Article

A study on alpha-terpineol in Alzheimer's disease with the use of rodent *in vivo* model, restraint stress effect and *in vitro* Amyloid beta fibrils

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Alzheimer's disease (AD) is a neurological disorder in which the neuronal degeneration is associated with inflammatory processes and oxidative stress. Since alpha-terpineol was shown to possess antioxidant and anti-inflammatory effects, the administration of this compound was studied on a rat model of AD. To create this model, Aβ1-42 was injected into the hippocampus of male Wistar rats. Generated AD models were divided into simple AD models and AD models in which short-term immobilization stress was added. Preventive and therapeutic (post-AD induction) effects of alpha-terpineol consumption (100 mg/Kg) were subsequently investigated in AD models, which were compared with control groups. Biochemical factors (superoxide dismutase and malondialdehyde), histological manifestations (amyloid plaques and neuron counts) and possible memory impairment (shuttle-box experiment) were investigated in all groups. For the *in vitro* experiment, alpha-terpineol effect was checked on Aβ1-42 fibril formation. In preventive and therapeutic modes, alpha-terpineol consumption could improve neurogenesis and long-term memory while reducing amyloid plaque counts and ameliorating biochemical factors (higher levels of superoxide dismutase and malondialdehyde and reduced levels of MDA). *In vitro*, shorter fibrillar structures were formed in the presence of alpha-terpineol, which indicates an anti-amyloid effect for this compound. In conclusion, alpha-terpineol significantly counteracted AD consequences.

Keywords: Amyloid plaques. Aβ42. Alpha-terpineol. Memory. Movement.

INTRODUCTION

Alzheimer's disease (AD) is an advanced, age-related, unstable disorder diagnosed with memory and cognitive impairment indicative of oxidative stress (Van Cauwenberghe, Van Broeckhoven, Sleegers, 2016. There is currently no cure for AD, and present treatments can only alleviate the symptoms. It is now widely accepted that an extracellular amyloid protein (Aβ) may cause AD (Majd, Power, Grantham, 2015). This peptide is a product of amyloid precursor protein (APP) processing in the brain which produces two main forms with 40 and 42 amino acids: Aβ(1-40) and Aβ(1-42 (Lambert *et al.*, 1998). Memory impairment in AD begins with the changes in the hippocampal synaptic function and then gradually progresses to neuronal destruction and loss. The aggregation of Aβ peptide was reported as one of the primary reasons for this memory loss in AD (Selkoe, 2002). On the other hand, there were the indications of oxidative stress and inflammation in the pathogenesis of AD (Van Cauwenberghe, Van Broeckhoven, Sleegers, 2016. Finally, a number of studies have emphasized that

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a stressful stimulus may damage synaptic plasticity and neurogenesis in the hippocampal region and subsequently affect memory (Kim *et al.*, 2006a; Grigoryan *et al.*, 2014). An association between psychological stress and the development of AD was shown. In fact, psychological stress can contribute to the development of AD and further exacerbation of disease (Justice, 2018).

Natural products are believed to show neuroprotective effects and bioactive properties in biochemical pathways involved in the neurodegenerative disorders (Essa *et al.*, 2012). Monoterpenes are the main chemical components of the essential oils of medicinal plants with therapeutic properties (Bakkali *et al.*, 2008**)**. Alpha-terpineol is volatile monoterpene alcohol and one of the main components of the essential oils of various herbal species, such as *Ravensara aromatica* (ravensara), *Laurusnobilis* (laurel), *Myrtuscommunis* (myrtle), *Eucalyptus globules* (eucalyptus), and *Croton sonderianus* (De Sousa, 2011), as well as *Abieskoreana wilson* (Kim, 2006b and *Salvia* spp. (Kennedy *et al.*, 2011). The orange flower was reported to contain the highest amount of α-terpineol. Meanwhile, distilled lime oil contains some relatively rare terpineol isomers, including β- and γ-terpineol (Tisserand, Young, 2013). Alpha-terpineol is the predominant isomer found in essential oils and virtually the only terpineol isomer which was separately tested (Tisserand, Young, 2013).

The extract of *Salvia* spp. (which contains alpha-terpineol) is used in traditional European medicine to strengthen memory and treat dementia (Kennedy *et al.*, 2011). Alpha-terpineol showed the antioxidant effect (Brand *et al.*, 2001), the potent inhibition of superoxide production, as well as selective cell regulation during inflammation (Held, Schieberle, Somoza, 2007). Furthermore, previous studies showed that alpha-terpineol has anticonvulsant (De Sousa, Quintans, Almeida, 2007), sedative (De Sousa *et al.*, 2007), analgesic (Quintans-Júnior *et al.*, 2011), hypotensive (Ribeiro *et al.*, 2010), antibacterial (Kotan, Kordali, Cakir, 2007), and anti-fungal activities (Pitarokili *et al.*, 2002).

