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Combating cognitive decline in a rat model of Alzheimer's disease: The role of high-intensity interval training and cannabidiol administration in modulating microRNA-124, BACE1, and nestin

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Keywords
Dementia; High-Intensity Interval Training; Cannabis; Cognitive Decline; Nestin

Abstract

Background: The global prevalence of Alzheimer's disease (AD) has emerged as a paramount concern due to the aging population. The current research examines the effects of six-week high-intensity interval training (HIIT), with and without cannabidiol (CBD) supplementation, on cognitive decline, micro ribonucleic acid (miRNA)-124 expression levels, and the protein expression of beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) and nestin in the hippocampus of rats with amyloid beta (Aβ)-induced AD.

Methods: 30 male Wistar rats were randomly divided into six groups: control (CNT), sham, Alzheimer (AD), Alzheimer + HIIT (H-AD), Alzheimer + CBD (C-AD), and Alzheimer + HIIT + CBD (CH-AD). Following the hippocampal injection of Aβ1-42, the HIIT protocol and CBD supplementation [20 mg/kg/day, per os (P.O.)] were initiated and conducted over six weeks. After the last intervention, the hippocampus tissues were collected to assess miRNA-124 expression levels and the protein expression of BACE1 and nestin.

Results: HIIT alone and in combination with CBD treatment significantly improved cognitive impairment induced by AD.

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Moreover, both treatments significantly reduced BACE1 protein expression and increased nestin protein expression in AD rats. However, despite decreased miRNA-124 expression levels in the AD group in comparison with the CNT group, HIIT and CBD administration did not alter miRNA-124 expression levels in other groups.

Conclusion: The findings can contribute to a higher understanding of the beneficial effects of HIIT combined with CBD administration in mitigating AD-induced cognitive impairment by reducing BACE1 protein expression and increasing nestin protein expression.

Introduction

Alzheimer's disease (AD) is considered a neurodegenerative condition associated with cognitive impairment caused by neuronal death and brain atrophy. AD is a progressive, age-related neurodegenerative disorder characterized by synaptic dysfunction and neuronal degeneration, which underlie its hallmark cognitive decline.[1,2] In recent years, researchers have indicated that the formation of amyloid beta (Aβ) plaque and tau protein deposition are the primary pathways in the development of AD.[3,4] Aβ contributes to extracellular calcium-dependent toxicity by generating reactive nitrogen species (RNS) and reactive oxygen species (ROS), which aggravate cellular damage, leading to impaired cellular respiration and changes in the formation of synapses associated with learning and memory.[5] In addition, micro ribonucleic acids (miRNAs) are a class of non-coding ribonucleic acids (RNAs) that can suppress or reduce messenger RNA (mRNA) translation.[6] It has been demonstrated that miRNAs play a fundamental role in the pathogenesis of AD.[7] Lagos-Quintana et al. discovered miRNA-124 in mice almost 20 years ago and revealed that miRNA-124 was a significant portion of the total miRNAs in the brain and was expressed primarily in neurons.[8] Previous studies have reported that miRNA-124 has a significant role in the pathogenesis of AD. In patients with AD, miRNA-124 acts as a potent inhibitor of beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), which might make a key contribution to the development of AD.[9] BACE1 has been among the primary therapeutic targets for AD and plays a critical role in regulating Aβ production. BACE1 can initiate Aβ formation and cause neuronal loss and memory deficits.[10] On the other hand, it has been established that Aβ production is suppressed in BACE1 knockout mice.[11] Furthermore, nestin, a neuroepithelial stem cell protein, is categorized as an intermediate filament cellular protein initially described as expressed in growing and adult brain neural stem cells (NSCs).[12] Nestin, an NSC marker, has been shown to reduce expression in AD and plays a significant role in neurogenesis and neurodegeneration. Typically, there is a significant level of nestin in NSCs and progenitor cells, contributing to the regeneration and repair mechanisms within the brain. However, in chronic AD, there is often a downregulation of nestin expression, which correlates with diminished neurogenesis and impaired neural repair mechanisms.[13,14]

