Research Article

The effect of aerobic training with saffron extract on plasma levels of apo E4 and presenilin gene in Alzheimer's rats by trimethyltin chloride

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Abstract

There is no treatment for Alzheimer's disease. However, some treatments can have a slowing and controlling effect on the disease process. The aim of this study was to investigate the effect of aerobic training with saffron extract on plasma levels of apo E4 and presenilin gene in Alzheimer's rats by trimethyltin chloride. In this experimental study, 32 male Sprague-Dawley rats with the weight of 180±20 g were selected and after Alzheimer's induction (by intraperitoneal injection of 80 mg/kg trimethyltin chloride) were randomly divided into 4 groups; control, aerobic training, saffron extract, saffron extract- aerobic training. Saffron extract was injected intraperitoneally at the dose of 25 mg/kg daily for eight weeks. The aerobic training program consisted of incremental running on the treadmill at a speed of 15 to 20 m/min and 15 to 30 min per session and 3 sessions per week for 8 weeks. Data were analyzed using two-way ANOVA at the P<0.05. The results showed that aerobic training (P=0.13), saffron extract (P=0.14) and the interaction of aerobic training and saffron extract (P=0.13) had no significant effect on apo E4in rats. Also, aerobic training (P=0.68), saffron consumption (P=0.67) and interaction of aerobic training and saffron extract (P=0.32) had no significant effect on persniline gene in rats. According to the results, it seems that aerobic training and saffron extract do not significantly alter the levels of apo E4 and the presenilin of Alzheimer's rats.

Key Words: **Alzheimer, Exercise, Saffron extract, Apo E4, Persniline**

Introduction

Alzheimer's disease is a neurodegenerative disease that is associated with differences in memory and cognitive functions and is also a very common cause of dementia, especially in the elderly. Clinical features of Alzheimer's disease include decreased cognitive function, disruption of normal daily activities, and changes in individual behavioral practices (Kim et al., 2018). Several genetic, environmental, and clinical risk factors may play a role in the onset and progression of Alzheimer's disease. APOEs are a class of apolipoproteins mainly found in the CNS and normally produced by astrocytes and microglia. Apolipoprotein E4 (ApoE4) is the most important genetic risk factor for the development of Alzheimer's disease (Riedel et al., 2016). ApoE4 is a glycoprotein involved in cholesterol transport and lipoprotein metabolism, and often occurs in Alzheimer's patients and regulates Aβ metabolism (Kanekiyo et al., 2014; Mahley, 2016). The presenilin gene is also the only gene involved in the late onset of the disease. In the early stages of Alzheimer's, genetic testing is appropriate for mutations in apolipoprotein and presenilin. The presenilin gene is present in intracellular membranes, including the nucleus accumbens, endoplasmic reticulum, and Golgi apparatus. Although the exact function of this protein has not yet been determined, the presenilin gene appears to be involved in the control of apoptosis (Lee et al., 2010). On the other hand, the protein presenilin is involved in the natural neurogenesis and life of neurons as well as the progenitor cells of specific areas of the brain. This protein is also involved in the activity of the enzyme gamma secretase and is required for the proteolytic division of amyloid precursor protein and beta amyloid production (Sepulveda-Falla et al., 2011). Studies have shown that dysfunction of apolipoprotein E, which also increases oxidative stress and is associated with Alzheimer's disease, potentiates the activity of the presenilin gene (Chan & Shea, 2006).

Environmental and pharmacological interventions have been investigated to see whether they delay or reduce the progressi-

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-on of Alzheimer's disease. One of the environmental interventions is physical activity. Physical activity generally means movement of the body due to muscle contraction, which increases the body's energy above the amount of rest and is one of the indicators of a healthy life. Long-term physical activity is associated with improved cognitive function and a lower risk of Alzheimer's disease in humans (Gronek et al., 2019). Observational studies in healthy elderly have reported that higher levels of physical activity are associated with a reduced risk of cognitive decline, better cognitive function, and hippocampal volume retention, and a stronger effect on APOE4 carriers (Smith et al., 2014). Exercise also prevents memory impairment caused by mutations in the presenilin genes and β-secretase activation, and reduces Aβ42 deposition in the cortex and hippocampus of older rat (Kang et al., 2013).

