nm in spectrophotometer. The enzyme activity was calculated using extinction coefficient 6.22 $mM^{-1}$ $cm^{-1}$. One unit of enzyme activity has been defined as the amount causing the oxidation of 1 μmol of NADH per minute. The enzyme activity is expressed as units/mg protein.

*2.7.4. Determination of hippocampal MDA level*

Malondialdehyde (MDA), which is an indicator of lipid peroxidation, was spectrophotometrically measured by using the thiobarbituric acid assay as previously described [59]. 200 μl of supernatant was added and briefly mixed with 1 ml of 50% trichloroacetic acid in 0.1 M HCl and 1 ml of 26 mM thiobarbituric acid. After vortex mixing, samples were maintained at 95 °C for 20 min. Afterwards, samples were centrifuged at 3000 rpm for 10 min and supernatants were read at 532 nm. A calibration curve was constructed using MDA as standard and the results were expressed as nmol/mg protein.

*2.7.5. Estimation of protein concentration*

Estimation of protein was done using a BCA protein assay kit (Sigma-Aldrich, Germany). The BCA protein assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantification of total protein, as previously described [60].

*2.8. Apoptosis*

Total DNA was isolated from the hippocampal samples using the phenol/chloroform method as previously described [61]. 50 mg tissue sample was digested overnight at 37 °C in 0.6 ml digestion buffer (100 mM NaCl, 10 mM Tris/HCl, 25 mM EDTA pH 8.00, 0.5% SDS) containing 0.1 mg/ml proteinase K (Boehringer Mannheim, Germany). The digest was extracted with equal volumes of Tris-saturated phenol (pH 8.0) (Roti-phenol, Roth, Germany) by shaking gently to completely mix the two phases. The phases were then separated by centrifugation and the aqueous phase (approx. 0.6 ml) was transferred to another tube avoiding interphase. The DNA was then precipitated by adding 300 μl of 7.5 M ammonium acetate (i.e., 1/2 of volume) and equal volume of 100% ethanol at room temperature and shaken gently to mix thoroughly. DNA seen as stringy precipitate was pelleted by centrifugation and washed with 70% ethanol to remove traces of sodium dodecyl sulfate and phenol. After removing ethanol, DNA was air-dried for 10 min at room temperature and suspended with 50 μl of 10 mM Tris (pH 8.0), 1 mM EDTA. DNA content was determined spectrophotometrically by absorbance at 260 nm and the purity of the DNA was confirmed by a ratio > 1.8 at 260/280 nm. Approx. 0.5 mg genomic DNA was dissolved in a mixture of 10 μl of Tris–EDTA and 5 μl of gel loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% (v/v) glycerol) and then loaded on a 1.5% agarose gel in Tris–boric acid–EDTA (TBE) buffer (89 mM Tris boric acid, 2 mM EDTA, pH 8.0). Electrophoresis was performed in TBE at 120 V until sufficient resolution was obtained. A 1-kb DNA ladder (New England Biolabs, Ipswich, MA) was used as a standard size marker. The bands were visualized by ethidium bromide staining under UV light.

*2.9. Histological analysis*

To detect the amyloid plaque burden, fixed brains from each group were examined histologically. Highman's Congo red stain was used to identify amyloid deposits in the hippocampal samples [62]. The slides were observed and photos taken under a microscope (LASER Confocal Scanning Microscope Leica DM 5500Q TCS SPE with DFC 290 camera). Sections from each treatment group were examined by an independent observer blind to treatments, following standard laboratory procedures.

*2.10. Statistical analysis*

The animal's behavioral activities in Y-maze task together with the results for the enzymes activity and MDA level were statistically analyzed with analysis of variance (ANOVA). In order to evaluate differences between groups in radial arm-maze task, separate repeated-measures ANOVA were calculated on number of working memory errors, number of reference memory errors and time taken to consume all five baits with group (Control, Aβ(1–42), CO1% + Aβ(1–42) and CO3% + Aβ(1–42)) as between-subject factor and days (1 to 7) as within-subjects factors. All results are expressed as mean ± S.E.M. F values for which $p < 0.05$ were regarded as statistically significant. Significant differences were determined by Tukey's post hoc test. Pearson's correlation coefficient and regression analysis were used in order to evaluate the connection between behavioral measures and oxidative stress markers and between the antioxidant defense and lipid peroxidation.

