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Short-Communication

Investigating the impact of prolonged swimming exercise along with aqueous *Artemisia annua* supplementation on hippocampal angiogenic and inflammatory biomarkers in trimethyltin-induced neurodegeneration in rats

Hakimeh Banavi Yasooj[1], Hadi Ghaedi[1,*], Omidreza Salehi[2]

[1]*Department of Physical Education, Lam.C., Islamic Azad University, Lamerd, Iran*
[2]*Department of Sport Sciences, Pishtazan Institute of Higher Education, Shiraz, Iran*

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**** Corresponding Author:***
Tel: +989177828667
Fax:+987152721232
ghaedi.Hadi@gmail.com

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Abstract

Objective: This study investigated the effects of eight-week swimming training (ST), combined with *Artemisia annua* supplementation, on hippocampal angiogenesis and inflammation in rats with trimethyltin (TMT)-induced Alzheimer's disease (AD).

Materials and Methods: Twenty-eight male Sprague-Dawley rats (16–18 months old) with TMT-induced AD were randomly assigned to four groups: TMT control, TMT+*A. annua*, TMT+ST, and TMT+ST+*A. annua*. Seven healthy rats served as healthy controls (HC). ST was performed for eight weeks, five sessions per week, with session durations ranging from 5-35 min. Rats in the supplementation groups received an aqueous extract of aerial parts of *A. annua* 50 mg/kg/day, oral administration.

Results: Compared with the TMT group, *vascular endothelial growth factor (VEGF)* and *endothelial nitric oxide synthase (eNOS)* expression were significantly increased, whereas *nuclear factor Kappa-B (NF-κB)* and *tumor necrosis factor-alpha (TNF-α)* expression were significantly decreased in the TMT+*A. annua*, TMT+ST, and TMT+ST+*A. annua* groups ($p<0.05$). The TMT+ST+*A. annua* group showed the greatest increase in *VEGF* and *eNOS* and the greatest reduction in *NF-κB*. In addition, *eNOS* expression was higher in TMT+ST than in TMT+*A. annua*, whereas *NF-κB* and *TNF-α* expression were lower in TMT+*A. annua* than in TMT+ST ($p<0.05$).

Conclusion: Eight weeks of ST and *A. annua* supplementation, alone or in combination, exerted anti-inflammatory and angiogenic effects in TMT-induced AD rats. Notably, *A. annua* showed stronger anti-inflammatory effects, whereas ST induced greater angiogenic responses. Their combination may synergistically enhance neuronal survival and vascular function, representing a promising neuroprotective strategy for neurodegenerative disorders.

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Introduction

Alzheimer's disease (AD) represents the most prevalent subtype of dementia, marked by amyloid beta (Aβ)-induced neurotoxicity. Neurofibrillary tangles, elevated tau protein levels, and microvascular dysfunction are key pathological features of the disease (Tsartsalis et al. 2024). These processes ultimately result in neuronal loss in neural structures involved in learning and memory, particularly the hippocampus (Kumari, Dhapola, and Reddy 2023). Evidence suggests that Aβ accumulation and vascular deposition reduce vascular elasticity and promote neuronal loss and neuroinflammation. Oxidative stress elevation triggers key inflammatory pathways, notably involving nuclear factor-κB (NF-κB) and interleukin-1β (IL-1β), and contributes to dysregulation of angiogenic markers, including decreased vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (eNOS) (Sheikh et al. 2023).

Considering the demand for safe and noninvasive treatment strategies, consistent physical exercise is increasingly recognized for its neuroprotective potential in degenerative brain disorders. Exercise has been shown to enhance cognitive function by improving cerebral blood flow, upregulating neurotrophic factors, and increasing antioxidant capacity (Sheikholeslami-Vatani, Salehi, and Hosseini 2021). Physical activity has been shown to stimulate nuclear factor erythroid 2–related factor 2 (Nrf2) and activate the PI3K signaling cascade, thereby enhancing the transcription of antioxidant genes and supporting cognitive performance (Hashemi et al. 2024). Exercise-driven enhancement of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) expression has been shown to upregulate hypoxia-inducible factor 1-alpha (HIF-1α) subsequently facilitating the transcription of VEGF and eNOS (Song et al. 2024). Several studies support these findings. For instance, eight weeks of swimming exercise (five sessions per week, 60 min per session at 15–60 m/min) significantly increased VEGF, Platelet-derived growth factor (PDGF), and angiopoietin-1 and -2 levels within the brain of rats following surgical ovariectomy (Yoon et al. 2023). A meta-analytic review reported that physical activity in older males enhances the expression of angiogenic and endothelial markers, including VEGF, fibroblast growth factor 2 (FGF2), and E-selectin (Song et al. 2024). In another human study, cerebral blood flow and VEGF increased, ultimately improving cerebrovascular function in patients with AD (Pedrinolla et al. 2020). In Pahlavani's study (2023), eight weeks of aerobic exercise at 50–75% VO_2max and high-intensity interval training both increased brain-derived neurotrophic factor (BDNF), tropomyosin receptor kinase B (TrkB), nerve growth factor (NGF), interleukin-1 receptor antagonist (IL-1RA), and IL-10 levels. Moderate-intensity training reduced NF-κB, TNF-α, C-reactive protein (CRP), interleukin-1 beta (IL-1β), and interferon-gamma (IFN-γ), while acute exercise elevated IL-6 levels (Pahlavani 2023). Thus, the effects of exercise on inflammatory and angiogenic mechanisms appear to be intensity-dependent.