In addition to environmental and pharmacological interventions, the use of some medicinal plants can play an important role in the prevention and control of Alzheimer's disease. Saffron is the most traditional spice and its plant is the most expensive plant cultivated in the world. Dried saffron has been used as a food seasoning for many years. Saffron has several medicinal and therapeutic properties and applications (Milajerdi et al., 2017). Saffron stigma contains more than 150 chemicals, among which crocin, picrocrocin and safranal have a more important role in creating the pharmacological effects of saffron. The beneficial effects of saffron and its active ingredients in various body tissues such as the central nervous system have been observed (Milajerdi et al., 2015). Transcrocin-4, dimethic biosyl ester crocetin (crocin) and dimethylcrocetin inhibit the formation of amyloid beta fibrils (Amyloid β 1-40) and inhibit the activity of acetylcholinesterase. Therefore, it has been suggested that the use of saffron and its active ingredients may be useful for the prevention or treatment of Alzheimer's disease (Kianbakht, 2008).

Alzheimer's disease (AD) can gradually get worse, and the more advanced the disease, the higher the costs. Therefore, different techniques can be used to stop the progression of the disease, slow down the course of the disease and prolong the distance from mild to severe Alzheimer's. If these measures are effective, they can remove a major economic burden from the family and the government and cause a sense of happiness and reduce depression in the patient, his family and those around him. There is no specific treatment for AD and this points to the importance of identifying preventive strategies that can reduce or delay the risk of AD. However, some treatments can have a slowing and controlling effect on the disease process. Therefore, despite the mentioned problems and the study of these cases, at least in the animal model and exercise training along with the administration of saffron extract, it may be possible to take a step to treat mental disorders caused by Alzheimer's disease. According to the mentioned studies, in none of them, the interactive effects of exercise training and saffron extract on apo E4 and presenilin genes in Alzheimer's rats have been studied and our information in this field is insufficient. Therefore, the present study intended to investigate the effect of aerobic exercise with consumption of saffron extract on the levels of apo E4 and the presenilin gene of trimethyltin chloride in Alzheimer's rats.

Materials and Methods

Animal and AD induction

The present study is an experimental and basic type performed in a laboratory method. The statistical population of the present study consisted of male Sprague Dawley rats, housed in Animal Care Center of Islamic Azad University, Marvdasht Branch, Iran. Thirty-two 4-week-old rats weighing 180 ± 20 g were selected for study. The statistical sample of this study was selected through purposful sampling according to weight and age conditions. After being transferred to the laboratory environment, rats were kept in a cage in the laboratory for seven days to adapt to the environment and were removed only when the cages were washed. Clear polycarbonate cages with autoclave capability were used to house the rats. The optimum temperature of the animal shelter was 20 to 24 oC and the relative humidity was about 55 to 65 percent. The lighting cycle was also precisely controlled every 12 hours by an electronic light regulator in the laboratory animal care hall. In this study, the food required by the subject in the form of pellets was prepared from the Center for Breeding and Reproduction of Laboratory Animal Stem Cells in Shiraz and was provided to the animals indefinitely. The required water was also supplied freely in milliliter bottles for laboratory animals. After transferring the rats to the laboratory and leaving them in a cage for one week to adapt to the environment, they were treated for Alzheimer's disease by trimethyltin. This neurotoxin specifically apoptosed neurons in the hippocampus in different areas. 8 mg / kg body weight of rats in the study groups was injected intraperitoneally during one injection phase (for example, for a 250 kg rats, 0.250 ml of trimethyltin solution was injected). After trimethyltin injection, a number of behavioral symptoms were observed in rats and these clinical symptoms included: 1) muscle tremors, 2) fever, 3) bleeding from the eyes and nose, 4) nausea, 5) convulsions and 6) twisting of the tail. Rats were purposefully and randomly divided into 4 groups of 8 series according to Morgan table to determine the sample size and to evaluate the effects of trimethyltin in induction of Alzheimer's animal model and to evaluate the effects of endurance training and saffron.

Trimethyltin chloride

To prepare trimethyltin chloride (TMT) solution, 80 mg of this substance (German company, Fluca) was dissolved in 10 ml of

Table 1. Endurance Training Protocol

Week	Speed (m/min)	Duration (min)
1	15	15
2	15	15
3	17	20
4	17	20
5	19	25
6	19	25
7	20	30
8	20	30

solvent (normal saline) and prepared for injection into rats. For each kilogram of rat weight, 1 ml of this solution was prepared (MA & SheikholeslamiM, 2018).

