

The effect of eight weeks of interval swimming training with galantamine on antioxidant capacity, inflammation and depression markers in rats exposed to post-traumatic stress disorder

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Abstract

Brain metabolic dysfunction is a key contributor to the pathophysiology of post-traumatic stress disorder (PTSD). Although exercise and antioxidant agents have individually demonstrated neuroprotective properties, their combined effects remain inadequately characterized. Therefore, this study investigated the impact of eight weeks of interval swimming training (ST) combined with galantamine (G) on antioxidant status, inflammatory mediators, and depression-related markers in a rat model of PTSD. Thirty-two male Wistar rats (280–320 g, 14–16 months) were exposed to PTSD through bottle immobilization (2 h), forced swimming (15 min), and ether anesthesia. Animals were randomly allocated into four groups: (1) PTSD, (2) PTSD + G (5 mg/kg/day, oral), (3) PTSD + ST (8 weeks, 3 sessions/week, 15–40 min interval swimming with 2-min intervals at 2% body weight load), and (4) PTSD + ST+G. Assessments included immobility duration, inflammatory markers (NF- κ B, TNF- α), oxidative stress indices (MDA, SOD, GPx, TAC), neurotransmitter receptors (Ser-R, Dop-R), and microRNAs (Let-7a, miR-124-3p). All intervention groups showed reduced immobility time, NF- κ B, TNF- α , and Let-7a levels, alongside elevated TAC, Ser-R, Dop-R, and miR-124-3p compared with PTSD ($P = 0.001$). MDA decreased in PTSD + ST and PTSD + G, while SOD and GPx increased in PTSD + ST+G. Galantamine exerted stronger anti-inflammatory effects than ST alone. The combined intervention produced superior improvements in inflammatory, antioxidant, neurotransmitter, and microRNA profiles versus either treatment alone ($P = 0.001$). Both ST and G exert neuroprotective effects following PTSD; however, galantamine demonstrates comparatively stronger anti-inflammatory and antioxidant influence. Their combined administration induces synergistic benefits through complementary mechanisms, supporting concurrent use to attenuate PTSD-associated neurobiological impairments.

Introduction

Data show that traumatic injuries associated with psychological disorders lead to disability and death; in Iran, a recent report also showed that a significant percentage of children and adolescents, as well as adults, have traumatic disorders such as anxiety and post-traumatic stress disorder (PTSD) (Hooshyari et al., 2024). In addition, these reports showed that after the outbreak of the Coronavirus disease 2019 (COVID-19) pandemic, the percentage of psychological diseases, including PTSD, increased in the world; in addition, this data showed that the incidence of this disease is also associated with the occurrence of physical disorders (Carmassi et al., 2024). Available information shows that the occurrence of PTSD occurs after difficult periods, enduring psychological stress, and the occurrence of a traumatic event in life, and is ultimately associated with severe impairment in physical and mental functioning (Alemu et al., 2024). On the other hand, it seems that physiological disorders such as oxidant-antioxidant imbalance, inflammatory factors, and body homeostasis can be mutually related to the occurrence of PTSD. It seems that the disorder in the hypothalamic-pituitary-adrenal (HPA) axis leads to the occurrence of disorders in hormones such as adrenocorticotropin (ACTH), corticotropin-releasing hormone (CRH), and thyroid-stimulating hormone (TSH), which ultimately leads to disruption in cell metabolism and increases free radicals in various parts of the body, including the brain. Therefore, the beginning of the process of cellular damage (which is the disruption of the oxidant-antioxidant balance) occurs. So that the

reduction of superoxide dismutase (SOD), glutathione peroxidase (GPx), total antioxidant capacity (TAC) and the increase of malondialdehyde (MDA) can cause disruption in the functioning of the inflammatory system. With the increase in oxidative stress, inflammatory factors such as nuclear factor kappa-B transcription factor (NF- κ B), interleukin (IL) – 1 beta (IL-1 β), tumor necrosis factor alpha (TNF- α), interferon gamma (IFN- γ), IL-6 and IL-12 increase. However, this is not the beginning and then there is a disruption in neurotransmitters, of which serotonin and dopamine are the most relevant to the topic of depression (Lushchak et al., 2023).

In addition, data show that non-coding RNAs such as microRNAs are also effective in this process, as previous studies have shown a relationship between miR-124-3P and Let-7a with a decrease in TNF- α and IL-1 β levels (Chen et al., 2022; Pawar et al., 2024). As studies have shown, increasing Let-7a levels through the toll-like receptor 7 (TLR7) activation pathway in microglia leads to the activation of inflammatory pathways, an increase in NF- κ B and TNF- α (Buonfiglioli et al., 2019). While researchers have reported a relationship between reduction in miR-124-3P and memory loss with cognitive impairment (Vuokila et al., 2020). Given the need to provide effective treatments for psychological disorders, it is believed that regular and long-term physical activity can be an effective solution to improve mental and physical health (Hosseini et al., 2022). So that exercises can be effective in improving anxiety and depression in psychological disorders in animal models by improving the function of neurotrophins, activating the transcriptional pathway of dopamine and serotonin receptors (Ghanbari et al., 2022). Studies have also shown that exercises can increase the expression of antioxidants in the nervous system by affecting non- coding RNAs effective in regulating antioxidant gene expression (Agahi et al., 2022). More specifically, it seems that exercises, by activating nuclear factor-erythroid 2 related factor 2 (Nrf2) and simultaneously activating miR-125b and miR-146, which have a favorable role in the transcription of antioxidant expression, lead to an increase in SOD, GPx, TAC, catalase, and ultimately improve cognitive function in animal models (Agahi et al., 2022). In addition, it seems that regular exercise, by the mechanism of activation of ephedrines and activation of Nrf2, can increase the expression of serotonin and dopamine receptors in brain tissue (Ghanbari et al., 2022). In addition, researchers have shown that exercise can reduce the symptoms of cognitive diseases such as PTSD (Kinsman et al., 2024). A review study also stated that exercises can lead to the transcription of Let-7a by affecting the PI3K/NRF1 pathway (Afzal et al., 2024). In addition to exercise, researchers believe that the use of natural antioxidants can play an effective role in the treatment of neurological diseases. Galantamine (G) is known as a specific acetylcholinesterase inhibitor; this antioxidant can reduce inflammatory factors in cognitive diseases by affecting and inhibiting NF- κ B (Kwon et al., 2024). It has been suggested that G can activate the PI3K/Akt pathway by affecting tryptophan, leading to transcription of antioxidant enzymes and ultimately leading to a decrease in TNF- α , NF- κ B and IL-1 β in Alzheimer's disease (Frost et al., 2024). In this context, a study also showed that G consumption led to an increase in SOD and GPx as well as a decrease in MDA and ultimately improved memory in a Parkinson's model (Lazarova et al., 2021). In a study, the results also indicated a favorable effect of G on memory and cognitive function in an animal model of AD (Lim et al., 2024).

