JOURNAL OF NUTRITION FASTING AND HEALTH

Effect of Eight Weeks of Positive Slope and Negative Slope Training, Along with Royal Jelly on The Hippocampal Expression Of B- Amyloid And Γ-Secretase in Trimethyltin-Induced Alzheimer's Disease Rats

Zahra Giti[1], Abdolali Banaeifar[1*], Sajad Arshadi[1], Mohammad Ali Azarbayjani[2]

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

ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 14 Jun 2020
Accepted: 20 Jul 2020
Published: 01 Jan 2021

Keywords:
Training
Royal jelly
Amyloid-beta
Gamma secretase
Alzheimer's disease

ABSTRACT

Introduction: One of the main causes of Alzheimer's disease is an increase in amyloid-beta (Aβ) peptides, which are produced by the activity of the enzyme gamma-secretase (γ-secretase). Although exercise and the use of royal jelly have protective effects on neurons, there is limited information about their interactive effects, so the present study was performed to investigate the effect of eight weeks of positive slope training (PST) and negative slope Training (NST), along with the use of royal jelly (RJ) on the hippocampal expression of Aβ and γ-secretase in Alzheimer's rats treated with trimethyltin (TMT).

Methods: In this experimental study, 42 male rats were injected intraperitoneally with 8 mg/kg TMT and were randomly assigned to seven groups of 6 subjects, including: (1) sham (royal jelly solvent), (2) PST, (3) NST, (4) PST+RJ, (6) NST+RJ and (7) RJ were divided. To assess the effects of Alzheimer's induction on variables, six rats were included in the healthy control group. Rats performed endurance training for eight-weeks, five days per week, and 60 minutes per session and the royal jelly groups received 100 mg/kg royal jelly peritoneally each day for eight weeks.

Results: PST, NST, and RJ decreased the expression of Aβ and γ-secretase ($P < 0.001$). Also, PST+RJ and NST+RJ decreased the expression of Aβ and γ-secretase in the hippocampal tissue of rats with Alzheimer's disease ($P < 0.001$).

Conclusion: It seems that PST, NST, and RJ can reduce the progression of Alzheimer's disease markers, and PST+RJ and NST+RJ synergistically reduce the progression of Alzheimer's disease in animal models.

► Please cite this paper as:
Giti Z, Banaeifar A, Arshadi S, Azarbayjani MA. Effect of Eight Weeks of Positive Slope and Negative Slope Training, Along with Royal Jelly on The Hippocampal Expression Of B- Amyloid And Γ-Secretase in Trimethyltin-Induced Alzheimer's Disease Rats. J Nutr Fast Health. 2021; 9(1): 29-34. DOI: 10.22038/jnfh.2020.49567.1272

Introduction

Alzheimer's disease (AD) is a rapidly increasing dementia that kills millions of people around the world or imposes heavy economic and psychological costs on these patients or their families (1). AD is associated with vascular disorders of the central nervous system, with previous studies showing pathological changes in the cerebral vascular system (1,2).

Various factors play a role in Alzheimer's disease, but one of the main causes of Alzheimer's disease is an increase in amyloid-beta (Aβ) peptides, especially Aβ 1-42, which is generated as a result of the sequential proteolytic breakdown of amyloid precursor protein by β- and γ-secretase enzymes. Subsequent accumulation of amyloid plaques on the cell surface disrupts the receptor function of neurotrophic hormones and growth hormone hormones and stops the repair and neurogenesis process (2). On the other hand, researchers believe that lifestyle changes and performing exercise have protective effects on the brain (3) in such a way that exercises can improve the cellular molecular function of the brain, increase blood flow to the brain, increase the brain's resistance to neurological damage, and thus improve cognitive and physical factors related to the brain (3). In this regard, the researchers pointed out that low-intensity and high-intensity exercises on the ergometer bike improve cognitive function, and increase resistance to injury in the elderly (3).