Nonetheless, the exact molecular pathways responsible for these effects are yet to be fully elucidated. Beyond physical activity, phytotherapeutic compounds have been suggested as adjunctive interventions owing to their antioxidative and anti-inflammatory potential. *Artemisia annua* (*A annua*), an annual herb native to Asia and the Middle East, may exert neuroprotective effects via these mechanisms. By inhibiting TNF-α, IL-6, IL-1β, toll-like receptor 4 (TLR4), and NF-κB, *A annua* may reduce Aβ1–42 accumulation and improve cognitive function (Zhao et al. 2022). Li et al. (2022) reported that *A annua* can increase cerebral blood flow, reduce hydrogen peroxide and Aβ, modulate the ERK/AMPK/GSK3/Nrf2 pathway, and enhanced cognitive performance (Li et al. 2022). de Faveri Favero et al. (2024) demonstrated that deoxyartemisinin and *A*

annua-derived artemisinin derivatives reduced TNF-α, IL-6, and IL-1β and modulated opioid receptors in brain tissue in an animal model of neurodegeneration (de Faveri Favero et al. 2024).

Despite evidence supporting the individual effects of exercise and *A annua* on inflammation and angiogenesis, no study has examined their combined impact on these markers in brain tissue following cognitive impairment. The integration of physical training with *A annua* administration may offer valuable insights into their potential synergistic impact on cerebral perfusion and neuroinflammatory modulation. Accordingly, this study aimed to evaluate the impact of an eight-week swimming training combined with *A annua* administration on hippocampal angiogenic and inflammatory markers in rats exposed to trimethyltin (TMT).

Materials and Methods

Animals

In this experimental and fundamental study, 35 male Sprague-Dawley rats (approximately 16–18 months old, weighing 300–340 g) were obtained from the Pasteur Laboratory Animal Breeding Center. The animals were then transferred to the Exercise Physiology Laboratory at the Pishtazan Institute of Higher Education in Shiraz. To ensure environmental adaptation, the animals underwent a one-week acclimatization period prior to experimentation. Throughout this phase, standard housing conditions were upheld, including a 12-hr light/dark photoperiod, ambient temperature maintained at 22–24°C, and relative humidity stabilized around 55%. Rats had unrestricted access to standard laboratory chow and water. They were housed in washable polycarbonate cages lined with sterile wood shavings to facilitate moisture absorption and maintain hygiene.

Induction of Alzheimer's disease

AD was induced by administering a single intraperitoneal dose of trimethyltin chloride (10 mg/kg of TMT; Sigma-Aldrich, USA) to 28 rats, after a 12-hr fasting period. Fourteen days post-injection, AD induction was confirmed by observing clinical signs—including aggression, tail twitching, and periorbital hemorrhage—and by conducting the Y-maze test to assess cognitive impairment. This behavioral test was used solely to validate disease induction by comparing TMT-exposed rats with healthy controls (Sheikholeslami-Vatani et al. 2021). Upon confirmation, the rats were randomly assigned to four groups: (1) TMT control, (2) TMT+*A annua*, (3) TMT+ST, and (4) TMT+ST+*A annua*. Additionally, seven non-treated rats were assigned to the healthy control (HC) group to enable comparative evaluation of AD-related alterations across experimental parameters (Figure 1).