Although the beneficial role of exercise and G on the nervous system and cognitive function has been investigated, the simultaneous effect of these two interventions on oxidant- antioxidant, inflammation, and cognitive impairment pathways is still not well understood. It seems that combining these two interventions could be an effective strategy for the treatment of PTSD and provide more comprehensive information for researchers in this field. Therefore, the present study aimed to investigate the effect of eight weeks of interval swimming training (ST) with G on antioxidant capacity, inflammation, and depression markers in rats exposed to PTSD.

Experimental Procedures

Keeping animals

In this experimental and fundamental study (post-test design with a control group), 40 male Wistar rats with a weight range of 280–320 g and mean age of 14–16 months were purchased from the Pasteur Institute of Iran. After preparation, the studied samples were transferred to the Exercise Physiology Laboratory Center of Pishtazan Institute of Higher Education, Shiraz. The rats were kept in the standard laboratory environment for one week for adaptation without any intervention. Throughout the study period, the samples were kept in standard conditions of a 12-hour light-dark cycle; temperature of 22 to 24 degrees Celsius, relative humidity of 55% in a quiet and noise-free environment. The samples were kept in washable polycarbonate cages during the study period. Also, sterile wood shavings were used to absorb urine and moisture on the floor of the cages, and access to water and food for the animals was free and available. The ethical principles of working with laboratory animals were observed according to the Ethics Committee for Biomedical Research of Pishtazan Institute of Higher Education, Shiraz.

Induction of PTSD

The following method was used to induce PTSD. In the first stage, rats were restrained for 2 hours using a 100 ml bottle with a diameter of 4 cm. In the second stage, following restraint and immobilization, rats were forced to swim in a special animal pool for 15 minutes. In the next stage, rats rested for 15 minutes and were anesthetized with ether for 3 minutes (Carmassi et al., 2024).

Grouping

Rats were randomly divided into 5 groups including: 1) PTSD control (the group that only received disease induction), 2) PTSD + Galantamine (PTSD + G) (the group that received galantamine at a dose of 5 mg/kg as an intraperitoneal injection in addition to disease induction), 3) PTSD + ST) (the group that received ST for eight weeks in addition to disease induction), 4) PTSD + ST+G (the group that received ST and galantamine in addition to disease induction), and 5) healthy control (HC) (that were kept in the laboratory without any intervention to investigate the effect of disease induction on the research variables).

Swimming training protocol

Swimming training protocol

Swimming training in this study was carried out in a special animal pool with dimensions of 110 cm in height and 45 cm in width in water at a temperature of 32 ° C. This protocol consisted of two phases of familiarization and the main protocol. In the familiarization phase, the rats were familiarized with the water environment and ST for 10 days. Subsequently, in the main protocol period, inspired by previous research and with slight changes, the rats trained for eight weeks, five sessions per week. In this protocol, the rats performed ST for 15 minutes in the first week. Subsequently, ST was gradually carried out from 15 minutes to 30 minutes until the sixth week of training. Then, in the final two weeks, the training duration was increased to 40 minutes. It is worth noting that in order to comply with the principle of overload; the rats carried a weight equivalent to 2% of their body weight attached to their tails during ST (Parsa et al., 2022).

Galantamine supplementation

In this study, G was obtained from Sigma-Aldrich. Rats received 5 mg/kg of their body weight of G per day by intraperitoneal injection. To prepare the solution, 27 mg of G per day was mixed with 4.8 ml of normal saline. After complete dissolution, 0.3 cc of the solution was injected intra- peritoneally into each rat with a 1 cc syringe (Sperling et al., 2019).

Assessment of Depression with Forced Swimming Test

The forced swimming test was used to measure the level of depression 24 hours after the last ST and G supplementation session. The forced swimming test is one of the most valid and common tests for examining depression in rodents. The duration of this test is 5 minutes and the behavior of the rat is recorded during this period. Conventionally, the cessation of arm and leg movements of the rat and its floating are considered as immobility and its duration as immobility time. Twenty- four hours before the test, the animal is placed in water for 15 minutes to gain experience in forced swimming test (Ghanbari et al., 2022).

Dissection and sampling

Forty- eight hours after the final ST session and in a 12-hour fast; rats were anesthetized with *ketamine* (55 mg/kg) and *xylazine* (25 mg/kg). After ensuring anesthesia, first 5 cc of blood was directly taken from the heart tissue of the rats. Then, after opening the skull cavity using a cutter, the rat brain structure was taken out. Then, the hippocampus tissue was extracted based on the rat brain atlas. Then, after washing the tissues, they were placed at -70°C to measure the variables.

