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The Effect of Royal Jelly and Endurance Exercise on Cognitive Function and Pathological Changes of Hippocampus Tissue in Rats with Experimental Autoimmune Encephalomyelitis

Sara Tavakolian[1], Maghsoud Peeri[1*], Forouzan Fattahi Masrour[1], Amir Hajghasem[1]

1. Department of Exercise Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran.

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ABSTRACT

Introduction: Although the positive effects of physical trainings as well as nutrition on cognitive function are reported, but there is limited information regarding their combined effect. Present study aimed to review the effect of royal jelly (RJ) and endurance exercise (EE) on cognitive function and pathological changes of hippocampus tissue in rats with experimental autoimmune encephalomyelitis (EAE).

Methods: Forty- nine Sprague- Dawley female EAE rats were selected as sample and were assigned to 1) EE+RJ100, 2) EE+RJ50, 3) EE, 4) RJ100, 5) RJ50, 6) Sham (Sh), and 7) EAE groups, also 7 healthy rats were assigned to healthy control group (HC). Rats in EE groups were trained 4 sessions per week (30 minutes with speed of 11-15 m/min) for five weeks and RJ was injected intra- peritoneally. One-way ANOVA along with *Tukey's post hoc* were used for data analysis (P≤0.05).

Results: The percentage of healthy cells in the C1 region (PHC), avoidance (AM) and spatial memory (SM) levels in the EE group were more favorable than the EAE group (P≤0.05). Also, the PHC in the C3 and C1 regions, the AM and SM values in the RJ50 and RJ100 groups were higher than those of the EAE group (P≤0.05). Similarly, AM and SM levels in the EE+RJ50 and EE+RJ100 groups were significantly more favorable than the EAE group (P≤0.05). The effects of EE+RJ50 and EE+RJ100 on improving SM and some subsections of AM were more favorable than the effects of RJ100 (P≤0.05).

Conclusion: EE and RJ (50 and 100mg/kg), alone and in combination, have favorable effects on improving memory and neurogenesis.

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Introduction

Multiple sclerosis (MS) disease is an inflammatory and chronic disease in which myelogenesis increases due to a disorder in the immune system (1). Despite the limitations in conducting human studies, the use of the experimental autoimmune encephalomyelitis (EAE) model can be a common method for research related to immunodeficiency issues. It seems that the similar mechanisms of this modeling with MS disease can contribute to the future of fundamental research in this field (2). This neurodegenerative disease in the central nervous system leads to impaired memory and cognitive function, and more than 40- 70% of people with MS suffer from cognitive disorders (3). In terms of the pathophysiology of MS, blood flow disorders, increased inflammatory factors, and increased oxidative stress lead to the destruction of the myelin sheath, and this affects the reduction of myelin-regenerating proteins in the gray and white matter of the brain (4). It is believed that the increase in the activity of immune cells (T helper cells) can rise the NF-κB and further this protein can induced the transcription of other inflammatory factors like IL-1 beta, IL-23, IL-17 and TNF-a in brain, including the hippocampus (5). Since the hippocampus is responsible for memory and learning, increased oxidative stress and inflammatory factors lead to neuron destruction and cell death, which is responsible for stronger encoding in fear-based memory, and the CA3 region, which have a more selective roles in encoding of this memory (5,6).

On the other hand, studies show that exercises are effective as a non- pharmacological solution for improvement of some diseases such as

* *Corresponding authors:* Maghsoud Peeri; Professor, Department of Exercise Physiology, Central Tehran Branch, Islamic Azad University, Tehran1465613111, Iran. Tel: +98 9121124434, Email: m.peeri@iauctb.ac.ir.

neurological impairments (7). In such a way that exercise by improving molecular- cellular pathways dependent on neurotrophins, improving neurogenesis and increasing blood flow to the brain can help in psychological disorders in animal models of neurological disorders (7), improving memory in the EAE model (2) as well as improve memory and learning in human subjects (8). Researchers have pointed out that exercise for four weeks and five sessions with an intensity of 17 m/min in the last week and a duration of 30 minutes led to an improvement in cognitive performance with the Morris Maze test (2). It has been noted that exercise leads to pain reduction, cognitive function improvement, and inflammatory factors reduction in the EAE model (9). Li *et al.,* reported that exercise for eight and ten weeks, three sessions a week, leads to improved memory in patients with MS. as well as aerobic exercises improved the memory of patients with MS (10).

