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Coenzyme Q10 supplementation and fored treadmill exercise: A combined intervention to improve cognitive function and reduce anxiety-like behavior in a PTSD rat model

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Abstract

OBJECTIVE: To investigate the combined effects of Coenzyme Q10 (CoQ10) supplementation and forced treadmill exercise on cognitive function and anxiety-like behavior in a rat model of post-traumatic stress disorder (PTSD).

METHODS: Adult male Wistar rats (n = 49) were randomly assigned to seven groups: sham, sham with exercise, sham with CoQ10, sham with CoQ10 and exercise, PTSD, PTSD with exercise, and PTSD with CoQ10 and exercise. PTSD was induced using the single prolonged stress (SPS) model. Anxiety-like behavior was assessed using elevated plus maze (EPM) and open field tests (OFT). Hippocampal BDNF levels, serum corticosterone, and SOD activity were measured. Expression of apoptosis-related genes (*Bax, Bcl-2, Caspase-3*) and microRNAs (miR-124, miR-21, miR-208) were analyzed using RT-PCR.

RESULTS: The PTSD with CoQ10 and exercise group showed significant improvements in EPM and OFT performance, indicating reduced anxiety-like behavior. This group also exhibited increased hippocampal BDNF levels, normalized corticosterone levels, and enhanced SOD activity. Gene expression analysis revealed a decreased *Bax/Bcl-2* ratio and upregulation of miR-124 in the PTSD with CoQ10 and exercise group. MiR-21 and miR-208 levels were also modulated, suggesting potential cardiovascular benefits.

CONCLUSION: The combination of CoQ10 supplementation and forced treadmill exercise significantly ameliorates anxiety-like behavior and cognitive deficits in a rat model of PTSD. This intervention appears to act through multiple mechanisms, including modulation of BDNF levels, oxidative stress reduction, and regulation of apoptotic pathways and microRNAs. These findings suggest a novel, multifaceted approach for PTSD treatment that warrants further investigation in clinical settings.

Introduction

Post-traumatic stress disorder (PTSD) is a debilitating mental health condition affecting approximately 12.5% of primary care patients, making it a prevalent public health concern (Bogolepova 2024). It ranks high among psychological responses to life-threatening experiences (Gualtieri *et al.* 2020). PTSD significantly impacts mental and emotional well-being, causing memory, attention, and executive function impairments alongside heightened anxiety and emotional dysregulation (Kim & Seo 2013). Mounting evidence suggests that PTSD disrupts brain function and structure, with the hippocampus being a particularly vulnerable region. Research indicates that PTSD alters hippocampal size and function by decreasing brain-derived neurotrophic factor (BDNF) production and promoting apoptosis (programmed cell death) through modulation of proteins like Bcl-2, Bcl-XL, BH3-only family members, and the *Bax* family (Yakhkeshi *et al.* 2022). Unfortunately, current pharmacological interventions for PTSD are not universally successful and often come with significant side effects, limiting their prescription (Yakhkeshi *et al.* 2022). Additionally, PTSD is associated with a range of co-occurring physical health problems including chronic pain, hypothalamic-pituitary-adrenal axis dysfunction, diabetes, hypertension, and dyslipidemia (Greene *et al.* 2024). The observed cognitive deficits in PTSD patients display a concerning degree of persistence, mirroring neuropsychological abnormalities found in neurodegenerative diseases (Bogolepova 2024). Despite the established benefits of physical activity for overall health, individuals with mental illness often engage less due to disease-related disengagement (Greene *et al.* 2024). While previous studies demonstrate the positive impact of exercise on mental health, including PTSD, further randomized controlled trials are needed to identify the most effective exercise types and intensities for promoting adherence in PTSD patients (Greene *et al.* 2024). Given the significant health risks associated with PTSD, researchers have explored both pharmacological and psychological therapies, with promising results (Malaktaris *et al.* 2019). The development of effective treatments to alleviate the cognitive and behavioral deficits associated with PTSD holds the potential to drastically improve the quality of life and functional outcomes for patients.

Coenzyme Q10 (CoQ10) exists in three redox states within eukaryotic cells: ubiquinol ($Q10H_2$), ubisemiquinone, and fully oxidized ubiquinone. It plays a vital role in mitochondrial function by facilitating electron transport in the oxidative phosphorylation pathway, ultimately contributing to ATP production. This multifaceted role has positioned CoQ10 as a potential therapeutic target for protecting the brain and increasing cognitive function and also, neurodegenerative diseases like Alzheimer's disease (AD), where oxidative stress is a key pathological factor (Grundman & Delaney 2002). Studies suggest CoQ10 supplementation may offer neuroprotective benefits and enhance cognitive function (Grundman & Delaney, 2002; Fernandes *et al.* 2023). Exercise represents another promising therapeutic strategy for various health conditions, including mental illnesses like PTSD. Exercise training can improve both core PTSD symptoms and physical health markers, potentially reducing the risk of co-occurring disorders such as cardiovascular disease (CVD) (Rosenbaum *et al.* 2011). Notably, abnormal blood pressure responses to exercise are recognized as a significant predictor of accelerated CVD progression (Weggen *et al.* 2024). Interestingly, PTSD, despite being a psychiatric disorder, manifests with physiological symptoms that elevate CVD risk. Individuals with PTSD often exhibit elevated blood pressure, a key CVD risk factor, and are more susceptible to developing CVD, particularly heart failure (Weggen *et al.* 2024).

MicroRNAs (miRNAs), a family of small non-coding RNAs, have recently garnered significant interest due to their potential role in cancer development by regulating gene expression after transcription and acting as either oncogenes or tumor suppressors (Modarresi Chahardehi *et al.* 2024). Among these, miR-124 is particularly prevalent in the brain, with high expression in the central nervous system (CNS) as well as other tissues like bone marrow, lymph nodes, thymus, and peripheral blood mononuclear cells (Gourishetti *et al.* 2023). Conversely, miR-21 plays a crucial role in the development of myocardial fibrosis and heart failure, as demonstrated by Chang *et al.* who linked miR-21 to increased heart size in older individuals with heart stress (Chang *et al.* 2022). Interestingly, studies suggest that miRNA levels, including miR-21, can be altered following traumatic brain injury (TBI), potentially impacting the underlying pathological processes (Ge *et al.* 2014). Recent research has explored the potential of serum miR-208 as a highly effective biomarker for early detection of acute myocardial infarction (AMI) based on CVD biomarkers (Wang *et al.* 2021). Shifting focus to the brain, the hippocampus is critical for cognitive functions such as learning and memory. This region is particularly susceptible to the effects of PTSD (Eichenbaum 2017; Liu *et al.* 2023). PTSD is characterized by symptoms like anxiety and depression-like behaviors (Preston & Kozicz 2022). Exercise has emerged as a promising therapeutic approach for depressive disorders due to its low cost, relative safety, and synergistic effects with antidepressants (Shafia *et al.* 2023). SPS is a suggested animal model for PTSD. Rats exposed to SPS exhibited heightened suppression of the hypothalamo-pituitary-adrenal (HPA) axis, a response that has been consistently observed in individuals with PTSD (Li *et al.* 2010). Studies consistently report the beneficial impacts of regular physical activity on brain function and plasticity (Nishijima *et al.* 2016). Notably, aerobic exercise has gained recognition as a viable non-pharmacological treatment for PTSD. Animal models

subjected to forced treadmill exercise exhibit neuroplastic changes, regulation of neurotransmitter systems, and decreased anxiety-like behavior, suggesting its potential therapeutic benefits in alleviating PTSD symptoms (Nishijima *et al.* 2016).

This study investigated the individual and combined effects of CoQ10 supplementation and forced treadmill exercise on cognitive function and anxiety-like behavior in a well-established PTSD rat model. Our findings demonstrate that the combination of CoQ10 and exercise can significantly improve cognitive function and reduce anxiety-like behavior in PTSD rats, suggesting its potential therapeutic value. Moreover, this study highlights the need for further research to elucidate the underlying mechanisms, including the potential role of CVD-related miRNA biomarkers, by which this combination intervention exerts its beneficial effects.

Materials and methods

Animals and experimental groups

This study employed male adult Wistar rats (2.5-3 months old, 200-250g body weight) obtained from the Animal House of AJA University of Medical Sciences, Tehran, Iran. All experiments were performed following guidelines established by the AJA University Ethics Committee and the National Institutes of Health guide for the care and use of laboratory animals. The ethics number was registered by the Council of Laboratory Animals of AJA University of Medical Sciences, Tehran, Iran (Approval No. IR.AJAUMS.REC.1402.10). Animals were housed under controlled conditions with a 12-hour light/dark cycle and a constant temperature of 24 ± 1°C. They had ad libitum access to standard laboratory chow and water. A total of 49 rats were randomly divided into seven groups (n = 7/group):

1. Sham (NSPS): Received no intervention, only olive oil (less than 0.5 mL) and saline (less than 0.5 mL) daily.
2. Sham with Exercise (NSPS/EXC): Subjected to forced treadmill exercise for four weeks.
3. Sham with CoQ10 (NSPS/CoQ10): Administered CoQ10 (100 mg/kg) orally in olive oil (less than 0.5 mL) daily for four weeks.
4. CoQ10 + Exercise (NSPS/CoQ10-EXC): Received CoQ10 (100 mg/kg) orally in olive oil (less than 0.5 mL) daily for four weeks and subjected to forced treadmill exercise. The CoQ10 solution (100 mg/125 mL) was obtained from America Mediac and Science Co., USA.
5. PTSD (SPS): Exposed to the single prolonged stress (SPS) model for PTSD induction and received no intervention.
6. PTSD with Exercise (SPS/EXC): Underwent SPS model for PTSD induction and participated in forced treadmill exercise for four weeks.
7. PTSD with CoQ10 and Exercise (SPS/CoQ10-EXC): Exposed to the SPS model, received CoQ10 (100 mg/kg) orally in olive oil (less than 0.5 mL) daily for four weeks, and participated in forced treadmill exercise.

