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

Neda Pardeh Shenas
Islamic Azad University Marvdasht
Omidreza Salehi (omidreza.67salehi@gmail.com)
University of Kurdistan https://orcid.org/0000-0001-5328-4658
Seyed Ali Hosseini
Islamic Azad University Marvdasht

Research article

Keywords: Training, Royal Jelly, Serotonin, Dopamine, Alzheimer's Disease

DOI: https://doi.org/10.21203/rs.3.rs-350557/v1

Abstract

Background

Studies have shown that exercises and the use of natural foods have favorable effects on Alzheimer's disease (AD). The purpose of this study was to investigate the effects of resistance training (RT) with royal jelly (RJ) on serotonin and dopamine receptors expression in hippocampal tissue of rats with AD.

Methods

In this experimental study, 56 rats with AD were randomly divided into (1) control, (2) RT, (3) RT + 100 mg/kg RJ, 4) RT + 200 mg/kg RJ, 5) 100 mg/kg RJ, 6) 200 mg/kg RJ2 and 7) sham. Also in order to review the effects of AD induction on serotonin and dopamine, 8 healthy rats selected as healthy control group. During 8 weeks the groups 3, 4, 5 and 6 received daily RJ with specific doses peritoneally and groups 2, 3 and 4 performed RT three sessions per week with 30–100 percentage of body weight. Independent sample t- test, One way ANOVA and two-way ANOVA was used to investigate the effect of RT, RJ and interaction of RT and RJ also Bonferroni's *post- hoc* test was used to evaluate the difference between the doses of RJ using SPSS software ($P \leq 0.05$).

Results

RT had a significant effect on the increase of dopamine ($p = 0.001$) and serotonin receptors ($p = 0.001$); RJ had a significant effect on the increase of dopamine receptor($p = 0.01$) and serotonin ($p = 0.001$) also RT simultaneously with RJ consumption had a significant interactive effect on the increase of serotonin receptor ($p = 0.001$).

Conclusion

It appears that RT and RJ can enhance serotonergic and dopaminergic function in hippocampal tissue of rats with AD, however, the effects of RJ seems to be dose dependent.

Background

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder that is associated with behavioral disorders such as anxiety and depression (1, 2). Studies have shown that in AD, the increased amyloid plaques and beta-amyloid formation, increase the oxidative stress, inflammation and neuronal death as well as impair the shape and function of receptors of dopaminergic and serotonergic system, acetylcholine, neuronal plasticity and the release of neurotransmitters in pre- synaptic and post-synaptic spaces in different parts of the brain such as the hippocampus (2). Indeed, researchers believe

that serotonin (5-hydroxytryptamine/5-HT) and dopamine neurotransmitters are important neurotransmitters in the plasticity and function of neurons, and AD reduces their hippocampal levels (3).

Regarding the importance of modulating these neurotransmitters and their role in preventing depression and anxiety in AD, studies have shown that regular exercises by reduce the inflammatory factors and modulate oxidative stress, have favorable effects on cognitive impairment and brain function (4). But it appears that the response of serotonin and dopamine to exercise are different, so that eight weeks of aerobic training increased serotonin, dopamine and tryptamine 5-hydroxylase (T5H) in cortex tissue of diabetic rats (4) also regular and long-term aerobic training significantly improved the neurotransmitters and markers of AD in women with dementia (5). Due to atrophy of neuronal nerves and their role in memory and learning in the elderly; nowadays resistance training (RT) has been introduced as an appropriate therapeutic approach to prevent AD complications (6). Although contradictory results have been reported, although eight weeks of RT significantly reduced anxiety and improved mood in the elderly (7) nevertheless 24 weeks of RT significantly reduced levels of serotonin, dopamine, epinephrine and norepinephrine in elderly women (6). Given the conflicting results regarding the effect of exercise on some neurotransmitters, researchers believe that these neurotransmitters are significantly affected by psychological factors; also, the effect of exercise on these variables depends on the type, intensity, and duration of the training period (6). Although the duration of the exercise was relatively long in study of Kim *et al.* (2019); it seems that the intensity of the exercises and the age of the subjects are also influential factors on the results. Given the inconsistency in the results, the researchers believe that the consumption of natural ingredients has positive effects on cognitive impairments in animal models of AD. Royal jelly (RJ) is a nutrient secreted from the working bee's jawbone and forms the queen's main food also has antioxidant and anti-inflammatory properties on neurons (8). Previous studies have shown that RJ consumption increased memory, learning and neurotrophins as well as decreased beta amyloid depletion in mouse (8); RJ consumption modulated serotonin and dopamine in the cortex tissue of the elderly rats, however, this effect was dose dependent, so that 100 mg/kg RJ had favorable effects than 50 mg/kg RJ (9). Also, RJ increased neuronal function, memory, and learning in an animal model of AD (10). Given the role of serotonin and dopamine as important neurotransmitters in the central nervous system and their role in the plasticity of hippocampal neurons, it seems that the use of noninvasive and non-pharmacological approaches may be an appropriate strategy to improve cognitive function in patients with AD. However, the mechanism of RT and RJ on serotonin and dopamine are still unclear. Therefore, the present study aimed to review the effect of eight weeks of RT with RJ on serotonin and dopamine receptors (5-HTR and DRD) gene expression in hippocampus tissue in an animal model of AD.

