The Effect of Aerobic Training with Royal Jelly Consumption on Health Related Anthropometric Markers in an Experimental Autoimmune Encephalomyelitis Model

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\textbf{Abstract}

\textbf{Background and Objective:} Interactive effects of Aerobic Training (AT) and Royal Jelly (RJ) consumption is still not well understood in Multiple Sclerosis (MS). Therefore, the aim of this study was determine the effect of aerobic training with royal jelly consumption on health related anthropometric markers in an Experimental Autoimmune Encephalomyelitis (EAE) model.

\textbf{Material and Methods:} 49 EAE induced by complete Freund’s Adjuvant, model female Sprague-Dawley rats were assigned to 7 groups: (1) EAE, (2) sham (Sh), (3) RJ50 mg/kg, (4) RJ100, (5) AT, (6) AT+RJ50, (7) AT+RJ100. Also, 7 healthy rats were put in the healthy control group (HC). AT was performed for five weeks, four sessions per week at a speed of 11-15 m/min for 30 minutes and RJ was IP injection for 50 and 100 mg/kg/day.

\textbf{Results:} In the AT, AT+RJ50, AT+RJ100 and RJ100 groups, weight, visceral fat weight and aerobic power were higher than the EAE group (P≥0.05); in the RJ50 group, weight and in the RJ100 and AT+RJ100 groups, BMI was lower than the EAE group (P≥0.05). AT+RJ100 reduced food intake compared to the EAE group (P≥0.05); also, aerobic power in the AT, AT+R50 and AT+RJ100 groups was higher than the RJ50 and RJ100 groups (P≥0.05).

\textbf{Conclusion:} Generally AT and RJ alone improved anthropometric markers in EAE model; also interaction of AT and RJ was dependent of dosage of RJ so that the effect of 100 mg/kg dosage was higher than 50 mg/kg.

\textbf{Keywords:} Exercise [MeSH], Royal Jelly [MeSH], Food [MeSH], Encephalomyelitis [MeSH].
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**Introduction**