Exercise also reduces Aβ by decreasing oxidative stress and inflammatory factors; also, short-term resistance exercise by inhibiting pro-inflammatory and inflammatory cytokines improves cognitive function and enhances the

* *Corresponding author:* Abdolali Banaeifar, Associate Professor, Department of Sports Physiology, South Tehran Branch, Islamic Azad University, Tehran, Iran. Tel: +989122251779, E-mail: Alibanaeifar@yahoo.com

function of cortical neurons and the hippocampus in mice with AD(4); Also, 5 months of endurance training with treadmill reduces Aβ levels in mice with AD (5). However, there are differences in the intensity of different exercises. Exercise intervention possibly with different effects on the oxidative-antioxidant stress system, can have different effects on the central nervous system and AD indicators (6). The type of contraction, the different intensities of exercise on the positive slope training (PST), and the negative slop training (NST) also appear to have favorable effects on brain protection (7). Thus, PST and NST increased the hippocampal expression of serotonin in rats with AD but did not alter dopamine levels (7); while in Hosseini et al.'s study, PST was only able to reduce the pain threshold of rats with AD (8); Also, moderate to high endurance training did not have a significant effect on Aβ increase in rats (6).

Due to the contradictory results regarding the different effects of endurance training on the central nervous system, researchers in the field of sports science have recently drawn attention to the use of natural antioxidants along with sports activities to improve the central nervous system (8). Among these natural ingredients, royal jelly (RJ), which is produced by the submandibular glands of bees, with its antioxidant and anti-inflammatory effects can have protective effects on neurons (9). In this regard, the treatment of N2a/APP695 cells with 1-9 μg/mL RJ decreased Aβ and inhibited γ-secretase (9). Optional and compulsory training combined with RJ consumption had an interactive effect on improving avoidance memory and spatial memory in rats with AD (10); endurance training combined with RJ consumption reduced inflammatory factors in the muscle tissue of rats with AD (11). Previous studies have shown the protective effects of exercise and RJ supplementation in animal models of AD. However, due to the increasing trend of aging and cognitive disorders in a wide range in people who will reach elderly age, and the limitation of information in line with the most effective method of exercise and antioxidant efficacy without side effects for the replacement of synthetic drugs, this study aimed to investigate the effect of eight weeks of PST and NST with RJ on Aβ and γ-secretase levels in the hippocampal tissue of Alzheimer's rats treated with trimethyltin (TMT).

Materials and Methods

In this experimental fundamental study, 42 rats (mean age 8.1 ± 2.34 weeks old and mean weight 200.35 ± 22.45 g) were prepared from the Center for Animal Reproduction and Breeding of the Islamic Azad University of Marvdasht and kept in the laboratory of animal sports physiology of this university branch for one week for adaptation. The study was approved by the Ethics Committee of Marvdasht Branch of Islamic Azad University.

During the protocol, rats were kept in the standard conditions of transparent polycarbonate autoclave cages at temperature of 22 ±2˚ C, the relative humidity of 55 to 65%, 12-hour light-dark cycle and free access to water and food. On the eighth day, 36 rats were injected intraperitoneally with 8 mg/kg neurotoxin TMT (8). Two weeks after the injection of neurotoxin to test for Alzheimer's induction, the shuttle box and Maze Y tests were performed, and after 24 hours of ensuring full effect on the hippocampus, Alzheimer's rats were randomly assigned to six groups of six subjects, including: (1) control + normal saline (royal jelly solvent) (Sh), (2) PST, (3) NST, (4) PST+RJ, (5) NST+RJ and (6) RJ. Also, to investigate the effects of Alzheimer's induction on Aβ levels and inhibition of γ-secretase, 6 rats were included in the healthy control group (HC). Then, rats in the PST group trained for eight weeks, five sessions per week for 60 minutes at a speed of 16 m/min, and rats in the NST trained for eight weeks, five sessions per week for 60 minutes at a speed of 16 m/min (7). The royal jelly group received 100 mg/kg RJ peritoneally dissolved in normal saline each day for eight weeks (7). Then 48 hours after the last training session, rats were anesthetized using a combination of ketamine 10% and xylazine 2% at a dose 50 to 10. After complete anesthesia, the hippocampal tissue of rats was isolated by laboratory specialists and immediately placed in special tissue-keeping cryo-tubes and transferred to -70˚ C environment.

