



Endurance Training and Royal Jelly Consumption Effects on Dopamine in Rats with Alzheimer's disease

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ARTICLE INFO	ABSTRACT
<p>Article type: Research Paper</p>	<p>Introduction: Alzheimer's disease may be a sort of amnesia with brain dysfunction that gradually degrades the patient's mental abilities. The purpose of this study was to research the effect of endurance training with royal jelly consumption on dopamine in the hippocampus tissue of Alzheimer's rats with trimethyltin.</p>
<p>Article History: Received: 05 Jul 2020 Accepted: 06 Sep 2020 Published: 02 Jun 2021</p>	<p>Methods: During this study, 30 rats underwent injection of 8 milligram / Kilograms trimethyltin chloride and were divided into groups of 6 rats: control, training, royal jelly consumption, and training with royal jelly consumption. 6 rats were included in the healthy control group to be evaluated the effects of Alzheimer's induction on dopamine levels. The training groups were active on the treadmill for 8 weeks. The royal jelly consumption groups received 100 milligram / Kilograms royal jelly per day peritoneally for 8 weeks. The Kolmogorov-Smirnov, one-way ANOVA, and Tukey's post hoc tests were used to analyze the findings. ($P \leq 0.05$).</p>
<p>Keywords: Alzheimer's Dopamine Training Positive slopes Royal jelly</p>	<p>Results: Alzheimer's induction with trimethyltin toxin had a Significant Impact on reducing dopamine gene levels ($P = 0.04$); royal jelly, training, and training with royal jelly consumption had a significant effect on increasing dopamine gene expression levels ($P = 0.001$). Also, the effect of training with Royal Jelly was greater than training or royal jelly consumption alone. ($P = 0.001$)</p> <p>Conclusions: Although training and royal jelly consumption improve dopamine gene expression levels in the hippocampus tissue of rats with Alzheimer's disease, the effects of training combined with royal jelly consumption appear to be greater than those of royal jelly consumption alone.</p>

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Introduction

Alzheimer's disease is a condition that comes with age and more people are experiencing every day. AD is a kind of neglected disease that impaired brain function analysis is that gradually the patient's mental abilities and gradually degrades the patient's mental abilities (1).

This disease, People often lose the ability to store information, speech power, recognize time. Eventually, death occurs due to respiratory problems (2).

Alzheimer's disease has been the leading cause of death in European and American countries after stroke, cancer, and heart disease in the last 20 years (3). The major component of the brain is the hippocampus of humans and other vertebrates. The Humans and other mammals have two hippocampi, one in each side of the brain. The hippocampus is part of the limbic system and it plays an important role in the

formation, organization, and maintenance of memory (4).

The disease likely develops from multiple effects, such as stress oxidative, inflammation of brain tissue, decrease in acetylcholine, a decrease of dopamine neurons (5).

In Alzheimer's disease, with the loss of neuron synapses in some areas of the brain, necrosis of brain cells in different areas of the nervous system, the formation of spherical protein structures called aging plaques outside the neurons of some areas of the brain and protein structures of its fibers in neuronal soma appears (6).

Dopamine (DA) is an organic compound of the catecholamines and *phenethylamines* family, which plays an essential role in the body and brain. Dopamine is synthesized from its precursors in the brain and kidneys, also in many plants and animals. It acts as a neurotransmitter in the brain and a hormonal agent in the blood.

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Also, dopamine is stored mainly on dopaminergic nerve cells in the adrenal glands (7).

Dopamine plays the most important role in creating pleasure and reward. The increase in this substance in certain areas of the brain is known as the reward center, causes a person to feel euphoria (rewarded), which is why dopaminergic drugs are largely abused. Dopamine is also involved in the control of the motor system, causing the emergence of symptoms such as Parkinson's and schizophrenia (psychosis) with the elimination of dopaminergic neurons or dysfunction of the dopamine system (8).

The secretion of dopamine decreases with growing old of cells, making it difficult to transfer information and correlation between cells. This problem is most evident in memorization, so that are forgotten memories and fade (9).

There has also been a lot of research on oral supplements such as royal jelly that have had a positive effect on many diseases. For example, royal jelly is a viscous substance secreted by young worker bees, on which beehive larvae feed. The worker larvae feeding on royal jelly stops since the third day of growth, but the larvae selected to become queens still go on to feed on this jelly. Queen bees aren't born queens but become queens based on postpartum nutrition. Feeding on royal jelly is the cause of larger size, high fertility, and longer life of the queen (10).

Royal jelly contains 12-15 percent of protein, 10-12 percent of carbohydrates, 3-7 percent of lipids, minerals, and vitamins. Recently, research has shown that royal jelly has a variety of biological activities in cells and tissues of the body, especially the nervous system. Royal jelly contains phosphorus compounds, especially acetylcholine, which is one of the carriers of neural messages from one cell to the next cell (11).

In a recent study by Zamani *et al.* on the "effects of royal jelly on learning and memory in rats after injecting streptozotocin into the lateral ventricles of the brain", it had been found that regarding memory problems, royal jelly had a positive effect on preventing and alleviating the Alzheimer's disease (12).