Control (NSPS) and PTSD-induced (SPS) groups were placed on the treadmill for the same duration as the exercise groups but without running.

Single prolonged stress (SPS) model

This study employed the well-established SPS model to induce PTSD-like symptoms in rats (Yakhkeshi *et al.* 2022). The SPS protocol consisted of three distinct stressors administered sequentially within a single day, from 9:00 AM to 2:00 PM: 1) Restraint: Rats were individually restrained in appropriately sized cages for 2 hours, 2) Forced Swim: Following restraint, rats were subjected to forced swim in a cylindrical container (240 mm diameter, 500 mm height) filled with water to two-thirds of its height for 20 minutes, and 3) Ether Anesthesia: After drying and a 15-minute recovery period, rats were anesthetized with ether vapor in a bell jar until unconsciousness. Following the SPS procedure, rats were group-housed (6 per cage) for 30 days. This model effectively replicates neuroendocrine and anxiety alterations characteristic of PTSD in humans (Mirjalili *et al.* 2022).

Exercise training protocol

To minimize stress associated with the treadmill, all animals underwent a habituation phase four days following the SPS procedure. During habituation, rats were placed on a stationary treadmill (0° incline) for three daily sessions (15 minutes/session) at a slow speed (3 m/min). Following habituation, exercise groups engaged in moderate-intensity exercise for four weeks, five days per week. The exercise protocol consisted of running on a treadmill at 10 m/min for 30 minutes per session with a 0° incline. Three animals displaying exercise aversion were excluded and replaced with new rats. Sedentary control groups were placed on the stationary treadmill for 5 minutes daily (Yakhkeshi *et al.* 2022).

Behavioral tests

Elevated plus-maze test (EPM)

Anxiety-like behavior was assessed on day 43 using the EPM test, a well-established method (Tarsaei *et al.* 2022). The EPM consisted of two open arms (50 x 10 cm) and two enclosed arms (50 x 10 x 40 cm) connected by a central platform (10 × 10 cm). The maze was elevated 50 cm from the floor. Each rat was placed individually in the center of the maze facing an open arm and allowed to explore freely for 5 minutes. The following behaviors were recorded: Open Arm Time (OAT%): Percentage of time spent in the open arms and Open Arms Entries (OAE%): Percentage of entries into the open arms. An increased OAT% and OAE% are indicative of anxiolytic effects (reduced anxiety), while decreased values suggest anxiogenic effects (increased

Tab. 1. The primary sequences of the selected genes

Gene	Primer sequence (direction 5´→3´)
Bax	Forward: GGCTGGACACTGGACTTC
	Revers: CAGATGGTGAGTGAGGCA
Bcl-2	Forward: GTGGACAACATCGCTCTG
	Reverse: AGACAGCCAGGAGAAATCA
Caspase3	Forward: GACAACAACGAAACCTCC
	Reverse: AGGGTAATCCATTTTGTAACTG
miR-124	Forward: ATAATTCGGTAAGGCACGCGGTG
	Reverse: ATCCAGTGCAGGGTCCGAGG
miR-21	Forward: TCCAGCCCCTCCTCAGTCA
	Reverse: AGCCCTTGCAGCCTTCACA
miR-208	Forward: GTCATCTAGAAAGCTTGATGCAGGAAA GAGCTTTGG
	Reverse: TGACAGATCTCAGCTGA CATCCTCTAGGCTGGGGTT
β-actin	Forward: TCCTCCTGAGCGCAAGTAC
	Reverse: CCTGCTTGCTGATCCACATCT
U6	Forward: CTCGCTTCGGCAGCACA
	Reverse: AACGCT TCACGAATTTGCGT

anxiety). The exploration behavior was video recorded for subsequent analysis. A camera was used to record each session. According to the following formula, the anxiety index was calculated:

$$\text{Anxiety index} = 1 - \left(\frac{\text{Open} - \text{arm time}}{\text{Total time}} \right) + \left(\frac{\text{Open} - \text{arm entries}}{\text{Total entries}} \right)/2$$

Open-field test (OFT)

Anxiety is believed to be quantified by the duration of time spent in the central area of the open field (Prut & Belzung, 2003). The open field consisted of a Plexiglas box (90 × 90 cm, 42 cm height) with a black checkered floor (18 × 18 cm squares). The floor was divided into a central zone (demarcated by gridlines 11 cm from the walls) and a peripheral zone. The study assessed dependent variables, including the duration of time spent in the center and the ratio of distance traveled in the center to the overall distance traveled. The observer, who was unaware of the treatment regimen, rated each video footage and noted the number of squares traversed and the number of times the subject reared. After the test, every rat was placed back into its respective cage. Following the completion of the test, every rat was placed back into its own cage.

Tissue collection and processing

Following the behavioral tests (EPM and optional OFT), animals were sacrificed in accordance with ethical guidelines. To minimize the impact of ether on corticosterone levels, anesthesia and blood collection were performed swiftly. All animals, regardless of group (SPS or non-SPS), were euthanized using a combination of ketamine (30-50 mg/kg) and xylazine (3-5 mg/kg) through intraperitoneal injection. After 40 to 50 seconds of administration, the animals were in a suitable anesthesia condition. After being washed with physiologic serum, the samples were immediately placed into special tubes. Blood was collected from the trunk and centrifuged at 4000× g for 20 minutes. Serum was separated and stored at -80°C for subsequent corticosterone assay and metabolic analyses. This consistent approach ensured that any potential effect of ether on hormone levels would be reflected equally across all groups.

Determination of BDNF levels and SOD activities

BDNF protein levels were quantified using a commercially available ELISA kit (ZB-10013S-M9648, Zellbio Co., Germany) following the manufacturer's instructions. Briefly, thawed samples were added to the ELISA plate wells. Subsequently, a horseradish peroxidase (HRP)-conjugated anti-BDNF antibody was added and incubated for 45 minutes at room temperature with shaking. The wells were then washed to remove unbound antibodies and antigens. Substrate solution was added and incubated for 15 minutes, followed by the stop solution. The absorbance was measured at 450 nm using a microplate reader (Inno, South Korea). BDNF concentration was determined based on a standard curve and expressed as pg/mg protein (Fatemi *et al.* 2018a).

SOD activity was measured using a commercially available assay kit (ZB-10290S-M9648, Zellbio Co., Germany) following the manufacturer's protocol. Briefly, thawed samples were combined with the chro-

mogen solution according to the kit instructions and mixed thoroughly. The absorbance was then measured at 420 nm using a microplate reader (e.g., RT-2100C, Rayto, China). SOD activity was determined based on a standard curve and expressed as units per milligram of protein (Fatemi *et al.* 2018b).

RT-PCR

Primers were designed using Primer3 software (http://primer3.ut.ee/) and confirmed for specificity using the NCBI BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Hippocampi were rapidly dissected and frozen at -80°C until real-time PCR (RT-PCR) analysis. β-actin mRNA served as an internal control and was co-amplified with BDNF mRNA using primers listed in Table 1.

To establish a linear working range for all PCR products, a preliminary experiment was conducted to determine the optimal cycle number and denaturation temperature. The experimental amplification protocol, as described by Mizuno *et al.* (2000), consists of an initial round at 94°C for 5 minutes, followed by 27 cycles of denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 2 minutes using a PCR Thermal Cycler (Dena Tajhiz, Iran).

Ethical consideration

All animal procedures were conducted in accordance with the guidelines established by the National Institutes of Health (NIH) for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). This included providing animals with ad libitum access to standard laboratory chow and water, maintaining them in a temperature-controlled environment with a 12-hour light/dark cycle, and minimizing stress during handling and experimentation.

Statistical analysis

Data are presented as mean ± standard deviation (SD) from three independent replicates. One-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test was used to compare differences between treatment groups. A p-value less than 0.05 was considered statistically significant. Two-way ANOVA test was applied for the analysis of EPM test, in which two independent variables (i.e., exercise and CoQ10) were used. Half maximal inhibitory concentration (IC50) values, when applicable, were determined using regression analysis of triplicate data points across various concentrations. GraphPad Prism 8.3 software (GraphPad Software, Inc., La Jolla, CA, USA) was used for all statistical analyses and graphical representations to ensure consistency and clarity.

Results

General body parameters

Changes in body weight can serve as a useful indirect measure of the body's physiological response to stress (Dehghani *et al.* 2022). No mortality was observed among the seven groups of rats over the four-week research period. Throughout the experiment, the body weights of the rats steadily increased across all groups. The schematic design of the study (Fig. 1A), data on weight changes, and weight gain are presented in Fig. 1.

