Heliyon 10 (2024) e32299

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

# The protective effect of biotin supplementation and swimming training on cognitive impairment and mental symptoms in a rat model of Alzheimer's disease: A behavioral, biochemical, and histological study

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ARTICLE INFO

*Keywords:*
Alzheimer's disease
Aβ plaque
Biotin supplementation
Swimming training

ABSTRACT

Vitamin B (Vit B) plays a regulatory role in cognitive memory and learning. We examined the biochemical and behavioral effects of biotin supplementation (BS) and swimming training (ST) on Alzheimer's disease (AD), the most common type of dementia, in male rats. Sixty rats were randomly assigned to six groups: control, sham (receiving phosphate-buffered saline), AD (receiving a single injection of Aβ into the lateral ventricle), ST (for 28 days and before Aβ injection), and BS (receiving BS through oral gavage for 28 days before Aβ injection). The treatments were continued until the end of the behavioral tests. Learning and memory functions were investigated through the Morris water maze (MWM) and depression and anxiety-like behaviors were tested by elevated plus-maze (EPM) and forced swimming tests. In addition, oxidative stress biomarkers, such as total thiol groups (TTG) and malondialdehyde (MDA) in serum were assessed and histological studies were performed using brain tissues. In the AD group, Aβ increased the distance traveled and escape latency in the MWM, but co-administration of BS and ST attenuated the results of the MWM, EPM, and FST tests. Furthermore, BS decreased the litigious biochemical effects of Aβ by enhancing the levels of TTG, in addition to reducing serum MDA levels. The use of BS as a potent antioxidant improved Aβ-induced memory impairment. It attenuated oxidative stress biomarkers in the brain (number of Aβ plaques) and serum of AD rats. We provide evidence for the use of BS in neurodegenerative disorders, such as AD, to elucidate the possible mechanisms.

## 1. Introduction

Oxidative damage plays a major role in the development of dementia, particularly AD [1]. The brains of AD patients have a

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https://doi.org/10.1016/j.heliyon.2024.e32299
Received 6 January 2023; Received in revised form 14 April 2024; Accepted 31 May 2024
Available online 7 June 2024

significant extent of oxidative damage associated with the unusually significant amyloid β (Aβ)accumulation and the deposition of neurofibrillary tangles [2]. Stress may lead to increased production of Aβ and its incorporation into Aβ plaques [3]. The risk of mental illness is higher in older adults with anxiety and depressive symptoms than in older adults with mild cognitive impairment and AD [4]. Aβ aggregation is a key event in the accumulation of Aβ in the brain, which is an early and central pathophysiological change within the biological continuum of AD [5]. Insomnia and sleep deprivation can also trigger Aβ aggregation [6]. Therefore, Aβ aggregation can trigger anxiety and depression, and preventing Aβ-aggregation could be a potential therapeutic strategy for these disorders.

Biotin, a water-soluble vitamin, belongs to the Vit B complex family. Biotin acts as a coenzyme for four carboxylases: pyruvate carboxylase, acetyl-CoA carboxylase, propionyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase [7]. During biotin deficiency, methylcrotonyl-CoA accumulates in the mitochondria and depletes succinyl-CoA and glycine, leading to the prevention of heme/cytochrome synthesis and resulting in mitochondrial ROS generation, premature cellular senescence [8], and heme deficiency, which are linked to cognitive dysfunction [9]. Biotinylation is also influenced by biotin deficiency. Biotins can regulate gene expression through the biotinylation of histones [10] and affect cell proliferation, gene silencing, cell death, and DNA repair pathways through apoptosis [11]. This suggests that correcting biotin deficiencies can increase mitochondrial metabolism in the aging brain, which can optimize ATP generation efficiency [12].

Regular exercise is a non-pharmacological approach that prevents memory deterioration in AD [13,14], and exercise or physical activity can improve cognitive impairment and memory decline and postpone the onset of most cases of dementia, such as AD [15]. Studies on rodents have shown that regular exercise training can improve neuroprotection, inflammation [16], and cognitive [17,18] and non-cognitive [19] functions by reducing Aβ and tau deposition. Nonetheless, physical activity can attenuate cognitive deficits through Aβ-dependent or -independent mechanisms [15]. Various types of exercise, such as swimming [20], treadmill running [21], and wheel running [22] have been studied in AD; however, all types of exercises improve behavioral impairment in AD by increasing the levels of neurotrophic factors. The neuroprotective role of biotin supplementation (BS) has recently gained considerable attention owing to its ability to prevent mitochondrial damage [23]. Thus, this study was done to determine the protective effect of BS and ST on cognitive impairment and mental symptoms in a rat model of AD.

## 2. Materials and methods

### 2.1. Animals and experimental design

Adult male rats (200–250 g) were purchased from the animal house of Hamadan University of Medical Sciences. Five animals were housed in each cage and after a week of adaptation to the new environment, 60 rats were randomly assigned to six groups (ten rats per group): 1) the control group: the rats that received a standard diet, 2) sham group: the rats that received phosphate-buffered saline (PBS) via intracerebroventricular (ICV) injection as a solvent of Aβ1– 40; 3) AD group: the rats that received a single injection of Aβ into the lateral ventricle; 4) BS group: the rats that received BS (IRAN DARU Co.) at 10 mg/kg/bw through oral gavage once a day for 28 days [24] before Aβ injection, 5) ST group: the rats that received ST for 28 days before Aβ injection, and 6) BS + ST: the rats that received BS (10 mg/kg/bw) through oral gavage once a day and performed ST for 28 days before Aβ injection. The treatments were continued until the end of the behavioral tests. All animal procedures were performed in accordance with the institutional and national guidelines (ARRIVE guidelines) for animal care and approved by the Animal Research Ethical Committee of the Shiraz University of Agriculture. The experimental design is illustrated in Fig. 1.

### 2.2. The main reagents and drugs

Aβ (1–40) peptide (Tocris Bioscience; UK) was dissolved in PBS (10 μg in 10 μl of sterile PBS) and incubated at 37 °C for seven days to induce peptide aggregation [25].

