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The Effect of 3-Carene on Memory, Histological, and Biochemical Parameters in Male Wistar Alzheimer's Rats

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ABSTRACT

Alzheimer's disease (AD), as the most common type of dementia, gradually leads to a decrease in memory, speech, and other cognitive skills that affect a person's ability to perform daily activities. With the increase in the incidence of this disease, especially in old ages, there is a need to identify factors and drugs for prevention or treatment. In the present study, the effect of 3-Carene was investigated on behavioral, biochemical and histological parameters in Alzheimer's-induced Wistar male rats by beta-amyloid (Aβ42) injection. Forty two Wistar male rats were randomly assigned to 7 groups (n = 6 rats/group), including control group without any treatments; PBS group receiving phosphate buffer (Aβ42 solvent); AD group with Aβ42 injection into rats' hippocampus; sham group receiving corn oil (3-Carene solvent); two treatment groups receiving 3-Carene at 200 and 400 µg/kg after being injected with Aβ42; the protective group receiving 3-Carene (400 µg/kg) before beta-amyloid injection in a protective mode. The results demonstrated that 3-Carene improved the memory, lipid profile, amyloid plaques, and superoxide dismutase activity and malondialdehyde level, whereas Alzheimer's-induced group showed impairment in all examined parameters. Protection with 3-Carene also demonstrated similar improvements against AD. Hence, 3-Carene has shown capability of improving the amyloid plaques, memory as well as biochemical factors associated with AD.

Keywords: Alzheimer's disease, 3-Carene, Amyloid plaques, Memory, Biochemical parameters

INTRODUCTION

Alzheimer's disease (AD) is a disease of brain degeneration and the most common cause of dementia [1]. The condition is characterized by decreased memory, speech, problem-solving, and other cognitive skills that affect a person's ability to perform daily activities due to damaged or destroyed cells in parts of the brain involved in cognitive function. People in the later stages will need to be hospitalized and cared for all the time. However, AD can be eventually fatal [2]. It has been proposed that synaptic loss, neuronal death, and cognitive dysfunction in AD occur as a result of Aβ accumulation in the brain [3-5]. Aβ42 peptide (containing 42 amino acids) is considered a toxic specie involved in AD pathophysiology and its presence in cerebrospinal fluid could be a reliable predictor of AD progression [5]. In order to study Aβ aggregation and further assess potential new candidates for AD therapy *in vivo*, intra-cerebroventricular administration of Aβ peptide into rat brain has been used to stimulate AD [6].

Essential oils are a diverse family of low-molecular-weight organic compounds with comprehensive biological activity. According to the chemical structure, these active compounds can be divided into different groups: terpenes, terpenoids, phenylpropenes and others [7]. Pine essential oil is mainly composed of monoterpenes such as α- and β-Pinene, 3-Carene, Limonene and Terpinene [8]. 3-Carene is a two-ring monoterpene composed of cyclohexane and cyclopropane linkages with a sweet and pungent odor (Fig. 1) [9]. As the second most abundant monoterpene of pine essential oils after α-Pinene, 3-Carene has shown an excellent inhibitory effect on AChE in human red blood cells [10,11]. However, the putative neuroprotective effects of 3-Carene is unknown. Therefore, the objective of this study was to assess the effects of 3-Carene, as a natural compound, on amyloid-β plaque accumulation, lipid profiled, and biochemical parameters in Alzheimer's disease rat models.

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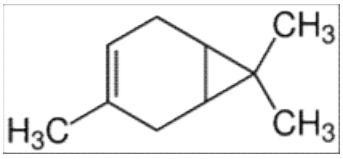

Fig. 1. Chemical structure of 3-carene.

MATERIALS AND METHODS

Compounds

Aβ42 and 3-Carene were obtained from Sigma (St. Louis, MO, USA). Aβ42 was dissolved in PBS and placed in an incubator at 37°C for 1 week before use [6,12]. 3-Carene doses (200 and 400 µg/kg) were prepared in corn oil. Commercial kits used for the evaluation of low-density lipoprotein cholesterol (LDL), high-density lipoprotein (HDL), triglycerides (TG), cholesterol, superoxide dismutase (SOD), and malondealdehyde (MDA) were purchased from Zist Shimi Company, Iran.

Aβ42 Preparation

Aβ42 was dissolved in phosphate buffer (1 mg ml^{-1}). The microtube containing Aβ42 solution (1 mg ml^{-1}) was covered with parafilm and kept in the incubator at 37 °C for 7 days to form Aβ fibrils. These conditions were previously proved to be the optimum conditions for Aβ42 fibril formation [6,12]. The solution was then stored at -20 °C.