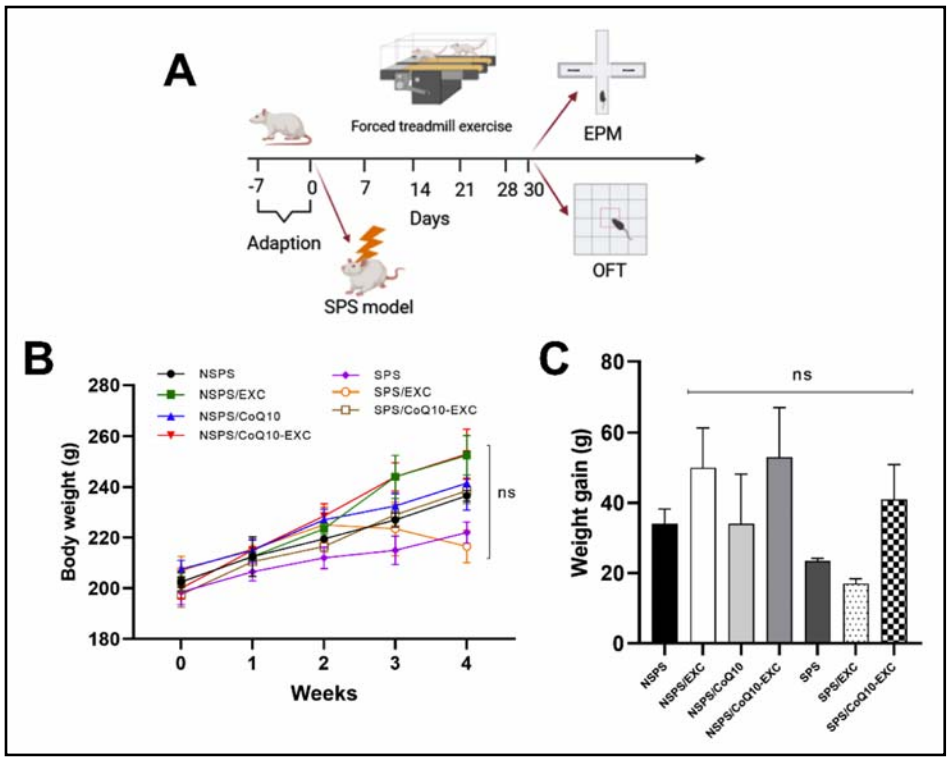

Fig. 1. The schematic design of the study **(A)**, body weight **(B)** and weight gain **(C)** in experimental groups over 4 weeks. Data represent the mean ± SD (n = 7 per group). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. 'ns' indicates no statistically significant differences between experimental groups and the control group.

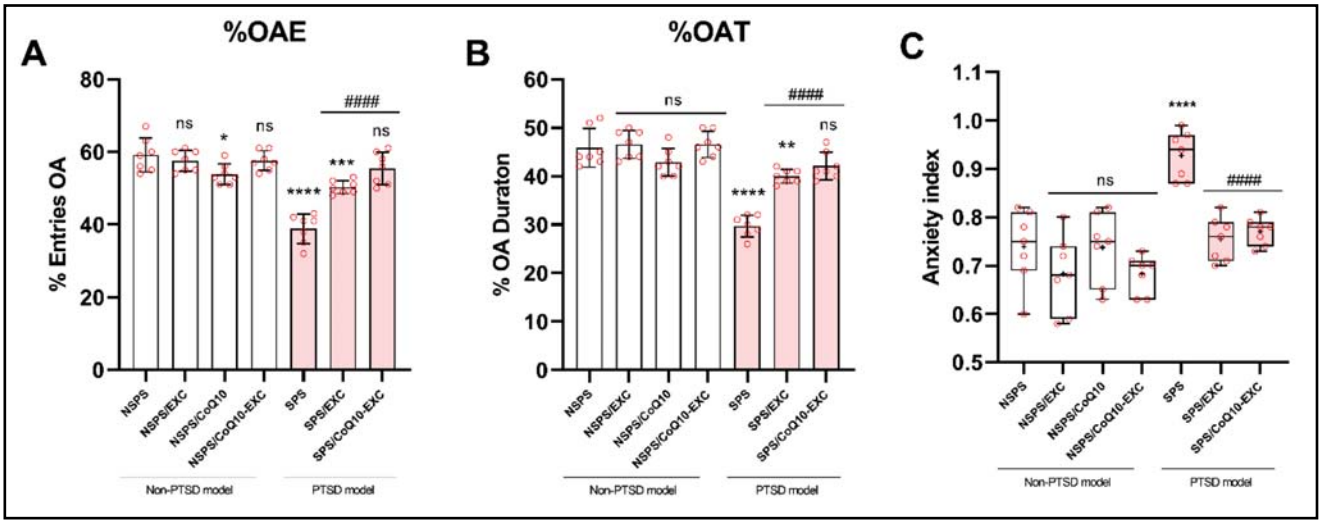

Fig. 2. Effects of Coenzyme Q10 (CoQ10) and exercise on PTSD and non-PTSD rat models in the EPM. **A)** Anxiety-like behaviors represented by the percentage of open arm entries, **B)** Percentage of time spent in the open arms, and **C)** Anxiety index. Data are shown as mean ± SD (n = 7 per group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to control by one-way ANOVA with Dunnett's multiple comparisons test. ns = no significant difference versus control (NSPS); ## $p < 0.01$, ### $p < 0.001$, and #### $p < 0.0001$ vs. SPS group.

As illustrated in Figures 1B and 1C, there was no significant differences in body weight and weight gain among the experimental rats ($p > 0.05$).

Elevated plus maze test

Anxiety-like behavior on EPM was assessed on day 30, one day after the last training session in SPS and NSPS male rats (Fig. 2). The data for open arm entries (OAE) in the EPM are presented in Fig. 2A. The percentage of entries into the open arms was measured across seven groups. The NSPS/EXC, NSPS/CoQ10-EXC, and SPS/CoQ10-EXC groups did not show significant differences compared to the NSPS group. In contrast, the NSPS/CoQ10, SPS, and SPS/EXC groups spent significantly less time in the open arms compared to the NSPS group ($p = 0.0340$, $p < 0.0001$, and $p < 0.0001$, respectively). Based on our results, SPS/EXC and SPS/CoQ10-EXC groups showed an increase in %OAE compared to the SPS group ($p < 0.0001$). A two-way ANOVA on the percentage of OAE revealed a significant main effect between SPS/NSPS groups and CoQ10/non-CoQ10 in exercise groups. Additionally, significant interactions were found between NSPS/EXC × SPS/EXC ($F_{1,24} = 16.1$, $p = 0.0005$) and NSPS/CoQ10-EXC × SPS/CoQ10-EXC ($F_{1,24} = 4.78$, $p = 0.0388$).

The time spent in open arms (OAT) was recorded in seconds for the NSPS and experimental groups (Fig. 2B). The NSPS/EXC, NSPS/CoQ10, NSPS/CoQ10-EXC, and SPS/CoQ10-EXC groups demonstrated no significant difference compared to the NSPS group. However, the SPS and SPS/EXC groups showed significant difference, with p-values of < 0.0001 and 0.0018, respectively.

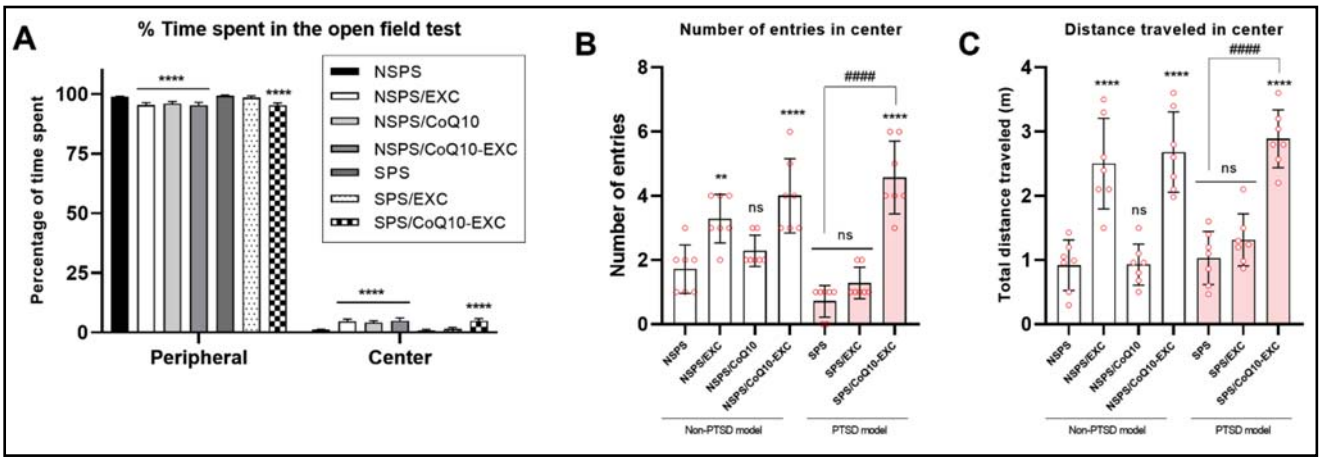

Fig. 3. An analysis of three behavioral factors in the open-field test across several groups. **A)** The percentage of time spent in the peripheral and central areas in the OFT. **B)** The number of entries into the center of the OFT. **C)** The total distance traveled in the center area. Data represent the mean ± S.D. (n = 7 per group). Statistical differences in the data obtained from the tested groups were determined using one-way ANOVA followed by Dunnett's post hoc test compared to the NSPS group. ** $p < 0.01$, **** $p < 0.0001$, and ns = not significant. #### $p < 0.0001$ vs. SPS group.