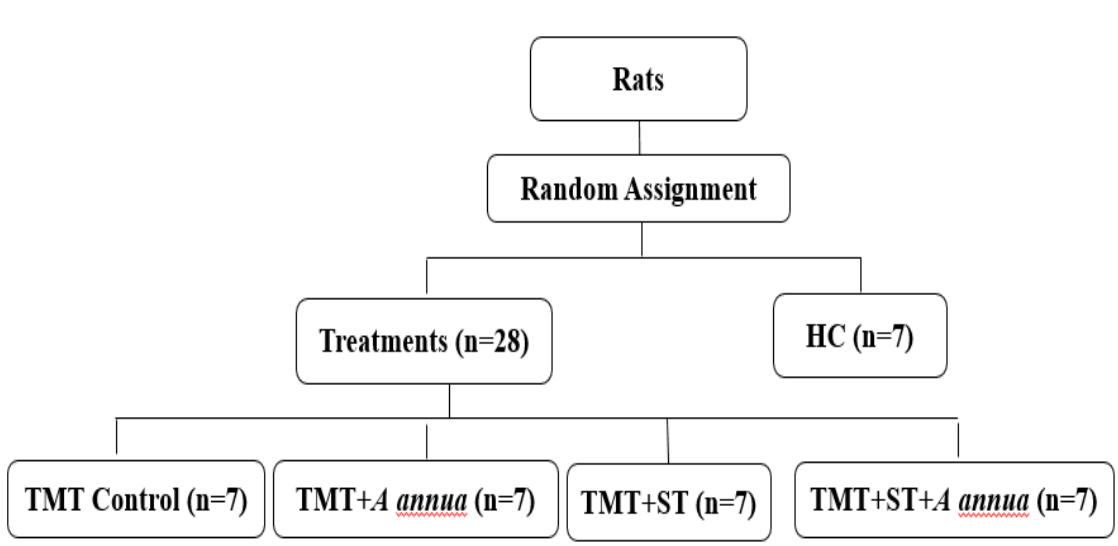

Figure 1. Experimental grouping and treatment allocation. Rats were randomly assigned to five groups: one healthy control (HC) group and four trimethyltin (TMT)-injected groups including TMT control, TMT+*A annua*, TMT+Swimming training (ST), and TMT+ST+*A annua*. Each group consisted of seven rats.

Swimming training protocol

The swimming protocol spanned eight weeks, comprising five sessions per week. Session duration progressively increased from 5 min in week one to 35 min by week eight. During the first two weeks, the rats performed continuous swimming without weights for 5–15 min per session. In the third and fourth weeks, a weight equivalent to 2% of body weight was attached to the tails of the rats. From the fifth to eighth weeks, the weight was progressively increased, reaching 4.95% of body weight by the eighth week (Lunz et al. 2008).

A annua extract

Artemisia annua (*A annua*) was collected from native populations in Marvdasht, Fars Province, Iran. The plant was authenticated and approved by the Agricultural Jihad Office of Marvdasht before use in the present study**.** The aerial parts of the plant (leaves and stems) were separated, cleaned, air-dried, and pulverized for extract preparation. To prepare the extract, the water distillation method was used based on the protocol described by Ebrahimi, Setorki, and Dastanpour (2019). A mixture containing 50 g of pulverized plant material and 500 ml of distilled water was transferred into a distillation flask and subjected to heating until a steady distillate flow of approximately 2–3 ml/min was achieved. Following a 4-hr distillation process, the essential fraction was harvested and subsequently dehydrated over anhydrous sodium sulfate for 24 hr. Prior to administration, the dried Artemisia extract was dissolved in distilled water to prepare a solution of 50 mg/kg. Rats in the extract groups received this solution orally once daily (Ebrahimi, Setorki, and Dastanpour 2019).

Sampling

Seventy-two-hour post training and supplementation, the animals underwent anesthesia with ketamine (75 mg/kg) and xylazine (25 mg/kg) following a 12-hr fasting period, implemented to reduce aspiration risk and stabilize metabolic parameters. After confirming complete anesthesia, brain tissue was carefully extracted using surgical instruments by

laboratory specialists, and the hippocampus was subsequently isolated.

Gene expression

To measure the variables, hippocampal tissue was first kept frozen. A homogeneous solution was then prepared from the tissue. RNA was extracted using a specialized extraction kit manufactured by ROSH company (Germany), Cat NO: R.41444A. The quality of the extracted RNA was assessed using a spectrophotometer at a wavelength of 420–510 nm, and upon confirming RNA purity, the samples were used for cDNA synthesis. At this stage, cDNA was synthesized following the Kiazyst kit protocol (Cat NO: K1296). The designed primers were validated using the NCBI database (Table 1), and after confirming their efficiency, the primers were mixed with cDNA and placed in a qReal-Time PCR device (made by Step One company, Italy) for amplification across multiple cycles. Once the expression threshold was reached, the device was stopped, and gene expression levels were measured relative to an internal control gene such as Tata binding protein (*TBP)*. The 2^–ΔΔCT formula was used to quantify the data.