The search for an AD cure is still a valid one, since the disease cannot be treated currently, and considering the globally longer life expectancy, the number of AD cases are predicted to grow. We aimed at testing alpha-terpineol potential in the treatment of AD. First, a rodent model of AD was used, and since stress is also a component in AD, groups undergoing restraint stress were added. Alpha-terpineol effect was observed in both preventive and therapeutic modes in the *in vivo* setting. Furthermore, an *in vitro* experiment was run in order to check the effect of alpha-terpineol on Aβ42preformed fibrils, based on the fact that Amyloid beta fibrils are involved in the pathology of AD (Selkoe, 2002). Checking the potential of alpha-terpineol on fibril formation and destabilization can provide details onto one of the mechanisms by which the compound is acting in the AD model. In fact, numerous *in vitro* studies aim at finding compounds which can either stop the formation of amyloid fibrils or act on the formed fibrils as anti-amyloid compounds could be further considered for their potential in treating AD (Ashrafian, Zadeh, Khan, 2021).

MATERIAL AND METHODS

Animals

For the experimental study, 72 male Wistar rats (200 ± 50 grams) were purchased from the Pasteur Institute of Iran. The rats were housed at six per cage (42 × 26 cm), at 20 ± 0.5°C, under a 12/12-h light/dark cycle. They were regularly monitored, the light cycle and temperature were checked, and their cages were cleaned. Animals were habituated to the environment for one week prior to the experiments start. During the whole period of the experiment, the animals had free access to standard pellet food and water. Shuttle-box experiments were carried out in a sound-attenuated room, to which the rats were habituated for at least 1 hour. All experiments were strictly performed in accordance with the Guide for the Care and Use of Laboratory Animals (Derrell Clark *et al.*, 1997) and approved by the Research and Ethics Committee of Science and Research Branch, Azad University.

Compounds

Alpha-terpineol and Aβ1-42 were purchased from Sigma (St. Louis, MO, USA). The 1mg vial of Aβ1-42 was dissolved in 200µL of double-distilled water and placed in an incubator at 37°C for 1 week before use (Yaghmaei *et al.*,

2014; Hu *et al.*, 2008). More details about the process are provided under the section entitled "*in vitro* experiments".

Experimental groups and AD model generation

The rats were randomly assigned to nine groups (n = 6 rats/group) as follows:

1. Control group (Ctr): Received regular water and food and did not undergo surgery for three weeks.
2. Second control group (sham-operated) (S+W): Underwent surgery (S), and distilled water (W) was injected into their brains once.
3. Restraint Group (R): Underwent restraint stress and did not go under any surgery for three weeks.
4. AD group (Aβ): Underwent surgery, and Aβ42 solution was injected into their brains once as described above.
5. Sham group (Aβ+ W): Prepared similar to group #4 and then received distilled water (as Aβ solvent) instead of alpha-terpineol once a day for two weeks after one week of AD induction.
6. AD and restraint stress (Aβ+R): Prepared similar to group #4, and after one week of AD induction, the animals underwent restraint stress for two weeks.
7. Experimental group 1 (Aβ+T+100): Prepared similar to group #4 and received a therapeutic dose of 100 mg/Kg of alpha-terpineol intraperitoneally (IP) once a day for two weeks after one week of AD induction.
8. Experimental group 2 (Aβ+P+100): Received protective dose of 100 mg/Kg of alpha-terpineol (IP) once a day for one week prior to AD induction and then underwent Aβ injection.
9. Experimental group 3 (Aβ+R+100): Prepared similar to group #4, underwent restraint stress, and received a therapeutic dose of 100 mg/Kg of alpha-terpineol (IP) once a day for a total of two weeks after one week of AD induction.

Practically, the injected volume was 0.5 ml of either solvent or compounds. As terpineol was used in the range from 50-200 mg/Kg in various studies (Russo, Marcu, 2017), we chose the 100 mg/Kg dose based on those experiments.

In summary: the experiment lasted 21 days, and animals were sacrificed on the 22nd day. Interventions, including treatment with alpha-terpineol, administration of water in the sham group, and restrained stress, were started on the 8th day, with a duration of two weeks, and lasted till the 21st day. In protection (or preventive) mode, alpha-terpineol was administered one week prior to AD model generation.

To induce AD, the rats were first anesthetized by ketamine and xylazin injection (Wellington, Mikaelian, Singer, 2013) and placed within a stereotactic device. After localizing the hippocampus using stereotaxy based on brain atlas (Paxinos, Watson, 2006), 2 μl of Aβ42 solution was slowly injected with a Hamilton syringe in the ventricle of animal's brain in the cornu ammonis (CA1) region on both sides of the hippocampus. The coordinates were the following: anterior-posterior (AP)= −4.8 mm, medial-lateral (ML)= ±3.5 mm, and dorsal-ventral (DV)= −4 mm. After one week, amyloid plaques were formed in the rats' brains which were visible using histological methods (detailed below). All experiments, including AD induction following administration of alpha-terpineol, restraint stress, or combination of restraint stress and alpha-terpineol administration, were done for a total of three weeks. Alpha-terpineol (100 mg/Kg) was prepared in double-distilled water. In the protective mode, alpha-terpineol was administered one week prior to Aβ42 injection.

Restraint stress test

The rats were raised under standard conditions until restraint stress was given, and they were introduced to the shuttle box a day prior to examination. To induce restraint stress, the rats were subjected to 5 hours of restraint in a polypropylene tube (3×3×10 cm) for two weeks (Yu *et al.*, 2010).