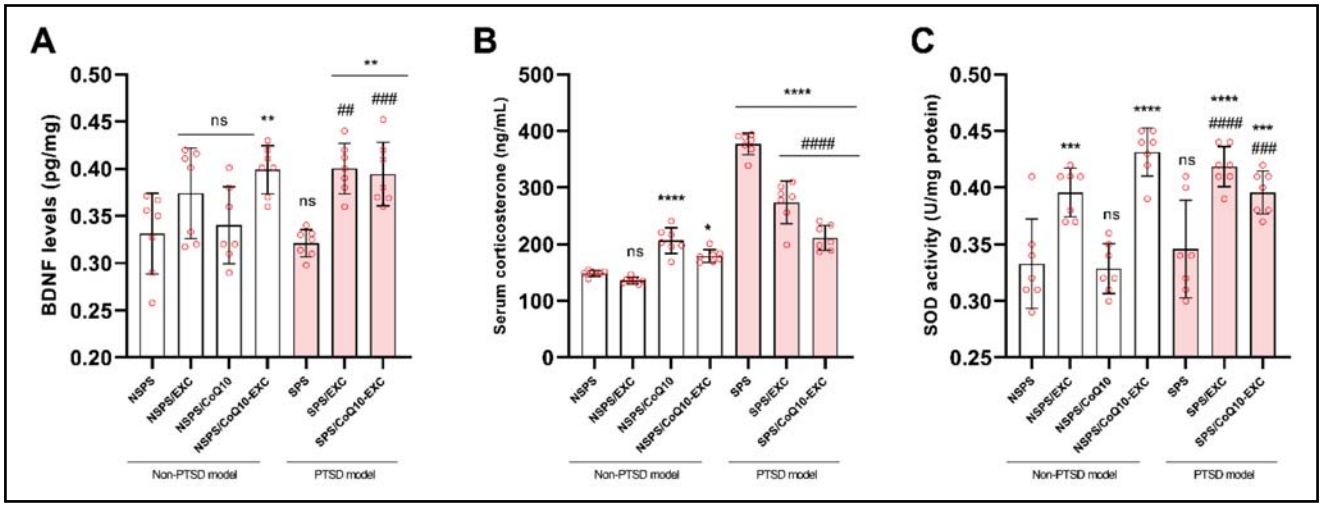

Fig. 4. A) The effect of PTSD model and non-PTSD model, exercise, and CoQ10 supplementation in various groups on the expression of BDNF in the hippocampal tissue. **B)** comparison of variations in serum CORT levels in rats across experimental groups. **C)** Activities of SOD in experimental groups of rats. Statistical differences in data obtained from the tested groups was determined using Dunnett's test. Values are expressed as mean ± SD (n = 7), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ against the control group; ## $p < 0.01$, ### $p < 0.001$, and #### $p < 0.0001$ vs. SPS group; ns = no significant.

A two-way ANOVA on the percentage of OAT revealed a significant difference only between NSPS × SPS groups ($F_{1,24} = 14.0$, $p = 0.0010$). Furthermore, the SPS/EXC and SPS/CoQ10-EXC groups showed an increase in %OAT compared to the SPS group ($p < 0.0001$).

An anxiety index score (ranging from 0 to 1) was computed by dividing the time spent in the open arm and the number of entries into the open arm by the total number of entries. In this study, the anxiety index ranged from 0.58 to 0.99, with the SPS group showing a significant increase compared to the NSPS group ($p < 0.0001$). The SPS/EXC and SPS/CoQ10-EXC groups demonstrated a reduced anxiety index compared to the SPS group ($p < 0.0001$).

Open-field test

The NSPS/EXC, NSPS/CoQ10, NSPS/CoQ10-EXC, and SPS/CoQ10 groups showed a significant decrease in the periphery/center area ($p < 0.0001$; Fig. 3A). The NSPS/EXC, NSPS/CoQ10-EXC, and SPS/CoQ10-EXC groups had a significantly higher number of entries into the center ($p = 0.0036$, $p < 0.0001$, and $p < 0.0001$, respectively). A Similar pattern was observed for center distance, with the NSPS/EXC, NSPS/CoQ10-EXC, and SPS/CoQ10-EXC groups showing the highest distance at 2.5, 2.68, and 2.89 meters ($p < 0.0001$; Fig. 3C). These were followed by the SPS/EXC, SPS, and NSPS/CoQ10 groups ($p > 0.05$). Based on our findings in Fig. 3B and C, only the SPS/EXC group showed an increase in the number of entries into the center and the distance traveled in the center of the OPT area compared to the SPS group ($p < 0.0001$).

BDNF, CORT levels and SOD activities

The groups exhibited statistically significant variations in BDNF levels ($F_{6,42} = 6.75$, $p < 0.0001$; Fig. 4A). Forced treadmill exercise in the non-SPS model (NSPS/EXC group), CoQ10 administration (NSPS/CoQ10), and the SPS group did not modify BDNF levels compared to the NSPS group ($p > 0.05$). However, the NSPS/CoQ10-EXC, SPS/EXC, and SPS/CoQ10-EXC groups showed significant increases in BDNF levels ($p = 0.0040$, $p = 0.0033$, and $p = 0.0080$, respectively). According to Fig. 4A, exercise enhanced BDNF levels in the SPS/EXC and SPS/CoQ10-EXC groups compared to the SPS group ($p < 0.0001$ and $p = 0.0001$, respectively).

A one-way ANOVA revealed a statistically significant increase in CORT levels across the tested groups compared to the NSPS group ($F_{6,42} = 115$, $p < 0.0001$; Fig. 4B). All SPS groups (SPS, SPS/EXC, and SPS/CoQ10-EXC) indicated a significant increase in plasma CORT levels compared to the other NSPS groups ($p < 0.0001$). Additionally, the NSPS/CoQ10 and NSPS/CoQ10-EXC groups demonstrated elevated CORT levels ($p < 0.0001$, $p = 0.0375$, respectively). Our results indicate that exercise significantly reduced CORT levels in the SPS/EXC and SPS/CoQ10-EXC groups compared to the SPS group ($p < 0.0001$).

Based on Fig. 4C, statistically significant differences in total SOD activity were observed among the groups ($F_{6,42} = 15.9$, $p < 0.0001$). Neither NSPS/CoQ10 nor SPS groups altered SOD activity compared to the NSPS groups. However, the NSPS/EXC and SPS/CoQ10-EXC groups exhibited increased SOD activity ($p = 0.0008$), similar to the NSPS/CoQ10-EXC and SPS/EXC groups ($p < 0.0001$). Interestingly, exercise did not reduce SOD activities in the SPS/EXC and SPS/CoQ10-EXC groups compared to the SPS group ($p = 0.0004$ and $p = 0.0090$, respectively).