## 3. Results

*3.1. Effect of coriander volatile oil on spatial memory in Y-maze task*

As can be seen in Fig. 1A, in the Y-maze task ANOVA revealed a significant drug effect ($F(3,36) = 3$, $p < 0.001$) on spatial working memory, as evidenced by spontaneous alternations percentage. Additionally, Tukey's post hoc analysis revealed a significant difference between the control and Aβ(1–42) groups ($p = 0.0001$), Aβ(1–42) and CO1% + Aβ(1–42) groups ($p = 0.0001$) and Aβ(1–42) and CO3% + Aβ(1–42) groups ($p = 0.0001$) for spontaneous alternations percentage, indicating that coriander volatile oil significantly improved spatial working memory (Fig. 1A). Furthermore, spontaneous alternation percentage in control ($t = 8.86$, $p = 0.001$), Aβ(1–42) ($t = 3.75$, $p = 0.02$), CO1% + Aβ(1–42) ($t = 3.67$, $p = 0.02$) and CO3% + Aβ(1–42) ($t = 4.63$, $p = 0.01$) groups were statistically different from the chance level (50%) (Fig. 1A).

The changes in the spontaneous alternation percentage of both CO1% + Aβ(1–42) and CO3% + Aβ(1–42) groups are not related to the changes in motor activity, as evidenced in Y-maze task by the number of arm entries (Fig. 1B).

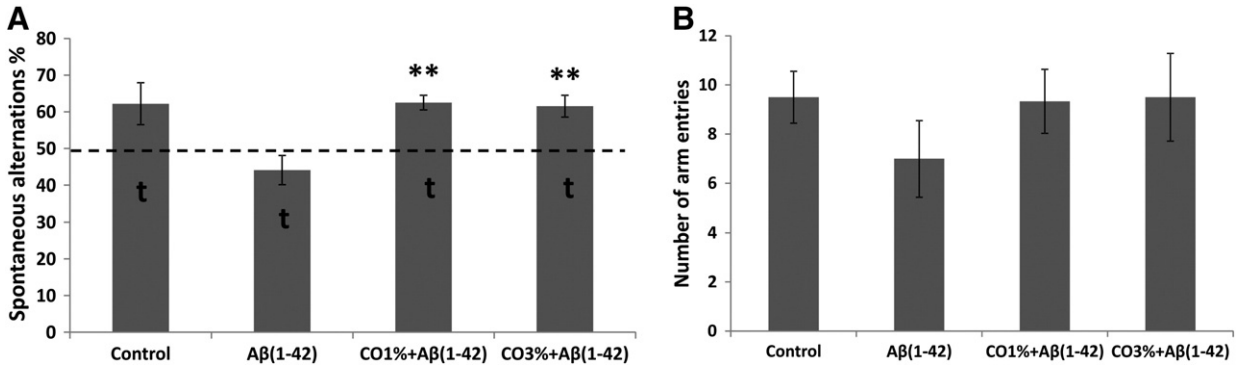


**Fig. 1.** Effects of the coriander volatile oil (CO1% and CO3%) on spontaneous alternations % (A) and number of arm entries (B) in the Aβ(1–42)-treated rats. Values are means ± S.E.M. (n = 10 animals per group), **p < 0.0001 vs. Aβ(1–42) alone treated-group.

### *3.2. Effect of coriander volatile oil on spatial memory in radial arm-maze task*

To investigate whether coriander volatile oil (CO1% and CO3%) affects spatial memory formation, the rats were further evaluated in the radial arm-maze task.

For working memory errors, repeated-measures ANOVA revealed a significant time difference ($F(6,175) = 5.26$, $p < 0.0001$), a significant group difference ($F(4,175) = 127$, $p < 0.0001$) and a significant time-group interaction ($F(24,175) = 2.74$, $p < 0.0001$) (Fig. 2A). For the time taken to consume all five baits, repeated-measures ANOVA revealed a significant time difference ($F(6,175) = 4.84$, $p < 0.0001$) and a significant group difference ($F(4,175) = 13.06$, $p < 0.0001$) (Fig. 2B). Additionally, Tukey's post hoc analysis revealed significant differences between control and Aβ(1–42) groups ($p = 0.0001$), Aβ(1–42) and CO1% + Aβ(1–42) groups ($p = 0.0001$), Aβ(1–42) and CO3% + Aβ(1–42) groups ($p = 0.0001$) and CO1% + Aβ(1–42) and CO3% + Aβ(1–42) groups ($p = 0.03$) for working memory errors (Fig. 2A) and between control and Aβ(1–42) groups ($p = 0.0001$), Aβ(1–42) and CO1% + Aβ(1–42) groups ($p = 0.0001$) and Aβ(1–42) and CO3% + Aβ(1–42) groups ($p = 0.0001$) for time taken to consume all five baits (Fig. 2B), indicating that coriander volatile oil significantly improved working memory during 7 days training in radial arm-maze task.