Animals

The experiments were conducted on forty two male Wistar rats (200 ± 5 g; Pasteur Institute, Tehran, Iran). The animals were kept in an animal room that was maintained at 20-25 °C with 50-70% relative humidity under a 12-h light-dark cycle. The rats had free access to food (rodent pellets) and water. During the first week, the rats were handled once a day to adapt to the existing conditions. To induce Alzheimer's disease (AD), animals were first anesthetized by injecting 2 ml combination of ketamine and xylasin (1:5 ratio) intraperitoneally to each animal to prevent muscle stiffness. The injection site was disinfected with alcohol before and after injection. After anesthesia, the animals' hair was cut between two eyes and two ears. Then it was placed in a stereotaxic device. Using stereotaxy and brain atlas, 2 µl of Aβ42 solution was injected with a hamilton syringe in the ventricle of the animal's brain. Injection was slowly carried out in the CA region on both sides of the hippocampus. After surgery, animals recovered for one hour in a warm box before they were returned to their cages. The animals were given 7 days of recovery [6]. After one week, amyloid plaques were formed in the animal's brain which were visible by the use of histological method. All experiments were performed in accordance with the international guidelines set in the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996) and approved by the Research and Ethics Committee of Science and Research Branch, Islamic Azad University (NO. IR.IAU.SRB.REC.1398.124).

Experimental Design

The rats were allowed to acclimate for seven days prior to their use in the studies. Forty two Wistar male rats were randomly assigned into 7 groups (n = 6 rats/group) as follows:

- Control group: receiving regular food and water with no Aβ42 injection
- PBS group: phosphate buffer (as Aβ42 solvent) was injected into rats' hippocampus instead of Aβ42
- AD group: Aβ42 was injected once into rats' hippocampus as described above
- Sham group: AD-induced group received corn oil (3-Carene solvent) intraperitoneally for four weeks
- AD + 3-Carene (200 µg/kg) group: AD-induced group received 3-cerene (200 µg/kg) intraperitoneally for four weeks in a treatment mode
- AD + 3-Carene (400 µg/kg) group: AD-induced group received 3-cerene (400 µg/kg) intraperitoneally for four weeks in a treatment mode
- 3-Carene + AD group: the rats first received 3-Carene at 400 µg/kg intraperitoneally for two weeks in a protective mode and then Aβ was injected into their hippocampus

Behavioral Test

The shuttle box has two compartments, each with a length and width of 20 and a height of 30 cm connected via a sliding door. One is a light chamber (safe area) with white walls, and the other one is a dark chamber (insecure room)

with black borders. Stainless steel shock bars are located on the dark area floor and connected to the electroshock device with wires to adjust the electric shock amount. It sends an electric shock (1 mA and a frequency of 50 Hz for 3 s). The phenomenon of this test in passive modeling is based on punishment. In this model, an electric shock is applied to the animal's foot and as a result, the animal learns not to enter a dark room [6].

All the rats were placed in the laboratory environment at least 30 min before starting the experiment to get used to it. Then, they were gently placed in the light compartment. After 5 s, the slider was opened between the two boxes and the animal was allowed to enter the dark box. The time it takes for the animal to enter the dark chamber from the light section is recorded as the initial delay time. Entering the hind legs into the dark enclosure was considered the animal's entry into the dark compartment, and then the sliding door was closed. After 10 s, the animal was returned to its cage. Also, at this stage, animals with an entry delay time of more than 100 s were excluded from the experiment.

The rat was placed in the light compartment after 30 min and returned to the slider after 5 s. Immediately after the animal entered the dark chamber, the door was closed. The animal was shocked (50 Hz, one mA, 3 s) and returned to the cage after 15-20 min. Two minutes later, the animal was placed in the light compartment again. If the animal re-entered the dark cell, it would be shocked too, but if it learned and did not enter the dark cell for two minutes, the test would be completed, and the mouse would be returned to its cage.

Finally, the animal's long-term memory was assessed 24 h after training. Each animal was placed in the light compartment, and the sliding door was opened after 20 s. The time it took for the animal to enter the dark chamber (Re-entry Latency) was recorded. The experiment ended with the animal entering the darkroom. However, if the animal has memory and refuses to join the darkroom, the investigation ends five minutes after being placed in the light section [13].

Measurement of Biochemical Parameters

The animals were first anesthetized. The blood was collected and transferred from the heart to separate falcons. Blood samples were centrifuged at 5000 × g for 10 min. Supernatant, which contained serum isolated from blood cells, was sampled and transferred to a microtubule. Samples were stored at -20 °C until the parameters were measured. Lipid profile parameters, including triglyceride, total cholesterol, HDL and LDL were then measured. SOD activity measurement was also made in the brain tissue based on the enzyme ability to inhibit the auto-oxidation of pyrogallol, which is checked at 420 nm [14]. Level of MDA was measured in the brain tissue in which MDA reacts with thiobarbituric acid (TBA) in serum to produce a state of MDA-TBA, which is then measured by colorimetric (OD = 532) method [15].

