Original Article

Effect of Linalool, Cineole, and β-Bourbonene Coupled with Aerobic Training on the Improvement of Presenilin-1/Amyloid Protein Precursor/Interleukin-1 beta/CASPASE 1 Network, Oxidative Capacity, and miRNA-210 in Mice with Alzheimer's Disease

Alimoradi, Z[1], Taghian, F[1*], Jalali Dehkordi, K[1]

1. *Department of Sports Physiology, Faculty of Sports Sciences, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran*

How to cite this article: Alimoradi Z, Taghian F, Jalali Dehkordi K. Effect of Linalool, Cineole, and β-Bourbonene Coupled with Aerobic Training on the Improvement of Presenilin-1/Amyloid Protein Precursor/Interleukin-1 beta/CASPASE 1 Network, Oxidative Capacity, and miRNA-210 in Mice with Alzheimer's Disease. Archives of Razi Institute Journal. 2024;79(3):629-638. DOI: 10.32592/ARI.2024.79.3.629

ABSTRACT

Alzheimer's is an advanced nervous disorder related to aging. The present study aimed to determine the effect of eight-week aerobic training, along with the consumption of Linalool, Cineole, and β-Bourbonene, on the prevention and improvement of Alzheimer's disease. Mice were randomly assigned to 8 groups: control group, mice induced with Alzheimer's disease treated with β-amyloid (Alzheimer group), Alzheimer's mice treated with bioactive compounds of herbal medicine (Linalool with a concentration of 25 mg/kg, Cineole with a concentration of 100 mg/kg, and β-Bourbonene with a concentration of 10 µg/ml) by gavage for 8 weeks (Alzheimer+Biocompounds group), Alzheimer's mice treated with aerobic exercise with a moderate intensity treadmill for 8 weeks (Alzheimer's+Training group), Alzheimer's mice treated with bioactive compounds of herbal medicine and aerobic exercise for 8 weeks (Alzheimer+Biocompounds+Training group), healthy mice initially treated with bioactive compounds of herbal medication (Linalool with a concentration of 25 mg/kg, Cineol with a concentration of 100 mg/kg, and β-Bourbonene with a concentration of 0.20 µg) by gavage for 8 weeks and then induced with Alzheimer's (Biocompounds+Alzheimer group), healthy mice initially treated with aerobic exercise using a treadmill with moderate intensity for 8 weeks and then induced with Alzheimer's disease (Training+Alzheimer group), and healthy mice initially treated with bioactive compounds of herbal medicine and aerobic exercise for 8 weeks and then induced with Alzheimer's disease (Biocompounds+Training+Alzheimer group). Compared to other groups, Interleukin-1 beta, CASPASE1, Presenilin-1, and amyloid protein precursor levels improved in mice initially treated with aerobic exercise and biocompounds. Oxidative capacity was improved by exercise training and bioactive compounds. In addition, exercise training and bioactive compounds regulated the miRNA-210 in the hippocampus of the mice with Alzheimer's. It can be concluded that the consumption of biocompounds and aerobic training can manage and prevent Alzheimer's.

Keywords: Aerobic exercise, Alzheimer's, Biocompounds, Herbal compounds

Article Info:
Received: 11 September 2023
Accepted: 13 November 2023
Published: 30 June 2024

Corresponding Author's E-Mail:
ft.taghian@gmail.com

1. Introduction

Various types of molecular and cellular damage accumulate and eventually disrupt the normal functioning of the body. Therefore, individuals' physical and mental abilities decline over time, making them more prone to diseases and reducing their life expectancy (1). Apart from the evident reduction in physical capabilities, aging is frequently concomitant with other crucial life modifications (2). According to studies, the decline in physical function related to aging is associated with the onset of chronic diseases. For decades, researchers in the field of aging have been monitoring different aging indicators to discover promising new approaches to enhance healthcare and elevate the quality of life throughout the lifespan. Alzheimer's disease has been recognized as a major global concern. According to the etiology reports of the National Health Service, it is estimated that Alzheimer's disease or other types of dementia affect 1 in 14 individuals over the age of 65 and 1 in every 6 individuals over 80 (3). A bulk of research is being conducted on molecular and biological markers of aging, including genomic instability, telomere shortening, lack of protein homeostasis, disruption of intercellular junctions, mitochondrial dysfunction, cellular degeneration, epigenetic changes, reduced nutrient sensing, and reduced age of stem cells. These markers aim to detect the onset of deterioration and death due to aging (4, 5). Reversing the symptoms of Alzheimer's disease can be achieved by synchronizing a combination of medication and lifestyle, helping to compensate signaling pathways and improve the quality of life (6). The overexpression of amyloid beta (Aβ) and the accumulation of Tau protein in the brain tissue of people over 65 are responsible for the manifestation of Alzheimer's disease, which is characterized by the build-up of extracellular senile plaques, as well as the development of mental disorders, dysphagia, apraxia, agnosia, and other disorders (7). A sedentary lifestyle and unhealthy diets are often associated with the manifestation of these signs and symptoms in most Alzheimer's patients (8, 9). Therefore, the analysis of biochemical and molecular markers of Alzheimer's disease is indispensable for observing aging and has great potential to generate new approaches and cures. Healthy aging is a strategic approach to preserving and enhancing mental and physical wellness across the lifespan and is increasingly recognized as an operative index of physical and mental health (6). Although chemical drugs are utilized to mitigate the complications of Alzheimer's disease, studies have demonstrated minimal effectiveness or adverse reactions (10). Consequently, the application of medicinal plants has been examined owing to their potential to deliver neuroprotection through inhibiting acetylcholinesterase enzyme or suppressing oxidative stress. Three bioactive compounds (Linalool, Cineole, and β-Bourbonene**)** with anti-inflammatory and antioxidant effects are used as preventive agents. They significantly reduce the levels of extracellular Aβ, tauopathy, astrogliosis, microgliosis, and pro-inflammatory markers (11, 12). Our goal in this bioinformatic-experimental analysis was to identify genes involved in Alzheimer's pathogenesis as genetic markers that show significant expression changes between Alzheimer's patients and healthy individuals. Moreover, this study investigated the gene network effectiveness in the treatment of genes with the ability to design drugs. Therefore, according to the role of exercise activity and herbal medicines, the present study aimed to evaluate the effect of 8-week intermittent exercise along with the consumption of three bioactive compounds, namely Linalool, Cineol, and β-Bourbonene, on the prevention and recovery of mouse models of Alzheimer's disease.

