Additive Beneficial Effects Of Aerobic Training And Royal Jelly On Hippocampal Inflammation And Function In Experimental Autoimmune Encephalomyelitis Rats

Maryam Kheirdeh
Shiraz University
Maryam Koushkie Jahromi (koushkie53@yahoo.com)
Shiraz University
Mohammad Hemmatinafar
Shiraz University
Javad Nemati
Shiraz University

Research Article

Keywords: aerobic exercise, Royal Jelly, Inflammation, Anxiety, Depression, Multiple Sclerosis

Posted Date: March 16th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1411482/v1

Abstract

Background: Although the beneficial role of training and the use of some antioxidants in physiological and psychological disorders in autoimmune diseases has been reported, the simultaneous effect of aerobic training (AT) and royal jelly (RJ) with different doses is not well understood. The present study aimed to investigate the effect of AT and RJ on inflammatory factors, hippocampus and psychological functions in the experimental autoimmune encephalomyelitis (EAE).

Methods: Sprague-Dawley rats with EAE were assigned to seven groups: (1) EAE, (2) sham (Sh), (3) 50 mg / kg RJ (RJ50), (4) 100 mg / kg RJ (RJ100), (5) AT, (6) AT + RJ50, and (7) AT + RJ100 and healthy control.

Results: AT decreased IL-17, TGF-β gene expression and immobilization time, while it increased IL-10, OAT% and OAR% compared to the EAE group. RJ50 and RJ100 decreased IL-17, IL-23 gene expression, and immobilization time, and increased IL-10 and OAR% compared to the EAE group. AT + RJ50 and AT + RJ100 decreased IL-17, IL-23, TGF-β, and immobilization time, while increased IL-10 and OAT% compared to the EAE group. The effect of AT + RJ100 on decreasing IL-17, IL-23, immobilization time, increasing TGF-β, IL-10, and OAR% was more favorable than RJ50.

Conclusion: AT and RJ improved inflammatory factors and reduced anxiety and depression. The synergistic effect of two interventions, especially using higher doses of RJ were more favorable.

Background

Autoimmune diseases are one of the most common diseases of the present century, and various factors such as age, gender, diet, lifestyle, physical activity, etc. are effective on the development of these disease[1]. Autoimmune diseases are associated with the development of physical-cognitive disorders, anxiety, depression and reduced quality of life through damage to various parts of the nervous system [2-4]. Increased proinflammatory and inflammatory cytokines in autoimmune diseases lead to dysfunction of B and T lymphocytes and their subsets such as T helper 1 (Th1), T helper 2 (Th2), T helper 17 (Th17), and T regulatory (Treg)[5]. In the pathology of multiple sclerosis (MS), it has been shown that Th1 activation leads to the production of cytokines such as IL-1, IL-6, IL-12, IL-23, the tumor necrosis factor alpha (TNF-α), and damages oligodendrocytes and neurons. Furthermore, Th1 activation, differentiates and develops Th17, and produces IL-17A and IL17F in addition to disrupting Treg function and impairing transforming growth factor- β (TGF-β) secretion,, leading to neuronal damage and physical-mental disorders[5, 6]. Increased inflammatory factors rise IL-23 and decline the expression of type 1 and 2 cannabinoid receptors 1/2 (CB1 and CB2), damage the hypothalamic-pituitary-adrenal axis, and cause depression, mental disorder and anxiety due to dysfunction of neurotransmitters[7, 8]. In addition, increased inflammatory factors are closely associated with the development of depression and anxiety in autoimmune diseases and MS by disrupting CB1 receptor [9-12].

Various pharmacological and non-pharmacological methods have been proposed to treat or reduce the progression of MS, and regular physical activity is one of the methods that has been reported to have beneficial effects on MS patients[13]. Exercise can reduce inflammatory factors [14-16] and improve psychological performance by increasing serotonergic activity, modulating free radicals, reducing inflammatory factors, and modulating the GABA pathway and cannabinoid receptors [17-19]. In this regard, one study showed that high intensity continuous training following induction of experimental autoimmune encephalomyelitis (EAE) model enhanced the resistance of brain cells against increased inflammatory factors, improved nitric oxide, and reduced reactive oxygen species in rats[20]. Also, 26 days of swimming training increased myelin synthesis, suppressed proinflammatory factors, adjusted weight, and improved IFN-β in EAE model[21], and eight weeks of combined training (aerobic-resistance) improved strength and balance, decreased IL -17 and IFN-γ in women with MS[22].

However, due to the contradictions in the role of high intensity exercise on oxidative stress and inflammation, it seems that the use of some natural antioxidants along with exercise activities has an enhancing role in treating diseases, or preventing injuries caused by training[23, 24]. Among these, royal jelly (RJ), made by bee's submandibular glands, is used in the treatment of some diseases, including autoimmune diseases, due to its anti-inflammatory, antioxidant, anti-apoptotic properties as well as improving metabolism [25]. RJ can improve immune function, and modulate Treg cell activity by neutralizing ROS, improving the function of neurotrophins, and regulating the pituitary-hypothalamic-adrenal axis. RJ also increase anti-inflammatory cytokines such as IL-10, and a decrease in IL-23 by improving TGF-β levels [25, 26]. In this regard, a study has shown that consumption of 2 grams of RJ daily modulates the function of Tregs markers such as $CD4^+$ and $FOXP3^+$ in patients with MS [25]. A review study indicated that royal jelly consumption improve the physical and psychological aspects of Alzheimer's patients [27]. In the study by Shahla et al., dose-dependent RJ consumption improved the function of Th17 and Th1 cells in the EAE model.[28] Consumption of 85 mg / kg RJ inhibited IL-1β, TNF-α and improved apoptotic markers in the cerebral cortex of cadmium-exposed mice [29]. Favorable effects of RJ on neuronal disease has been found to be dose-dependent, so that among the doses of 150, 300 and 450 mg / kg, higher doses had the best effect [30]. Consumption of 100 mg / kg RJ decreased depression and anxiety in rats with Alzheimer's disease[31], while according to another study, despite the favorable effects of RJ at doses of 100 and 200 mg / kg, the dose of 200 mg / kg was more favorable than the dose of 100 mg / kg [32]. In addition, studies that examined the effect of RJ consumption with exercise training showed that the interaction of both interventions improves motor balance, cognitive function, memory and neurotrophins in rats with trimethyltin neurotoxin-induced neurological disorders[24, 33].