Histological Studies

At the end of experiment, the rats were decapitated under anesthesia and their brain were removed for histological assessments. The brains were fixed in 10% formalin and consequently embedded with paraffin. Serial sections of 6 μm-thickness were then prepared and stained with hematoxylin and eosin. The slides were examined by light microscopy. Furthermore, Thioflavin-S method was applied for staining of amyloid plaques [6], in hippocampus cells, for which photomicrographs were prepared and amyloid plaques were counted using Image J 1.8.0-112 software.

Statistical Analysis

In the present study, Anova and Tukey's analyses of variance were used to evaluate the significant differences. SPSS and Graphpad Prism 8 softwares were applied to analyse the data and draw graphs, respectively. The results are expressed as Mean ± SEM with significance levels of $p < 0.05$, $p < 0.01$ and $p < 0.001$ for groups at all stages.

RESULTS

Passive Avoidance Test

Alzheimer’s-induced group showed loss of long-term memory by entering the dark chamber on an average time of 75 seconds. However, all experimental groups being treated with both doses of 3-Carene showed significantly longer step-through latency compared to the Alzheimer’s group ($p < 0.001$) in the test day (Fig. 2). However, the group that received 3-Carene in a protective mode exhibited similar step-through latency to the Alzheimer’s group.

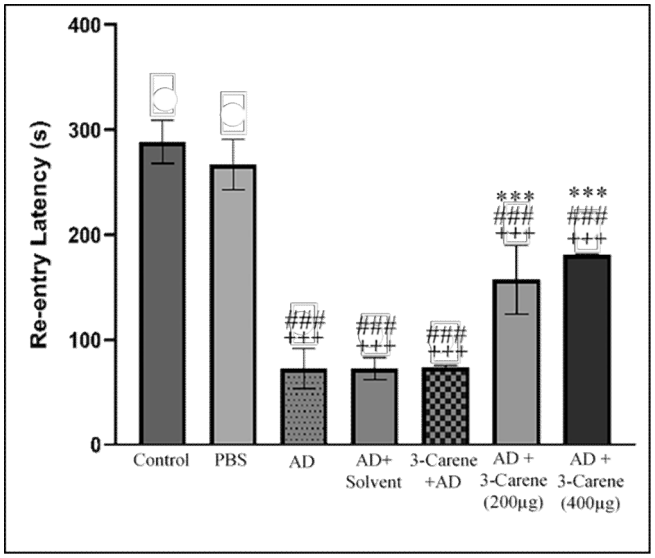

Fig. 2. The mean latency to enter the dark chamber on the test day. Control: rats received only regular water and food; PBS: rats underwent surgery with PBS being injected into brain; AD: Aβ1–42 was injected into brain to induce Alzheimer's disease (AD); AD+solvent: Alzheimer's-induced group treated with coil oil as 3-carene solvent; 3-carene+AD: rats received 3-carene 400 µg/kg in a protective mode before induction of AD; AD+3-cerene 200 µg/kg: Alzheimer's-induced group treated with 200 µg/kg 3-carene; AD+3-carene 400 µg/kg: Alzheimer's-induced group treated with 400 µg/kg 3-carene. +++: $p < 0.001$ compared with the control group. ###: $p < 0.001$ compared with the PBS group. ***: $p < 0.001$ compared with the Alzheimer's disease (AD) group receiving β-amyloid.

