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Interactive Effects of Endurance Training With Royal Jelly Consumption on Motor Balance in an Experimental Encephalomyelitis Model

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ABSTRACT

Introduction: Multiple sclerosis (MS) is a chronic inflammatory demyelinating factor of the central nervous system that leads to decreased balance and increased risk of falls. The aim of this study was to investigate the interactive effects of endurance training (T) with royal jelly (RJ) consumption on the motor balance of rats with MS.

Methods: In this experimental study, 56 rats with MS (using complete Freund's adjuvant) were divided in 7 groups of eight animals, including: 1) control (MS), 2) Sham (royal jelly solvent), 3) 50 mg/kg RJ, 4) 100 mg/kg RJ, 5) T, 6) T+RJ50, 7) T+RJ100. Rats in the royal jelly consumption groups received the prescribed doses of royal jelly peritoneally each day for 5 weeks. Also, rats in the endurance training groups performed endurance training on a rat treadmill for five weeks, five sessions per week, each session 30 minutes at a speed of 11 m/min. At the end of 48 hours after the last training session and royal jelly consumption, the motor balance of rats was measured using a rotarod device. Also, the weight of brain cerebellum tissue was measured by a digital scale. The one-way analysis of variance with Tukey's *post hoc* test were used to analyze the findings ($P \leq 0.05$).

Results: The duration of motor balance in the T, T + RJ50 and T + RJ100 groups was significantly higher than the MS group ($P = 0.001$); also, in the T + RJ50 and T + RJ100 groups, it was significantly higher than the RJ50 and RJ100 groups ($P = 0.001$)

Conclusion: It appears that training and royal jelly consumption have an interactive effect on improving motor balance, and improving motor balance is training-dependent. Given the existence of effective physiological mechanisms, it seems necessary to conduct further studies by examining the pathological and physiological aspects.

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating factor of the central nervous system that is associated with inflammation of tissues and apoptosis of nerve cells . The prevalence of this disease in women is 2 to 3 times that of men and it often occurs in the age group of 20 to 40 years (1). MS is the most common neurodegenerative autoimmune neurological condition in young adults and affects more than 2.3 million people worldwide. Due to the high probability of disability and decreased nerve function, there are concerns about the role of exercise in MS patients with a fear of increased injury (2). MS is a multifocal inflammatory disease of the brain and spinal cord. The underlying cause of MS is unknown, although genetic and environmental factors have been shown to play a role (3). It is a neurodegenerative disease characterized by the formation of a lesion in the nerve center, inflammation, and destruction of myelin sheaths. Given that patients with MS have poorer balance in most field balance tests than healthy individuals, it seems that maintaining balance in patients with MS is of great importance (4). Although balance is defined as the ability to maintain a position to perform voluntary activities and deal with disturbances (internal and external) and, biomechanically, to maintain the body mass center within the level of reliance, having a natural balance requires coordination between different bodily systems such as the musculoskeletal, visual, atrial, nervous and

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cardiovascular systems. Balance disorder causes a person to reduce their social participation for fear of falling (5). Prescribing exercise is currently considered as a useful treatment strategy to minimize the lack of functional ability in chronic diseases. In this regard, it has been reported that both endurance and resistance training involve balance systems in patients with MS. Both types of training are effective in preventing falls, stimulating deep receptors, increasing muscle strength, flexibility and movement control (6).

Studies have been conducted in this regard, for example, a study by Hosseini et al. (2020) showed that induction of Alzheimer's with trimethyltin disturbed the motor balance of rats. However, performing eight-weeks of regular exercise improved the motor balance time of Alzheimer's rats (7); another study showed that rehabilitation exercise training improved brain function and improved balance and quality of life in patients with MS (Chen, 2021). In addition, the results of another study showed that balance exercises at home increased the quality of gait test, increased balance and improved the quality of life of men and women with MS (8).