Method of measuring physiological variables

The measurement of gene expression levels of variables in this study was done using the qReal Time PCR method. First, 50 mg of tissue was separated from the hippocampus and incubated in the medium, then the homogenized tissue was used for RNA extraction. For RNA extraction, the manufacturer's protocol (Qiagen, Germany) was used, and to ensure the quality of RNA, agarose gel electrophoresis and optical absorption at a wavelength of 260 nm were used with the Sigma Pico Drop device (made in the USA). Then, after cDNA synthesis using the manufacturer's protocol in the Fermentase kit (K1621) and using the designed primers (Table 1) based on the guide to the genes studied on the PUBMED site, reverse transcription reaction was performed. To determine the efficiency and specificity of the primers, the pre-primers were evaluated using software available on the NCBI website. Also, the TBP internal control gene was used to measure the gene expression levels of the research variables. After ensuring that the qReal Time PCR device was completed and the samples reached the expression threshold (Cycle Threshold), the $2^{-\Delta\Delta CT}$ formula was used to quantify the ratio of the target gene to the reference gene.

To measure SOD levels, a special kit using the ELISA method and manufactured by Navand Lab Kit version 0.6, made in Iran, and a U/ml scale was used. To measure TAC, an ELISA method with a special kit manufactured in Germany, Zell Bio Company with economic code CAT No. ZB-TAC-96A and a scale of 0.1mM was used. Also, to measure MDA, an ELISA method and a kit prepared by Zell Bio Company, made in Germany, with economic code ZB-MDA-96A and a scale of 0.1mM was used. Also, to measure GPx, an ELISA method was measured using a special kit manufactured in KiaZist, made in Iran, with economic code KGPX96 and a U/ml scale.

Table 1
Sequences of primers used in the present study

Genes	Primer Sequences	Sizes (bp)
<i>TBP</i>	Forward: 5'-GCGGGGTCATGAAATCCAGT-3' Reverse: 5'-AGTGATGTGGGGACAAAACGA - 3'	147
<i>NFkB</i>	Forward: 5'-GCTGCCAAAGAAGGACACGACA - 3' Reverse: 5'-GGCAGGCTATTGCTCATCACAG - 3'	131
<i>TNF-α</i>	Forward: 5'-AGGCACTCCCCCAAAGATG-3' Reverse: 5'-CCACTTGGTGGTTTGTGAGTG - 3'	213
<i>Sero R (5-HT Receptor)</i>	Forward: 5'- TTAGGAACTTCGTCGGCACC - 3' Reverse: 5'- CCATCTTGCGCTTTGCTTCA - 3'	283
<i>Dopa R</i>	Forward: 5'-CGGTACCTCATCGCTGCATA - 3' Reverse: 5'-AAACACTGTTGCAATGCCCC - 3'	210
<i>Let-7a</i>	Forward:AGGTAGTAGGTTGTATAGTTG Reverse (Universal primer): CCCAGTTATGGCCGTTTA	
<i>miR-124-3p</i>	Forward:TCTTTAAGGCACGCGGTG Reverse: TATGGTTTTGACGACTGTGTGAT	
U6	Forward: CTCGCTTCGGCAGCACA Reverse: AACGCTTCACGAATTTGCGT	

Statistical analysis of data

The data were presented using the mean and standard deviation. Also, Shapiro- Wilk test were used to examine the distribution of data. Then, considering the parametric nature of the data, one-way analysis of variance (ANOVA) test was used to examine the differences between groups. Also, Tukey's *post- hoc* test was used to determine the location of the differences between groups. The data of the present study were analyzed in GraphPad Prism 8.0.0 software. Also, a significance level of 0.05 was considered for all calculations.

Results

Inflammatory markers

The results of one-way ANOVA showed that there was a significant difference in the levels of NF-KB (P = 0.001) and TNF-α (P = 0.001) in the research groups.

Furthermore, the results of Tukey's *post-hoc* test showed that the levels of NF-KB in the PTSD group were significantly higher than the HC group ($P = 0.001$). However, in the PTSD + G ($P = 0.001$), PTSD + ST ($P = 0.001$) and PTSD + ST+G ($P = 0.001$) groups were significantly lower than PTSD group. Also, in the PTSD + G group were significantly lower than PTSD + ST group ($P = 0.001$); in addition, in the PTSD + ST+G group were significantly lower than PTSD + G ($P = 0.001$) and PTSD + ST ($P = 0.001$) groups (Fig. 1-A).

TNF- α levels in the PTSD group were significantly higher than HC group ($P = 0.001$). However, TNF- α levels in the PTSD + G ($P = 0.001$), PTSD + ST ($P = 0.001$) and PTSD + ST+G ($P = 0.001$) groups were significantly lower than PTSD group. Also, in the PTSD + G group were significantly lower than PTSD + ST group ($P = 0.001$); Also, in the PTSD + ST+G group were significantly lower than PTSD + G ($P = 0.001$) and PTSD + ST ($P = 0.001$) groups (Fig. 1-B).

Figure – 1. Gene expression levels of inflammatory factors in the brain tissue of rats in the research groups

*** ($P = 0.001$) significant increase compared to the HC group; ### ($P = 0.001$) significant decrease compared to the PTSD group; \$\$\$ ($P = 0.001$) significant decrease compared to the PTSD + G group; ^^^ ($P = 0.001$) significant decrease compared to the PTSD + ST group

Oxidant- antioxidant markers

There was a significant difference in the levels of SOD ($P = 0.001$), GPx ($P = 0.001$), TAC ($P = 0.001$), and MDA ($P = 0.001$) in the study groups. SOD levels in the PTSD group were significantly lower than HC group ($P = 0.001$), but were significantly higher in the PTSD + G ($P = 0.001$) and PTSD + ST+G ($P = 0.001$) groups than PTSD group. Also, in the PTSD + G group, SOD levels were significantly higher than PTSD + ST group ($P = 0.0102$) and in the PTSD + ST+G group were significantly higher than PTSD + G ($P = 0.004$) and PTSD + ST ($P = 0.001$) groups (Fig. 2-A).