Methods

In this experimental study, 64 male Sprague-Dawley rats with an average age of eight weeks, and a mean weight of 250±20.12 g were purchased from the Animal Breeding Center of Islamic Azad University, Marvdasht Branch. After transferring the rats to the Sport Physiology Laboratory of this academic unit, for adaptation to the laboratory environment, all rats were kept for one week with free access to water and food. On the eighth day, 56 rats were injected a single dose of 8 mg/kg of trimethylethyltin (TMT)

(Sigma- Aldrich, MERK Company, CAS Number: 1065-45-1) peritoneally (11). Fourteen days later rats with AD were randomly divided into 7 groups of 8 rats including 1) AD control, 2) RT, 3) 100 mg/kg RJ (RJ100), 4) 200 mg/kg RJ (RJ200), 5) RT+RJ100, 6) RT+RJ200, and 7) sham. Also in order to review the effects of TMT on serotonin and dopamine, 8 healthy rats selected as healthy control group. Before start the research protocol, for confirmation of AD induction by TMT, spiritual memory measured by Y- maze test in health control and AD control groups. Then, after confirmation of AD induction research protocol started. During 8 weeks the groups 3- 6 received daily RJ with specific doses peritoneally (12); groups 2, 5 and 6 performed RT three sessions per week with 30- 100 percentage of body weight (13) and group 7 received RJ solvent (saline) peritoneally. It should be noted that, the only difference between the control and the sham groups is that the sham group received the RJ solvent (saline); however the control group did not receive any material. RJ purchased from Jihad Daneshgahi and transferred to animal lab. RJ was kept at a temperature of 4 C throughout the period, and the daily dose was dissolved with saline and injected peritoneally (12).

RT protocol

RT protocol consisted of an eight-week climb up on a ladder (with 1 meter high, 4 cm distance between 2 steps and vertical slope). Before each training session, rats climbed on ladder, three repetitions without weight and without rest between repetitions to warm up. RTs were designed based on the weights of rats, so that in the first week, rats lifted 30 percent of their body weight in four sets of two repetitions with a rest interval of 30- 45 seconds between each repetition and a recovery interval of 1- 2 minutes between each set. This trend continued until in the eighth week, rats lifted 100 percent of their body weight (14). Indeed weights selected at the start of the training were 30% of the body weight of rats and increased by 100% of body weight in the final week (14).

Y maze test for spiritual memory evaluation

The Y maze test consists of three arms made of MDF. Each arm is 46cm long, 15cm high and 15cm wide, with equal angles to each other and the arms are connected through a central enclosure. To do this test, the rat was first placed at the end of an arm and allowed access to all areas of the maze within a 5-min interval. The number of times which the animal entered each arm was observed and recorded. The animal enters the arm when the hind legs of the animal were fully enclosed in the arm, alternating behaviors as successful and serial entry into all arms in the three overlapping sets was intended. Thus the percentage of alteration (PA) observed to maximum frequency (the total number of arms entered) was multiplied by 100.

Sampling method

Forty eight hours after the last training session and RJ injection, all rats were anesthetized with ketamine (70 mg/kg) and xylosine (3-5 mg/kg). After anesthesia, the hippocampus tissues of rats were extracted. After washing and weighing, the hippocampus tissues were transferred to cryopreserved specimens for storage and frozen at -80 ° C.

Measurement of serotonin and dopamine receptors

For molecular analysis at the gene expression level, first, extraction of RNA from the hippocampus tissue was carried out according to the manufacturer's protocol (RNA extraction kite; Yekta Tajhiz Company), then; drawing on the light absorbance property at wavelength of 260 nm, the concentration and degree of purity of the RNA sample was quantitatively obtained using the following equation:

C (µg/µl) = A260× □× 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 (K1622; Fermantaz Company), and then the synthesized cDNA was used for reverse transcription reaction. Initially, the designed primers for genes were examined, and then genes expressions were examined by quantitative q-RT PCR method. The sequence of primers presented in Table 1.

