RESEARCH PAPER

Acta Neurobiol Exp 2024, 84: 218–229
DOI: 10.55782/ane-2024-2587

N-acetylcysteine prevents hypothyroidism-induced impairment of learning and memory in adolescent male rats *via* affecting oxidative status, inflammatory response and BDNF in hippocampal tissues

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The present study was assumed that N-acetylcysteine (AC) might improve cognitive function in adolescent rats with hypothyroidism through various mechanisms. Sixty adolescent rats were randomly divided into the following groups: Vehicle (received normal saline intraperitoneally (IP)); Propylthiouracil (PTU)-induced hypothyroidism (0.05%, dissolved in drinking water); Hypothyroid rats were IP treated with different doses of AC (50, 100, and 150 mg/kg/day) for a period of six weeks; Normal rats treated with the highest doses of AC (150 mg/kg/day). Behavioral and biochemical analyses were studied for all groups. In the Morris water maze test, AC significantly reduced both the time to find the hidden platform and the distance travelled as compared to non-treated hypothyroid rats. In the passive avoidance test, the latency of entering the dark chamber was significantly increased by AC, whereas decreased the time spent in the darkroom of the chamber compared to the hypothyroid rats. In biochemical results, AC reduced both malondialdehyde content and nitrite while increased the thiol content, catalase and superoxide dismutase enzymes activity in both the cortex and the hippocampus, and a notable improvement in brain-derived neurotrophic factor (BDNF) levels in hippocampal tissues of the hypothyroid rats, while decreasing the level of interleukin-6 in rat hippocampal region. Therefore, based on the results, the beneficial effects of AC on cognitive impairment in adolescent hypothyroid rats are probably related to its anti-oxidant properties and notable improvement in BDNF levels.

Key words: hypothyroidism, N-acetylcysteine, learning and memory, BDNF, oxidative stress, interleukin-6

INTRODUCTION

Thyroid hormones (THs), including triiodothyronine (T3) and thyroxin (T4) as an important part of endocrine system are responsible for several important functions including homeostasis, growth, organismal development, and definitely essential for proper neurodevelopment (Shahid et al., 2018). In fact, thyroid hormone deficiency affects all tissues, particularly the central nervous system (CNS) in which THs are important modulators of brain metabolism (Mitchell & Klein, 2004). Adult-onset hypothyroidism may not cause concerning symptoms in the early stage of the disease. Gradually, untreated hypothyroidism can cause a number of health problems including weight

Received 6 June 2023, accepted 19 June 2024

gain, tiredness, lethargy, cold intolerance, dry skin, constipation, infertility, heart disease and minimal brain dysfunction has also been reported, however, it is not as serious as neurological impairment in children exposed to thyroid deficiency in either prenatal or childhood (Vaidya & Pearce, 2008).

In fact, most of the negative effects of hypothyroidism on cognition and memory have been attributed to biochemical and biophysical changes in the hippocampus which is considered as an important brain region involving in cognition. Even though, the exact mechanism has not been understood yet (Mulat et al., 2021). Several studies have demonstrated the possible and close relationship between thyroid hormone deficiency and antioxidant imbalance which means hypothyroidism affects antioxidant defense system in various regions of rat brain (Mishra et al., 2021; Chaalal et al., 2019). All of these events have been considered as an important factors involving learning, memory, and cognitive impairments in fetuses and neonates exposed to hypothyroidism associated with both hippocampal and cortical tissues oxidative damage (Baghcheghi et al., 2017; Khordad et al., 2018; de Souza Cardoso et al., 2021; Blas-Valdivia et al., 2021). In this regard, several studies have been associated with enhanced the expression of the nitric oxide synthase (*NOS* gene) of which leads to overproduction of nitric oxide in hypothyroidism (Barreiro Arcos et al., 2006). So, reactive nitrogen and oxygen species (RNS, ROS; respectively) have been considered as important mediators in hypothyroidism-associated neurotoxicity (Robello et al., 2016). Besides, recent studies have indicated strong relationship between activation of pro-inflammatory pathway such as interlukin-6 (IL-6) and Alzheimer's disease which means IL-6 pathway might be associated with cognition and memory impairment (Lyra e Silva et al., 2021).

Furthermore, brain-derived neurotrophic factor (BDNF), a vital protein for the proliferation, survival, and growth of neurons which has crucial role in learning and memory is reduced in hypothyroidism as well (Hryniewicz et al., 2007).