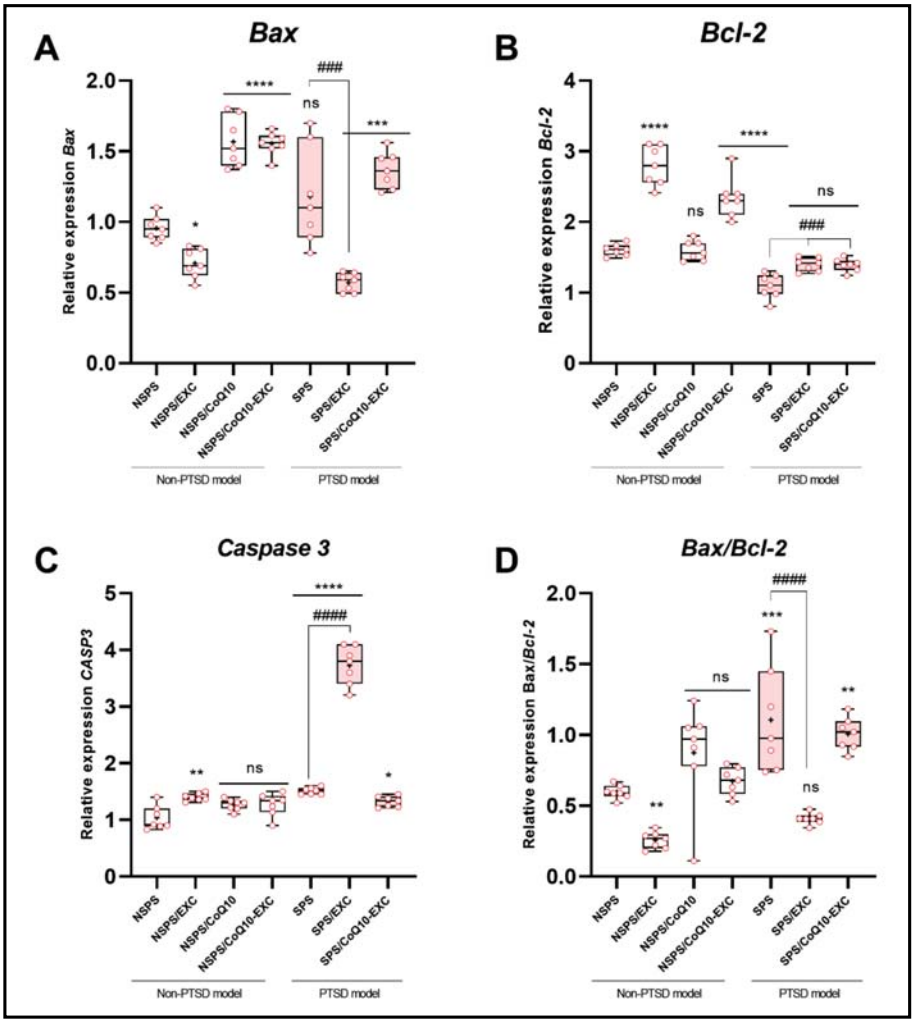

Fig. 5. Relative genes expression of Bax **(A)**, Bcl-2 **(B)**, Casp3 **(C)**, and Bax/Bcl-2 ratio **(D)**. Results are expressed as mean ± SD (n = 7). Statistical significance was assessed using a one-way ANOVA followed by Dunnett's test. Compared with the control group: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, **** $p < 0.0001$, and ns = not significant; compared with the SPS group: ### $p < 0.001$, and #### $p < 0.0001$ vs. SPS group.

Apoptotic related hippocampal mRNAs and the level of miR-124, miR-21, and miR-208

Data on the mRNA expression of *Bax, Bcl-2*, and *Casp3* are presented in Fig. 5. The effects of exercise and SPS on the expression of *Bax* are depicted in Fig. 5A, revealing significant difference among groups ($F_{6,42} = 38.7$, $p < 0.0001$). The NSPS/EXC, NSPS CoQ10, and SPS/CoQ10-EXC groups showed significant increases ($p < 0.0001$), whereas the NSPS/EXC and SPS/EXC groups exhibited notable decreases ($p = 0.0423$ and $p = 0.0003$, respectively) compared to the NSPS group. The SPS group did not show a significant difference compared to the NSPS group ($p > 0.05$). A two-way ANOVA demonstrated a significant difference among the four groups (NSPS/EXC, SPS/EXC, and SPS/CoQ10-EXC). Additionally, significant interactions were found between NSPS/EXC × SPS/EXC ($F_{1,24} = 18.7$, $p = 0.0002$) and NSPS/CoQ10-EXC × SPS/CoQ10-EXC ($F_{1,24} = 484$, $p < 0.0001$). However, no significant differences were found between NSPS × SPS and CoQ10 × EXC ($p > 0.05$).

The findings on the impacts of exercise and SPS on the expression of *Bcl-2* are presented in Fig. 5B, showing significant differences among groups ($F_{6,42} = 75.8$, $p < 0.0001$). The NSPS/EXC, NSPS/CoQ10-EXC, and SPS groups demonstrated significant increases in *Bcl-2* expression ($p < 0.0001$). In contrast, the NSPS/CoQ10, SPS/EXC, and SPS/CoQ10-EXC did not exhibit significant differences compared to the NSPS group ($p > 0.05$). A two-way ANOVA conducted among the four groups (NSPS/EXC, SPS/EXC, and SPS/CoQ10-EXC) revealed a significant effect of SPS ($F_{1,24} = 206$, $p < 0.0001$), NSPS/CoQ10-EXC × SPS/CoQ10-EXC ($F_{1,24} = 7.09$, $p = 0.0139$), and overall interaction ($F_{1,24} = 7.96$, $p = 0.0097$).

Data on the mRNA expression of *Casp3* are depicted in Fig. 5C. There was a significant enhancement observed in NSPS/EXC, SPS, SPS/EXC, and SPS/CoQ10-EXC groups ($p = 0.0024$, < 0.0001, < 0.0001, and $= 0.0226$). Only NSPS/CoQ10 and NSPS/CoQ10-EXC showed no significant difference ($p > 0.05$). A two-way ANOVA conducted among the four groups (NSPS/EXC, SPS/EXC, and SPS/CoQ10-EXC) revealed a significant effect of SPS ($F_{1,24} = 230$, $p < 0.0001$), NSPS/CoQ10-EXC × SPS/CoQ10-EXC interaction ($F_{1,24} = 261$, $p < 0.0001$), and overall interaction ($F_{1,24} = 209$, $p < 0.0001$).

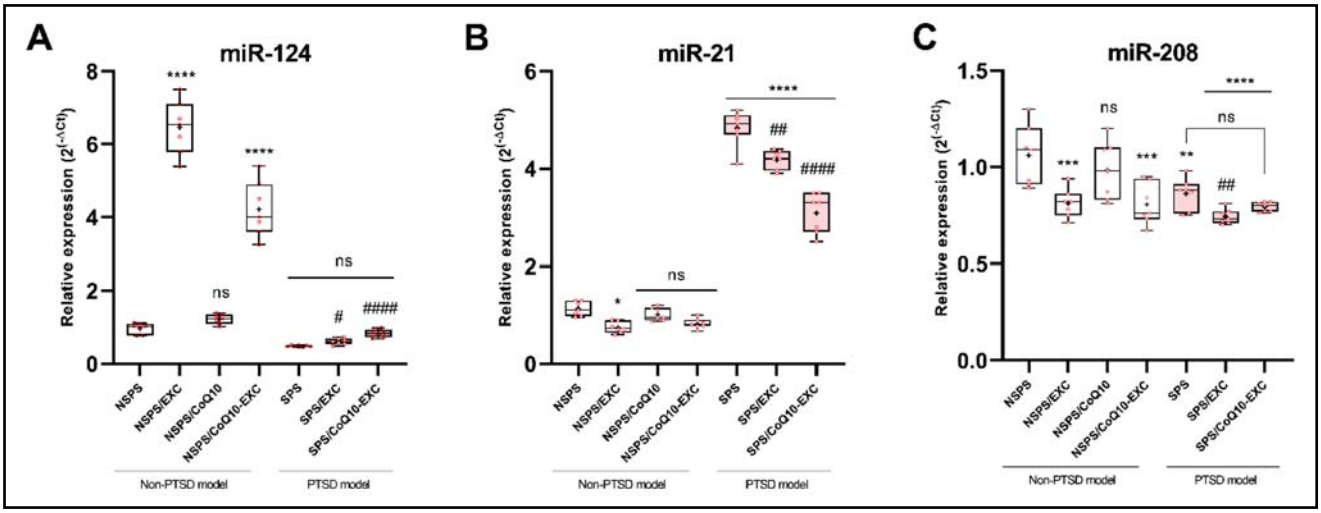

Fig. 6. The level of miRNAs. **A)** miR-124, **B)** miR-21, and **C)** miR-208 in various groups; Results of experiments are expressed as mean ± SD (n = 7). Statistical significance was assessed using a one-way ANOVA followed by Dunnett's test. Compared with the control group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, **** $p < 0.0001$, and ns = not significant; # $p < 0.05$, ## $p < 0.01$ ### $p < 0.001$, and #### $p < 0.0001$ vs. SPS group.

In order to investigate the potential impact of miR-124 on PTSD-like behavior in rats exposed to SPS, hippocampal tissue was analyzed followed several behavioral tests (Fig. 6A). Significant differences were revealed among groups ($F_{6,42} = 225$, $p < 0.0001$). Only the NSPS/EXC and NSPS/CoQ10-EXC groups showed significant increases ($p < 0.0001$). A two-way ANOVA analysis revealed significant differences among the four groups (NSPS/EXC, SPS/EXC, and SPS/CoQ10-EXC) indicating a notable impact of SPS ($F_{1,24} = 532$, $p < 0.0001$), NSPS/CoQ10-EXC × SPS/CoQ10-EXC ($F_{1,24} = 25.7$, $p < 0.0001$), and their interaction ($F_{1,24} = 37.7$, $p < 0.0001$). Additionally, a one-way ANOVA among SPS groups (SPS, SPS/EXC, and SPS/CoQ10-EXC) revealed that the SPS/EXC and SPS/CoQ10-EXC groups showed increased fold change compared to the SPS group ($p = 0.0181$ and $p < 0.0001$, respectively).

Two miRNA, miR-21 and miR-208, were selected to quantify hypertrophy in our experimental rat groups (Fig. 6B and C). For analysis miR-21 (Fig. 6B), except for the NSPS/EXC group, which showed a significant reduction ($p = 0.0236$), other groups, including SPS, SPS/EXC, and SPS/CoQ10-EXC, exhibited significant increases ($p < 0.0001$). A two-way ANOVA carried out among four groups (NSPS/EXC, SPS/EXC, and SPS/CoQ10-EXC) exhibited significant effects of SPS ($F_{1,24} = 56.9$, $p < 0.0001$), NSPS/CoQ10-EXC × SPS/CoQ10-EXC ($F_{1,24} = 993$, $p < 0.0001$), and their interaction ($F_{1,24} = 42.4$, $p < 0.0001$). According to a one-way ANOVA among SPS groups, the SPS/EXC and SPS/CoQ10-EXC groups demonstrated considerable declines compared to the SPS group ($p = 0.0039$ and $p < 0.0001$, respectively).