### 2.3. Aβ injections and surgery

To establish the AD model, anesthetization was done with an intraperitoneal injection of ketamine (100 mg/kg, Behbod Darou,

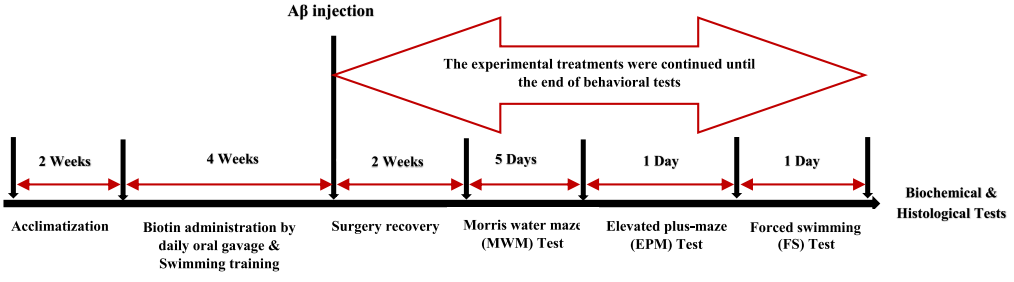


**Fig. 1.** The experimental design.

Tehran, Iran) and xylazine (10 mg/kg, Alfasan, Woerden, The Netherlands), and the animals were placed in a stereotaxic device. The aggregated Aβ (5 μl) was injected gently within 5 min into the lateral ventricle (4 mm ventral to the cortex surface, 2 mm lateral to the midline, and 1.2 mm posterior to the bregma) [26]. The sham group only received PBS. Following injections, the skin was sutured, and the animals were allowed to recover in their home cages [27].

### 2.4. Swimming exercise

Animals performed a four-week ST program in a cylindrical tank (148 cm × 50 cm), containing temperature-controlled water (30–32 °C), which included two phases: 1) adaptation phase (water depth: 5 cm, 10 min) and 2) ST phase (two or three sessions of 10 min, water depth: 5–15 cm) with an interval of 10 min between sessions [28,29].

### 2.5. Behavioral study

#### 2.5.1. Evaluation of spatial memory

Learning and memory were assessed by the Morris water maze (MWM) test [30,31]. The device was a black circular pool (diameter: 160 cm and height: 80 cm) filled with water to a depth of 40 cm, which was kept at room temperature. The pool was divided into four equal quadrants. A black square platform (10 cm in diameter) was centered in the northeast quadrant of the pool and submerged 1.5 cm below the water level. The walls of the room had different extra-maze cues so that the animals could learn the platform position. The pool had four quadrants (north (N), south (S), east (E), and west (W)) [32]. There was a video camera in the ceiling above the pool to record the sessions. The spatial probe test was done 24 h after the fourth session, during which the platform was removed, and the animals could swim for 60 s before being removed from the pool. The rats were released in the water exactly opposite from the platform location and their behavior, including escape latencies and swim speed was recorded for subsequent analysis. Thirty minutes following the probe test, the platform covered with aluminum foil was raised above the water surface, and in another zone, the animals could swim and find it within 60 s to test their visual ability. A digital camera positioned above the water maze was used to capture images per second and transmit them to a personal computer running the Neurovision software (Iran).

#### 2.5.2. Elevated plus-maze (EPM) test

The EPM was used to evaluate anxiety-like behaviors [33]. The apparatus consisted of two open and two closed arms (length: 70 cm, width: 10 cm), crossing perpendicularly in the center. The closed arms were surrounded by opaque walls (30 cm) and the whole maze was elevated 50 cm above the floor. Each rat was gently placed in the central area with its nose facing one of the arms and allowed to freely explore it for 10 min. The device was cleaned with a 5 % ethanol solution and dried with a cloth before testing the next animal. All sessions were recorded by a camera attached to a 60 W bulb placed 180 cm above the maze (22 lx in the central square of the maze).

#### 2.5.3. Forced swimming test (FST)

FST is a widely used test to assess anti-depressant-like activity [34], which was used as previously described [28]. Animals were individually placed in a clear plastic cylinder filled with water (25 ± 1 °C) for 6 min. During the test phase, the swimming time, struggling time, and time spent floating with minimal movements to keep the head above the water were recorded as behavioral responses.

### 2.6. Determination of MDA and TTG

After the experiments, the rats were anesthetized with ketamine (100 mg/kg, Behbod Darou, Tehran, Iran) and xylazine (10 mg/kg, Alfasan, Woerden, The Netherlands). Blood samples were taken from the portal vein, followed by centrifugation at 3000 rpm for 10 min at 4 °C. Total thiol group (TTG) and malondialdehyde (MDA) levels were measured using commercially available kits (Navandsalamat Co., Iran.

### 2.7. Histology

The animals' brains were fixed in 10 % neutral buffered formalin for at least 48 h. They were then washed, dehydrated in alcohol, and embedded in paraffin blocks. For histopathological assessment, tissue sections (4 μm) were stained with Hematoxylin and Eosin (H&E) and Congo Red, and the results were observed under an optical microscope at × 40 and × 400 magnifications. The survival rate was considered and the number of Aβ plaques was calculated using ImageTool software [35].

### 2.8. Statistical analysis

Statistical analysis was conducted using SPSS 26. The Kolmogorov-Smirnov test was applied to indicate the normal distribution of data. A one-way ANOVA followed by Tukey's post hoc test was used for all statistical comparisons, with a significance level set at $p < 0.05$.

## 3. Results

### 3.1. Effects of BS and ST on MWM performance

A significant difference was found between the BS + ST, BS, and other experimental groups in terms of traveled distance and escape latency ($p < 0.001$). As shown in Fig. 2 (a, b), all treated rats that received BS with ST and BS alone for four weeks showed a decrease in escape latency and traveled distance ($p < 0.001$). The BS + ST and BS groups spent more time in the target quadrant compared to other groups (sham, control, ST, and AD groups) ($p < 0.001$) [Fig. 2 (c)]. The AD group was found with an increase in escape latency and traveled distance compared to other groups (Fig. 2). The post hoc analysis indicated no significant difference between the ST group and the sham and control groups in terms of traveled distance, escape latency, and the mean time spent in the target quadrant ($P > 0.05$) [Fig. 2 (a-c)].

### 3.2. EPM results

Different parameters were regarded for the measurement of the rat's locomotor activity in the EPM. The number of entries into the closed arms and the time elapsed in closed arms indicated the locomotion state of the animals [Fig. 3 (a, b)]. In the AD group, the time spent in the open arms and the number of entries into the open arms significantly decreased. This group was found with an increase in the time spent in the closed arms of the EPM and the number of entries into the closed arms ($P < 0.001$). In addition, there was a significant difference between the BS + ST, BS alone, and ST groups and other experimental groups (sham, control, and AD groups) in the time spent in the open and closed arms of the EPM and the number of entries into the closed arms ($P < 0.001$). As shown in Fig. 3 (a), the BS + ST group showed a significant increase in the time spent in the open arms of the EPM and the number of entries into the open arms. This group showed a decrease in the time spent in the closed arms of the EPM and the number of entries into the closed arms ($P < 0.001$). The BS + ST group showed no significant difference in the time spent in open and closed arms and the number of entries into the open and closed arms ($P > 0.05$).