Many studies have shown that physical activity changes the release of endogenous opioids, neurotransmitters such as dopamine and acetylcholine in the brain. In this respect, researchers such as Arazi *et al* found that hybrid exercise can raise blood dopamine levels and

improve health-related fitness indicators in men addicted to methamphetamine. exercise also can be useful as a treatment during rehabilitation (9). Also, it has been specified that exercise can reduce symptoms of Alzheimer's disease. That is, it increases the neurogenesis of the hippocampus and prevents memory loss (2). However, in an 8-12-week study, exercise had no significant difference effect on dopamine concentration (13). The purpose of this study was to investigate the effect of endurance training with royal jelly consumption on dopamine in the hippocampus tissue of Alzheimer's rats with trimethyltin.

Materials and Methods

In this experimental study, 30 rats (5-6-month old male Wistar) were prepared from the center of Islamic Azad University of Shiraz, Marvdasht Branch, and transferred to the physiology Laboratory of this unit. They weighed 230-300 g at the beginning of the study. Rats were kept for one week in a special cage in optimum (21 to 23 ° C) with a relative humidity of about 54 to 64 Percent, 12-hour darkness-light cycle under standard conditions. They also had adequate access to water and food.

Then 24 rats underwent intraperitoneal injection of 8 milligrams/kilograms trimethyltin chloride (14); and after ensuring Alzheimer's disease were divided into groups of 6 subjects: control, training, royal jelly consumption, and training with royal jelly consumption. With the appearance of Alzheimer's symptoms such as seizures, an increase in body temperature, nausea, and tail twisting of rats and to evaluate the effects of Alzheimer's disease on rats, 6 rats were included in the healthy control group. The training groups were active on the treadmill for 8 weeks, 3 sessions per week, and 60 minutes each session. The royal jelly consumption groups received 100 milligrams/kilograms royal jelly per day peritoneally for 8 weeks.

The exercise group of rats first ran on the treadmill speed of 8 meters per minute with zero slopes for 10 minutes for warm-up; then, the rats ran at a speed of 16 meters per minute in positive upward slope, afterwards, the rats started the exercises at a speed of 15 centimeters per second in the first week at +15 percent slopes. After 4 weeks, 5 centimeters per second is added to the treadmill speed each week (16). While the Royal Jelly Group received a dose of 100 milligrams/kilograms (17) daily for eight weeks. Almost 48 hours after exercise, rats were

anesthetized with ketamine 10 percent (50 milligrams per kilogram dose) and xylazine two percent (ten milligrams per kilogram dose) after approximately five minutes. Then, after extracting the hippocampus tissue, they were stored in a special device at -17°C for further investigation (15).

To analyze gene expression, Pidgin Iran Company, first extracted RNA from hippocampal tissue in the brain. Then, the concentration and

degree of purity of the RNA sample drawing on the light absorbance property at a wavelength of 260 nm, was quantitatively obtained using the special equation. After all RNA samples were extracted and by taking CDNA synthesis, was used reverse transcription reaction. Then genes were examined by the quantitative q-RT PCR method. Also, B2m was selected as a housekeeping gene. Table 1 shows the sequence of primers used.

Table 1. Reference genes and Sequence of forward-reverse primers of Dopamine for Real-time PCR reaction

Gene	Forward (5'-3')	Reverse (5'-3')	Product Size, bp
B2m	5'- CGTGCTTGCCATTCAGAAA -3'	5'-ATATACATCGGTCTCGGTGG-3'	244
Dopamine	5'- CGGTACCTCATCGCTGCATA -3'	5'- AAACACTGTTGCAATGCCCC -3'	210

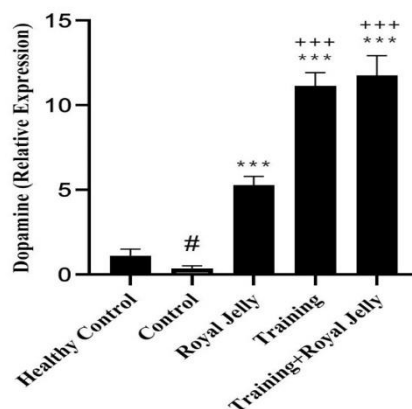
To examine the normal distribution of data, the Kolmogorov-Smirnov test, and Tukey's post hoc tests were used to analyze the findings. ($p \leq 0.05$).

Results

As the expression level of the dopamine gene is presented in Figure 1. The results of the one-way ANOVA test showed that dopamine gene levels in the control group were significantly lower than the healthy group. ($P = 0.04$), however, dopamine gene levels in the groups of training on Positive Slopes, royal jelly consumption, and training on

Positive Slopes with royal jelly consumption groups were significantly higher than the control group. Also, dopamine gene expression levels in the training (Positive Slopes) and training (Positive Slopes) with royal jelly consumption groups were significantly higher than the royal jelly consumption group alone.

($P = 0.001$) (Figure1). The PCR product is shown in Figure 2.



