Original Article

The Effect of Eight Weeks of Voluntary Wheel Running Exercise with Royal Jelly Consumption on Behavioral Disorders and Antioxidant Capacity in Rats with Trimethytin Model of Alzheimer's Disease

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Abstract

Background & Objective: Alzheimer is a neurodegenerative disease in which numerous changes occur in the patient's brain, since it is suggested that royal jelly (RJ) and Physical activity have several pharmacological activities, including neuroprotective and improvement of cognitive function. This study aimed to investigate the effect of eight weeks of voluntary wheel running exercise with royal jelly consumption on behavioral disorders and antioxidant capacity in rats with Trimethytin model of Alzheimer's disease.

Materials & Methods: This experimental study was performed on 48 male rats; Alzheimer's Trimethytin was induced on 40 rats. The rats were then randomly divided into six groups each group consisting of 8 members: healthy control group (HC), Alzheimer control group (ADC), voluntary exercise (VW) (wheel running), sham (SH), voluntary exercise combined with the royal jelly intake (VWJ) and royal jelly intake (RJ). Voluntary wheel running was done for eight weeks, three sessions per week and 60 minutes each session. To determine depression and anxiety, The Forced Swimming Test (FST) and Elevated Plus-Maze (EPM) were used respectively. Real-Time PCR was used to determine Gene expression of Superoxide dismutase (SOD) and Glutathione peroxidase (GPX). Data analysis was performed by the multivariate analysis of variance (MANOVA) test and post hoc Scheffe at the significance level of $P < 0.05$ using SPSS software version 20.

Results: The results of multivariate analysis of variance show that there is a significant difference in all five variables of OAT, SOD, GPX, OAE and immobility in at least one group. According to the results of Scheffe post hoc test the findings showed that immobility time decreased in the VWJ, RJ, HC groups. The anxiety related indices increased in VWJ, RJ, HC groups. Although the differences in SOD antioxidant index were not significant, Gene expression of GPX increased in VWJ and RJ groups.

Conclusion: In this study, voluntary exercise alone did not reduce anxiety and depression, and this highlights the role of Royal Jelly in it, and the results with the effect size of 0.757 showed the greatest effect on reducing the depression index. It seems that the combination of voluntary exercise with royal jelly supplementation can modulate behavioral disorders and antioxidant capacity in rats with trimethylettin-induced Alzheimer's disease.

Keywords: Trimethyltin, Royal Jelly, Depression, Anxiety, Antioxidant, Alzheimer

Introduction

Alzheimer's disease (AD) is the most frequent form of dementia, where behavioral and cognitive disruption symptoms coexist (1). Depression, apathy, anxiety, and other conduct disorders are the complaints most often reported by caregivers (2). Dementia usually leads to a marked decrease in the cognitive, mental and physical skills of the affected person, who, over time requires an increased amount of care, aid and support (3).

It has been shown that reactive oxygen species (ROS) from mitochondria can cause many neurological diseases and are associated with nerve cell death and neuronal destruction (4). Recent human and animal studies indicate that