For reference memory errors, repeated-measures ANOVA revealed a significant time difference ($F(6,175) = 3.33$, $p < 0.004$), a significant group difference ($F(4,175) = 3.62$, $p < 0.007$) and a significant time-group interaction ($F(24,175) = 1.70$, $p < 0.02$) (Fig. 2C). Additionally, Tukey's post hoc analysis revealed significant differences between control and Aβ(1–42) groups ($p = 0.004$) and Aβ(1–42) and CO1% + Aβ(1–42) groups ($p = 0.006$) for reference memory errors

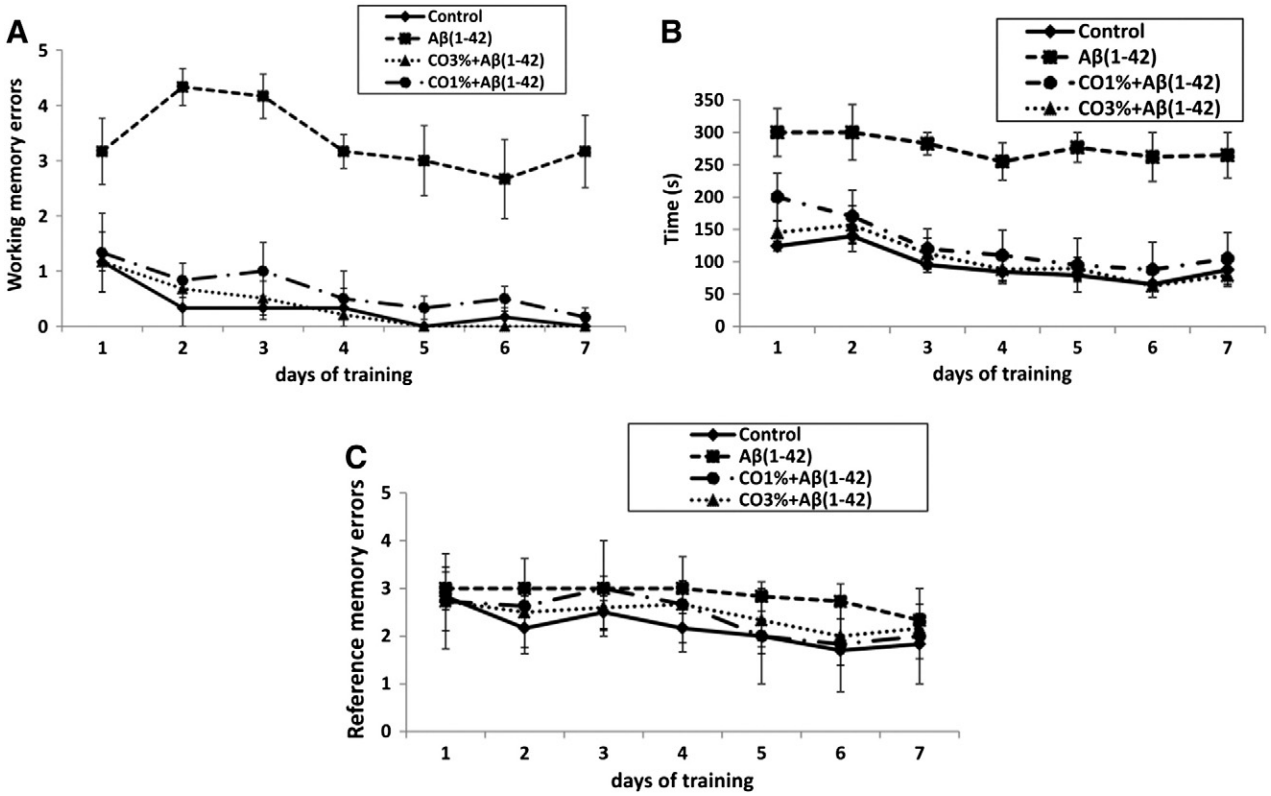


**Fig. 2.** Effects of the coriander volatile oil (CO1% and CO3%) on the working memory errors (A), the time taken to consume all five baits (B) and the reference memory errors (C) during 7 days training in radial arm-maze task. Values are means ± S.E.M. (n = 10 animals per group).