### 3.3. FST results

The statistical analysis showed a significant interaction between the use of BS in the ST, ST alone, BS groups, and swimming and struggling time in the FST (Fig. 4). The time of swimming and struggling significantly increased in the BS + ST group ($P < 0.001$), but

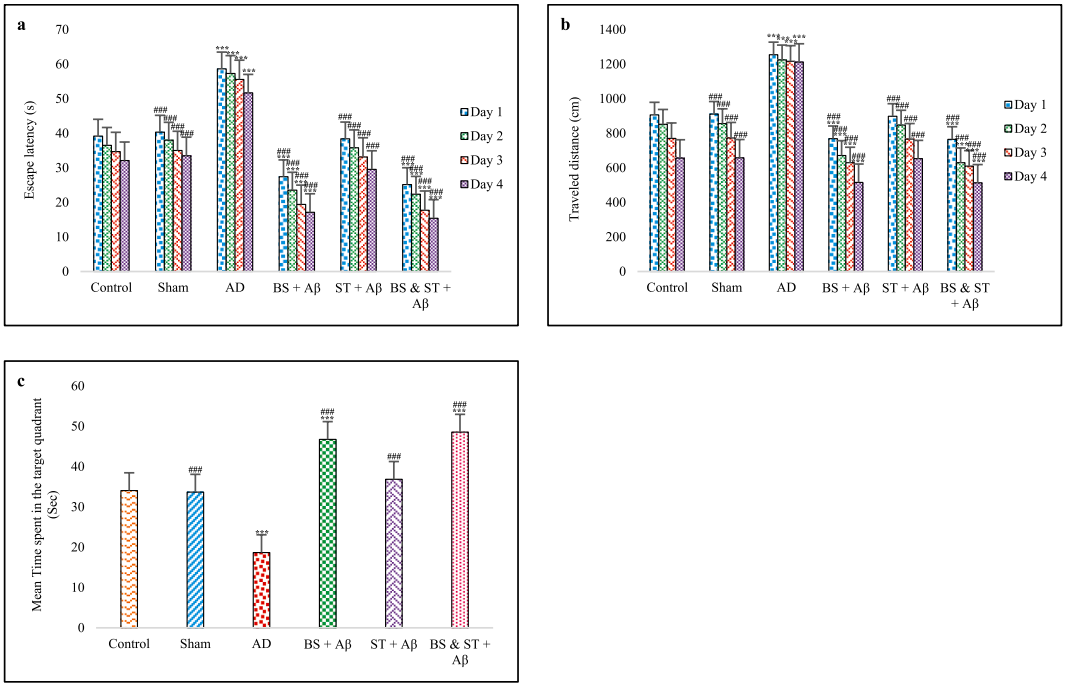


**Fig. 2.** Morris water maze (MWM) test results. Escape latency **a**, traveled distance **b**, the mean time spent in the target quadrant **c**. Sham group: the rats that received phosphate-buffered saline (PBS); Alzheimer's group (AD) (biotin supplementation (BS) + Aβ): the rats that received BS for 28 days before Aβ injection; swimming training (ST) + Aβ group: the rats that performed ST for 28 days before Aβ injection, and BS + ST + Aβ group: the rats that received both BS and ST before Aβ injection. Data are shown as mean ± standard error of the mean (SEM). ***$p < 0.001$ compared to the control group and ###$p < 0.001$ compared to the AD group.

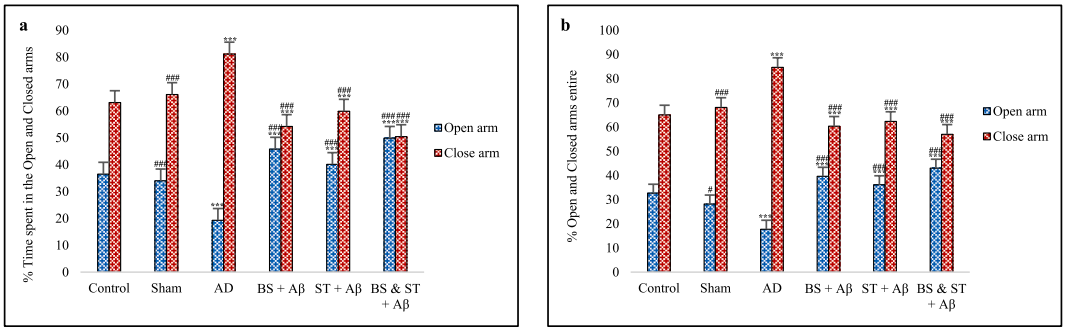


**Fig. 3.** Effects of the biotin supplementation (BS) and swimming training (ST) on the time spent in the open and closed arms (**a**) and open and closed arms entries (**b**) in the experimental groups using the elevated plus-maze test. Sham group: the rats that received phosphate-buffered saline (PBS); AD: Alzheimer's group (BS + Aβ): the rats that received BS for 28 days before Aβ injection; ST + Aβ group: the rats that performed ST for 28 days before Aβ injection; and BS + ST + Aβ group: the rats that received BS and ST before Aβ injection. Values are presented as mean ± SEM (n = 10 animals per group). ***$p < 0.001$ compared to the control group and #$p < 0.05$ and ###$p < 0.001$ compared to the AD group.

there was no significant difference between these groups (BS group versus the sham and control groups) in the swimming, struggling, and floating time in the FST ($P > 0.05$). Furthermore, the rats of the AD group showed an increase in the floating time compared to other groups ($P < 0.001$), while the floating time significantly decreased in the BS + ST and ST groups ($P < 0.001$).

### 3.4. Effects of BS and ST on oxidative stress markers in rats

MDA is the most frequently used biomarker of oxidative stress in many health problems. The impact of BS and ST on TTG and MDA serum concentrations was evaluated [Fig. 5 (a, b)]. Our results showed that the levels of MDA in the blood of the AD group significantly increased compared to other experimental groups ($P < 0.001$). Therefore, Aβ caused an increase in the levels of MDA in the AD group [Fig. 5 (b)]. In addition, BS significantly decreased the levels of MDA in the blood compared to the AD, sham, control, and ST groups ($P < 0.001$). These administration of BS synergistically returned serum MDA levels to healthy levels in rats. Also, serum TTG levels were higher in the BS group ($P < 0.001$), while TTG levels in the AD rats were lower than in all experimental groups ($P < 0.001$) [Fig. 5 (a)]. There were no significant differences between the BS + ST group and the ST group in the levels of MDA and TTG in the blood ($p > 0.05$).