Today, evidence shows that immune system defects in the central nervous system are associated with a wide range of physiological and pathological disorders in the function of neurons and cause many diseases such as Multiple Sclerosis (MS), Parkinson's, Huntington, Alzheimer's, etc. (1). Also, following autoimmune encephalomyelitis, neuronal damage in different areas of the brain can reduce memory, cognition, depression, anxiety, psychotic symptoms and reduce the quality of life in these patients by damaging the limbic and extra-limbic parts (2). But the most common of these autonomic disorders is MS, which researchers said that it is an inflammatory disease of the central nervous system that currently affects 2.3 million people worldwide (3). Several pathological factors simultaneously destroy the autonomic nervous system and expose these people to several other diseases such as depression, anxiety, sleep disorders, fatigue, severe headaches, migraine and peripheral nervous system disorders (4) With increased inflammation in (this patients) neurotransmitter disorders are associated with chronic fatigue and further this fatigue is associated with decreased physical activity, which in turn leads to weight gain, obesity, changes in lipid profile, decreased motor ability, increased risk of cardiovascular disease, muscle weakness and decreased quality of life (5, 6). Previous studies have reported a decrease in the physical and mental health of these people (7) and the progression of the disease is associated with physical disabilities, weight gain, loss of body mineral mass and cardiovascular health (8). However, many advances have been made in pharmacological and non-pharmacological treatment methods in these patients, the multidimensionality of this disease and the irreversible side effects of synthetic drugs in this disease have led researchers to perform non-invasive and safe methods for prevention and improving the health of these people. Therefore, physical activity has long been introduced by the World Health Organization as a desirable way to improve physical and psychological disorders in patients with MS and the elderly (9, 10). As a result, researchers have shown that endurance, continuous, interval exercise and resistance training are associated with improved body composition of these patients (9): In a review study, Misse et al. stated that although research is needed on fatigue and pain after exercise, nevertheless regular and long-term exercise has beneficial effects on muscle strength, aerobic strength, and endurance in people with systemic autoimmune myopathy (11); also, it has been showed that high intensity interval training and high intensity continuous training increased muscle mass, decreased fat mass, promoted muscle strength, VO2max, (12) neurotrophins and amended heart disease markers in patients with MS (13). Despite the favorable role of physical activity in these patients, it seems that genetic factors, gender, type of diet are also influential factors in the health of patients (with autoimmune) (14). Nowadays, the use of antioxidants in the treatment of diseases has been considered by researchers, so that researchers believe that natural antioxidants can improve cell function and resistance of nerve cells against neuronal disorders- induced oxidative stress via strengthening the enzymatic and non-enzymatic antioxidant system (15, 16). Consequently, the use of natural antioxidants along with exercise is recommended for neurodegenerative disorders (10). Among these natural antioxidants royal jelly, which is produced from the bee's submandibular glands, contains isoflavones, flavonoids, phenols and amino acids with strong antioxidant effects that can reduce oxidative stress, inhibit systemic inflammation, improve fat metabolism, increase skeletal muscle volume, and cause favorable
changes in body composition (17). In this regard, researchers have shown the effect of royal jelly on reducing chronic pain, increasing motor balance, reducing inflammatory factors in the muscle tissue of rats with central nervous system disorders such as Alzheimer (15-18); royal jelly also reduced inflammatory factors and improved immune function in patients with MS (19). Therefore, the use of royal jelly along with physical activity has recently been considered by researchers in diseases of the central nervous system; for example, exercise along with royal jelly consumption increases motor balance, reduces inflammatory factors, improves neurotrophins in rodents with Alzheimer disease (15-18). Also, aerobic training and royal jelly consumption reduced inflammatory factors in patients with MS (19). Although the role of exercise training and royal jelly consumption in diseases of the nervous system and MS has been investigated, the subjects’ limitations, differences in the extent of motor disorders, and inability to perform physical activity have limited detailed studies in human specimens. In addition, the limitation of accurate information about the effect of aerobic activity along with the use of royal jelly on body composition, aerobic power and caloric intake in these patients enhances the need for such studies. Thus, the aim of this study was to investigate the effect of five weeks of aerobic training with royal jelly consumption on body composition, aerobic power and food intake in the animal model of experimental autoimmune encephalomyelitis.

Materials and Methods

• Maintenance of laboratory animals

In this experimental study, 63 female Sprague-Dawley rats with an age range of 8-10 weeks and a weight range of 180-220 g were prepared from the Laboratory Animal Breeding Center of Marvdasht Islamic Azad University and were kept in the laboratory for a week for adaptation after transferring to the Animal Sports Physiology Laboratory of this university. It is noteworthy that all the ethical principles of working with animals in this study were done according to the ethical principles of working with animals at Marvdasht University and the Helsinki Convention. During the study, the animals were kept in standard conditions in terms of light (12-hour dark-light cycle), temperature (22-24° C), humidity (55-60%) in transparent polycarbonate cages with autoclave capability; also, grated sterile wood was used to change the animal bed and the animals had free access to water and food throughout the study.

• Induction of EAE

After the adaptation period, in order to induce EAE, 20 guinea pigs were first prepared from the Pasteur Institute of Iran and transferred to the animal laboratory of Islamic Azad University. The guinea pigs were then dissected following anesthesia with ketamine and xylazine and their spinal cord tissue was extracted. The spinal cord tissues of the guinea pigs were immediately immersed in a nitrogen tank and then pounded in a nitrogen-filled mortar. Then, to homogenize the spinal cord tissues, they were mixed with an equal amount of normal saline and placed in a shaker at 5° C until completely homogenized. The homogenized solution was then converted to an emulsion solution at 1:1 ratio with Complete Freund’s Adjuvant (CFA). It is noteworthy that for the preparation of this suspension, two glass syringes were used, which were connected by a steel interface. One of the syringes contained homogenized guinea pig brain and spinal cord and the other syringe contained the same volume of Freund's complete adjuvant, mixed in equal proportions, and became uniform and white, using the solution shaker. After rats’ complete anesthesia with ketamine and xylazine, 400 μl of the antigen and adjuvant mixture was injected subcutaneously in the back and 100 μl into the bolster area of each animal with needle number 25.