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oxidative stress may play a role in the etiology and pathogenesis of depression and anxiety (5). Anxiety, a main contributor to various psychiatric and neurodegenerative disorders, leads to cellular death through destruction of biomolecules such as DNA. Stress-induced detrimental effects are mediated by oxidative stress i.e., increased lipid peroxidation and production of free radicals such as ROS. In addition, anxiety suppresses hippocampal neurogenesis. Significantly, smaller hippocamps of depressed patients compared with healthy individuals indicate sub-optimal neurogenesis (6-8). Researchers have shown that antioxidants are important compounds for the treatment of memory impairment and behavioral disorders caused by oxidative stress (9). For physiological reasons, it is believed that the central nervous system (CNS), especially the hippocampus, is highly sensitive to oxidative stress. The hippocampus plays an important role in processing emotions and this area of the brain is impaired during periods of depression and anxiety (10). In addition to medication, it is important to pay attention to the patient's environment and lifestyle. Therefore, the use of proper diet and physical exercise are non-pharmacological methods to prevent or treat behavioral disorders caused by AD (3). One of the antioxidant-rich foods with anti-inflammatory properties is Royal Jelly (RJ) (9). RJ is a creamy secretion of the hypopharyngeal and mandibular glands of bee workers of the Apis mellifera L. species, and it has shown significant neuroprotective actions. Because it is a rich mixture of proteins, lipids, sugars, vitamins, and minerals, RJ is considered a target nutraceutical, and it has been used to treat various health problems (10, 11). RJ contains biologically active amino acids such as aspartic acid, cysteine, cystine, tyrosine, glycine, lysine, leucine, valine, and isoleucine. As indicated by previous researchers, the antioxidant effect of RJ may be related to its free amino acid content (11, 12)**.** Few experimental studies were conducted to test the effect of RJ on neuropsychiatric disorders, and it seems that lipids in it may counteract stress-induced depression or anxiety effects. A unique RJ component, cAMP-N1 oxide, not found in any other materials, acts directly on neuronal differentiation and stimulates the formation of different brain cells (10). Also, RJ facilitates the differentiation of all types of brain cells: neurons, astrocytes, and oligodendrocytes and ameliorates neuronal function by regenerating hippocampal granule cells that function in the cognition process (9, 12).

In addition to the type of nutrition, the evidence suggests that lifestyle practices that could potentially deter or slow disease progression are especially important considering that neurodegeneration in AD begins up to a decade or more before the appearance of clinical symptoms (3). It is recommended by the Alzheimer's Association to clinicians as a way to maintain cognitive functioning in AD and enhance a patient's quality of life (e.g., by lowering depression). Evidence suggests that physical exercise may improve memory in Alzheimer's patients and perhaps slow cognitive decline and attenuate behavioral deficits in old age (1). Regular physical activity has clear health benefits. Some studies have reported the effects of physical exercise such as wheel running and treadmill running on increasing the neurogenesis in hippocampal region. It indicated that physical exercise induces neuroplasticity of the brain and improves cognitive functions, as evidenced by animal and human studies. Neurogenesis in the adult hippocampus is known to be decreased by various factors, such as aging, depression, stress, depression and neurodegenerative disease (13). There appears to be some evidence that exercise training improves behavior, although which type of behavior is unclear and which type of training exercise shows the most benefit has not been established yet. The results of epidemiological studies of physical activity in patients with dementing disorders, such as AD, have been inconsistent, some reporting an inverse association between physical activity and cognitive decline, while others report no relationship (14-18). In depression, Aman and Thomas found no improvements in depressed mood after three weeks of engaging in exercise (16) and a pilot study by Steinberg et al. found evidence of higher depression scores and poorer quality of life after 12 weeks of daily aerobic exercise (16). Williams and Tappen found that comprehensive exercise as well as walking for 16 weeks reduced depressed mood (18, 19). Although Edwards et al. found no effect of chair-based exercise in reducing levels of behavioral disorders after 12 weeks (15), Aman and Thomas found an improvement after only three weeks of aerobic exercise and strength training (16). In a research by Zhixiong Zhang et al, it was

suggested that pretreatment by voluntary wheel running showed significant neuroprotective effects on the ischemic-reperfused rat brain, decrease oxidative stress, repair DNA damage and reduce cell apoptosis and showed that voluntary exercise may modulate cell apoptosis in chronic restraint stress (20). Carla M et al, examined the effects of voluntary (16 weeks of wheel running) and forced (16 weeks of treadmill running) exercise on mouse model of Alzheimer's disease. The results indicate that voluntary exercise is more beneficial for mitigating the behavioral and neuropathological components of the AD process than forced exercise (21). However, the beneficial effect of exercise type, its duration, intensity and frequency is also unclear (14).