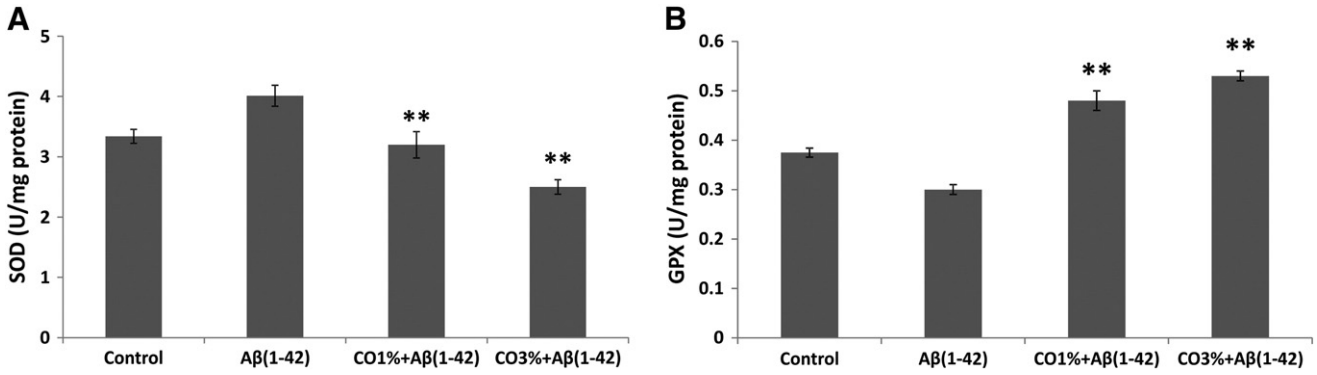


**Fig. 3.** Effects of the coriander volatile oil (CO1% and CO3%) on SOD (A) and GPX (B) specific activities in the rat hippocampal homogenates. Values are means ± S.E.M. (n = 10 animals per group), **p < 0.001 vs. Aβ(1–42) alone treated-group.

(Fig. 2C), indicating that coriander volatile oil significantly improved long-term memory during 7 days training in radial arm-maze task.

### 3.3. Effect of coriander volatile oil on SOD and GPX specific activities

For SOD and GPX specific activities estimated in the rat hippocampal homogenates, ANOVA revealed a significant drug effect for SOD ($F(3,36) = 8.31$, $p < 0.001$) (Fig. 3A) and for GPX ($F(3,36) = 8.69$, $p < 0.001$) (Fig. 3B) specific activities. Additionally, Tukey's post hoc analysis revealed significant differences between control and CO1% + Aβ(1–42) groups ($p = 0.009$), control and CO3% + Aβ(1–42) groups ($p = 0.03$), Aβ(1–42) and CO1% + Aβ(1–42) groups ($p = 0.03$), Aβ(1–42) and CO3% + Aβ(1–42) groups ($p = 0.01$) and CO1% + Aβ(1–42) and CO3% + Aβ(1–42) groups ($p = 0.05$) for SOD specific activity (Fig. 3A) and between control and Aβ(1–42) groups ($p = 0.008$), control and CO1% + Aβ(1–42) groups ($p = 0.0001$), control and CO3% + Aβ(1–42) groups ($p = 0.0001$), Aβ(1–42) and CO1% + Aβ(1–42) groups ($p = 0.0001$), Aβ(1–42) and CO3% + Aβ(1–42) groups ($p = 0.0001$) and CO1% + Aβ(1–42) and CO3% + Aβ(1–42) groups ($p = 0.003$) for GPX specific activity (Fig. 3B), suggesting that coriander volatile oil possesses strong antioxidant proprieties.

### 3.4. Effect of coriander volatile oil on LDH specific activities

As can be seen in Fig. 4, for LDH specific activity, ANOVA revealed significant drug effect ($F(3,36) = 2.78$, $p < 0.0001$). Additionally, Tukey's post hoc analysis revealed significant differences between control and Aβ(1–42) groups ($p = 0.002$), control and CO1% + Aβ(1–42) groups ($p = 0.002$), control and CO3% + Aβ(1–42) groups ($p = 0.01$), Aβ(1–42) and CO3% + Aβ(1–42) groups ($p = 0.01$) and CO1% + Aβ(1–42) and CO3% + Aβ(1–42) groups ($p = 0.007$) for LDH activity, suggesting anticytotoxic effects.

### 3.5. Effect of coriander volatile oil on hippocampal MDA level

For MDA level estimated in the rat hippocampal homogenates, ANOVA revealed a significant drug effect ($F(3,36) = 4.22$, $p < 0.0001$) (Fig. 5). Also, Tukey's post hoc analysis revealed significant differences between control and Aβ(1–42) groups ($p = 0.0001$), control and CO1% + Aβ(1–42) groups ($p = 0.02$), control and CO3% + Aβ(1–42) groups ($p = 0.0001$), Aβ(1–42) and CO1% + Aβ(1–42) groups ($p = 0.0001$), Aβ(1–42) and CO3% + Aβ(1–42) groups ($p = 0.0001$) and CO1% + Aβ(1–42) and CO3% + Aβ(1–42) groups ($p = 0.0001$) for MDA level (Fig. 5), suggesting that coriander volatile oil exhibited attenuation in lipid peroxidation.