Recently, evidence from several experimental studies has identified various therapeutic tools to alleviate AD. Among these tools, exercise training has emerged as a promising non-pharmacological approach for AD management.[15-17] Among various training methods, high-intensity interval training (HIIT) has gained popularity among researchers. HIIT has been found to be more efficacious in promoting aerobic fitness and maximal oxygen consumption than continuous training.[18] Additionally, the primary reason why many people nowadays avoid exercise training is a lack of enough time for exercise. Compared to moderate-intensity continuous training (MICT), HIIT effectively stimulates physiological adaptations despite having a lower overall training volume and shorter duration. Therefore, HIIT is not only more time-efficient than MICT for patient populations, but also may improve exercise compliance through enhanced enjoyment.[19] Moreover, HIIT may have a pivotal role in combating AD via modulating brain-derived neurotrophic factor (BDNF), oxidative stress, inflammation, and decreasing Aβ plaques.[20-22] Besides HIIT, it has been established that cannabidiol (CBD), one of the cannabinoids in cannabis plants, can be beneficial in AD treatment.[23] Despite lacking clinical approval, CBD has emerged as a powerful agent to reduce neurotoxicity induced by Aβ and has been indicated to have neuroprotective, anti-oxidative, and anti-inflammatory effects against AD.[23,24] Furthermore, CBD has been reported to reduce tau hyperphosphorylation, neurodegeneration, and Aβ accumulation.[25-28]

To date, there has been a notable paucity of studies describing the impact of HIIT and

CBD administration on cognitive impairment and AD induced by Aβ, focusing specifically on miRNA-124 expression levels, BACE1, and nestin protein expression.

Accordingly, this study tests the hypothesis that combined HIIT and CBD supplementation attenuates AD-related cognitive impairment in a rat model through regulation of miRNA-124, BACE1, and nestin expression.

Materials and Methods

Animals and ethical statement: Thirty adult male Wistar rats (10 weeks old, 220-280 g) were used in this study. Rats were randomly assigned to groups, and the researchers collecting data were blinded to the group assignments. They were housed in pairs (3 per cage) under standard conditions: 12-hour light/dark cycle, 22-25 °C, 40%-60% humidity, with free access to food and water. The sample size was chosen based on similar previous studies to ensure reliable results. The health and well-being of the rats were checked daily. All procedures followed the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals and fully adhered to ARRIVE 2.0 guidelines. The study was approved by the Ethics Committee of University of Tehran, Tehran, Iran (approval number: IR.SSRC.REC.1399.139).

Study design: After two weeks of acclimatization to a new environment and an animal treadmill, the animals (n = 5 per group) were randomly assigned to six groups:

1. ***Control (CNT):*** This group received no intervention.
2. ***Sham operation (Sham):*** This group was administered dimethyl sulfoxide (DMSO) ($A\beta_{1-42}$ vehicle) via stereotaxic hippocampal injection and received sesame oil (CBD vehicle) orally.
3. ***Alzheimer (AD):*** This group received an $A\beta_{1-42}$ injection (intrahippocampal) and subsequently was administered sesame oil (CBD solvent) as the vehicle used for CBD.
4. ***Alzheimer + HIIT (H-AD):*** This group received an $A\beta_{1-42}$ injection (intrahippocampal), was administered sesame oil (CBD solvent) as the vehicle used for CBD, and was subjected to HIIT.
5. ***Alzheimer + CBD (C-AD):*** This group received an $A\beta_{1-42}$ injection (intrahippocampal) and was administered CBD dissolved in sesame oil at the dosage of 20 mg/kg/day.[29,30]
6. ***Alzheimer + HIIT + CBD (CH-AD):*** This group received an $A\beta_{1-42}$ injection (intrahippocampal), was administered CBD (20 mg/kg/day) dissolved in sesame oil,[29,30] and was subjected to HIIT.

Following the two-week acclimatization phase, the interventions were performed over six weeks.

AD induction in rats: In this study, to induce AD, initially, the rats were anesthetized by an intraperitoneal injection of ketamine and xylazine (100 mg/kg and 10 mg/kg, respectively). Following anesthesia, the rats' heads were placed in a stereotaxic apparatus to facilitate surgical procedures. Afterward, according to the Paxinos atlas, the injection site was localized 3.8 mm posterior to bregma, 2.2 mm lateral, and 2.7 mm ventral to the brain surface,[31] and the desired location was marked. $A\beta_{1-42}$ was bilaterally injected (2.5 µl per side) into the hippocampal Cornu Ammonis-1 (CA1) region using a Hamilton microsyringe. For each microliter of injection, a duration of 60 seconds was considered. Moreover, the same surgical procedure (as in the AD group) was carried out on the rats in the sham group receiving vehicle (DMSO). To prepare $A\beta_{1-42}$, initially, the $A\beta_{1-42}$ peptide (Sigma-Aldrich, USA; Catalog No. SCP0038) was prepared at 5 µg/µl in a 3% DMSO buffer solution. Afterward, in order to allow Aβ to fibrillate, it was incubated at 37 °C for seven days.[32]