While physical exercise has long been seen to contribute to physical and psychological health, there is now interest in whether it can delay the progression of neurodegenerative diseases, such as AD (22). The intensity of activity under one's control can be beneficial. Due to the fact that AD is a disease of the elderly, and due to their physical and mental disabilities, it seems that the use of voluntary exercise, which is a type that regulates intensity under the control of the individual, can be beneficial, because during voluntary exercise (the rotating wheel), the subjects (animal samples), suffer less pressure and stress, and there is a reduction in oxidative damage to the brain than in forced exercise (23). However, some studies have not shown these positive effects of exercise and physical activity in this area (24,25).

There are various methods for inducing experimental AD in laboratory samples, one of which is the intra-peritoneally injection of trimethyltin chloride (TMT). TMT is known to be highly neurotoxic compared to other organotin compounds. Accidental exposure to TMT causes neuropathological symptoms, cognitive impairments, hyperactivity, aggressive behavior, and seizures (26). Studies showed that TMT increased expression of reactive oxygen species (ROS) and inducible nitric oxide synthase (iNOS). On the other hand, pharmacologic inhibition studies showed that the iNOS-mediated NO generation and ROS increased expression of Bax and then mitochondrial-mediated apoptosis which then activates the caspase protease cascade to execute apoptosis (20). So, TMT is considered a useful tool to study the molecular mechanisms that occur in the human neurodegenerative diseases, in particular for the hippocampal damage that the neurotoxic triggers (27).

Considering the effect of organotin toxins in the environment and food chain, and in addition to the side effects of chemical drugs that are used to reduce the psychological effects of Alzheimer's disease, the aim of this study was to answer the question that whether the voluntary exercise protocol and RJ consumption as an antioxidant dietary supplement could be used as a non-pharmacological and natural solution to improve behavioral symptoms and antioxidant capacity and reduce the process of nerve damage caused by Alzheimer's induced by TMT injection.

Materials & Methods

The current experimental study was conducted on 48 male Sprag Dawley rats aged 8 weeks, obtained in December 2018 from the Animal Breeding Center of the Islamic Azad University, Marvdasht Branch, Iran. Animals were transferred to the sports physiology lab in Azad University, Marvdasht and maintained under standard conditions for seven days in transparent polycarbonate autoclaving cages with the optimum temperature of 20°C - 24°C, relative humidity of 55% - 65%, 12:12 hour light-dark cycle, and free access to water and standard food pellets. On the 8th day, 40 rats were injected 8mg/kg TMT intra-peritoneally and (28). Three days later after confirmation of hippocampus degeneration, AD symptoms were observed by a number of behavioral changes in rats. These clinical symptoms included muscle tremors, elevated body temperature, nausea, seizure, and tail twists. The rats with AD were equally divided into six groups each group consisting of 8 members including AD control (ADC), VW (Voluntary wheel running), sham (RJ solvent) (SH), VW + RJ (VWJ), RJ and healthy control (HC) group. The RJ groups received 100 mg/kg RJ daily for eight weeks (29); 48 hours after the last training session, the depression and anxiety were measured.

Training Protocol: The voluntary exercise group was placed in a rotating wheel, and the distance traveled per day was recorded by the device in meters per minute. VW (Voluntary wheel running, for eight weeks, three sessions per week and 60 minutes each session) had done (21).

Forced Swimming Test (FST): This test appears to be suitable for detecting depressive-like behavior in rodents. Rats were individually placed into a glass cylinder (30 cm diameter ×40 cm height) filled with water at 23–27°C to a depth of 28 cm. The rats were submitted to forced swimming for 15min (pre-test), and the next day, the rats were put back into the water and underwent the FST. All animals were forced to swim for 5 min, and the immobility time was recorded by competent observers manually. Immobility time was defined as the time spent by the mouse floating in the water without struggling, and making only those movements necessary to keep its head above the water. After the forced swimming, the rats were gently dried and returned to their home cages. (30).