Measurements of SOD and MDA

Blood samples were collected after rats were sacrificed (on day 22 after the experiment had started). The samples were first allowed to clot for 30 min at room

temperature and then centrifuged at 3000 rpm at 37 °C for 10 min to separate the serum. The serum levels of SOD (superoxide dismutase) and MDA (Malondialdehyde) were determined using a photometric method and kits from Pars Azmoon Co. Karaj, Iran.

Shuttle-box testing

For the shuttle-box test, a box was used that consisted of two chambers of equal size (26 × 26 cm) and separated by a sliding door (8 x 8 cm). Each experiment began with a pre-test in which the rat was first placed in the chamber for 5 seconds. Then, the sliding door was raised, and the rat was allowed to remain in the dark chamber for 10 seconds. The rat was then returned to its own cage and left inside the cage for 30 minutes. Afterward, the rat was put into the shuttle box and received a shock in the feet area (50HZ, 1Ma for5 seconds) after entering the dark area; then the rat was brought back into its cage and stayed there for 120 seconds. Right after, the rat was put into the shuttle box. If a 300-second delay was observed before entering the dark area, a passive avoidance pass was registered. To assess long-term memory, a similar process was used 24 hours after the training period. The basis of that experiment is the fact that latency could be considered an increase or decrease of memory retention (Guaza, Borrell, 1985; Hosseinzadeh, Roshan, Pourasghar, 2013). We performed these tests once on day one, before treatment, and once on day 21 after the experiment had started.

Histological testing

To remove the rats' brains for histological evaluation at the end of experiment, animals were sacrificed by anesthesia on day 22 after the experiment had started. Brains were then stored in formalin 10% for 24 hours and later processed for paraffin embedding. To assess neurogenesis, hematoxyllin eosin staining was done. Moreover, thioflavin S staining method was employed to detect amyloid plaques, and the images were observed by a fluorescence microscope (Gandy, 2005).

In vitro experiment

Aβ42 peptide was first dissolved in deionized water (DW) to a 1 mg/ml final concentration. To make mature fibrils, tubes containing Aβ42 monomers were incubated at 37° C for 2 and 4 days while the water bath containing the tubes was being gently stirred by a Teflon magnetic bar. Afterward, to check the destabilization potential of alpha-terpineol, aliquots of 1 mg/ml of four-day-old preformed Aβ fibrils were further incubated with alpha-terpineol (100 µM) at 37° C for 3 weeks (Ghobeh *et al.*, 2014). In all experiments, the water bath containing the samples tubes was gently stirred by a Teflon magnetic bar.

Transmission Electron Microscopy

Five µl of 1 mg/ml samples were adsorbed onto copper 400 mesh F-C grids. After 2 minutes, excess fluid was removed with a paper filter, and then 5 µl of 1% uranyl acetate was added onto the grid. Excess dye was removed after 2 minutes. After being completely dried out, the samples were observed by a Hitachi HU-12A electron microscope (Hitachi, Japan) operated at 75 kV.

Statistical Analysis

Data are expressed as mean ± SEM. After analyzing the normal distribution of data and homogeneity of variances (Kolmogorov–Smirnov), one-way ANOVA was used to assess the statistical significance among groups, and Tukey test was used as post-hoc. The values of $p \leq 0.05$ were considered statistically significant.

RESULTS

Therapeutic effects of alpha-terpineol on biochemical factors, passive avoidance learning, and brain tissue histology

As shown in Figure 1, the serum level of SOD was significantly lowered ($p < 0.001$) in the disease-induced groups (Aβ and Aβ+W) compared with control groups (Ctr and S+W). Meanwhile, the SOD level of group treated with alpha-terpineol (Aβ+T+100) was significantly increased

compared to the disease-induced groups. Regarding the serum level of MDA, the treated group with alpha-terpineol (Aβ+T+100) showed a significant decrease (p<0.001) in the enzyme level compared with the disease-induced groups (Aβ and Aβ+W), showing, the efficiency of treatment with alpha-terpineol. A significant reduction in the MDA level was also observed in the control groups (Ctr and S+W) compared with the disease-induced groups (Figure 2).
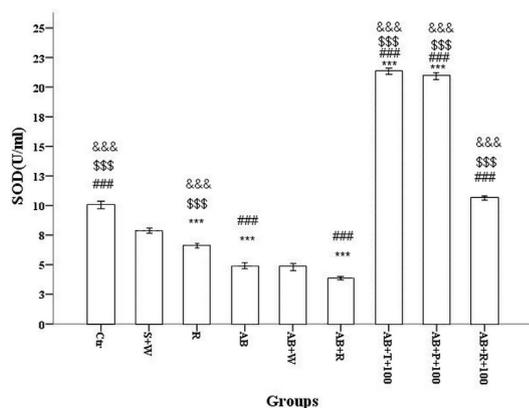