GPx levels in the PTSD group were significantly lower than HC group ($P = 0.001$), but were significantly higher than PTSD + ST+G group ($P = 0.001$) (Fig. 2-B).

TAC levels in the PTSD group were significantly lower than HC group ($P = 0.001$); but were significantly higher than PTSD + G ($P = 0.001$), PTSD + ST ($P = 0.001$) and PTSD + ST+G ($P = 0.001$) groups and were significantly higher than PTSD + ST+G group ($P = 0.001$) (Fig. 2-C).

MDA levels in the PTSD group were significantly higher than HC group ($P = 0.001$); but were significantly lower than PTSD + G ($P = 0.001$) and PTSD + ST ($P = 0.001$) groups and were significantly lower in the PTSD + G group than PTSD + ST+G group ($P = 0.001$) (Fig. 2-D).

*** ($P = 0.001$) significant change compared to the HC group; ### ($P = 0.001$) significant change compared to the PTSD group; \$\$\$ ($P = 0.001$) significant change compared to the PTSD + G group; ^^^ ($P = 0.001$) significant change compared to the PTSD + ST group

= 0.001) significant change compared to the PTSD + ST group; €€€ (P = 0.001) significant change compared to the PTSD + ST+G group

Neurotransmitter markers

The results showed that there was a significant difference in the levels of Ser-R (P = 0.001), Dop-R (P = 0.001), Let-7a (P = 0.001) and miR-124-3p (P = 0.001) in the study groups.

The levels of Ser-R in the PTSD group were significantly lower than HC group (P = 0.001); however, in the PTSD + G (P = 0.001), PTSD + ST (P = 0.001) and PTSD + ST+G (P = 0.001) groups were significantly higher than PTSD group and in the PTSD + ST+G group were significantly higher than PTSD + G (P = 0.001) and PTSD + SIT (P = 0.001) groups (Fig. 3-A).

Dop-R levels in the PTSD group were significantly lower than HC group (P = 0.001); however, in the PTSD + G (P = 0.001), PTSD + ST (P = 0.001) and PTSD + ST+G (P = 0.001) groups were significantly higher than PTSD group; in the PTSD + G group were significantly higher than PTSD + ST group (P = 0.001) and in the PTSD + ST+G group were significantly higher than PTSD + G (P = 0.001) and PTSD + ST (P = 0.001) groups (Fig. 3-B).

Let-7a levels in the PTSD group were significantly higher than HC group (P = 0.001); however, in the PTSD + G (P = 0.001), PTSD + ST (P = 0.001) and PTSD + ST+G (P = 0.001) groups were significantly lower than PTSD group; in the PTSD + G group were significantly lower than PTSD + ST group (P = 0.03) and in the PTSD + SIT+G group were significantly lower than PTSD + G (P = 0.001) and PTSD + ST (P = 0.001) groups (Fig. 3-C).

The miR-124-3p levels in the PTSD group were significantly lower than HC group (P = 0.001); however, in the PTSD + G (P = 0.001), PTSD + ST (P = 0.001) and PTSD + ST+G (P = 0.001) groups were significantly higher than PTSD group and in the PTSD + ST+G group were significantly higher than PTSD + G (P = 0.001) and PTSD + ST (P = 0.001) groups (Fig. 3-D).

*** (P = 0.001) significant change compared to the HC group; ### (P = 0.001) significant change compared to the PTSD group; \$\$\$ (P = 0.001) significant change compared to the PTSD + G group; ^^ (P = 0.001) significant change compared to the PTSD + ST group

Depression

A significant difference in the results of the forced swimming test was observed in the study groups (P = 0.001). The results of the *post-hoc* test showed that the immobility levels in the PTSD group were significantly higher than HC group (P = 0.001) and in the PTSD + G (P = 0.001), PTSD + ST (P = 0.001) and PTSD + ST+G (P = 0.001) groups the immobility levels were significantly lower than PTSD group (Fig. 4).

*** (P = 0.001) significant change compared to the HC group; ### (P = 0.001) significant change compared to the PTSD group