Still again, in another study it was shown that aerobic training and Pilates resulted in a significant increase in serum BDNF in patients with MS (9). Studies have shown that proper nutrition along with exercise can have beneficial effects on disorders caused by MS. Royal jelly is a viscous substance secreted by young worker bees, containing 12-15% protein, 10-12% carbohydrates, 7-3% lipids (including sterols and fatty acids), minerals and vitamins. Royal jelly has been shown to have a variety of biological activities in various cells and tissues of the body, especially the nervous system. Royal jelly contains phosphorous compounds, especially acetylcholine, which is one of the carriers of neural messages from one cell to the next. Royal jelly increases oxygen delivery to brain tissue. Another component of royal jelly is 10-hydroxy-trans-2-decanoic acid (HDEA), an unsaturated fatty acid that facilitates neurogenesis by NSCs (Neural stem / progenitor cells). Royal jelly has also been shown to increase oxygen delivery to brain tissue (10).

Studies show that royal jelly also plays a role in improving neuronal cell functions by mechanisms similar to insulin-like growth hormone (IGF-1), growth hormone (GH), and improving neurotrophins (7). In this regard, the study of Hosseini et al. showed that consumption of 100 mg / kg RJ for eight weeks improved the motor balance of rats with Alzheimer's disease (7); royal jelly consumption also reduced muscle atrophy during aging and improved the quality of life of the elderly with MS (11).

Studies have shown that the use of antioxidants in different doses along with exercise training in conditions of neurological disorders can be a good way to improve the patients' health.

It also appears that keeping balance and muscle strength in people with diseases of the nervous system is an important factor in preventing reduced movement and physical activity. Therefore, the use of antioxidants along with exercise training can be a novel idea in the prevention of muscle atrophy and nervous system that needs to be investigated in more details.

In this vein, it seems that a combination of voluntary exercise with royal jelly supplementation can improve functional and behavioral disorders in MS patients (12). Therefore, the present study seeks to investigate the interactive effects of endurance training with royal jelly consumption on motor balance in an experimental encephalomyelitis model.

Material and Method

In this experimental study, fifty-six Sprague-Dawley female rats with an age range of 8-10 weeks and a weight range of 220-2200 g were provided from the Laboratory Animal Breeding Center of Marvdasht Islamic Azad University and kept in the laboratory for one week for adaptation.

During the research period, the animals were kept in standard conditions of light (12:12 hour dark-light cycle), temperature (22-24 ° C), and humidity (55-60%). Also, in this study, ethical considerations according to the ethical principles of working with animals of Marvdasht University and the Helsinki Agreement were observed.

On the eighth day, 20 guinea pigs were prepared from the Iranian Pasteur Institute to induce EAE. After anesthesia, the spinal cord of guinea pigs was extracted for use as antigen and immediately immersed in nitrogen tank and crushed. For homogenization, spinal cord tissue was mixed with an equal amount of normal saline and placed in a shaker at 5° C.

The homogenized solution was then converted to emulsion solution in a 1: 1 ratio with complete Freund`s adjuvant (CFA). To prepare this suspension, two glass syringes were connected by a stainless steel interface and equal amounts of adjuvant and homogenized spinal cord solution were used in two syringes.

After complete anesthesia of rats with ketamine and xylazine, 400 µl of the antigen and adjuvant mixture was injected subcutaneously in the dorsal and 100 µl into the cushion area of each animal with needle number 25 (1,13).

To diagnose induction of the disease, the daily morbidity process was evaluated and the morbidity scale was assessed based on the following: zero: no disease, 1: tail movement disorder, 2: tail paralysis, 3: gait disorder, 4: one-leg paralysis, 5: two-leg paralysis, 6: paralysis of all legs and hands, and 7: death (Mousavi, 2018; Abedi, 2017).

After ensuring EAE induction, 49 rats with EAE were divided into seven groups, including: (1) MS control, (2) sham, (3) 50 mg / kg royal jelly (RJ50), (4) RJ100, (5) aerobic training (T), (6) T + RJ50, and (7) T + RJ100. Also, 8 healthy rats were included in the healthy control (HC) group to investigate the effects of EAE induction on the research variables. Rats in the RJ groups, received peritoneally royal jelly from the Marvdasht Agricultural Jihad Center for 5 weeks daily at doses of 100 mg / kg and 50 dissolved in normal saline (14).