FIGURE 1 - Overall serum level of SOD in different groups. Comparison of control group (Ctr) with other groups (*** p<0.001); comparison of restraint (R) group with other groups (### p<0.001); comparison of AD group (AB) with others ($$$ p<0.001); comparison of AD and restraint stress group (AB +R) with other groups (&&& p<0.001). Please refer to the Methods and Materials section for the groups' definitions. F-value: 826.2.
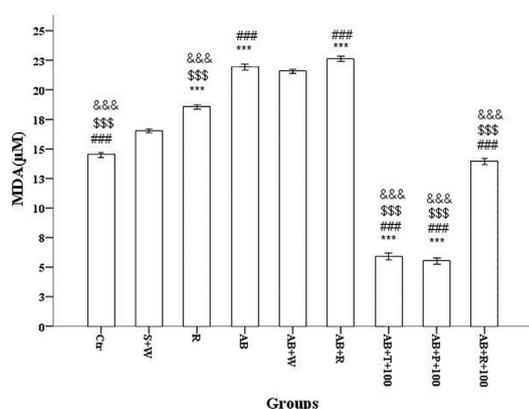

FIGURE 2 - Overall serum level of MDA in different groups. Comparison of control group (Ctr) with other groups (*** p<0.001); comparison of restraint (R) group with other groups (### p<0.001); comparison of AD group (AB) with others ($$$ p<0.001); comparison of AD and restraint stress group (AB +R) with other groups (&&& p<0.001). Please refer to the Methods and Materials section for the groups' definitions. F value : 775.1.

The statistical analysis of shuttle-box test showed that there was a significant difference (p<0.001) between the group treated with alpha-terpineol and AD-induced groups (Aβ and Aβ+W). The same significant difference was also observed among Ctr and S+W groups and Aβ and Aβ+W groups, showing that consumption of alpha-terpineol seemed to restore the long-term memory (Figure 3).
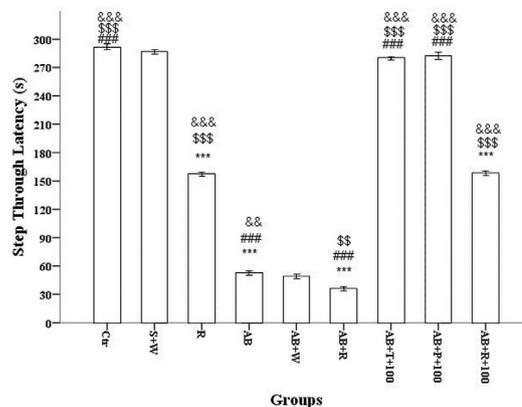

FIGURE 3 - The mean latency to enter the shuttle box. Comparison of control group (Ctr) with other groups (*** p<0.001); comparison of restraint (R) group with other groups (### p<0.001); comparison of AD group (AB) with others ($$$ p<0.001); comparison of AD and restraint stress group (AB +R) with other groups (&& p<0.01, &&& p<0.001). Please refer to the Methods and Materials section for the groups' definitions. F-value: 1885.8.

The induction of Alzheimer's disease (in Aβ and Aβ+W groups) resulted in amyloid plaques formation and reduced neurogenesis, whereas treatment with alpha-terpineol significantly (p<0.001) increased the number of neurons (Figure 4 and Figure S1 of supplementary data) and decreased the amount of plaques (Figure 5 and Figure S2 of supplementary data) in the treated group (Aβ+T+100).
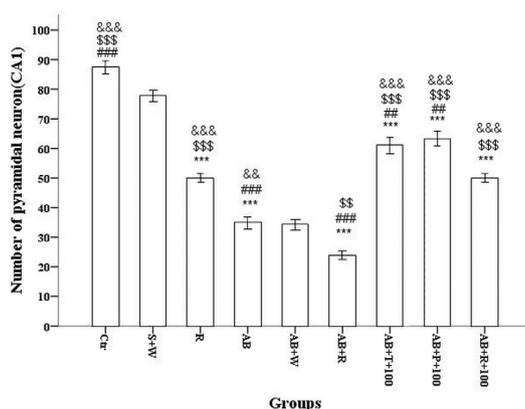
FIGURE 4 - Neuron numbers in different groups Comparison of control group (Ctr) with other groups (*** $p<0.001$); comparison of restraint (R) group with other groups (### $p<0.001$); comparison of AD group (AB) with others ($$$ $p<0.001$); comparison of AD and restraint stress group (AB +R) with other groups (&&& $p<0.001$). Please refer to the Methods and Materials section for the groups' definitions. F-value: 113.3.
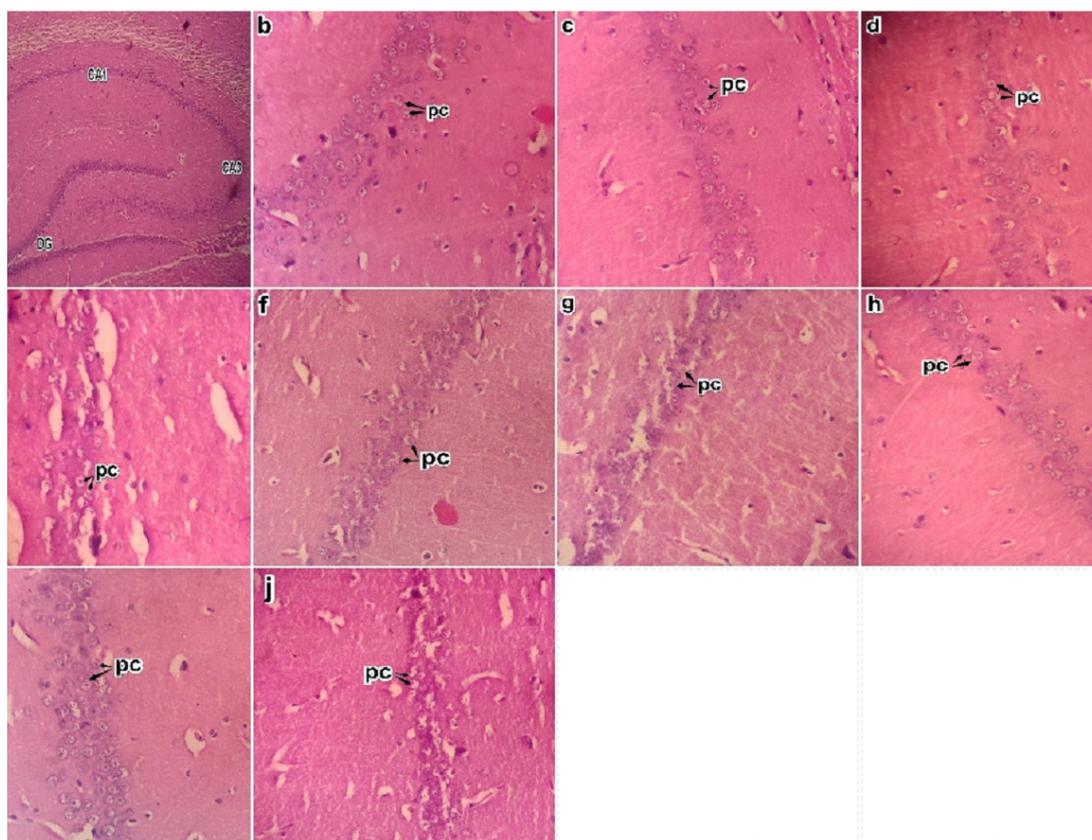