Table 1. Primers Sequence Used in This Study		
5’- CGTGCTTGCCATTCAGAAA -3’	Forward	B2m
5’-ATATACATCGGTCTCGGTGG -3’	Reverse	
5’- CGGTACCTCATCGCTGCATA -3’	Forward	DRD
5’- AAACACTGTTGCAATGCCCC -3’	Reverse	
5’- TTAGGAACTTCGTCGGCACC -3’	Forward	5-HTR
5’- CCATCTTGCGCTTTGCTTCA -3’	Reverse	

Statistical analysis

Independent sample t- test was used for confirmation of AD induction by compare the results of Y maze test between healthy control and AD control groups. One way ANOVA and Bonferroni’s *post- hoc* tests were used to investigate the effects of AD and RJ solvent on the research variables. Also, two-way ANOVA was used to review the effect of RT, RJ and interaction of RT and RJ also Bonferroni’s *post- hoc* test was used to evaluate the difference between the doses of RJ using SPSS software ($P \leq 0.05$).

Results

The results of Y maze test, genes expression levels of 5-HTR and DRD are presented in Fig. 1–4 respectively. The results of independent sample t- test showed that percentage of alteration ($P = 0.001$) (Fig. 1) and total number of arm entries ($P = 0.02$) (Fig. 2) in AD control group was significantly lower than healthy control group.

The results of one-way ANOVA test showed that there were significant differences in DRD (P = 0.001) and 5-HTR (P = 0.001) levels in healthy control, AD control and sham groups.

The results of Bonferroni's *post- hoc* test showed that DRD and 5-HTR genes expression levels in AD control and sham groups were significantly lower than healthy control group (P = 0.001) (Figs. 3 and 4).

The results of two-way ANOVA test showed that RT (F = 15.06, P = 0.001, Eta = 0.67) and RJ (F = 4.93, P = 0.01, Eta = 0.19) had a significant effect on increase of DRD; however, the interaction of RT and RJ on DRD was not significant (F = 1.48, P = 0.23, Eta = 0.06) (Fig. 4). Also the results of Bonferroni's *post- hoc* test showed that 200mg/kg RJ had more effect on increase of DRD rather than 100 mg/kg RJ (P = 0.01) (Fig. 3).

The results of two way ANOVA test showed that RT (F = 39.66, P = 0.001, Eta = 0.48) and RJ (F = 76.60, P = 0.001, Eta = 0.78) had a significant effect on increase of 5-HTR, also the interaction of RT and RJ on increase of 5-HTR was significant (F = 9.71, P = 0.001, Eta = 0.31). The results of Bonferroni's *post- hoc* test showed that 100 mg/kg RJ (P = 0.04) and 200 mg/kg RJ (P = 0.001) had a significant effect on increase of 5-HTR nevertheless 200mg/kg RJ had more effect on increase of 5-HTR rather than 100 mg/kg RJ (P = 0.001) (Fig. 4).

Discussion

The results showed that induction of AD with TMT significantly decreased the 5-HTR and DRD genes expression levels in hippocampus tissue of rats. TMT is a neurotoxin that has been widely used to simulate AD, so that with the mechanism of increased inflammation and oxidative stress, induces apoptosis in microglia and astrocytes also it is associated with decreased neuronal plasticity and neurotransmitter dysfunction (15). Studies show that TMT causes a dopaminergic, serotonergic hippocampal system defect (15, 16). On the other hand, the results of present study showed that RT increased DRD and 5-HTR genes expression in hippocampus tissue of rats with AD. Dopamine and serotonin are neurotransmitters that exhibit different changes in response to various exercise activities, so that changes in the ratio of secretion of these hormones following short and vigorous exercise activities can cause fatigue. However, long-term activities have positive effects on resting and basal levels of these neurotransmitters as a factor for neuronal plasticity (17). Exercises by increase in brain-derived neurotrophic factor (BDNF) levels, sensitivity of neurotransmitter receptors in synaptic space, neurogenesis, endorphin release, dopamine carriers, serotonin as well as modulated tyrosine hydroxylase receptor activity, increase the expression of serotonin and dopamine receptors (18, 19). In line with the present study, it has been shown that eight weeks of RT significantly increased the expression of type 2 and 3 dopamine receptors (19) as well as increased serum levels of serotonin and dopamine (18) in methamphetamine users. In addition serotonin and dopamine levels were also higher in active subjects than inactive individuals (20); nevertheless 24 weeks of RT significantly reduced serum levels of serotonin, dopamine, epinephrine and norepinephrine in elderly women (6). It appears that the differences

in the results of noted study with the present study can be due to differences in measurement method, baseline levels of variables as well as intensity of exercise.