Also, in recent years the researchers pay attention to consumption of antioxidant supplements along with exercise to reduce some risk factors of neurodegenerative diseases (11). Royal jelly (RJ) is a whitish yellow slimy substance and produced worker bee. This material contains nutrients that are the main food of the queen and the larvae of the hive. RJ contains 12- 15% protein, 10- 12% carbohydrate, 3- 7% sterol, fatty acid, mineral elements and various vitamins (12). Studies have shown that this substance acts as a neurotransmitter due to its properties similar to acetylcholine, and by improving the neuron's antioxidant function, it leads to increased oxygen supply to the brain tissue, facilitating neurogenesis, neuronal differentiation, and improving brain glucose metabolism (13). It has been reported that 50 and 100 mg/kg RJ consumption led to the reduction of inflammatory factors and the increase of anti-inflammatory markers, as well as the improvement of anxiety and depression in rats with EAE. In addition the same effect of two doses on the variables has been reported (13). However, 3% of the diet RJ did not improved learning and memory in rats with Alzheimer's disease (AD) (12). Also, in patients with MS, the RJ consumption reduced inflammatory factors (14).

Although in previous studies, the physical trainings simultaneously with RJ supplementation improved inflammatory factors (13,14), anxiety, depression (15), balance, pain tolerance threshold (11), myelin reproduction (16), physical performance and anthropometric characteristics (17) in the conditions of neurodegenerative disorders, but few studies have investigated their simultaneous effect on cognitive markers and tissue pathology in the hippocampus. It appears that the effect of exercise along with consumption of antioxidants should be further investigated (18). Therefore, using different doses can lead to more information in this field. Considering the growing trend of neurodegenerative diseases in societies; conducting fundamental studies to better understand the synergistic effect of exercise and the appropriate dose of RJ along with exercise can lead to providing a suitable solution for conducting human studies. Therefore, this manuscript reviewed the effect of RJ consumption and endurance exercise (EE) with two doses of 50 (RJ50) and 100 (RJ100) mg/kg on cognitive function and changes in hippocampus tissue pathology in an animal model of EAE.

Materials & Methods

Present study was experimental study. Fifty-three female rats (weighing 200-220 g and aged 8- 10 weeks) selected as sample. For keeping rats in standard laboratory situation, the 12-hour light- dark cycle controlled by a timer as well as all rats access to food and water freely. Relative humidity was 55% and temperature was between 22- 24°C.

Grouping and EAE induction

To induction of EAE model on the eighth day, the spinal cords of 20 guinea pigs (obtained from Center of Iranian Pasteur Institute) were extracted after anesthesia with ketamine and xylosin, then the spinal cord tissue of the guinea pigs were immediately immersed in a nitrogen tank and then were beaten in an oven filled with nitrogen. They were then mixed with normal saline and placed in a shaker until it is completely homogenized then mixed with compound freund's adjuvant (CFA) in a ratio of 1 to 1 to form an emulsion solution. After preparing the solution, 53 rats were injected subcutaneously with 400 microliters of antigen and adjuvant mixture in the back area and 100 microliters in the pillow area of each rat with a needle (No. 25) after complete anesthesia. After the injection of

CFA solution and antigen (guinea pig marrow); the daily course of the disease was evaluated.
All animal categorized based on the disorder. The classification was as follows: 7: death, 6: paralysis four limbs, 5: paralysis of both legs, 4: paralysis of one leg, 3: walking disorder, 2: tail paralysis and 1: tail movement disorder (19,20). Also 2 rats died after 14 days of emulsion injection and 2 rats were removed from the research due to inability to move and complete paralysis. Finally, 49 rats with EAE were included in the study.

Then animal were divided in research groups according to the disease scale; So that all seven disease scales were placed in all groups so that there is no difference in terms of clinical evidence in the research groups. 49 rats assigned in 1) EE+RJ100, 2) EE+RJ50, 3) EE, 4) RJ100, 5) RJ50, 6) Sham (Sh), and 7) EAE groups also 7 healthy rats assigned in healthy control group (HC). During 5 weeks the groups 3 and 6 received 50 mg/kg of RJ intra- peritoneal, and groups 4 and 7 received 100 mg/kg of RJ daily by intra-peritoneal injection (21), in addition, groups 5, 6 and 7 performed 30 minutes EE at a speed of 11 m/min for five sessions per week (22,23).

Endurance Training Protocol

ET was started 10 days after confirmation of EAE induction. To acquaint the rats with ET in the first week, the rats trained daily for 5- 25 minutes (slope of 11% and speed of 6 m/min). The training protocol in the first week was 25 minutes (speed of 11 m/min). Then two minutes were added to the training time every week until that in fifth week the time reached 35 minutes. During the training protocol, the slope of the treadmill was 11% as well as speed was considered to be 11 m/min (22,23).

