



The Effect for Eight Weeks of Resistance Training with Royal Jelly Consumption on Anxiety and Depression in A Rat Model for Alzheimer's Disease

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ARTICLE INFO	ABSTRACT
<p><i>Article type:</i> Research Paper</p>	<p>Introduction: Today, due to the prevalence and spread of Alzheimer's disease (AD) and negative impact on life and health, effective methods have been considered for treating the disease. This study aimed to investigate the effect of eight weeks for resistance training (RT) with royal jelly (RJ) on anxiety and depression in rats with AD.</p>
<p><i>Article History:</i> Received: 03 Sep 2020 Accepted: 16 Nov 2020 Published: 05 Sep 2021</p>	<p>Methods: In the experimental study, 56 male Sprague-Dawley rats with AD (induced by 8 mg/kg Trimethyltin chloride) divided into seven groups of eight rats including (1) Alzheimer's control (AD), (2) sham (Sh), (3) RT, (4) RT+ 100 mg/kg RJ (RT+RJ100), (5) RT+ 200 mg/RJ (RT+RJ200), (6) RJ100, and (7) RJ200. Eight rats were assigned into the healthy control (HC) group to investigate the effect of AD induction on research variables. The groups of 3, 4 and 5, performed RT with an intensity of 30 to 100% of body weight three sessions per week for eight weeks, and the groups of 4-7 received the selected doses of RJ peritoneally daily. Anxiety-like behaviors and depression were measured by the elevated plus-maze test and forced swim test respectively. To analyze the findings, one-way ANOVA was used with Tukey's <i>post-hoc</i> test, and two-way ANOVA with Bonferroni's <i>post-hoc</i> test ($p \leq 0.05$).</p>
<p><i>Keywords:</i> Resistance training Royal jelly Anxiety Depression Alzheimer's</p>	<p>Results: Eight weeks of RT ($p \leq 0.05$) and RJ ($p \leq 0.05$) could significantly reduce anxiety and depression in rats with AD. 200 mg/kg RJ had a more favorable effect on reducing anxiety ($p \leq 0.05$) and depression ($p \leq 0.05$) than 100 mg/kg RJ. In addition, RT and RJ had an interactive effect on reducing depression in rats with AD ($p \leq 0.05$).</p> <p>Conclusion: Apparently, the RT and RJ consumption have interactive effects on reducing anxiety; Besides, the anti-anxiety and anti-depression effects of RJ can be dependent on dosage.</p>

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Introduction

The human brain is a collection and organ to store, recall, and process all the necessary information. Its destruction by Alzheimer's disease (AD) is very similar to a computer memory, which has lost its information (1,2). The damage process in AD begins in the hippocampus of the brain (3). The hippocampus, the part of the brain that is responsible for storing information about short-term memory, plays an important role in learning. By interfering with other parts of the brain, AD usually begins in the area, it also interferes with speaking, reading, calculating, making decisions, and coordinating body movements (3). As the levels of serotonin in the brain in AD patients decreased, it leads to forgetfulness, learning disabilities, and aging (4). Most prescribed drugs in each part of the world are drugs to change the level of serotonin. These

drugs are used in depression, general anxiety disorder, and social phobia (2).

Exercise is proved to have beneficial effects on physical and mental health as it reduces the prevalence of diseases, promotes neuroprotection, neuroplasticity, increases cognitive function, and has anti-anxiety and anti-depressant properties (3). Moreover, Exercise reduces the symptoms of anxiety and depression and improves mood and the feeling of well-being. positive effects on fatigue and anger could be gained by only one session of aerobic and resistance training (RT) (5). The researchers mentioned that regular aerobic physical activity is a good tool to prevent or treat many diseases, reduce the baseline risk factors for stroke and heart attack, prevent neurological disorders such as anxiety, depression, degenerative and weakening diseases of the central nervous system including Parkinson's, AD and improving