2. Materials and Methods

This research was approved by the Ethics Committee of the Islamic Azad University of Isfahan branch (Khorasgan) in compliance with all the protocols for keeping and working with laboratory animals (IR.IAU.KHUISF.REC.1401.010). In this experimental study, 48 C57BL/6 female mice in the weight range of 20-22 g and an average age of 15 weeks were purchased and transferred to the laboratory. C57BL/6 mice were kept under a standard 12/12-h light/dark cycle in temperature-controlled cages (22°C-24°C) with a relative humidity of 65±5%. In addition, the animals were fed with a standard diet. It is worth noting that the purpose of this study was to investigate the performance of aerobic exercise and the consumption of bioactive compounds of herbal medicine (Linalool, Cineol, and β-Bourbonene) by gavage for 8 weeks before and after the mice were infected with Alzheimer's disease. After a week of acclimatization to the animal nest environment, mice were randomly assigned to the following 8 groups (5 mice in each group):

1. Control group;

2. Mice induced with Alzheimer's disease and treated with Aβ (Alzheimer group);

3. Alzheimer's mice treated with bioactive compounds of herbal medicine (Linalool with a concentration of 25 mg/kg, Cineole with a concentration of 100 mg/kg, and β-Bourbonene with a concentration of 10 µg/ml) by gavage for 8 weeks (Alzheimer+Biocompounds group);

4. Alzheimer's mice treated with aerobic exercise with a moderate intensity treadmill for 8 weeks (Alzheimer's+Training group);

5. Alzheimer's mice treated with bioactive compounds of herbal medicine and aerobic exercise for 8 weeks (Alzheimer+Biocompounds+Training group);

6. Healthy mice initially treated with the bioactive compounds of the herbal medicine (Linalool with a concentration of 25 mg/kg, Cineol with a concentration of 100 mg/kg, and β-Bourbonene with a concentration of 0.20 µg by gavage for 8 weeks) and then induced with Alzheimer's (Biocompounds+Alzheimer group);

7. Healthy mice initially treated with aerobic exercise using a treadmill with moderate intensity for 8 weeks and then induced with Alzheimer's disease (Training+Alzheimer group); and

8. Healthy mice initially treated with the bioactive compounds of the herbal medicine and aerobic exercise for 8 weeks and then induced with Alzheimer's disease (Biocompounds+Training+Alzheimer group). This study mainly focused on management and treatment. Therefore, for the treatment goal, mice were induced with Alzheimer's disease, treated with aerobic exercise and consumption of bioactive compounds of herbal medicine (Linalool, Cineole, and β-Bourbonene) for 8 weeks, and were then induced with Alzheimer's disease. Finally, 48 h after the last training and treatment session, the mice were anesthetized with chemical drugs (Ketamine & Xylazine), and the brain tissue was isolated and stored at -80°C for further experiments.

2.1. Alzheimer's induction

Aβ1-42 oligomer was purchased from Sigma-Aldrich. In brief, synthetic Aβ1-42 (Sigma-Aldrich, USA) was dissolved in cold hexafluoroisopropanol (HFIP) (Sigma-Aldrich, USA) for 20 min, and the solution was then vortexed for 10 min to form Aβ1-monomers. Aβ1-42 monomers were vacuum spun, precipitated, and finally dissolved in a 10% HFIP solution. The Aβ1-42 solution was centrifuged for 20 min at 4°C after incubation for 48 h under constant stirring at room temperature. The supernatants were separated and transferred to pre-chilled tubes. A volume of 50 µM of soluble Aβ1-42 oligomers was collected after complete evaporation of HFIP. Aβ1-42 oligomer solution was stored at 4°C until use (13, 14). Female C57BL/6 mice were generally anesthetized by intraperitoneal administration of sodium pentobarbital 0.2% (50 mg/kg) and then placed in a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). Aβ1-42 oligomer at a concentration of 50 µM or 0.9% sterile saline was injected at a concentration of 1 mM (1 µL per side, at a rate of 0.2 µL/min) into the bilateral dentate gyrus region of the dorsal hippocampus of C57BL/6 mice (13).

2.2. Aerobic exercise protocol

Aerobic exercises were performed using a mouse treadmill. Moderate exercise with a treadmill was started by walking slowly for 5 min at a speed of 7 m/min. Subsequently, the speed was increased every 3 min to reach moderate intensity. The speed started at 7-10 m/min in the first week and rose to 10-15 m/min for 45 min by the eighth week (15).

2.3. Preparation of bioactive compounds Linalool, Cineol, and β-Bourbonene

Linalool and Cineol compounds were purchased from Sigma-Aldrich (USA) and β-Bourbonene from J&H Produc (USA). The safe dose used for each compound was determined based on previous studies; therefore, Linalool was administered at a concentration of 25 mg/kg (11), Cineol at a concentration of 100 mg/kg (16), and β-Bourbonene at a concentration of 0.20 µg/capita/day.