Discussion

The results showed that in the PTSD group, inflammatory markers (NF-KB and TNF- α), MDA and Let-7a levels were significantly higher and antioxidant markers (SOD, GPx, TAC), neurotransmitters (Ser-R, Dop-R) and miR-124-3p levels were significantly lower than HC group. In addition, the duration of immobility in the PTSD group was significantly longer than HC group. With the onset of PTSD, disruption of the HPA axis leads to disruption of cell metabolism and a decrease in the function of the oxidant-antioxidant and inflammatory system. As a result, this leads to disruption of neurotransmitters related to anxiety and depression with the involvement of miR-124-3P and Let-7a (Chen et al., 2022; Lushchak et al., 2023; Pawar et al., 2024). However, the results showed that in the PTSD + ST group, inflammatory markers (NF-KB and TNF- α), oxidative (MDA), Let-7a and depression were lower, and the TAC levels, neurotransmitters (Ser-R and Dop-R) and miR-124-3p were significantly higher than PTSD group. It seems that ST can have antidepressant effects by increasing myokines leading to stimulation of the 5-HT3-IGF-1 mechanism. It seems that ST leads to a reduction in anxiety and depression by increasing the sensitivity of 5-HT2 receptors, increasing the sensitivity of 5-HT1A receptors, and increasing tryptophan hydroxylase (TPH), 5-HT, beta-endorphin, enkephalin, dynorphin, dopamine type 1 and 2 receptors, increasing the expression of brain-derived neurotrophic factor (BDNF), orexin, TrkB, increasing nerve growth factor, and decreasing IFN- γ , TNF- α , IL-1 β , and IL-6 (Pahlavani, 2024). In this context, researchers showed that exercise, through the mechanism of TLR4/MyD88/NF- κ B modulation, leads to inhibition of gene expression of inflammatory factors such as TNF- α , IL-1 β , modulation of epinephrine and norepinephrine in brain tissue; and through this pathway leads to a decrease in cerebral blood pressure in the paraventricular nucleus of the hypothalamus (Qi et al., 2019). Also, in a study, the results indicated the inhibition of apoptosis in brain tissue following exercise through the inhibition of miR-124/STAT3/BCL-2/BAX in an animal model of cerebral ischemia. So that an increase in miR-124 increased the expression of BCL-2 (Song et al., 2025). In a study, researchers also stated that exercise can increase plasma kynurenic acid by increasing PGC-1 α with the type 1 subunit. This leads to an increase in tryptophan expression in the brain, and tryptophan can subsequently modulate neurotransmitters such as 5-HT and norepinephrine, and ultimately is associated with a decrease in depression (Ross et al., 2023). Also, in a study, the results showed that voluntary exercise increased the expression of miR-874, miR-92a, miR-21 and decreased the expression of miR-138, let-7c and miR-124 in the cerebral cortex tissue of rats with cognitive impairment (Miao et al., 2015). These researchers suggested that ultimately these changes could have protective effects on inflammatory function and oxidative stress. On the other hand, it seems that in a study, researchers showed that 20 days of continuous training had no significant effect on changes in some miRs such as Let-7a, miR16, miR-21, and miR15b in an animal model of spinal cord injury (Liu et al., 2010). It seems that the duration of the training period and the type of injury are reasons for the contradiction in the results. Although the results of the present study show that ST has a limited effect on increasing antioxidants such as SOD, GPx, and TAC. However, it seems that exercises can initially lead to the activation of nuclear factor- erythroid 2 related factor 2 (Nrf2) by inducing oxidative stress and transcribing antioxidant enzymes in DNA. This protective effect against a single training session eventually becomes an adaptation with regular exercise and improves antioxidant function. However, it

seems that the intensity, duration and type of exercise are also important factors affecting changes in the antioxidant system (Souza et al., 2022).

The results showed that in the PTSD + G group, the levels of NF-KB, TNF- α , MDA and Let-7a were significantly lower and the levels of SOD, TAC, Ser-R, Dop-R, and miR-124-3p were significantly higher than PTSD group. In addition, the duration of immobility in this group was significantly shorter than PTSD group. In this context, a study showed that eight weeks of G consumption led to an increase in SOD, Cat and GPx, improving the lipid profile, regulating adipokines and improving cardiac function (Sangaleti et al., 2021). In another study, the results showed that 7 days of G at a dose of 5 mg/kg led to the suppression of inflammatory factors such as IL-1 β , IL-6, TNF- α , HMGB1, NF- κ B and the modulation of acetylcholine transferase in the brain tissue of laboratory mice with brain injury (Zaghloul et al., 2023). In another study, the results showed a reduction in oxidative stress, reduced inflammation and improved acetylcholinesterase levels in conditions of oxidative stress caused by lipids in obese African American peoples (Parsa et al., 2022). In another study, researchers showed that the consumption of 5 mg/kg of G led to a decrease in NF- κ B, cyclooxygenase-2, MDA, Bax, and caspase 3, as well as increase in SOD, GSH and Bcl-2 in doxorubicin-induced brain toxicity in an animal model (Aldubayan et al., 2024). In a case study, the results showed that G consumption led to a reduction in depression in depressed women with Alzheimer's disease (Alagiakrishnan, 2010). Another study showed that G consumption, by modulating nicotinic acetylcholine receptors (nAChRs), modulating acetylcholinesterase, and improving dopamine, improved cognitive function in scopolamine-induced toxicity (Schilström et al., 2007). However, according to the studies, although the anti-inflammatory and antioxidant effects of G are well established; however, no study has been conducted to investigate the effects of this drug on miRs involved in neuro- inflammation. Therefore, this study is innovative in this regard. In general, it seems that G can reduce neural stress by inhibiting acetylcholinesterase, and through this pathway, it can modulate neurotransmitters such as epinephrine, acetylcholine, inhibit apoptosis, inhibit oxidative stress, and ultimately improve cognitive function in conditions of cognitive impairment (Tewari et al., 2018).

The results showed that in the PTSD + ST+G group, the levels of NF-KB, TNF- α , and Let-7a were significantly lower, and the levels of SOD, GPx, TAC, Ser-R, Dop-R, and miR-124-3p were significantly higher than PTSD group. In addition, the duration of immobility in this group was significantly shorter than PTSD group. Studies show that exercise can lead to improved cognitive function by increasing myokines, stimulating 5-HT₃-IGF-1, increasing the sensitivity of serotonin and dopamine receptors, improving neurotransmitters, increasing the expression of NRF2 to increase the transcription of antioxidants, inhibiting miR-124/STAT3/BCL-2/BAX, and modulating macrophages, leading to a reduction in inflammatory factors and ultimately reducing anxiety and depression (Pahlavani, 2024; Song et al., 2025; Souza et al., 2022). G also reduces depression by increasing enzymatic and non-enzymatic antioxidants, improving lipid metabolism (Sangaleti et al., 2021), suppressing IL-1 β , IL-6, TNF α , HMGB1, NF- κ B, and modulating acetylcholine transferase in brain tissue (Zaghloul et al., 2023), reducing Bax, and caspase 3 (Aldubayan et al., 2024). Although the cellular-molecular mechanisms seem to be somewhat different from each other, it seems that these two interventions can enhance each other's effects and contribute to improving cognitive function and reducing anxiety. In this context, a study showed that G