The results also showed that RJ significantly increased 5-HTR and DRD genes expression in hippocampus tissue of rats with AD and effects of RJ on serotonin and dopamine was dose dependent so that high dose had greater effect on increase of serotonin and dopamine rather than low dose. Researchers believed that consumption of RJ in stress conditions modulates catecholamines, in other words, RJ consumption by increasing neurotrophins levels is effective in neuronal plasticity, which increased the levels of serotonin and dopamine and its receptors in the hippocampus and improved the spatial memory of 18-month-old rats (9). Studies have shown that RJ consumption has a protective effect on dopaminergic neurons by increasing the gene expression of glial cell-line derived neurotrophic factor (GDNF) (21). Consumption of honey and RJ also appears to increase serotonin levels by reducing inflammatory factors such as interferon gamma (IFN-γ), reducing apoptotic factors such as caspase-3, improving noradrenaline levels, and improving neurotrophins levels; This can improve the performance of the serotonergic system (22). In line with the present study, 5 days of RJ consumption regulated neurotransmitters, notably that 500 mg/kg RJ had more favorable effects on neurotransmitters than 100 mg/kg RJ (9); Also, 50 mg/kg RJ consumption for eight weeks had a significant effect on modulating levels of alanine, aspartic acid, glutamic acid and histidine but decreased gamma- aminobutyric acid (GABA) levels (23). Although there is limited information on the effect of RJ on dopamine and serotonin, but Almeer *et al.* showed that RJ consumption increased antioxidant enzymes, anti-apoptosis markers, and serotonin also decreased oxidative stress cortex tissue of cadmium-poisoned mice (24).

In present study the interaction of RT and RJ consumption on increase of 5-HTR was significant; however, this interaction was not significant on DRD levels. Despite numerous reviews, no studies have been found to investigate the interactive effect of RT and RJ on neurotransmitters, so the present study was limited in comparison with the results of other studies. However, it seems that exercises can enhance the increase of expression of type 1–5 dopamine receptors and serotonin receptors by increasing neurotrophins, dopamine and serotonin transporter (18, 19); also RJ by antioxidant and anti-apoptotic effects increases the expression of dopamine and serotonin receptors in hippocampus tissues of rats with AD (9, 23, 24). Therefore, RT and RJ consumption seem to have synergistic effects on increased expression of 5-HTR by noted mechanisms; But regarding the lack of interactive effect of RT and RJ on increase of dopamine, the researchers showed that RJ at low and short doses has no significant effect on dopamine and noradrenaline modulation (9). Regarding the antioxidant pathway of RJ on neurotransmitters, it seems that lack of measurement of oxidative and antioxidant pathways along with evaluating survival of CA1 hippocampal neurons following TMT injection (for confirmation of AD induction) were the limitations of this study. Therefore, it is recommended that these factors be measured in future studies along with dopamine and serotonin.

Conclusions

It seems that RT and RJ can enhance serotonergic and dopaminergic functions in hippocampal tissue of rats with AD, however, the effects of RJ seems to be dose dependent.

Abbreviations

Resistance training
RT
Royal jelly
RJ
Alzheimer's disease
AD
Serotonin /5-hydroxytryptamine
5-HT
Tryptamine 5-hydroxylase
T5H
Brain- derived neurotrophic factor
BDNF
Glial cell-line derived neurotrophic factor
GDNF
Interferon gamma
IFN-γ
Gamma- aminobutyric acid
GABA
Dopamine Receptor
DRD
Serotonin Receptor
5-HTR

Declarations

Ethics approval and consent to participate

All ethical aspects of this research are approved by the ethics committee of Islamic Azad University, Marvdasht branch

Consent for publication

The authors of this study express their consent to the publication of the research in the journal Journal of the International Society of Sports Nutrition.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

Competing interests are not reported for this research.

Funding

Part of the funding for this research is provided by the Islamic Azad University, Marvdasht Branch.

Authors' contributions

All authors have an equal share in this research.

Acknowledgments

The authors like to appreciate the vice-chancellor of Marvdasht Branch of Islamic Azad University for support of this study.

Conflict of interest

The authors declare that they have no conflict of interests.

References

1. Morgese MG, Trabace L. Monoaminergic System Modulation in Depression and Alzheimer's Disease: A New Standpoint? Front Pharmacol [Internet]. 2019;17:10:483. https://doi.org/10.3389/fphar.2019.00483.
2. Martorana A, Koch G. Is dopamine involved in Alzheimer's disease? Front Aging Neurosci. 2014;6:252. https://doi.org/10.3389/fnagi.2014.00252.
3. Meneses A. Serotonin, neural markers, and memory. Front Pharmacol. 2015;6:143. https://doi.org/10.3389/fphar.2015.00143.
4. Amirsasan R, Esmaeili A, Dabbagh Nikokheslat S, Karimi P. The effect of aerobic training on serotonin and tryptophan hydroxylase of prefrontal cortex in type 2 diabetic rats. J Urmia Univ Med Sci. 2017; 27 (12): 1058–1067. URL: http://umj.umsu.ac.ir/article-1-3433-en.html.
5. George EK, Reddy PH. Can Healthy Diets, Regular Exercise, and Better Lifestyle Delay the Progression of Dementia in Elderly Individuals? J Alzheimer's Dis. 2019;(Preprint):1–22. DOI:10.3233/JAD-190232.
6. Kim Y-S, O'sullivan DM, Shin S-K. Can 24 weeks strength training reduce feelings of depression and increase neurotransmitter in elderly females? Exp Gerontol. 2019;115:62–8. https://doi.org/10.1016/j.exger.2018.11.009.