HIIT protocol: A week following the $A\beta_{1-42}$ injection, the rats of the training groups were familiarized with a motorized animal treadmill (Tajhiz Gostare Omide Iranian, Iran) by walking on the treadmill at a speed of 5-10 m/min for 5-10 minutes for five days. Afterward, to adjust the training load, the rats' maximum volume of oxygen (VO_{2max}) was assessed according to the relationship between treadmill speed and VO_{2max}, as previously described.[33,34] Each training session consisted of a six-minute warm-up at 50%-60% VO_{2max}, three intervals of 4 minutes at 90%-100% VO_{2max}, separated by 2 minutes at 50%-60% VO_{2max}, and a six-minute cool-down at 50%-60% VO_{2max}.[35] The running speed was gradually increased over six weeks of training based on the animals' capacity (Table 1).

Morris Water Maze (MWM): 48 hours following the final intervention, the MWM was performed. The MWM apparatus consisted of a circular black pool with dimensions of 150 cm in diameter and 50 cm in wall height. The tank was filled with water at a temperature of 25 ± 2 °C. The circular tank was divided into four equal quadrants (designated as first, second, third, and fourth) for spatial navigation analysis.

Table 1. Schema of high-intensity interval training (HIIT) protocol

	Warm-up	High-intensity interval	Recovery interval	Cool-down
Time (minute)	1 × 6	3 × 4	3 × 2	1 × 6
Intensity (VO_{2max}) (%)	50 to 60	90 to 100	50 to 60	50 to 60
Week 1 (m/min)	15	34	15	15
Week 2 (m/min)	18	37	18	18
Week 3 (m/min)	21	40	21	21
Week 4 (m/min)	24	43	24	24
Week 5 (m/min)	27	47	27	27
Week 6 (m/min)	30	50	30	30

VO_{2max}: Maximum volume of oxygen

The hidden platform, measuring 11 cm in diameter and 30 cm in height, was positioned in the middle of the fourth quadrant, with its bottom surface 1 cm below the water level. Animal behavior and maze parameters were recorded using a video camera interfaced with Noldus EthoVision XT video tracking software. The testing protocol comprised two distinct phases:

- ***Acquisition phase:*** The rats were put in the pool and given the opportunity to swim and explore for four trials each day over a period of four days in order to locate the concealed escape platform. The animals were given a duration of 60 seconds to explore the tank. If they were unable to locate the escape platform within this timeframe, the researcher directed them toward the escape/hiding platform, and they stayed on it for 20 seconds. The distance traveled to reach the platform was recorded as an assessment of the learning process. This measurement was obtained by averaging the results of four trials.
- ***Probe phase:*** On day 5, animals underwent a probe trial in which the escape platform was removed from the pool to assess spatial memory retention. The animals were permitted to swim in the tank for a duration of 1 minute, and time spent in the target quadrant was recorded.[36]

It is vital to note that the distance traveled to reach the platform in the acquisition trial and time spent in the target quadrant in the probe trial were measured to assess cognitive function.

Real-time polymerase chain reaction (RT-PCR): The expression levels of miRNA-124 was assessed using an RT-PCR quantitative polymerase chain reaction (qPCR). Firstly, we extracted 1 µg of RNA using the RNeasy Mini Kit from Qiagen Company, Iran, in combination with Trizol from Kiazist Company (Iran). The extracted RNA was then converted into complementary deoxyribonucleic acid (cDNA) using the Easy cDNA Synthesis Kit from Parstous (Iran). Afterward, qPCR analysis was conducted using the SYBR Green Master Mix from AddBio on an Applied Biosystems (ABI) StepOne qPCR thermocycler system.