Propylthiouracil (PTU) which inhibits the enzyme thyroid peroxidase was introduced in 1947 for the treatment of hyperthyroidism and also widely used to produce hypothyroidism animal model (Zoeller & Crofton, 2005). Now, it is well known that cognitive impairment due to PTU- induced hypothyroidism is accompanied by brain tissue oxidative damage (Beheshti et al., 2017). Several studies have been reported the protecting effect of natural products on memory deficiency associated with hypothyroidism through reducing oxidative reagents in the brain tissue (Baghcheghi et al., 2018).

N-acetylcysteine (AC), N-acetyl derivative of natural amino acid (l-cysteine), as a known exogenous antioxidant, which penetrates the blood-brain barrier and breaks the disulfide bonds leading to stabilization of the extracellular proteins by which can protect brain tissues against reactive oxygen species (ROS) (Joy et al., 2019). In fact, its main biological effects are attributed to providing cysteine for glutathione (GSH) synthesis cysteine (Aldini et al., 2018; Aggarwal et al., 2022). Moreover, it has been used as antidote for acetaminophen toxicity through reduced glutathione (GSH) precursor which is a well-known endogenous antioxidant and a substrate of several antioxidant enzyme (Joy et al., 2019). It is a well-tolerated drug that is used for many neurological and mental diseases (Slattery et al., 2015).

Considering the results of our prior studies (Baghcheghi et al., 2019; Beheshti et al., 2017) have stated that one of main proposed mechanism involving in the learning and memory disorders caused by hypothyroidism is the occurrence of oxidative stress, it was assumed that AC have a strong potential to improve the cognition impairment induced by hypothyroidism. Therefore, the present study aimed to evaluate whether AC has an efficacy to improve learning and memory impairment induced by hypothyroidism in adolescent rats and also investigate the oxidative stress pathway and BDNF as an important factor demonstrating pivotal role in neuron plasticity and memory.

METHODS

Animal groups and drugs

Sixty adolescent Wistar rats, 21 days old, (weighed 50 ± 5 g) were provided from the laboratory animal centre of Mashhad University of Medical Sciences. In the following experimental study, rats (n=10 rats/group) were housed in animal cages with room condition: 12 h/12 h light/dark cycles starting at 7:00 a.m. and the environmental temperature of 25°C with unlimited access to food and water.

Animals were randomly divided into six groups including vehicle received saline intraperitoneally (IP), and five experimental groups: Vehicle: received normal drinking water; hypothyroid groups (Hypo): received 0.05% PTU dissolved in drinking water (Farrokhi et al., 2014); Hypo-AC 50, 100, 150 mg/kg: hypothyroid group received three different doses of AC 50/100/150 mg/kg IP; AC 150 mg/kg: received AC 150 mg/kg/day IP without induction of hypothyroidism.

All the vehicle and other experimental groups received the treatment for 6 consecutive weeks. After the period of the treatment, the Morris water maze (MWM) and passive avoidance (PA) tests were performed and eventually they were killed for subsequent biochemi-

cal analysis. All experimental protocols were approved by the Ethics Committee of the Torbat Heydariyeh University of Medical Sciences (permission code for this study: IR.THUMS.REC.1400.035).

Behavioral tests

MWM test

This method was applied to assess the spatial learning and memory function in rats. Basically, the test includes the "space navigation" and "space probe" steps, both of which are carried out in a water pool (150 cm in diameter, 60 cm high and water temperature: 22 ± 1°C). At the centre of this pool, 1.0 cm below the water surface, there is a platform (9 cm in diameter) which enables the swimming rat to escape. As for the test trials, "space navigation test" was done during 5 consecutive days (at 8 a.m.) including four independent trials a day. For this purpose, animal is placed into the water and the time taken for the rat to find the platform is recorded. In case it fails to find the escape platform during 60 s, the experimenter manually guides the animal to the platform and the time is reported as 60 s. In "spatial probe test" rats are allowed to have free swimming in the pool for 60 s in 4 consecutive trials and the cumulative time spent in the target quadrant is recorded.

PA test

In this experimental protocol, animals learn to avoid an environment in which they have faced an aversive stimulus. In this method, the test apparatus has two light and dark compartments separated by a guillotine door. In the first protocol, the guillotine door is open and animals can freely explore throughout the apparatus for 5 min. Then, an electric shock (2 mA, 2 s) is applied to the animals' feet once they enter the dark chamber (acquisition phase). After 1/24/48 h, animals are transferred to the light compartment. Finally, duration of delay to enter the dark section, duration of stay in darkness and number of entries into the dark section were recorded for each rat.

Biochemical assessments

At the end of behavioral experiments, animals were sacrificed by CO_2. First, blood samples were taken by cardiac puncture for subsequent analyses of thyroid function then rapidly decapitated and brains were carefully excised. Thyroid function was measured through using the radioimmunoassay method, serum T4 levels were measured by the Navid Medical Laboratory (Mashhad, Iran). Hippocampal tissues were dissected and kept frozen for biochemical sassy. Biological indicators of oxidative stress including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and total thiol content were measured. In addition, the tissue levels of IL-6, BDNF and nitrite level were determined.