7. Lee SW, Youm Y, Lee WJ, Choi W, Chu SH, Park Y-R, et al. Appendicular skeletal muscle mass and insulin resistance in an elderly Korean population: the Korean social life, health and aging project-health examination cohort. Diabetes Metab J. 2015;39(1):37–45. doi:10.4178/epih/e2014003.
8. Kunugi H, Mohammed Ali A. Royal Jelly and Its Components Promote Healthy Aging and Longevity: From Animal Models to Humans. Int J Mol Sci. 2019;20(19):4662. https://doi.org/10.3390/ijms20194662.
9. Pyrzanowska J, Piechal A, Blecharz-Klin K, Joniec-Maciejak I, Graikou K, Chinou J. Administration of Greek Royal Jelly produces fast response in neurotransmission of aged Wistar male rats. J Pre-Clin Clin Res. 2015;9(2):151–7. https://doi.org/10.5604/18982395.1186497.
10. Zamani Z, Reisi P, Alaei H, Pilehvarian AA. Effect of royal jelly on spatial learning and memory in rat model of streptozotocin-induced sporadic Alzheimer's disease. Adv Biomed Res. 2012;1:26. doi:10.4103/2277-9175.98150.
11. Bazyar Y, Rafiei S, Hosseini A, Edalatmanesh MA. Effect of endurance exercise training and gallic acid on tumor necrosis factor-α in an animal model of Alzheimer's disease. Shefaye Khatam. 2015; 3 (3): 21–26. URL: http://shefayekhatam.ir/article-1-718-en.html.
12. Arzi A, Houshmand G, Goudarzi M, Khadem Haghighian H, Rashidi Nooshabadi MR. Comparison of the analgesic effects of royal jelly with morphine and aspirin in rats using the formalin. J Babol Univ Med Sci. 2015;17(2):50–6. URL: http://jbums.org/article-1-5285-en.html.
13. Dehghan F, Hajiaghaalipour F, Yusof A, Muniandy S, Hosseini SA, Heydari S, et al. Saffron with resistance exercise improves diabetic parameters through the GLUT4/AMPK pathway in-vitro and in-vivo. Sci Rep. 2016;6:25139. DOI:10.1038/srep25139.
14. Ghodsbin S, Farsi S, Hosseini SA. The Effect of resistance training with genistein on interleukin-6 and C- reactive protein in induced diabetic rats. IJDO. 2019; 11 (1): 56–66 URL: http://ijdo.ssu.ac.ir/article-1-467-en.html.
15. Kaur S, Sharma N, Nehru B. Anti-inflammatory effects of Ginkgo biloba extract against trimethyltin-induced hippocampal neuronal injury. Inflammopharmacology. 2018;26(1):87–104. https://doi.org/10.1007/s10787-017-0396-2.
16. Bina A, Hourizadeh S. The Most Important Factors of Causing Hearing loss Following Central Auditory System Disorder and Central Nervous System (CNS) Disorder. J Otolaryngol ENT Res. 2015;2(6):48. DOI:10.15406/joentr.2015.02.00048.
17. Cordeiro LMS, Rabelo PCR, Moraes MM, Teixeira-Coelho F, Coimbra CC, Wanner SP. Physical exercise-induced fatigue: the role of serotonergic and dopaminergic systems. Brazilian J Med Biol Res. 2017; 50 (12): e6432 **https**://**doi**.**org**/**10**.**1590**/**1414-431x20176432**.
18. Robertson CL, Ishibashi K, Chudzynski J, Mooney LJ, Rawson RA, Dolezal BA, et al. Effect of exercise training on striatal dopamine D2/D3 receptors in methamphetamine users during behavioral treatment. Neuropsychopharmacology. 2016;41(6):1629. doi:10.1038/npp.2015.33.
19. Arazi H, Dadvand SS, Fard MT. Neurotransmitters and cardiovascular responses to aerobic and resistance exercise in men addicted to methamphetamine. Baltic J Sport Health Sci. 2017;3(106):2–

10. .