After five minutes of the denaturation step at 95 °C, the polymerase chain reaction (PCR) process started with 40 amplification cycles. Each cycle comprised denaturation at 95 °C for 15 seconds, annealing at 60 °C for 15-20 seconds, and extension at 72 °C for 15-30 seconds. A melting curve analysis was performed by heating at 95 °C for 15 seconds and then at 65 °C for one minute. The qPCR procedure concluded with a cooling phase at 30 °C for 20 seconds. The miRNA-124 expression level was normalized against the U6 housekeeping gene. The specific primer sequences for miRNA-124 used in this study were miRNA-124 forward: TGCTTCGGCAGCACATATAC, and miRNA-124 reverse: AGGGGCCATGCTAATCTTCT. The normalized expression level was ultimately calculated using the $2^{-\Delta CT}$ method.[37,38]

Western blot analysis: Hippocampal tissues were immediately flash-frozen in liquid nitrogen post-collection. Samples were homogenized in ice-cold radioimmunoprecipitation assay (RIPA) lysis buffer (CytoMatinGene, CMGRIP50, Iran) supplemented with protease/phosphatase inhibitors, then centrifuged (12000 rpm, 20 minutes, 4 °C) to obtain total protein extracts from the supernatant. Protein aliquots (20-30 µg) were mixed with Laemmli buffer, heat-denatured (100 °C, 5 minutes), and resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoresis was performed at 100 V for 1-2 hours until the dye front reached the gel bottom.

Proteins were transferred to nitrocellulose membranes using a semi-dry system, followed by blocking with 5% skim milk/tris-buffered saline with Tween-20 (TBST) (0.1% Tween-20; Sigma-Aldrich, USA) for 1 hour at real-time (RT). Membranes were incubated overnight at 4 °C with primary antibodies [diluted in 1% bovine serum albumin (BSA)/TBST] under gentle agitation. After three TBST washes, horseradish peroxidase

(HRP)-conjugated secondary antibodies were applied (2 hours, RT). Protein bands were visualized using enhanced chemiluminescence (ECL) substrate, captured on X-ray film, and quantified via ImageJ. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the loading control for normalization.

A one-way analysis of variance (ANOVA) statistical method followed by Tukey's multiple comparison tests was utilized to analyze the data. Normality of data was assessed using the Shapiro-Wilk test, and homogeneity of variances was examined with the Brown-Forsythe test; except for RT-PCR (miRNA-124), all other datasets met normality assumptions. Because the data obtained from RT-PCR did not pass the normality test, a Kruskal-Wallis test was used to examine miRNA-124 expression levels. Moreover, a two-way repeated measures ANOVA was used to evaluate the distance to reach the platform in the acquisition phase of MWM. $P < 0.05$ was considered statistically significant. Furthermore, all data were reported as the mean ± standard error of the mean (SEM), and all statistical analyses were performed using the GraphPad Prism 9 software.

Results

All behavioral tests, western blot analysis, and RT-PCR revealed that no statistically significant differences between the control and Sham groups were evident. Therefore, to simplify data interpretation in this study, we merely compared all groups to the CNT group, and the Sham group was eliminated from the analysis.

Evaluation of spatial learning and reference memory in the MWM

The distance traveled to reach the platform was assessed to evaluate memory acquisition. As shown in figure 1A, the findings of the two-way repeated measures ANOVA revealed that there was no significant difference in the distance traveled to reach the platform between the experimental groups [treatment factor ($F_{5,12}$ = 2.348, P = 0.105) and time factor ($F_{1.973,23.68}$ = 5.391, P = 0.012)], except for the C-AD group from day 1 to day 2 (P = 0.035). There was no significant difference in the distance traveled to reach the platform in any of the groups from day 1 to day 4 (CNT: P = 0.549, -36.02%; AD: P = 0.934, -17.61%; C-AD: P = 0.266, -37.63%; H-AD: P = 0.540, -22.98%; CH-AD: P = 0.496, -43.76%).

Furthermore, to evaluate reference memory at the end of the interventions, a probe trial was conducted on day five; during this phase, the hidden platform was eliminated, and the time spent in the target quadrant was recorded (Figure 1B). The findings of the one-way ANOVA illustrated significant differences among different groups ($F_{5, 12}$ = 17.85, P < 0.0001).

In summary, the results indicated a significant reduction in the time spent in the target quadrant in the AD group in comparison with the CNT group (P < 0.001). As expected, the H-AD and CH-AD groups revealed a significant increase in the time spent in the target quadrant in comparison with the AD group (P = 0.011 and P = 0.003, respectively).