Saffron extract

To prepare saffron extract, 9.2 g of saffron was poured into 1000 cc of deionized distilled water and the mixture was incubated for 16 hours at 50 ° C, then the solution was passed through a strainer and stored at 4 ° C for injection. It was injected intraperitoneally at a dose of 25 mg / kg (Azarian et al., 2020).

Endurance training protocol

The endurance training protocol included 8 weeks of incremental running on a non-sloping treadmill (zero slope) at a speed of 15 to 20 meters per minute for 15 to 30 minutes per session and 3 sessions per week. To warm up the animals in the training group, first after placing the animals on the treadmill, the animals ran at a speed of 8 meters per minute for 5 to 10 minutes, then the training program was performed. At the end of the training program, in order to run the cooling down, the speed of the device was reduced inversely to zero speed. The program lasted about five to seven minutes (Asishirazi et al., 2017) (Table 1).

Blood and tissue sampling and measurement of laboratory variables

48 hours after the last training session (10 to 12 hours fasting), the rats in each group were anesthetized by intraperitoneal injection of ketamine (30-50 mg / kg) and xylazine (3-5 mg / kg).

Table 2. Real-time PCR Primer Pequences

Gene	Primer	Sequence	Amplicon
pers1	Forward	5´GGCCACCATCAAGTCAGTCA 3´	**104 bp**
	Reverse	5´ GATGGCGGCATTCAAGATCG 3´	
GAPDH	Forward	5´AGTGCCAGCCTCGTCTCATA3´	**104 bp**
	Reverse	5´GAGAAGGCAGCCCTGGTAAC3´	

By splitting the animal's chest, a blood sample was collected from the right ventricle with a syringe soaked in EDTA fluid and poured into a tube containing EDTA, and the collected plasma was analyzed with ELISA method for apo E4 (Cat no: RK03509, ZellBio GmbH, Germany). The skull was then split open with a razor blade and the brain was carefully removed. The healthy brain was divided exactly in half by a razor blade and separated from the limbic system by the clear atlas of the hippocampus. Rat hippocampal tissue was sampled and after washing in physiological serum, it was immersed in 1.8 microtubes containing 20% RNAlaterTM fluid and transferred to the laboratory for genetic testing. The expression of pers1 gene was measured by Real time - PCR technique and after quantification, the gene expression values were analyzed by the formula $2^{-\Delta\Delta Ct}$. PCR reaction was performed using (Applied Biosystems) PCR master mix and SYBR Green in ABI Step One (Applied Biosystems, Sequence Detection Systems. Foster City, CA) according to the manufacturer's protocol. Primers were designed based on pers1 gene information in NBCI Gene Bank by Macrogen Inc. (Seoul, Korea). The sequence of primers used is listed in Table 2.

Statistical analysis

After the normality of the data was confirmed by Shapiro-Wilk test, two-way analysis of variance was used to examine the changes in anti-inflammatory indices between groups. All statistical operations of the study were performed using SPSS software version 23 and the significance level was considered at $p < 0.05$.

Results

To evaluate the effect of endurance training with saffron consumption on apoE4 of rats with Alzheimer's disease, the results of bilateral analysis of variance were analyzed showing that endurance training (P = 0.13) and saffron consumption (P = 0.14) had a no significant effect on apo E4. Also, the interaction of endurance training with saffron consumption is not significant (P = 0.13) (Figure 1).

Data analysis also showed that exercise (P = 0.68), saffron consumption (P = 0.67) and the interaction of endurance training with saffron consumption (P = 0.32) had no significant effect on reducing the rat presenilin gene (Figure 2).