### 3.5. Histological studies

Hippocampal neuronal damage and the neuronal survival rate in the rats were observed by Congo Red and H&E staining [Fig. 6 (a-f)]. The hippocampal neuronal cells in the AD group were dead and the intercellular space became larger. In this experimental group, some cells were disordered and the pyramid-like multilayer structures were disintegrated. In addition, neuronal loss in the first region in the hippocampal circuit (CA1) area and hippocampal histopathological damage were observed [Fig. 7 (a, b)]. The number of damaged neurons significantly increased in the AD group than in the control group ($P < 0.001$).

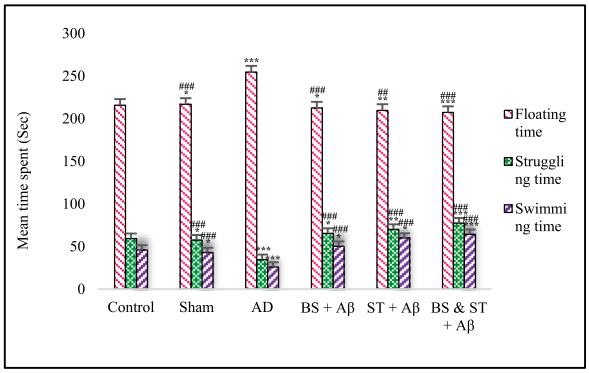


**Fig. 4.** Assessment of anxiety-like behaviors. Effects of the biotin supplementation (BS) and swimming training (ST) on floating, struggling, and swimming time in the experimental groups within 6 min in the forced swimming test. Sham group: the rats that received phosphate-buffered saline (PBS); AD: Alzheimer's group (BS + Aβ): the rats that received BS for 28 days before Aβ injection; ST + Aβ group: the rats that performed ST for 28 days before Aβ injection; and BS + ST + Aβ group: the rats that received BS and ST before Aβ injection. Values are presented as mean ± S.E.M for 10 rats. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared to the control group and ##$p < 0.01$ and ###$p < 0.001$ compared to the AD group.

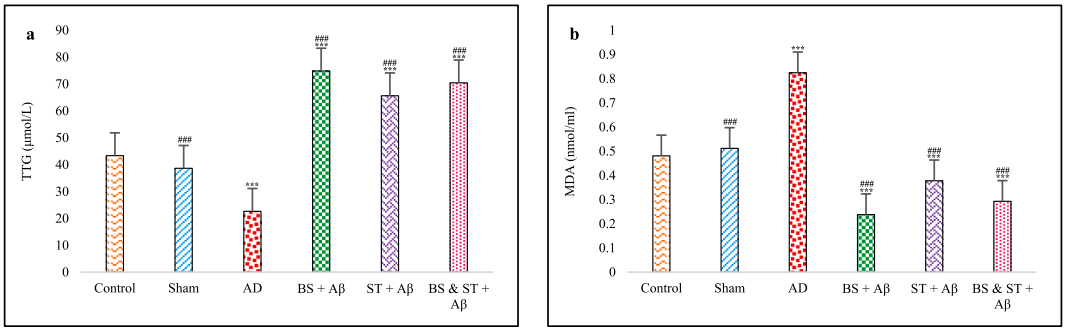


**Fig. 5.** Plasma levels of total thiol groups (TTG) and malondialdehyde (MDA) in all groups. Sham group: the rats that received phosphate-buffered saline (PBS); AD: Alzheimer's group (BS + Aβ): the rats that received BS for 28 days before Aβ injection; ST + Aβ group: the rats that performed ST for 28 days before Aβ injection; and BS + ST + Aβ group: the rats that received BS and ST before Aβ injection. Data are presented as mean ± SEM (n = 10 per group). ***$p < 0.001$ compared to the control group and ###$p < 0.001$ compared to the AD group.

The neuronal survival rate assessed in the CA1 area indicated variation among different groups. The AD group showed a marked reduction in the neuronal survival rate compared to other experimental groups ($P < 0.001$) [Fig. 7 (b)].

BS significantly increased large, round, and regular neurons in the CA1 area ($P < 0.001$) (Fig. 6). In addition, the number of survival neurons significantly increased in the BS + ST, BS, and ST groups than in other experimental groups ($P < 0.001$) [Fig. 6 (*d*-*f*)]. H&E staining revealed no significant neuronal abnormalities in the hippocampus of the BS group and the pyramidal cells in the CA1 area were arranged tightly and neatly with no cell loss. The BS group indicated a significant improvement in the neuronal survival rate in the CA1 area than in other groups ($P < 0.001$) [Fig. 7 (b)].

Neuronal damage in the BS and BS + ST groups was rare compared to the AD group, which indicated the neuroprotective effect of BS [Fig. 7 (a)].

## 4. Discussion

In our study, the rats in the BS alone and BS + ST groups exhibited a significant decrease in MDA levels compared to other experimental groups. Also, the TTG levels in these groups were significantly higher than in other experimental groups; however, these results were different for the AD group. In many animal studies, the role of oxidative stress and antioxidant concentrations have been assessed in neurodegenerative disorders [36] because mitochondrial damage and oxidative stress lead to neurodegeneration [37]. Therefore, a diet rich in antioxidant vitamins may slow the progression of neurodegenerative diseases, such as AD [38,39]. There are two major groups of micronutrients necessary for optimal cognitive performance: water-soluble vitamins (B complex) and minerals [40]. There are four major mechanisms, by which micronutrients affect cognitive function: through their role in neurotransmitter production, receptor modification and neuronal membrane, affecting brain energy requirements, and through their role in Homocysteine (Hcy) metabolism [40]. Nonetheless, these micronutrients have a direct effect on cognitive function via their involvement in the energy metabolism of glial cells and neurons, receptor binding, the production of neurotransmitters, and the maintenance of ion pumps of the membrane. A marginal deficiency of these micronutrients causes nonspecific symptoms, which are more associated with cognitive performance [40]. ST along with BS significantly decreased anxiety and depression-related behaviors. B vitamins can protect mitochondrial and other enzymes because these vitamins are cofactors, like biotin, or precursors of mitochondrial cofactors [41]. Biotin can act as a coenzyme for biotin-dependent carboxylases [42] and biotin deficiency reduces the activity of these enzymes. The antioxidant capacity of biotin [43] is attributed to its protective effects on neurons [42]. Exercise training also reduced stress oxidative, as the main cause of memory deficit in patients [44,45]. Various mechanisms have been suggested for the ameliorative effect of exercise training on reversing neurological disorders, including synaptic plasticity and the management of energy metabolism [46]. In accordance with our results, aerobic exercise training had anxiolytic effects and protected the animals from stress [47], and reduced anxiety-related behavior [48]. Regular swimming exercise, which was performed before Aβ (1–40) infusion, could protect against Aβ (1–40)- induced neurotoxicity [49].