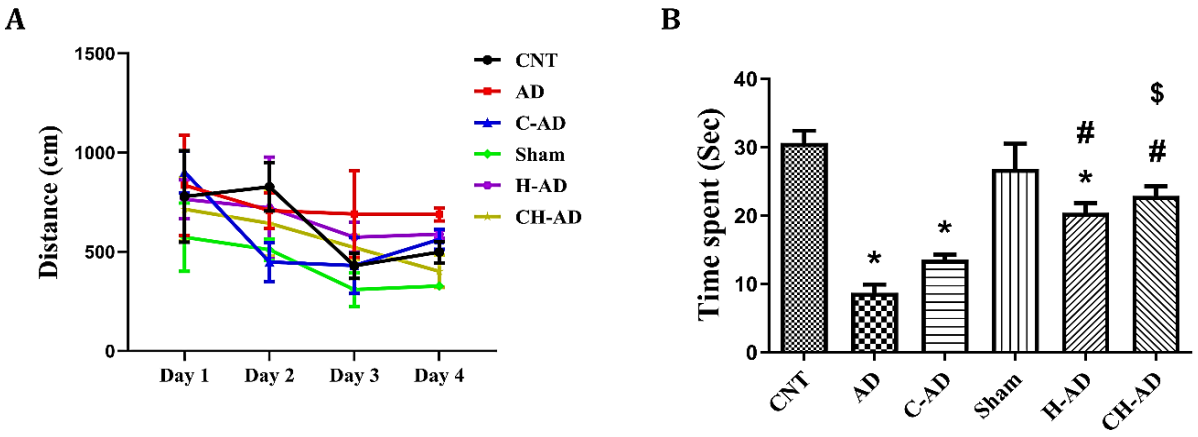

Figure 1. Spatial learning and memory deficit in the Morris Water Maze (MWM) test in rats after treatments (n = 3 per group): A) The distance traveled to reach the platform in the acquisition trial; B) The spending time in the target quadrant in the probe trial

Data are presented as mean ± standard error of the mean (SEM). $^{*}P < 0.05$ vs. the CNT group; $^{\#}P < 0.05$ vs. the AD group; $^{\$}P < 0.05$ vs. the C-AD group

CNT: Control group; AD: Alzheimer's group; H-AD: Alzheimer's + high-intensity interval training (HIIT) group; C-AD: Alzheimer's + cannabidiol (CBD); CH-AD: Alzheimer's + HIIT + CBD

The C-AD group showed no significant difference compared to the AD and H-AD groups (P = 0.523 and P = 0.195, respectively). Furthermore, the CH-AD group significantly enhanced the time spent in the target quadrant compared to the C-AD group (P = 0.046), but no statistically significant difference was detected compared to the H-AD group (P = 0.941). What is interesting about the data in figure 1B is that the CH-AD group demonstrated no significant differences in the time spent in the target quadrant compared to the CNT group (P = 0.120).

Assessment of miRNA-124 expression levels

RT-PCR was utilized to assess the expression levels of miRNA-124. The results of the Shapiro-Wilk test showed that the data obtained from RT-PCR did not pass the normality test. Therefore, the Kruskal-Wallis test was employed to analyze miRNA-124 expression levels. The results demonstrated that the AD group significantly reduced miRNA-124 expression levels compared to the CNT group (P = 0.034). However, the other groups did not reveal any significant differences (Figure 2).

Measurement of BACE1 and nestin protein expression

Concerning BACE1 protein expression, the results of the one-way ANOVA demonstrated significant differences among different experimental groups ($F_{5,12}$ = 266.7, P < 0.001).

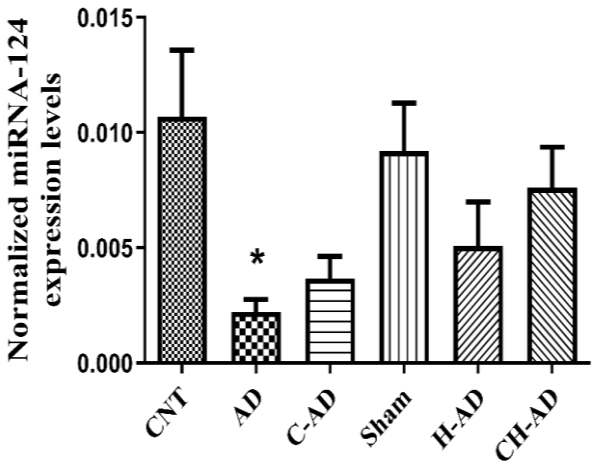

Figure 2. Micro ribonucleic acid (miRNA)-124 expression levels in the experimental groups (n = 5/group)

Data are reported as mean ± standard error of the mean (SEM). *P < 0.05 vs. the CNT group