FIGURE S1 - H&E staining of pyramidal cells (pc) in the sagittal sections of hippocampus CA1 region. a: X40. In the following images X400 was applied: b (Ctr); c (S+W); d (R); e (Aβ); f (Aβ+W); g (Aβ+R), h (Aβ+T+100), i (Aβ+P+100), and j (Aβ+R+100). Please refer to the Methods and Materials section for the groups' definitions. pc: pyramidal cells.
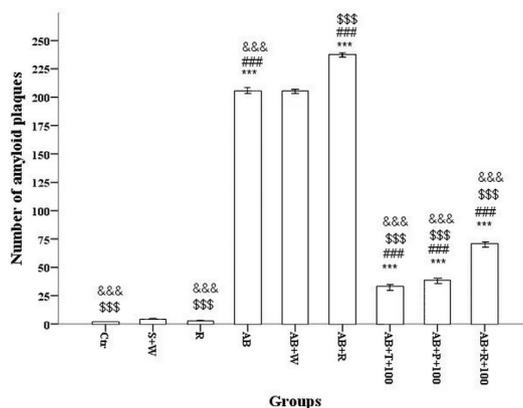
FIGURE 5 - Amyloid plaque numbers in different groups. Comparison of control group (Ctr) with other groups (*** p<0.001); comparison of restraint (R) group with other groups (### p<0.001); comparison of AD group (AB) with others ($$$ p<0.001); comparison of AD and restraint stress group (AB +R) with other groups (&&& p<0.001). Please refer to the Methods and Materials section for the groups' definitions. F-value: 3181.4.
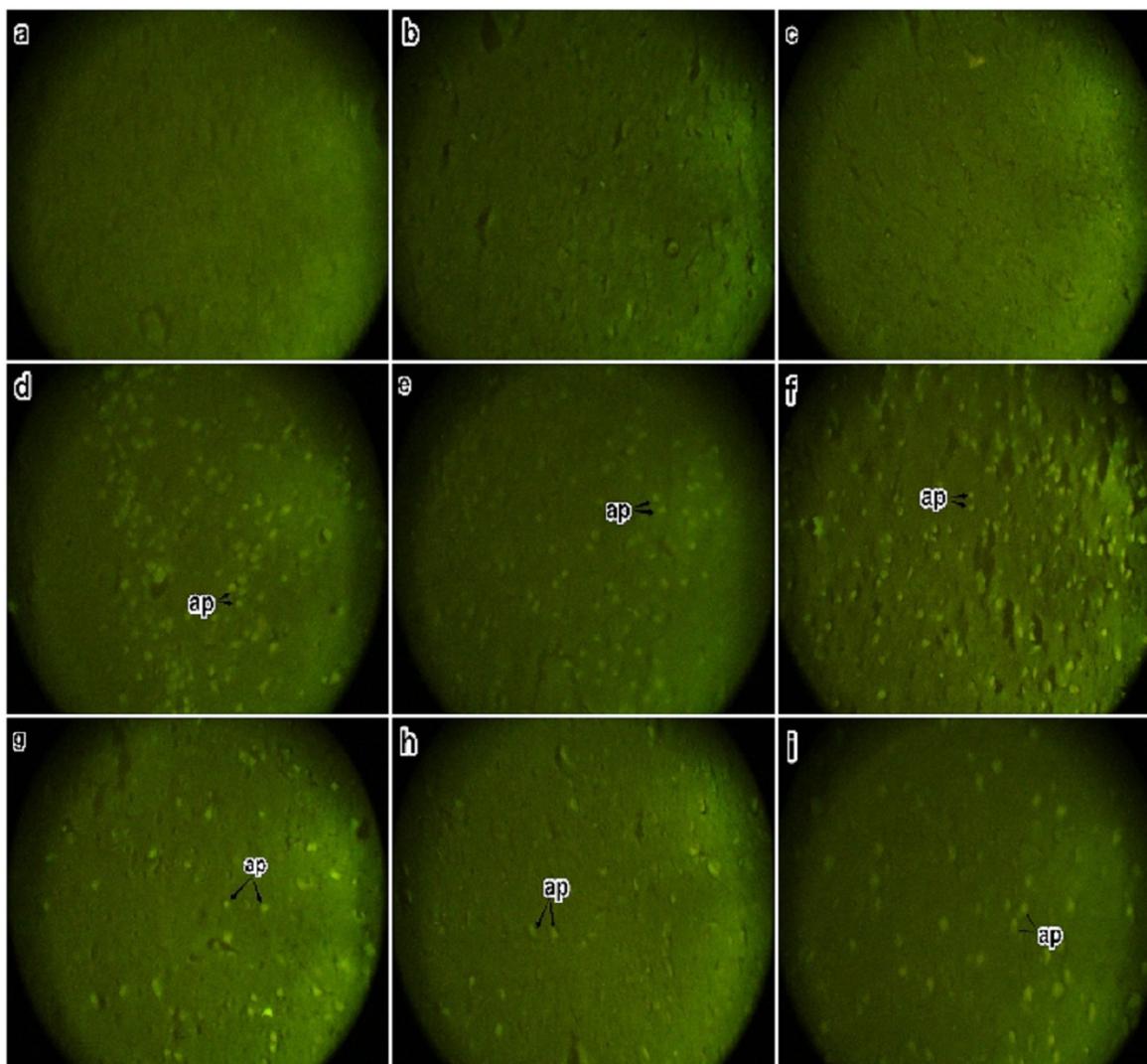