Exercise Training Protocol

The training groups ran three sessions a week for one week to familiarize themselves with the treadmill at a speed of 5 to 10 meters per minute for 5 to 10 minutes. Rats in the PST group trained for eight weeks, five sessions per week for 60 minutes at a speed of 16 m / min, and in the NST group trained for eight weeks,

five sessions per week for 60 minutes at a speed of 16 m / min speed. The training was such that the positive and negative slopes were considered 9 degrees for the first week, and one degree was added to the positive and negative slopes each week. It also took 5 minutes to warm up and cool down at 8 m/min at the beginning and end of the workout (7).

Measuring the Expression Levels of Aβ and γ-Secretase

For molecular analysis at the gene expression level, firstly, extraction of RNA from the hippocampus tissue was carried out according to the manufacturer's protocol (Sinagen, Iran); secondly, using light absorbance at a wavelength of 260 nm, the concentration and degree of purity of the RNA sample were quantitatively obtained using the following equation:

$$C\ (\mu g/\mu l) = A260\times \varepsilon \times d/1000$$

After extracting RNA with high purity and high concentration from all of the samples, cDNA synthesis steps were taken according to the manufacturer's protocol, and then the synthesized cDNA was used for reverse transcription reaction. Initially, the designed primers for genes were examined, and then gene expressions were examined by quantitative q-RT PCR method. It should be noted that B2m was selected as the housekeeping gene. The sequence of the primers used is shown in Table 1.

Table 1. The sequence of the primers used in the present study

Genes	Primer Sequences	Sizes (Bp)
B2m	Forward: 5'- CGTGCTTGCCATTCAGAAA -3' Reverse: 5'-ATATACATCGGTCTCGGTGG -3'	244
amyloid beta	Forward: 5'- AATGAAGGGTCTGGGTTGAC-3' Reverse: 5'- CTTCTGCAAAGAACACCAGTTT-3'	124
γ-Secretase	Forward: 5'- GCAAAGAACTTGAGTTCATCACG -3' Reverse: 5'- GGCCTTGGCGTTGATACAATA -3'	89

Data Analysis Procedure

The Shapiro-Wilk test was used to test the normal distribution of the findings. Also, to investigate the differences between the groups, one-way analysis of variance (ANOVA) and Tukey's *post- hoc* test were used in Graph pad prism 8.3.0 software (P≤ 0.05).

Results

The levels of hippocampal amyloid-beta and gamma-secretase in the research groups are presented in Figures 1 and 2. The results of the one-way analysis of variance showed a significant difference in the levels of Aβ (P = 0.001) and γ-secretase (P = 0.001) in the research groups.

The results of Tukey's *post hoc* test showed that the levels of Aβ in the Sh group were significantly higher than the HC group (P = 0.001). However, the levels of Aβ in the RJ (P = 0.001), PST (P = 0.001), NST (P = 0.001), PST+RJ (P = 0.001) and NST+RJ (P=0.001) groups were significantly lower than the Sh group. Also, Aβ levels in the NST+RJ group were significantly lower than the PST group (P = 0.016) (Figure 1).

γ-secretase levels in the Sh group were significantly higher than the HC group (P = 0.001). However, the levels of γ-secretase in the RJ (P = 0.001), PST (P = 0.001), NST (P = 0.03), PST+RJ (P = 0.001) and NST+RJ (P = 0.001) groups were significantly lower than the Sh group. Also, the levels in the RJ group were significantly lower than the PST (P = 0.004) and NST (P = 0.001) groups. γ-secretase levels in the PST+RJ (P = 0.007) and NST+RJ (P = 0.001) groups were significantly lower than the PST group. γ-secretase in the PST + RJ (P = 0.001) and NST+RJ (P = 0.001) groups were significantly lower than the NST group (Figure 2).