According to previous studies, the effect of training and different doses of RJ consumption, individually or in combination, is not clear. Thus, the questions aroused whether the of training and two doses of royal jelly, separately or in combination, have favorable effects on the tissue and function of the hippocampus following myelin damage through the mechanisms and indices of modulating oxidative stress as well as inflammatory and anti-inflammatory cytokines.

Methods

Animals and general procedures

In this experimental study, 63 female Sprague-Dawley rats with an age range of 8-10 weeks and a weight range of 200-220 gr were provided from the Center for Breeding and Reproduction of Laboratory Animals and after transferring to the sports physiology animal laboratory of the university, they were kept in the laboratory for one week for adaptation. Inclusion criteria were being able to perform treadmill running (trainability) and not being affected with any kind of disease and clear diagnose of EAE in EAE groups. Exclusion criteria were, not being able to continue exercise programs and affecting with any kind of disease. All ethical principles of working with animals in this study were regarded according to the ethical principles of working with animals of Shiraz University as well as the Helsinki Agreement. The graduate and ethic committee at Shiraz University approved the study proposal and procedures (number: 34579). During the study, the animals were kept in standard conditions of light (12-hour dark-light cycle), temperature (22-24°C), and humidity (55-60%) in clear polycarbonate cages with autoclave capability. Sterile shaved woods were used to change the bedding of the animals, and they had *ad libitum* access to water and food throughout the study.

Induction of EAE disease

After a seven-day adaptation period, to induce EAE, 20 guinea pigs were provided from the Pasteur Institute of Iran and transferred to the animal laboratory. The guinea pigs were anesthesized by ketamine and xylazine, and they were then dissected and their spinal cord tissues were extracted. The spinal cord tissues were immediately immersed in a nitrogen tank and were then pounded in a nitrogen-filled mortar. Considering the previous studies, guinea pig's spinal cord was used as an antigen to further enhance the complete freund's adjuvant in the nervous system. To homogenize the spinal cord tissue, it was mixed with an equal amount of normal saline, and placed in a shaker at 5°C until it was completely homogenized. The homogenized solution was then made into an emulsion solution in a ratio of 1 to 1 with complete freund's adjuvant (CFA). To prepare this suspension, two glass syringes were used, being connected by a steel interface. One of the syringes contained the homogenized guinea pig's brain and spinal cord and the other syringe contained the same volume of complete CFA; the solution was mixed in equal proportions and its color was uniformed and whitened using a 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 cushion area of each animal with needle number 25. To diagnose induction of the disease, the daily disease process was evaluated and the disease scale was set as follows: zero: no disease, 1: tail movement disorder, 2: tail paralysis, 3: gait disorder, 4: one-leg paralysis, 5: two-leg paralysis, 6: hands and legs paralysis, and 7: death[34, 35]. Due to the research requirement for animals' minimal daily activities, rats on scales 6 and 7 were typically excluded from the study.

Grouping and research design

After ensuring the induction of EAE in rats, based on the standards and their homogenization and on mobility and disability scale as well as inclusion and exclusion criteria, 49 rats with EAE were divided into seven groups of equal number (n=7), including: (1) experimental autoimmune encephalomyelitis (EAE), (2) sham (Sh), (3) 50 mg / kg royal jelly consumption (RJ50), (4) 100 mg / kg royal jelly consumption (RJ100), (5) aerobic training (AT), (6) AT + RJ50, and (7) AT + RJ100. Allocation to each group was through simple randomization method. The number of animals in each group was according to statistical approach for animal studies [36]. In addition, eight healthy rats were included in the healthy control (HC) group to evaluate the effects of EAE induction on the research variables. Rats in the royal jelly consumption groups received peritoneally doses of the prescribed royal jelly (dissolved in normal saline) every day for 5 weeks [37]. Rats in the endurance training groups performed endurance training on the treadmill at a speed of 11 meters per minute, five sessions per week and each session of 30 minutes for 5 weeks [38, 39].

Familiarization of animals to treadmill training

Endurance training began approximately 10 days after induction of the EAE experimental model. To familiarize animals to treadmill, rat performed endurance training on the treadmill every day at a speed of 6 meters per minute and a slope of 11 degrees for 5 to 25 minutes for a week [38, 39].

Main aerobic training protocol

Rats performed endurance training every day at a speed of 11 meters per minute for 25-35 minutes for five weeks. In other words, the training period was 25 minutes in the first week, and 2 minutes were added to the duration of exercise each week so that duration of training reached 35 minutes in the fifth week. One of the reasons for choosing this training protocol was the neuroprotective effects of this type of training in rats with cognitive impairments and rats and mice with the experimental model of Parkinson's encephalomyelitis [38, 39].

Royal jelly consumption

In this study, royal jelly was used with doses of 100 and 50 mg / kg during five weeks. RJ was prepared from Marvdasht Agricultural Jihad Center and dissolved daily in normal saline as required; then it was injected peritoneally into rats similar to a recent study[37].

Evaluation of anxiety-like behaviors

Elevated plus-maze behavioral model was used to measure anxiety. This evaluation was based on a model first proposed by Pellow et al, using an elevated + -maze consisting of two open arms and two enclosed arms. The dimensions of the open and closed arms are 10 × 50, with two sides and the end of the enclosed arms height is 40 cm. The four arms lead to a central area are 10 × 10 cm. The maze was placed at a height of 50 cm above the ground. The rats were placed in the central area of the maze, facing an open arm. During the 5 minutes, animals moved freely in different parts of the maze, and the frequency of entries to the open arm and closed arm, and the duration of placement in the open and

closed arm were measured[40]. The test and related evaluations were performed by a research assistant who were blind about the group allocations.

Forced swimming test

Forced swimming as a valid test was used to measure depression. 24 hours before the test, the animals were placed in water for 15 minutes. The behavior of rat was recorded during five minutes testing. Inertness of the mouse's limbs and its buoyancy were considered as immobility and its duration is considered as immobility time. All test procedures were performed according to the available guidelines[41]. The test and related evaluations were performed by a research assistant who were blind about the group allocations.

Measurement procedures

Rats weight was were measured once a week throughout the study. 48 hours after the last training session, the rats were anesthetized with ketamine (15 mg/kg) and xylazine (70 mg/kg) and killed by the drug, and their hippocampus tissue was isolated and homogenized and stored in nitrogen fluid at -80 ° C for further analysis. Real time PCR was used to measure the gene expression levels of the research variables.