Discussion

The findings of the present study showed that eight weeks of aerobic exercise, consumption of saffron extract and also the interaction of aerobic exercise and consumption of saffron extract were associated with no significant decrease in apoE4 and presenilin gene in Alzheimer's rats compared to the control group. The effect of exercise protection on neurons has been demonstr-

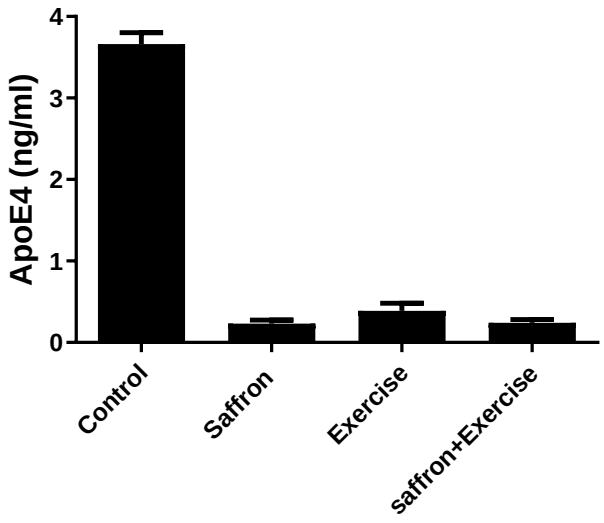

Figure 1. Mean Changes of CpoE4 of Alzheimer's Rats in Different Groups.

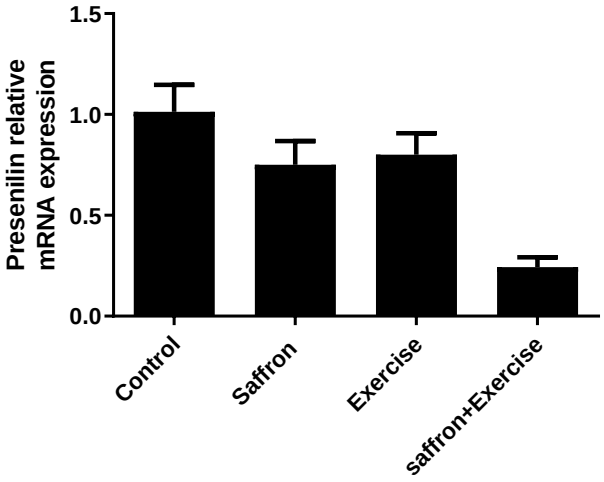

Figure 2. Mean Changes in the Presenilin Gene Expression of Alzheimer's Rats in Different Groups

-ated, in animal and human studies, particularly in the hippocampus and dentate gyrus, both of which show pathological changes in Alzheimer's disease (Pereira et al., 2007). However, very little is known about the response of apoE4 and the presenilin gene to exercise. ApoE4 is a protein that regulates cholesterol, lipid metabolism, and cellular compensatory processes in the bloodstream and in the central nervous system (Mahley & Huang, 2012). Studies show that there is an association between changes in APOE4 and exercise (Smith et al., 2016). Regular physical activity has been shown to prevent or delay the symptoms of dementia and Alzheimer's disease, especially in people with apoE4 (Rovio et al., 2005). Schuit et al. (2001) found that the risk of cognitive decline was higher APOE4 and exercise (Smith et al., 2016). Regular physical activity has been shown to prevent or delay the symptoms of dementia and Alzheimer's disease, especially in people with apoE4 (Rovio et al., 2005). Schuit et al. (2001) found that the risk of cognitive decline was higher APOE4 in participants who exercised less than 1 hour daily. Amyloid levels were found to be higher in the brains of physically inactive APOE4 allele carriers but lower in physically active APOE4 carriers (Head et al., 2012). Neuroimaging studies have also reported that physical activity can be more effective in APOE4 carriers (Deeny et al., 2012). Also, there is an inverse relationship between the risk of dementia and physical activity in non-carriers of APOE4, which is less than carriers of APOE4 (Rovio et al., 2005). In addition to amyloid-beta-dependent pathways, physical activity can improve cognitive function in APOE4 carriers through other mechanisms such as improved perfusion and neurogenesis, as well as neuroprotective processes (Raichlen & Alexander, 2014).