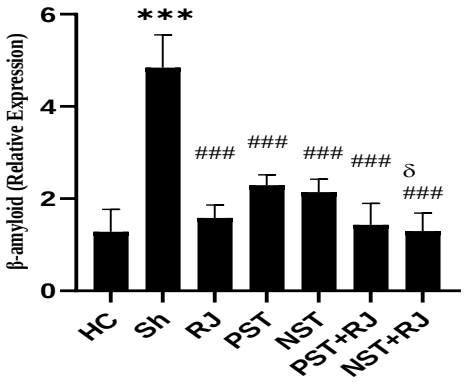

Figure 1. Levels of β-amyloid expression in the seven research groups
HC: healthy control, Sh: Sham, RJ: royal jelly, PST: positive slope training, NST: negative slope training
***P (<0.001) Significant increase compared to the HC group
###P (<0.001) Significant decrease compared to the Sh group
δ (P <0.05) significant decrease compared to the PST group

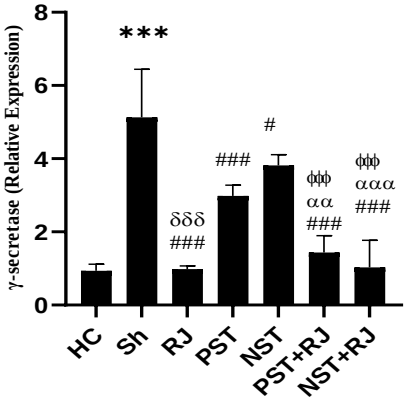

Figure 2. Levels of γ -secretase expression in the seven research groups
HC: healthy control, Sh: Sham, RJ: royal jelly, PST: positive slope training, NST: negative slope training
***(P <0.001) Significant increase compared to the HC group
#(P <0.05), ### (P = 0.001) significant decrease compared to the Sh group
δδδ (P <0.001) Significant decrease compared to the PST and NST groups
αα (P <0.01), ααα (P <0.001) Significant decrease compared to the PST group
φφφ (P <0.001) Significantly decrease compared to the NST group

Discussion

The results showed that PST and NST reduced the expression of Aβ and γ-secretase in the hippocampal tissue of rats with AD. Fundamental studies have shown that exercise enhances α-secretase activity as an enzyme that activates non-amyloidogenic pathways and inhibits the pathway of amyloid precursors in the inner membrane of the nerve cell, releasing a substance that has protective effects on nerve cells. More importantly, exercise breaks down amino acids in the beta-amyloid protein and reduces APP protein, inhibiting β-secretase, and γ-secretase (6). In another study, the researchers found that regular exercise was associated with a mechanism for decreased levels of reactive oxygen species (ROS), modulation of cellular redox, decreased neuronal inflammation, decreased tumor necrosis factor-alpha (TNF-α) and interleukin-1 alpha (IL-1α), an increase in nitric oxide synthase in the endothelial vessels of the brain, as well as an increase in brain-derived neurotrophic factor (BDNF), and hence reduces APP protein and inhibits β-secretase, and γ-secretase (12). In this regard, researchers have shown that long-term aerobic training lowers amyloid-beta levels in the hippocampal tissue of older laboratory rats, and that exercise improves memory and learning in older rats

(13). In one study, researchers found that exercise with a mechanism for increasing BDNF increased neuronal plasticity and decreases levels of Aβ and APP (14). Optional exercise also reduced beta-amyloid and APP in the hippocampal tissue of rats with Alzheimer's AD (5); however, one study found that 16 weeks of moderate-intensity and high-intensity exercise with walking and running did not significantly reduce beta-amyloid levels in patients with AD (6). Also, in one study, researchers found that although aerobic and stretching training improved cognitive function for 12 months, they did not have a significant effect on reducing amyloid plaques in patients with AD (15). It seems that the type and intensity of exercise and the different statistical population are the reasons for differences between these two studies and the present study.

