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Research Article

The effect of five weeks of aerobic training with royal jelly consumption on glycemic indices in multiple sclerosis rats

Abdolhamid Mosalla nezhad [1], Farzaneh Taghian [2]*, Khosro Jalali Dehkordi [3], Seyed Ali Hosseini [4]

1,2,3. Department of Physical Education and Sport Sciences, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.

4. Department of Sport Physiology, Marvdasht Branch, Islamic Azad University, Marvdasht, Iran.

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Abstract

Background: Although the beneficial role of exercise and antioxidants in multiple sclerosis (MS) has been reported, the interactive effect of aerobic training (AT) and royal jelly (RJ) consumption is still not well known. Therefore, the aim of the present study was to investigate the effect of five weeks of ET and RJ consumption on glycemic indices of MS rats.

Materials and Methods: In this experimental trial, 49 female Sprague-Dawley rats of EAE model weighing 180-210 gr were divided into seven groups based on their motor disability, including (1) experimental autoimmune encephalomyelitis (EAE), (2) sham (Sh), (3) 50 mg /kg of royal jelly consumption (RJ50), (4) 100 mg /kg of royal jelly consumption (RJ100), (5) aerobic training (AT), (6) AT+RJ50, and (7) AT+RJ100. In order to investigate the effects of EAE on the variables, 7 healthy rats were included 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 injected intraperitoneally with the determined dose daily. To analyze the findings, one-way analysis of variance and Tukey's *post hoc* test were used ($P \leq 0.05$).

Results: Insulin levels in the RJ50, RJ100, ET, ET+RJ50 and ET+RJ100 groups were significantly lower than the EAE group (P=0.01).

Conclusion: It seems that aerobic training combined with royal jelly consumption has a synergistic and favorable effect on improving glycemic indices of MS rats.

Keywords:
Training, Royal Jelly, Glycemic Indices, Multiple Sclerosis.

***Corresponding author:** Farzaneh Taghian
Address: Department of Physical Education and Sport Sciences, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.
Email: f.taghian@khuisf.ac.ir **Tell**: 0098313535001-9
F T: 0000-0001-9531-2952

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1. Introduction

Multiple sclerosis (MS) is an inflammatory disease in the central nervous system, and having this disease is associated with metabolic disorders such as obesity (1). Despite the multifactorial nature of MS disease, studies show that weight gain, metabolic disorders, central and peripheral immune system disorders, and axonal demyelination are associated with the incidence of cardiac disorders in MS patients (1,2). Since the central nervous system controls the vessels and internal organs of the body through the sympathetic and parasympathetic system, there seems to be a strong connection between the nervous system and immune cells; because the disorders of the nervous system due to peripheral disruptions can lead to the reaction of the inflammatory system through the increase of free radicals, and by increasing the circulating levels of circulating pro-inflammatory proteins such as C-reactive protein, interleukin 1 (IL-1), interferon gamma (IFN-γ) lead to damage to the vagus nerves, the adrenergic system, as well as the beta-adrenergic receptor (β-AR) (3,4). Subsequently, the systemic increase of inflammation through disruption in peripheral endothelial is associated with disruption in nitric oxide (NO) levels and ultimately causes disruption in low-density lipoprotein (LDL), cholesterol (Chol) and a decrease in high-density lipoprotein (HDL) levels (4,5). On the other hand, studies show that various synthetic drugs have been developed today to moderate the destructive effects of disorders associated with experimental autoimmune encephalomyelitis (EAE); but despite the advances, these drugs are always associated with various side effects in patients (6).

Therefore, due to the restrictions of the research on human patients, researchers use EAE animal modeling to improve treatment and find the best way to reduce and treat the complications of neuroimmune system deficiency diseases (7). Researchers believe that changing lifestyle from inactive to active lifestyle is one of the non-invasive methods to improve neurotrophins, immune system function and improve quality of life (8). It seems that exercise training improves the metabolism of energy substrates, improves the function of the immune system, reduces inflammation and improves cardiovascular function and finally improves the quality of life of these patients by creating different adaptations (9). Researchers believe that in addition to exercises, proper diet, and the use of natural antioxidants lead to the modulation of immune system function and reduction of inflammation in patients with nervous system disorder (10). One of these natural antioxidants is royal jelly (RJ), which is secreted by the submandibular glands of honey bees and is recommended to patients due to its antioxidant, anti-inflammatory, neurotrophin-improving properties in the nervous system (11). Also, because of its anti-inflammatory and antioxidant properties, RJ improves fat profile and reduces CRP in overweight elderly people (12). Therefore, the potential of RJ in improving the fat profile and improving neurotrophins can be a favorable method in the prevention or treatment of nervous and metabolic system diseases. According to noted sentences present study aimed to investigate the effect of five weeks of aerobic training (AT) along with the consumption of two different doses of RJ on glycemic indices of MS rats.