To diagnose the induction of the disease, the disease process was evaluated daily and was scaled as follows: 0: no disease, 1: tail movement disorder, 2: tail paralysis, 3: gait disorder, 4: one-leg paralysis, 5: paralysis of both legs, 6: paralysis
of all four legs and hands, and 7: death (20, 21). It is noteworthy that 15 days after daily evaluation of the condition of rats, induction of the disease was diagnosed and rats in grades 1 to 3 were included in the study as sample and rats that were unable to perform the activity during research process were removed from the research process. In addition, in order to homogenize the research groups, after diagnosing the degree of disease in rats, the researchers placed the rats in the research groups in such a way that at the beginning of the research, the rats in different groups had the same disease percentage. Also, 4 rats in this study had severe disease and 3 rats in this study died due to the severity of the disease. Finally, the number of samples remained so that 7 rats were placed in each group.

- Grouping and research design

20 days After ensuring the induction of EAE in rats according to the scales and their homogenization based on motor ability and disability scale, 49 rats with EAE were divided into (1) EAE control, (2) sham (Sh), (3) 50 mg/ kg RJ (RJ50), (4) 100 mg/ kg RJ (RJ100), (5) aerobic training (AT), (6) AT + RJ50, (7) AT + RJ100. It is also noteworthy that 7 healthy rats were included in the Healthy Control (HC) group to investigate the effects of EAE induction on the research variables. Rats in the royal jelly consumption groups received doses of the prescribed royal jelly (dissolved in normal saline) peritoneally per day for 5 weeks (22). Also, rats in the endurance training groups performed endurance training on a rat treadmill for 5 weeks, five sessions per week and each session for 30 minutes at a speed of 11 meters per minute (23, 24).

- Aerobic training protocol

Endurance training began approximately 10 days after induction of the MS experimental model. The rats performed endurance training on a rat treadmill for one week every day for 5 to 25 minutes at a speed of 6 meters per minute, and a slope of 11 degrees, and then they performed endurance training for 5 weeks, every day at speed 11 meters per minute for 30 minutes. One of the reasons for choosing this training protocol is the neuroprotective effects of this type of training in mice with cognitive impairment and rats with the experimental model of Parkinson's And Encephalomyelitis (EAE) (23, 24).

- Consumption of royal jelly

To consume royal jelly at doses of 100 and 50 mg/ kg during five weeks, the daily required royal jelly prepared from Marvdasht Agricultural Jihad Center was dissolved in normal saline and was then injected peritoneally into rats (22).

- Maximum running speed (aerobic power)

To determine aerobic power or maximum speed, rats ran on a treadmill for 5 minutes at a speed of 8 m / min to warm up. Then they ran for 8 minutes at a speed of 10 to 15 meters per minute; in the third stage, they ran for 5 minutes at a speed of 20 meters per minute; in the fourth stage, they ran for 10 minutes at a speed of 25 meters per minute, and then they ran for 20 minutes at a speed of 30 meters per minute. In the final stage, running at a speed of 35 m/min until the rats hit the end of the treadmill three successive times per minute was considered exhaustion (maximum oxygen consumption (Vo2max)) (25).

- Food intake

In order to evaluate the food intake in rats, they were placed separately in single cages and the specified amount of weighted food was placed in the cages. The next day, the remaining food was accurately measured using German Kern 0.01 g scale. The remaining food was then deducted from the total food that had been placed in the cage the day before to obtain the amount of food consumed (26).

- Measurement of body composition parameters

To measure the weight of rats in pre-test and post-test, German Kern scale with a precision of 0.01 g were used. Also, to measure Body Mass Index (BMI) and visceral fat weight, 48 hours after the last training session and in the fasting state, rats were anesthetized with ketamine and xylazine, and after ensuring complete anesthesia using a tape measure, the distance from the tip of the nose
to the anus was accurately measured and recorded in the state of complete anesthesia. Then, the weight of rats (grams) was divided by their height (cm) to calculate their body mass index (27). Then the abdominal cavity was opened using a surgical razor and all visible fats in the abdominal cavity were extracted and measured using a Kern scale with a precision of 0.001 g (28).