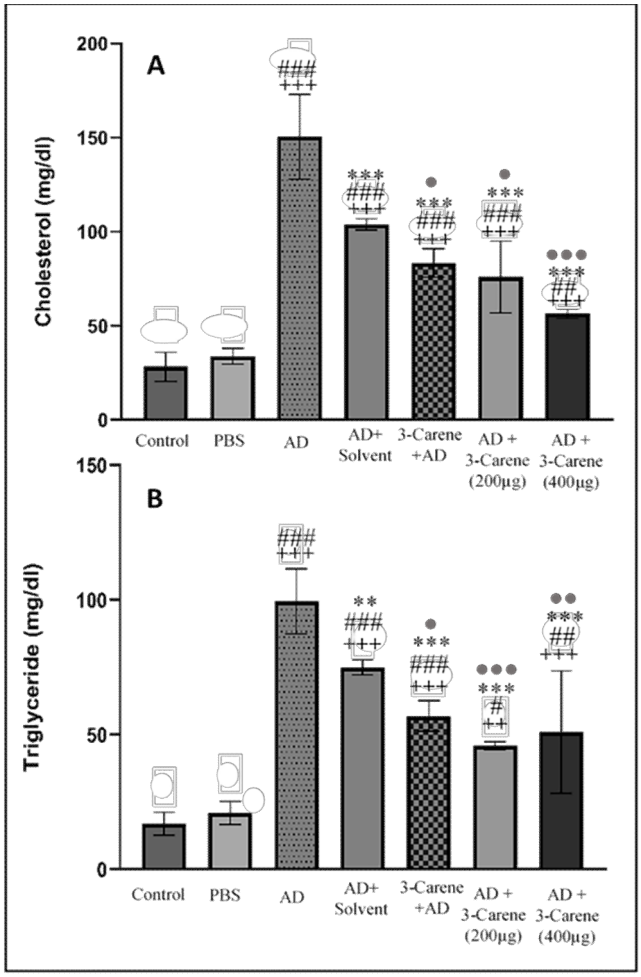

Fig. 3. Overall serum levels of cholesterol (A) and triglyceride (B) in different groups. Control: rats received only regular water and food; PBS: rats underwent surgery with PBS being injected into brain; AD: Aβ1–42 was injected into brain to induce Alzheimer's disease (AD); AD+solvent: Alzheimer's-induced group treated with coil oil as 3-carene solvent; 3-carene+AD: rats received 3-carene 400 µg/kg in a protective mode before induction of AD; AD+3-cerene 200 µg/kg: Alzheimer's-induced group treated with 200 µg/kg 3-carene; AD+3-carene 400 µg/kg: Alzheimer's-induced group treated with 400 µg/kg 3-carene. +++: $p < 0.001$ compared with the control group. ###: $p < 0.001$, ##: $p < 0.01$ and #: $p < 0.05$ compared with the PBS group. ***: $p < 0.001$ and **: $p < 0.01$ compared with the Alzheimer's disease (AD) group receiving β-amyloid. ●●●: $p < 0.001$, ●●: $p < 0.01$ and ●: $p < 0.05$ compared with the Alzheimer's disease (AD) group receiving corn oil as 3-carene solvent.

Effects of 3-Carene Treatment and Protection on Lipid Profiles

A few factors of lipid profile, including cholesterol, triglyceride, LDL, and HDL levels were evaluated. As shown in Fig. 3A, the level of cholesterol was significantly high in the disease-induced group, injected with β-amyloid, compared to the control group ($p < 0.001$). However, when the disease group received 3-Carene in both treatment and protective modes, the amount of cholesterol was significantly lowered ($p < 0.001$). In the AD-solvent group, the usage of corn oil improved the cholesterol level compared to the disease group ($p < 0.001$) [16]. However, the AD-induced groups receiving 3-Carene as both

therapeutic and protective modes demonstrated significant difference compared to the AD-solvent group receiving corn oil. The serum level of triglyceride was also considerably higher in the AD-induced group compared to the control group ($p < 0.001$). On the other hand, as compared to the disease group, triglyceride level was notably improved in the groups receiving 3-Carene in both treatment and protective modes ($p < 0.001$) (Fig. 3B). Interestingly, the usage of corn oil improved triglyceride level compared to the disease group ($p < 0.01$) [13]. However, the AD-induced groups receiving 3-Carene as both therapeutic and protective modes demonstrated significant difference compared to the AD-solvent group receiving corn oil.

Regarding the serum level of LDL, it was high in the disease-induced group compared to the control group ($p < 0.001$) while its level was notably lowered in the groups receiving 3-Carene in both treatment and protective modes compared to the AD-induced group ($p < 0.001$) (Fig. 4A). With respect to HDL, only the group treated with the highest dose of 3-Carene (200 µg/kg) showed the most increase in the HDL level compared to the disease-induced group ($p < 0.001$), in which the HDL level was notably lower than the control group ($p < 0.001$). The group receiving 3-Carene in a protective mode also demonstrated an increase in HDL level ($p < 0.001$) (Fig. 3B). The usage of corn oil improved the levels of HDL and LDL [13]. However, the AD-induced groups receiving 3-Carene as both therapeutic and protective modes demonstrated significant difference compared to the AD-solvent group receiving corn oil.