Elevated Plus-Maze (EPM): The elevated-plus maze was used to assess anxiety-like behavior and locomotor activity. This apparatus consisted of four arms (two open and two closed arms) arranged in the shape of a plus sign, and elevated to a height of 50 cm from the floor. The open arms had no walls (50×10 cm), but to prevent the rats from falling a rim of Plexiglas (0.5 cm high), surrounded the perimeter of the open arms. The closed arms were enclosed by walls with 40 cm height (50×10×40 cm). At the intersection of four arms, there was a square platform of 10×10 cm without any walls. Each animal was individually placed in the center of the maze facing an open arm, and allowed 5 minutes of free exploration. The number of entries (with all four paws) into open and closed arms, and the total times that the animal spent in the open and closed arms were separately recorded. The percentage of open arm time (%OAT) and open arm entries (%OAE) that are used as the standard anxiety indices were calculated using the following formulae:

- (a) %OAT (the ratio of the total time spent in the open arms to the total time spent in four arms × 100)
- (b) %OAE (the ratio of the total entries into the open arms to the total entries in four arms × 100)

The sum of all the open and closed arms entries was used as the index of general locomotor activity (7).

Measurement of gene expression: Forty-eight hours after the last training session, the rats were anesthetized with ketamine 10% (50 mg/kg dose) and xylazine 2% (10 mg/kg dose) after approximately 5 minutes. The hippocampus tissue was extracted by specialists and after setting in cryotube it was placed in liquid nitrogen and stored at -70ºC for further investigation. For molecular analysis of the gene expression level, firstly, total RNA was isolated from the tissues using an RNA extraction kit (Cinnagen Inc., Iran). The quantity and quality of RNA were examined by spectrophotometer, 260/280 optical absorption and agarose gel electrophoresis, respectively. When preparing the cDNA from the purified sample after absorption reading, a volume of 1 lg of RNA was removed and prepared according to the instructions in the cDNA Fermentas Kit (Fermentas Inc.). Reverse transcription reaction was performed using the RevertAid M-MuLV Reverse Transcriptas enzyme. The cDNA prepared was used for PCR (or transferred to a freezer for maintenance at a temperature of -20ºC). PCR reactions were performed in the system (ABI, Step One, USA), Applied Bio Systems, Step One, using the Real Q Plus 2x kit Master Mix Green (Ampliqon Inc), according to the brochure instructions. Primers design was performed using primer 3 software (Table 1).

Table1. The primer Sequences

Genes	Primer Sequences	Sizes (bp)
B2m	Forward: 5'- CGTGCTTGCCATTCAGAAA -3' Reverse: 5'-ATATACATCGGTCTCGGTGG -3'	244
SOD	Forward: 5'- CAAGGAACCACAGGCCTTAT -3' Reverse: 5'- GGCTAACATTCTCCCAGTTGA-3'	133
Gpx1	Forward: 5'- CATTGAGAATGTCGCGTCCC-3' Reverse: 5'- TTGCCATTCTCCTGATGTCCG-3'	141

B2m gene was used as an internal control in real-time PCR reactions. Real time PCR reactions were performed for 10 min at 94ºC followed by 40 cycles of 15 s at 94 ºC and 60 s at 60 ºC. After each real-time PCR run, gel electrophoresis and melting curve analysis were performed to confirm the specific amplification of the target. Negative control with no pattern was used in all reactions as well. The amplification signals of various samples were normalized with B2m Ct (cycle threshold). After real-time- PCR cycles were over, the amount of gene expression was measured, using the $2^{-\Delta\Delta CT}$ method.

Results

Statistical Analyses: For statistical analysis, multivariate analysis of variance (MANOVA) was used and post hoc Scheffe at the significant level ($P \leq 0.05$) with SPSS software version 20 was performed. Because of the presuppositions for multiple analysis of variance the normality of data distribution with Shapiro Wilk test and assess equality of variances with Levene's Test had done; F ($P > 0.05$).

