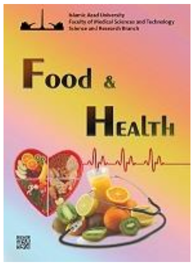
Food & Health 2020, 3(1): 6-10



Food & Health Journal

Journal homepage: fh.srbiau.ac.ir

The effect of endurance training with royal jelly consumption on dopamine in the hippocampus tissue of rats with Alzheimer's disease

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A R T I C L E I N F O

Short Communication

Article history:
Received 21 November 2019
Revised 09 February 2020
Accepted 25 February 2020
Available online 15 March 2020

Keywords:
Endurance Training
Royal Jelly
Dopamine
Alzheimer's disease

A B S T R A C T

Alzheimer's disease is an age-related ailment that affects more and more people every day. It is a type of amnesia with brain dysfunction that gradually degrades the patient's mental abilities. The aim of the present study was to investigate the effect of endurance training with royal jelly consumption on dopamine in the hippocampus tissue of Alzheimer's rats treated with trimethyltin. In this experimental study, 30 rats underwent intraperitoneal injection of 8 mg/kg trimethyltin (TMT) chloride and after ensuring Alzheimer's disease were divided into groups of 6 subjects: control, training, royal jelly consumption, and training with royal jelly consumption. In order to investigate the effects of Alzheimer's induction on dopamine levels, 6 rats were included in the healthy control group. The training groups ran on the treadmill for eight weeks, five sessions a week, and 60 minutes each session. The royal jelly consumption groups received 100 mg/kg royal jelly per day peritoneally for eight weeks. The Kolmogorov-Smirnov, one-way ANOVA, and Tukey's mean comparison tests were used to analyze the findings ($p \leq 0.05$). Alzheimer's induction with trimethyltin toxin had a significant effect on reducing dopamine gene expression 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, training and training with royal jelly consumption had a greater effect on increasing dopamine gene expression levels than royal jelly consumption ($p=0.001$). 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.

1. Introduction

Alzheimer's disease is a type of age-related illness that affects more and more people day by day. It is a type of amnesiac disorder with dysfunction of the brain in which the patient's mental ability is gradually dissipated. The most obvious type of dementia is memory disorder (1). Memory disorder usually develops and progresses gradually. Research shows that this neurodevelopmental disease is one of the main causes of dementia (a condition where the ability to think, understand and remember is impaired); it begins in old age with the loss of information retention power, especially temporary memory, and gradually ends with the loss of the ability to recognize the time, depression, loss of speech power, loneliness and eventually death due to respiratory disorders (2). Alzheimer's disease is currently the most common cause of death in Western countries after heart disease, cancer, and stroke (3). In the living beings' brain, there is a special memory system, called the hippocampus, which is responsible for learning and storage of vital information and is a structure for learning and memories (4). The hippocampus (also known as the seahorse due to its resemblance to the seahorse) is a curved neural structure in the brain located at the base of the middle horn of the lateral ventricle of the brain. The seahorse is a structure of a lateral system that is shaped like a seahorse and is located deep in the temporal lobe (5). Various factors such as oxidative stress, glutamate-dependent toxicity, decrease in acetylcholine and serotonin, decrease or disappearance of

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dopamine neurons and inflammation of brain tissue are involved in the development of Alzheimer's disease (6). 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 (7). Various factors such as oxidative stress, glutamate-dependent toxicity, decrease in acetylcholine and serotonin, decrease or disappearance of dopamine neurons, and inflammation of brain tissue are involved in the development of Alzheimer's disease (6). Dopamine (DA) is an organic compound of the catecholamines and phenethylamines family, which plays a vital role in the body and brain. Dopamine is synthesized from its precursors in the brain and kidneys, as well as in most plants and animals. It acts as a neurotransmitter in the brain and a hormonal agent in the blood. Dopamine is stored mainly in the vesicles of dopaminergic neurons as well as in the adrenal glands (8). Dopamine plays the most important role in creating pleasure and reward. Increasing this substance in certain areas of the brain, 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 (9). With growing old and aging of cells, the secretion of dopamine decreases, making it difficult to communicate between cells and transfer information. This problem is most evident in the segment of retention and memories so that memories are faded or forgotten (10). 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 are not born queens but become queens based on postpartum nutrition. This jelly plays an important role in feeding the queen. Feeding on royal jelly is the cause of larger size, high fertility, and longer life of the queen (11). Royal jelly contains 12-15% protein, 10-12% carbohydrates, 3-7% lipids (including sterols and fatty acids), minerals, and vitamins. Recently, royal jelly has been shown to have 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 (12). 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 was found that regarding memory problems, royal jelly had a positive effect on preventing and alleviating the Alzheimer's disease (13). Another study also examined the jelly and found that royal jelly could improve cognitive processes by having a positive effect on neurological functions and possibly play a significant role in the prevention and treatment of some neurological disorders (14). Several studies have reported that exercise can alter the release of neurotransmitters such as dopamine, glutamate, acetylcholine, serotonin, and endogenous opioids in the brain. In this regard, Arazi et al. (10) found that combined training could advance levels of blood serotonin and dopamine, and improve health-related fitness indicators in methamphetamine-addicted men and could be helpful as a non-pharmacological treatment during rehabilitation; Fang Yu et al. (15) reported that 3 sessions of moderate-intensity cycling aerobic training for 6 months could prevent memory loss. It has also been reported that aerobic training can reduce symptoms and disease, increase hippocampal neurogenesis, and stop memory loss in animal models (2). However, in an 8-week study aerobic training had no significant effect on dopamine concentration (16). Therefore, considering the contradictory results of the research conducted and the possible effects of training on a positive slope on preventing the progression of Alzheimer's and also considering that no study has been performed on training and royal jelly consumption, the aim of this study was to investigate the effect of endurance training on a positive slope on dopamine gene expression in the hippocampus tissue of Alzheimer's rats treated with trimethyltin chloride (TMT).