Anxiety, depression, and cognitive impairment are all associated. According to Donovan et al. higher levels of brain Aβ were linked to more anxious-depressive symptoms [50]. Another study found that anxiety and depression were linked to subjective cognitive decline, which may be mediated by aggregation of Aβ and tau in the brain [51]. Depressive and anxious symptoms cause an increase in the risk of cognitive decline [52]. Similarly, Aβ1-42 levels might be associated with mental hindrance [53]. Aβ aggregation and anxiety in AD have a complicated relationship, and this relationship can cause anxiety [54]. According to recent research, exercise may have positive effects on AD and mental disorders. Basic subatomic tools of these effects include neurogenesis, brain-derived neurotrophic factor (BDNF), and miR-129 [55]. Exercise improved cognitive function and psychological conditions, like insomnia and depression [56]. Several underlying mechanisms related to muscle function, brain function, and neuroinflammation are linked to the beneficial effects of exercise on the brain [57]. Incorporating an exercise program into patients' lifestyles was found to be beneficial and rewarding [58]. According to another study, swimming improved the cognitive and behavioral functioning of mice with AD [28]. Active work may reduce age-related diseases, including amyloidosis [59]. Physical activity improves memory and cognitive function in

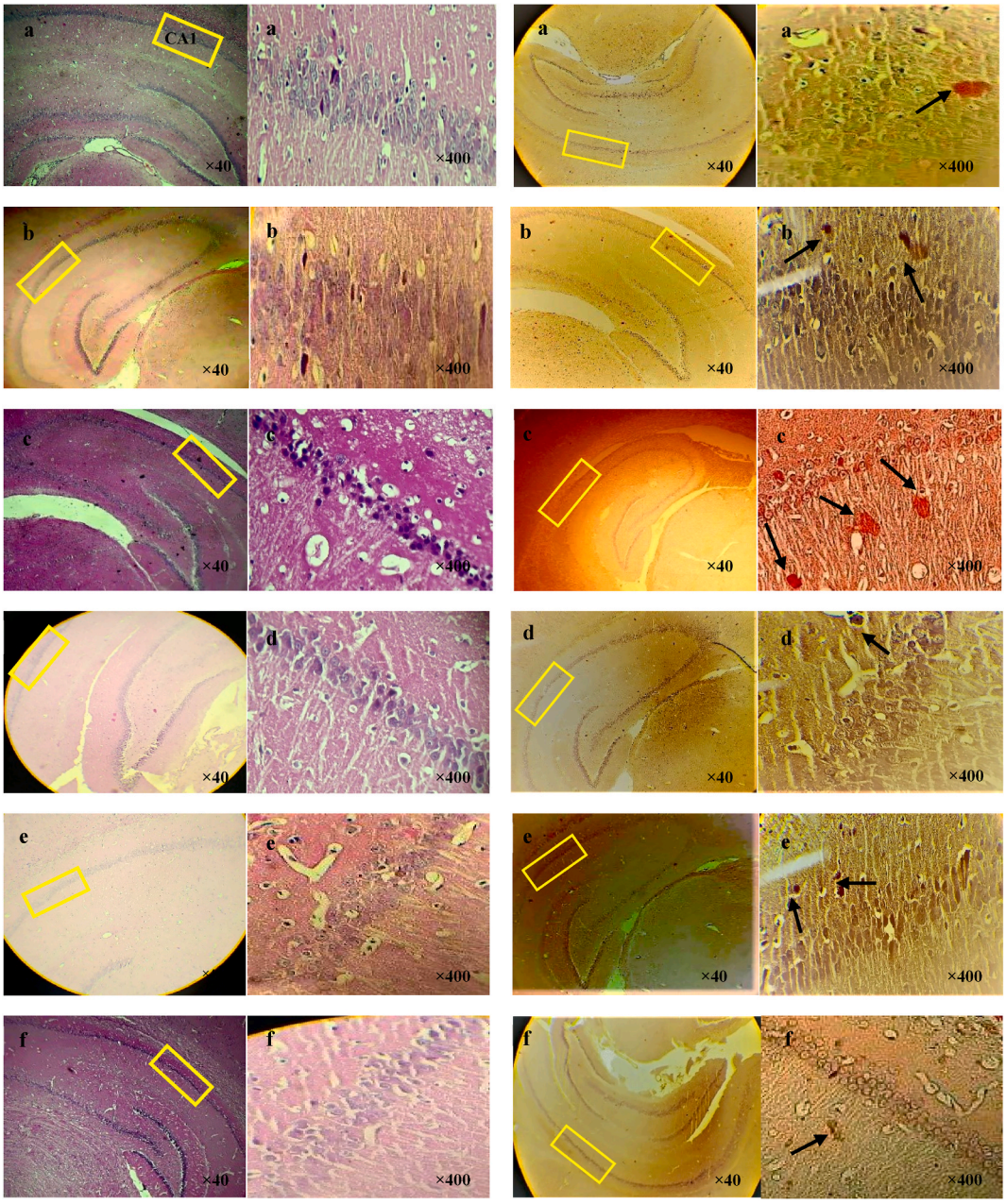


**Fig. 6.** H&E (two left columns) and Congo Red (two right columns) staining of the sections of the hippocampal CA1 region. Control (**a**), Sham (**b**), AD (**c**), BS + Aβ (**d**), ST + Aβ €, BS + ST + Aβ (**f**) groups. Sham group: the rats that received phosphate-buffered saline (PBS); AD: Alzheimer's group (BS + Aβ): the rats that received BS for 28 days before Aβ injection; ST + Aβ group: the rats that performed ST for 28 days before Aβ injection; and BS + ST + Aβ group: the rats that received BS and ST before Aβ injection ( × 40 and × 400). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

AD [60]. Several studies have discussed the molecular mechanisms of AD and the potential benefits of exercise and natural antioxidants in alleviating the disease [61]. In addition, an enriched environment has been shown to improve cognition in tauopathy models in rats [62]. Antioxidant vitamins have potential therapeutic effects against AD [63]. These vitamins have antioxidant and anti-inflammatory properties leading to protection against neurodegeneration and reducing Aβ formation [64]. The molecular mechanisms, by which these vitamins enhance AD include their ability to act synergistically against lipid peroxidation and reduced oxidative stress, neuroinflammation, and apoptosis [65].