2. Materials and Methods

In this experimental study with a post-test design along with a control group, 58 female Sprague-Dawley rats with an approximate age of 9 ± 2 weeks, and an approximate weight of 200 ± 20 grams were prepared and transferred to the Animal Physiology Laboratory of the Islamic Azad University, Marvdasht Branch. The samples were kept in the laboratory for one week to adapt to the environment. It is worth mentioning that during the research period, all the ethical principles of working with laboratory animals were observed in compatibility with the Helsinki Agreement and under the supervision of the University Biomedical Ethics Committee. During the whole research period, all standard conditions including 12-12 hours of light-darkness, approximate humidity of 55-60%, and the standard temperature of 22-24° C were observed. Also, during the research protocol, animals had free access to water and special food for rats. In addition, to keep the samples, washable cages and sterile grated soil were used to absorb the urine and moisture in the cages.

Induction of experimental autoimmune encephalomyelitis (EAE)

In order to induce EAE in this research, 20 guinea pigs were prepared at the same time as rats and transferred to the laboratory. Three days later, the guinea pigs were anesthesized with ketamine and xylazine at a dose of 20 mg/kg and 55 mg/kg, respectively. After anesthesia, the spinal cord of guinea pigs was carefully extracted and used as an antigen. Thus, first, the guinea pig spinal cord was placed in a liquid nitrogen tank immediately after extraction and was crushed after a few minutes. Next, to homogenize, guinea pig spinal cord was mixed with an equal amount of normal saline and dissolved for five minutes at room temperature.

Then the homogenous solution obtained was mixed with an equal proportion of complete Freund's Adjuvant (Compound Freund's Adjuvant = CFA) and was dissolved for 10 minutes to turn into a white and uniform solution. This solution, comprising 400 microliters of antigen solution and Freund's complete adjuvant, which finally became a suspension, was then injected subcutaneously after anesthesia in the area next to the spinal cord of rats. Also, 100 microliters of the suspension was injected into the leg pad area of each animal with a No. 25 needle to 50 rats. One week after the injection, the first signs of EAE induction emerged to follow up the condition of the disease. Thus, in order to classify the disease, the following scaling was considered in animals: 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 It is worth mentioning that one rat died at this stage due to the severity of the disease (13,14).

Grouping and research design

Given the disease scales and homogenization, 49 rats with EAE were divided into seven groups of seven animals, including: (1) EAE control, (2) Sh, (3) consumption of 50 mg/kg of royal jelly (RJ50), (4) consumption of 100 mg/kg of royal jelly (RJ100), (5) aerobic training (AT), (6) AT+RJ50 and (7) AT+RJ100 were divided. It is also worth mentioning that seven healthy rats were included in the healthy control group (HC) to investigate the effects of EAE induction on the research variables.

Endurance aerobic training protocol

To perform endurance training 10 days after EAE induction, rats were first introduced to the treadmill for 5 to 25 minutes every day for a week at a speed of 6 m/min and an incline of 11 degrees. Next, they did endurance training every day at a speed of 11 m/min for 30 minutes for 5 weeks (15,16).

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 (17).

Dissection

48 hours after the last training session, rats were anesthetized using a combination of ketamine and xylazine in a 12-hour fasting state. After ensuring complete anesthesia, 4-5 cc of blood was taken directly from the heart tissue of the samples using a 5 cc Supa syringe made in Iran. It is worth mentioning that to separate the serum from the plasma, the samples were left at room temperature for 2 hours to clot. Next, the blood samples were centrifuged for 10 minutes in an eight-channel centrifuge manufactured by Behdad Company in Iran at 13,000 revolutions per minute to separate serum and plasma. These serum samples were kept at a temperature of -21° C until the time of measurement.

Method of measuring glycemic indices

To measure glucose the kit of Pars Azmoun company with mg/dL scale, and to measure insulin, the kit of Pars Azmoun company with µIU/mL scale were used.

Statistical analysis

The Shapiro-Wilk , one-way analysis of variance test along with Tukey's *post-hoc* tests were used for statistical analysis of data (P≤0.05).

3. Results

The results of one-way analysis of variance showed a significant difference in the levels of glucose (P=0.001 and F=6.41) and insulin (P=0.001 and F=11.01) in the research groups. The results showed that blood glucose levels in the EAE group were significantly higher than the HC group (P=0.001), but no significant difference was observed in the Sh and EAE groups (P=0.99). However, in the ET+RJ50 (P=0.02) and ET+RJ100 (P=0.01) groups, the levels were significantly lower than the Sh group (D-1). Insulin levels in the EAE group were significantly higher than the HC group (P=0.001); However, no significant difference was observed in the Sh and EAE (P=0.99) groups. Insulin levels in the RJ50 (P=0.003), RJ100 (P=0.002), ET (P=0.005), ET+RJ50 (P=0.001) and ET+RJ100 (P=0.001) groups were significantly lower than the EAE group (D-2).