Royal Jelly Consumption

In order to prepare the RJ, 462 mg of fresh RJ (purchased from the Jihad agricultural center of Marvdasht city) was dissolved in 6.3 cc of normal saline. In the following, 30 international units of the solution were injected intraperitoneally to the 100 mg/kg dose groups and 15 international units to the 50 mg/kg groups (21).

Assessment of Passive Avoidance Memory (AM)

This test was conducted over two consecutive days, which included three stages of familiarization with the environment, acquisition and recovery.

At first stage which is phase of familiarization with the environment, animal kept in the lab environment 30 minutes before the beginning of this phase. Next, the animal placed in the light compartment, then after 5 seconds, the guillotine door of the shuttle box was opened, and the animal allowed exploring the environment of the shuttle box. After the rat entered the dark compartment and explored; after 10 seconds, the animal returned to the cage. The criterion for entering the dark compartment for the rat was 100 seconds, and if the rat did not enter the dark compartment during this period of time, the rat was excluded from the memory test process.

Next, in the second stage, which was known as the training and memory acquisition phase (30 minutes after the first stage), the animal again placed in the light compartment in the same way, and after 5 seconds the guillotine door was opened and the rat was allowed to enter the dark compartment. At this time, after the rat entered the dark compartment, the guillotine door was closed and an electric shock of 50 Hz, 1 milliamp was given to the rat for 3 seconds. Then the rat was returned to her cage after 15- 20 seconds. After two minutes, the rat was again placed in the light compartment, and if she entered the dark compartment, she was given the same amount of electric shock through the metal bars on the bottom of the device. But if she did not enter the dark compartment for two minutes, the test ended and the rat was slowly returned to her cage.

After 24 hours of the memory acquisition phase, the final phase was performed. At this stage, the rat was placed in light compartment and after 5 seconds the guillotine door was opened and the rat was allowed to move freely in the shuttle box rooms for five minutes. During this period, the time it takes for the rat to enter the dark compartment (secondary latency); the time the rat spent in the dark compartment and the numbers of times the rat entered and exited the dark compartment were recorded (24,25).

Assessment of Spatial Memory (SM)

Y-maze device was used for evaluation of spatial memory. It is worth mentioning that the Y-shaped maze was made of three MDF arms and each arm was 15 cm wide, 15 cm high and 46 cm long. Also, the arms of the Y maze are equilateral and are connected through a central area. To perform this test, animal placed at the end of arm A in such a way that it could access all areas of the maze within an 8-minute time frame. During this time, the movement time and the path of the animal were recorded. The entry of the animal into the arm was considered when the rear legs of the animal were completely placed inside an arm and interval behaviors were considered as consecutive successful entries (serial) into all arms in sets of 3. For calculate the percentage of non- repetitive intervals, the below formula was used.

100 × (2 - total number of intervals/ number of non- repeating intervals) (24,25).

Sampling

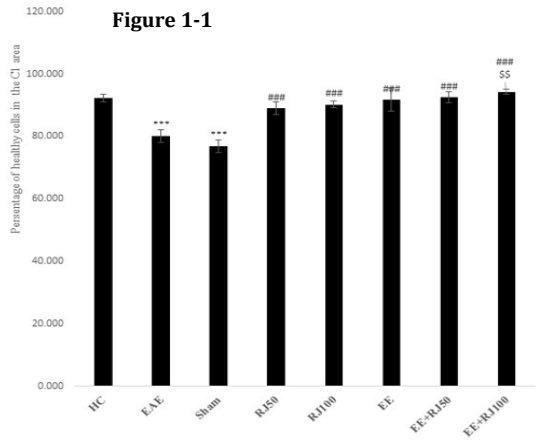
All rats were treated with 15 mg/kg xylosin and 80 mg/kg ketamine (Alfasan Company; Netherlands) in 12 hours of fasting. First upper part of the skull was removed and brain tissue was separated then hippocampus tissue after extraction was placed in 10% formalin solution to evaluate their pathology. It is worth mentioning that 24 hours later, the buffer solution of 10% formalin was changed again and after being transferred to the laboratory, it was used to check the pathological changes.

Hematoxylin-eosin Method

In this method, in order to evaluate the tissue, it was done using a microtome device. First, paraffin blocks were prepared from the hippocampus tissue. In this method, 5 micron thick blocks were prepared from the hippocampus tissue separately in coronal sections and then placed on the slide. Then the tissue was used by staining with hematoxylin, which changes the color of the nucleus to pink, and eosin, which is a special dye for the cytoplasm, which changes the color of the cytoplasm to blue. In the following, healthy cells in the C3 and C1 regions were checked by an optical microscope with 100 times magnification.