To determine whether miR-208 is related to hypertrophy response in rats, we measured the levels of this miRNA. As shown in Fig. 6C, there was a significant difference between groups ($F_{6,42} = 8.68$, $p < 0.0001$). The NSPS/EXC, NSPS/CoQ10-EXC, SPS, SPS/EXC, and SPS/CoQ10-EXC groups demonstrated significant decreases ($p = 0.0003$, $p = 0.0002$, $p = 0.0042$, $p < 0.0001$, and $p = 0.0001$, respectively). Only the NSPS/CoQ10 group did not show a significant change ($p > 0.05$). A two-way ANOVA among specific groups (NSPS/EXC, SPS/EXC, and SPS/CoQ10-EXC), indicated no significant effect of SPS ($F_{1,24} = 0.707$, $p = 0.4088$), NSPS/CoQ10-EXC × SPS/CoQ10-EXC interaction ($F_{1,24} = 2.39$, $p = 0.1352$), and overall interaction ($F_{1,24} = 1.12$, $p = 0.3007$). Based on one-way ANOVA among SPS groups, only SPS/EXC group showed a significant reduction compared to the SPS group ($p = 0.0013$).

Discussion

The present study provides compelling evidence for the therapeutic efficacy of combining Coenzyme Q10 (CoQ10) supplementation with forced treadmill exercise in improving cognitive impairments and reducing anxiety-related behaviors associated with PTSD in a rat model. CoQ10 acts as an electron carrier in the mitochondrial respiratory chain and plays a crucial role as an antioxidant within cells (Hargreaves & Mantle 2019). Our results demonstrate intricate relationships among PTSD, physical activity, and CoQ10 supplementation, impacting various aspects of behavior, biochemistry, and molecular processes. The current study used SPS to induce PTSD, a reliable method eliciting numerous behavioral, biochemical, and physiological deficits commonly reported in individuals with PTSD.

The EPM test, utilized to quantify anxiety-like behavior in rats, is a reliable and frequently employed

test for evaluating anxiety-related behavior and the anxiolytic effects of medicines and steroid hormones in rats (Barbar Shemirani *et al.* 2022; Tarsaei *et al.* 2022). The EPM and OFT results demonstrate significant anxiolytic effects of the combined CoQ10 and exercise intervention in PTSD-modeled rats. The SPS/CoQ10-EXC group showed marked improvements in open arm entries and time spent in open arms, indicating reduced anxiety-like behavior. This aligns with previous studies showing exercise-induced anxiolytic effects in PTSD models (Yakhkeshi *et al.* 2022). Interestingly, our results suggest that CoQ10 supplementation may enhance these exercise-mediated benefits, possibly due to its antioxidant properties and role in mitochondrial function (Hargreaves & Mantle 2019; Mantle *et al.* 2023). The ethical concerns surrounding molecular studies in humans have led to the suggestion of using animal models such as predator stress or single prolong stress to investigate the causes of hippocampus atrophy in PTSD. Behavioral differences in rats on the EPM apparatus are now recognized as symptoms of this disorder (Alani *et al.* 2013).

The neurotrophic protein BDNF plays a crucial role in regulating synaptic plasticity, a potential neurobiological process underlying memory and learning (Rovny *et al.* 2023). The observed elevation in BDNF levels in the SPS/CoQ10-EXC group is particularly remarkable. BDNF is essential for neuroplasticity and has been linked to the development of PTSD. In relation to the processing of trauma, the degree and occurrence of PTSD is directly linked to the presence of the BDNF met allele (Miller & Wiener, 2014; Notaras *et al.* 2015). Previous studies have demonstrated that the BDNF gene affects navigational behavior in addition to the prevalence and severity of PTSD (Miller *et al.* 2017). The synergistic impact of exercise and CoQ10 on BDNF levels indicates a possible explanation for the observed enhancements in behavior. This discovery expands upon prior studies regarding the increase in BDNF caused by exercise (Modarresi *et al.* 2021; Haghighi *et al.* 2023), demonstrating a potential enhancing effect of coQ10.

However, it is worth mentioning that the combination of exercise with SPS resulted in a more significant elevation in corticosterone levels compared to SPS alone. This study demonstrated an increase in CORT levels after exposure to SPS. Li *et al.* found that exercise can counteract the effects of SPS on the process of apoptosis in male rats (Li *et al.* 2010). Nevertheless, CoQ10 could reduce the CORT level significantly compared to the SPS/EXC group. Our study corroborated previous research findings that demonstrated a decrease in stress hormone levels, including corticosterone and cortisol, as a result of exercise (Droste *et al.* 2006; Khajehnasiri *et al.* 2021). The SPS/CoQ10-EXC group showed a modification of CORT levels, suggesting a restoration of normal function in the HPA axis. This axis is frequently disrupted in individuals with PTSD (Chen *et al.* 2020). The normalization of stress response systems may contribute to the observed changes in behavior, indicating that the combined intervention has the potential to repair these systems.

The reduction in oxidative stress markers, as evidenced by increased SOD activity, further highlights the potential of CoQ10 and exercise to mitigate the detrimental effects of PTSD. This is consistent with the known antioxidant properties of CoQ10 and the ability of exercise to enhance antioxidant defenses. The increased SOD activity in the NSPS/Co-Q10-EXC and SPS/CoQ10-EXC groups reflects an enhanced antioxidant defense system. This finding aligns with other research that demonstrates an increase in antioxidant enzymes as a result of exercise (Kim *et al.* 2019). CoQ10 is the sole antioxidant that is produced naturally inside the body and can dissolve in fat. It is found in tissues in its active state, regardless of dietary intake (Kalayci *et al.* 2011). CoQ10, a powerful antioxidant, seems to enhance this impact, perhaps reducing oxidative stress linked to PTSD. Our gene expression analysis reveals complex modulation of apoptotic pathways. The decreased *Bax/Bcl-2* ratio in the SPS/CoQ10-EXC group suggests a shift towards anti-apoptotic signaling. This is particularly relevant given the hippocampal atrophy observed in PTSD model (Goswami *et al.* 2013; Abd El-Aal *et al.* 2017). Exercise and CoQ10 may modulate these pathways, leading to neuroprotection and potentially reversing structural abnormalities in the hippocampus caused by PTSD (Goswami *et al.* 2013). CoQ10 is recognized for its role as an antioxidant and its ability to eliminate free radicals. It has the capability to decrease and counteract free radicals and reactive oxygen species (ROS) and is also engaged in enhancing the effectiveness of the electron transport chain and the regeneration of vitamin E and C (Kiani *et al.* 2024). Furthermore, CoQ10 enhances the functionality of antioxidant enzymes such as SOD, GPx, and CAT by absorbing free radicals and promoting the production of genes responsible for these enzymes (Sangsefidi *et al.* 2020). However, in our study, CoQ10 combining with exercise could enhance SOD activity, whereas only CoQ10 did not change significantly.

This study examined apoptosis-related parameters including *caspase-3, Bcl-2*, and *Bax*. *Caspase-3* is an important effector in the cascade of caspases that regulate cell death, and its cleavage initiates the execution phase of apoptosis. As part of the mitochondria-mediated apoptotic process, *caspase-3* plays a critical role (Yakhkeshi *et al.* 2022). Additionally, in the hippocampus of SPS rats, increased apoptotic cells, increased *caspase-9* and *caspase-3* expression, a drop in the *Bax/Bcl-2* ratio, and the release of cytochrome c from the mitochondria into the cytoplasm were observed. The findings point to the involvement of the mitochondrial route in the process of SPS-induced apoptosis in the hippocampus of rats with PTSD (Li *et al.* 2010). In their study on the hippocampus of rats, Li *et al.* demonstrated

that all stressed groups under one month had significantly higher amounts of apoptotic *Bax* and *caspase-3* proteins and a higher ratio of *Bax/Bcl-2* compared to the control group. In contrast, the amount of anti-apoptotic Bcl-2 protein decreased significantly compared to the control group. This was demonstrated using a single prolong stress model (Li *et al.* 2010).

The upregulation of miR-124 in the SPS/CoQ10-EXC group is a novel finding that may have significant implications for PTSD treatment. However, we observed increasing levels in the NSPS/EXC and NSPS/CoQ10-EXC groups as well. The involvement of miR-124 in neurogenesis and synaptic plasticity has been demonstrated to be critical (Liu *et al.* 2019; Rahmani *et al.* 2022). In a paradoxical twist, miR-124 regulates autophagy, neuroinflammation, oxidative stress, neuronal excitability, neurodifferentiation, Aβ deposition, and hyperphosphorylation of tau protein in age-related intellectual disabilities. On the other hand, it may have a dual function by regulating apoptosis and negatively impacting synaptic plasticity and growth of individual neurons (Liu *et al.* 2019). The increase seen in our study indicates that the combination intervention has the potential to improve neuroplasticity, which might help overcome the brain changes caused by PTSD. Chen *et al.* found that increasing the expression of miR-124 in the hippocampus might ameliorate PTSD-like behaviors observed in SPS rats. The impact of miR-124 may be accomplished by targeting TNF receptor-associated Factor 6 (TRAF6), a gene that is regulated by miR-124 and has a significant function in immunological and inflammatory responses via modulating nuclear factor kappa-B (NF-κB) (Chen *et al.* 2022). While aggressively suppressing glialogenesis, miR-124 promotes the positive neurogenesis of neural cell lineage growth. Polypyrimidine Tract Binding Protein 1 (PTBP1), Sry-related HMG box (Sox9), and Small C-terminal domain Phosphatase 1 (SCP1) suppress anti-neuronal gene targets from miRNA and thereby drive basal cell differentiation toward nerve and play a crucial role in neural differentiation (Mojtahedi *et al.* 2012). According to previous studies, exercise at both low and high intensities increases neurogenesis in the hippocampus. Based on the Mojtahedi *et al.*'s results and the present study, both applied intensities significantly increased the level of expression miR-124 (Mojtahedi *et al.* 2012).