2.4. Bioinformatics data mining

In this study, different bioinformatics algorithms and protein-protein interaction networks were used to identify the most important genes involved in Alzheimer's pathogenesis. To this end, we used the DisGeNET database to obtain a list of genes related to Alzheimer's (C0002395) with a score of gda > 0.01. The Protein-Protein Interaction (PPI) network was drawn in the STRING 11.0 database, and network analysis was performed based on degree = 40, betweenness centrality = 0.005, and closeness centrality = 0.5 parameters. Molecular and key pathways related to genes were identified based on a data mining algorithm.

2.5. RNA extraction and cDNA synthesis

RNAs were extracted from the brain tissue according to the RNA extraction kit protocol (Parstous, Iran). It is worth noting that we used a fresh brain, and the tissue was separated immediately after sacrifice. A nanodrop spectrophotometer evaluated the concentration and purity of the mRNA sample at 260/280 absorbance (Thermo Scientific, USA). In addition, cDNA synthesis was conducted based on the protocol. Moreover, the cDNA was synthesized according to reverse transcription polymerase chain reaction (RT-PCR). mRNA expression was evaluated using the quantitative RT-PCR method (CYBR Green TaKaRa, Japan). Gene expression analysis was performed based on the 2-ΔΔCT method. The reference gene in this study was 18 S ribosomal RNA. In addition, the primers were purchased from Macrogen (South Korea). The sequences of the genes are presented in table 1.

2.6. Data analysis method

The data were analyzed in GraphPad Prism software for descriptive and inferential statistics. The Shapiro-Wilk test was performed to homogenize the distribution. In addition, data were calculated using a one-way analysis of variance (ANOVA) with Tukey's post hoc test. Descriptive statistics were described as mean ± standard deviation (SD). The level of statistical significance in all analyses was set at $P < 0.05$.

3. Results

3.1. Bioinformatic Analysis

Among the key genes in the pathogenesis of Alzheimer's disease, 58 nodes as hub genes were obtained based on bioinformatics data analysis. Differential expression of interleukin-1 beta (IL-1β), CASPASE1 (*CASP1*), amyloid protein precursor (*APP*), presenilin-1 (*PSEN1*) genes, as well as the concentration levels of catalase (CAT) and glutathione peroxidase 4 (GPx4), were selected for investigation in the laboratory. Hub genes in the protein network were identified as proteins with the ability to design potential drugs in aging and Alzheimer's management through in-silico methods (Figure 1a).

3.2. Regulation of *PSEN1/APP* by exercise training and bioactive compounds

The level of the *PSEN1* significantly reduced, while the *APP* significantly enhanced in mice with Alzheimer's treated with Aβ (Alzheimer group) compared to the control group (Figures 2a and b). Previous studies have indicated that the expression level of the *PSEN1* and *APP* significantly decreased and amplified in mice with

Alzheimer's treated (by exercise training and bioactive compounds) (Figures 2a and b). Moreover, aerobic training and the consumption of the biocompounds significantly regulated the expression level of PSEN1/APP in the Alzheimer's mice treated with bioactive compounds of herbal medicine (Alzheimer+Biocompounds group) and Alzheimer's mice treated with aerobic exercise (Alzheimer's+Training group) with moderate intensity for 8 weeks (Figures 2a and b). In Alzheimer's mice receiving treatment intervention with bioactive compounds of herbal medicine along with aerobic exercise for 8 weeks (Alzheimer+Biocompounds+Training group), the expression level of the *APP* increased, whereas that of decreased *PSEN1* decreased (Figures 2a and b).

On the other hand, healthy mice initially treated with the bioactive compounds of the herbal medicine and induced with Alzheimer's disease (Biocompounds+Alzheimer group), as well as the healthy mice treated with aerobic exercise and then induced with Alzheimer's disease (Training+Alzheimer group) exhibited a significant enhancement in the expression level of the *PSEN1* and a reduction in the expression level of the *APP* (Figures 2a and b). We found a synergetic effect in the Biocompounds+Training+Alzheimer group. Therefore, the level of *PSEN1* significantly amplified and that of *APP* decreased in the Biocompounds+Training+Alzheimer group, compared to the other groups (Figures 2a and b).

Table 1. Primer list

Gene	Forward primer (5`-3`)	Reverse primer (5`-3`)	Annealing temperature (°C)	Accession Number
IL1β	TGGACCTTCCAGGATGAGGACA	GTTCATCTCGGAGCCTGTAGTG	60	NM_008361
CASPASE 1	GGCACATTTCCAGGACTGACTG	GCAAGACGTGTACGAGTGGTTG	60	NM_009807
APP	TCCGTGTGATCTACGAGCGCAT	GCCAAGACATCGTCGGAGTAGT	60	NM_007471
PSEN1	GAGACTGGAACACAACCATAGCC	AGAACACGAGCCCGAAGGTGAT	58	NM_008943
miRNA-210	TGCGTGTGACAGCGGCT	GAACATGTCTGCGTATCTC	60	MI0000695
18s rRNA	CGGACACGGACAGGATTG	TCGCTCCACCAACTAAGAAC	59	NR_003278.1