Statistical Analysis

In this research, the data were reported based on the mean and standard deviation. Shapiro- Wilk, one-way ANOVA along with Tukey's *post- hoc* tests were used for statistical analysis of data (P≤0.05).



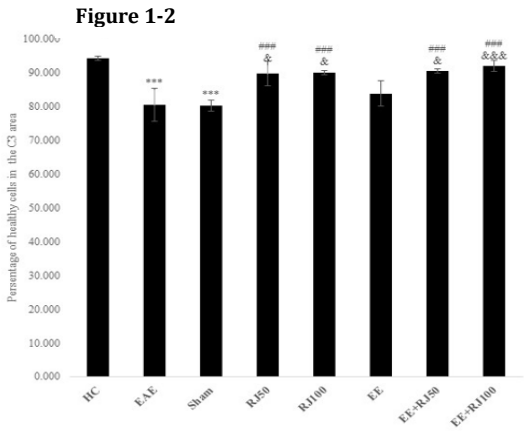


Figure 1. Percentage of healthy cells in C1 (1-1) and C3 (1-2) regions in research groups
*** (P=0.001) significant decrease compared to HC group; ### (P=0.001) significant increase compared to EAE group; $$ (P=0.01) significant increase compared to RJ50 group; & (P=0.05) and &&& (P=0. 001) significant increase compared to EE group

Results

Percentage of Healthy Cells in C1 and C3 Regions

The results showed that percentage of healthy cells in C3 and C 1 regions in the research groups were different (P≤0.05). Tukey's *post- hoc* test showed that in the EAE group the percentage of healthy cells in the C1 region was less than the HC group (P≤0.05). Percentage of healthy cells in the C1 region in EE+RJ100, EE+RJ50, EE, RJ100 and RJ50 groups was more than EAE group (P≤0.05) as well as in EE+RJ100 group was high in compare to RJ50 group (P≤0.05) (Figure 1-1).

In the C3 region, percentage of healthy cells in the EAE group was less than HC group (P≤0.05), nevertheless in EE+RJ100, EE+RJ50, RJ100 and RJ50 groups was more than EAE and EE groups (P≤0.05) (Figure 1-2).