Moreover, the mechanism of exercise-induced changes in APOE4 and the exercise-induced presenilin gene is not well understood. It has been suggested that APOE4 and the presenilin gene can lead to the pathogenesis of Alzheimer's disease in several ways: (1) damage to the cerebral vascular system; (2) initiation or acceleration of A-beta accumulation in the brain; (3) impaired cholesterol transport; and (4) increased neuritis (Liu et al., 2013). It has been shown that patients who carry APOE4 and the presenilin gene carry more benefit from exercise intervention by maintaining cognitive function and improving physical function (Jensen et al., 2019). Therefore, it is possible that exercise changes the level of APOE4 by affecting the above pathways and is beneficial for Alzheimer's patients. Smith et al. (2014) suggested that understanding the changes in APOE could play a key role in providing physical activity recommendations for the elderly as a tool to prevent future cognitive decline and brain atrophy (Smith et al., 2014). Overall, these results suggest that people who are genetically predisposed to Alzheimer's disease are much more likely to benefit from physical activity. However, the effect of exercise on Apo E4 is different. Bernstein et al. (2002) suggested that high-intensity physical activity with high energy expenditure may neutralize the atherogenic effects of the APO E4 allele on fat profiles. People with the APOE4 allele who do a lot of strenuous physical activity with a lot of energy have shown higher HDL-C and lower TG levels than people with APOE3. Prolonged exercise may have a positive effect on risk factors by affecting APOE4 (Lee et al., 2018). Therefore, it is possible that a longer period of aerobic exercise in the present study could be associated with a significant change in apoE4.

Some traditional herbal antioxidants also have the potential to treat Alzheimer's. It has been shown that DNA methylation is impaired as a result of S-adeniosyl methionine deficiency in older

mice and leads to high expression of presenilin and antioxidant supplementation (apple juice concentrate) by altering the amount of S-adenyosyl methionine high PS-1 expression (Chan & Shea, 2006). Furthermore, in a study by Chavadari et al. (2014) investigating the effect of antioxidant supplementation (tocopherol alpha acetate and 1.65 mg per kg body weight of ascorbic acid and vitamin E) and moderate-intensity endurance training on apolipoprotein E4 in 16-week-old rats, the results showed that apolipoprotein E4 levels improved the use of antioxidants. The results also showed that moderate-intensity exercise improved apolipoprotein E4 levels in rats (Chaudhari et al., 2014). In our study, saffron extract caonsumption and the interaction of aerobic exercise and saffron extract consumption were associated with non-significant decrease in apoE4 and the presenilin gene in Alzheimer's rats compared to the control group. It seems that saffron and its active components are effective in diseases related to the nervous system. These functions are attributed to its antioxidant, anti-inflammatory and anti-apoptotic properties (Milajerdi et al., 2015), which are performed by its flavonoids, tannins, saponins and crocins (Ochiai et al., 2007). Another study reported that crocin was ineffective at low doses (Sugiura et al., 1995), however, high doses of crocin in intraventricular injection alone improved hippocampal function (Abe & Saito, 2000). As the results of studies show, the effects of saffron depend on its dose and duration of use, so if the current intervention continued for a longer period, clearer results were obtained. Our study has some limitations, including the fact that the model used may not be generalizable to Alzheimer's patients. Other limitations of the present study include the lack of measurement of beta-amyloid, cholesterol and lipid levels, and other Alzheimer's-related factors. By manipulating the dose of saffron extract in samples with Alzheimer's disease, clearer results may be achieved.

Conclusion

According to the research findings, it seems that aerobic exercise and saffron extract do not significantly alter the levels of apo E4 and the presenilin of Alzheimer's rat. Due to the small number of studies conducted in this regard, research on the effect of exercise and herbal supplementation on the levels of apo E4 and the presenilin in patients with Alzheimer's disease seems necessary.

What is already known on this subject?

The interactive effects of exercise training and saffron extract on apo E4 and presenilin genes in Alzheimer's rats have been studied and our information in this field is insufficient.

What this study adds?

It seems that aerobic exercise and saffron extract do not significa-ntly alter the levels of apo E4 and the presenilin of Alzheimer's rat.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All experimental protocols were approved by the Ethics Committee of Islamic Azad University, Marvdasht Branch, Shiraz, Iran.

Informed consent Animal study.

Author contributions

Conceptualization: S.Sh., A.R.; Methodology: S.Sh., A.R.; Software: S.Sh., A.R.; Validation: S.Sh., A.R.; Formal analysis: S.Sh.; Investigation: S.Sh., A.R.; Resources: A.R.; Data curation: S.Sh.; Writing - original draft: S.Sh., A.R.; Writing - review & editing: A.R.; Visualization: S.Sh.; Supervision: S.Sh.; Project administration: A.R.; Funding acquisition: S.Sh.

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