To use MANOVA, it is ideal that variances and covariance of the variables be equal. According to the table 2 Leven's test for Immobility and SOD shows variance equality ($p > 0.05$), and for GPX, OAE, OAT the Leven's test was significant; with neglecting of some non-equal variance Parametric statistics can be tolerantly used to analyze data. As the table 2 shows the non-significance of Box's test with p=.073 ($p > 0.05$) the equality of covariance matrices confirms the covariance equality (31).

Immobility: immobility time in the forced swimming test, SOD: Superoxide dismutase, GPX: Glutathione peroxidase, OAE: The percentage of open arm entries, OAT: The percentage of open arm time.

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After evaluation the mean and standard deviation (Table 3), According to Table 4, the overall results of multiple variance analysis in all four indicators were significant ($P \leq 0.05$) which showed the significance of the indicators at least in one of the groups and variables.

Table 5 shows the results of the multivariate variance analysis test separately for the

Table 2. The result of equality the covariance and variances

Box's Test of Equality of Covariance Matrices			
	F	**Sig.**	**Box's M**
	1.592	.073	55.601
Leven's test of Equality of variances			
	F		**sig**
SOD	1.410		.254
GPX	6.112		.001
OAE	12.676		.000
OAT	3.716		.011
Immobility	.178		.968

Immobility: immobility time in the forced swimming test, SOD: Superoxide dismutase, GPX: Glutathione peroxidase, OAE: The percentage of open arm entries, OAT: The percentage of open arm time.

Table 3. The descriptive indices (mean and standard deviation) of the variables in the 6 groups.

variables	Group	Mean	Std. Deviation
Immobility	VW	189.6000	28.46577
	VWJ	92.6000	17.11140
	RJ	134.6667	26.60576
	SH	169.8000	23.63684
	HC	125.2000	25.60664
	ADC	207.1667	25.31732
SOD	VW	.5640	.45269
	VWJ	.6560	.46597
	RJ	1.2700	.66504
	SH	.4360	.33027
	HC	1.3120	.65148
	ADC	.4333	.31328
GPX	VW	.4220	.18445
	VWJ	.5382	.33404
	RJ	.5983	.28701
	SH	.0488	.04263
	HC	.3840	.19882
	ADC	.0480	.02086
OAE	VW	53.3360	13.94354
	VWJ	69.3340	11.82023
	RJ	57.9167	10.05195
	SH	41.9360	8.35121
	HC	56.6680	9.13054
	ADC	25.0000	27.38613
OAT	VW	29.8000	34.07638
	VWJ	58.6000	24.70425
	RJ	58.0000	22.20811
	SH	14.4000	7.36885
	HC	62.2000	37.14431
	ADC	10.5000	6.18870

Immobility: immobility time in the forced swimming test, SOD: Superoxide dismutase, GPX: Glutathione peroxidase, OAE: The percentage of open arm entries, OAT: The percentage of open arm time, VW: Voluntary wheel running, VWJ: VW + RJ, RJ: royal jelly, SH: sham, HC: healthy control, ADC: AD (Alzheimer) control group.

Table 4. General results of multiple variance analysis

Effect		F	Sig.	Partial Eta Squared
Group	Pillai's Trace	2.666	.000	.339
	Wilks' Lambda	3.975	.000	.442
	Hotelling's Trace	5.497	.000	.574
	Roy's Largest Root	27.887[c]	.000	.843

Table 5. Multivariate analysis of variance test results by dependent variables

Source	Dependent Variable	F	Sig.	Partial Eta Squared
Group	Immobility	16.165	.000	.757
	SOD	3.569	.014	.407
	GPX	7.052	.000	.576
	OAE	5.530	.001	.515
	OAT	5.071	.002	.494

Immobility: immobility time in the forced swimming test, SOD: Superoxide dismutase, GPX: Glutathione peroxidase, OAE: The percentage of open arm entries, OAT: The percentage of open arm time

dependent variables. As can be seen in the table, for SOD, GPX, OAE, OAT and immobility variables, the F index has significance ($p < 0.05$). Interventions have had the greatest effect on immobility.