- **Data analysis procedure**

  In this study, the Shapiro-Wilk test was used to investigate the normality of the data. Also, using Graphpad Prism 8.3.6, dependent sample t-test was used to investigate changes in the pre-test and post-test of groups; one-way analysis of variance was run to investigate the difference between groups, and Tukey’s post hoc test was used to determine the place of difference between groups (P≥0.05).

**Results**

The results of dependent t-test showed that the levels of weight and food intake in the post-test of HC, EAE, Sh, RJ50, AT, AT + RJ50 and AT + RJ100 increased compared to the pretest. But there was no significant difference in BMI levels in the pre-test and post-test of these groups; in the RJ100 group, food intake levels in the post-test increased compared to the pretest, while BMI levels in the post-test of this group decreased compared to the pretest. The results of analysis of covariance to eliminate the effect of pretest showed a significant difference in the post-test levels of weight (P = 0.001), food intake (P = 0.002) and BMI (P = 0.003). Also, the results of one-way analysis of variance showed that there was a significant difference in the levels of visceral fat weight (P = 0.001) and aerobic power (P = 0.001) in the rats of the study groups.

The results of Bonferroni’s post hoc test showed that there was no significant difference in the post-test weight levels of the HC and EAE groups (P = 0.99); there was also no significant difference in the post-test weight levels of the Sh group compared to the EAE group (P = 0.99). Post-test weight levels in the RJ100 (P = 0.001), AT + RJ50 (P = 0.031) and AT + RJ100 (P = 0.029) groups were significantly lower than the Sh group (Figure 1). There was no significant difference in the post-test levels of food intake in the HC and EAE groups (P = 0.99). Also, the post-test levels of food intake in the Sh (P = 0.99), RJ50 (P = 0.99), RJ100 (P = 0.99), AT (P = 0.99), AT + RJ50 (P = 0.46) groups were not significantly different compared to the EAE group; however, the post-test levels of food intake in the AT + RJ100 group was significantly lower than the EAE group (P = 0.03) (Figure 2). There was no significant difference in the post-test levels of BMI in the HC and EAE groups (P = 0.40), also no significant difference was observed in the Sh (P = 0.75), RJ50 (P = 0.21), AT (P = 0.08) and AT + RJ50 (P = 0.09) groups in comparison with the EAE group, but the post-test levels of BMI in the RJ100 (P = 0.001) and AT + RJ100 (P = 0.004) groups were lower than the EAE group (Figure 3).

The results of Tukey’s post hoc test showed that there was no significant difference in visceral fat weight levels in the HC and EAE groups (P = 0.10), also no significant difference was observed in the Sh (P = 0.55) and RJ50 (P = 0.13) groups compared to the EAE. However, the levels in the RJ100 (P = 0.001), AT (P = 0.001), AT + RJ50 (P = 0.001) and AT + RJ100 (P = 0.001) were less than the EAE group; and the weight of visceral fat in the RJ100 (P = 0.006), AT (P = 0.001), AT + RJ50 (P = 0.018) and AT + RJ100 (P = 0.001) groups was significantly lower than the RJ50 group (Figure 4). Aerobic power levels in rats in the EAE group were significantly lower than the HC group (P = 0.001), and no significant difference was observed in the Sh (P = 0.66) and RJ50 (P = 0.06) groups compared to the EAE group. But the levels in the RJ100 (P = 0.009), AT (P = 0.001), AT + RJ50 (P = 0.001) and AT + RJ100 (P = 0.001) groups were significantly higher than the EAE group, and no significant difference was observed in aerobic power levels in the RJ50 and RJ100 groups (P = 0.99).
However, the levels in the AT (P = 0.001), AT + RJ50 (P = 0.001) and AT + RJ100 (P = 0.001) groups were significantly higher than the RJ50 group, also in the AT + RJ50 (P = 0.001) and AT + RJ100 (P = 0.001) groups were significantly higher than the RJ100 group (Figure 5).