FIGURE S2 - Thioflavin S staining of amyloid plaques in the hippocampus CA1 region. a (Ctr); b (S+W); c (R); d (Aβ); e (Aβ+W); f (Aβ+R), g (Aβ+T+100), h (Aβ+P+100), and i (Aβ+R+100). Please refer to the Methods and Materials section for the groups' definitions. ap: amyloid plaques; ×400.

Protective effects of alpha-terpineol on biochemical factors, passive avoidance learning, and brain tissue histology

The serum level of SOD in the group receiving 100 mg/Kg alpha-terpineol in the protective mode was significantly higher than control (Ctr and S+W) and AD-induced (Aβ and Aβ+W) groups. (Figure 1). On the contrary, serum level of MDA was significantly lower in the protected group (Aβ+P+100) than Ctr, S+W, Aβ, and Aβ+W groups (Figure 2).

Besides, the statistical analysis of behavioral test showed that the group receiving alpha-terpineol in a protective mode (Aβ+P+100) indicated significant improvement in long-term memory than Ctr, S+W, Aβ, and Aβ+W groups (Figure 3).

The histological examination of brain tissue showed that the number of neurons and plaques notably increased and decreased, respectively, in the Aβ+P+100 group compared with Ctr, S+W, Aβ, and Aβ+W groups (Figures 4 and 5 and Figures S1 and S2 of supplementary data).

Effect of restraint stress on Alzheimer's disease model combined with treatment

Three groups of rats underwent restraint stress in different modes: one group underwent restraint stress and did not go under any AD surgery (R); in one group, Alzheimer's disease was induced and underwent restraint stress (Aβ+R); in one group AD was induced in rats and they underwent restraint stress and received a therapeutic dose of 100 mg/Kg of alpha-terpineol (Aβ+R+100).

The serum levels of SOD in the R and control groups (Ctr and S+W) were significantly different (p<0.001) from the disease-induced group (Aβ) (Figure 1). Even the group which received a therapeutic dose of compound (Aβ+R+100) indicated a notably higher level of SOD compared with the disease-induced groups (p<0.001). On the contrary, there was no difference between Aβ and Aβ+R groups. Regarding MDA levels, there was also a significant difference (p<0.001) between AD-induced groups (Aβ and Aβ+R) and groups R and Aβ+R+100

(Figure 2). The overall result was that the presence of Alzheimer's disease overcame the restraint stress, whereas treatment with alpha-terpineol demonstrated improvement.

In the behavioral tests, as shown in Figure 3, there was a significant difference (p<0.001) in long-term memory between R and Aβ groups which showed that the R group was reasonably equivalent to the control group. The same difference was also observed between Aβ+R+100 and Aβ groups. Furthermore, Aβ+R group demonstrated inferior long-term memory than Aβ group (Figure 3).