CNT: Control group; AD: Alzheimer's group; H-AD: Alzheimer's + high-intensity interval training (HIIT) group; C-AD: Alzheimer's + cannabidiol (CBD); CH-AD: Alzheimer's + HIIT + CBD

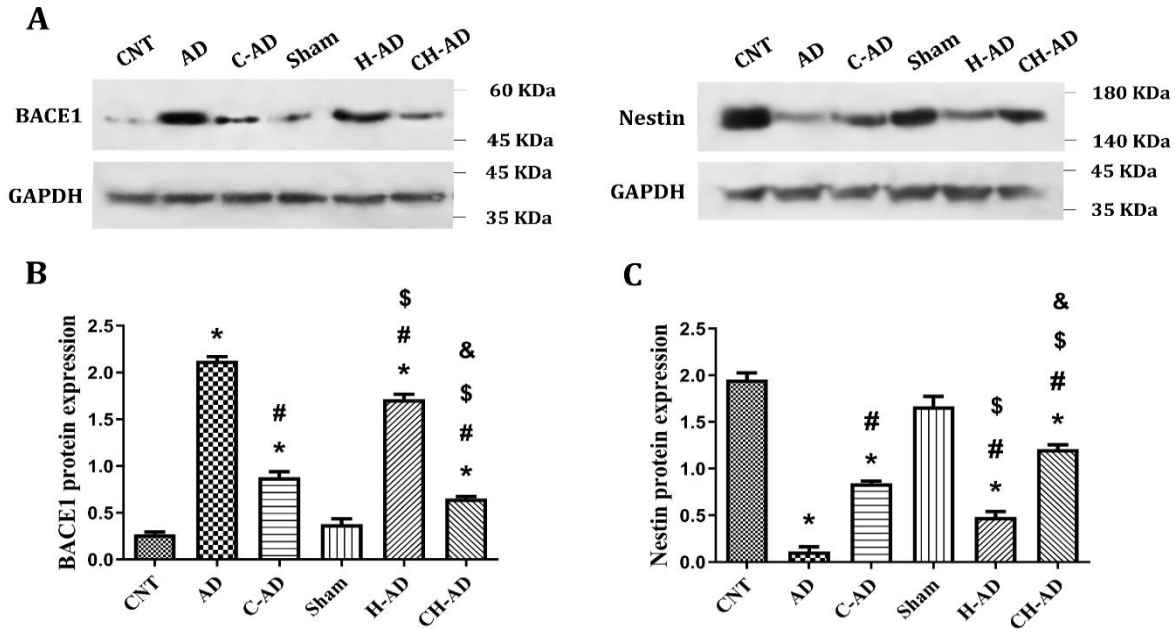

Figure 3. The results of western blot analysis (n = 3/group): A) Representative western blot; B) Beta-site amyloid precursor protein cleaving enzyme 1 (BACE1)/glyceraldehyde-3-phosphate dehydrogenase (GAPDH); C) Nestin/GAPDH

Data are presented as mean ± standard error of the mean (SEM). *P < 0.05 vs. the CNT group; #P < 0.05 vs. the AD group; $P < 0.05 vs. the C-AD group; &P < 0.05 vs. the H-AD group

BACE1: Beta-site amyloid precursor protein cleaving enzyme 1; CNT: Control group; AD: Alzheimer's group; H-AD: Alzheimer's + high-intensity interval training (HIIT) group; C-AD: Alzheimer's + cannabidiol (CBD); CH-AD: Alzheimer's + HIIT + CBD

As indicated in figure 3, western blot analysis revealed that, in comparison with the CNT group, the AD group exhibited a significant increase in BACE1 protein expression ($P < 0.001$).

As expected, the C-AD, H-AD, and CH-AD groups significantly reduced the expression of BACE1 compared to the AD group ($P < 0.001$, $P = 0.005$, and $P < 0.001$, respectively). Furthermore, the H-AD group illustrated significantly higher BACE1 protein expression compared to the C-AD group ($P < 0.001$). However, the combination of HIIT and CBD (CH-AD group) yielded additional benefits compared to the C-AD and H-AD groups ($P = 0.0394$ and $P < 0.001$, respectively).