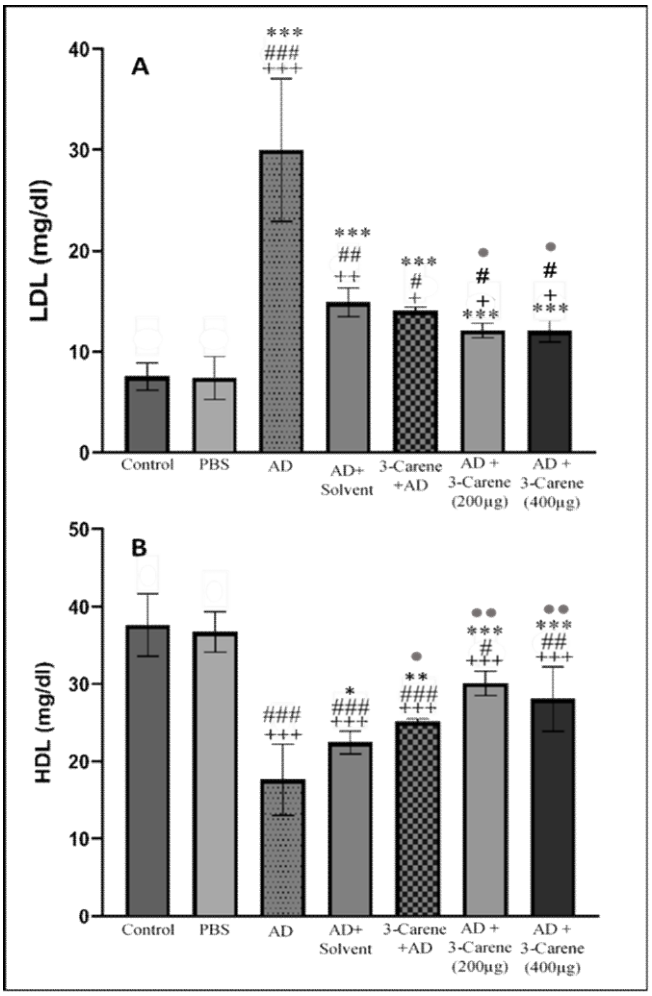

Fig. 4. Overall serum levels of LDL (A) and HDL (B) in different groups. Control: rats received only regular water and food; PBS: rats underwent surgery with PBS being injected into brain; AD: Aβ1–42 was injected into brain to induce Alzheimer's disease (AD); AD+solvent: Alzheimer's-induced group treated with coil oil as 3-carene solvent; 3-carene+AD: rats received 3-carene 400 µg/kg in a protective mode before induction of AD; AD+3-cerene 200 µg/kg: Alzheimer's-induced group treated with 200 µg/kg 3-carene; AD+3-carene 400 µg/kg: Alzheimer's-induced group treated with 400 µg/kg 3-carene. +++: $p < 0.001$, ++: $p < 0.01$, and +: $p < 0.05$ compared with the control group. ###: $p < 0.001$, ##: $p < 0.01$ and #: $p < 0.05$ compared with the PBS group. ***: $p < 0.001$, **: $p < 0.01$, and *: $p < 0.05$ compared with the Alzheimer's disease (AD) group receiving β-amyloid. ●●: $p < 0.01$ and ●: $p < 0.05$ compared with the Alzheimer's disease (AD) group receiving corn oil as 3-carene solvent.

Effects of 3-Carene Treatment and Protection on Biochemical Factors

The level of MDA and SOD activity were assessed before and after treatment with 3-Carene in blood serum of different groups. The AD-induced group demonstrated a notable reduction in the SOD activity compared to the control group ($p < 0.01$). As compared to the disease group, SOD activity was improved in the groups receiving 3-Carene in both treatment ($p < 0.001$) and protective ($p < 0.01$) modes (Fig. 5). On the other hand, serum level of MDA was increased in the disease-induced group compared with the control group ($p < 0.001$), while the MDA levels of the treated and protected groups significantly decreased

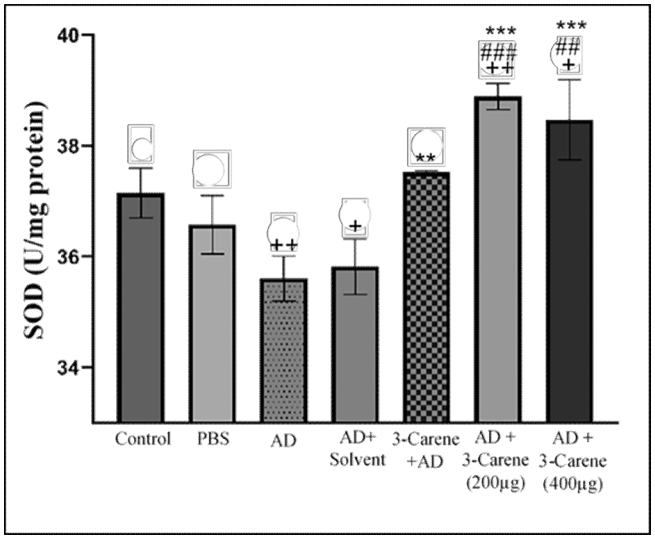

Fig. 5. Overall activity of SOD in the brain of different groups. Control: rats received only regular water and food; PBS: rats underwent surgery with PBS being injected into brain; AD: Aβ1–42 was injected into brain to induce Alzheimer's disease (AD); AD+solvent: Alzheimer's-induced group treated with coil oil as 3-carene solvent; 3-carene+AD: rats received 3-carene 400 µg/kg in a protective mode before induction of AD; AD+3-cerene 200 µg/kg: Alzheimer's-induced group treated with 200 µg/kg 3-carene; AD+3-carene 400 µg/kg: Alzheimer's-induced group treated with 400 µg/kg 3-carene. +++: $p < 0.001$, ++: $p < 0.01$, and +: $p < 0.05$ compared with the control group. ###: $p < 0.001$ and ##: $p < 0.01$ compared with the PBS group. ***: $p < 0.001$ and ** $p < 0.01$ compared with the Alzheimer's disease (AD) group receiving β-amyloid.