To measure IL-17, IL-23, IL-10 and TGF-β gene expression levels by qPCR, 20 mg of tissue was isolated from the hippocampus; RNA extraction from tissues in all study groups was performed according to the protocol of the manufacturer (Kiagen, Germany). To ensure the quality of RNA, electrophoresis was performed using agarose gel and light absorption property at 260 nm with Sigma's Picop Drop device (made in USA). Also, the formula (C (µg / µl) = A260 × ε × d / 1000) was used to evaluate RNA quality. Following cDNA synthesis, a reverse transcription reaction was performed using the fermentase kit manufacturer protocol (K1621) and implementing the designed primers (Table 1). To determine the efficacy and specificity of the primers, the pre-primers were evaluated using the software available on the NCBI site. To measure the gene expression levels, the research variables were used using the TBP internal control gene. After confirming the completion of the qPCR and after reaching the expression threshold (Cycle Treshold), the formula $2^{-\Delta\Delta CT}$ was used to quantify the ratio of the desired gene to the reference gene.

Insert table 1

Statistical analysis

The Shapiro-Wilk test was used to investigate the normality of the findings of the study. Regarding the normal distribution of findings, in order to investigate difference between the groups, one-way analysis of variance (ANOVA) was used and in the case of significant difference, for paired group comparisons, Tukey's *post hoc* test were used in Graphpad Prism 8.3.6 software ($P \leq 0.05$).

Results

The results of one-way analysis of variance showed that there were significant differences in the levels of IL-17 (P = 0.001 and F = 40.18), IL-23 (P = 0.001 and F = 25.27), IL -10 (P = 0.001 and F = 42.76), TGF-β (P = 0.001 and F = 30.33) gene expression, percentage of open arm time (OAT%) (P = 0.001, F = 94.22), percentage of the number of open arm entries (OAR%) (P = 0.001 and F = 6.78) and duration of immobility (P = 0.001 and P = 0.27) F) in the study groups.

The results of Tukey's *post hoc* test showed that IL-17 gene expression levels in the EAE group were significantly higher than the HC (P = 0.001) and Sh (P = 0.001) groups. However, IL-17 in the RJ50 (P = 0.001), RJ100 (P = 0.001), AT (P = 0.001), AT + RJ50 (P = 0.001) and AT + RJ100 (P= 0.001) were significantly lower than the EAE group.

IL-17 in the AT + RJ50 (P = 0.01) and AT + RJ100 (P = 0.001) groups were significantly lower than the RJ50 group, while in the AT + RJ100 group were significantly lower than the RJ100 group (P = 0.001). Besides, IL-17 in the AT + RJ50 (P = 0.03) and AT + RJ100 (P = 0.001) groups were significantly less than the AT group. However, no significant difference was observed between the AT + RJ50 and AT + RJ100 groups (P = 0.90) (Figure 1-A).

IL-23 gene expression in rats' hippocampus tissue in the EAE group were significantly higher than the HC (P = 0.001) and Sh (P = 0.001) groups. There was no significant difference in the AT group with EAE (P = 0.99) and Sh (P = 0.13) groups. IL-23 gene expression in the RJ50 (P = 0.007), RJ100 (P = 0.001), AT + RJ50 (P = 0.001) and AT + RJ100 (P = 0.001) were significantly less than the EAE group. IL-23 gene expression in the RJ50 (P = 0.02), RJ100 (P = 0.001), AT + RJ50 (P = 0.001) and AT + RJ100 (P = 0.001) groups were significantly lower than the AT group. There was no significant difference in IL-23 gene expression levels in the RJ100 and AT + RJ100 (P = 0.11), as well as, in AT + RJ50 and AT + RJ100 (P = 0.58) groups compared to the RJ50 group. IL-23 gene expression in AT + RJ100 group were significantly less than the RJ50 group (P = 0.001), however, no significant difference was observed between the AT + RJ50 and AT + RJ100 (P = 0.11) (Figure 1-B).

IL-10 gene expression levels in the EAE (P = 0.008) and Sh (P = 0.007) groups were significantly less than the HC group, but in the RJ50 (P = 0.001), RJ100 (P = 0.01), AT (P = 0.002), AT + RJ50 (P = 0.001) and AT + RJ100 (P = 0.001), the levels were significantly higher than the EAE group. IL-10 gene expression levels in the AT + RJ100 group were significantly higher than the RJ50 group (P = 0.001). Also, in the AT + RJ50 (P = 0.001) and AT + RJ100 (P = 0.001) groups, it was significantly higher than the AT group. Besides, in the AT + RJ100 group, IL-10 gene expression was significantly higher than the AT + RJ50 group (P = 0.001) (Figure 1-C).

TGF-β gene expression levels in the EAE (P = 0.001) and Sh (P = 0.001) groups were significantly higher than the HC group. There was no significant difference in TGF-β gene expression of the RJ100 group with EAE (P = 0.55) and Sh (P = 0.99) groups, however, in the RJ50 (P = 0.001), AT (P = 0.001), ET + RJ50 (P = 0.001) and AT + RJ100 (P = 0.001) groups, the levels were significantly less than the EAE group. TGF-β

gene expression levels were also lower in the AT + RJ100 group than in the RJ50 group (P = 0.01). In the RJ50 (P = 0.004), AT (P = 0.001), AT + RJ50 (P = 0.001) and AT + RJ100 (P = 0.001) groups, TGF-β gene expression levels were significantly less than the RJ100 group. No significant difference was observed in the AT + RJ50 (P = 0.88) and AT + RJ100 (P = 0.94) groups compared to the AT group; while no significant difference was observed between the AT + RJ50 and AT + RJ100 groups (P = 0.23) (Figure 1-D).

The duration of immobility in the EAE (P = 0.001) and Sh (P = 0.001) groups was significantly longer than the HC group; however, in the RJ50 (P = 0.001), RJ100 (P = 0.001), AT (P = 0.001), AT + RJ50 (P = 0.001) and AT + RJ100 (P = 0.001) groups, the levels were significantly lower than the EAE group. There was no significant difference in the duration of immobility between the RJ100 (P = 0.12) and AT (P = 0.13) groups compared to the RJ50 group. In the AT + RJ50 (P = 0.001) and AT + RJ100 groups (P = 0.001), it was significantly lower than the RJ50 group, however, no significant difference was observed in the AT + RJ50 and AT + RJ100 groups (P = 0.99) (Figure 2-A).