The histological investigations showed that the numbers of neurons in the R and Aβ+R+100 groups were significantly higher (p<0.001) than those in the Aβ group (Figure 4 and Figure S1 of supplementary data). By applying restraint stress to Aβ group (Aβ+R), neurogenesis diminished notably compared to Aβ group (p<0.01).

Meanwhile, the counts of plaques in the R and Aβ+R+100 groups were significantly lower than that of the Aβ group while showing similarity with normal animals (Ctr and S+W) (Figure 5 and Figure S2 of supplementary data).

Destabilizing effects of alpha-terpineol on pre-formed Aβ42 fibrils *in vitro*

This study's in vitro part involved monitoring the destabilizing effect of alpha-terpineol 100 µM for 3 weeks of incubation with preformed Aβ42 fibrils *in vitro*. Aβ42 fibrillation proceeded from monomeric state to typical fibrils after 4 days (Figure 6a and b). Based on TEM images, fibrils removal by alpha-terpineol is not remarkable during the first week of incubation (Figure 6c). After three weeks (Figure 6d), images taken from incubated samples revealed that shorter fibrillar structures were formed in the presence of alpha-terpineol (Aβ42 fibrils are used for comparison). It is thus suggested that in order to destabilize fibril formation, longer times of incubation are needed in the presence of alpha-terpineol 100 µM. This observation could be related to the removal of fibrillary plaques after *in vivo* treatment with the compound.
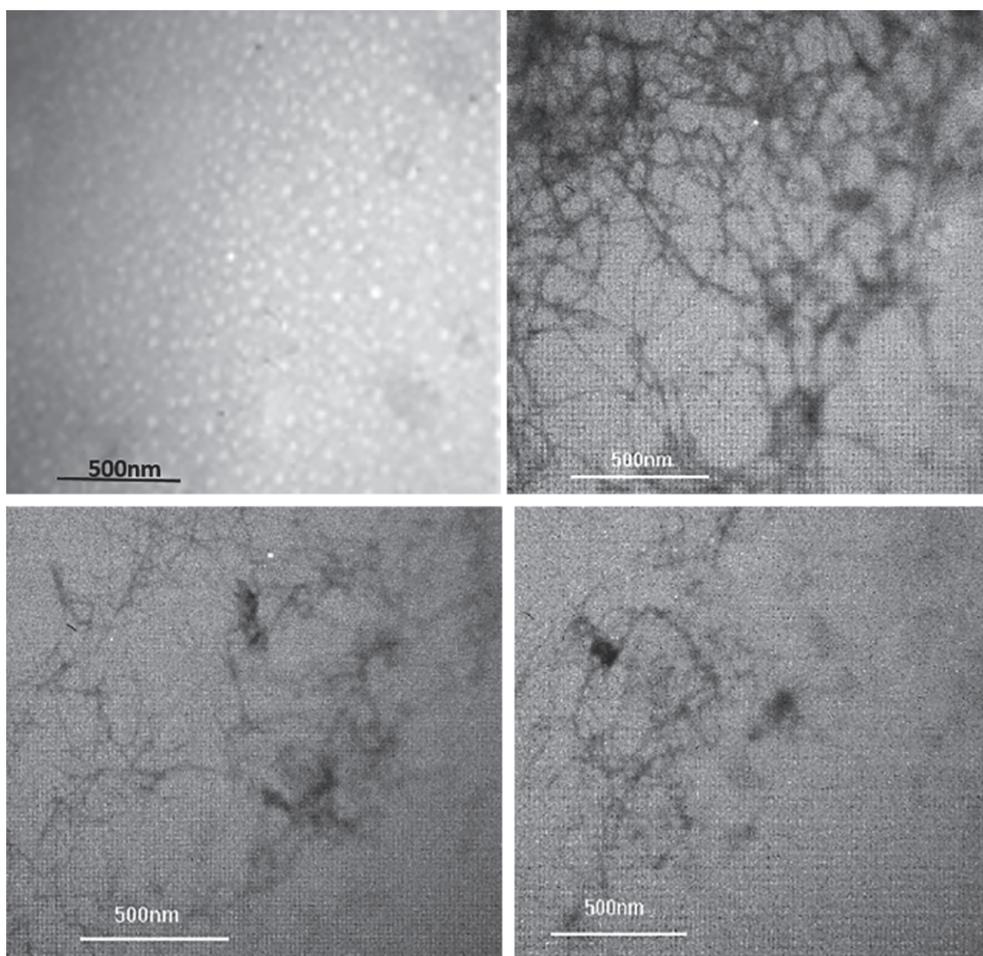
FIGURE 6 - Electron microscope analysis of Aβ42 fibrillation with and without alpha-terpineol. TEM images of Aβ42 fibrillation after immediate incubation (a) and 4 days (b) in the absence of alpha-terpineol. The second row indicates the four-day-old Aβ42 fibrils incubated with alpha-terpineol 100 μM for 1 week (c) and 3 weeks (d).