**Figure 1.** The pre-test and post-test levels of weight in the eight research groups

*(P≤0.05): Significant increase in the post-test levels of weight compared to the pre-test

#(P≥0.05), ## (P≤0.01) and ### (P≤0.001): Weight loss in the post-test (or elimination of the pre-test effect) compared to the EAE group

**Figure 2.** The pre-test and post-test levels of food intake in the eight research groups

*(P≤0.05): Significant increase in the post-test levels of weight compared to the pre-test

#(P≥0.05): Weight loss in the post-test (or elimination of the pre-test effect) compared to the EAE group
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Figure 3. The pre-test and post-test levels of BMI in the eight research groups

* (P≤0.05): Significant increase in the post-test levels of weight compared to the pre-test
& (P≤0.05): Significant decrease in the post-test levels of weight compared to the pre-test
## (P≥0.01) and ### (P≥0.001): Weight loss in the post-test (or elimination of the pre-test effect) compared to the EAE group

Figure 4. The pre-test and post-test levels of visceral fat in the eight research groups

### (P≤0.001): Weight loss in the post-test compared to the EAE group

Figure 5. Levels of aerobic power in the eight research groups

*** (P≤0.001): Weight loss in the post-test compared to the HC group
## (P≥0.01) and ### (P≥0.001): Significant increase compared to the EAE group
&&& (P≥0.001): Significant increase compared to the RJ50 group
++ (P≤0.001): Significant increase compared to the RJ100 group