Regarding the nestin protein expression, the one-way ANOVA results displayed a significant difference among the study groups ($F_{5,12} = 122.2$, $P < 0.001$). The results, as shown in figure 3, demonstrated a significant reduction in nestin protein expression in the AD group compared to CNT group ($P < 0.001$). As expected, the rats in the C-AD, H-AD, and CH-AD groups significantly decreased nestin protein expression in comparison with the AD group ($P < 0.001$, $P = 0.014$, and $P < 0.001$, respectively). The C-AD group caused a significant increase in the expression of nestin compared to the H-AD group ($P = 0.016$). Moreover, the CH-AD group exhibited increased expression of nestin compared to the C-AD and H-AD groups ($P < 0.050$ and $P < 0.001$, respectively).

Discussion

The main objective of the current research was to uncover the promising potential of HIIT and CBD supplementation for reducing cognitive impairment caused by AD through the regulation of miRNA-124, BACE1, and nestin expression. Our research revealed that both HIIT and CBD showed potential to mitigate cognitive decline, as evidenced by improved performance in the probe trial of the MWM test, though acquisition phase results were less consistent across groups. Moreover, HIIT and CBD administration demonstrated the ability to attenuate cognitive impairment by reducing the protein expression of BACE1 and increasing nestin protein expression without affecting miRNA-124 expression levels. The most significant finding was that HIIT combined with CBD produced additional benefits.

Firstly, the cognitive improvement observed following HIIT, particularly in the MWM probe trial, suggests this intervention may enhance certain aspects of neuroplasticity and cognitive function in AD models. These data are in line with recent studies confirming that exercise training can enhance brain health by promoting neurogenesis, synaptic plasticity, and vascularization in the hippocampus, a critical region for memory and learning that is severely affected in AD.[39-41] HIIT has been confirmed to play a vital role in behavioral improvement through increasing antioxidant capacity.[42] Furthermore, previous research has suggested that engaging in 8-minute HIIT daily over a period of 8 weeks can potentially alleviate cognitive deficits. This improvement, which is in line with our findings, is likely due to the promotion of a positive redox balance and enhanced activity of the BDNF/tyrosine kinase B (TrkB) pathway, which may contribute to the reduction of neuroplasticity.[21] However, previous studies by Hajizade Ghonsulakandi et al.[43] and Kodali et al.[44] reported that moderate-intensity exercise training failed to produce significant improvements in AD-related cognitive deficits. This inconsistency may be due to inadequate training intensity, which plays a key role in training adaptation. Regarding the CBD effect, our findings reflect those of Chen et al., who also found that CBD made a crucial contribution to mitigating memory and cognitive impairments induced by Aβ in an animal model of AD.[45] Our results align with the findings of Amini and Abdolmaleki, who reported that CBD administration significantly enhanced spatial memory and attenuated cognitive impairment in an AD rodent model.[46] Eventually, The greater improvement in retention compared to acquisition may reflect a stronger influence of HIIT on neurogenesis-related processes, as indicated by increased nestin expression, rather than on mechanisms underlying initial learning.

The decrease in the protein expression of BACE1 after HIIT and CBD administration is particularly intriguing. BACE1 is a crucial enzyme involved in the formation of Aβ peptides, which accumulate to form plaques, a hallmark of AD pathogenesis.[4] The reduced expression of BACE1 indicates a possible mechanism by which HIIT and CBD might alleviate the amyloidogenic pathway, leading to reducing Aβ plaque formation and its related neurotoxic effects. These results highlight the therapeutic potential of targeting BACE1 in the management of AD. Consistent with Alkadhi and Dao[47] report, our data demonstrate that MICT significantly reduces hippocampal BACE1 expression in Aβ-induced AD rats. Accordingly,

the reduced levels of BACE1 resulting from MICT can suppress Aβ formation, thereby alleviating neuronal loss and memory deficits induced by AD. This finding was also reported by Xia et al., suggesting that moderate-intensity treadmill exercise could decrease Aβ accumulation in amyloid precursor protein (APP)/presenilin 1 (PS1) transgenic mice by modulating protein kinase RNA-like endoplasmic reticulum kinase (PERK)/eukaryotic initiation factor 2-alpha (eIF2α) signaling, reducing BACE1 and PS1 expression, and potentially offering a preventive and therapeutic strategy for AD.[48] Although several studies have confirmed the efficacy of MICT in reducing BACE1 in the hippocampus of animals suffering from AD, the effect of HIIT on BACE1 protein expression is still unknown. With respect to CBD's impact on BACE1 protein expression, few in vivo studies have been carried out. However, in vitro studies have established that the gene expression of BACE1 was downregulated after CBD treatment, leading to reduced Aβ deposition.[49] Thus, our findings and the results of recent research further support the idea that both HIIT and CBD administration may reduce AD-induced cognitive impairment by decreasing BACE1 protein expression. Additionally, we found that the combined HIIT and CBD intervention demonstrated enhanced efficacy in suppressing BACE1 expression compared to either treatment alone.