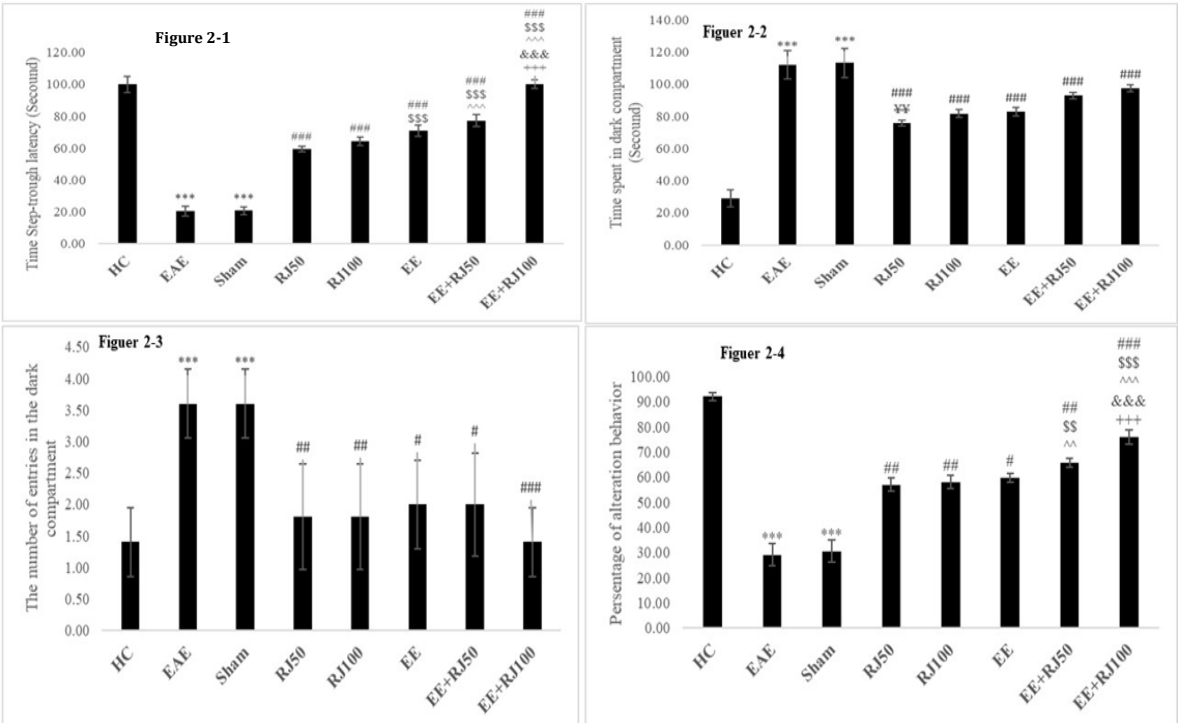


Figure 2. Latency in entering the dark compartment (2-1), the time of staying in the dark compartment (2-2), the number of entering the dark compartment (2-3), the percentage of non-repetitive intervals (2-4) in research groups
*** (P=0.001) significant difference compared to HC group; # (P=0.05), ## (P=0.01) and ### (P=0.001) significant difference compared to EAE group; $$$ (P=0.001) significant difference compared to RJ50 group; & (P=0.05) and &&& (P=0.001) significant difference compared to EE group, ^^^ (P=0.001) significant difference compared to RJ100 group; +++ (P=0.001) significant difference compared to EE+RJ50 group and ¥¥ (P=0.01) significant difference compared to EE+RJ100 group

Avoidance and Spatial Memory

The levels of the percentage of non-repetitive intervals, the number of entering the dark compartment, the time spent in the dark compartment and the latency in entering the dark compartment in the research groups were different (P≤0.05). The latency in entering the dark compartment HC group was higher than EAE (P≤0.05), nevertheless in EE+RJ100, EE+RJ50, RJ100, RJ50 and EE groups was more than EAE group (P≤0.05) also in EE+RJ100, EE+RJ50 and EE groups was more than RJ50 group (P≤0.05). In EE+RJ100 and EE+RJ50 groups was more than RJ100 group (P≤0.05) as well as in the EE+RJ100 group was more than EE+RJ50 and EE groups (P≤0.05) (Figure 1-2).

The time spend in the dark compartment in HC group was less than EAE group (P≤0.05), nevertheless in EE+RJ100, EE+RJ50, EE, RJ100 and RJ50 groups was less than EAE group (P≤0.05) also in EE+RJ100 group was higher than EE group (P≤0.05) (Figure 2-2).

The number of entering the dark compartment in HC group was lower than EAE group (P≤0.05), nevertheless in EE+RJ100, EE+RJ50, EE, RJ100

and RJ50 groups was less than EAE group (P≤0.05) (Figure 3-2).
The percentage of non-repetitive intervals in HC group was higher than EAE group (P≤0.05), nevertheless in EE+RJ100, EE+RJ50, EE, RJ100 and RJ50 groups was less than EAE group (P≤0.05), in EE+RJ100 and EE+RJ50 groups was more than RJ50 group (P≤0.05), in EE+RJ100 and EE+RJ50 groups was less than RJ100 group (P≤0.05) as well as in the EE+RJ100 group was higher EE+RJ50 and EE groups (P≤0.05) (Figure 4-2).
Healthy cells: The nucleus is purple in color and completely visible; the cell is round and has a definite range. Arrow in EAE group indicates cell damage.
Unhealthy cells: Damaged cells of the nucleus are not visible, and the shape of the cell is changed from round to spherical or triangular, with no apparent range. In the EAE group, the percentage of healthy cells decreased, EE and RJ led to an improvement in the percentage of healthy cells. But this improvement was more favorable in the simultaneous EE and RJ groups.