OAT% levels in the EAE group (P = 0.001) and Sh (P = 0.001) were significantly lower than the HC group. No significant difference was observed in the RJ50 group compared to the EAE group (P = 0.69). In the RJ100 (P = 0.001), AT (P = 0.001), AT + RJ50 (P = 0.001) and AT + RJ100 (P = 0.001), the levels were significantly higher than the EAE group. OAT% levels in the RJ100 (P = 0.001), AT (P = 0.001), AT + RJ50 (P = 0.001) and AT + RJ100 (P = 0.001) groups were significantly higher than the RJ50 group, and the levels in the AT + RJ100 group were significantly higher than the AT group (P = 0.046). No significant difference was observed in AT + RJ50 and AT + RJ100 groups (P = 0.07) (Figure 2-B).

OAR% levels in the EAE group were significantly lower than the HC group (P = 0.002). No significant difference was observed in the AT + RJ50 and EAE groups (P = 0.12). In the RJ50 (P = 0.001), RJ100 (P = 0.004), AT (P = 0.004) and AT + RJ100 (P = 0.004) groups, the levels were significantly higher than the EAE group. In addition, no significant difference was observed in the AT + RJ50 and AT + RJ100 groups (P = 0.07) (Figure 2-C).

Discussion

The results showed that, IL-17 and TGF-β gene expression decreased in AT while immobility time, IL-10, and OAT% increased in AT compared to the EAE group. Consistent with the present study findings, it was shown that high intensity continuous training increased the number of microglia and decreased reactive oxygen species (ROS) and some inflammatory factors in the brain tissue of EAE rats[20]. Also, aerobic training improved the anthropometric and physiological markers of EAE rats[23]. 26 days of swimming training, 30 minutes daily increased myelin synthesis, suppressed proinflammatory factors, adjusted weight, improved interferon beta (IFN-β) in EAE model[21]. Eight weeks of combined training (aerobic-resistance), three sessions per week, and each session lasting 45 minutes improved strength and balance, and decreased IL-17 and IFN-γ in women with MS[22]. Eight weeks of combined training (endurance-resistance) decreased C-reactive protein (CRP), IL-6 and increased IL-10 in women with

MS[42]. 16 weeks of aerobic training increased BDNF and neuroplasticity, and decreased anxiety and depression in patients with MS[17]. A meta-analysis study also reported that exercise improved cognitive function, reduced anxiety and depression, and improved health-related physical fitness in patients with MS[43]. Regarding the effect of exercise on autoimmune diseases, exercise seems to increase brain-derived neurotrophic factor (BDNF) and TrkB by improving blood flow to the brain, and by activating antioxidants, it can increase the regulatory T family, increase IL-10 and TGF-β. Furthermore, improvement of neurotrophins can improve mood and reduce anxiety and depression[44]. Intestinal-brain axis adaptation following exercise seems to be of particular importance in regulating the immune system in EAE patients, because favorable changes in the microbiota of the gastrointestinal tract following resistance training lead to a decrease in inflammatory cytokines such as IL-17 in the animal model of MS[45]. Increased intestinal microbial activity and increased reabsorption of short-chain fatty acids reduce the permeability of nervous and gastrointestinal cells to Th17 proteins and increase regulatory T, and by improving the pathway of mitogen-activated protein kinases (MAPK), P38MAPK and JNK can reduce TNF-α, IFN-γ, IL-17, prostaglandin E2 (PGE2) in brain tissue in EAE disease[45]. Regarding the lack of significant changes in IL-23 and the decrease in TGF-β, two points should be considered; first, the function of TGF-β as a post-translational agent for activating helper cells varies in different parts of the nervous system, because it simultaneously plays a role in the proliferation and differentiation of cells expressing inflammatory and anti-inflammatory factors[46]. Second, it appears that performing exercise and the body's oxidative response to any exercise session should be considered with caution in EAE patients. Because, the type of inflammatory adaptation to high-intensity training is not yet well known and contradictory results have been reported in this regard[44]. Therefore, despite the beneficial effects of exercise on inflammatory factors in the nervous system, the adaptation of the immune system to this intervention, especially in brain tissue, is still not well known.

The results of the present study showed that RJ50 and RJ100 decreased IL-17, IL-23 gene expression, decreased immobility time, increased IL-10 and OAR% compared to the EAE group;

TGF-β decreased only in the RJ50 group and OAT% increased only in the RJ100 group. Consistent with the present study, the researchers showed that consumption of RJ at doses of 100 and 500 mg / kg and its main constituent (10-hydroxy-2 decanoic acid (10-H2DA)) at doses of 3 and 6 mg / kg increased myelination, TGF-β, IL-4, IL-10, and decreased IL-17 and TNF-α in the hippocampal tissue of EAE model[28]. It should be noted that these changes were dose dependent and a higher effect was observed at higher doses[28]. Nonetheless, in our study no significant difference was observed in different doses and it seems that the difference of the consumption period is one of the reasons for the discrepancy in the results. In the study of Shahla et al, 25 days of royal jelly consumption was evaluated, while in our study, eight weeks of royal jelly consumption was evaluated.It was found in another study that daily consumption of RJ 500 mg / kg reduced the levels of IL-6, TNF-α and IL-6 in overweight women[47]. In addition, in the previous studies, the anti-anxiety and anti-depression effects of neurotrophins[33] and improvement of neurotransmitters following RJ administration[32, 48] has been reported. It appears that consumption of royal jelly due to the presence of fatty acids, such as 10-H2DA subunits, increases regulatory Th2 and T cells, while inhibits Th1 and Th17; moreover, the antioxidant property of RJ is

another mechanism that can make neurons resistant to oxidative damage and can improve neuroplasticity by increasing antioxidant factors[28]. Furthermore, RJ appears to inhibit capillary permeability against the inflammatory phase. RJ also inhibits lipopolysaccharides and IFN-γ by stimulating macrophages. The most important point is that RJ contains large amounts of manus-rich sugar chains and directly activates macrophages and suppresses pro-inflammatory factors and increases anti-inflammatory factors [47, 49, 50].