Figure 1

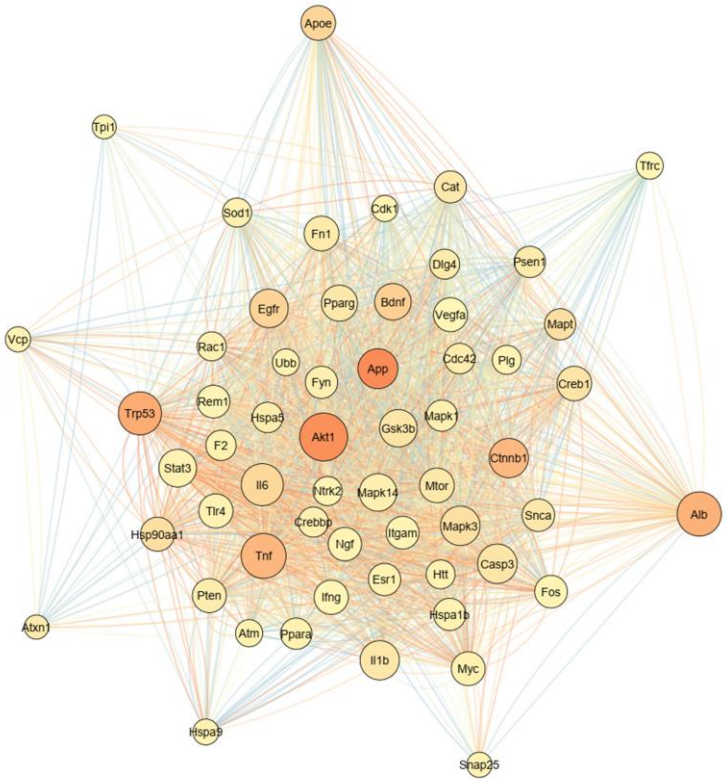

Figure 1: Protein-protein interactions network involved in pathomechanism of Alzheimer's.

Figure 2

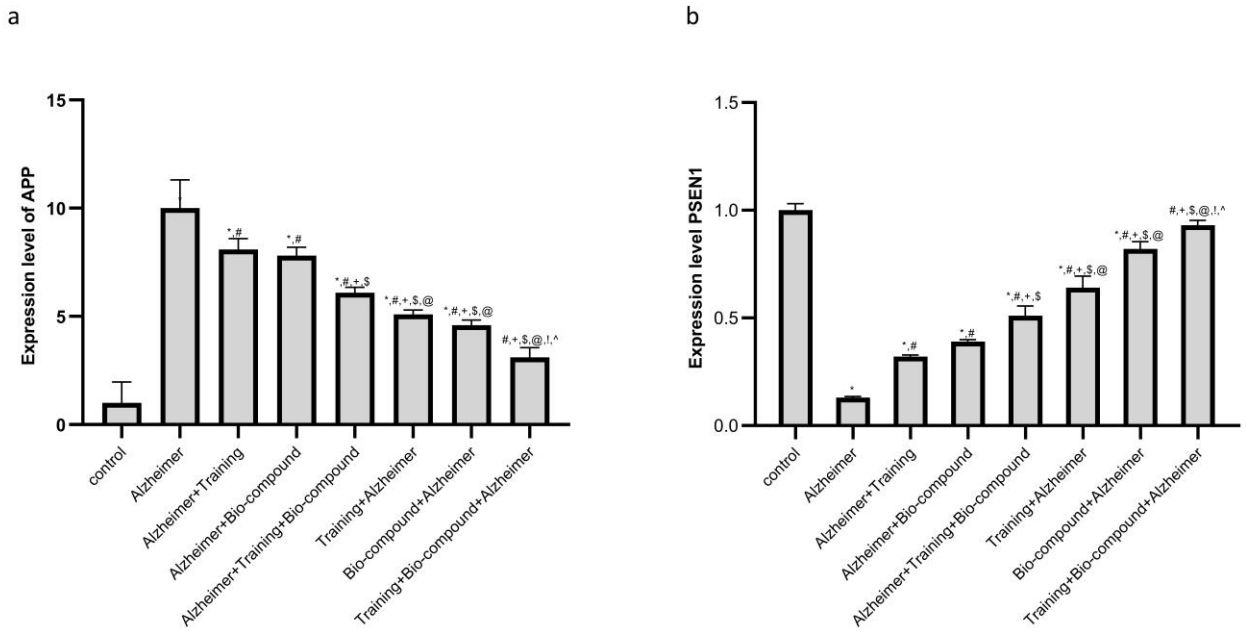

Figure 2: (a and b) APP and PSEN1 were regulated by exercise training and bioactive compounds in the mice involved with Alzheimer.

3.3. Improvement of the *IL1β/CASPASE1* networks by exercise training and bioactive compounds

The expression level of the *IL1β/CASPASE 1* was significantly increased in the Alzheimer's group, in comparison to the control group (Figures 3a and b). Moreover, the relative expression of the *IL1β/CASPASE1* was regulated in the Alzheimer's mice treated with bioactive compounds of herbal medicine (Alzheimer+Biocompounds group) and Alzheimer's mice treated with aerobic exercise (Alzheimer's+Training group) (Figures 3a and b). Therefore, these results demonstrated that exercise training, as well as the consumption of Linalool, Cineol, and β-Bourbonene, improved the inflammation pathway (Figures 3a and b). Based on our data, the expression level of the *IL1β/CASP1* was reduced in the Biocompounds+Alzheimer and Training+Alzheimer groups, compared to the other groups (Figures 3a and b). In addition, the level of the *IL1β/CASPASE 1* was significantly decreased in the Biocompounds+Training+Alzheimer group, in comparison to the other groups (Figures 3a and b).

3.4. Improvement of oxidative capacity by exercise training and bioactive compounds

The concentration of CAT and GPx was reduced in the Alzheimer's mice treated with Aβ (Alzheimer group), compared to the control group (Figures 4a and b). Moreover, the bioactive compounds of herbal medicine (Alzheimer+Biocompounds group) and aerobic exercise (Alzheimer+Training group) reduced the concentrations of the CAT and GPx in the Alzheimer's mice (Figures 4a and b). It was found that when the healthy mice were initially treated with the bioactive compounds of the herbal medicine (Biocompounds+Alzheimer group) and aerobic exercise (Training+Alzheimer group), the concentrations of the CAT and GPx significantly increased in comparison with the other groups (Figures 4a and b).