Discussion

In present study the percentage of healthy cells in the C1 region, AM and SM in the EE group improved compare to EAE group. Nowadays, due to the limitations in human studies, EAE modeling is used for more detailed investigations of MS disease. The animal model with EAE is one of the most widely used models for investigating inflammatory mechanisms in MS. Researchers believe that regular exercise for more than three weeks can suppress inflammatory factors, reduce axonal demyelination, improve neurogenesis, improve neuronal plasticity, increase regulation of neurotrophins and increase antioxidants in hippocampus tissue in EAE models (9). Researchers showed that ET strengthened the action of immune cells (B and T cells) as well as improved the function of dendritic cells (26). Also TGF-β, IL-17, anxiety and depression in EAE rats reduced following ET (13). These researchers attributed the mechanism of improvement in cognitive performance following exercise to the improvement of the function of immune cells such as regulatory T and stated that exercise are effective in modulating the function of type 1 and 2 macrophage cells, this ultimately leads to the reduction of inflammatory cytokines and improvement in myelination and neurotrophins (13). In study of Lohrasbi *et al.,* ET reduced demyelination markers in the hippocampus of rats with EAE via reducing inflammatory receptors (16). In a study, researchers showed that ET reduced the time to reach the platform of the water Morris maze (improving memory) in rats with EAE (2). Also, early exercise training reduced cell death in the C1 region and memory (27). Also researchers showed that aerobic training reduced apoptosis and necrosis in the C1 hippocampus of rats with diabetes disease (28). These researchers attributed the improvement of memory to the function of neurotrophins such as increasing the expression of BDNF and its receptor in hippocampal neurons, regulating glucocorticoids, modulating the function of the HPA axis. In addition, the available information regarding changes in the percentage of healthy cells following exercise in the hippocampus of EAE models is limited. But it seems that exercises increase catecholamines, increase cerebral blood flow, increase the density of capillaries in the hippocampus, and reduce IL-1β and TNF- α which lead to reduction of apoptosis and ultimately increase of the percentage of healthy hippocampus cells (29).
Also, healthy cells in the C3 and C1 regions, avoidance and spatial memory in the RJ100 and RJ50 groups improved compare to EAE group. Available data show that 10-hydroxy-2-decanoic acid (10HDA) is one of the main components of RJ and this substance has the most neurobiological effects. So that; this material can accept electrons and neutralize free radicals due to its empty capacity. Therefore, this substance can act directly as an antioxidant and can be involved in neuronal protection (30). In addition, it seems that RJ, due to its estrogenic effects, can activate protein synthesis pathways, and from this pathway, increase the expression of BDNF, NGF, IGF-1, and improve spatial memory in animal models with AD (31). The researchers showed that 150 mg/kg/day RJ increases the healthy cells in the C1 region of rats with temporal lobe epilepsy (32). In this context, researchers have shown that 150 and 300 mg/kg RJ can improve the antioxidant system, increase the expression of BDNF, serotonin, dopamine, acetylcholine, reduce apoptotic markers and improve tissue conditions by examining the pathology (30). In addition, similar to the present study, these researchers did not report a

significant difference in the two doses. Silva *et al.,* reported that 100 mg/ml RJ improved antioxidants, improved spatial memory, reduced neuronal damage, and improved neuronal differentiation in rats with AD (31). Researchers showed that 50 and 100 mg/kg RJ consumption led to improvement of spatial memory, fear-based memory (avoidance), improvement of antioxidants, reduction of free radicals, reduction of Aβ in AD rats (33). It has been reported that the 50 and 100 mg/kg RJ consumption improved exercise performance and anthropometric indicators in rats with EAE model (16). Although other studies have also shown that RJ with the mechanism of modulating the function of B and T cells, reducing IL-17, IL-23, and myelin damage markers can improve the cognitive functions (depression and anxiety) (13,16).

The results showed that healthy cells in the C3 and C1 regions, the avoidance and spatial memory in the EE+RJ100 and EE+RJ50 groups improved compare to EAE group. Also, the effects of EE+RJ50 and EE+RJ100 on improving spatial memory and some subsections of memory were more favorable than the effect of EE, RJ50 and RJ100. Data show that exercise and RJ can be effective in improving neurogenesis with the mechanism of increasing the transcription of antioxidants, improving some neurotrophins such as cannabinoids (15), improving myelination, reducing oxidative stress, and improving some micro RNAs (16). It also seems that exercises are effective in improving memory and learning performance by improving peripheral BDNF, increasing myokines, and increasing the expression of the TrKB receptor (2). While RJ can improve memory and learning by neutralizing free radicals, improving antioxidant capacity, stimulating the transcription of antioxidants, and increasing neurotrophins in the central nervous system (30). The data shows that these two interventions simultaneously with similar mechanisms can improving memory and reducing hippocampal cell damage. Researchers showed that combination of ET and RJ led to improved expression of cannabinoid receptor type 1 and improved pain tolerance in rats with EAE model (15). Indeed the combination EE and RJ on these indicators was more favorable than the effect of each one alone. Lohrasbi *et al.,* reported that ET+RJ100 had a more favorable effect on reducing demyelination markers than ET, RJ and ET+RJ50 (16). Dehkordi *et al.,* (2022) reported that the combination effects of ET and RJ in improving anthropometric indicators and exercise performance of rats with EAE model was far more favorable than the effect of each one alone (17). Therefore, the beneficial effect of these two interventions on improving neurogenesis and neuronal protection is not far from expected. Considering the effect of neurotrophins in improving memory and neurogenesis, lack of evaluation of neurotrophins such as BDNF and NGF along with their molecular- cellular mechanisms can be the limitations of this research. Thus measure these proteins in future studies can be suggested as well as according to the role of the oxidant-antioxidant system in neurobiology, it appears that the lack of evaluation of the factors of this system is another limitation of this research. Therefore, evaluate these indicators in future studies can be suggested. As the formation of amyloid plaques and inflammatory markers play a special role in memory impairment in EAE conditions; therefore, enabling to measure these indicators is another limitation of the this research; therefore evaluate these variables in future studies for a better understanding of molecular- cellular mechanisms can be suggested.