Table 1. Primers sequences in the present study

Genes	Primer Sequences	Sizes (bp)
TBP	Forward: 5'- GCGGGGTCATGAAATCCAGT-3' Reverse: 5'- AGTGATGTGGGGACAAAACGA -3'	147
VEGF	Forward: 5'- ACTTGAGTTGGGAGGAGGATGTC-3' Reverse: 5'- GGATGGGTTTGTCGTGTTTCTGG-3'	183
eNOS	Forward: 5'- TGACCCTCACCGATACAACA -3' Reverse: 5'- CGGGTGTCTAGATCCATGC -3'	60
NF_kB	Forward: 5'-CATGAGCTGACCCTACCCTG-3' Reverse: 5'-TTTCTTCGATCCGATGGCGA-3'	128
TNF-α	Forward: 5'- ATGGGCTCCCTCTCATCAGT-3' Reverse: 5'- GCTTGGTGGTTTGCTACGACG-3'	106

Statistical analysis

Descriptive data is expressed as mean ± standard deviation. The Shapiro–Wilk test was utilized to examine the normality of variable distribution. Upon confirmation of normality, intergroup differences were analyzed using one-way ANOVA, followed by Tukey's post hoc test for pairwise comparisons. To further evaluate the independent and combined effects of ST and *A annua* administration, a two-way ANOVA was conducted with Bonferroni correction applied for multiple comparisons. Also, independent t-test was used to analyze Y-maze data between the HC and TMT groups. All statistical analyses were performed using SPSS software (version 22), with significance defined at $p<0.05$.

Results

Spontaneous alternation percentage (SAP) in the Y-maze test

As depicted in Figure 2, independent t-test analysis revealed a markedly reduced spontaneous alternation percentage in the TMT group compared to healthy controls (HC), with a statistically significant difference ($t = 33.62$, $p = 0.001$).

Analysis of angiogenic markers

One-way ANOVA revealed a statistically significant difference in the expression of *VEGF* (F=141.65 and p=0.001) and *eNOS* (F=165.57 and p=0.001) in the study groups. Tukey's post hoc analysis further revealed that *VEGF* expression in the TMT group was significantly reduced compared to the HC group (p=0.001); but in the TMT+*A annua* (p=0.001), TMT+ST (p=0.001) and TMT+ST+*A annua* (p=0.001) groups were significantly higher than the TMT group. No significant difference were detected between the TMT+*A annua* and TMT+ST groups (p=0.69); but in the TMT+ST+ *A annua* group *VEGF* levels were significantly higher than the TMT+*A annua* (p=0.001) and TMT+ST (p=0.001) groups. Also, the results of two-way analysis of

variance showed that ST (p=0.001, F=242.22, and effect size 0.91) and *A annua* (F=193.16, p=0.001, and effect size 0.88) had significant effect on increasing *VEGF* levels in the hippocampus tissue of rats with AD. ST and *A annua* also had a synergistic effect on increasing *VEGF* levels (F=31.35, p=0.001, and effect size 0.56) (Figure 3-A).

The TMT group exhibited a significant reduction in *eNOS* expression compared to the HC group (p=0.001); however, in the TMT+*A annua* (p=0.001), TMT+ST (p=0.001) and TMT+ST+*A annua* (p=0.001) groups were significantly higher than TMT group. Also, in the TMT+ST group were significantly higher than TMT+*A annua* group (p=0.001); in addition, in the TMT+ST+*A annua* group were significantly higher than TMT+*A annua* (p=0.001) and TMT+ST (p=0.001) groups. The results of two-way analysis of variance showed that ST (F=299.90, p=0.001, and effect size 0.92) and *A annua* (F=110.20, p=0.001, and effect size 0.82) had significant effect on increasing eNOS levels in the hippocampus tissue of rats with AD. However, the interactive effect of ST and *A annua* on *eNOS* changes was not significant (F=1.21, p=0.28, and effect size 0.046) (Figure 3-B).

Analysis of inflammatory markers

NF-κB gene expression levels were significantly higher in the TMT group compared with the HC group (p=0.001). However, *NF-κB* expression was significantly reduced in the TMT+*A annua* (P = 0.001), TMT+ST (P = 0.001), and TMT+ST+*A annua* (p=0.001) groups compared with the TMT group. Moreover, *NF-κB* expression in the TMT+*A annua* group was significantly lower than that in the TMT+ST group (P = 0.04). In addition, the TMT+ST+*A annua* group exhibited significantly lower *NF-κB* expression than both the TMT+*A annua* (p=0.001) and TMT+ST (P = 0.001) groups. Two-way ANOVA revealed that ST (p = 0.001, F = 29.04, effect size = 0.54) and *A annua* (p = 0.001, F = 84.36, effect size = 0.77) exerted significant main effects on reducing *NF-κB* expression in the hippocampal tissue of AD rats. However, the interaction effect between ST and *A annua* on *NF-κB* expression was not statistically significant (p = 0.40, F = 0.71, effect size = 0.029) (Figure 4-A).