We found that taking BS alone and in combination with ST significantly improved cognitive abilities. According to EPM, FST, and MWM tests, concomitant intake of BS alone and in combination with ST reduced depression and anxiety-like behaviors in rats, but in

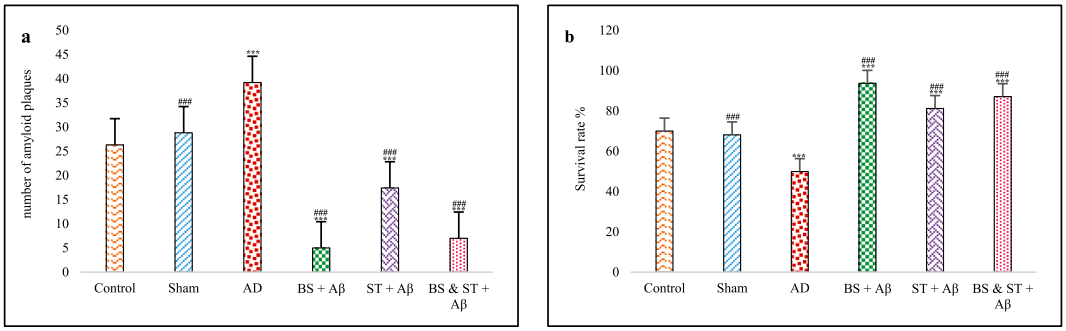


**Fig. 7.** Effect of treatments on the number of amyloid plaques and the neuronal survival rate in the hippocampus (CA1) using Congo Red and H&E staining. Control (**a**), Sham (**b**), AD (**c**), BS + Aβ **(d)**, ST + Aβ €, BS + ST + Aβ **(f)** groups. Sham group: the rats that received phosphate-buffered saline (PBS); AD: Alzheimer's group (BS + Aβ): the rats that received BS for 28 days before Aβ injection; ST + Aβ group: the rats that performed ST for 28 days before Aβ injection; and BS + ST + Aβ group: the rats that received BS and ST before Aβ injection. The black arrows in the right pitchers indicate pyramidal cells and the black arrow in the left pitchers shows amyloid plaques in the hippocampal CA1 region. Data are presented as mean ± S.E.M (n = 10 for each group). ***$p < 0.001$ compared to the control group and ###$p < 0.001$ compared to the AD group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

tissues, BS was more effective in reducing the number of Aβ plaques and increased neuronal survival in the hippocampus of AD rats. This improvement could be attributed to the reduced Aβ deposition and MDA levels and increased GSH and SOD concentrations in the brain homogenate [42]. Biotin reduces lipid peroxidation and oxidative stress in the brain, kidney [66], and liver [66,67]. In our study, Aβ (1–40) caused significant learning impairments in the MWM in rats, but we showed that simultaneous intake of BS alone and in combination with ST reversed the memory impairments in the experimental groups. Biotin can protect the brain and prevent AD. According to Ref. [68], high-dose biotin supplementation may neutralize promotion-related concealment of a system that separates Aβ. Biotin plays a role in the digestion of mitochondria and can reestablish glucokinase activity through a cyclic guanosine monophosphate (cGMP)-intervened system [69]. High-segment biotin has been shown to fabricate cGMP levels, which can restore fruitful long-stretch potentiation in rodent models [68]. Additionally, neuronal apoptosis and Aβ production can be inhibited by cGMP, indicating that high-dose biotin may have the potential to treat and prevent AD. By inhibiting the soluble guanylyl cyclase (sGC) activity that is involved in the production of cGMP, high-dose biotin for AD reduces Aβ levels [68]. Biotin as a vital vitamin for lipogenesis, is the coenzyme of acetyl-CoA carboxylase (ACC), the rate-limiting enzyme for fatty acid biosynthesis. Due to the high lipid content of the brain, biotin deficiency has been linked to neurological disorders [70].

The main finding of our study was that concomitant administration of BS and ST reversed memory impairment in the BS + ST group. The concomitant administration of BS and ST was more effective than that of BS or ST alone, and the concomitant administration of BS and ST may be more effective in suppressing oxidative stress in AD rats. Therefore, the concomitant administration of AD may cause neurogenic effects in the brain of rats.

## 5. Conclusion

The current study is the first on the neuroprotective role of BS with ST against AD by modulating oxidative stress and Aβ accumulation. Our data indicated the role of BS in preventing or protecting AD through its antioxidant activity. Our results suggest the efficacy of biotin as a dietary supplement and ST in preventing the major components of AD. B vitamins are essential for all aspects of brain function; therefore, natural therapeutic compounds based on BS can be used to treat and prevent oxidative stress-related diseases, such as AD. However, further clinical trials are needed to determine their potential and efficacy. Future research on AD patients should investigate the effects of different doses of BS on patients' cognitive performance.

## Funding statement

This research was supported by Shiraz University (Grant No.: 0gcb3m148075).

## Data availability statement

Data will be made available on request.

## Ethical approval

All animal procedures were performed in accordance with the institutional and national guidelines (ARRIVE guidelines) for animal care and approved by the Animal Research Ethical Committee of the Shiraz University of Agriculture (Ethics Code: IR.SHZU.REC. 0gcb3m148075).

## CRediT authorship contribution statement

**Shadi Almasi:** Conceptualization, Data curation, Investigation, Methodology, Software, Writing – original draft. **Mohammad Reza Jafarzadeh Shirazi:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Visualization, Writing – original draft. **Mohammad Reza Rezvani:** Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Writing – original draft. **Mahdi Ramezani:** Data curation, Methodology, Software, Validation, Visualization, Writing – original draft. **Iraj Salehi:** Conceptualization, Formal analysis, Investigation, Resources, Validation, Visualization, Writing – original draft. **Atefeh Pegah:** Data curation, Methodology, Software, Visualization, Writing – original draft. **Alireza Komaki:** Conceptualization, Formal analysis, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Alireza Komaki reports was provided by Hamadan University of Medical Sciences. Alireza Komaki reports a relationship with Hamadan University of Medical Sciences that includes: funding grants.

## Acknowledgments

The authors are grateful to the staff of the Neurophysiology Research Center, Hamadan University of Medical Sciences for supporting this study.