20. Arazi H, Mollazadeh R, Dadvand SS, Davaran M. The circulatory levels of Serotonin and Dopamine and physical fitness factors in active and inactive men addicted to opium during rehabilitation. Phys Act Rev. 2016;4:1–8. http://dx.doi.org/10.16926/par.2016.04.01.
21. Taherianfard M, Ahmadi Jokani S, Khaksar Z. Royal jelly can modulate behavioral and histomorphometrical disorders caused by Parkinson's disease in rats. Physiol Pharmacol. 2017;21(2):120–128. URL: http://ppj.phypha.ir/article-1-1242-en.html.
22. Al-Salem HS, Bhat RS, Al-Ayadhi L, El-Ansary A. Therapeutic potency of bee pollen against biochemical autistic features induced through acute and sub-acute neurotoxicity of orally administered propionic acid. BMC Complement Altern Med. 2016;16(1):120. https://doi.org/10.1186/s12906-016-1099-8.
23. Pyrzanowska J, Wawer A, Joniec-Maciejak I, Piechal A, Blecharz-Klin K, Graikou K, et al. Long-term administration of Greek Royal Jelly decreases GABA concentration in the striatum and hypothalamus of naturally aged Wistar male rats. Neurosci Lett. 2018;675:17–22. https://doi.org/10.1016/j.neulet.2018.03.034.
24. Almeer RS, Kassab RB, AlBasher GI, Alarifi S, Alkahtani S, Ali D, et al. Royal jelly mitigates cadmium-induced neuronal damage in mouse cortex. Mol Biol Rep. 2019;46(1):119–31. https://doi.org/10.1007/s11033-018-4451-x.

Figures

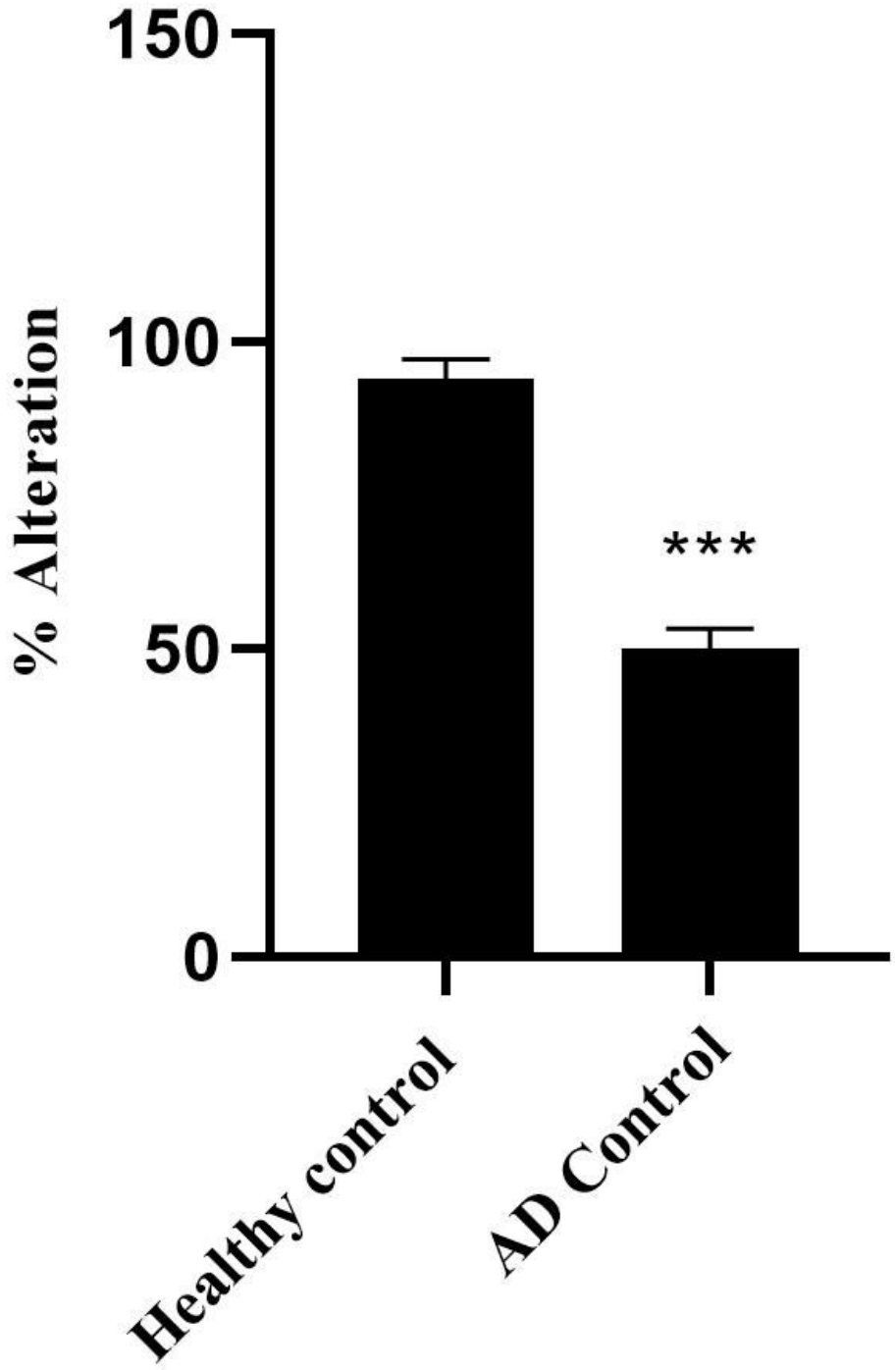

Figure 1

Percentage of alteration in healthy control and AD control groups *** P≤0.001 Significant decrease compared to healthy control group