and exercise led to inhibition and modulation of cerebrospinal fluid, reduction of TNF α , Bax, and caspase 3, increase of BDNF and Bcl-2 in an experimental autoimmune encephalomyelitis model. However, exercise led to an increase in the anti-apoptotic effect of G in this pathway (El-Emam et al., 2021). Therefore, the result that in the ST and G combination group, the levels of NF-KB and TNF- α were significantly lower and the levels of SOD, Ser-R, Dop-R, and miR-124-3p were significantly higher than the PTSD + ST and PTSD + G groups; is justifiable. Despite the reviews, this study was the only one that simultaneously examined the effects of ST and G on neuro- protection. Therefore, comparison of the results with the results of other studies is limited. However, it seems that the difference in the mechanism of exercise, which leads to improved antioxidant and anti-inflammatory function through the stimulation of oxidative stress and inflammation and then the creation of adaptations (Souza et al., 2022), has a somewhat more limited effect than the effect of G, which can specifically suppress inflammatory factors (Aldubayan et al., 2024). Therefore, the results of the present study that in the PTSD + G group, the levels of NF-KB, TNF- α and Let-7a were significantly lower and the levels of SOD and Dop-R were significantly higher than in the PTSD + ST group; can be justified. It seems that the transcription mechanism of antioxidants (PI3K/NRF2) is an important pathway in the function of the antioxidant system, and its lack of evaluation is recognized as a limitation in this study. Therefore, it is suggested that the upstream pathways of antioxidants should also be evaluated in future studies. It also seems that the evaluation of myokines as proteins affecting neurotrophins is another limitation of the present study. Therefore, it is suggested that the myokine pathway and their relationship with changes in neurotrophins and neuronal protection should be evaluated in future studies.

Conclusion

It seems that although ST and G both have neuro- protective effects; however, the effect of G on reducing inflammation and increasing antioxidants is somewhat more effective than the effect of ST. In addition, it seems that ST and G, although through separate pathways, lead to the enhancement of each other's effects in protecting the nervous system following PTSD. Therefore, their simultaneous use is recommended in conditions of cognitive disorders.

Declarations

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Author Contribution

• Study concept and design: Iman Fathi and Omidreza Salehi • Acquisition of data: Seyed Hossein MirDehghan, Fatemeh Rezaei Homden, Zahra Momeni Raghavadi, Ismaeil Kanaani, • Analysis and interpretation of data: Omid Reza Salehi and Seyed Hossein MirDehghan • Drafting of the manuscript: Omidreza Salehi, Iman Fathi • Critical revision of the manuscript for important intellectual content: Iman

Data Availability

The data underlying this manuscript have been published on Mendeley Data and are available at: <https://data.mendeley.com/drafts/tddrfms8yt>

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Figures

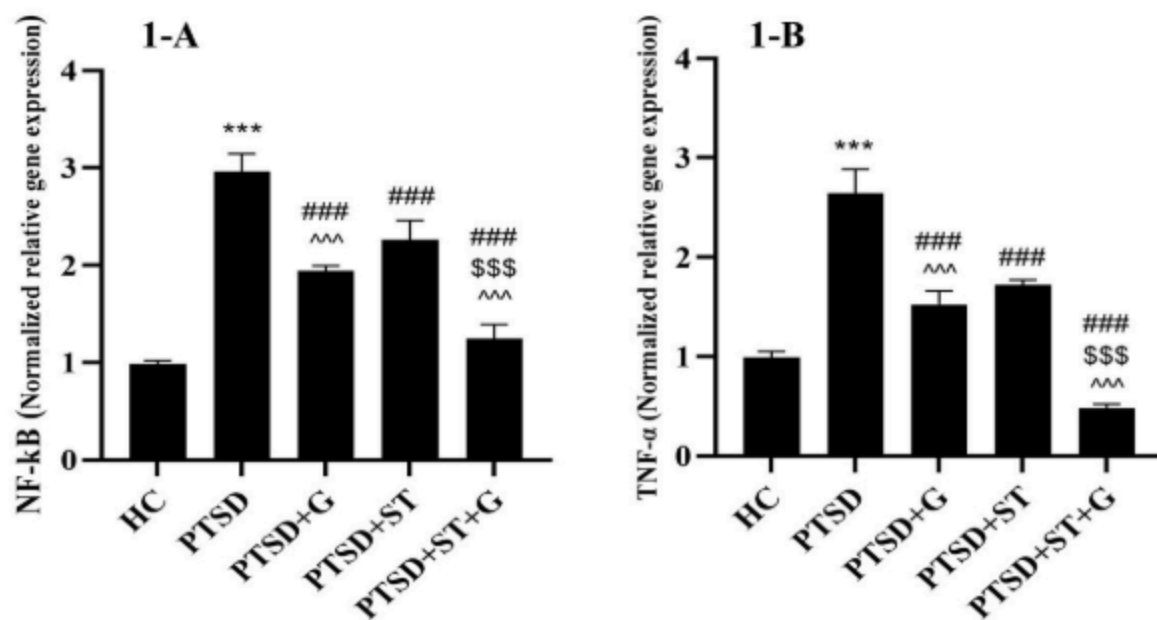


Figure 1

Gene expression levels of inflammatory factors in the brain tissue of rats in the research groups