Conclusion

Although EE and RJ (50 and 100mg/kg) alone and their interactive effects have favorable effects on improving memory and neurogenesis.

Declarations

Acknowledgments

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Conflict of Interest

The authors declare the existence of any conflict of interest in this study.

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References

1. Tacchino A, Podda J, Bergamaschi V, Pedullà L, Brichetto G. Cognitive rehabilitation in multiple

sclerosis: Three digital ingredients to address current and future priorities. Front Hum Neurosci. 2023;17:1130231.
2. Hafedh M, Parnow A. Exercise training improves memory and produces changes in the adrenal gland morphology in the experimental autoimmune encephalomyelitis. Endocr Regul. 2022;56(1):31–7.
3. Jamoussi H, Ali N Ben, Missaoui Y, Cherif A, Oudia N, Anane N, et al. Cognitive impairment in multiple sclerosis: Utility of electroencephalography. Mult Scler Relat Disord. 2023;70:104502.
4. Theodosis-Nobelos P, Rekka EA. The Multiple Sclerosis Modulatory Potential of Natural Multi-Targeting Antioxidants. Molecules. 2022;27(23):8402.
5. Charabati M, Wheeler MA, Weiner HL, Quintana FJ. Multiple sclerosis: Neuroimmune crosstalk and therapeutic targeting. Cell. 2023;186(7):1309–27.
6. Yazdanfar SK, Edalatmanesh MA, Hosseini SE. The Effect of Gallic Acid on Passive Avoidance Memory, Working Memory, and Dark Neuron Cell Density in CA1/CA3 Areas in Rats Hippocampal Degeneration Model. J Anim Biol. 2022;14(4):263–74.
7. Sheikholeslami-Vatani D, Salehi OR, Hosseini SA. Psycho-physiological effects of high intensity interval training and vitamin E consumption in elderly trimethyltin-treated Alzheimer's rats. Metab Exerc. 2021;11(2):57–76.
8. Sandroff BM, Wylie GR, Baird JF, Jones CD, Diggs MD, Genova H, et al. Effects of walking exercise training on learning and memory and hippocampal neuroimaging outcomes in MS: A targeted, pilot randomized controlled trial. Contemp Clin Trials. 2021;110:106563.
9. Parnow A, Hafedh M, Tsunoda I, Patel DI, Baker JS, Saeidi A, et al. Effectiveness of exercise interventions in animal models of multiple sclerosis. Front Med. 2023;10.
10. Riemenschneider M, Hvid LG, Petersen T, Stenager E, Dalgas U. Exercise Therapy in Early Multiple Sclerosis Improves Physical Function But Not Cognition: Secondary Analyses From a Randomized Controlled Trial. Neurorehabil Neural Repair. 2023;15459683231159660.
11. Hosseini SA, Salehi OR, Farzanegi P, Farkhaie F, Darvishpour AR, Roozegar S. Interactive effects of endurance training and royal jelly consumption on motor balance and pain threshold in animal model of the alzheimer disease. Arch Neurosci.2020; 7(2).
12. Zamani Z, Reisi P, Alaei H, Pilehvarian AA, Zamani Z. Effect of royal jelly (RJ) on learning and memory in rats after intracerebroventricular injection of streptozotocin (icv-STZ). J Isfahan Med Sch. 2011;28(123).
13. Kheirdeh M, Jahromi MK, Hemmatinafar M, Nemati J. Additive beneficial effects of aerobic training and royal jelly on hippocampal inflammation and function in experimental autoimmune encephalomyelitis rats. Mult Scler Relat Disord. 2023;70:104527.
14. Molaei R, Vahidian-Rezazadeh M, Moghtaderi A. Effect of 6 weeks aerobic exercise and oral Royal Jelly consumption on inflammatory factors' multiple sclerosis patients. Med J mashhad Univ Med Sci. 2019;62(3):1524–35.
15. Kheirdeh M, Koushkie Jahromi M, Brühl AB, Brand S. The effect of exercise training and royal jelly on hippocampal cannabinoid-1-receptors and pain threshold in experimental autoimmune encephalomyelitis in rats as animal model of multiple sclerosis. Nutrients. 2022;14(19):4119.
16. Lohrasbi M, Taghian F, Jalali Dehkordi K, Hosseini SA. The functional mechanisms of synchronizing royal jelly consumption and physical activity on rat with multiple sclerosis-like behaviors hallmarks based on bioinformatics analysis, and experimental survey. BMC Neurosci. 2022;23(1):1–18.
17. Jalali Dehkordi K, Hosseini SA. The Effect of aerobic training with royal jelly consumption on health related anthropometric markers in an experimental autoimmune encephalomyelitis model. Jorjani Biomed J. 2021;9(4):1–12.
18. Sellitto C, Corbi G, Stefanelli B, Manzo V, Trucillo M, Charlier B, et al. Antioxidant supplementation hinders the role of exercise training as a natural activator of SIRT1. Nutrients. 2022;14(10):2092.
19. Weissert R. Actively induced experimental autoimmune encephalomyelitis in rats. Mult Scler Methods Protoc. 2016;161–9.
20. Kalkowski L, Golubczyk D, Kwiatkowska J, Domzalska M, Walczak P, Malysz-Cymborska I. Local autoimmune encephalomyelitis model in a rat brain with precise control over lesion placement. PLoS One. 2022;17(1):e0262677.
21. Malekinejad H, Ahsan S, Delkhosh-Kasmaie F, Cheraghi H, Rezaei-Golmisheh A, Janbaz-Acyabar H. Cardioprotective effect of royal jelly on paclitaxel-induced cardio-toxicity in rats. Iran J Basic Med Sci. 2016;19(2):221.
22. Tajiri N, Yasuhara T, Shingo T, Kondo A, Yuan W, Kadota T, et al. Exercise exerts neuroprotective effects on Parkinson's disease model of rats. Brain Res. 2010;1310:200–7.
23. Bernardes D, Oliveira ALR de. Regular exercise modifies histopathological outcomes of pharmacological treatment in experimental autoimmune encephalomyelitis. Front Neurol. 2018;9:950.
24. Bozorgi A, AliHosseini S, Rasoli MH. Effect of Voluntary and Forced Training with Royal Jelly Consumption on Learning and Spatial Memory of Rat Model of Alzheimer's Disease. Jundishapur J Chronic Dis Care. 9(1).
25. Hosseini SA, Salehi O, Keikhosravi F, Hassanpour G, Ardakani HD, Farkhaie F, et al. Mental health benefits of exercise and genistein in elderly rats. Exp Aging Res. 2022;48(1):42–57.
26. Schiffmann D, Lampkemeyer V, Lindner M, Fleck A-K, Koch K, Eschborn M, et al. Endurance exercise attenuates established progressive experimental

