The Effects of Resistance Training With the Consumption of Saffron Extract on Spatial Memory and Tau Accumulation in the Hippocampal Tissue of Male Alzheimer's Induced Rats

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Abstract:

Introduction: Alzheimer's disease is a progressive, degenerative brain disease that causes severe thinking and memory impairment, and there is no definitive treatment for this disease. The present study aimed to investigate the effect of resistance training along with the consumption of saffron extract on spatial memory and tau accumulation in the hippocampal tissue of Alzheimer's male rats.

Material & Methods: In this experimental study, 40 adult male rats were randomly divided into 4 Alzheimer's groups, including control, resistance exercise, resistance exercise, and supplement. Alzheimer's was induced by injecting amyloid beta 42-1 into the hippocampus. Resistance exercises were performed for 12 weeks, including three sessions per week. Y Maze test was used to measure spatial memory, and Tau protein was measured by the ELISA method. One-way analysis of variance tests was used to analyze the data.

Results: The results showed that after 12 weeks of resistance training with saffron supplement, there was a significant increase in spatial memory performance in the intervention groups compared to the control group ($P < 0.5$). Also, regarding the amount of tau protein accumulation in the aerobic exercise group, aerobic exercise, and extract showed a significant decrease compared to the control group ($P < 0.5$).

Conclusion: It seems that resistance exercise and consumption of saffron extract improve spatial memory performance and reduce the accumulation of tau protein in the hippocampal tissue of Alzheimer's male rats.

Keywords: Resistance exercises, Saffron, Alzheimer's, Spatial memory, Tao protein

1. Introduction

Alzheimer's disease is a type of brain dysfunction that gradually affects memory and other mental abilities such as thinking, reasoning, and judgment; it is known as a dementia disease and the most common age-related neurological disorder. For several decades, amyloid beta and tau protein have been thought to be two prominent factors in the development of Alzheimer's disease (1). The formation of amyloid plaques around nerve cells and neurofibrillary filaments inside brain cells are the leading causes of Alzheimer's disease (2). Researchers believe that the factors affecting Alzheimer's include age, genetics, and gender (3). Also, the symptoms of Alzheimer's disease include memory loss, personality changes, depression, anxiety, and other mental disorders (4).

There are different reasons for Alzheimer's, including oxidative stress deposition of beta-extracellular amyloid plaques (5). The formation of intracellular neurofibrillary tangles is the hyperphosphorylation of tau protein .Two main reasons for this disease have been widely investigated: the accumulation of amyloid beta peptide and tau phosphorylated proteins (6,7). Alzheimer's rat model will be beneficial for investigating new treatment methods with the hope that these findings can be generalized to humans (6). Proteins that stabilize microtubules are abundantly found in neurons of the central nervous system and, to a lesser extent, in astrocytes and oligodendrocytes. Taking proteins that have lost their function in stabilizing microtubules is the leading cause of dementia-related diseases such as Alzheimer's and Parkinson's. In Alzheimer's disease, abnormal Tao is separated from the microtubules and causes the microtubules to disintegrate. The occurrence of these problems inside the neuron turns off cell movement and transport and causes cell death. This causes disconnection and disconnection of neurons in specific brain parts and, eventually, memory loss (7,8).

In this regard, there are two critical hypotheses. The first hypothesis states that a decrease in the binding of phosphorylated tau protein to the microtubule may cause the loss of function. As a result, it causes instability in the microtubule and disruption of axon transmission. The second hypothesis also states that the increase in tau phosphorylated protein leads to the accumulation of toxic effects in nerve cells. In recent years, research results have shown that the increase in phosphorylated tau protein causes a decrease in nerve cells and memory impairment, and suppressing its expression leads to an improvement in memory and an increase in synaptic connections (7). Currently, there is no definitive treatment for Alzheimer's disease, and drug therapy solutions are only focused on the symptoms and signs of the disease, which often do not lead to satisfactory results (9,10). On the other hand, research has shown that people with less exercise and physical activity during their lives are more prone to Alzheimer's disease, and compared to active people, they have double the risk of developing (10,11). Exercise increases synaptic variability, improves the antioxidant system, improves signaling, plasticity, and synaptic transmission, and reduces apoptosis (12). Meanwhile, resistance exercises with mechanisms such as increasing BDNF levels as a mediator of synaptic effects, neural connections, and plasticity in the brain increase memory and learning. Also, exercise improves the function of nerve cells through the proliferation of cells in the hippocampus, inhibiting apoptosis in the dentate gyrus of the hippocampus and increasing the synaptic space in different parts of the brain. The information obtained from the findings of past researchers believed that sports activities increase the cognitive performance of people in old age. In contrast, some other researchers believe that physical activity and sports have little effect on this performance (13). Increasing physical activity enhances brain activity, especially in the hippocampus area, and thus reduces the secondary impact of Alzheimer's disease (14). Some studies have demonstrated that long-term resistance training can significantly reduce the level of hyperphosphorylated tau protein and, in parallel, increase the level of dephosphorylated tau protein (15). In this regard, Ebrahimi et al. stated that eight weeks of resistance training improved spatial memory (16). In a review study of the effect of resistance training on Alzheimer's disease, Azevedo et al. reported that resistance training could reduce the amount of tau protein deposition in the hippocampal tissue. Suppress, the decrease in Tau hyperphosphorylated protein could be due to inhibition or activation of tau kinases (17). On the other hand, some drugs are used to prevent the progression of this disease; these drugs have few effects in the treatment or have many side effects (18,19).