Discussion

The results showed that AT decreased weight, visceral fat weight and increased aerobic power of EAE rats. Researchers have shown that decreased aerobic power and reduced motor ability is one of the most common maladies of MS and autoimmune disease, but the main cause is chronic fatigue in these people, which is not easily detected. Thus, chronic fatigue due to increased inflammation and oxidative stress in the central nervous system, spinal cord and peripheral nervous system, myelin destruction and neuropathic apoptosis as a disturbing factor can cause reduced quality of life, reduced motor ability, social and occupational personality disorders, cognitive disorders, depression, obesity and heart disease in these people (11, 29); however, exercise can improve neurotransmitters in the nervous system and advance physical function by the mechanism of enhancing muscle strength and increasing mitochondrial biogenesis in skeletal muscle and heart tissue (30). In addition, other studies have shown that exercise is associated with an increased mechanism of metabolism of energy substrates along with an increase in muscle capacity for their metabolism and an increase in the capacity of the lungs to exchange oxygen-carbon dioxide, ultimately increasing aerobic power, fat loss and improved body composition in patients with autoimmune disease (5). In this regard, eight weeks of yoga and resistance training increased motor capacity and muscle strength in patients with MS (30); Three months of exercise at home, three sessions per week, and each session lasting 40 minutes to the point of fatigue improved the quality of life and aerobic power of the elderly with autoimmune myasthenia gravis (31); also in a case study, researchers showed that 12 weeks of combined training (endurance-resistance) improved muscle strength, aerobic capacity, functional capacity, chronic fatigue in a 67-year-old woman with relapsing polychondritis as an autoimmune disease (32) as well as performing aerobic training at an intensity of 60-80% of the maximum Heart Rate Reserve (HRR) improved aerobic power and increased walking ability in patients with MS; however, it did not have a significant effect on their body composition (5), so it seems that the severity, type and basic ability of patients with MS are the reasons for the effective change in their body composition. The lack of change in BMI can also be attributed to the increase in muscle weight. On the other hand, it appears that neuronal damage to various parts of the brain following autoimmune disease inhibits the ability of exercise to control appetite and caloric intake (33, 34). The results showed that RJ50 and RJ100 reduced the weight of rats in the EAE model; also, RJ100 decreased BMI, visceral fat weight and increased aerobic power in EAE rats. Researchers have reported that RJ, or 10-hydroxy-trans-2-decanoic acid, is an unsaturated fatty acid that exerts its antioxidant effects by crossing the blood-brain barrier. It also mimics the effects of brain-derived neurotrophic factor, and is effective in regenerating nerve cells, neurogenesis, improving neuronal function in the hypothalamus and regulating appetite (15, 35). RJ also improves fat profile and improves sugar metabolism. Researchers have shown that RJ reduces cholesterol synthesis, increases high-density lipoprotein, and increases insulin sensitivity by reducing the expression of squalene epoxidase, and thus improves fat metabolism by improving glucose metabolism; it also improves the function of T cells and neutrophils by reducing inflammatory factors (19). Therefore, it seems that weight loss after eight weeks of RJ at 50 and 100 mg / kg is due to this mechanism; however, researchers believe that changes in fat profile following RJ use depend on dosage, duration of use, and underlying disorder (type of disease). Thus, in a study, the researchers suggested that 9 days of RJ consumption at a dose of 350 mg had a significant effect on improving the fat profile; however, no significant change in body composition was achieved (36). Also, daily consumption of 1000 RJ mg reduced inflammatory factors in patients with MS (19); in addition, the anti-inflammatory effects of RJ at a dose of 100 mg / kg in skeletal muscle tissue have been reported in animal models with nervous system disorders (18). Hence, reducing
inflammatory factors and improving fat profile can also be the reasons for reducing fat mass and increasing aerobic power. However, at lower dosage and shorter duration, this may not occur due to insufficient biological effects. Therefore, it seems that in addition to the ability to inhibit inflammatory factors and reduce oxidative stress, RJ100 can activate other beneficial biological effects in patients with MS. However, the effect of royal jelly on caloric intake, muscle mass and body composition is still not well understood. The results showed that AT + RJ50 and AT + RJ100 decreased weight, visceral fat weight and increased aerobic power in EAE rats; AT + RJ100 also reduced food intake and BMI in EAE rats; in addition, the increase in aerobic power is training-dependent, so that it was higher in the AT, AT + RJ50 and AT + RJ100 groups than the RJ50 and RJ100 groups. Studies show that exercise by stimulating the synthesis of muscle proteins in striated muscle tissue, improves neuronal function, improves the metabolism of fats and sugars, improves physical function and reduces the risk of cardiovascular disease in patients with metabolic syndrome as well as patients with nervous system disorders (30); it also increases muscle capacity for substrate metabolism, increases lung capacity for oxygen, increases aerobic power, reduces fat weight, and improves body composition in patients with neurological disorders (5, 11, 31, 32). Also, dose-dependent RJ with the mechanism of increasing antioxidants, improving fat profile, increasing insulin sensitivity, improving neurotrophin function, improving neurogenesis and improving neuronal function helps to lose weight, improve BMI, reduce visceral fat weight and increase aerobic power in rats with neurological disorders (15, 19, 35). Therefore, it seems that AT and RJ can enhance each other’s effects from almost similar pathways and improve anthropometric markers in patients with MS. In this regard, the study of Molaei et al. (2019) showed that exercise and royal jelly simultaneously reduced inflammatory factors and improved neutrophil function in patients with MS (19). Also, the interactive effect of royal jelly and exercise in positive and negative slopes for four weeks at a dose of 100 mg/kg was associated with increased muscle strength and motor balance in Alzheimer’s rats (15). In addition, eight weeks of endurance training with consumption of 100 mg/kg RJ was associated with a reduction in inflammatory factors in skeletal muscle tissue of Alzheimer’s rats (18). Besides, aerobic training and crocin consumption in previous studies reduced anxiety and increased aerobic power in rats with Alzheimer’s disease (25). Due to numerous studies, researchers could not find a study in which human subjects were studied under controlled conditions in terms of similar nutrition and lifestyle. Therefore, considering the objectives of this study on investigating the interactive effect of an antioxidant and aerobic training, one of the strengths of this study is the detailed study of the effect of each independent variable on dependent variables. On the other hand, the lack of study of physiological and pathological variables related to the present variables is also one of the limitations of the present study, thus it is suggested that this limitation should be removed in future studies.

Conclusion

It appears that AT and RJ alone has a favorable effect on anthropometric markers, but RJ100 is more desirable than RJ50. Also, depending on the dosage consumed, the interactive effect of AT and RJ on anthropometric markers of obesity, aerobic power and food intake is more favorable than the effect of either one alone in the EAE model.

Ethical Approval

Researchers received introduction letters from Isfahan (Khorasgan) Branch of Islamic Azad University with code IR.IAU.KHUISF.REC.1400.027.

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