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brain function (6). Rashidi *et al.*, (2017) compared the effect of aerobic and anaerobic exercise on students' depression and anxiety. In the end, the results showed that both aerobic or anaerobic exercises have a significant effect on reducing of depression and anxiety (7). Exercise causes brain cells to proliferate, especially in the hippocampus. The structure (hippocampus) is involved in the transfer of information from short-term to long-term memory. Choosing an effective and appropriate exercise for the treatment and prevention of depression, AD and other diseases for the nervous system is vital (8). Besides, royal jelly (RJ), or its compounds have been reported to facilitate neurogenesis in the hippocampus and to differentiate different types of brain cells such as neurons and neuroglial cells from precursor stem cells in the area of the dentate gyrus (9). One of the compounds that have been proven to have protective and anti-apoptotic effects on nerve cells is RJ. RJ is a light yellowish-white gelatinous substance, colloidal, and sticky with a special odor and a bitter taste (10). This jelly is a viscous substance which have been produced by young worker bees in the genus *Apis mellifera*. It helps the hive larvae to be fed to become queens (11). In recent decades, several researches investigated the role of physical activity in reducing anxiety and stress and indicate the importance and benefits of physical activity on brain function (12). Considering the importance of the role of exercise in AD and the uncertain effects of RJ on improving the symptoms of this disease, the current study was conducted to investigate the effect of eight weeks of RT with RJ consumption on anxiety and depression in rats with AD.

Methods

In this experimental study, 64 Sprague-Dawley rats were purchased from the animal lab in Marvdasht branch of Islamic Azad University and as for the adaptation to a new environment, all rats kept in sport physiology lab of the noted university for one week in the standard situation (22- 24 C temperature, 12 hours light and darkness cycle and 55- 60 percent relative humidity) with free access to food and water. In all of the research periods, ethical considerations were according to guidelines of the animal ethics committee. Then 56 rats were induced with AD with 8 mg/kg Trimethyltin chloride (TMT) (Sigma- Aldrich, MERK Company, CAS Number: 1065-45-1). After two weeks, to diagnose AD

disease, in addition to physical examination such as aggression, bleeding around the eyes, tail twisting, the memory, and learning tests were performed using a shuttle box device (13). When AD induction was confirmed, rats were randomly divided into seven groups of eight rats including (1) Alzheimer's control (AD), (2) sham (Sh), (3) RT, (4) RT+ 100 mg/kg RJ (RT+RJ100), (5) RT+ 200 mg/RJ (RT+RJ200), (6) RJ100, and (7) RJ200. It is noteworthy that to investigate the effects of AD induction on the research variables, 8 rats were assigned to the healthy control group (HC). Groups of 3, 4 and 5, performed RT with an intensity of 30 to 100% of body weight three sessions per week for eight weeks (14), and the groups of 4-7 received 100 and 200 mg/kg RJ dissolved in physiological saline peritoneally for eight weeks (12).

Resistance training protocol

The training in each session consisted of four sets (first set 50%, second set 75%, third set 90%, and fourth set 100% weight set for that week) and two repetitions (climbing stairs twice). The interval between each set was 2 to 3 minutes and the interval between each repetition was 40 to 60 seconds (14).

Measurement of variables

A forced swimming test was used to measure depression. The forced swimming test is among most valid and common tests for rodent depression. The time of this test is 5 minutes and the behavior of rats is recorded during the period. Conventionally, the cessation of the movement for the rat's limbs and its floating is considered as immobility and its duration is considered as immobility time. To gain the experience of forced swimming, the animal is placed in water for 15 minutes in twenty- four hours before the test (15).

Elevated plus-maze was used to measure behavioral model anxiety. The evaluation was based on a model first proposed by Plooy *et al.*, The number of times the animal moved freely in different parts of the plus-maze during 5 minutes; the number of times the animal entered different parts of the open arm; the number of times the animal entered the closed arm; the length of time the animal stayed in the open arm; and finally, the length of time the animal remained in the closed arm of the plus-maze were measured by video recording. Only when all four legs of the animal were in the arm meant

they enter an open or closed arm. The time spent in each arm was calculated accordingly. For each animal, the percentage of entry into the open arm and the percentage of time spent in the open arm was calculated (3,16).

Data analysis procedure

To evaluate the normality in data distribution, the Shapiro-Wilk test was used, and to evaluate the effect of TMT on the research variables, one-

way ANOVA with Tukey's *post-hoc* tests were used. Also, to evaluate the effect of RJ supplement and RT on the research variables two-way ANOVA with Bonferroni's *post-hoc* tests in SPSS 22 were used ($p \leq 0.05$).

Findings

Table 1 presents the mean and standard deviation for anxiety-like behaviors and depression in rats in the study groups.