2. Material and methods

In this experimental study, 24 rats were prepared from the animal breeding and proliferation center of the Islamic Azad University of Marvdasht and transferred to the physiology laboratory of this university. To adapt, rats were kept for one week under standard conditions in transparent autoclavable polycarbonate cages with optimum temperature (20 to 24˚C), the relative humidity of about 55 to 65%,12-hour darkness-light cycle, and had free access to food and water. Researchers received introduction letters from Islamic Azad University, Eslamshahr Branch with number 23121408971002. Then on the eighth day, 18 rats were intraperitoneally injected with 8mg/kg trimethyltin chloride (TMT) (15); after 24 hr, being assured of the complete effect of TMT on the hippocampus, (Alzheimer's symptoms were observed by some behavioral symptoms in rats. These clinical symptoms were included: 1) muscle tremors, 2) elevated body temperature, 3) nausea, 4) seizures, and 5) tail twists), rats with Alzheimer's disease were randomly divided into three groups of 6 subjects, including: (1) control, (2) training group, (3) royal jelly, (4) training with royal jelly consumption. It is noteworthy that to investigate the effects of Alzheimer's induction on dopamine levels, 6 rats were placed in the healthy control group. To train, the training groups ran on the treadmill for one week, three sessions per week, at a speed of 5 to 10 m / min for 5 to 10 min. Then, the rats in the positive training group (at a speed of 16 m / min in positive upward slope) performed endurance training for four weeks, five sessions per week, and 60 minutes per session (16). To perform training, rats initially ran on the treadmill with zero slopes for 5 min at a speed of 8 m/min for warm-up; afterward, the training group performed training at a speed of 15 cm/s in the first week at +15% slopes, adding 5 cm/s to the treadmill speed each week. At the end of each training session, rats cooled again for 5 min at 8 m/min on the treadmill with zero slopes. Training were performed such that rats trained for one hour as the sum of warm-up and cool-down. While the Royal Jelly Group received a dose of 100 mg/kg (17) daily for eight weeks. 48 hours after the last training session, rats were anesthetized with ketamine 10% (50mg/kg dose) and xylazine 2% (10mg/kg dose) after approximately 5 minutes. The hippocampus tissue was then extracted by specialists and after setting in a cryotube was placed in liquid nitrogen and stored at -70˚C for further investigation (17). For molecular analysis at the gene expression level, first, extraction of RNA from the hippocampus tissue was carried out according to the manufacturer's protocol (Sinagen, Iran), then, drawing on the light absorbance property at the wavelength of 260 nm, the concentration and degree of purity of the RNA sample was quantitatively obtained using the following equation:

$$C\ (\mu g/\mu l) = A260 \times \varepsilon \times d/1000$$

After extracting RNA with high purity and high concentration from all of the samples, cDNA synthesis steps were taken according to the manufacturer's protocol, and then the synthesized cDNA was used for reverse transcription reaction. Initially, the designed primers for genes were examined, and then gene expressions were examined by quantitative q-RT PCR method. It should be noted that *B2m* was selected as the housekeeping gene. The sequence of primers used is shown in Table 1. To examine the normal distribution of data, the Kolmogorov-Smirnov test, and to analyze the findings, one-way ANOVA along with Tukey's comparison of means were used (p≤0.05).

Table 1. The sequence of forward-reverse primers of Dopamine and reference genes for the real-time PCR reaction.