The modulation of miR-21 and miR-208 levels in the SPS/CoQ10-EXC group offers valuable information on the possible cardiovascular impacts of the intervention, considering the heightened susceptibility to cardiovascular illness in those with PTSD. These findings indicate that the combination treatments may have wider health advantages beyond neuropsychiatric problems. Empirical data strongly indicates that the dysregulation of miR-21, leading to differential expression of its target genes, enhances the vulnerability to numerous central nervous system illnesses (Bai & Bian, 2022). This study employs a well-established SPS model for PTSD and employs behavioral testing and genetic analysis to thoroughly assess the effects of the intervention. We provide new discoveries on the synergistic impact of CoQ10 and exercise on the expression of miR-124 and miR-208, which may enhance our comprehension of the pathophysiology of PTSD and prospective therapeutic approaches.

Limitations and future directions

While our study presents strong data supporting the effectiveness of combining CoQ10 supplements and exercise in a PTSD model, there are some limitations that need to be addressed in future research. Using only one PTSD model, such as the SPS, may restrict the capacity to apply the findings to a broader population. In order to verify the strength and reliability of the results, it is advisable for future research to incorporate several models of PTSD. Furthermore, there is a need for additional clarification about the molecular pathways that contribute to the combined benefits of CoQ10 and exercise. Utilizing pathway analysis and proteomics methods might offer a more comprehensive understanding of the molecular mechanisms behind the reported effects.

Furthermore, it is essential to examine potential variations in responsiveness to the intervention based on sex, considering the well-documented differences in the prevalence and manifestation of PTSD across genders (Heyn *et al.* 2022). Conducting longitudinal studies to investigate the lasting impact of the intervention, including any epigenetic alterations, might provide useful insights.

Compliance with ethical standards

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Author's contribution

AMC and MG wrote and organized the first draft; NPG collected data and wrote some sections; AMC analyzed the data; MG checked the latest version of manuscript. All authors read and approved the final manuscript.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data availability statement

This manuscript has no associated data.

Conclusion

The results of this study have significant consequences for the treatment of PTSD. The concurrent use of CoQ10 supplements and exercise offers a potentially safe and

non-pharmacological approach that might enhance existing therapy for PTSD. The observed impacts on several systems, including as behavior, neurochemistry, and gene expression, indicate that a comprehensive strategy to treating PTSD should consider both the psychological and physiological elements of the condition. Our study concludes that combining CoQ10 supplementation with exercise has significant therapeutic promise in treating PTSD. This intervention is designed to address many aspects of PTSD pathophysiology, providing a new and innovative therapy method. Further clinical trials are necessary to apply these findings to individuals with PTSD and improve treatment regimens

REFERENCES

1 Abd El-Aal SA, Abd El-Fattah MA, El-Abhar HS (2017). CoQ10 augments rosuvastatin neuroprotective effect in a model of global ischemia via inhibition of NF-kappaB/JNK3/Bax and activation of Akt/FOXO3A/Bim Cues. *Front Pharmacol.* **8**: 735. doi:10.3389/fphar.2017.00735

2 Alani B, Maghsoudi N, Khatibi A, Noureddini M, Asefifar F, Shams J (2013). Study of the variations in apoptotic factors in hippocampus of male rats with posttraumatic stress disorder. *Adv Biomed Res.* **2**: 42. doi:10.4103/2277-9175.109757

3 Bai X, Bian Z (2022). MicroRNA-21 is a versatile regulator and potential treatment target in central nervous system disorders. *Front Mol Neurosci.* **15**. doi:10.3389/fnmol.2022.842288

4 Barbar Shemirani S, Nasiri Khalili M, Mahdavi S, Modarresi Chahardehi A (2022). The effect of mirtazapine on reducing chronic stress in male rats. *Int Clin Neurosci J.* **9**: e21.

5 Bogolepova AN (2024). [Cognitive impairment in post-traumatic stress disorder]. *Zh Nevrol Psikhiatr Im S S Korsakova.* **124**(5): 69–74. doi:10.17116/jnevro202412405169

6 Chang WT, Shih JY, Lin YW, Huang TL, Chen ZC, Chen CL (2022). miR-21 upregulation exacerbates pressure overload-induced cardiac hypertrophy in aged hearts. *Aging (Albany NY).* **14**(14): 5925–5945. doi:10.18632/aging.204194

7 Chen J, Ye W, Li L, Su J, Huang Y, Liu L, et al. (2020). Paeoniflorin regulates the hypothalamic-pituitary-adrenal axis negative feedback in a rat model of post-traumatic stress disorder. *Iran J Basic Med Sci.* **23**(4): 439–448. doi:10.22038/ijbms.2020.41214.9738

8 Chen Y, An Q, Yang ST, Chen YL, Tong L, Ji LL (2022). MicroRNA-124 attenuates PTSD-like behaviors and reduces the level of inflammatory cytokines by downregulating the expression of TRAF6 in the hippocampus of rats following single-prolonged stress. *Exp Neurol.* **356**: 114154. doi:10.1016/j.expneurol.2022.114154

9 Dehghani Z, Mahdavi SM, Chahardehi AM, Mansouri V, Sherafat SJ (2022). The effect of 2.45 ghz electromagnetic fields on fear memory extinction in male rats. *J Lasers Med Sci.* **13**: e52-e52. doi: 10.34172/jlms.2022.52

10 Droste SK, Schweizer MC, Ulbricht S, Reul JM (2006). Long-term voluntary exercise and the mouse hypothalamic-pituitary-adrenocortical axis: impact of concurrent treatment with the antidepressant drug tianeptine. *J Neuroendocrinol.* **18**(12): 915–925. doi:10.1111/j.1365-2826.2006.01489.x

11 Eichenbaum, H. (2017). Prefrontal-hippocampal interactions in episodic memory. *Nat Rev Neurosci.* **18**(9): 547–558. doi:10.1038/nrn.2017.74

12 Fatemi I, Khaluoi A, Kaeidi A, Shamsizadeh A, Heydari S, Allahtavakoli MA (2018a). Protective effect of metformin on D-galactose-induced aging model in mice. *Iran J Basic Med Sci.* **21**(1): 19–25. doi:10.22038/IJBMS.2017.24331.6071

13 Fatemi I, Heydari S, Kaeidi A, Shamsizadeh A, Hakimizadeh E, Khaluoi A, Allahtavakoli M (2018b). Metformin ameliorates the age-related changes of d-galactose administration in ovariectomized mice. *Fundam Clin Pharmacol.* **32**(4): 392–399. doi:10.1111/fcp.12364

14 Fernandes MSS, Fidelis D, Aidar FJ, Badicu G, Greco G, Cataldi S, et al. (2023). Coenzyme Q10 supplementation in athletes: A systematic review. *Nutrients.* **15**(18). doi:10.3390/nu15183990

15 Ge XT, Lei P, Wang HC, Zhang AL, Han ZL, Chen X, et al. (2014). miR-21 improves the neurological outcome after traumatic brain injury in rats. *Sci Rep.* **4**(1): 6718. doi:10.1038/srep06718

16 Goswami S, Rodriguez-Sierra O, Cascardi M, Pare D (2013). Animal models of post-traumatic stress disorder: face validity. *Front Neurosci.* **7**: 89. doi:10.3389/fnins.2013.00089

17 Gourishetti K, Balaji Easwaran V, Mostakim Y, Ranganath Pai KS, Bhere D (2023). MicroRNA (miR)-124: A promising therapeutic gateway for oncology. *Biology (Basel).* **12**(7). doi:10.3390/biology12070922

18 Greene DR, Holland-Winkler AM, Petruzzello SJ (2024). Enjoyment and affective responses to moderate and high-intensity exercise: A randomized controlled trial in individuals with subsyndromal PTSD. *Sports (Basel).* **12**(5). doi:10.3390/sports12050138

19 Grundman M, Delaney P (2002). Antioxidant strategies for Alzheimer's disease. *Proc Nutr Soc.* **61**(2): 191–202.

20 Gualtieri G, Ferretti F, Masti A, Pozza A, Coluccia A (2020). Post-traumatic stress disorder in prisoners' offspring: A systematic review and meta-analysis. *Clin Pract Epidemiol Ment Health.* **16**: 36–45. doi:10.2174/1745017902016010036

21 Hargreaves IP, Mantle D (2019). Coenzyme Q10 Supplementation in Fibrosis and Aging. *Adv Exp Med Biol.* **1178**: 103-112. doi:10.1007/978-3-030-25650-0_6