TNF-α gene expression were significantly elevated in the TMT group compared with the HC group (p = 0.001). In contrast, TNF-α expression was significantly decreased in the TMT+*A annua* (p = 0.001), TMT+ST (p = 0.001), and TMT+ST+*A annua* (p = 0.001) groups relative to the TMT group. Additionally, *TNF-α* levels in the TMT+*A annua* group were significantly lower than those in the TMT+ST group (P = 0.001). Furthermore, the TMT+ST+*A annua* group showed significantly lower *TNF-α* expression compared with the TMT+ST group (P = 0.001). Statistical analysis demonstrated significant main effects of ST (p = 0.001, F = 30.08, effect size = 0.55) and *A annua* (P = 0.001, F = 131.05, effect size = 0.84) on reducing *TNF-α* expression in the hippocampus of AD rats. Notably, a significant synergistic interaction between ST and *A annua* was observed for TNF-α reduction (p = 0.001, F = 16.25, effect size = 0.40) (Figure 4-B).

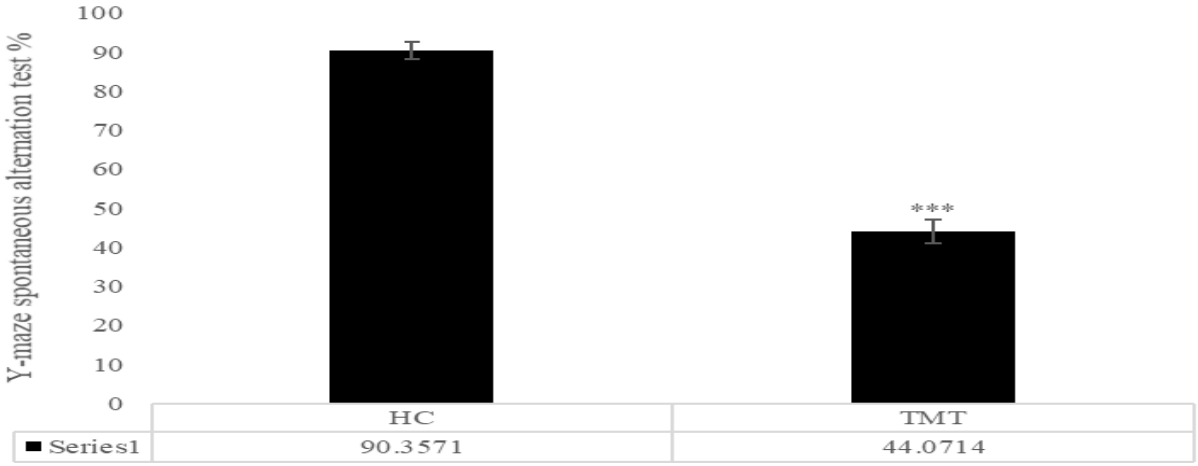

Figure 2. Spontaneous alternation percentage (SAP) in the Y-maze test. *** ($p<0.001$) indicates a statistically significant reduction in SAP in the trimethyltin group (TMT) group compared to the healthy control (HC) group.