The results also showed that AT + RJ50 and AT + RJ100 decreased IL-17, IL-23, TGF-β,IL-10 and OAT% while decreased duration of immobility compared to the EAE group. OAR% increased in the AT + RJ100 group compared to all other groups. Few studies are available on the simultaneous effect of exercise training and RJ consumption on nervous system disorders. For example, in a study it was reported that AT and RJ improved anthropometric markers and decreased visceral fat weight in the EAE animal model, and combination of AT and RJ had a synergistic effect on improving these markers, although the effect of higher dose of RJ combined with training was reported to be more favorable[23]. Therefore, the synergistic effect of training and RJ on some variables in this study can be attributed to their common mechanisms such as improved antioxidants, physical function and cerebral blood flow and enhancing weight loss. Also in a study, six weeks of resistance training combined with vitamin D consumption reduced IL-17 levels. However, these two did not have a significant effect on granulocyte-monocyte growth factor and NF-κB at the same time[34]. Exercise appears to enhance BDNF and TrkB by improving microbiota, reduce inflammatory cytokines, increase the reabsorption of short-chain fatty acids, reduce the permeability of nervous and gastrointestinal cells to Th17 proteins, and increase regulatory T, improve blood flow to the brain, which lead to improved mood and reduced anxiety and depression by improving immune system function[44, 45]. Consumption of RJ increases the differentiation of Th2 and T cells, decreases Th1 and Th17, increases antioxidants, neuroplasticity[28], stimulates macrophages, suppresses pro-inflammatory factors and increases anti-inflammatory factors[47, 49, 50].

It seems that common mechanisms in the antioxidant pathway, intestinal-cerebral pathway, and macrophage differentiation in these two interventions have been able to synergistically improve inflammatory factors and anxiety and depression in the EAE model. However, in some pathways, the lack of evaluation of cell-molecular pathways to ensure the exact mechanism is one of the limitations of the present study. Therefore, it is suggested that the study of intestinal-cerebral pathway and microbiota be considered in future studies. The lack of assessment of P38MAPK and JNK pathways in the modulation of inflammation in this study was another limitation of our study. Therefore, the study of these pathways and mechanisms are recommended for future studies.

Conclusion

Aerobic training and RJ, separately and in combination appear to improve inflammatory factors, reduce anxiety, and depression in the EAE model. It is noteworthy that the combination of aerobic training and RJ, especially with a higher dose of 100 mg, induces a synergistic favorable effect on some inflammatory variables. However, more future studies are needed for further clarification.

Abbreviations

AT: Aerobic training; RJ: royal jelly; EAE: Experimental autoimmune encephalomyelitis; Sh: Sham; RJ50: 50 mg / kg royal jelly; RJ100: 100 mg / kg royal jelly; IL: Interleukin; Th: T helper Treg: T regulatory; TNF: Tumor necrosis factor alpha; TGF-β: Transforming growth factor- β, CB1 and CB2: Cannabinoid receptors 1/2 , MS: Multiple sclerosis , CFA: freund's adjuvant; BDNF: Brain-derived neurotrophic factor; IFN-β:interferon beta; CRP: C-reactive protein.

Declarations

Ethical approval and consent to participants

All experiments were performed according to the ethical principles of animal welfare of Shiraz University in Iran as well as accordance with the Helsinki Treat (Laboratory Animal Ethics). The method for euthanasia and anaesthesia were approved by the Animal Ethics Committee. The graduate and ethic committee at Shiraz University approved the study proposal and procedures (number: 34579). The study is reported in accordance with ARRIVE guidelines.

Consent to participants is not applicable.

Consent for publication

Not Applicable.

Data availability

The datasets generated and analysed during the current study are not publicly available due to importance of its novelty during manuscript publication, but are available from the corresponding author on reasonable request.

Authors' contributions

MK, MKJ, MH and JN participated in research design. MK performed the study. MK and MKJ contributed in analysis and writing first draft of manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no competing interests.

Funding

Postgraduate and research grants by Shiraz University supported this study financially.

Acknowledgement

We thank the Marvdasht lab stuff and management for their cooperation in the study.

References

1. Nataf S, Hunot S, Dorothée G, Liblau R: **Brain-Targeted Autoimmunity: Beyond Multiple Sclerosis**. Frontiers in Immunology 2021, **12**:1085.
2. Bingham KS, Rozenbojm N, Chong-East M, Touma Z: **Exploring the Mental Health Needs of Persons With Autoimmune Diseases During the Coronavirus Disease 2019 Pandemic: A Proposed Framework for Future Research and Clinical Care**. ACR Open Rheumatology 2021, **3**(1):25–33.
3. Bhagavati S: **Autoimmune disorders of the nervous system: pathophysiology, clinical features, and therapy**. Frontiers in Neurology 2021, **12**:539.
4. Mitchell D, Shireman J, Sierra Potchanant EA, Lara-Velazquez M, Dey M: **Neuroinflammation in Autoimmune Disease and Primary Brain Tumors: The Quest for Striking the Right Balance**. Frontiers in Cellular Neuroscience 2021:321.
5. Moudgil KD, Choubey D: **Cytokines in autoimmunity: role in induction, regulation, and treatment**. Journal of Interferon & Cytokine Research 2011, **31**(10):695–703.
6. Schinocca C, Rizzo C, Fasano S, Grasso G, La Barbera L, Ciccia F, Guggino G: **Role of the IL-23/IL-17 pathway in rheumatic diseases: an overview**. Frontiers in immunology 2021, **12**:321.
7. Yuan X-C, Zhu B, Jing X-H, Xiong L-Z, Wu C-H, Gao F, Li H-P, Xiang H-C, Zhu H, Zhou B: **Electroacupuncture potentiates cannabinoid receptor-mediated descending inhibitory control in a mouse model of knee osteoarthritis**. Frontiers in Molecular Neuroscience 2018, **11**:112.
8. Hammell D, Zhang L, Ma F, Abshire S, McIlwrath S, Stinchcomb A, Westlund K: **Transdermal cannabidiol reduces inflammation and pain-related behaviours in a rat model of arthritis**. European journal of pain 2016, **20**(6):936–948.
9. Bruno A, Dolcetti E, Rizzo FR, Fresegna D, Musella A, Gentile A, De Vito F, Caioli S, Guadalupi L, Bullitta S: **Inflammation-associated synaptic alterations as shared threads in depression and multiple sclerosis**. Frontiers in Cellular Neuroscience 2020, **14**:169.
10. Mustafa W, Elgendy N, Salama S, Jawad M, Eltoukhy K: **The Effect of Cannabis on the Clinical and Cytokine Profiles in Patients with Multiple Sclerosis**. *Multiple sclerosis international* 2021, **2021**.
11. Milovanovic J, Arsenijevic A, Stojanovic B, Kanjevac T, Arsenijevic D, Radosavljevic G, Milovanovic M, Arsenijevic N: **Interleukin-17 in chronic inflammatory neurological diseases**. Frontiers in Immunology 2020, **11**:947.
12. Mecha M, Carrillo-Salinas FJ, Feliú A, Mestre L, Guaza C: **Perspectives on cannabis-based therapy of multiple sclerosis: a mini-review**. Frontiers in Cellular Neuroscience 2020, **14**:34.
13. So W-Y, Kalron A: **The association between body mass index and leisure-time physical activity in adults with multiple sclerosis**. International journal of environmental research and public health 2020, **17**(3):920.