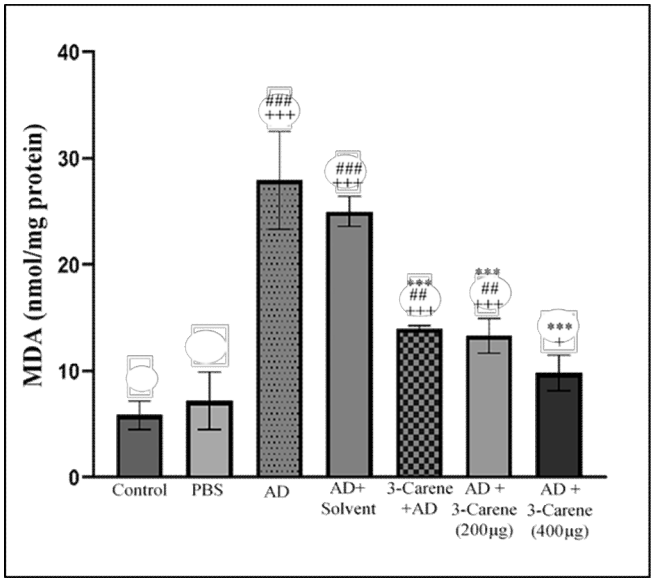

Fig. 6. Overall level of MDA in the brain of different groups. Control: rats received only regular water and food; PBS: rats underwent surgery with PBS being injected into brain; AD: Aβ1–42 was injected into brain to induce Alzheimer's disease (AD); AD+solvent: Alzheimer's-induced group treated with coil oil as 3-carene solvent; 3-carene+AD: rats received 3-carene 400 µg/kg in a protective mode before induction of AD; AD+3-cerene 200 µg/kg: Alzheimer's-induced group treated with 200 µg/kg 3-carene; AD+3-carene 400 µg/kg: Alzheimer's-induced group treated with 400 µg/kg 3-carene. +++: $p < 0.001$ and +: $p < 0.05$ compared with the control group. ###: $p < 0.001$ compared with the PBS group. ***: $p < 0.001$ compared with the Alzheimer's disease (AD) group receiving β-amyloid.

($p < 0.001$) compared to the Alzheimer's-induced group (Fig. 6).

Amyloid Plaque Formation

Upon intra-hippocampal injection of Aβ42, a high number of plaques formed in the brain tissue of disease-induced group compared with the control group ($p < 0.001$) (Figs. 7 and 8C). Amyloid plaques were investigated by Thioflavin S staining, which results into a fluorescence in amyloid plaques that could be distinguished as bright spots (Fig. 6). Consumption of 3-Carene in both treatment ($p < 0.01$) and protective ($p < 0.001$) modes caused a significant reduction in the plaque numbers compared to the AD-induced group (Figs. 7 and 8).

DICUSSION

Investigations have proved that Aβ aggregation plays an important role in the pathophysiology and the neurodegeneration process of AD [17] resulting in oxidative toxicity on neuronal cells [18,19]. Studies have shown that beta-amyloid increases neurons' apoptosis by increasing oxidative stress, as one of the principle events in Alzheimer's disease [19]. Alzheimer's disease has also