Also, rats in the training groups performed endurance training at a speed of 11 meters per minute on a special treadmill for five weeks, five sessions per week and each session for 30 minutes. The endurance training protocol started approximately 10 days after induction of the MS experimental model. To perform endurance training, first the rats were introduced to the treadmill every day for 5 to 25 minutes at a speed of 6 meters per minute and an inclination of 11 degrees for a week, then they performed endurance training at a speed 11 meters per minute every day for 30 minutes, for 5 weeks. One of the reasons for choosing this training protocol was the neuroprotective effects of this type of training on rats and mice with cognitive impairments in the experimental model of Parkinson's and encephalomyelitis (EAE) (15,16).

At the end of 48 hours after the last training session and royal jelly consumption, the motor balance of rats was measured using a rotarod device. Also, 24 hours later, rats were anesthetized with ketamine and xylazine and the cerebellar tissue of rats was extracted and measured by digital scales. In order to measure the motor balance, rats were first placed on the rotarod device for 3 minutes for familiarization and were trained to move on it according to the main protocol (at a rotation speed of 10 rpm with acceleration of 7 rpm^2) and 30 minutes later, the balance test was performed (at a speed of 10 rpm with acceleration of 7 rpm^2). The duration of the animal resistance to maintain balance on the wheel was recorded in seconds. The total duration of this test was considered to be 300 seconds. This test was measured three times for each rat with repeated intervals of approximately 30 minutes and their mean was calculated for analysis.

Statistical Analysis Procedure

The Kolmogorov-Smirnov test and one-way analysis of variance with Tukey’s *post hoc* test were used to analyze the findings (P≤0.05).

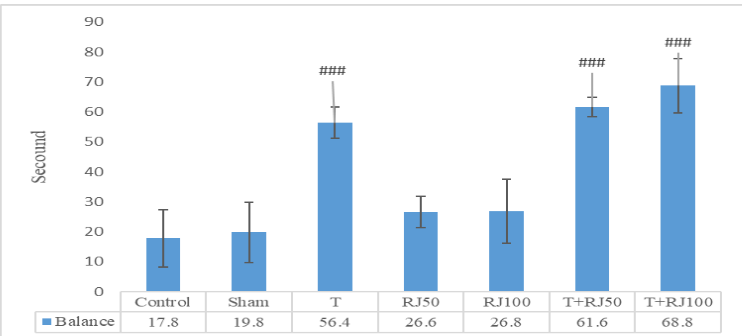

Figure 1. Balance levels in the research groups
(P≤ 0.001) Significant increase compared to the control, sham, 50 mg / kg royal jelly consumption, and 100 mg/kg royal jelly consumption groups