These results support the hypothesis that the coriander volatile oil may have induced a decrease in neuronal oxidative stress.

More importantly, when linear regression was determined, significant positive correlations between working memory errors vs. MDA ($n = 40$, $r = 0.847$, $p = 0.0001$) (Fig. 6A) and reference memory errors vs. MDA ($n = 40$, $r = 0.434$, $p = 0.03$) (Fig. 6B) were observed. In Y-maze task, no significant correlation between behavioral response and indicators of oxidative stress was observed (data not shown). Additionally, a significant positive correlation was evidenced by determination of the linear regression between SOD vs. MDA ($n = 40$, $r = 0.514$, $p = 0.01$) (Fig. 6C). These data suggest that decrease of working memory errors, reference memory errors and MDA level could be related to the antioxidant effects of coriander volatile oil. Moreover, the increase

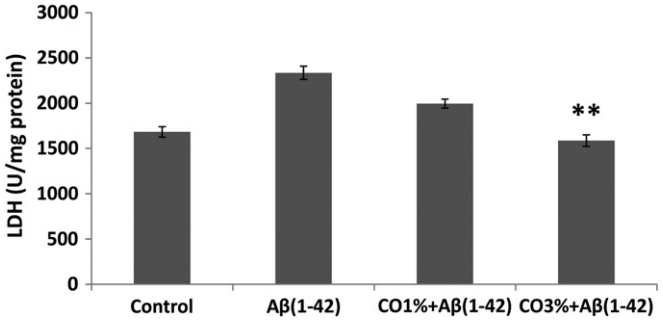


**Fig. 4.** Effects of the coriander volatile oil (CO1% and CO3%) on LDH specific activity in the rat hippocampal homogenates. Values are means ± S.E.M. (n = 10 animals per group), **p < 0.01 vs. Aβ(1–42) alone treated-group.

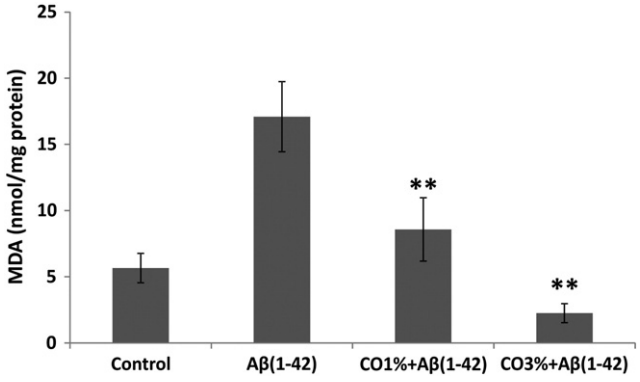


**Fig. 5.** Effects of the coriander volatile oil (CO1% and CO3%) on MDA in the rat hippocampal homogenates. Values are means ± S.E.M. (n = 10 animals per group), **p < 0.0001 vs. Aβ(1–42) alone treated-group.