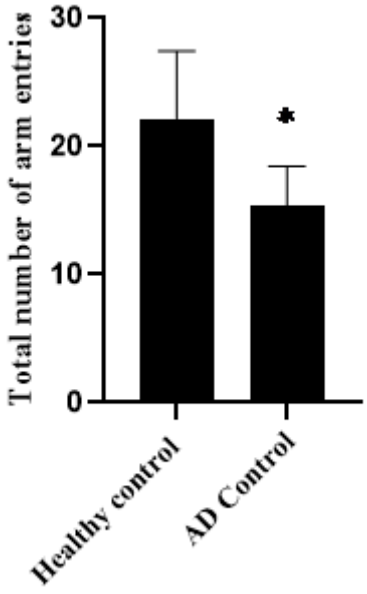

Figure 2

Total number of arm entries in healthy control and AD control groups * P≤0.05 Significant decrease compared to healthy control group

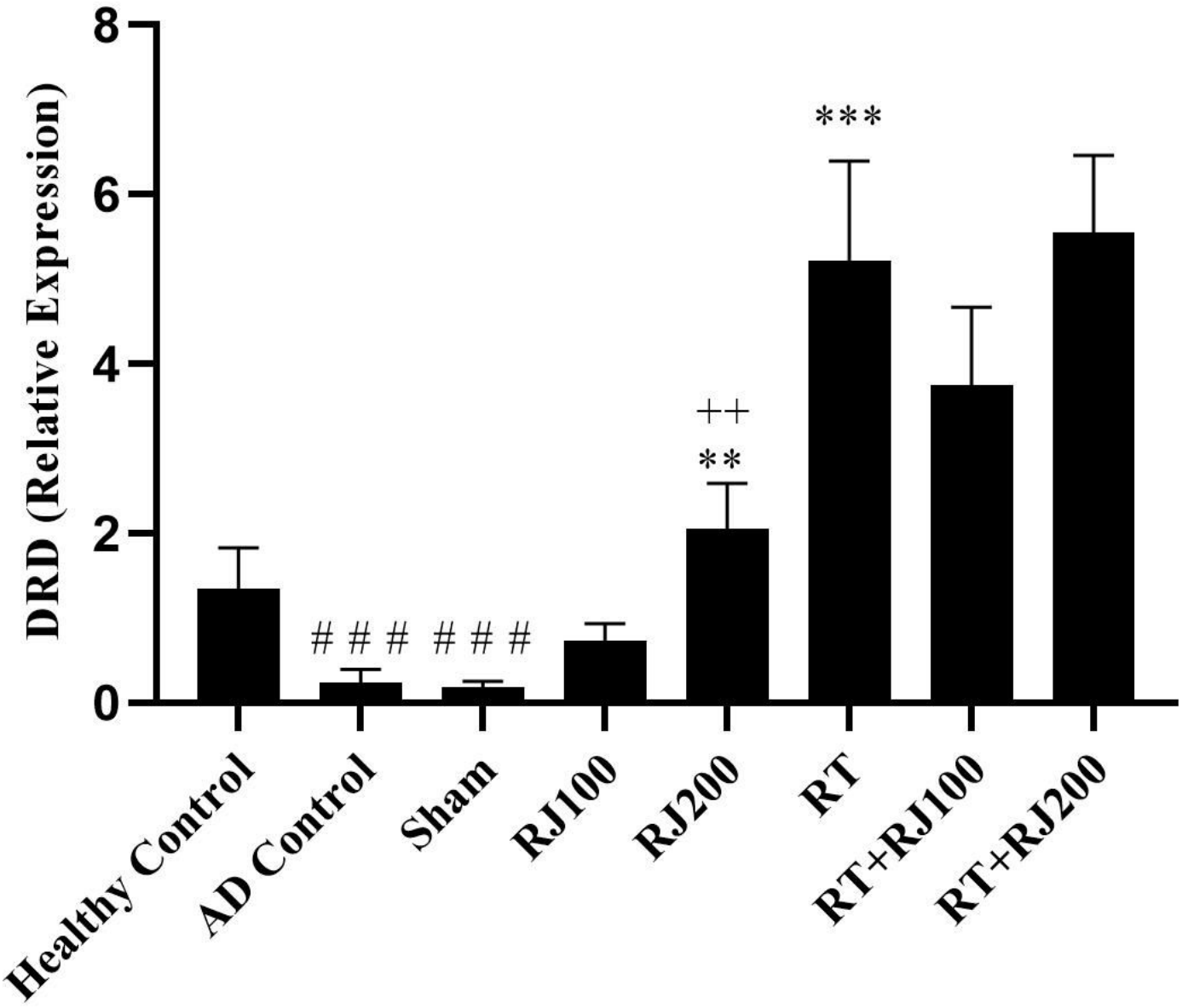

Figure 3

DRD gene expression levels in eight groups of study # # # P≤0.001 Significant decrease compared to healthy control group *** P≤0.001; ** P≤0.01 Significant effect on increase of DRD compared to AD control group + + P≤0.01 Significant effect on increase of DRD compared to RJ100