3.5. Regulation of the miRNA-210 by exercise training and bioactive compounds in the hippocampus of mice with Alzheimer's

The data mining and literature review revealed that miRNA-210 could be a potential target for Alzheimer's conditions. These studies have indicated that miRNA-210 might be considered a diagnostic and therapeutic marker in Alzheimer's disease (17, 18). Moreover, evidence has demonstrated that miRNA-210 could contribute to cognitive and neurogenesis impairment. Therefore, based on these data, we selected miRNA-210 to assess its relative expression in mice with Alzheimer's (17, 19). miRNA-210 expression was elevated in Alzheimer's condition in comparison with the control group (Figure 5a).

Figure 3

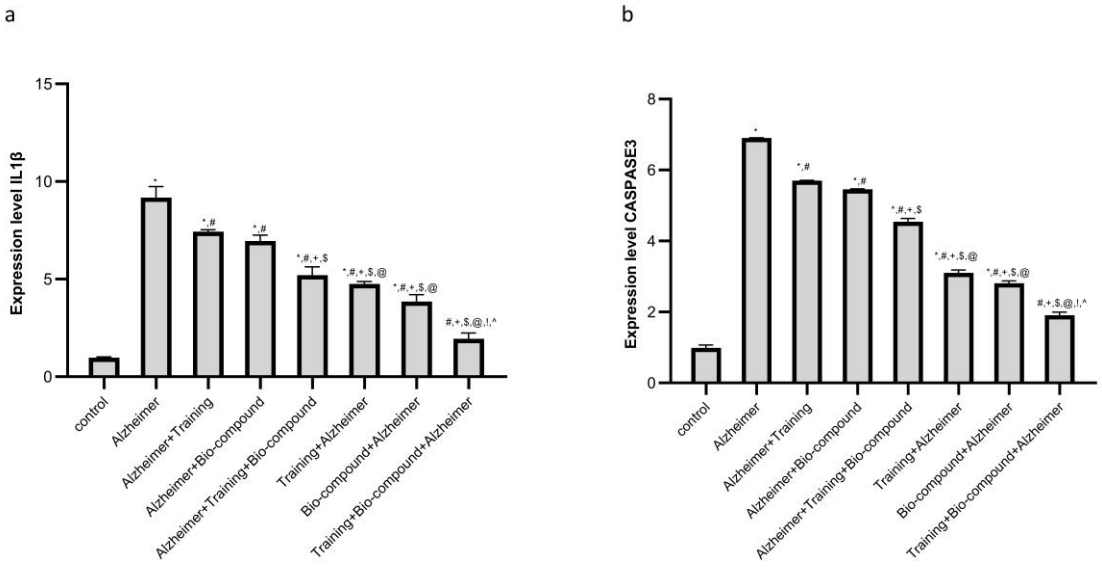

Figure 3: (a and b) The expression level of the IL1β and CASPASE1 in the mice involved with Alzheimer treated with exercise training and bioactive compounds.

Figure 4

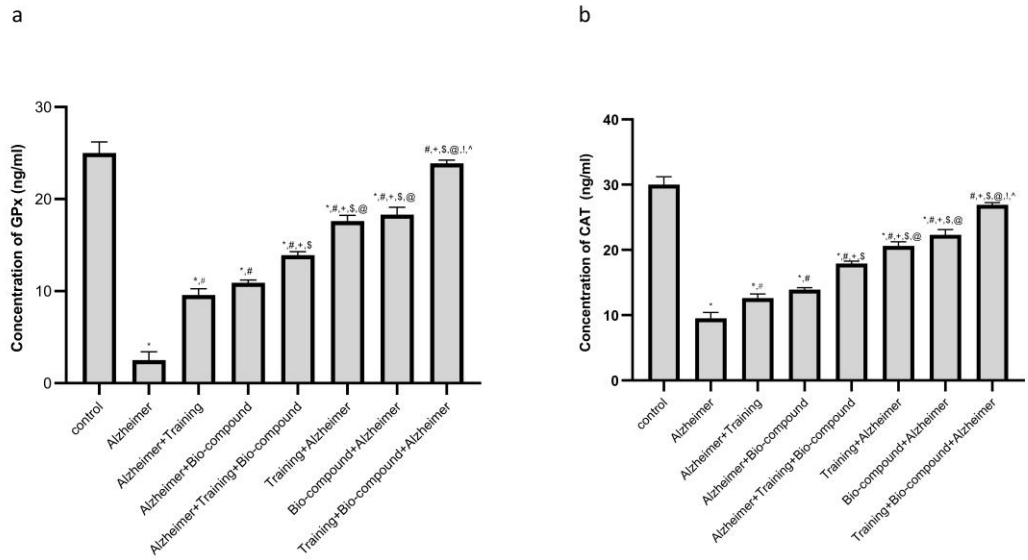

Figure 4: (a and b) The alternation of GPx and SOD concentration in the mice involved with Alzheimer treated with exercise training and bioactive compounds.

Figure 5

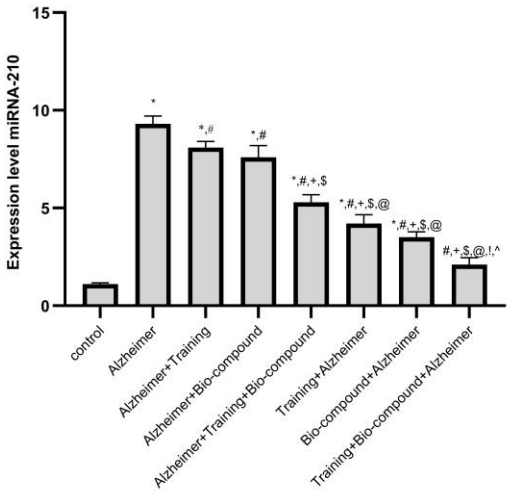

Figure 5: The expression level of the miRNA-210 was improved by aerobic exercise and bioactive compounds in the mice involved with Alzheimer.