For this reason, medicinal plants have attracted researchers' attention due to their ability to exert neuron protection through the control pathway of acetylcholinesterase or by inhibiting oxidative stress (18,19). Among these plants, saffron has antioxidant effects (20). Some research results showed that Saffron, as an antioxidant, significantly strengthens memory (19). Considering the benefits of the saffron plant on Alzheimer's disease and the beneficial effects of exercise on the improvement of neurological symptoms, there are still many ambiguous points in this field, and investigating these issues, at least in an animal model, can help progress towards improving this disorder. Therefore, the present study aims to examine resistance exercises and the consumption of saffron plant extract on spatial memory and the amount of tau protein accumulation in the hippocampal tissue of male Alzheimer's induced rats.

2. Materials and Methods

Subjects

This research was of an experimental type with four groups as a post-test. The research samples included 40 adult male rats 6-7 months old, weighing 260-270 gr, obtained from the Pasteur Institute of Iran. After being transferred to the Vira Armanian animal house in Rasht, The animals were kept under controlled conditions with a cycle of 12 hours of darkness and 12 hours of light, with a temperature of 22-24 (°c) and a humidity of about 50-60%. They had free access to food. These animals were placed in special cages for two weeks to adapt to the new environment (21).

For the induction of Alzheimer's disease, amyloid beta 42-1 (Sigma-Aldrich) was used, which was dissolved in twice sterile distilled water and incubated for one week at 37°C. Terpinolene at a 100 mg/kg dose was dissolved in 0.5 cc of twice sterile distilled water and injected intraperitoneally into rats through an insulin syringe. Animals were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg), and after being placed in the stereo tax machine, shaving the hair on the head and making a sagittal incision, the bregma and lambda sutures were clearly defined. Next, the skull was slowly pierced with a drill. For the injection of amyloid beta, two microliters of amyloid beta were injected bilaterally and slowly through the holes created in the brain with a Hamilton syringe. To fully absorb the drug, the injection lasted for 60 seconds. It is worth noting that the needle remained in place for 2 minutes (22,23). Further, the rats were divided into four groups: control, resistance training, and resistance training and supplemental. Forty-eight hours after the last intervention, all rats were fasted for 8-10 hours and weighed before the tissue removal. Then, anesthesia was done by inhalation with chloroform; after complete anesthesia and pain test and ensuring lack of consciousness, tissue was removed and kept in a -80 Celsius freezer for evaluation (26).

Also, the ethical points of maintaining and working with laboratory animals were observed according to the instructions of the National Institute of Health for the care and use of laboratory animals during the entire work period.

Exercise Training

The resistance training protocol included climbing a 110 cm long ladder at an angle of 80 degrees to the horizon. A box was placed on the top of the ladder to rest between the sets. The familiarization program included placing the rats in the upper third of the ladder to climb, then placing them in the middle half, and finally at the lowest height to climb the ladder. The training program started one week after the familiarization phase, including eight sets of climbing the ladder with increasing overload. Each group consisted of 8 to 12 dynamic repetitive movements until reaching the upper rest box. In the first and second sets, the weight added to the animal's tail was 50% of the body weight, and the overload was 75% for the set. The third and fourth sets, 90% for the fifth and sixth sets, and 100% for the seventh and eighth sets were calculated and applied based on the animal's weight. In between, the animals were placed in the upper box for 60 seconds and rested at the top of the ladder. At the same time, a tape tied the weights to the rat's tail near the trunk. The rats were weighed daily before starting the training, and the resistance training program was implemented for 12 weeks and three sessions per week (25).

Supplement

The rats in the extract group and the exercise group received the extract at 100 mg per kilogram of body weight once a day at 8:00 am for seven days a week for 12 weeks through gavage.