Measurement of MDA, nitrite and thiol concentrations plus SOD and CAT activities hippocampal concentration of MDA, as a marker of lipid peroxidation, was measured as described previously. MDA reacts with thiobarbituric acid (TBA) and constitutes a red chemical complex. Nitrite concentration, as another pro-oxidant marker, was measured by Griess reagent protocol. In brief, 100 µl supernatant was mixed with Griess reagent; then, this combination was transferred to a microplate and the absorbance rate was read at 520 nm. Finally, the obtained values were calculated from the standard calibration plot (Baradaran et al., 2021). Furthermore, total thiol content was measured using a method in which the reaction occurs between DTNB (2,2'-dinitro-5,5'-dithiolbenzoic acid) and thiol groups to form a yellow chemical substance. At the final step, the absorbance index was obtained at λ=412 nm. Activity of SOD and CAT enzymes was quantified using a previously established method (Azizi-Malekabadi et al., 2018). In brief, SOD activity was measured at 570 nm based on a colorimetric technique (Madesh & Balasubramanian, 1998). In this regard, one unit of SOD equals to the amount of enzyme that should be suppressed by 50% of the MTT reduction rate. Moreover, Aebi protocol was applied to assess the CAT enzymatic activity in which hydrogen peroxide (30 mM) acts as a substrate (Aebi et al., 1976; Asgharzadeh et al., 2018).

Measurement of BDNF and IL-6

Hippocampal levels of BDNF and IL-6 were all quantified by rat ELISA kits (IBL International, Hamburg, Germany and MyBioSource, San Diego, CA, USA), according to the instructions provided by the manufacturer. The absorption rates were recorded by a micro plate reader (Biotech, USA) and results were assessed *versus* the standard curve for the same experiment (Abareshi et al., 2017).

Statistical analysis

The raw data were transferred to GraphPad Prism software (version 6) for statistical analyses, one way

ANOVA (time spent in target quadrant in MWM test and biochemical parameters) and two-way ANOVA (time to find the platform in MWM test and PA test) were applied followed by Tukey's *post hoc* tests. Results were expressed as means ± standard error of the mean (SEM and an alpha (α) at 95% confidence interval (i.e., p=0.05) was considered statistically significant.

RESULTS

The effect of AC treatment on serum T4 level

Significant reduction of serum T4 levels ($F_{(5,54)}$=19.17, P<0.001) in adolescent rats was demonstrated in six-week administration of 0.05% PTU. Hypothyroid groups receiving daily dosage administration of AC (50 and 100 mg/kg) had no significant effects on serum T4 levels, while administration of AC (150 mg/kg) was significantly increased T4 levels in the hypothyroid rats (P<0.001), and approximately similar to normal limit of vehicle (P<0.05). So, the highest dose of AC (150 mg/kg) significantly increased serum T4 level close to the expected reference range compared to groups received dose of AC at 50 and 100 mg/kg (P<0.001 and P<0.05, respectively).

Behavioral findings

The negative effect of hypothyroidism on spatial memory was reversed by AC

The results of Two-way ANOVA revealed a significant effect for the group ($F_{(5,1050)}$=17.3, P<0.001) and day ($F_{(5,1050)}$=19.431; P< 0.001). There was no significant interaction between the factors (group × day) $F_{(20,1050)}$=0.532, P=0.9541) in the 5 days of learning. Furthermore, the *post hoc* test results illustrated significant increase of time spent to locate the hidden platform during the five days of learning trials in MWM in hypothyroid rats (P<0.05 – P<0.001; Fig. 2A). Treatment with 150 mg/kg AC significantly attenuated the effect of hypothyroidism which was evident by the less time spent to locate the platform during the learning trials compared to the hypothyroid rats (P<0.05; Fig. 2A). However, there was insignificant difference among different doses of AC.

The results of the probe trial showed that hypothyroidism reduced the target quadrant exploration time compared to the vehicle group ($F_{(5,218)}$=5.235, P<0.001; Fig. 2B), whereas treatment of the hypothyroid rats with 100 and 150 mg/kg doses of AC increased the searching time by rats in the target quadrant compared to the Hypothyroid group (P<0.05, P<0.01; Fig. 2B).