*** (P=0.001) significant increase compared to the HC group; ### (P=0.001) significant decrease compared to the PTSD group; \$\$\$ (P=0.001) significant decrease compared to the PTSD+G group; ^^^ (P=0.001) significant decrease compared to the PTSD+ST group

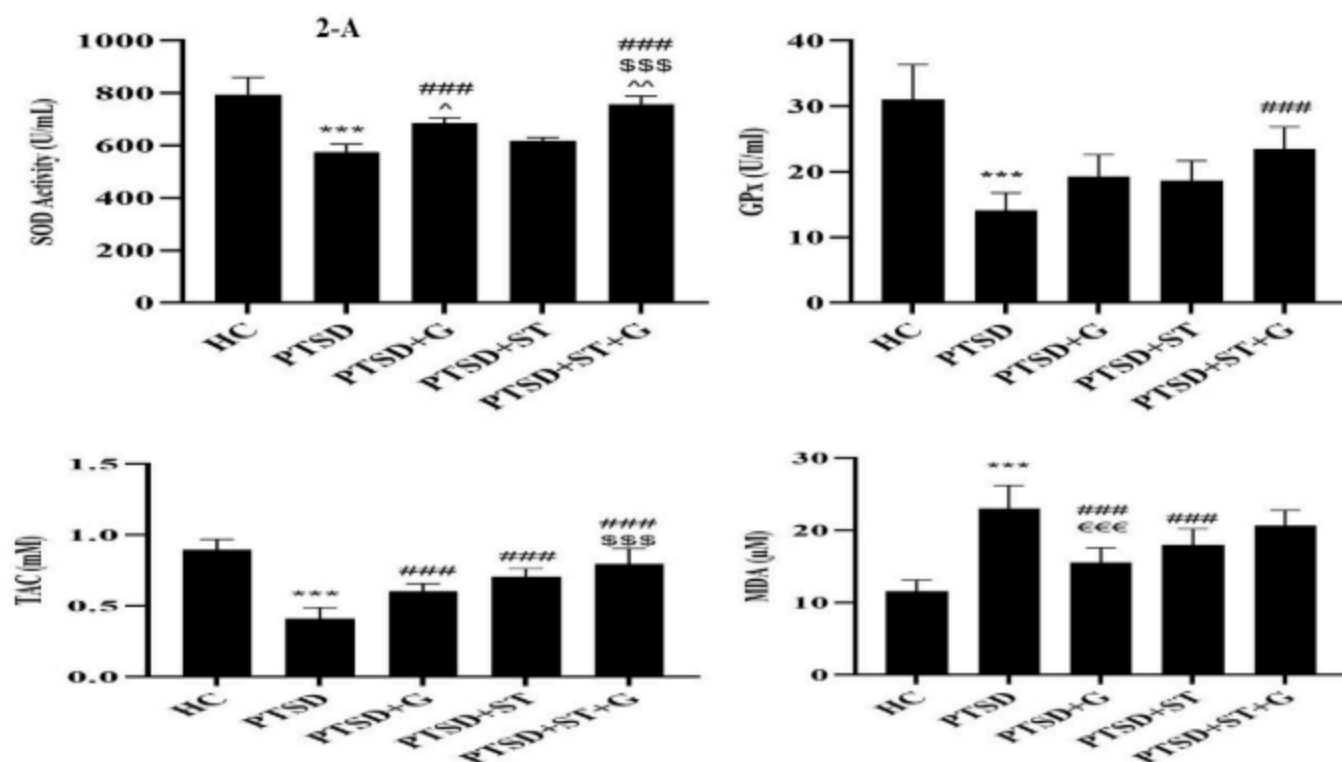


Figure 2

Oxidant- antioxidant markers in the brain tissue of rats in the research groups

*** (P=0.001) significant change compared to the HC group; ### (P=0.001) significant change compared to the PTSD group; \$\$\$ (P=0.001) significant change compared to the PTSD+G group; ^^^ (P=0.001) significant change compared to the PTSD+ST group; €€€ (P=0.001) significant change compared to the PTSD+ST+G group