Measurements

Y-maze test for memory evaluation

A behavioral test called Y Maze was used to examine memory. The memory performance of the animals in this test was investigated by observing and measuring the animal's spontaneous alternation behavior in a working session for 8 minutes. The corresponding maze is made of black Plexiglas and has three arms with dimensions of 40 × 30 × 15 cm connected through a central area. To perform this test, we placed the rats in the end part of an arm, and unrestricted access to all maze areas was possible. The number of times the Rat entered each component was recorded by observation. The animal's entry into an arm was when the rear legs of the animal were entirely inside the arm. Alternating behavior was considered consecutive successful entries into all arms in sets of three. Based on this, the rotation percentage was calculated from the ratio of the observed rotation to the maximum rotation (2 - the total number of included arms) ×100 (24).

Tau protein measurement method

The subject's head was separated from the neck area by special scissors to collect hippocampal samples. First, using a scalpel, the skull was split, and the brain was carefully removed. Then, the healthy brain was divided into two halves by a surgical blade (exactly), and according to the coordinates of the hippocampus, the hippocampus was separated from the limbic system with the help of a precise sinus atlas. Then, the hippocampal samples were collected and stored at -70°C for further measurement (27). Next, 50 mg of hippocampal tissue was placed in a cold saline-citrate buffer solution to measure the tau protein level. A microhomogenizer homogenized this tissue for 10 minutes. Then, the homogenized tissue was centrifuged, and the supernatant was transferred into the Eppendorf. This solution was used to measure tau protein in hippocampal tissue. The level of tau protein in the hippocampus tissue was measured by the ELISA method using a special research kit for rats manufactured by Case Bio Company, Germany, according to the manufacturer's instructions. It is worth mentioning that the sensitivity of the measuring kit was 0.1 Pico gram per milligram, and its coefficient of variation was 6.8% (27,28).

Statistical analysis

Descriptive analysis was reported as the mean and standard deviation. The Shapiro-Wilk test was used for the normality of the population, and according to the results, all the data had a normal distribution. To compare the results of the desired experiments, they were analyzed by one-way analysis of variance using SPSS version 20 software.

3. Results

To observe the subjects' weight, weighing was done when the issues entered the research. The average and standard deviation of weight changes of the groups during eight weeks are shown in Table 1.

Table 1. Changes in weight of rats.

variable	group Control	group Resistance exercises	group supplement, resistance exercises	group supplement
Weight of the first week of training (gr)	265.5±12.3	271±3.97	278 ± 7.8	272±3.97
Weight of twelve weeks of training (gr)	290.7±16.5	273.4±10.9	280±0.54	279±4.99

According to the obtained results, there were weight changes in all groups; in other words, there was an increase in the control and supplement groups, and the weight increase was more in the control group.

However, in the resistance training and the resistance training and supplement, weight changes were associated with a decrease, and this weight loss in the resistance training + supplement is more than in the resistance training group. To analyze the data, the analysis of variance test was used.

Table 2. Results of analysis of variance.

ANOVA Welch test results	Test statistics	Degree of freedom 1	Degree of freedom 2	The significance of the test
tau protein (Pg./ per milligram of tissue)	0.938	10	12.98	0.002*
Spatial memory (percent frequency)	0.699	10	121.87	0.001*

The results of one-way analysis of variance (Welch test in the case of inequality of variances) showed that there was a significant difference between the research groups.