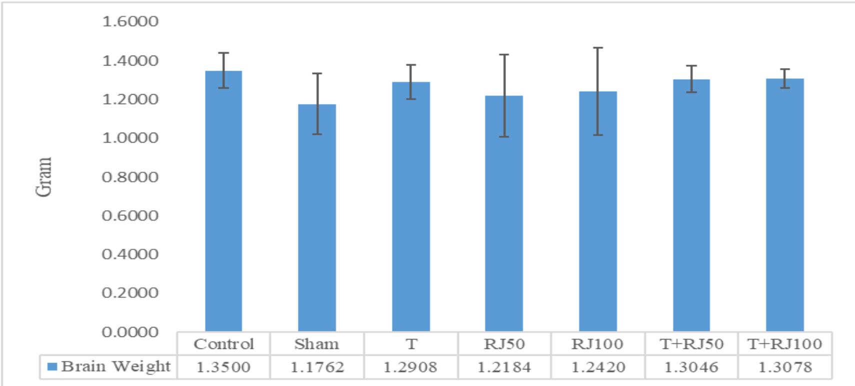

Figure 2. Brain weight levels in the research groups

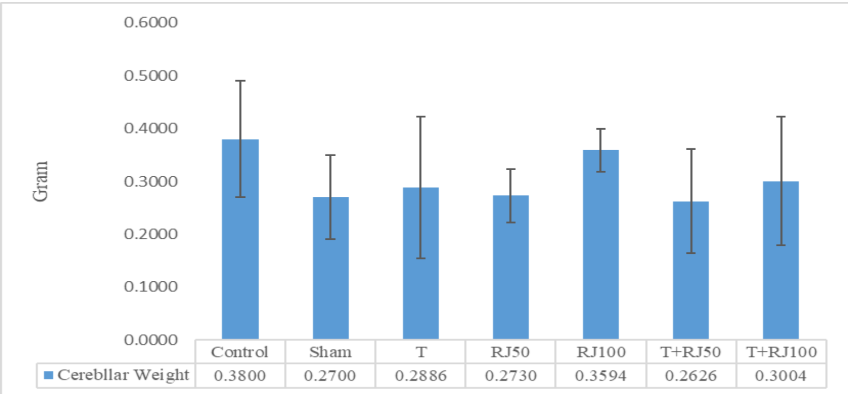

Figure 3. Cerebellum weight levels in the research groups

Results

The mean and standard deviation of motor balance, brain and cerebellum weight are reported in Figures 1 to 3, respectively. The results of one-way analysis of variance showed that there was a significant difference in the amount of motor balance in the 7 groups of the study (P = 0.001). However, there was no significant difference in the weight of the brain (P = 0.50) and cerebellum (P = 0.37) in the 7 research groups. The results of Tukey's *post hoc* test showed that the balance levels in the training, training + 50 mg / kg royal jelly consumption and training + 100 mg / kg royal jelly consumption groups were significantly higher than the control, sham, 50 mg / kg royal jelly consumption, and 100mg / kg royal jelly consumption groups (P = 0.001) (Figure 1).

Discussion

The results of the present study showed that 5 weeks of training improved balance in rats with MS, however it had no significant effect on the brain and cerebellum weight of rats with MS.

Studies show that with increasing inflammatory and oxidative factors, damage occurs to various parts of the central nervous system, so that these factors with damage to regions such as the motor cortex and pre-motor decrease motor power, decline the ability to perform more subtle actions, and decrease motor balance in patients with neurological disorders (7); in addition, aging and impaired physical fitness, weight gain, fat gain, decreased aerobic capacity, systemic inflammation, and nerve cell impairment can cause damage to motor neurons; also, systemic inflammatory factors even on the motor plate can disrupt the function of neurotransmitters and affect the patient's balance (7,17). On the other hand, using neurotoxins and modeling neurological disorders with toxins such as trimethyltin, adjuvant and myelin oligodendrocyte glycoprotein with increased oxidative stress, increased inflammatory factors, decreased neurotrophins, degradation of myelin and finally degradation of neurotransmitters in animal model may bring about disorders of the central and peripheral nervous system, cause chronic pain and decrease balance (7,17).

Regarding the effects of exercise on MS patients, Perese Kila *et al.,* (2017) showed that regular moderate to high intensity exercise consistently attenuates the progression and pathological symptoms of EAE, thus indicating an important non-pharmacological intervention for the improvement of immune diseases such as MS (18). Also, the findings of the studies of Kargarfard and Shariat (2017) and Divasahaiam *et al.,* (2021) indicate the favorable effects of exercise on improving balance in patients with MS (9,19). In fact, sports activities improve the balance in these patients by improving the nervous system and prevent the occurrence of most disorders as well as problems of this disease such as falls. In this regard, Papalia *et al.,* (2020) showed that physical exercise is an effective treatment to improve balance and reduce falls in the elderly (20). Khani and Kazemi (2021) also reported that regular exercise balances the activity of the sympathetic nerves (21). In other words, it modulates the hypothalamic-pituitary-adrenal (APH) axis response and leads to increased adaptation mechanisms. Smith *et al.,* (2020) found that in MS, there is an imbalance between the levels of proinflammatory and anti-inflammatory cytokines, which is associated with higher levels of proinflammatory cytokines associated with demyelination (22). Physiological benefits of exercise include reduced levels of reactive oxygen species (ROS), modulation of cellular redox, reduction of neuroinflammation, reduction of inflammatory factors such as tumor necrosis factor alpha (TNF-α), interleukin-1 alpha (IL-1α), as well as increased brain-derived neurotrophic factor (BDNF). Also, the results of the present study showed that 5 weeks of 50 and 100 mg/kg royal jelly consumption had no significant effect on improving motor balance, brain and cerebellum weight of rats with MS. Regarding the therapeutic effects of RJ, it has been reported that royal jelly consumption can improve the liver enzymes of patients with MS (23). In addition, Deh Bozorgi *et al.,* (2020) showed that royal jelly consumption has a significant effect on increasing NGF gene expression in the hippocampal tissue of rats with Alzheimer's disease (24).