It was also revealed that the expression level of the miRNA-210 was decreased by the consumption of the bioactive compounds (Alzheimer+Biocompounds group) and aerobic exercise with moderate intensity (Alzheimer's+Training group) for 8 weeks in mice with Alzheimer's (Figure 5a). In addition, our results indicated that the miRNA-210 level regulated in healthy mice initially treated with the bioactive compounds for 8 weeks and then induced with Alzheimer's disease (Biocompounds+Alzheimer group) (Figure 5a). Moreover, a significant decrease was observed in the relative expression of the miRNA-210 in healthy mice initially treated with eight-week moderate-intensity aerobic exercise and then induced with Alzheimer's disease (Training+Alzheimer group) (Figure 5a). Healthy mice initially treated with the bioactive compounds and aerobic exercise for 8 weeks and then induced with Alzheimer's disease (Biocompounds+Training+Alzheimer group) exhibited a significant decline in the expression level of the miRNA-210, compared to the other groups (Figure 5a).

4. Discussion

As evidenced by the results of this study, the relative expression of the *IL1β/CASPASE 1* was improved in the mice initially treated with aerobic exercise and biocompounds. The oxidative capacity was improved by exercise training and bioactive compounds. In addition, exercise training and bioactive compounds regulated the miRNA-210 in the hippocampus of mice with Alzheimer's. Moreover, based on our data, the expression level of the *PSEN1/APP* significantly decreased in mice initially treated with aerobic exercise and biocompounds compared to that in other groups. In the present study, bioinformatic analysis highlighted the proteins involved in Alzheimer's disease by considering the highest visualization parameters in the PPI network. Moreover, the review of traditional herbal medicines as complementary and alternative medicine to improve dementia led to the creation of a library of bioactive compounds. Our analysis suggested that effective compounds derived from plants and plant ligands, including Linalool, Cineole, and β-Bourbonene, could be effective in the prevention and treatment of dementia. This study assessed the simultaneous effect of aerobic exercise and the oral consumption of bioactive compounds of herbal medicine (Linalool with a concentration of 5.2 mg/kg, Cineole with a concentration of 100 mg/kg, and β-Bourbonene with a concentration of 72 mg/kg) before and after Alzheimer's disease. This study aimed to gain a deeper understanding of the mechanisms of exercise and bioactive compounds in mitigating the incidence and symptoms of Alzheimer's disease. In a bioinformatics study by Hajibabaie et al., a medicinal cocktail, including effective compounds derived from garlic, black pepper, sage, black cumin, black seed, fig, gooseberry, fenugreek, citrus, *Tetradium*, ginger, berries, red grapes, onions, green tea, apples, fruits, and vegetables, was introduced as an effective compound that potentially modulated hub proteins and molecular signaling pathways in Alzheimer's. Moreover, they investigated the genomics dataset to determine the efficacy of physical activity in old age and Alzheimer's conditions. They demonstrated that high-intensity physical activity, compared to inactivity, could significantly regulate the expression level of hippocampal genes and make them approach normal conditions (6). Therefore, it can be concluded that physical