$P < 0.05$ decrease of significant compared to the healthy control group

*** $P < 0.001$ increase of significant compare to control group

+++ $P < 0.001$ increase of significant compare to the royal jelly group

Figure 1. In the hippocampus tissue of rats study dopamine gene expression levels in four groups

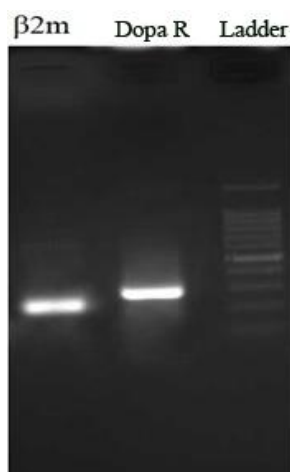


Figure 2. Electrophoresis of dopamine gene expression in the rats PCR method

Discussion

The purpose of this study was to Analysis the effect of endurance exercise (with positive upward slope) with royal jelly consumption on dopamine in the hippocampus tissue of Alzheimer's rats with trimethyltin.

In this research, it was shown that the induction of Alzheimer's with trimethylation toxin led to a decrease in dopamine gene expression in the Brain hippocampus tissue of rats. However, endurance exercise on a positive slope and consuming royal jelly each led to increased levels of dopamine gene in rats with Alzheimer's. Also, there was a significant difference in the levels of dopamine gene expression between the two groups of training with royal jelly consumption and royal jelly consumption alone.

Dopamine is a hormone and a neurotransmitter that is synthesized in plants and most animals. Also In the brain, functions as a neurotransmitter and a hormonal agent in the blood (16). Research has shown that Increasing dopamine to improve memory and spatial learning as well as a sense of euphoria and vitality and play an important role in creating pleasure and reward in individuals. However, dopamine secretion decreases with increasing age and cell aging, and in this way, communication between the cell and the transmission of information becomes difficult. This problem is most evident in the memory and recollection section; therefore, by reduction of dopamine secretion in the brain, the memories fade or are forgotten and it causes dementia (17).

Regarding the mechanism of royal jelly, it has been reported that increasing the amount of royal jelly due to containing phosphorus compounds, especially acetylcholine, which is one of the carriers of neural messages from one cell to the next cell, is likely to increase dopamine and serotonin in the brain and intestines (18).

Pantothenic acid in royal jelly can also affect the chemical compositions of the brain. Pantothenic acid, or vitamin B5, often known as "vitamin stress," stimulates the synthesis of coenzyme A, which is probably needed to produce neurotransmitters such as acetylcholine and stress relievers (19).

The results of this study show that effected of endurance training (with positive upward slope) and consuming royal jelly on the improvement of dopamine gene expression. In line with current research, the results of the study, the results of most researchers such as Rojas *et al.* (2016), (20), Rammes *et al* (2017), (21), and O'dell *et al* (2012), (22) showed that "Alzheimer's induction can lead to a significant reduction in dopamine. Such a decrease in Alzheimer's induction is probably due to impaired or impaired dopamine receptors in the brain, and this decrease may lead to dopaminergic and serotonergic disorders and serotonin and dopamine transmitters" (7).

In line with current research, Compared to the effects of exercise on dopamine, The researchers reported that " Physical exercises had a significant effect on increased dopamine levels" (2), (22).

It seems physical exercises appear to affect levels of dopamine receptors and leads to an increase

in dopamine in the blood. Due to its fatty acid-tryptophan competition function, it increases the absorption of fatty acids in the blood and thus increases dopamine synthesis in the brain (23). It seems likely that in sports activities, especially running on a positive slope, stimulation of muscular spindle receptors and Golgi tendon organs in a positive slope increases the speed of sending nerve impulses by increasing the amount of traction, and these impulses are sent to the spinal cord and thereby to the brain through a variety of mechanisms, including neurogenesis, mood elation, and endorphin release in the brainstem and hypothalamus, where the levels of release of many neurotransmitters such as acetylcholine, dopamine and glutamate change (24).

Exercise may also stimulate angiogenesis by directly affecting the neurotrophic growth factor, identified as a protective factor for dopaminergic neurons, and further stimulate dopaminergic terminals in the positive slope, which can induce an increase in vascular endothelial growth factor it can help improve recover angiogenesis-induced injury and repair of monoaminergic damage to dopamine (25).

In the present study, there are limitations, one of the limitations of the present study is not using different dopamine measurement methods such as ELISA and Western blotting. Therefore, it is suggested that, In future research measure dopamine and protein levels serum. Given that intensity of exercise may play an important role in increasing dopamine levels. This should be an effect of exercise with different intensities in the positive range on the expression level of the dopamine gene considered.

Conclusion

Although training and royal jelly consumption improve dopamine gene expression levels in the brain hippocampus tissue of rats with Alzheimer's disease, the effects of training combined with royal jelly consumption appear to be greater than those of royal jelly alone.

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evaluated Islamic Azad University of Shiraz, the proposal of this research was confirmed by the Marvdasht branch. Approval ID: IR.IAU.M.REC.1398.011.

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