autoimmune encephalomyelitis and is associated with an amelioration of innate immune responses in nod mice. Int J Mol Sci. 2023;24(21):15798.
27. Seydyousefi M, Fallahmohammadi Z, Moazzami M, Yaghoubi A, Faghfoori Z. Impact of early endurance training on improvement of brain damage in CA1 region of Hippocampus and expression of A2A protein following ischemic stroke in rats. J Isfahan Med Sch. 2019;37(526):485–92.
28. Shamsaei N, Abdi H, Shamsi M. The Effect of a continuous training on necrosis and apoptosis changes in the hippocampus of diabetic rats. J Ilam Univ Med Sci. 2017;25(1):1–11.
29. Yaghoubi M, Kordi M, Gaeini A. The effect of forced and voluntary exercise before induction of experimental autoimmune encephalomyelitis on the integrity of the blood-brain barrier and gene expression of some of tight junction proteins. J Appl Exerc Physiol. 2020;16(32):87–101.
30. Ibrahim DS, Shahen EMS. Effect of royal jelly on acrylamide-induced neurotoxicity in rats. J Chem Neuroanat. 2023;134:102358.
31. e Silva TG de S, da Silva JRM, da Silva Alves A, Britto LRG, Xavier GF, Sandoval MRL. Oral treatment with royal jelly improves memory and presents neuroprotective effects on icv-STZ rat model of sporadic Alzheimer's disease. Heliyon. 2020;6(2).
32. Hashemi P, Moloudi MR, Vahabzadeh Z, Izadpanah E. Anticonvulsant effects of royal jelly in kainic acid-induced animal model of temporal lobe epilepsy through antioxidant activity. Neurochem Res. 2023;48(7):2187–95.
33. Raoufi S, Salavati Z, Komaki A, Shahidi S, Zarei M. Royal jelly improves learning and memory deficits in an amyloid β-induced model of Alzheimer's disease in male rats: Involvement of oxidative stress. Metab Brain Dis. 2023;38(4):1239–48.