Royal jelly is mainly composed of proteins, sugars, fats (including sterols and fatty acids) and small amounts of mineral salts and vitamins. These substances have been shown to exhibit different pharmacological activities such as antitumor, antimicrobial, vasodilator, antihypertensive as well as growth stimulant, infections resisting, anti-hypercholesterolemic and anti-inflammatory activities. 10-Hydroxy-trans-2-decanoic acid (an unsaturated fatty acid) has been shown to be one of the fats in royal jelly, mimicking the effects of BDNF and possibly stimulating neurogenesis in the adult brain. Royal jelly is said to contain 10-hydroxy-2-decanoic acid (10-HDA). Because 10-HDA is an unsaturated fatty acid, it can cross the blood-brain barrier. 10-HDA has been reported to mimic the effects of BDNF and possibly stimulate neurogenesis in the brain (24).

In this regard, a study by Hosseini et al. (2020) showed that eight weeks of royal jelly consumption increased the motor balance of neurotoxin trimethyltin-induced Alzheimer's rats (7). Neurotoxin-induced damage and varying effectiveness of antioxidant supplements seem to be the reasons for the discrepancies in the results. Because adjuvant, with the mechanism of inflammatory factors, leads to the destruction of myelin, but trimethyltin has a role in neurological disorders by increasing the oxidative stress. However, this should be given more attention by future researchers. In addition, due to the insignificance of brain and cerebellum weight following the interventions of the present study, it seems that the period of this study was not sufficient for general and structural changes. None the less, regarding the interactive effects, the present study showed that although training + 50 mg/kg royal jelly consumption as well as training + 100 mg / kg royal jelly consumption had no significant effect on the brain and cerebellum of rats with MS, they had a significant effect on improving balance in rats with MS. Also, training + 50 mg / kg royal jelly consumption as well as training + 100 mg/kg royal jelly consumption had a greater effect on improving balance in MS rats than consuming 50 and 100 mg / kg royal jelly. Therefore, it seems that training with royal jelly consumption has interactive effects on improving motor balance in MS patients. Research limitations include the lack of ability to control physical activity outside of training time and failure to calculate calorie intake and consumption in rats during the study period;

therefore, it is suggested that in future studies, in addition to examining the effects of sports activities and royal jelly consumption, the amount of calories received and consumed during the research period should also be calculated.

Given the role of inflammatory factors in myelin destruction in the cerebellum and brain and the affectability of balance on these regions, it appears that the lack of measurement of these factors in the present study is one of the limitations of this study. Therefore, it is suggested that in future studies, in addition to motor balance, inflammatory factors should also be evaluated. Also, considering the role of brain volume and brain tissue weight, as well as due to the pathological and physiological reasons for increasing or decreasing the weight of the nervous system, it seems that lack of pathological measurments such as the percentage of healthy cells in different areas, lack of microscopic examination of oxidative damage, lack of examination of nucleus specificity as well as nerve cell size are some other limitations of this study. Therefore, it is suggested that in future studies, pathological studies be placed next to the field data.

Conclusion

According to the results of the present study, it seems that although training, training + 50 mg / kg royal jelly consumption and training + 100 mg / kg royal jelly consumption lead to improved balance in MS patients, training with royal jelly consumption has interactive effects on improving motor balance in MS and has more favorable effects than using royal jelly alone.

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