## References

[1] A. Grimm, A. Eckert, Brain aging and neurodegeneration: from a mitochondrial point of view, J. Neurochem. 143 (4) (2017) 418–431.
[2] Y. Christen, Oxidative stress and Alzheimer disease, Am. J. Clin. Nutr. 71 (2) (2000) 621S–629S.
[3] H. Dong, J.G. Csernansky, Effects of stress and stress hormones on amyloid-β protein and plaque deposition, J. Alzheim. Dis. 18 (2) (2009) 459–469.
[4] R.H. Pietrzak, et al., Amyloid-β, anxiety, and cognitive decline in preclinical Alzheimer disease: a multicenter, prospective cohort study, JAMA Psychiatr. 72 (3) (2015) 284–291.
[5] H. Hampel, et al., The amyloid-β pathway in Alzheimer's disease, Mol. Psychiatr. 26 (10) (2021) 5481–5503.
[6] S. Sadeghmousavi, et al., The effect of insomnia on development of Alzheimer's disease, J. Neuroinflammation 17 (1) (2020) 289.
[7] D.A. Bender, Optimum nutrition: thiamin, biotin and pantothenate, Proc. Nutr. Soc. 58 (2) (1999) 427–433.
[8] B.N. Ames, H. Atamna, D.W. Killilea, Mineral and vitamin deficiencies can accelerate the mitochondrial decay of aging, Mol. Aspect. Med. 26 (4–5) (2005) 363–378.
[9] H. Atamna, Heme, iron, and the mitochondrial decay of ageing, Ageing Res. Rev. 3 (3) (2004) 303–318.
[10] N. Kothapalli, et al., Biological functions of biotinylated histones, J. Nutr. Biochem. 16 (7) (2005) 446–448.
[11] F. Depeint, et al., Mitochondrial function and toxicity: role of the B vitamin family on mitochondrial energy metabolism, Chem. Biol. Interact. 163 (1–2) (2006) 94–112.
[12] M. Fenech, Vitamins associated with brain aging, mild cognitive impairment, and Alzheimer disease: biomarkers, epidemiological and experimental evidence, plausible mechanisms, and knowledge gaps, Adv. Nutr. 8 (6) (2017) 958–970.
[13] E. Duzel, H. van Praag, M. Sendtner, Can physical exercise in old age improve memory and hippocampal function? Brain 139 (3) (2016) 662–673.
[14] S.M. Ryan, Á.M. Kelly, Exercise as a pro-cognitive, pro-neurogenic and anti-inflammatory intervention in transgenic mouse models of Alzheimer's disease, Ageing Res. Rev. 27 (2016) 77–92.
[15] K. Ebrahimi, et al., Physical activity and beta-amyloid pathology in Alzheimer's disease: a sound mind in a sound body, EXCLI journal 16 (2017) 959.
[16] Y. Lu, et al., Treadmill exercise exerts neuroprotection and regulates microglial polarization and oxidative stress in a streptozotocin-induced rat model of sporadic Alzheimer's disease, J. Alzheim. Dis. 56 (4) (2017) 1469–1484.
[17] J. Cho, et al., Treadmill running reverses cognitive declines due to Alzheimer disease, Med. Sci. Sports Exerc. 47 (9) (2015) 1814–1824.
[18] E. Maliszewska-Cyna, K. Xhima, I. Aubert, A comparative study evaluating the impact of physical exercise on disease progression in a mouse model of Alzheimer's disease, J. Alzheim. Dis. 53 (1) (2016) 243–257.
[19] A.T. Dao, et al., Regular exercise prevents non-cognitive disturbances in a rat model of Alzheimer's disease, Int. J. Neuropsychopharmacol. 17 (4) (2014) 593–602.
[20] M. Belviranlı, N. Okudan, Voluntary, involuntary and forced exercises almost equally reverse behavioral impairment by regulating hippocampal neurotrophic factors and oxidative stress in experimental Alzheimer's disease model, Behav. Brain Res. 364 (2019) 245–255.
[21] M. Azimi, et al., Moderate treadmill exercise ameliorates amyloid-β-induced learning and memory impairment, possibly via increasing AMPK activity and up-regulation of the PGC-1α/FNDC5/BDNF pathway, Peptides 102 (2018) 78–88.
[22] M.A. Alomari, et al., Forced and voluntary exercises equally improve spatial learning and memory and hippocampal BDNF levels, Behav. Brain Res. 247 (2013) 34–39.
[23] F. Sedel, et al., Targeting demyelination and virtual hypoxia with high-dose biotin as a treatment for progressive multiple sclerosis, Neuropharmacology 110 (2016) 644–653.
[24] J. Komorowski, et al., The absorption and activity of a novel form of biotin, JDS-MB-001, in a hippocampal demyelination animal model, in: MULTIPLE SCLEROSIS JOURNAL, vol. 1, SAGE PUBLICATIONS LTD, 2018. OLIVERS YARD, 55 CITY ROAD, LONDON EC1Y 1SP.
[25] F. Mirzaei, et al., Multitarget effects of coconut oil (virgin type) on aβ-induced alzheimer's disease animal model, Archives of Neuroscience 6 (2) (2019).
[26] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates: Hard Cover Edition, Elsevier, 2006.
[27] M. Ramezani, et al., Therapeutic effects of melatonin-treated bone marrow mesenchymal stem cells (BMSC) in a rat model of Alzheimer's disease, J. Chem. Neuroanat. 108 (2020) 101804.
[28] H. Bashiri, et al., Swimming exercise improves cognitive and behavioral disorders in male NMRI mice with sporadic Alzheimer-like disease, Physiol. Behav. 223 (2020) 113003.
[29] H. Parsa, et al., Swimming training and Plantago psyllium ameliorate cognitive impairment and glucose tolerance in streptozotocin–nicotinamide-induced type 2 diabetic rats, J. Physiol. Sci. 71 (1) (2021) 1–12.
[30] E. Zarrinkalam, et al., Effects of endurance, resistance, and concurrent exercise on learning and memory after morphine withdrawal in rats, Life Sci. 157 (2016) 19–24.
[31] F. Heidari Soureshjani, et al., Effect of donepezil and hyoscyamoside on improving spatial memory in rats with Alzheimer's disease, J. Arak Univ. Med. Sci. 23 (4) (2020) 524–539.