14. Mokhtarzade M, Shamsi MM, Abolhasani M, Bakhshi B, Sahraian MA, Quinn LS, Negaresh R: **Home-based exercise training influences gut bacterial levels in multiple sclerosis**. Complementary Therapies in Clinical Practice 2021, **45**:101463.
15. Zhou Q, Shi C, Lv Y, Zhao C, Jiao Z, Wang T: **Circulating microRNAs in response to exercise training in healthy adults**. Frontiers in genetics 2020, **11**:256.
16. Deckx N, Wens I, Nuyts AH, Hens N, De Winter BY, Koppen G, Goossens H, Van Damme P, Berneman ZN, Eijnde BO: **12 weeks of combined endurance and resistance training reduces innate markers of inflammation in a randomized controlled clinical trial in patients with multiple sclerosis**. *Mediators of inflammation* 2016, **2016**.
17. Gravesteijn A, Beckerman H, De Jong B, Hulst H, De Groot V: **Neuroprotective effects of exercise in people with progressive multiple sclerosis (Exercise PRO-MS): study protocol of a phase II trial**. BMC neurology 2020, **20**(1):1–11.
18. Geva N, Defrin R: **Enhanced pain modulation among triathletes: a possible explanation for their exceptional capabilities**. PAIN® 2013, **154**(11):2317–2323.
19. Fingleton C, Smart KM, Doody CM: **Exercise-induced hypoalgesia in people with knee osteoarthritis with normal and abnormal conditioned pain modulation**. The Clinical journal of pain 2017, **33**(5):395–404.
20. Zaychik Y, Fainstein N, Touloumi O, Goldberg Y, Hamdi L, Segal S, Nabat H, Zoidou S, Grigoriadis N, Katz A: **High-intensity exercise training protects the brain against autoimmune neuroinflammation: regulation of microglial redox and pro-inflammatory functions**. Frontiers in cellular neuroscience 2021, **15**:27.
21. Kim J-Y, Yi E-S, Lee H, Kim J-S, Jee Y-S, Kim S-E, Kim C-J, Ko I-G: **Swimming exercise ameliorates symptoms of MOG-induced experimental autoimmune encephalomyelitis by inhibiting inflammation and demyelination in rats**. International Neurourology Journal 2020, **24**(Suppl 1):S39.
22. Golzari Z, Shabkhiz F, Soudi S, Kordi MR, Hashemi SM: **Combined exercise training reduces IFN-γ and IL-17 levels in the plasma and the supernatant of peripheral blood mononuclear cells in women with multiple sclerosis**. International immunopharmacology 2010, **10**(11):1415–1419.
23. 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 Biomedicine Journal 2021, **9**(4):1–12.
24. 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**. Archives of Neuroscience 2020, **7**(2).
25. Zahran AM, Elsayh KI, Saad K, Eloseily EM, Osman NS, Alblihed MA, Badr G, Mahmoud MH: **Effects of royal jelly supplementation on regulatory T cells in children with SLE**. Food & nutrition research 2016, **60**(1):32963.
26. Hosseini SA, Salehi O, Keikhosravi F, Hassanpour G, Ardakani HD, Farkhaie F, Shadmehri S, Azarbayjani MA: **Mental health benefits of exercise and genistein in elderly rats**. Experimental aging

research 2021:1–16.

27. Guo J, Wang Z, Chen Y, Cao J, Tian W, Ma B, Dong Y: **Active components and biological functions of royal jelly**. Journal of Functional Foods 2021, **82**:104514.
28. Shahla J, Dariush H, Bijan SM, Majid E, Zahra A, Bahman Y: **Comparative immunomodulatory effects of jelly royal and 10-H2DA on experimental autoimmune encephalomyelitis**. Gene Reports 2021, **24**:101217.
29. Almeer RS, Kassab RB, AlBasher GI, Alarifi S, Alkahtani S, Ali D, Abdel Moneim AE: **Royal jelly mitigates cadmium-induced neuronal damage in mouse cortex**. Molecular Biology Reports 2019, **46**(1):119–131.
30. You M, Miao Z, Sienkiewicz O, Jiang X, Zhao X, Hu F: **10-Hydroxydecanoic acid inhibits LPS-induced inflammation by targeting p53 in microglial cells**. International Immunopharmacology 2020, **84**:106501.
31. Azimpour M, Fathi M, Dezfoulian O: **The Effect of Royal Jelly on Depression and Anxiety in an Animal Model of Alzheimer's Disease**. The Neuroscience Journal of Shefaye Khatam 2021, **9**(2):79–90.
32. Khani A, Kazemi N: **The Effect for Eight Weeks of Resistance Training with Royal Jelly Consumption on Anxiety and Depression in A Rat Model for Alzheimer's Disease**. Journal of Nutrition, Fasting and Health 2021, **9**(3):202–206.
33. Bozorgi A, Hosseini 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 2020, **9**(1):e97261.
34. Mousavi S, Fallahmohammadi Z, Hajizadeh Moghaddam A: **Evaluating the protective effect of 6 weeks resistance training and vitamin D intake on brain neuro-inflammatory factors in female rats with experimental autoimmune encephalomyelitis**. Feyz Journal of Kashan University of Medical Sciences 2018, **22**(6):573–580.
35. Abedi E, Khezri S, Abtahi SM: **Evaluation of the chlorpromazine effect on experimental autoimmune encephalomyelitis in male rats**. Journal of Shahrekord Uuniversity of Medical Sciences 2017, **18**.
36. Arifin WN, Zahiruddin WM: **Sample size calculation in animal studies using resource equation approach**. The Malaysian journal of medical sciences: MJMS 2017, **24**(5):101.
37. 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**. Iranian journal of basic medical sciences 2016, **19**(2):221.
38. Tajiri N, Yasuhara T, Shingo T, Kondo A, Yuan W, Kadota T, Wang F, Baba T, Tayra JT, Morimoto T: **Exercise exerts neuroprotective effects on Parkinson's disease model of rats**. Brain research 2010, **1310**:200–207.
39. Bernardes D, Oliveira ALRd: **Regular exercise modifies histopathological outcomes of pharmacological treatment in experimental autoimmune encephalomyelitis**. Frontiers in Neurology 2018:950.