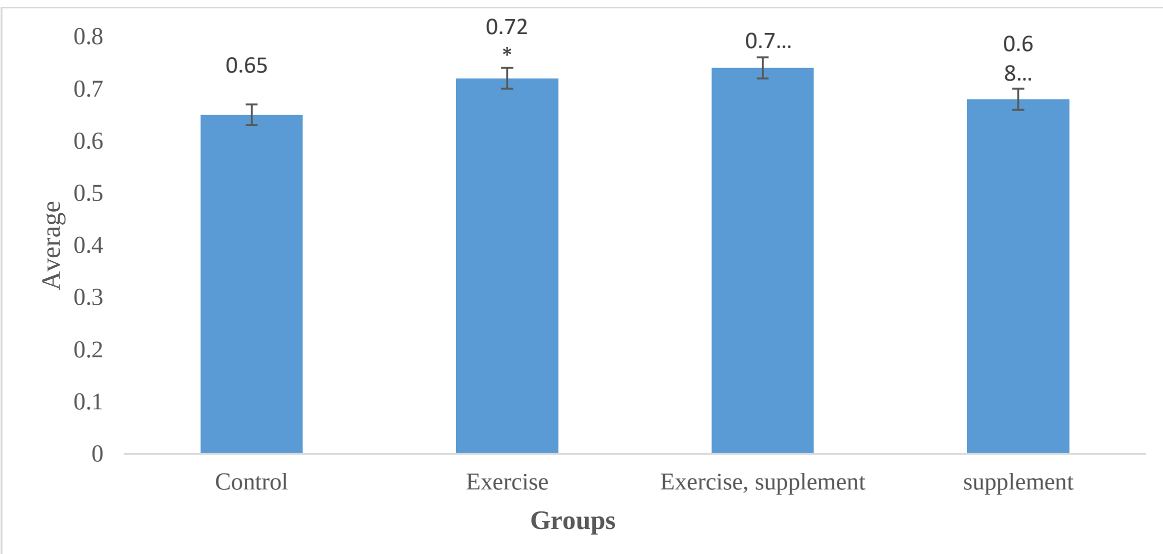

Figure 1. The average difference in spatial memory compared to the control group in the research groups
* significant difference compared to the control group (P≤0.5)

According to the, there is a significant difference between the control group, the resistance training group, resistance training + supplement; other words, resistance exercises, resistance exercises along with supplement use and supplement use improves spatial memory. It should be noted that the amount of effect in the group of rats that received resistance training with supplements was higher than in other groups, and this difference was present in all groups.

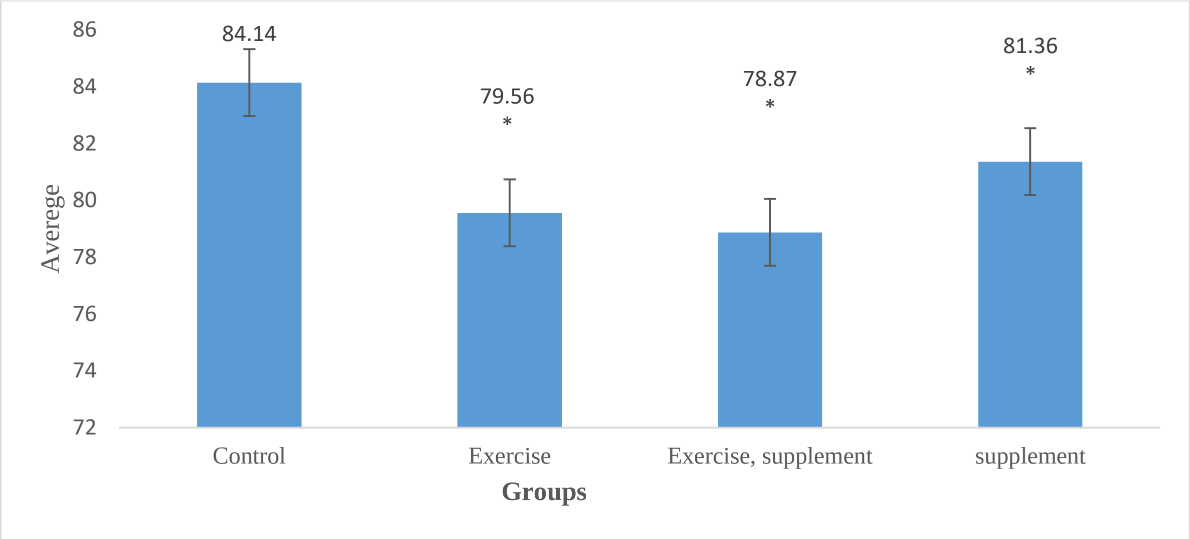

Figure 2. The average difference in tau protein accumulation compared to the control group in the research groups
*significant difference compared to the control group (P≤0.5)

According to the findings obtained from the results of the present study, there was a significant difference between the control group, the resistance training group, the resistance training group, and the supplement group, as well as the supplement group, in the changes in the amount of tau protein accumulation, It can be said that resistance training reduces the accumulation of tau protein in the hippocampus tissue. At the same time, this difference is insignificant in resistance training and resistance training with saffron extract supplements. It can be said that there is a difference, but this difference is not significant and equal to the results obtained. It has been found that the amount of tau protein accumulation in the hippocampal tissue of the Alzheimer's group of rats was lower than in all other groups with exercise training and supplemental consumption of saffron extract.