Gene	Forward (5'-3')	Reverse (5'-3')	Product Size, bp
B2m	5'-CGTGCTTGCCA TTCAGAAA -3'	5'-ATATACATCGG TCTCGGTGG-3'	244
Dopamine	5'-CGGTACCTCAT CGCTGCATA -3'	5'-AAACACTGTTG CAATGCCCC -3'	210

3. Results

Dopamine gene expression levels are shown in Fig.1. The results of one-way analysis of variance showed a significant difference in the levels of dopamine gene expression in the four groups of research (p=0.001, F=9.01). The results of Tukey's post hoc test showed that dopamine gene expression levels in the control group were significantly lower than the healthy group (p=0.04), however, dopamine gene expression levels in the training, royal jelly consumption, and training with royal jelly consumption groups were significantly higher than the control group. Also, dopamine gene expression levels in the training and training with royal jelly consumption groups were significantly higher than the royal jelly consumption group alone. (p=0.001) (Fig. 1). A figure of PCR product presented in Fig. 2.

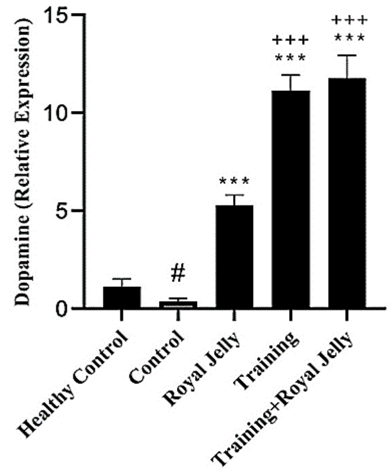

Fig. 1. Dopamine gene expression levels in the hippocampus tissue of rats in four groups of study. # p<0.05 significant decrease compared to the healthy control group. *** p<0.001 significant increase compared to the control group. +++ p<0.001 significant increase compared to the royal jelly group.

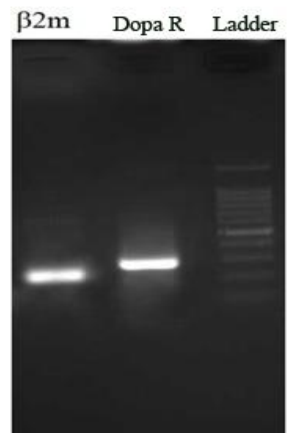

Fig. 2. Electrophoresis of serotonin gene expression in rats Real Time PCR method.

4. Discussion

The aim of this study was to investigate the effect of endurance training along with royal jelly consumption on dopamine in the hippocampus tissue of Alzheimer's rats treated with trimethyltin. The results of the present study showed that the induction of Alzheimer's with trimethylthin toxin led to a decrease in dopamine gene expression in the hippocampus tissue of rats. However, running on a positive slope and consuming royal jelly each led to increased levels of dopamine gene expression 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 synthesized from its precursors in the brain and kidneys, as well as in most plants and animals. It acts as a neurotransmitter in the brain and a hormonal agent in the blood. Dopamine is mainly stored in the vesicles of dopaminergic neurons as well as in the adrenal glands (18). Increasing dopamine has been reported 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 (19). 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 (20). 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 (21). The results of the present study indicate a significant effect of running on a positive slope and consuming royal jelly on the improvement of dopamine gene expression. Consistent with the findings of the present study, the results of the study by Tapia-Rojas et al. (22), Rammes et al. (23), and O'dell et al. (24) revealed that Alzheimer's induction can lead to a significant decrease in dopamine. Such a decrease in Alzheimer's induction may result in impairment of dopaminergic and serotonergic probably due to disturbance in the dopamine receptors in the brain or their damage, and this may decrease the serotonin and dopamine transmitters (8). Concerning the effects of exercise on dopamine in line with the findings of the present study, Avila et al. (1), García-Mesa et al. (2), and O'dell et al. (24) reported that exercise activities had a significant effect on increased dopamine levels. Exercise activity seems to specifically influence specific levels of serotonin and dopamine transmitters that increase fatty acid recruitment and, thus, the level of free tryptophan in blood increase due to the fatty acid-tryptophan competition in binding to albumin. Thus, the rate of dopamine synthesis in the brain increases and rises (25). 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 dopamine, glutamate, and acetylcholine change (26). 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 and it may contribute to recover angiogenesis-induced injury and have a direct effect on neurotrophic growth factor, leading to the restoration and repair of monoaminergic damage to dopamine and serotonin (27). Given that the intensity of exercise activity may play an important role in increasing dopamine levels, it is suggested that in future studies, the effect of exercise training with different intensities in negative and positive slopes on dopamine gene expression levels should be considered. Besides, the lack of use of various methods of measuring dopamine such as ELISA and western blot are of the limitations of the present study. Therefore, it is recommended that future studies measure dopamine levels at the levels of gene and protein expression.

5. Conclusion

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 alone.

Acknowledgments

Given that the present research is the result of a doctoral dissertation approved by Islamic Azad University, Islamshahr Branch, the authors appreciate the spiritual support of the Research Office of this academic branch.

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