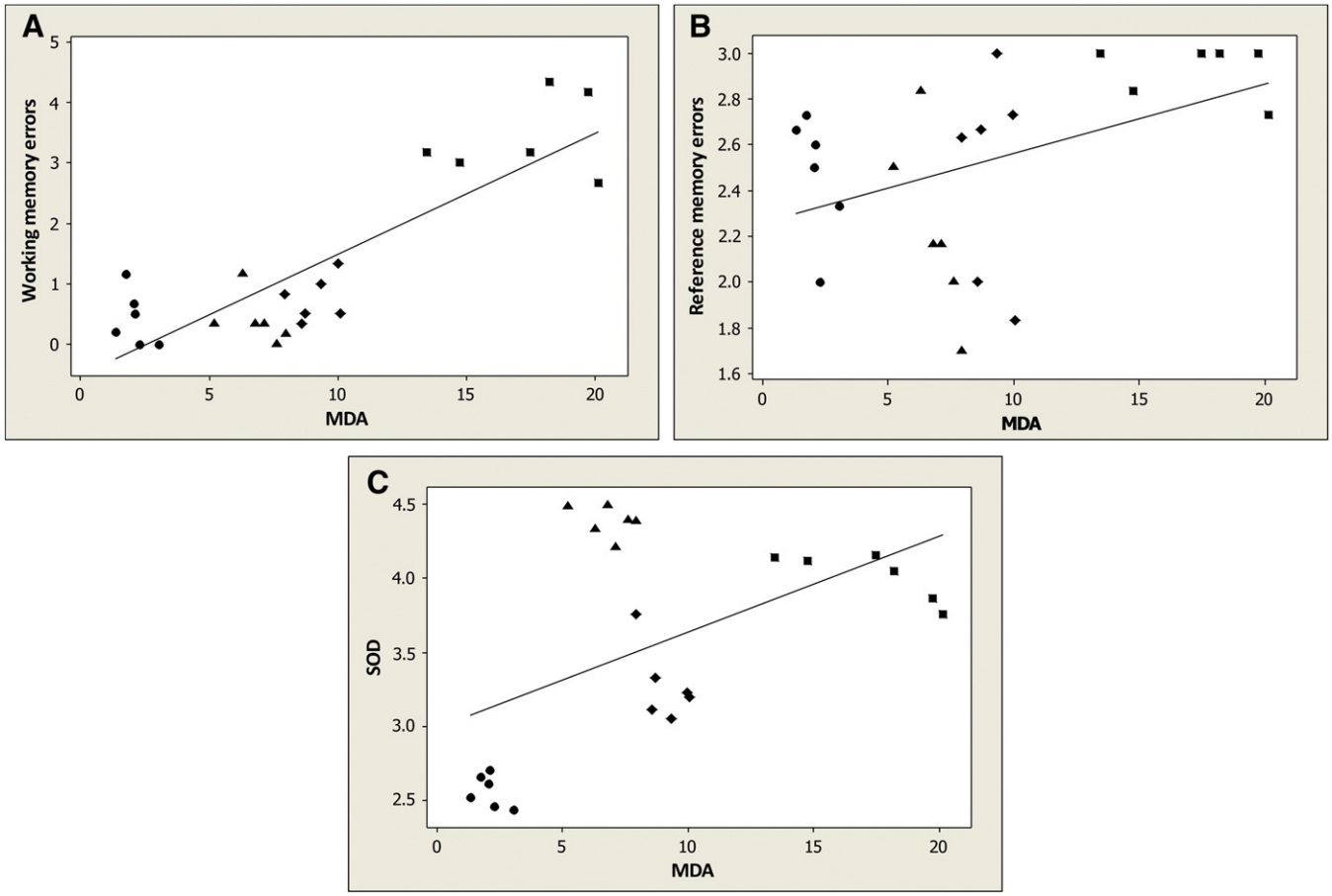


**Fig. 6.** Correlation between working memory errors vs. MDA (A), reference memory errors vs. MDA (B) and SOD vs. MDA (C) in control group (▲), Aβ(1–42) alone treated-group (■), CO1% + Aβ(1–42) group (♦) and CO3% + Aβ(1–42) group (●).

of the antioxidant defense and decrease of lipid peroxidation could be correlated with the involvement of coriander volatile oil in neuroprotection against Aβ(1–42)-induced neuronal oxidative stress generation.

### 3.6. Aβ deposits

As can be seen from the panel in Fig. 7, while the amyloid deposits are obvious and abundant in the hippocampus of rats treated with i.c.v. Aβ(1–42) (Fig. 7B), deposit are scarce in rats treated with CO1% (Fig. 7C) and CO3% (Fig. 7D).

### 3.7. Effect of coriander volatile oil on DNA fragmentation

In our study, DNA cleavage patterns were absent in the coriander groups (Fig. 8), suggesting that the coriander volatile oil protects against neurotoxicity and this effect could be related to their antioxidant activities.

## 4. Discussion

The present study was designed to examine the spatial memory formation following chronic exposure to coriander volatile oil (CO1% and CO3%) of rats subjected to intracerebroventricular injection of Aβ(1–42). Intracerebroventricular injection of Aβ(1–42) interferes with memory function and subsequently causes impairment of spatial memory within Y-maze and radial arm-maze tasks, in accordance with previous investigation using rats [14,21].

In the present study we used two well-characterized hippocampus-dependent spatial memory tasks: Y-maze and radial arm-maze. Our results showed that coriander volatile oil sustains memory formation in a rat model of AD Aβ(1–42)-induced.

The GC–MS analysis determined the main volatile component of our coriander volatile oil sample to be linalool (69.358%), so this is probably the constituent responsible for the observed cognitive-enhancing effects in Aβ(1–42)-treated rats. It has been suggested that inhaling linalool rich essential oils can be useful as a mean to counteract anxiety [63]. Furthermore, numerous studies indicated that coriander volatile oil has anxiolytic effects in the elevated plus maze behavior of mice [38] and facilitate memory formation [39].