Furthermore, our data indicated that the HIIT and HIIT combined with CBD administration had higher protein expression of nestin, indicating increased neurogenesis. Nestin is essential for regenerating and repairing neural tissue, as it is a hallmark of NSCs.[12] The enhanced expression of nestin implies that both HIIT and CBD treatments promote the proliferation and differentiation of neural progenitor cells, which helps to restore the integrity and function of the hippocampus. Our results are consistent with the increasing amount of research that supports the significance of exercise training and CBD in promoting neurogenesis and neural repair.[50-52] While existing evidence has confirmed the effectiveness of exercise training and CBD in enhancing neurogenesis through upregulating nestin protein expression, no empirical study has been performed to examine the impact of HIIT combined with CBD on the expression of nestin and the molecular mechanisms involved in a rat model of AD.

Surprisingly, the expression levels of miRNA-124, which modulates neuroinflammation and neuronal differentiation, did not show any significant differences following HIIT or CBD treatments. Although miRNA-124 has been documented to be downregulated in AD, our study did not detect any significant alteration in the treatment groups. This implies that longer intervention durations or other training methods may be necessary to cause changes in miRNA-124 expression levels or that the therapeutic potential of HIIT and CBD may be mediated via different signaling pathways.[53] This outcome is contrary to that of Mojtahedi et al., demonstrating that high-intensity training has higher effectiveness in regulating miRNA-124 in AD rats.[54] It is difficult to explain this result, but it might be related to the weekly training frequency, which was higher in Mojtahedi et al.'s study, and miRNA-124 may exhibit different reactions to different training frequencies. Furthermore, tissue-specific miRNA-124 regulation may explain these results, as effects could be localized to unexamined brain regions. Alternatively, homeostatic mechanisms may preserve miRNA-124 levels despite BACE1/nestin alterations.

A number of limitations need to be noted regarding the current study. A key limitation of this study is the relatively small sample size. While this sample size allowed for preliminary observations of trends, it may affect the generalizability of our results, reduce statistical power, and increase susceptibility to both type I and type II errors. Although our results align with mechanistic hypotheses, the findings must be interpreted cautiously. In addition, the precise mechanisms through which HIIT and CBD interventions influence the assessed variables need to be explored further in detailed studies. Additionally, a limitation of this study is the lack of direct Aβ plaque quantification, which would have provided complementary evidence linking BACE1 changes to amyloid pathology. While we found reduced BACE1 and increased nestin expression, we did not measure Aβ plaques. Future studies should quantify Aβ deposition to confirm these changes reduce amyloid pathology. Eventually, our CBD dose (20 mg/kg) was literature-derived but not empirically optimized for HIIT synergy in this AD model. A critical next step is combinatorial dose-response testing to establish: a) CBD's therapeutic window, and b) its exercise-dependent efficacy thresholds. Moreover, due to CBD's highly promising effects in

preclinical Alzheimer's research, we selected this compound for its neuroprotective potential in preclinical studies – though its optimal dosage, long-term safety, and clinical efficacy require further investigation. Finally, the lack of significant changes in miRNA-124 expression, despite BACE1 and nestin alterations, may reflect insufficient intervention duration, regional specificity of regulation, or compensatory mechanisms maintaining miRNA-124 levels. Future work should examine additional brain regions, such as the cortex, to better characterize these relationships. Therefore, our findings need to be interpreted with caution. Further investigations are required to elucidate the interactive mechanisms of HIIT and CBD and optimize their combined use for therapeutic objectives.

Conclusion

Our findings suggest that both HIIT and CBD alone can partly counteract AD-related impairments, while their combination produces stronger effects. The observed reduction in BACE1 and increase in nestin expression indicate that these interventions not only help limit Aβ-related damage but may also support neurogenesis and neuronal repair. Importantly, beyond the experimental setting, this combined approach has potential clinical relevance: time-efficient HIIT and non-psychoactive CBD could together represent a practical, accessible strategy to help mitigate cognitive decline in populations at risk for AD.

Conflict of Interests

The authors declare no conflict of interest in this study.

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