4. Discussion

Considering that in the new era, the use of drugs and nerve-supporting supplements is an important and everyday occurrence for some people (29). Physical activity and exercise improve learning and memory, affect cognitive function neurogenesis, and reduce brain damage caused by Alzheimer's disease and even the risk of Alzheimer's and dementia. It is beneficial for stimulating blood vessels and nerves in different organs (30). Also, saffron plant extract has been proven as a supplement with antidepressant effect and therapeutic effects in reducing anxiety and mental health during a clinical trial (31). Based on the findings of this study, 12 weeks of resistance training and the consumption of saffron plant extract in induced Alzheimer's rats in the control group significantly had a lower delay time and frequency percentage than other groups. it was also determined that the rats that performed resistance training better than the group that only took supplements, while the rats that used resistance training and supplements simultaneously performed better than the other groups. Previous studies have stated that the lack of energy in the brain is associated with cognitive performance disorders because the functions of brain cells, such as learning and memory, depend on cellular energy metabolism. Reducing available energy can disrupt the production of amyloid precursor proteins and proteins Tao and thus cause their accumulation in the brain (32). During recent decades, the effect of physical activity, especially resistance exercises, in improving cognitive function and memory as a preventive strategy against Alzheimer's in healthy people and people with Alzheimer 's-related diseases has been well proven (33,34). The results of studies by de las Her et al. in 2018 show that sports activities help maintain brain health, improve cognitive performance, effectively protect the central nervous system, and improve learning performance (35). One of the possible mechanisms in the protective ability of sports training is the capacity to block free radicals (36). Past studies have shown that regular sports activities improve learning and memory in rats by activating the antioxidant system. In another part, the neuroprotective effect of sports activity may occur through the increased regulation of the expression of neurotrophies. These proteins increase the regeneration ability of nerve cells (37). A study proved that eight weeks of exercise training improved memory performance and spatial learning in Alzheimer's rats. They stated that exercise seems to be a promising non-pharmacological strategy to prevent dementia in Alzheimer's disease (34). Meanwhile, saffron does not strengthen animals with healthy memory, but it can reduce the destructive effects on memory (38). Past studies have stated that the saffron plant improves the learning and memory of mice and reduces memory disorders to a large extent (39). There are many documents that Alzheimer's disease is caused by cell damage caused by the activities of free radicals, and the saffron plant extract contains many carotenoids that have strong antioxidant effects and can protect the nerve cells of the central nervous system from damage (40). Other researchers stated that the saffron plant and its effective ingredients, including crocin, can improve certain memory disorders with different mechanisms (38). Further, tau is one of the main proteins in the axon that stabilizes the microtubules that make up nerve pathways and nerve transmission

Tau is mostly present in the axons of neurons. The hyperphosphorylated forms of tau are insoluble; in this case, they tend to bind to reduce microtubules (42). The results of the present study showed that the level of phosphorylated tau protein in the hippocampal tissue of rats in the resistance training and supplementation group, rats in the resistance training group, and rats in the supplementation group was reduced compared to the control group. This difference is significant, But this difference in rats The results of the resistance training and supplementation group are not significant with the resistance training group. Tau protein accumulation was less observed in the group of resistance exercise and supplementation rats. In confirmation of these findings, Leki et al. and Qarari Arefi et al. stated in their results that sports activity can improve performance in Alzheimer's patients. In their results, they stated that the high volume of exercise activity reduces the loss of neurons and the level of phosphorylated tau in the hippocampal tissue of Alzheimer's rats (28,43). In this regard, Biod et al. 2011 investigated the effects of sports training on some neuroprotective variables in the hippocampus and cerebral cortex of healthy and Alzheimer's rats. Their findings indicated that 36 weeks of aerobic training caused a significant decrease in protein accumulation. Tau was found in the hippocampal tissue of Alzheimer's rats (44). Also, in another study, the effects of 12 weeks of exercise training on the pathology of tau protein in Alzheimer's mice and based on their findings, resistance exercise improves general movement and recognition activity, as well as a significant reduction of tau protein in the hippocampal tissue (45). Also, in another study, the effects of 12 weeks of exercise training on the pathology of tau protein in Alzheimer's mice and based on their findings, resistance exercise improves general movement and recognition activity, as well as a significant reduction of tau protein in the hippocampal tissue. The current research had limitations, such as examining different amounts of saffron plant extract supplements and the intensity and intermittent duration of resistance exercises; although many researchers have suggested the use of different drugs for treating Alzheimer's, generally, each one has side effects. Therefore, it seems that the use of saffron extract supplement and resistance exercises as complementary methods along with drug treatment in animal samples should be studied again so that if positive results are obtained, and the results of this clinical trial study are repeated, it can be used by Alzheimer's patients Take.

5. Conclusion

Based on the findings of this study, resistance exercises and supplemental consumption of saffron extract increase spatial memory efficiency compared to the control group. Also, the study results indicate that resistance exercise and saffron supplementation decrease the amount of tau protein accumulation in the hippocampus tissue compared with the control group of induced Alzheimer's male rats.

6. Acknowledgment

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