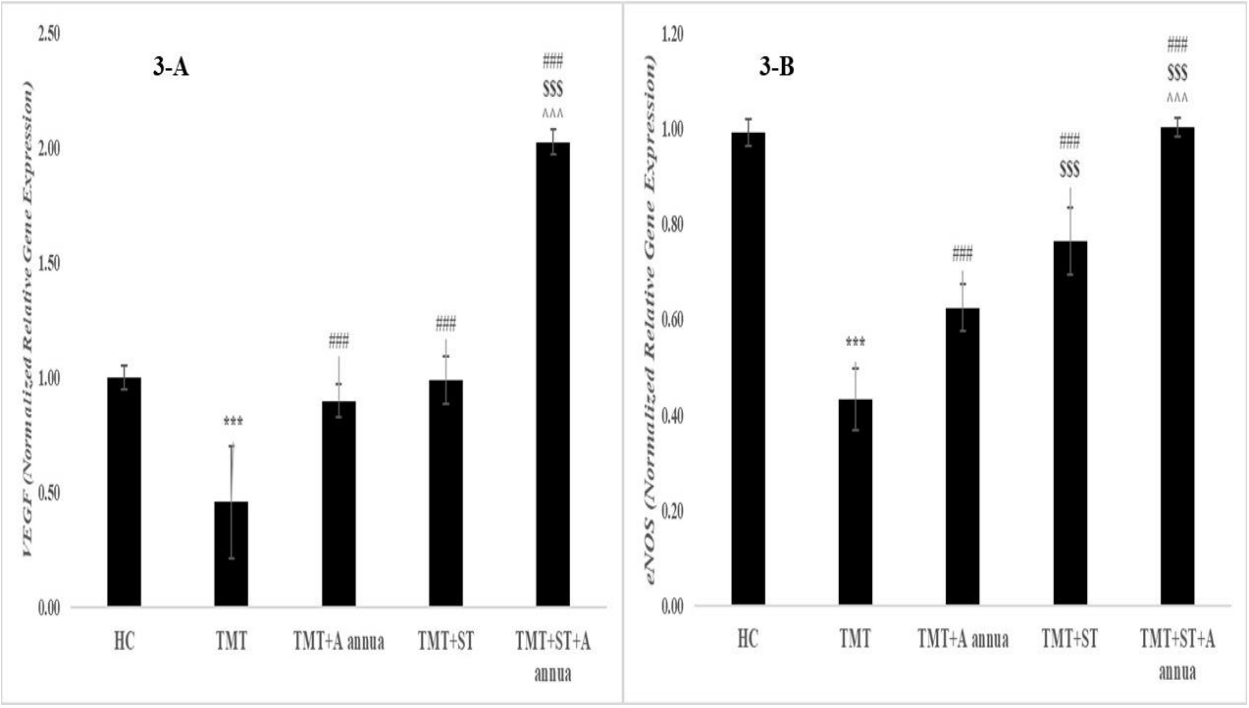

Figure 3. *VEGF* (3-A) and *eNOS* (3-B) gene expression levels in the hippocampus tissue of rats in the study groups. HC: healthy control; TMT: trimethyltin group; TMT+ST: trimethyltin+Swimming training; TMT+A annua: trimethyltin+Artemisia annua; TMT+ST+ A annua: trimethyltin+Swimming training+Artemisia annua.

*** ($p\leq0.001$) significant decrease compared to the HC group; ### ($p\leq0.001$) significant increase compared to the TMT group, $$$ ($p\leq0.001$) significant increase compared to the TMT+A *annua* group, and ^^^ ($p\leq0.001$) significant increase compared to the TMT+ST group.