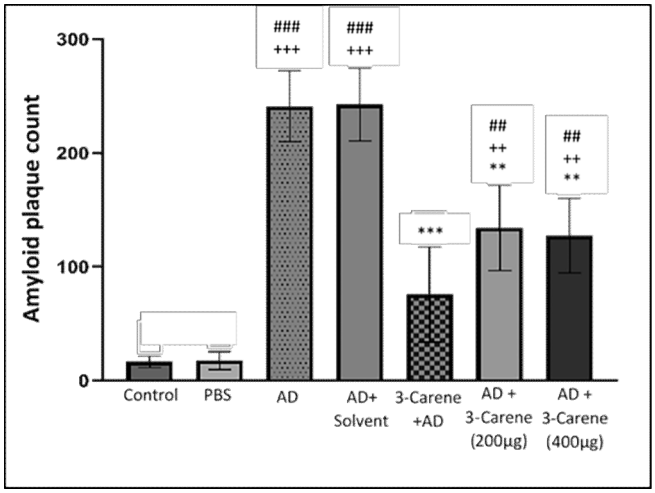

Fig. 7. Number of amyloid plaques in the hippocampus CA1 region in different groups. Control: rats received only regular water and food; PBS: rats underwent surgery with PBS being injected into brain; AD: Aβ1–42 was injected into brain to induce Alzheimer's disease (AD); AD+solvent: Alzheimer's-induced group treated with coil oil as 3-carene solvent; 3-carene+AD: rats received 3-carene 400 µg/kg in a protective mode before induction of AD; AD+3-cerene 200 µg/kg: Alzheimer's-induced group treated with 200 µg/kg 3-carene; AD+3-carene 400 µg/kg: Alzheimer's-induced group treated with 400 µg/kg 3-carene. +++: $p < 0.001$ and ++: $p < 0.01$ compared with the control group. ###: $p < 0.001$ and ##: $p < 0.01$ compared with the PBS group. ***: $p < 0.001$ and **: $p < 0.01$ compared with the Alzheimer's disease (AD) group receiving β-amyloid.

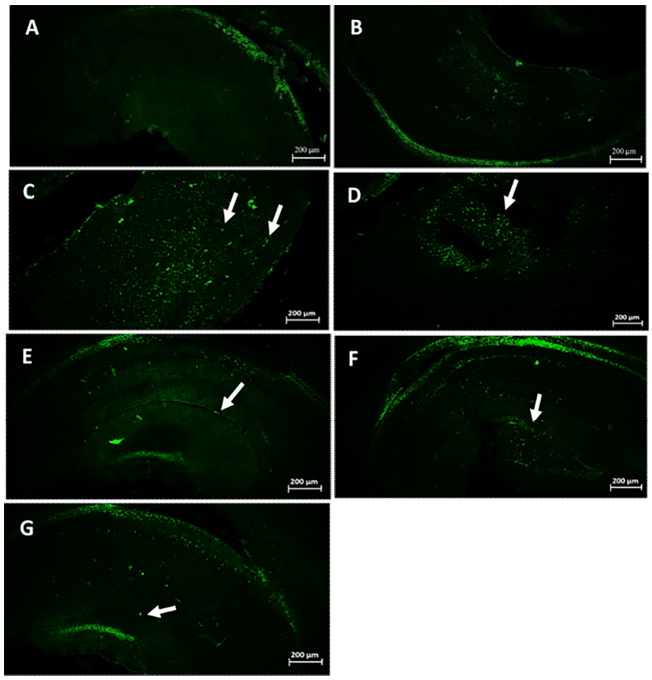

Fig. 8. Thioflavin S staining of amyloid plaques in the hippocampus CA1 region. A: (Control) rats received only regular water and food; B: (PBS) rats underwent surgery with PBS being injected into brain; C: (AD) Aβ1–42 was injected into brain to induce Alzheimer's disease (AD); D: (AD+solvent) Alzheimer's-induced group treated with coil oil as 3-carene solvent; E: (3-carene+AD) rats received 3-carene 400 µg/kg in a protective mode before induction of AD; F: (AD+3-cerene 200 µg/kg) Alzheimer's-induced group treated with 200 µg/kg 3-carene; G: (AD+3-carene 400 µg/kg) Alzheimer's-induced group treated with 400 µg/kg 3-carene.White arrows represent amyloid plaques in the tissue. Images are magnified at X100.

shown to disturb brain cells leading to memory, thinking, and behavior failures [20,21]. In agreement with the present study, it has been reported that Aβ41 injection in rat's hippocampus can lead to impaired learning and memory and amyloid plaque formation after one week [22]. Several studies have worked on the effects of small natural molecules and their anti-oxidative properties against Aβ fibril formation in AD [23,24]. 3-Carene is a bicyclic monoterpene and one of the major components of the pine tree essential oils. It has shown anti-inflammatory, antimicrobial, and anxiolytic effects [25]. In the present study, injection of Aβ42 into the rat's hippocampus also resulted in amyloid plaque formation, memory failure, and reduction of antioxidant strength. Applying 3-Carene to AD-induced rats, in both treated and protective modes, exhibited a notable reduction in the number of amyloid plaques leading to significant improvement of learning and memory impairment, proven by the histopathological assessment and behavioral test. Linalool, another similar monoterpene, has also shown improvement on cognitive functions [26] and on some antioxidant enzymes (SOD, GPX) as well as anti-inflammatory action by suppressing levels of pro-inflammatory proteins [27]. Limonene, another monoterpene has shown activity against neurodegeneration symptoms, namely memory impairment and hippocampal

damage. Limonene's probable mechanism of action has been stated based on its antioxidant and anti-inflammatory activities [28]. Seifi-Nahavandi *et al*. (2020) assessed the activity of p-cymene against Aβ formation in a rat model of AD. They showed that p-cymene, a monoterpene, could positively influence learning and memory functions (behavioral tests) and reduce amyloid plaque deposition [29]. Several other monoterpenes such as Thymol, Carvacrol, alpha-Pinene, and alpha-Terpenen have also exhibited memory and learning improvement by various mechanisms, including antioxidant and anti-inflammatory activities [30].