Table 1. The mean and standard deviation of research variables in rats in the research groups

Group	Percentage of the number of open arm entries	Percentage of time spent in the open arm	Movement time (seconds)
HC	41.40±3.34	30.43±2.72	152.85±18.89
AD	21.37±6.94	15.40±4.67	37.79±6.17
Sham	30.53±4.44	13.05±2.17	44.00±7.37
RT	38.14±3.08	23.19±2.41	79.02±5.81
RT+RJ100	38.14±3.08	27.29±3.26	109.28±9.67
RT+RJ200	41.91±5.77	29.10±2.64	144.00±10.45
RJ100	29.00±2.88	19.44±1.35	80.54±8.57
RJ200	29.78±2.76	20.45±1.28	100.42±8.57

The findings of the present study showed that AD induction significantly increased anxiety-like behaviors and depression in rats ($P = 0.001$). Eight weeks of RT significantly reduced anxiety-like behaviors ($P = 0.001$) and depression ($P = 0.001$) in rats with AD. Eight weeks of RJ consumption significantly reduced anxiety-like behaviors ($P = 0.001$) and depression ($P = 0.001$) in rats with AD.

Consumption of 200 mg / kg RJ had a more favorable effect on reducing anxiety ($P = 0.001$) and depression ($P = 0.001$) than 100 mg/kg RJ. RT and RJ consumption had significant interactive effects in reducing depression in rats with AD ($P = 0.007$). RT and RJ consumption did not have significant interactive effects in reducing anxiety-like behaviors in rats with AD ($P = 0.61$).

Discussion

The obtained results in the current study showed that AD induction significantly increased anxiety and depression in rats. AD is the leading cause of dementia and characterized by progressive loss of memory and other cognitive functions. Evidently oxidative stress plays a significant role in depression and anxiety disorders in AD patients. Anxiety is one of the behavioral symptoms of AD (17). In depression and anxiety, the individual's neurotransmitters of the brain are reduced, which the individuals feel depressed and lonely due to the decline of their physical and mental abilities, (13). The use of

TMT in laboratory animals is known to induce severe and selective neuronal death associated with microglial and astroglial activity, selectively in the limbic region and especially in the hippocampus; as a result, it provides a valuable tool for studying the function of the hippocampus during neurodegenerative events (18).

The results showed that eight weeks of RT and RJ consumption significantly decreased anxiety-like behaviors and depression in rats with AD. The previous studies show that regular exercise balanced sympathetic nerve activity; in other words, modulates the hypothalamic-pituitary-adrenal (APH) axial response (19). It is observed that physical activity can exert its effects to improve depression and anxiety by affecting the release of beta-endorphins and monoamines, reducing cortisol levels, and increasing self-efficacy in the individual (20). Researches have shown that exercise for 9 weeks and three 20-minute sessions per week reduces depression and increases adaptation mechanisms (21). Besides, it has been observed that the activity of the hypothalamic-pituitary-adrenal axis decreases in some patients with depression, and therefore, improving the function of the hypothalamic-pituitary-adrenal axis as a result of physical activity also plays a useful role in improving depression (19).

It seems that balance levels in serotonin and norepinephrine during exercise can reduce depression and moderate anxiety. In other

words, exercise can affect the human spirit in two ways, which are increasing the release of endorphins and reducing the levels of cortisol that are released into the blood due to stress (22). Consistent with the present study, researchers reported that 10 weeks, three sessions per week, and each session of 60 to 90 minutes of incremental exercise had a significant effect on reducing stress, anxiety, depression, and improving systolic blood pressure in kidney transplant patients (22); 12 weeks, three sessions per week of exercise in 5 sets reduced anxiety and depression for the patients with metabolic syndrome (23); eight weeks, three sessions per week and 60 minutes of water exercise per session had a significant effect on reducing anxiety and stress in older men (16). As could be seen on the literature review, most of the studies were in line with the present study, so it seems that a variety of long-term sports activities with different intensities reduce anxiety.

RJ, as the main food of the queen, is known as the richest biological nutrient and seems that have been used in traditional medicine to treat many diseases(24,25). The results of several studies show that RJ significantly enhanced the memory and learning of AD patients (25). The substance is absorbed into the blood after consumption and reaches the gray cells of the brain (26). RJ neutralizes free radicals by interrupting the oxidation chain reaction (27), which can prevent the destruction of nerve cells from this pathway. 10 days Consumption of food containing RJ (3% by weight) significantly improved memory and learning indices in the diabetic rats (28). It seems that differences in the amount of RJ consumption can have different effects on cognitive function and the central nervous system, as the results of this study are more favorable in a significant reduction in depression at a dose of 200 mg compared to a dose of 100 mg RJ.

Conclusion

It seems that RT and RJ consumption can reduce depression and anxiety in rats with AD. Therefore, it is suggested to use the RT and RJ in the diet plan to reduce the harmful effects of AD such as anxiety and depression.

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