40. Azarian F, Farsi S, Hosseini SA, Azarbayjani MA: **The effect of endurance training and crocin consumption on anxiety-like behaviors and aerobic power in rats with Alzheimer's**. *Iranian journal of psychiatry and behavioral sciences* 2019, **13**(4).
41. Yankelevitch-Yahav R, Franko M, Huly A, Doron R: **The forced swim test as a model of depressive-like behavior**. JoVE (Journal of Visualized Experiments) 2015(97):e52587.
42. Zadeh FT, Amini H, Habibi S, Shahedi V, Isanejad A, Akbarpour M: **The Effects of 8-Week Combined Exercise Training on Inflammatory Markers in Women with Multiple Sclerosis**. Neurodegenerative Diseases 2020, **20**(5–6):212–216.
43. Barahona-Fuentes G, Huerta Ojeda Á, Chirosa-Ríos L: **Effects of Training with Different Modes of Strength Intervention on Psychosocial Disorders in Adolescents: A Systematic Review and Meta-Analysis**. International Journal of Environmental Research and Public Health 2021, **18**(18):9477.
44. Fanara S, Aprile M, Iacono S, Schirò G, Bianchi A, Brighina F, Dominguez LJ, Ragonese P, Salemi G: **The Role of Nutritional Lifestyle and Physical Activity in Multiple Sclerosis Pathogenesis and Management: A Narrative Review**. Nutrients 2021, **13**(11):3774.
45. Chen H, Shen L, Liu Y, Ma X, Long L, Ma X, Ma L, Chen Z, Lin X, Si L: **Strength exercise confers protection in central nervous system autoimmunity by altering the gut microbiota**. Frontiers in immunology 2021, **12**.
46. Zhang S: **The role of transforming growth factor β in T helper 17 differentiation**. Immunology 2018, **155**(1):24–35.
47. Etemad Z, Zohali S: **The Effect of Aerobic Training and Royal Jelly Supplementation on Some Inflammatory Markers in Overweight Women**. Middle Eastern Journal of Disability Studies 2021, **11**(0):21–21.
48. Shenas NP, Salehi O, Hosseini SA: **The Effect of Resistance Training with Royal Jelly on Serotonin and Dopamine Receptors Genes Expression in the Hippocampus of a Rat Model of Alzheimer's Disease**. 2021.
49. Mohamed AA-R, Galal AA, Elewa YH: **Comparative protective effects of royal jelly and cod liver oil against neurotoxic impact of tartrazine on male rat pups brain**. Acta Histochemica 2015, **117**(7):649–658.
50. Collazo N, Carpena M, Nuñez-Estevez B, Otero P, Simal-Gandara J, Prieto MA: **Health promoting properties of bee royal jelly: Food of the queens**. Nutrients 2021, **13**(2):543.

Table

Table 1. Primer sequence of the study variables

Gene	Primer sequence	(bp)
TBP	Forward: 5’- GCGGGGTCATGAAATCCAGT-3’	147
	Reverse: 5’- AGTGATGTGGGGACAAAACGA -3’	
IL-17	Forward: 5’- CTGAAAGTCCTCAACTCCCTTAG -3’	99
	Reverse: 5’- CTCATTGCGGCTCAGAGT -3’	
IL-23	Forward: 5’- ATCCGCCAAGGTCTGGTGTT -3’	140
	Reverse: 5’- AGTGGTGATCCTCTGGCTGGA -3’	
TGFB	Forward: 5’- AGCAACAATTCCTGGCGTTACCT-3’	131
	Reverse: 5’- CGAAAGCCCTGTATTCCGTCTCC -3’	
IL-10	Forward: 5’- GTAGAAGTGATGCCCCAGGC -3’	124
	Reverse: 5’- CACAGGGGAGAAATCGATGACAG -3	