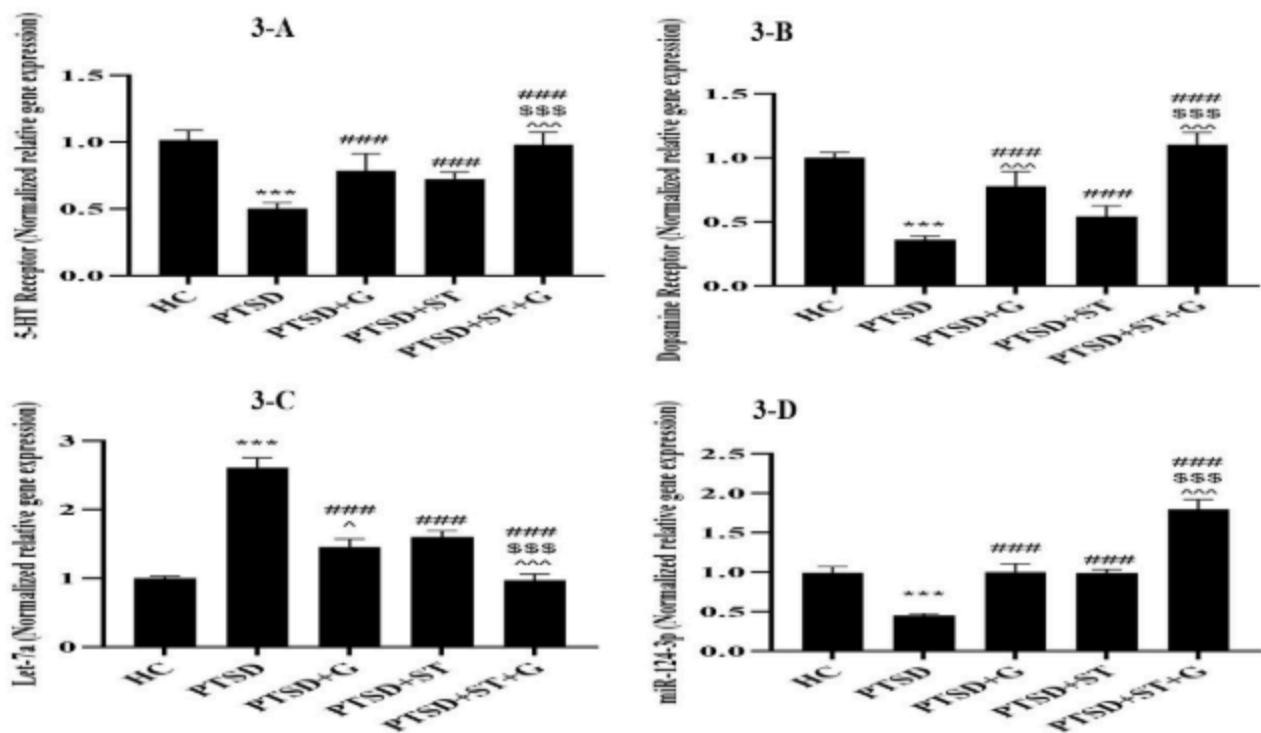


Figure 3

Neurotransmitter markers in the brain tissue of rats in the study groups

*** (P=0.001) significant change compared to the HC group; ### (P=0.001) significant change compared to the PTSD group; \$\$\$ (P=0.001) significant change compared to the PTSD+G group; ^^^ (P=0.001) significant change compared to the PTSD+ST group

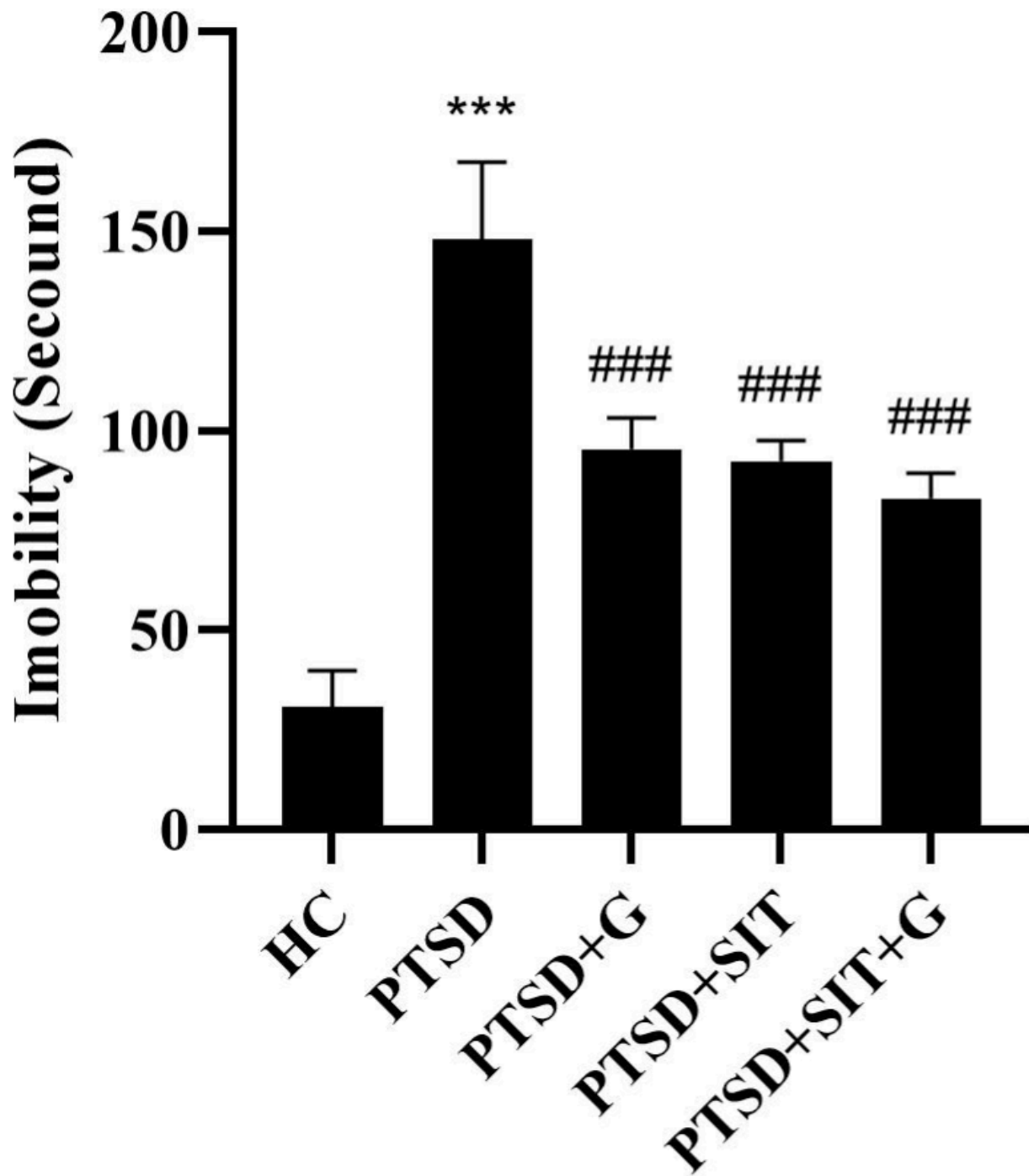


Figure 4

Duration of immobility in rats in the study groups

*** (P=0.001) significant change compared to the HC group; ### (P=0.001) significant change compared to the PTSD group