[32] M. Shekarian, et al., The protective and therapeutic effects of vinpocetine, a PDE1 inhibitor, on oxidative stress and learning and memory impairment induced by an intracerebroventricular (ICV) injection of amyloid beta (aβ) peptide, Behav. Brain Res. 383 (2020) 112512.
[33] C.P. Pellow S, S.E. File, M. Briley, P. Chopin, S.E. File, M. Briley, Validation of open: closed arm entries in an elevated plus maze as a measure of anxiety in the rat, J. Neurosci. Methods 14 (1985) 149–167.
[34] J.F. Cryan, A. Markou, I. Lucki, Assessing antidepressant activity in rodents: recent developments and future needs, Trends Pharmacol. Sci. 23 (5) (2002) 238–245.
[35] A. Sirwi, et al., Umuhengerin neuroprotective effects in streptozotocin-induced alzheimer's disease mouse model via targeting Nrf2 and NF-kβ signaling cascades, Antioxidants 10 (12) (2021) 2011.
[36] M. Asadbegi, et al., Effects of thymol on amyloid-β-induced impairments in hippocampal synaptic plasticity in rats fed a high-fat diet, Brain Res. Bull. 137 (2018) 338–350.
[37] D. Nuzzo, et al., Insulin resistance as common molecular denominator linking obesity to Alzheimer's disease, Curr. Alzheimer Res. 12 (8) (2015) 723–735.
[38] A. Mielech, et al., Vitamins in alzheimer's disease—review of the latest reports, Nutrients 12 (11) (2020) 3458.
[39] S. Shahidi, et al., Influence of N-acetyl cysteine on beta-amyloid-induced Alzheimer's disease in a rat model: a behavioral and electrophysiological study, Brain Res. Bull. 131 (2017) 142–149.
[40] E. Huskisson, S. Maggini, M. Ruf, The influence of micronutrients on cognitive function and performance, J. Int. Med. Res. 35 (1) (2007) 1–19.
[41] J. Liu, B.N. Ames, Reducing mitochondrial decay with mitochondrial nutrients to delay and treat cognitive dysfunction, Alzheimer's disease, and Parkinson's disease, Nutr. Neurosci. 8 (2) (2005) 67–89.
[42] H. Attia, et al., Biotin, coenzyme Q10, and their combination ameliorate aluminium chloride-induced Alzheimer's disease via attenuating neuroinflammation and improving brain insulin signaling, J. Biochem. Mol. Toxicol. 34 (9) (2020) e22519.
[43] B.A. Aldahmash, D.M. El-Nagar, K.E. Ibrahim, Attenuation of hepatotoxicity and oxidative stress in diabetes STZ-induced type 1 by biotin in Swiss albino mice, Saudi J. Biol. Sci. 23 (2) (2016) 311–317.
[44] B.K. Mehta, K.K. Singh, S. Banerjee, Effect of exercise on type 2 diabetes-associated cognitive impairment in rats, Int. J. Neurosci. 129 (3) (2019) 252–263.
[45] L.S.d.B. Amaral, et al., Previous exercise training reduces markers of renal oxidative stress and inflammation in streptozotocin-induced diabetic female rats, J. Diabetes Res. (2018) 2018.
[46] F. Gomez-Pinilla, C. Hillman, The influence of exercise on cognitive abilities, Compr. Physiol. 3 (1) (2013) 403.
[47] P. Salmon, Effects of physical exercise on anxiety, depression, and sensitivity to stress: a unifying theory, Clin. Psychol. Rev. 21 (1) (2001) 33–61.
[48] A. Pietrelli, et al., Aerobic exercise prevents age-dependent cognitive decline and reduces anxiety-related behaviors in middle-aged and old rats, Neuroscience 202 (2012) 252–266.
[49] L.C. Souza, et al., Neuroprotective effect of physical exercise in a mouse model of Alzheimer's disease induced by β-amyloid 1–40 peptide, Neurotox. Res. 24 (2) (2013) 148–163.
[50] N.J. Donovan, et al., Longitudinal association of amyloid beta and anxious-depressive symptoms in cognitively normal older adults, Am. J. Psychiatr. 175 (6) (2018) 530–537.
[51] M. Akinci, et al., Subjective cognitive decline and anxious/depressive symptoms during the COVID-19 pandemic: what is the role of stress perception, stress resilience, and β-amyloid? Alzheimer's Res. Ther. 14 (1) (2022) 126.
[52] S. Perin, et al., Elucidating the association between depression, anxiety, and cognition in middle-aged adults: application of dimensional and categorical approaches, J. Affect. Disord. 296 (2022) 559–566.
[53] Z. Liu, et al., Correlation between Aβ1–42, Dnmt3a2, urinary AD7c-NTP and cognitive dysfunction in first-episode and recurrent MDD: a case–control study, Indian J. Psychiatr. 64 (6) (2022) 560–566.
[54] R.P. Archer, Relationships between locus of control and anxiety, J. Pers. Assess. 43 (6) (1979) 617–626.
[55] Y. Guo, et al., Multi-omics studies reveal ameliorating effects of physical exercise on neurodegenerative diseases, Front. Aging Neurosci. 14 (2022).
[56] Y. Lu, et al., Recent advances on the molecular mechanisms of exercise-induced improvements of cognitive dysfunction, Transl. Neurodegener. 12 (1) (2023) 9.
[57] K. Nay, et al., Molecular mechanisms underlying the beneficial effects of exercise on brain function and neurological disorders, Int. J. Mol. Sci. 22 (8) (2021) 4052.
[58] M. Parle, M. Vasudevan, N. Singh, Swim everyday to keep dementia away, J. Sports Sci. Med. 4 (1) (2005) 37.
[59] X. Cui, et al., Exercise suppresses mouse systemic AApoAII amyloidosis through enhancement of the p38 MAPK signaling pathway, Disease Models & Mechanisms 15 (3) (2022) dmm049327.
[60] K.K. Siddappaji, S. Gopal, Molecular mechanisms in Alzheimer's disease and the impact of physical exercise with advancements in therapeutic approaches, AIMS Neurosci. 8 (3) (2021) 357.
[61] A. Varesi, et al., Non-enzymatic antioxidants against alzheimer's disease: prevention, diagnosis and therapy, Antioxidants 12 (1) (2023) 180.
[62] V. Mate, et al., Enriched environment ameliorates propagation of tau pathology and improves cognition in rat model of tauopathy, Front. Aging Neurosci. 14 (2022).
[63] Y. Feng, X. Wang, Antioxidant therapies for Alzheimer's disease, Oxid. Med. Cell. Longev. 2012 (2012).
[64] J.-H. Heo, Hyon Lee, K.-M. Lee, The possible role of antioxidant vitamin C in Alzheimer's disease treatment and prevention, Am. J. Alzheimer's Dis. Other Dementias 28 (2) (2013) 120–125.
[65] M. Talebi, et al., Zingiber officinale ameliorates Alzheimer's disease and cognitive impairments: lessons from preclinical studies, Biomed. Pharmacother. 133 (2021) 111088.
[66] C. Orhan, et al., Effect of supplementing chromium histidinate and picolinate complexes along with biotin on insulin sensitivity and related metabolic indices in rats fed a high-fat diet, Food Sci. Nutr. 7 (1) (2019) 183–194.
[67] A. Aguilera-Mendez, et al., Protective effect of supplementation with biotin against high-fructose-induced metabolic syndrome in rats, Nutr. Res. (N.Y.) 57 (2018) 86–96.
[68] M.F. McCarty, J.J. DiNicolantonio, Neuroprotective potential of high-dose biotin, Med. Hypotheses 109 (2017) 145–149.
[69] D.O. Kennedy, B vitamins and the brain: mechanisms, dose and efficacy—a review, Nutrients 8 (2) (2016) 68.
[70] J.A. Hamilton, et al., Brain uptake and utilization of fatty acids, lipids and lipoproteins: application to neurological disorders, J. Mol. Neurosci. 33 (2007) 2–11.