activity in Alzheimer's patients can be considered a complementary treatment approach. The results of the present study pinpointed that regular physical activity before the induction of Alzheimer's could exert protective physiological effects on the brain and memory. Furthermore, the obtained findings demonstrated that aerobic exercise after Alzheimer's disease in female mice could reduce the effects and symptoms of Alzheimer's and modulate the expression of hub genes involved in Alzheimer's pathogenesis. The presence of extracellular amyloid plaques and intracellular tau-containing neurofibrillary tangles upon histopathological evaluation is a hallmark of Alzheimer's disease, leading to the loss of hippocampal neurons and synaptic degeneration. Amyloidogenic Aβ peptide (38 to 43 amino acids) is generated through the sequential cleavage of amyloid precursor protein (APP) by β- and γ-secretase enzymes; however, Aβ formation can be prevented by APP cleavage with α-secretase, followed by γ-secretase (20, 21). miRNAs play a crucial role in regulating the expression of key genes involved in Alzheimer's disease pathogenesis, including β-site APP-cleaving enzyme 1, APP, tau, presenilin, and brain-derived neurotrophic factor (20-22). The regulation of complex functions in neurodevelopment, neuroplasticity, and cognitive processing is significantly affected by microRNAs through their post-transcriptional control over eukaryotic genomes (23, 24). Alzheimer's Disease has been shown to dysregulate miR-210, a highly conserved, hypoxia-regulated miRNA. According to a recent study by Watts et al., the human transcriptome indicated a marked increase in neurodegenerative diseases among miR-210 targets. To provide additional insight into the role of miR-210 in neuronal function and cognitive behavior, Watts et al. utilized conditional miR-210 knockout mice to characterize miR-210 regulation and function in primary hippocampal neurons and assessed visual discrimination and reversal learning via rodent touchscreen assays. Neuronal activation was found to induce miR-210 expression, and the absence of neuronal miR-210 *in vitro* caused an increase in oxidative metabolism, reactive oxygen species (ROS) production, dendritic density, and branching. Moreover, the absence of miR-210 in knockout mice resulted in improved behavioral flexibility and decreased perseverative responses during reversal learning, necessitating feedback and information updating. The data support that miR-210 has a conserved and activity-dependent function in cognitive processes through the modulation of neuronal metabolism (25). As supported by multiple pieces of evidence, synaptic function failure is believed to occur at the preclinical stages of Alzheimer's disease before the neuronal loss and the classical pathological hallmarks of Alzheimer's disease. In the modern era, it is of utmost importance to distinguish credible biomarkers that can be acquired through non-invasive approaches to enhance the diagnosis of Alzheimer's disease during its initial stages. In a cohort study consisting of cognitive healthy controls, mild cognitive impairment, and Alzheimer's disease subjects, Siedlecki-Wullich et al. examined plasma levels of a group of miRNAs related to synaptic proteins. An analysis of plasma and brain levels of miRNAs was performed on two separate cohorts, comprising 38 healthy controls, 26 individuals with mild cognitive impairment, 56 patients with Alzheimer's disease dementia, and 27 cases with frontotemporal dementia. The normality of the data was evaluated using the Shapiro-Wilk and D'Agostino-Pearson tests. A two-sided nonparametric Mann-Whitney test was conducted to compare the miRNA levels between the groups, whereas the receiver operating characteristic curve analysis was utilized to determine the sensitivity and specificity. A significant upregulation was observed in the plasma of mild cognitive impairment and Alzheimer's disease subjects for miR-92a-3p, miR-181c-5p, and miR-210-3p. Elevated levels of these miRNAs were detected in the plasma of mild cognitive impairment patients who later progressed to Alzheimer's disease. On the contrary, plasma samples taken from a cohort of frontotemporal dementia showed no variations in the levels of miR-92a-3p, miR-181c-5p, or miR-210-3p. The results of the study carried out by Siedlecki-Wullich indicated that the molecular signatures of plasma miR-92a-3p, miR-181c-5p, and miR-210-3p were unique and had the potential to serve as a biomarker for Alzheimer's disease (26). Recent research has pointed to the effect of 1,8-cineole pretreatment on 25-35 Aβ-induced inflammation in differentiated PC12 cells. In their study, Khan et al. treated cells with different doses of 1,8-cineole for 24 h and then replaced them with media containing 25-35 Aβ for another 24 h (27). Cell viability was reduced in cells treated with 25-35 Aβ and significantly restored by Cineole pretreatment. Cineol successfully reduced mitochondrial membrane potential, ROS, and nitric oxide levels in the cells treated with 25-35 Aβ. Cineol also reduced the levels of pro-inflammatory cytokines, IL-1β, tumor necrosis factor alpha, and IL-6 in cells treated with 25-35 Aβ. It also succeeded in reducing the expression of Cyclooxygenase-2 (COX-2), Nitric oxide synthase-2 (NOS2), and Nuclear factor kappa B. This study demonstrated the protective effects of cineole on inflammation and provided further evidence for its potential therapeutic use as an anti-inflammatory agent in neurological diseases (29). On the other hand, Sabogal et al. assessed the effects of oral administration of Linalool (25 mg/kg) every 48 h for 3 months in aged mice (21-24 months) and a triple transgenic model of mice with Alzheimer's (11). Alzheimer's triple transgenic mice treated with linalool showed more significant spatial learning, memory, and risk assessment behaviors during the elevated plus maze. The hippocampus and amygdala of triple transgenic mice treated with linalool exhibited a significant decrease in extracellular β-amyloidosis, tauopathy, astrogliosis, and microgliosis, as well as a significant decrease in the level of inflammatory markers P38 MAPK, NOS2, COX2, and *IL-1β*. Their findings suggested that linalool reversed the histological signs of Alzheimer's disease and restored cognitive and emotional functions through an anti-inflammatory effect. Therefore, linalool may be a candidate for the prevention of Alzheimer's disease in clinical studies (11). This study identified novel candidate proteins for Alzheimer's prognosis,

diagnosis, and therapeutic approaches based on bioinformatics analysis and *in vitro* studies. Among the notable limitations of this study, we can refer to the failure to evaluate the gain-of-function and lost-of-function in miRNA-210 in Alzheimer's conditions. In addition, different doses of Linalool, Cineole, and β-Bourbonene were not compared in this study. Furthermore, differences in intensity, duration, and repetition of aerobic exercise training were not evaluated. A new analysis of results examined the effects of complementary and alternative medicines, such as moderate-intensity aerobic exercise, and presented effective compounds derived from herbal medicines as a therapeutic cocktail. Based on the obtained results, moderate and high-intensity aerobic physical activities and the consumption of effective compounds derived from herbal medicines can be suggested as a new complementary and alternative medicine effective on memory function and mitigation of Alzheimer's symptoms and the aging process. On the other hand, differential expression of hub genes is recommended as new biomarkers for tracking and monitoring aging.

Acknowledgment
The authors would like to thank their colleagues for their association and helpful discussions in this study.

Authors' Contribution
Z.A., F.T., and K.J.D. performed the research study. Z.A., and F.T. conducted the data analysis. Z.A. and F.T. wrote the manuscript.

Ethics
This research was approved by the Ethics Committee of the Islamic Azad University of Isfahan branch (Khorasgan) in compliance with all the protocols for keeping and working with laboratory animals (IR.IAU.KHUISF.REC.1401.010).

Conflict of Interest
None of the authors has any conflicts of interest to disclose.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