The Y-maze task is a specific and sensitive test of spatial recognition memory in rodents. The test relies on an innate tendency of rats to explore a novel environment [64]. The Y-maze used in this study involves no aversive stimuli and was considered suitable for evaluating memory. The specific part of the brain involved in performance of this task includes the hippocampus [18]. As shown in Fig. 1, CO1% and CO3% in Aβ(1–42)-treated rats significantly improved spatial working memory, as evidenced by increase of spontaneous alternation percentage as compared to Aβ(1–42) alone-treated rats. This result suggests that both doses of coriander volatile oil (CO1% and CO3%) used in this study display an improvement effect on acquisition of the short-term memory of the Aβ(1–42)-treated rats within Y-maze task. However, no differences were observed between both doses of coriander volatile oil on spatial working memory in Y-maze task. Also, coriander volatile oil (CO1% and CO3%) increased both the locomotor activity as well as short-term memory in Aβ(1–42)-treated rats within Y-maze task. This effect of the coriander volatile oil observed in short-term memory cannot be attributed exclusively to increased locomotor activity, because the percentage of spontaneous alternation was also improved and the

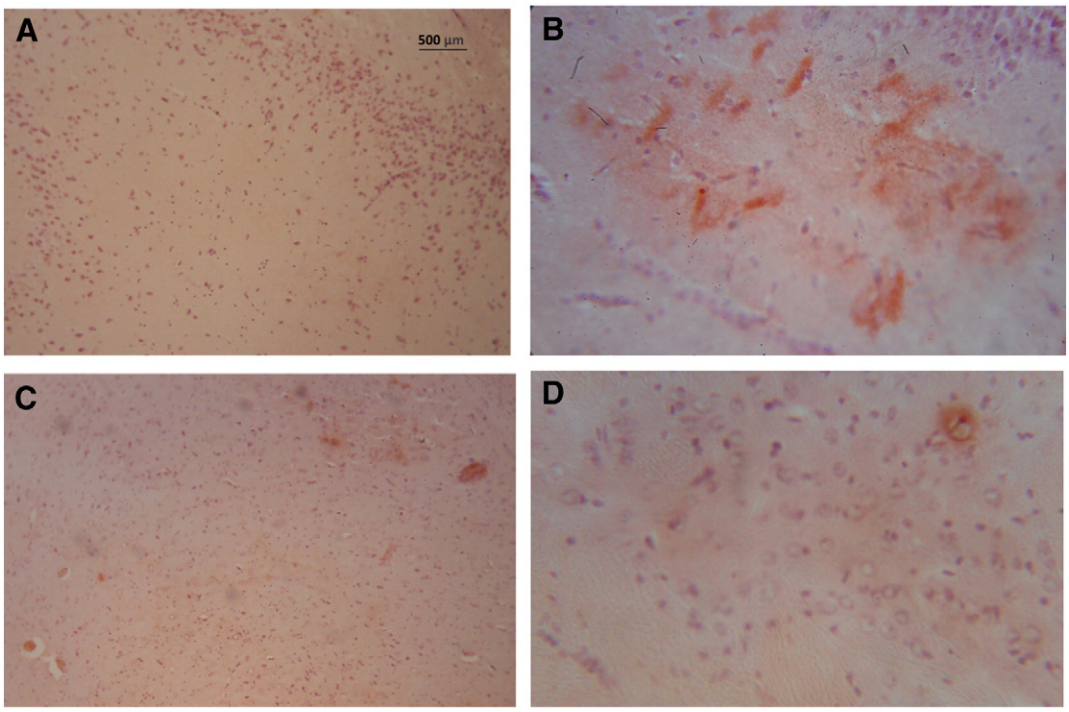


**Fig. 7.** Highman's Congo red staining of the hippocampus samples in control (A), Aβ(1–42) alone treated-group (B), CO1% + Aβ(1–42) group (C) and CO3% + Aβ(1–42) group (D). The staining was used to identify amyloid deposits in the hippocampal samples. Red patches indicated amyloid plaques formed 20 days after Aβ(1–42) injection. Scale bar, 500 µm.

effect of improving working and reference memory is also observed in radial arm-maze task. Therefore, the improvement of short-term memory observed in Aβ(1–42)-treated rats exposed to CO1% and CO3% is not an artifact due to the concomitant increase in motor activity.