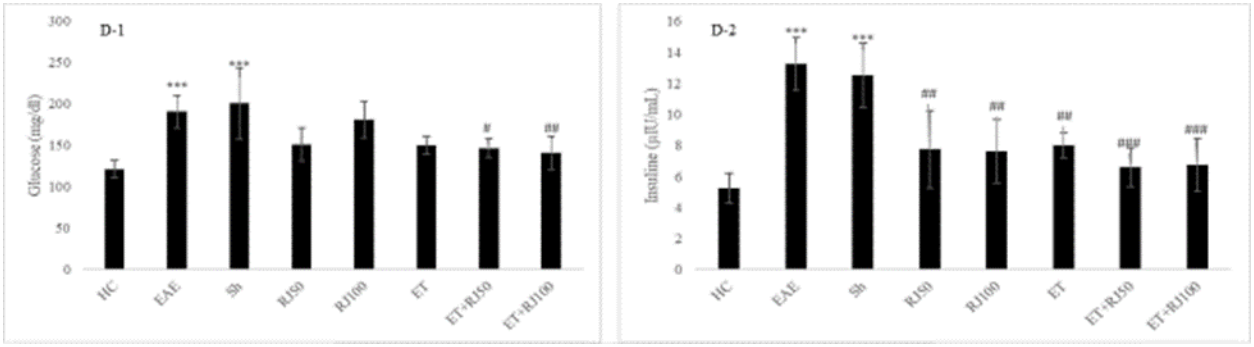

Figure 1: Levels of glycemic indices in research groups: Blood glucose (D-1) and insulin (D-2) in the research groups rats

***(P=0.001), significant change compared to the HC group

#(P=0.05), ## (P=0.01) significant change compared to the Sh and EAE group

4. Discussion

The results of the present study showed that blood glucose levels in the ET+RJ50 and ET+RJ100 groups were significantly lower than the Sh group. Insulin levels in the RJ50, RJ100, ET, ET+RJ50 and ET+RJ100 groups were significantly lower than the EAE group. In an study, swimming training reduced LDL, VLDL, TG, cholesterol and insulin resistance levels in diabetic rats (18). Also, in the field of metabolic indicators in patients with MS, researchers showed that three weeks of aerobic training reduced fatty acids, total cholesterol, and TG levels. In addition to this, the results of this study showed that following exercise training, fatty acid oxidation increased, and this improved metabolism and physical performance in MS patients (19). Eight weeks of aerobic training decreased total cholesterol, LDL, TG, VLDL and body fat percentage in women with MS (20). Also, in a previous study, the results showed that aerobic training improved weight loss, visceral fat weight, and aerobic capacity in an EAE model (21).

In a pilot study, researchers showed that 12 weeks of moderate-intensity endurance training reduced LDL, VLDL, and intermediate-density lipoprotein particle count in patients with MS, however no significant change was reported following high intensity endurance training. Also, 12 weeks of moderate-intensity training improved the blood glucose of these patients (22). In a study, consumption of 100 mg/kg of RJ led to a decrease in visceral fat weight, improved aerobic capacity and caloric intake in the EAE model, and the interactive effect of training and RJ with a dose of 100 mg/kg was far more favorable than training or RJ alone (21). In addition, RJ consumption led to improvement of adipokines, reduction of CRP, reduction of LDL, increase of HDL and increase of total antioxidant capacity in overweight elderly people (12). In another study, researchers showed that daily consumption of 1000 mg of RJ increased HDL, decreased TG, decreased hs-CRP and total cholesterol in women with type 2 diabetes (23). Studies show that doing aerobic training and taking RJ, depending on the dosage, duration of treatment, intensity, type and duration of training can lead to the improvement of metabolic indicators.

Conclusion

According to findings of present study it appears that aerobic training combined with royal jelly consumption has a synergistic and favorable effect on improving glycemic indices of MS rats.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: A.M., F.T., KH.J.D., S.A.H.; Methodology: A.M., F.T., KH.J.D.; Software: A.M., F.T., KH.J.D., S.A.H.; Validation: A.M., F.T., KH.J.D., S.A.H.; Formal analysis: F.T., KH.J.D., S.A.H.; Investigation: F.T., KH.J.D., S.A.H.; Resources: A.M., F.T., S.A.H.; Data curation: A.M., F.T., KH.J.D., S.A.H.; Writing - original draft: A.M., F.T., KH.J.D., S.A.H.; Writing - review & editing: A.M., F.T., KH.J.D.; Visualization: A.M., F.T., S.A.H.; Supervision: A.M., F.T., KH.J.D., S.A.H.; Project administration: A.M., KH.J.D., S.A.H.; Funding acquisition: A.M., F.T., KH.J.D., S.A.H.

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