22 Heyn SA, Bailowitz S, Russell JD, Herringa RJ (2022). Sex-based variations of prefrontal structure and longitudinal symptoms in pediatric posttraumatic stress disorder. *Depress Anxiety.* **39**(12): 902–912. doi:10.1002/da.23296

23 Haghighi HA, Ahmadi A, Carotenuto A, Askari R, Nikkhah K, Bagherzadeh-Rahmani B, et al. (2023). Effects of concurrent training and CoQ10 on neurotrophic factors and physical function in people with Multiple Sclerosis: a pilot study. *Eur J Transl Myol.* **33**(2). doi:10.4081/ejtm.2023.11253

24 Kalayci M, Unal MM, Gul S, Acikgoz S, Kandemir N, Hanci V, et al. (2011). Effect of Coenzyme Q10 on ischemia and neuronal damage in an experimental traumatic brain-injury model in rats. *BMC Neurosci.* **12**(1): 75. doi:10.1186/1471-2202-12-75

25 Khajehnasiri N, Dehkordi MB, Amini-Khoei H, Mohammadabadi MSM, Sadeghian R (2021). Effect of exercise intensity and duration on the levels of stress hormones and hypothalamic-pituitary-gonadal axis in adult male rats: an experimental study. *Hormones (Athens).* **20**(3): 483–490. doi:10.1007/s42000-021-00303-4

26 Kiani Z, Khorsand N, Beigi F, Askari G, Sharma M, Bagherniya M (2024). Coenzyme Q10 supplementation in burn patients: a double-blind placebo-controlled randomized clinical trial. *Trials.* **25**(1): 160. doi:10.1186/s13063-024-08006-y

27 Kim BK, Seo JH (2013). Treadmill exercise alleviates post-traumatic stress disorder-induced impairment of spatial learning memory in rats. *J Exerc Rehabil.* **9**(4): 413–419. doi:10.12965/jer.130058

28 Kim HN, Jeon DG, Lim Y, Jang IS (2019). The effects of coenzyme Q(10) supplement on blood lipid indices and hepatic antioxidant defense system in SD rats fed a high cholesterol diet. *Lab Anim Res.* **35**: 13. doi:10.1186/s42826-019-0013-1

29 Li XM, Han F, Liu DJ, Shi YX (2010). Single-prolonged stress induced mitochondrial-dependent apoptosis in hippocampus in the rat model of post-traumatic stress disorder. *J Chem Neuroanat.* **40**(3): 248–255. doi:10.1016/j.jchemneu.2010.07.001

30 Liu DB, Shi Y, Zheng SP, Zhou HR, Zhao LW (2023). [Effects of post-traumatic stress disorder on the excitability of glutamatergic and GABAergic neurons in dorsal and ventral hippocampus in mice]. *Sheng Li Xue Bao.* **75**(3): 369–378.

31 Liu X, Feng Z, Du L, Huang Y, Ge J, Deng Y, Mei Z (2019). The potential role of microRNA-124 in cerebral ischemia injury. *Int J Mol Sci.* **21**(1). doi:10.3390/ijms21010120

32 Malaktaris AL, Buzzella BA, Siegel ME, Myers US, Browne KC, Norman SB, Angkaw AC (2019). OEF/OIF/OND veterans seeking PTSD Treatment: Perceptions of partner involvement in trauma-focused treatment. *Mil Med.* **184**(3–4): e263–e270. doi:10.1093/milmed/usy231

33 Mantle D, Millichap L, Castro-Marrero J, Hargreaves IP (2023). Primary Coenzyme Q10 Deficiency: An Update. *Antioxidants (Basel).* **12**(8). doi:10.3390/antiox12081652

34 Miller JK, McDougall S, Thomas S, Wiener J (2017). The impact of the brain-derived neurotrophic factor gene on trauma and spatial processing. *J Clin Med.* **6**(12). doi:10.3390/jcm6120108

35 Miller JK, Wiener JM (2014). PTSD recovery, spatial processing, and the val66met polymorphism. *Front Hum Neurosci.* **8**: 100. doi:10.3389/fnhum.2014.00100

36 Mirjalili R, Shokouh E, Dehkordi NS, Afsari R, Shafia S, Rashidy-Pour A (2022). Prior short-term exercise prevents behavioral and biochemical abnormalities induced by single prolonged stress in a rat model of posttraumatic stress disorder. *Behav Brain Res.* **428**: 113864. doi:10.1016/j.bbr.2022.113864

37 Mizuno M, Yamada K, Olariu A, Nawa H, Nabeshima T (2000). Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. *J Neurosci.* **20**(18): 7116–7121.

38 Modarresi Chahardehi A, Afrooghe A, Emtiazi N, Rafiei S, Rezaei NJ, Dahmardeh S, et al. (2024). MicroRNAs and angiosarcoma: are there promising reports? *Front Oncol.* **14**: 1385632. doi:10.3389/fonc.2024.1385632

39 Modarresi F, Fatemi PR, Razavipour SF, Ricciardi N, Makhmutova M, Khoury N, et al. (2021). A novel knockout mouse model of the noncoding antisense Brain-Derived Neurotrophic Factor (Bdnf) gene displays increased endogenous BDNF protein and improved memory function following exercise. *Heliyon.* **7**(7): e07570. doi:10.1016/j.heliyon.2021.e07570

40 Mojtahedi S, Kordi M, Soleimani M (2012). Effect of different intensities of short term aerobic exercise on expression of miR-124 in the hippocampus of adult male rats. *Zahedan Journal of Research in Medical Sciences.* **14**(2).

41 Nishijima T, Torres-Aleman I, Soya H (2016). Exercise and cerebrovascular plasticity. *Prog Brain Res.* **225**: 243–268. doi:10.1016/bs.pbr.2016.03.010

42 Notaras M, Hill R, van den Buuse M (2015). The BDNF gene Val66Met polymorphism as a modifier of psychiatric disorder susceptibility: progress and controversy. *Mol Psychiatry.* **20**(8): 916–930. doi:10.1038/mp.2015.27

43 Preston G, Kozicz T (2022). A protocol for the induction of posttraumatic stress-disorder (PTSD)-like behavior in mice. *J Vis Exp.* **185**. doi:10.3791/63803

44 Prut L, Belzung C (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol.* **463**(1–3): 3–33. doi:10.1016/s0014-2999(03)01272-x

45 Rahmani S, Kadkhoda S, Ghafouri-Fard S (2022). Synaptic plasticity and depression: the role of miRNAs dysregulation. *Mol Biol Rep.* **49**(10): 9759–9765. doi:10.1007/s11033-022-07461-7

46 Rosenbaum S, Nguyen D, Lenehan T, Tiedemann A, van der Ploeg HP, Sherrington C (2011). Exercise augmentation compared to usual care for post traumatic stress disorder: a randomised controlled trial (the REAP study: Randomised exercise augmentation for PTSD). *BMC Psychiatry.* **11**: 115. doi:10.1186/1471-244X-11-115

47 Rovny R, Marko M, Michalko, D, Mitka M, Cimrova B, Vancova, Z, et al. (2023). BDNF Val66Met polymorphism is associated with consolidation of episodic memory during sleep. *Biol Psychol.* **179**: 108568. doi:10.1016/j.biopsycho.2023.108568

48 Sangsefidi ZS, Yaghoubi F, Hajiahmadi S, Hosseinzadeh M (2020). The effect of coenzyme Q10 supplementation on oxidative stress: A systematic review and meta-analysis of randomized controlled clinical trials. *Food Sci Nutr.* **8**(4): 1766–1776. doi:10.1002/fsn3.1492

49 Shafia S, Nikkhah, F, Akhoundzadeh K (2023). Effect of combination fluoxetine and exercise on prefrontal BDNF, anxiety-like behavior and fear extinction in a female rat model of post-traumatic stress disorder (PTSD): a comparison with male animals. *Behav Brain Funct.* **19**(1): 1. doi:10.1186/s12993-023-00204-z

50 Tarsaei M, Peyrovan ZS, Mahdavi SM, Modarresi Chahardehi A, Vafaee R, Haidari MH (2022). Effects of 2.45 GHz non-ionizing radiation on anxiety-like behavior, gene expression, and corticosterone level in male rats. *J Lasers Med Sci.* **13**: e56. doi:10.34172/jlms.2022.56

51 Wang J, Xu L, Tian L, Sun Q (2021). Circulating microRNA-208 family as early diagnostic biomarkers for acute myocardial infarction: A meta-analysis. *Medicine (Baltimore).* **100**(51): e27779. doi:10.1097/MD.0000000000027779

52 Weggen JB, Darling AM, Autler AS, Hogwood AC, Decker KP, Richardson J, et al. (2024). Lower vascular conductance responses to handgrip exercise are improved following acute antioxidant supplementation in young individuals with post-traumatic stress disorder. *Exp Physiol.* **109**(6): 992–1003. doi:10.1113/EP091762

53 Yakhkeshi R, Roshani F, Akhoundzadeh K, Shafia S (2022). Effect of treadmill exercise on serum corticosterone, serum and hippocampal BDNF, hippocampal apoptosis and anxiety behavior in an ovariectomized rat model of post-traumatic stress disorder (PTSD). *Physiol Behav.* **243**: 113629. doi:10.1016/j.physbeh.2021.113629