In behavioral neuroscience trial, radial arm-maze task is widely used [54,65]. These RAM tests are useful in evaluating the effect of drugs, stress and various other environmental factors on learning and memory

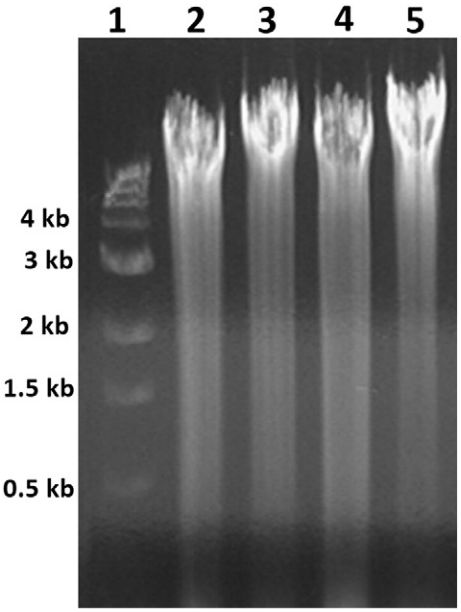


**Fig. 8.** Effects of the coriander volatile oil (CO1% and CO3%) on DNA fragmentation by agarose (1.5%) gel electrophoresis. Lane 1: DNA ladder; lane 2: control group; lane 3: Aβ(1–42) alone treated-group; lane 4: CO1% + Aβ(1–42) group and lane 5: CO3% + Aβ(1–42) group.

[66]. Working memory and reference memory are the two variables that report the physiological status of the brain [66]. Thus, Aβ(1–42)-treated rats exposed to CO1% and CO3% exhibited an improvement of working memory (Fig. 2A) as compared to Aβ(1–42) alone-treated rats, during 7 days training in radial arm-maze task. On the other hand, both CO1% and CO3% significantly improved long-term memory of Aβ(1–42)-treated rats, explored by reference memory (Fig. 2C) in radial arm-maze task. These findings could suggest that coriander volatile oil plays an important role in spatial memory formation, especially on working and reference memories. However, significant differences were observed between both doses of coriander volatile oil on working memory in radial arm-maze task.

Consequently, short response time in Aβ(1–42)-treated rats exposed to CO1% and CO3% could be a cause of cognitive improvement, evidenced by a decrease of time taken to consume all five baits (Fig. 2B) in radial arm-maze task (increased motivation of rats searching for food), during 7 days training, compared to Aβ(1–42) alone-treated rats.

It has previously been demonstrated that i.c.v. infusion of Aβ(1–42) in rats resulted in significant decrease of the antioxidant capacity [14,67]. Therefore, measurements of the activity of antioxidant enzymes are important when comparing the alterations induced by Aβ(1–42) with those found in AD patients. Analysis of AD brains demonstrates an increase in lipid peroxidation products in the amygdala, hippocampus and parahippocampal gyrus of the AD brain compared with age-matched controls [13].

In our study, Aβ(1–42) alone-treated rats exhibited an increase of SOD and LDH specific activities, an elevated MDA level and a decrease GPX activity in the hippocampal homogenates. The increases of the SOD activity appeared to parallel increases in MDA in the hippocampal homogenates suggesting that these events are needed to scavenge superoxide radicals induced by Aβ(1–42). MDA is the most abundant individual aldehyde resulting from lipid peroxidation and can be considered a marker of lipid peroxidation [13]. Consistently, increased lipid peroxidation was observed in an animal model of Alzheimer amyloidosis [68]. Furthermore, both doses of coriander volatile oil

(CO1% and CO3%), but especially CO3%, restored the activity of SOD and increased GPX activity in the hippocampal homogenates of Aβ(1–42)-treated rats. As expected for the antioxidant agents, both doses of coriander volatile oil (CO1% and CO3%), but especially CO3%, decreased the MDA level in the hippocampal homogenates.

The present study support the hypothesis that decreased SOD activity in Aβ(1–42)-treated rats exposed to coriander volatile oil can lead to decreased production of intracellular of $H_2O_2$ with a simultaneous increase of GPX activity. This could decrease the stimulation of lipid peroxidation and protein oxidation, implying that coriander volatile oil possesses strong antioxidant property.

Moreover, we found a significant positive correlation between working memory errors vs. MDA, reference memory errors vs. MDA and SOD vs. MDA when linear regression was determined. These results could suggest that increase of behavioral parameters in radial arm-maze task and the antioxidant defense along with decrease of lipid peroxidation could be correlated with involvement of coriander volatile oil in neuroprotection against Aβ(1–42)-induced oxidative stress generation in the rat hippocampus. Also, we reported that decrease of LDH activity (especially in CO3% + Aβ(1–42) group) together with the absence of DNA cleavage patterns in the Aβ(1–42)-treated rats exposed to coriander volatile oil, suggesting that coriander volatile oil possesses neuroprotective and antiapoptotic activities.

## 5. Conclusion

In summary, the present study indicated that multiple exposures to coriander volatile oil could effectively restore antioxidant brain status and may confer neuroprotection due to alleviation of oxidative damage induced by Aβ(1–42). Therefore, coriander volatile oil could be a potential candidate for further preclinical study aimed at the treatment of cognitive deficits in AD.

## Conflict of interest

The authors declare that they have no conflicts of interest in the research.

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