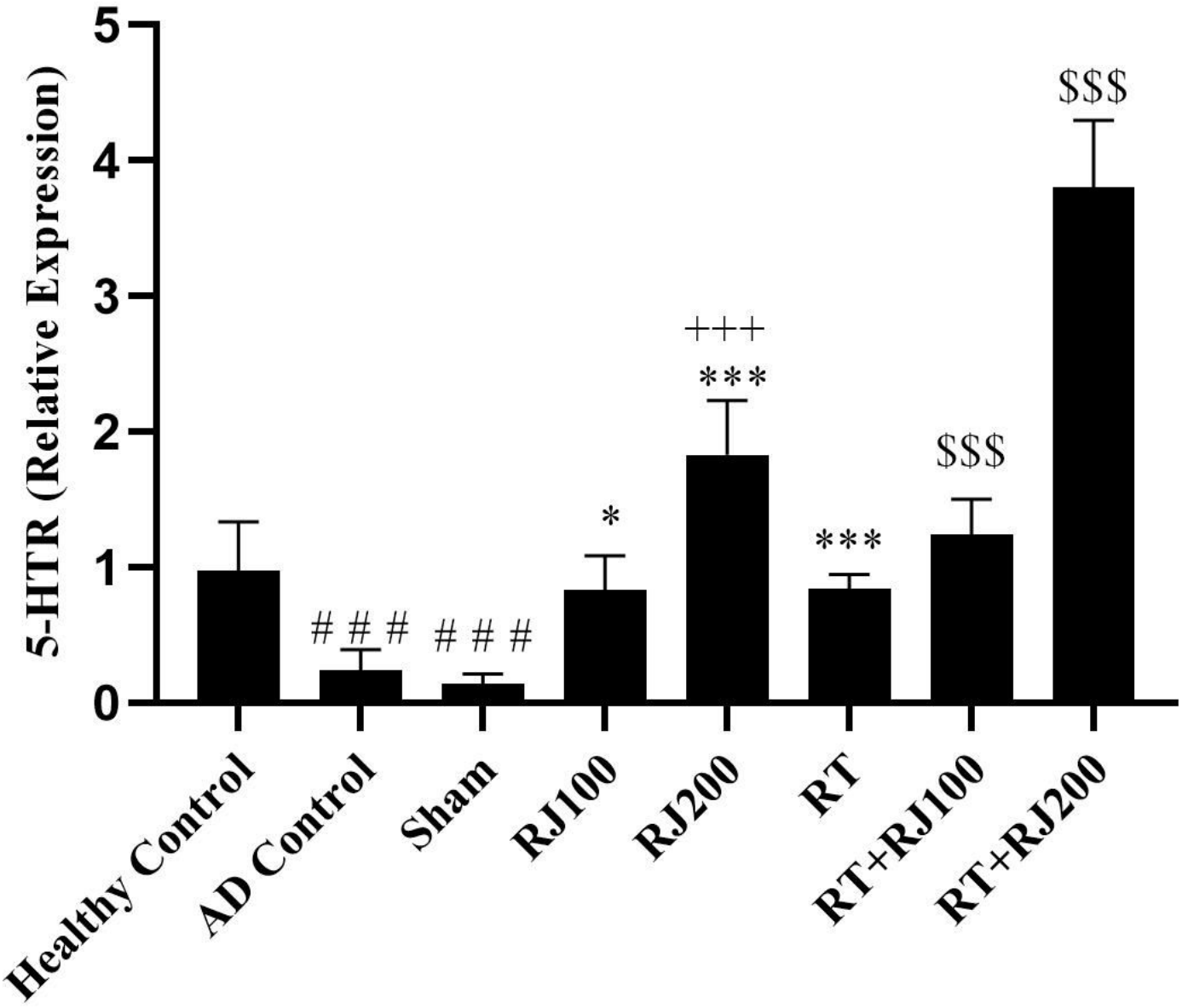

Figure 4

5-HTR gene expression levels in eight groups of study # # # P≤0.001 Significant decrease compared to healthy control group *** P≤0.001; * P≤0.05 Significant effect on increase of 5-HTR compared to AD control group + + P≤0.01 Significant effect on increase of 5-HTR compared to RJ100 $$$ P≤0.001 Interactive effects of RT and RJ on increase of 5-HTR