References

1. Ogrodnik M, Salmonowicz H, Gladyshev VN. Integrating cellular senescence with the concept of damage accumulation in aging: Relevance for clearance of senescent cells. Aging cell. 2019;18(1):e12841.
2. Foreman J. Exercise is Medicine: How Physical Activity Boosts Health and Slows Aging: Oxford University Press; 2019.
3. Sommerlad A, Perera G, Singh-Manoux A, Lewis G, Stewart R, Livingston G. Accuracy of general hospital dementia diagnoses in England: sensitivity, specificity, and predictors of diagnostic accuracy 2008–2016. Alzheimer's & Dementia. 2018;14(7):933-43.
4. Anand R, Gill KD, Mahdi AA. Therapeutics of Alzheimer's disease: Past, present and future. Neuropharmacology. 2014;76:27-50.
5. Knopman DS, Amieva H, Petersen RC, Chételat G, Holtzman DM, Hyman BT, et al. Alzheimer disease. Nature reviews Disease primers. 2021;7(1):33.
6. Hajibabaie F, Abedpoor N, Taghian F, Safavi K. A cocktail of polyherbal bioactive compounds and regular mobility training as senolytic approaches in age-dependent alzheimer's: the in silico analysis, lifestyle intervention in old age. Journal of Molecular Neuroscience. 2023;73(2-3):171-84.
7. Liang Y-J, Su Q-W, Sheng Z-R, Weng Q-Y, Niu Y-F, Zhou H-D, et al. Effectiveness of physical activity interventions on cognition, neuropsychiatric symptoms, and quality of life of Alzheimer's disease: An update of a systematic review and meta-analysis. Frontiers in aging neuroscience. 2022;14:155.
8. Kivimäki M, Singh-Manoux A, Pentti J, Sabia S, Nyberg ST, Alfredsson L, et al. Physical inactivity, cardiometabolic disease, and risk of dementia: an individual-participant meta-analysis. bmj. 2019;365.
9. Lubitz I, Ricny J, Atrakchi-Baranes D, Shemesh C, Kravitz E, Liraz-Zaltsman S, et al. High dietary advanced glycation end products are associated with poorer spatial learning and accelerated Aβ deposition in an Alzheimer mouse model. Aging Cell. 2016;15(2):309-16.
10. Delagarza VW. Pharmacologic treatment of Alzheimer's disease: an update. American Family Physician. 2003;68(7):1365-73.
11. Sabogal-Guáqueta AM, Osorio E, Cardona-Gómez GP. Linalool reverses neuropathological and behavioral impairments in old triple transgenic Alzheimer's mice. Neuropharmacology. 2016;102:111-20.
12. Hajibabaie F, Abedpoor N, Javanmard SH, Hasan A, Sharifi M, Rahimmanesh I, et al. The molecular perspective on the development of melanoma and genome engineering of T-cells in targeting therapy. Environmental Research. 2023:116980.
13. Li W-y, Gao J-y, Lin S-Y, Pan S-t, Xiao B, Ma Y-t, et al. Effects of involuntary and voluntary exercise in combination with Acousto-optic stimulation on adult neurogenesis in an Alzheimer's mouse model. Molecular Neurobiology. 2022;59(5):3254-79.
14. Xiang S, Liu F, Lin J, Chen H, Huang C, Chen L, et al. Fucoxanthin inhibits β-amyloid assembly and attenuates β-amyloid oligomer-induced cognitive impairments. Journal of agricultural and food chemistry. 2017;65(20):4092-102.
15. Abedpoor N, Taghian F, Ghaedi K, Niktab I, Safaeinejad Z, Rabiee F, et al. PPARγ/Pgc-1α-Fndc5 pathway up-regulation

in gastrocnemius and heart muscle of exercised, branched chain amino acid diet fed mice. Nutrition & metabolism. 2018;15(1):1-15.
16. Santos ECd, Silva LS, Pinheiro AS, Teixeira DE, Peruchetti DB, Silva-Aguiar RP, et al. The monoterpene 1, 8-cineole prevents cerebral edema in a murine model of severe malaria. Plos one. 2022;17(5):e0268347.
17. Liu S, Fan M, Zheng Q, Hao S, Yang L, Xia Q, et al. MicroRNAs in Alzheimer's disease: Potential diagnostic markers and therapeutic targets. Biomedicine & Pharmacotherapy. 2022;148:112681.
18. Ren Z, Yu J, Wu Z, Si W, Li X, Liu Y, et al. MicroRNA-210-5p contributes to cognitive impairment in early vascular dementia rat model through targeting Snap25. Frontiers in Molecular Neuroscience. 2018;11:388.
19. Nunomura A, Perry G. RNA and oxidative stress in Alzheimer's disease: focus on microRNAs. Oxidative medicine and cellular longevity. 2020;2020.
20. Ramakrishna S, Muddashetty RS. Emerging role of microRNAs in dementia. Journal of molecular biology. 2019;431(9):1743-62.
21. Smith PY, Hernandez-Rapp J, Jolivette F, Lecours C, Bisht K, Goupil C, et al. miR-132/212 deficiency impairs tau metabolism and promotes pathological aggregation in vivo. Human molecular genetics. 2015;24(23):6721-35.
22. Li Y, Zhang T, Zhou Y, Sun Y, Cao Y, Chang X, et al. A Presenilin/Notch1 pathway regulated by miR-375, miR-30a, and miR-34a mediates glucotoxicity induced-pancreatic beta cell apoptosis. Scientific reports. 2016;6(1):36136.
23. Hajibabaie F, Kouhpayeh S, Mirian M, Rahimmanesh I, Boshtam M, Sadeghian L, et al. MicroRNAs as the actors in the atherosclerosis scenario. Journal of physiology and biochemistry. 2020;76:1-12.
24. Hajibabaie F, Abedpoor N, Assareh N, Tabatabaiefar MA, Shariati L, Zarrabi A. The importance of SNPs at miRNA binding sites as biomarkers of gastric and colorectal cancers: a systematic review. Journal of Personalized Medicine. 2022;12(3):456.
25. Watts M, Williams G, Lu J, Nithianantharajah J, Claudianos C. MicroRNA-210 Knockout Alters Dendritic Density and Behavioural Flexibility. bioRxiv. 2019:762450.
26. Siedlecki-Wullich D, Català-Solsona J, Fábregas C, Hernández I, Clarimon J, Lleó A, et al. Altered microRNAs related to synaptic function as potential plasma biomarkers for Alzheimer's disease. Alzheimer's research & therapy. 2019;11:1-11.
27. Khan A, Vaibhav K, Javed H, Tabassum R, Ahmed ME, Khan MM, et al. 1, 8-cineole (eucalyptol) mitigates inflammation in amyloid Beta toxicated PC12 cells: relevance to Alzheimer's disease. Neurochemical research. 2014;39:344-52.