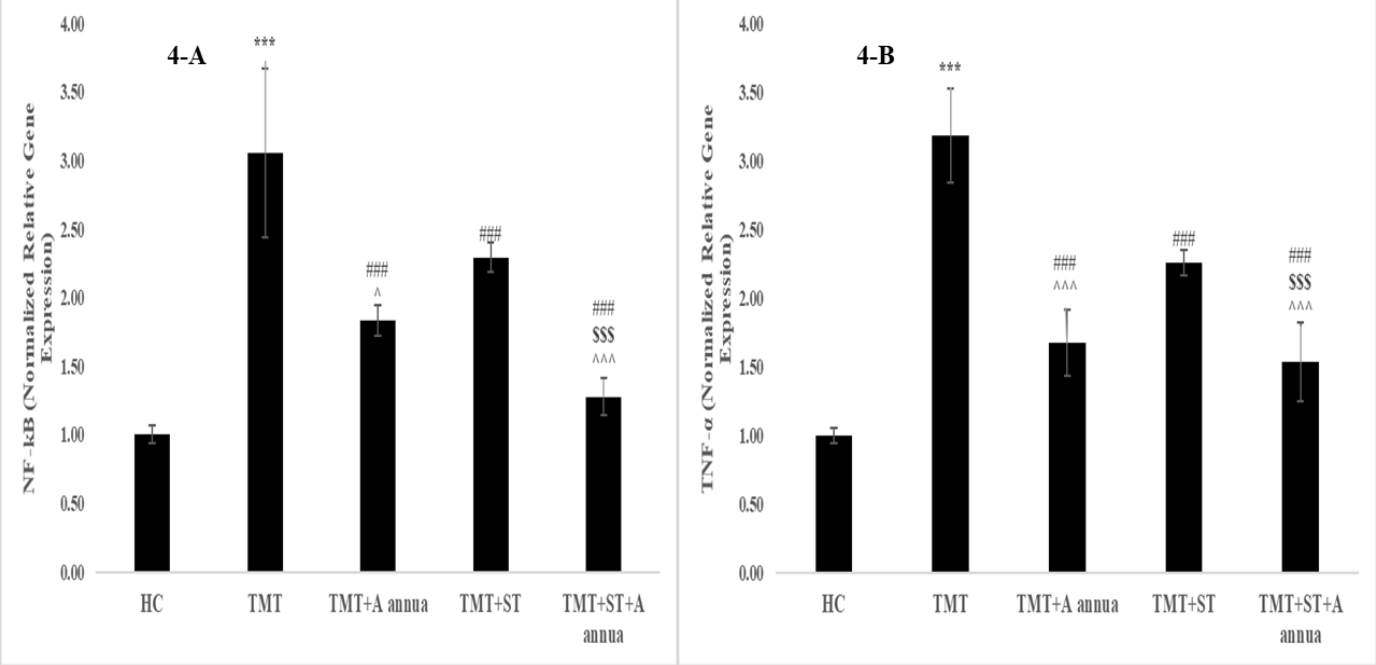

Figure 4. Gene expression levels of NF-kB *(4-A) and* TNF-α *(4-B) in the hippocampus tissue of rats in the research. HC: healthy control; TMT:* trimethyltin group*; TMT+ST:* trimethyltin+*Swimming training; TMT*+A annua*:* trimethyltin+Artemisia annua*; TMT+ST*+ A annua*:* trimethyltin+*Swimming training*+Artemisia annua. groups.*** ($p \leq 0.001$) significant increase compared to the HC group; ### ($p \leq 0.001$) significant decrease compared to the TMT group, $$$ ($P \leq 0.001$) significant decrease compared to the TMT+*A annua* group, ^^^ ($p \leq 0.001$) and ^ ($p \leq 0.05$) significant decrease compared to the TMT+ST group.

Discussion

The results showed that, the TMT group exhibited a significant downregulation of *VEGF* and *eNOS*, alongside elevated levels of *NF-κB* and *TNF-α*, when compared to the HC group**.** In the TMT+ST group, *VEGF* and *eNOS* expression levels were significantly upregulated, whereas *NF-κB* and *TNF-α* concentrations were markedly attenuated relative to the TMT group. These findings reflect a shift toward an angiogenically impaired and pro-inflammatory neurovascular profile following TMT exposure, whereas swimming training appears to partially reverse these pathological alterations. As mentioned earlier, increased oxidative stress and Aβ accumulation activate inflammatory signaling cascades. Accumulation of amyloid-β (Aβ) has been shown to activate the mitogen-activated protein kinase (MAPK) signaling cascade, which in turn promotes NF-κB transcriptional activity. This transcription factor plays a central role in modulating the expression of pro-inflammatory cytokines, including TNF-α, IL-1β, and other downstream mediators. Activation of this pathway simultaneously inhibits eNOS synthesis, resulting in vascular fibrosis and reduced vascular elasticity. Following the increase in TNF-α, angiogenic factors such as VEGF-VEGFR and FGF2 are suppressed, leading to impaired angiogenesis (Tsartsalis et al. 2024). Collectively, these mechanisms describe a convergent inflammatory–vascular dysfunction axis underlying TMT-induced neurodegeneration.

Conversely, evidence indicates that exercise exerts neuromodulatory effects partly through the upregulation of muscle-derived myokines—including BDNF, irisin, cathepsin B, clusterin, and glycosylphosphatidylinositol specific phospholipase D1 (GPLD1) which collectively contribute to neuroprotection, synaptic plasticity, and cognitive resilience.

The elevation of these myokines activates the PI3K/Akt pathway in neurons, enhancing their resistance to oxidative stress. In addition, exercise increases cerebral blood flow by increasing vasodilation, and this occurs by reducing lipopolysaccharides and TLR4, and as a result, NF-Kb and TNF-α are inhibited (Hu, Huang, and Chen 2024). In line with this, prior research has shown that three months of moderate-intensity exercise can reduce IL-1β, IL-6, p-tau181 protein, and TNF-α levels, thereby contributing to enhanced cognitive function in individuals diagnosed with mild cognitive impairment (Katsipis et al. 2024). In a study by Pahlavani *et al*., high-intensity endurance and interval training performed at 50–75% VO_2max was shown to elevate neurotrophin levels, suppress inflammatory mediators—including NF-κB, IL-1β, TNF-α, and CRP—and promote mitochondrial biogenesis in individuals with AD (Pahlavani 2023).