The results of multivariate analysis of variance show that there is a significant difference in all five variables of OAT, SOD, GPX, OAE and immobility in at least one group. Scheffee post hoc test has been used to examine which groups have significant differences. In Table 5, our significant differences between groups are identified by separating the dependent variables with ($p < 0.05$). However, the difference between some groups has border significance, which is due to the low volume of the sample in each group, therefore the borderline of this meaning can be overlooked (32).

Overall, the findings of the present study according to table 6 show that the dependent variable of immobility in the VWJ, RJ, HC, groups has decreased compared to the VW group, which shows the positive effect of exercise with RJ in reducing depression. this index has also increased in the ADC, SH groups in compared to the VWJ group, and in the ADC group in compared to the RJ and HC groups has also increased; This has been shown to have a positive effect on RJ in reducing depression, also according to the increase in ADC group in compared to the HC group; Thus, it may indicate the destructive effect of TMT on depression; although differences in SOD antioxidant index did not make sense, But another indicator, GPX, in Sham and ADC groups compared to VW, showed a decrease, and in the RJ group compared to the SH and ADC groups, it showed an increase, which indicates a positive effect of RJ consumption on GPX increase; and in terms of anxiety indicators, that include OAE and OAT, in VWJ, RJ and HC groups it has increased

compared with ADC group. This indicates a positive effect of exercise, especially RJ consumption, on reducing anxiety.

anxiety (26). It is reported that the administration of 8 mg/kg of TMT significantly reduces the density of the

Table 5. Scheffee post hoc test

Dependent Variable	(I) Group	(J) Group	Sig.
Immobility	VW	VWJ	.000
		RJ	.045
		HC	.018
	VWJ	SH	.003
		ADC	.000
	RJ	ADC	.002
	HC	ADC	.001
GPX	VWJ	SH	.044
		ADC	.031
	RJ	SH	.012
		ADC	.007
OAE	VWJ	ADC	.004
	RJ	ADC	.042
	HC	ADC	.075
OAT	VWJ	ADC	.095
	RJ	ADC	.077
	HC	ADC	.060

Immobility: immobility time in the forced swimming test, SOD: Superoxide dismutase, GPX: Glutathione peroxidase, OAE: The percentage of open arm entries, OAT: The percentage of open arm time, VW:Voluntary wheel running , VWJ: VW + RJ, RJ: royal jelly, SH: sham, HC: healthy control, ADC: AD (Alzheimer) control group.

Discussion

According to the results of this study, the Immobility index in the ADC group was higher than the HC group, and the indicators of anxiety (OAE, OAT) in the HC group were higher than the ADC group, which indicates the induction of anxiety and depression by TMT. TMT is an organotin compound that causes selective destruction in the CNS (27). The hippocampus is the most susceptible brain region. Several studies have shown that a common pathogenesis is seen between psychological and neurodegenerative disorders, and in fact, there is a direct relationship between disorders such as depression and anxiety with neuronal death and inflammatory factors in the hippocampus (33). TMT-induced hippocampal damage results in behavioral and learning disabilities such as hyperactivity, aggression, depression and granular cells in the rat follicles (34), increases the level of reactive oxygen species linked with apoptotic death, and causes behavioral disorders (27). Several studies showed that TMT-induced cell death occurs mainly by apoptosis as demonstrated by the presence of DNA fragmentation and chromatin condensation in TMT-treated cells and involves the activation of the pro-apoptotic genes. It is widely accepted that oxidative stress is an initiator of TMT-induced apoptotic cell death due to generation of both reactive oxygen species and nitrogen species (26). The cell death can be reduced by antioxidants and by increased levels of intracellular Glutathione (27).