Figures

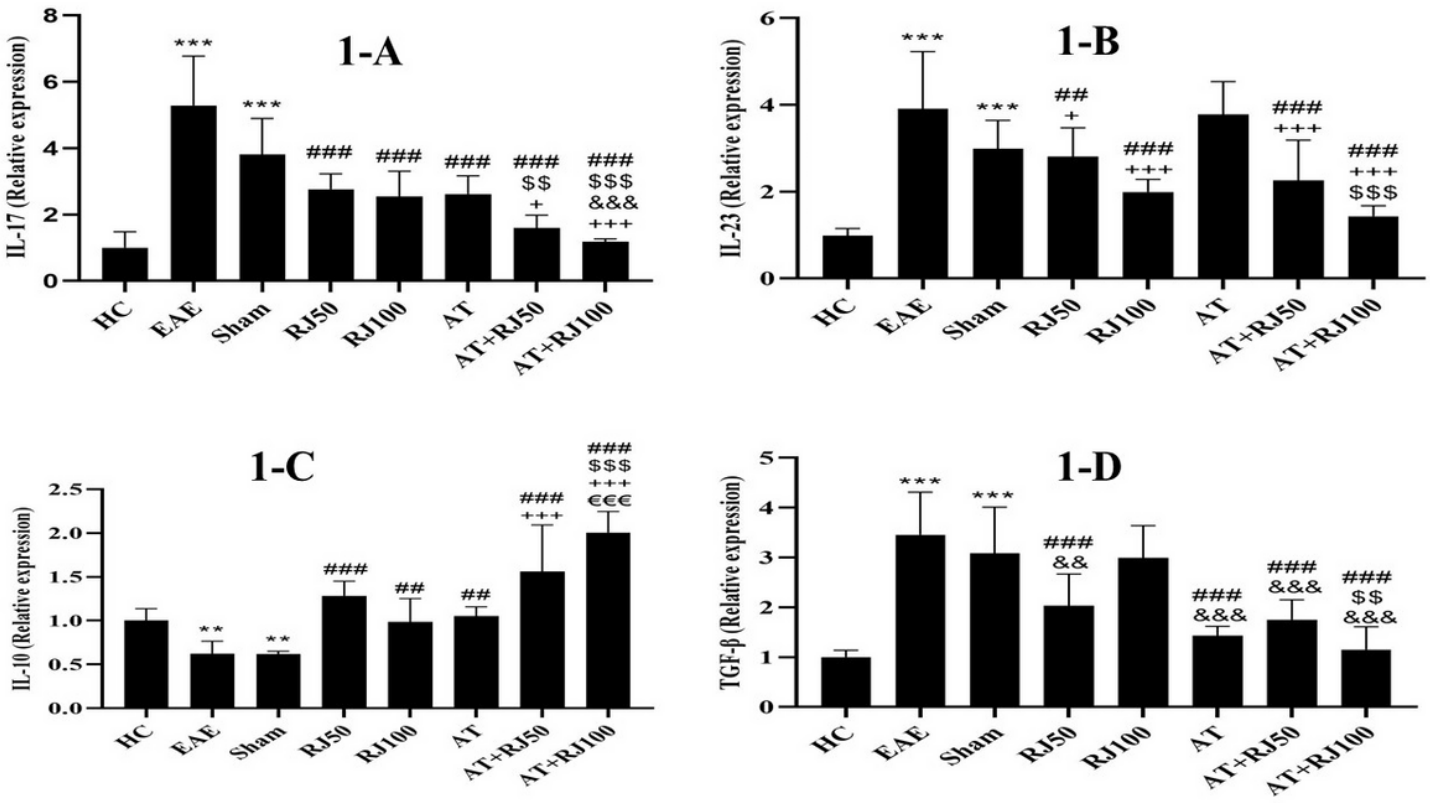

Figure 1

Comparison of chemical biomarkers between the study groups A: IL-17 and B: IL-23, C: IL-10, D: TGF-β gene expression

(P≥0.01), *(P≥0.001), Significant changes in the EAE and Sham groups compared to the HC group.## (P≥0.01) and ### (P≥0.001): Significant changes compared to the EAE group;

$$(P \geq 0.01) and$$

$ (P ≥0.001): Significant changes compared to the RJ50 group; &&& (P ≥0.001): Significant changes compared to the RJ100 group; +(P≥0.05) and +++ (P≥0.001): Significant changes compared to the AT group;€€€ (P≥0.001): Significant changes compared to the AT+RJ50 group.

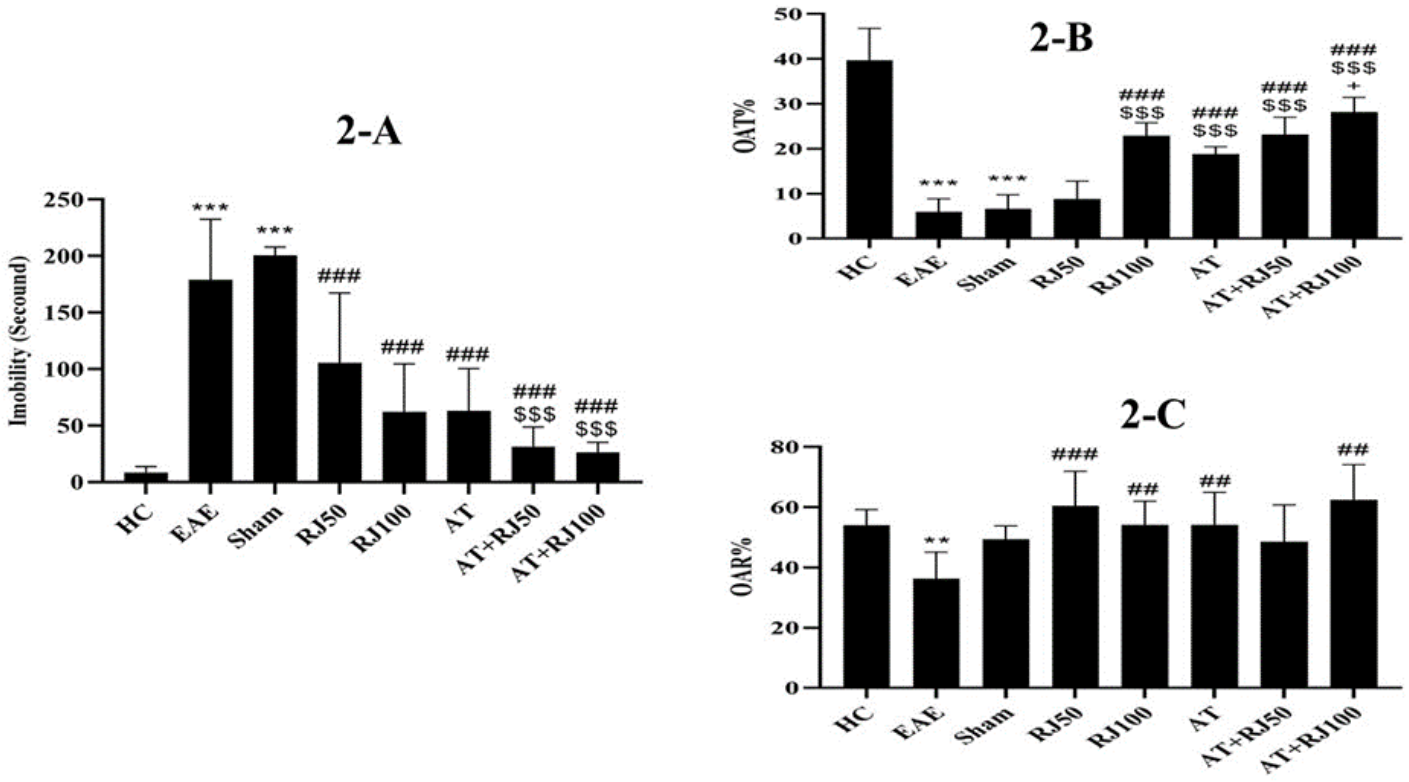

Figure 2

Levels of immobility (depression) and Anxiety in the research groups. A: Immobility, B: OAT %(the percentage of open arm time), C: OAR %(the percentage of open arm entries).(P≥0.01) and *** (P≥0.001): Significant changes in the EAE and Sham groups compared to the HC group;## (P≥0.01) and ### (P≥0.001): Significant changes compared to the EAE group;$$$ (P ≥0.001): Significant changes compared to the RJ50 group;+(P≥0.05) Significant changes compared to the AT group.