The results showed that in the TMT+*A annua* group, *VEGF* and *eNOS* levels were significantly elevated, while *NF-κB* and *TNF-α* levels were significantly reduced compared to the TMT group. It is believed that artemisinin, the main active component of *A annua*, is primarily responsible for the plant's biological and anti-inflammatory effects. In other words, this isoflavone can potentially neutralize reactive oxygen species (ROS), thereby inhibiting TLR4 and myeloid differentiation factor 88, leading to reduced levels of NF-κB, TNF-α, cyclooxygenase, and prostaglandin E2 (de Faveri Favero et al. 2024). Additionally, in the study by Kim et al., increased expression of Nrf2/Kelch-like ECH-associated protein 1 (Keap1), antioxidants, and Sirt1/2, along with decreased intravascular adhesion molecules, was observed in a neurodegenerative animal model (Kim et al. 2023). Another study, researchers showed that *A annua* increased ERK/cAMP response element-binding protein (CREB) signaling as well as decreased caspases, oxidative stress, and neuronal apoptosis in an animal model of neurodegenerative disorder (Zhao et al. 2020). In another study, researchers stated that *A annua* can directly lead to ROS scavenging, caspase 3 reduction, Aβ1–42 reduction, YAP signaling inhibition, and neuronal apoptosis in a neurodegenerative animal model (Zhao et al. 2022). In a review study, researchers also noted that *A annua* exerts antioxidant and anti-apoptotic effects in neurodegenerative, activation of ERK/CREB/B-cell lymphoma 2 (Bcl-2) and NRF2 signaling pathways (Sailike et al. 2022). Despite these findings, limited research has explored the angiogenic mechanisms of *A annua*, making the investigation of this pathway one of the novel aspects of the present study.

The results also showed that in the TMT+ST+*A annua* group, *VEGF* and *eNOS* levels were significantly higher, and *NF-κB* and *TNF-α* levels were significantly lower compared to the TMT group. Notably, *VEGF* and *eNOS* expression in the TMT+ST+*A annua* group exceeded that of the healthy control group. This pattern suggests a robust transcriptional activation of angiogenic signaling alongside a pronounced suppression of inflammatory mediators following combined intervention. Such supra-physiological increases in mRNA levels are plausible in qRT-PCR studies and indicate a potential enhancement of angiogenic signaling beyond baseline, although actual protein levels may differ. With respect to the synergistic impact of exercise and *A annua* supplementation, the only available evidence indicates that a four-week intervention combining physical training with A annua administration significantly upregulated mitochondrial biogenesis (D Allerton et al. 2021). Exercise-related adaptations are generally associated with neurotrophic and metabolic reprogramming, involving downstream activation of survival-related signaling networks and redox-sensitive regulatory pathways (Hu et al. 2024). Meanwhile, *A annua* exerts its anti-inflammatory effects

by antioxidants and anti-inflammatory can improve brain function (de Faveri Favero et al. 2024). *A annua* also induces upregulating Nrf2/Keap1, enhancing Sirt1/2 expression (Kim et al. 2023), while promoting ERK/CREB signaling and reducing caspase activity and oxidative stress (Zhao et al. 2020). Activation of ERK/CREB/Bcl-2 and NRF2 signaling contributes to its antioxidant and anti-apoptotic effects (Sailike et al. 2022).

Collectively, these complementary biological effects may converge to enhance angiogenic signaling while attenuating inflammatory responses, thereby supporting neuronal survival through partially overlapping but distinct molecular pathways. Moreover, eNOS expression was significantly higher in the TMT+ST group compared to the TMT+*A annua* group, indicating a more pronounced angiogenic response following exercise intervention. This differential response may be attributed to exercise-induced vascular shear stress and redox adaptation, which promote nitric oxide synthase activity and vascular responsiveness, ultimately facilitating VEGF upregulation (Huang and Nan 2019). In contrast, NF-κB and TNF-α gene expression levels in the TMT+*A annua* group were significantly lower than in the TMT+ST group.

Mechanistically, A annua may interfere with upstream inflammatory signaling cascades, including MAPK/JNK-associated pathways, thereby suppressing TLR4-mediated activation of NF-κB and downstream pro-inflammatory mediators (Wang et al. 2024). The comparative analysis revealed that exercise was more effective in enhancing eNOS levels, while *A annua* showed superior anti-inflammatory potential by reducing NF-κB and TNF-α. This indicates that the two interventions act through distinct nodes within their respective signaling pathways. Considering the pivotal role of oxidative stress and antioxidant defense systems in modulating inflammatory and apoptosis-related signaling cascades, the absence of direct assessment of these molecular pathways constitutes a notable limitation of the present study**.** Future research should therefore include assessments of oxidant-antioxidant signaling. Moreover, given the relevance of clinical validation and cognitive enhancement, a further limitation of the present study is the lack of direct assessment of cognitive function across the experimental groups. Future studies are recommended to incorporate cognitive performance evaluations to strengthen translational relevance.

According to the results, ST, *A annua*, and their combination appear to exert anti-inflammatory and angiogenic effects. However, the more pronounced anti-inflammatory effect of *A annua* and the greater angiogenic effect of ST is a notable finding. Therefore, the simultaneous use of these two interventions may be advantageous in conditions associated with neurodegenerative disorders.

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Conflicts of interest

The authors declare that they have no competing interests

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