In anxiety test with the device (EMP) the anxious rats are afraid of being in open arms without a wall, preferring to stay in the closed arms were enclosed by walls. On the other hand, the reduction in the number of animals entering the open arms was

accompanied by a decrease in the time of their presence, which is itself an indicator of the presence of anxiety behaviors in rats (7). According to the results of the study, it was shown that there was more anxiety in ADC group than in the RJ and VWJ group (by reducing OAE, OAT). It has been shown that voluntary exercise along with RJ consumption has a positive effect on reducing anxiety caused by TMT poisoning. However, the study, conducted by Baghan et al., concluded that voluntary exercise could be useful as a non-pharmacological treatment for mental and emotional disorders such as anxiety, and showed that voluntary exercise was significantly better than forced exercise and it eliminated anxiety and reduced gene expression of inflammatory factors in the brain (6). Studies have shown that exercise has a big effect on the structure and function of the CNS, possibly by increasing the expression and production of brain-derived neuronal growth factor (BDNF), neurogenesis and synaptogenesis, reducing inflammatory factors, increasing serotonergic system activity and reducing stress (1, 21, 35, 36).

Reducing oxidative stress leads to morphological and behavioral changes and has anti-anxiety effects (37). Since the hippocampus is one of the most important structures in the brain associated with depressive and anxiety behaviors (30), it can also be noted that reducing anxiety following exercises can result in changes in the hippocampus because it has been shown that running increases the number of new stimulatory neurons and more dendritic branches throughout the hippocampal circuit (8).

The findings of the present study show that in the forced swimming test, the immobility dependent variable of the VWJ, RJ, HC groups decreased, which shows the positive effect of exercise with RJ on reducing depression. Researchers in the Department of Psychiatry and Suicide Studies, McGill Group in Quebec, Canada., have found that some brain cells in patients affected by depression become larger and more inflamed, which can be seen as confirmation of the neurological inflammation theory in depression (38).

According to our results, previous studies have reported that voluntary exercise due to the arbitrary nature of the activity, unlike forced activity, prevents stress and, in fact, a stress-free sport that is performed at the animal's own discretion activates the pleasure pathways (36). Many studies have made voluntary exercise as one of the Environmental Enrichment factors (EE) (39). The role of serotonin as a key factor in neurogenesis due to exercise has been well-known for decreasing anxiety and depression. and serotonin receptors are expressed in the hippocampus and regulate neuronal excitability, synaptic plasticity, and memory (40). Greenwood et al., showed that eight weeks of exercise has caused the serotonin receptors to transmit messages and improve depression (17).

However, according to the results of this study, the voluntary exercise group alone did not show a decrease in stress and anxiety, so we can point to the prominent role of RJ. However, it should be noted that the effect of exercise on anxiety is multifactorial and is affected by the intensity of exercise, rest time after exercise and the level of anxiety before exercise. Therefore, the inconsistency of some research results in this field is due to the difference in the duration of time, the intensity of exercise and the time of performing anxiety and depression tests after exercise.

In the present study, an increase in GPX levels was observed in RJ and VWJ groups compared to sham and ADC groups, which indicates the antioxidant effect of RJ on controlling the causes of oxidative stress. RJ has previously been reported to have high antioxidant activity and the ability to inhibit free radicals such as superoxide and radical hydroxyl ions (11). The preconditioning actions of RJ can exert protective effects, by upregulation of the antioxidant system and the reduction in reactive oxygen species. It

may propose that RJ is an activator of antioxidant enzymes (4). Studies have shown that AD is also associated with high cholesterol levels because cholesterol buildup can accelerate the formation of beta amyloid plaque, so since RJ can also reduce fat and blood cholesterol, possibly by activating the beta form Estrogen Receptor (ER) receptors can also have a positive effect on behavioral disorders (5). In this regard, Zamani et al. reported that the consumption of food that contained 3% RJ for 10 days could improve learning and memory; cognitive processes by exerting positive effects on neural functions and therapy and preventing some neuronal disorders (41). Tiago Guardia evaluated the effect of RJ long-term oral administration on differentiation of new neurons in the hippocampus. The data obtained in this study demonstrate that the prolonged treatment with RJ reduced the deleterious effects on cognition, neurodegeneration and oxidative stress in animals submitted to the rat model of sporadic Alzheimer's disease. And the results of the Elevated Plus Maze Test show that there were no significant differences in any of the observed parameters and the RJ oral treatment did not produce changes in the anxiety levels and exploratory behavior of the animals (9).