The results showed that RJ reduced the expression of Aβ and γ-secretase in the hippocampal tissue of rats with AD. Researchers believe that RJ peptides, with their antioxidant and anti-inflammatory mechanisms, can improve the metabolism of neurons, so that by reducing the expression of low-density lipoprotein receptor (LDLR) receptors, the genes for presenilin 1 and 2, and APP inhibit β-secretase and γ-secretase enzymes (9). RJ also inhibits oxidative stress by the mechanism of kinase N-terminal c-Jun, and inhibits phosphorylation-dependent apoptotic mechanism. More importantly, the RJ-dependent Aβ reduction mechanism can be attributed to activation of the pathway of cyclic adenosine monophosphate (cAMP), phosphorylation protein kinase A (p-PKA), p-CREB and BDNF, and inactivation of APP, β-secretase, and γ-secretase (16). In line with the present study, 1-9 μg/Ml RJ inhibited extracellular Aβ in N2a/APP695 cells (9); 12 weeks of RJ consumption decreased Aβ levels, acetylcholinesterase, and decreased oxidative stress and APP in the hippocampal tissue of ovariectomized rabbit (17); RJ consumption also improved hippocampal metabolism, decreased Aβ, increased total antioxidant capacity, and β-site APP cleaving enzyme-1 in rabbits with AD (18).

The results showed that PST + RJ and NST + RJ reduced the expression of Aβ and γ-secretase in the hippocampal tissue of rats with Alzheimer's disease. Also, the effects of the γ-secretase reduction in the NST + RJ group were more favorable than in the PST group. No study has been found to investigate the interactive effect of exercise along with the use of royal jelly on amyloid-beta and gamma-secretase levels, but exercise with ROS reduction mechanism, decreased neuronal inflammation, increased eNS, increased BDNF, increased α-secretase activity and decomposition of Aβ reduces APP protein and inhibits γ-secretase (7,12). Also, RJ with kinase mechanism N-terminal c-Jun, ROS inhibition, inhibition of neuronal inflammation, and activation of cAMP/p-PKA/p-CREB/BDNF pathway causes inactivation of APP and γ-secretase (16,17). Although information about the interactive effect of exercise and RJ on Aβ and γ-secretase is limited, previous studies have shown that exercise and RJ interactively increase serotonin gene expression levels (7) and pain tolerance threshold (8); decrease inflammatory factors in muscle tissue (11) and increase memory and learning (10) in rats with AD. Also, NST appears to be able to induce good antioxidant system adaptations depending on the type of acentric contraction (7), so NST+RJ appears to have more favorable effects than PST in interaction with both RJ consumption and the type of contraction.

Due to the role of neurotrophic in the protective effects of neurons, the inability to measure the neurotrophic factors seems to be one of the limitations of the present study. Therefore, it is suggested that neurotrophic should be evaluated in future studies. Also, due to the need for cognitive and memory evaluation after amyloid-beta and γ-secretase reduction, it seems that lack of behavioral evaluation of memory and learning in this Alzheimer's model is another limitation of the present study. Therefore, it is suggested that researchers evaluate memory and learning in future studies.

Conclusion

PST, NST, and RJ appear to reduce the progression of AD indicators, and PST+RJ and NST+RJ synergistically reduce the progression of AD indicators in the animal model.

Acknowledgments

Given that the present study is the result of a doctoral dissertation approved by the Islamic Azad University, South Tehran Branch, the authors appreciate the spiritual support of the Research Office of this branch.