DISCUSSION

According to the biochemical and behavioral indices and histological investigations, both therapeutic and protective modes of alpha-terpineol consumption effectively diminished brain plaques and improved neurogenesis and memory in the animal model. Furthermore, the injection of aqueous solvent alone exhibited no particular effect on AD-related consequences (i.e., biochemical indices, plaque formation in the brain, and memory impairment). Meanwhile, based on the *in vitro* experiment, alpha-terpineol seemed to offer the potential to destabilize pre-formed fibrils.

Moreover, short-term immobilization strengthened the consequences of AD induction on memory as well as neurogenesis and biochemical indices, while the symptoms were improved when the restraint stress was accompanied by treatment with alpha-terpineol. Previous studies showed that chronic immobilization causes biochemical, pharmacological, and morphological changes in the hippocampus, especially in CA1 and CA3 regions (Magariños, McEwen, 1995; McEwen, 1999). Several studies have shown that acute and chronic stress produces undesired effects in memory and learning (Ghadrdoost *et al.*, 2011; Mohammadi *et al.*, 2014).

The injection of Aβ42 fibrils into rat brain is now an established method of generating AD model. Aβ42, is a potent neurotoxic peptide and a major structure of elderly plaques that result in neuronal dysfunction and

memory impairment in AD disease (Zhang *et al.*, 2016), resulting in inflammation and oxidative stress (Zhang *et al.*, 2012).

AD signs could be counteracted by potential anti-amyloid natural compounds possessing aromatic or other polycyclic characteristics (Yaghmaei *et al.*, 2013; Bag *et al.*, 2013). Many studies showed that essential oils, including basil, tarragon, lavender, Spanish sage, tea tree, and rosemary have significant anticholinesterase inhibitory activities (Geiger, 2018). The single chemical constituents of the mentioned essential oils such as 1,8-cineole, alpha-pinene, eugenol, alpha-terpineol and terpien-4-ol have also shown anticholinesterase inhibitory effects but to a lesser degree. These significant differences in anticholinesterase inhibitory activities between the whole plant essential oils and the single chemical constituents have implied possible synergies and antagonisms generated by secondary messenger chemical constituents of essential oils (Geiger, 2018).

One of the biologically active plant compounds are monoterpenes which are part of natural compounds called terpenes (Bakkali *et al.*, 2008; Aprotosoaie *et al.*, 2014). Among monoterpenes, alpha-terpineol has exhibited antioxidant (Brand *et al.*, 2001) and anti-inflammatory (Held, Schieberle, Somoza, 2007) properties. Studies have shown that alpha-terpineol has inhibitory properties for NF-κB and protein kinase (Hassan *et al.*, 2010). It is also found to be a selective inhibitor of agonist stimulated-superoxide production by monocytes (and does not act on the process related to neutrophils during an inflammatory response (Brand *et al.*, 2001; Held, Schieberle, Somoza, 2007). In a study, it was indicated that Abieskoreana, which contains a high level of alpha-terpineol, can increase the memory of scopolamine-induced amnesia in mice (Kim *et al.*, 2006b).

Moreover, it was shown that alpha-terpineol reduces oxidative stress by inhibiting lipid oxidation (Moghimi *et al.*, 2016). MDA, as a by-product of lipid peroxidation, is an extremely reactive and toxic aldehyde (Taso *et al.*, 2019), causing an irreversible modification of phospholipids, proteins, and DNA (Esterbauer, Cheeseman, 1990). In accordance with our study, MDA levels were elevated in AD patients compared to controls in other studies (Bradley-Whitman, Lovell, 2015), and its levels significantly decreased when treated with alpha-terpineol. Therefore, alpha-terpineol seem to be capable of diminishing lipid peroxidation leading to MDA level reduction.

The main causative factors of AD, namely abnormal deposition of Aβ peptide and intracellular accumulation of neurofibrillary tangles of hyperphosphorylated tau protein, are reported to be mainly initiated and enhanced by oxidative stress, a process caused by elevation of oxidants and reduction of the antioxidant defense system, such as SOD (Huang, Zhang, Chen, 2016). More specifically, Casado *et al.* (2008) have found impaired antioxidant defense enzymes expression or activity in AD patients. These results are in accordance with our study showing the diminished level of antioxidant SOD enzyme in AD group whereas alpha-terpineol showed the capability of increasing the level of SOD. It was reported that some of the pharmacological effects of alpha-terpineol are due to the inhibition of nitric oxide production while holding anti-tumor and analgesic effects (de Oliveira *et al.*, 2012).

Further than antioxidant and anti-inflammatory properties, we believe that the results of this experiment show that alpha-terpineol could also act on this AD model via its anti-fibril effect which was indicated in the *in vitro* experiment. Anti-fibrillation effect of various compounds, including aromatic compounds and especially larger polycyclic chemicals (such as flavonoids like myricetin and biochanin A as well as curcumin), was reported on various types of fibrils (Ono *et al.*, 2012; Ghobeh *et al.*, 2014).

In conclusion, with regard to its effect in diminishing amyloid plaques and improving learning and memory in AD model, which could be attributed in part to its anti-oxidant and anti-fibrillar properties, alpha-terpineol could be suggested as a structure with potential to be further developed as an anti-AD therapeutic.

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