In corroboration with our study, recently a study using a rabbit model of AD showed that RJ long-term oral administration, reduced the neuronal loss and enhanced anti-oxidative capacities in AD rabbits cortex and hippocampus brain areas (5). RJ can also be considered as a result of considerable neuroprotective value exert great influence on the dynamics of the hippocampal circuitry by the increase in the number of proliferating new neurons as well as impact on cognitive and behavioral aspects related to learning and memory, pattern recognition, stress and mood disorders (9). We could hypothesize that it plays an important role in the recovery of memory performance, acting together with the others beneficial effects, reduction in the levels of oxidative stress and the neuronal degeneration. These beneficial brain effects might be assigned to some RJ compounds that can be absorbed by gastro-intestinal tract and are able to pass through hematoencephalic barrier. In this regard, among the active substances in the RJ we highlight small peptides (obtained from the hydrolysis of major royal jelly proteins, MRJPs), free amino acids, the 10- carbon atoms fatty acids, 10-hydroxy-2-decenoic acid (10-HDA) and 10- hydroxydecanoic acid, besides AMP-N1 oxide (11). It has been shown that small peptides, with 2–4 amino acids residues isolated from RJ containing Tyr residues at C-terminal present strong hydroxyl-radical scavenging activity that is suggested act as free-radical scavengers (12). Concerning the 10-hydroxy-2-decenoic acid (10-HDA), the main and unique fatty acid found specifically in RJ demonstrated that it participates in the production of important molecules for brain function as brain-derivate neurotrophic factor (BDNF) and neurogenesis in neural progenitor cells (11). 10-hydroxy-2-decenoic acid increased the viability and growth of primary hippocampal neurons and using in vitro models of age-related neurodegeneration showed that this fatty acid was able to reduce cell death. Moreover, a decreased anxiety in aged male rats treated with 10-HDA was observed (9). Another RJ component that could respond, at least in part, for the neuronal benefits of RJ is the adenosine monophosphate (AMP) N1-oxide, which is found only in RJ, and present a neurotrophic factor. Thus, AMP N1-oxide induces neurite process, and stimulated expression of a specific protein of mature neurons, demonstrating its stimulatory activity to induce neuronal differentiation of CNS. In addition, it has been suggested that AMP N1-oxide acts by adenylyl cyclase-coupled adenosine receptors and could play a role in modulating neuronal function via adenosine receptors (9, 11). In line with this study RJ oral administration has been shown both to prevent trimethyltin induced acute neurodegeneration and to increase the

number of granule cells in the dentate gyrus, with concurrent improvement of cognitive functions (42). Taken together, the data obtained in this study demonstrate that the prolonged treatment with RJ reduced the deleterious effects on cognition, neurodegeneration, lipid peroxidation created by reactive oxygen and oxidative stress in animals submitted to the TMT injection model. Thus, RJ presents a potential therapeutic value for the treatment of cognitive deficits and a beneficial action in neurodegenerative processes**.**

Conclusion

The present study showed that the combination of voluntary exercise with RJ supplementation has the ability to moderate mood in the current model and can be used as a useful human method. Undoubtedly, the results of this research and similar research will help to clarify the mechanisms that affect the body and give more accurate advice on sports activities along with the use of dietary supplements, to specific groups.

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Conflicts of Interest

The authors claim that they have no conflict of interest in conducting this study.

The present study has been approved by research ethics committee of Shiraz University of Medical Sciences. Approval ID is “IR.SUMS.REC.1399.362”.

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