In the present study, the level of SOD was decreased in AD-induced rats. SOD activity is essential for inactivation of superoxide anions produced during oxidative stress in cells [31]. Abramov *et al.* reported that beta amyloid enters the nerve cells and produces oxygen free radicals [32]. In aging brain, the memory impairment is also thought to be due to an increased oxidative stress [33] causing the development and progression of Alzheimer's disease [34]. Both aged humans and rodents have exhibited cognitive impairment due to the accumulation of oxidative damage to major macromolecules [35]. There are endogenous systems, including antioxidant enzymes to protect cells from the adverse effects of free oxygen species [30]. In agreement with the present study, it has been shown that injection of intra-hippocampal Aβ also leads to reduced antioxidant activity [36]. Herein, 3-Carene demonstrated the ability of increasing SOD level and therefore could offer protection against Aβ-induced oxidative stress in the hippocampus. Linalool, a monoterpene, has also shown to reverse cognitive deficits [37,38] and has altered the level of the antioxidant (SOD) activity in mice injected with Aβ [37].

One way to directly measure the free radicals *in vivo* is to quantify the lipids reacting with the free radicals [39]. An increase in free radicals causes overproduction of malondialdehyde (MDA), the final products of polyunsaturated fatty acids peroxidation in the cells [40]. Therefore, MDA level is commonly known as a marker of oxidative stress [39]. In the present study, decreased activity of SOD was consistent with an increase in the level of MDA in the Alzheimer's-induced rats. Applying 3-Carene in both treated and protective modes exhibited a considerable increase in SOD activity leading to a notable decrease in MDA level. In accordance with our study, different studies have also demonstrated a great potential for other monoterpenes such as Oleuropein and Paeoniflorin to reduce MDA level in AD rat models [41].

In the present study, Lipid profile, including levels of TG, LDL, and cholesterol were elevated in the AD rats. Presečki *et al*. have reported that high serum levels of TG, cholesterol and LDL could be involved in the progress of AD [42]. The higher levels of LDL and lower levels of HDL have shown to be associated with brain amyloid [40]. A meta-analysis performed on patients with mild cognitive impairment has also revealed high TG and LDL-C [44]. Regarding the possible mechanism, it has been suggested that cholesterol may increase the activity of the β- or γ-secretase enzymes that generate Aβ from APP [45]. Another study has demonstrated that high levels of cholesterol could cause Aβ dissociation from the cell surface leading to Aβ aggregation in the extracellular space [46]. In accordance with our study regarding 3-Carene, Thymol and Carvacrol, as monoterpenes, demonstrated significant effects on serum levels of TG and cholesterol [47]. Interestingly, corn oil demonstrated the ability of improving the lipid profile. Corn oil contains the highest naturally occurring phytosterol levels of the refined vegetable oils, and is rich in polyunsaturated fatty acids [16]. Diets rich in unsaturated fatty acids have shown to reduce total cholesterol, LDL, and triglyceride and improve HDL levels [16]. However, the groups receiving 3-Carene in both treatment and protective modes demonstrated significant difference to the group receiving corn oil alone signifying the capability of 3-Carene in the prevention and treatment of AD-related parameters.

The therapeutic potential of monoterpenes in the treatment of AD is reported to be based on inhibition of Aβ-induced neurotoxicity, decreased tau-protein phosphorylation, promotion of Aβ clearance, attenuation of oxidative stress by boosting antioxidant defenses, and reduction of neuroinflammation [30]. Among possible mechanisms of actions for monoterpenes, the upregulation of the Aβ degrading enzymes such as Aβ proteases, LDL receptor-related protein 1, and the ApoE systems have been proposed [48,49]. Another established mechanism of monoterpene action is likely to be related to their anti-inflammatory effect. Monoterpenes suppress synthesis of

the key inflammatory mediators, including TNF-α, IL-1, COX, and NOS. They also regulate pro-inflammatory cytokines, such as NF-κB, thus playing a key role in the AD pathogenesis [50-52]. Monoterpenes and their derivatives constitute a very attractive treatment category for AD due to their multi-targeted mechanism of action on the central nervous system as well as antioxidant and anti-inflammatory properties [30]. Therefore, they can play an important role in the creation of new biologically active compounds including drugs.

CONCLUSION

It has been demonstrated that the intra-hippocampal injection of Aβ42 induced significant learning deficits, antioxidant impairment, and amyloid plaque formation. In this case, administration of 3-Carene, as a novel monoterpene, significantly improved long-term memory, amyloid plaque formation, and imbalances of antioxidant system and lipid profile associated with AD. Therefore, valuable therapeutic and protective potential for 3-Carene seems interesting enough to be further investigated.

ACKNOWLEDGEMENTS

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