References

1. Mouchlis VD, Melagraki G, Zacharia LC, Afantitis A. Computer-Aided Drug Design of β-Secretase, γ-Secretase and Anti-Tau Inhibitors for the Discovery of Novel Alzheimer's Therapeutics. Int J Mol Sci. 2020;21(3): 703-28;
2. Durrant CS, Ruscher K, Sheppard O, Coleman MP, Özen I. Beta secretase 1-dependent amyloid precursor protein processing promotes excessive vascular sprouting through NOTCH3 signaling. Cell Death Dis. 2020;11(2):1–15.
3. Veldema J, Jansen P. Ergometer training in stroke rehabilitation: systematic review and meta-analysis. Arch Phys Med Rehabil. 2020;101(4):674–89.
4. Liu Y, Chu JMT, Yan T, Zhang Y, Chen Y, Chang RCC, et al. Short-term resistance exercise inhibits neuroinflammation and attenuates neuropathological changes in 3xTg Alzheimer's disease mice. J Neuroinflammation. 2020;17(1):1–16.
5. Adlard PA, Perreau VM, Pop V, Cotman CW. Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. J Neurosci. 2005;25(17):4217–21.
6. Frederiksen KS, Madsen K, Andersen BB, Beyer N, Garde E, Høgh P, et al. Moderate-to high-intensity exercise does not modify cortical β-amyloid in Alzheimer's disease. Alzheimer's Dement Transl Res Clin Interv. 2019;5:208–15.
7. Hassanlouei F, Tabrizi L, AliHosseini S, Rasoli MH. The Effect of Running on Positive and Negative Slopes on Serotonin Levels in the Hippocampus Tissue of Rats with Alzheimer's Disease. Gene, Cell Tissue. 2019;7(1):e97076.
8. Hosseini SA, Salehi OR, Farzanegi P, Farkhaie F, Darvishpour AR, Roozegar S. Interactive effects of endurance training and royal jelly consumption on motor balance and pain threshold in animal model of the alzheimer disease. Arch Neurosci. 7(2):e91857.
9. Zhang X, Yu Y, Sun P, Fan Z, Zhang W, Feng C. Royal jelly peptides: potential inhibitors of β-secretase in N2a/APP695swe cells. Sci Rep. 2019;9(1):1–11.
10. Bozorgi A, Hosseini SA, 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.
11. Noura M, Arshadi S, Zafari A, Banaeyfar A. Effect of endurance training with royal jelly on crp gene expression in muscle tissue of rats with alzheimer's disease. Middle East J Rehabil Heal Stud. 2020;7(1):e99754.
12. Ebrahimi K, Majdi A, Baghaiee B, Hosseini SH, Sadigh-Eteghad S. Physical activity and beta-amyloid pathology in Alzheimer's disease: A sound mind in a sound body. EXCLI J. 2017; 16:959–72.
13. Thomas R, Zimmerman SD, Yuede KM, Cirrito JR, Tai LM, Timson BF, et al. exercise training results in lower amyloid plaque load and greater cognitive function in an intensity dependent manner in the tg2576 mouse model of alzheimer's disease. Brain Sci. 2020;10(2):88-94.
14. Nigam SM, Xu S, Kritikou JS, Marosi K, Brodin L, Mattson MP. Exercise and BDNF reduce Aβ production by enhancing α-secretase processing of APP. J Neurochem. 2017;142(2):286–96.
15. Tarumi T, Rossetti H, Thomas BP, Harris T, Tseng BY, Turner M, et al. Exercise training in amnestic mild cognitive impairment: a one-year randomized controlled trial. J Alzheimer's Dis. 2019;71(2):421-33.
16. You M, Pan Y, Liu Y, Chen Y, Wu Y, Si J, et al. Royal jelly alleviates cognitive deficits and β-amyloid accumulation in APP/PS1 mouse model via activation of the cAMP/PKA/CREB/BDNF pathway and inhibition of neuronal apoptosis. Front Aging Neurosci. 2019;10:428-40.
17. Pan Y, Xu J, Chen C, Chen F, Jin P, Zhu K, et al. Royal jelly reduces cholesterol levels, ameliorates Aβ pathology and enhances neuronal metabolic activities in a rabbit model of Alzheimer's disease. Front Aging Neurosci. 2018;10:50-64.
18.Pan Y, Xu J, Jin P, Yang Q, Zhu K, You M, et al. royal jelly ameliorates behavioral deficits, cholinergic system deficiency, and autonomic nervous dysfunction in ovariectomized cholesterol-fed rabbits. Molecules. 2019;24(6):e1149.