The negative effect of hypothyroidism on non-spatial memory was reversed by AC

It was realized from the two-way ANOVA that the time after the shock did not affect the delay time ($F_{(3,216)}$=66.64; P=0.064). The results also revealed significant effect for group ($F_{(5,216)}$=20.48, P<0.001). Insignificant interaction was observed between the ac-

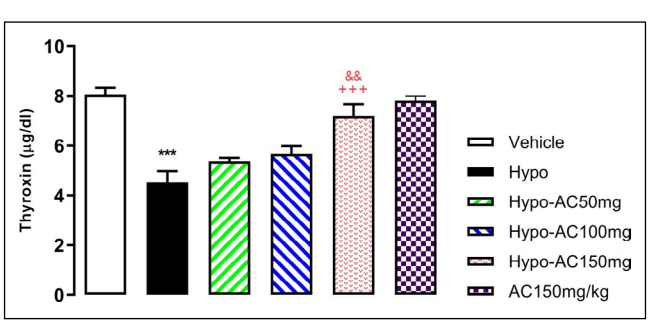

Fig. 1. The thyroxin concentration in the serum (n=10 rats/group). ***P<0.001 *vs.* Vehicle group, $^{+++}$P<0.001 *vs.* Hypothyroid group, $^{\&\&}$P<0.01 *vs.* Hypothyroid-AC50 group.

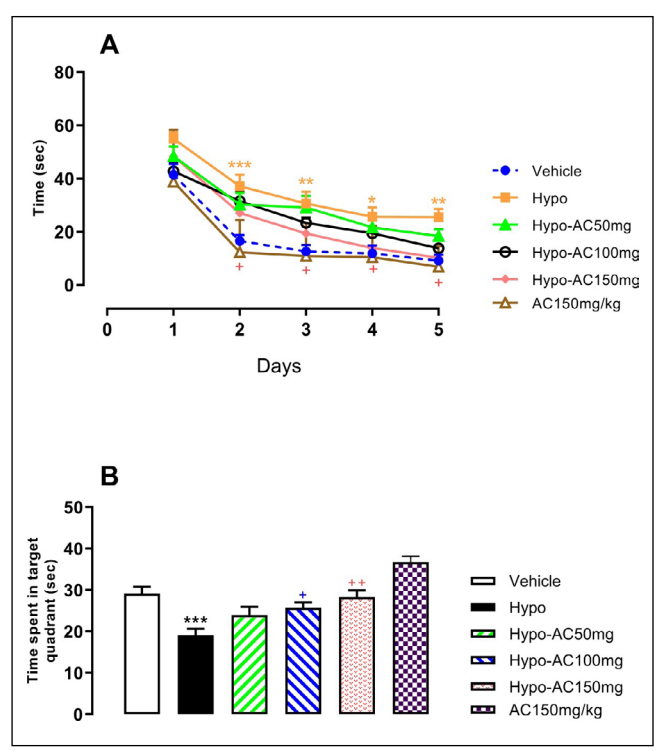

Fig. 2. The time spent (A) and time spent the distance in the target quadrant (B) of the MWM (*n*=10 rats/group). *P<0.05, **P<0.01 and ***P<0.001 *vs.* Vehicle group, $^{+}$P<0.05 and $^{++}$P<0.01 *vs.* Hypothyroid group.

tors (group × the time after the shock) ($F_{(15,216)}$=2.442, P=0.087). Animals in hypothyroid group spent lower time to enter to dark chamber as compared to vehicle (P<0.001; Fig. 3A). All hypothyroid groups treated with 100, 150 mg/kg of AC, the delay time to enter the dark compartment of the PA apparatus was lower than the hypothyroid group at 1 h, 24 h, and 48 h post-shock delivery (P<0.05, P<0.01, P<0.001; Fig. 3A). Treatment of hypothyroid rats with 150 mg/kg AC showed significant difference as compared to hypo-AC50 group (P<0.01 and P<0.001; Fig. 3A). Interestingly, groups only received 150 mg/kg dose of AC increased this time *versus* the vehicle at 1, 24, and 48 h after the shock (P<0.05, P<0.001, and P<0.001 respectively; Fig. 3A).

The results of the two-way ANOVA model demonstrated that the time spent in dark chamber after the shock did not affect the delay time ($F_{(2,162)}$=2.110; P=0.1272). The results revealed significant effect for group ($F_{(5,216)}$=64.49, P<0.001) as well. No significant interaction was observed between the actors (group × the time after the shock) ($F_{(10,162)}$=1.095, P=0.3749). Hypothyroidism also increased the amount of time spent in the dark part of the PA apparatus at 1, 24, and 48 h after the shock delivery compared to the vehicle group (P<0.001 for all; Fig. 3B). The doses of 100 and 150 mg/kg of AC significantly decreased this time at 1 h, 24 h, and 48 h as compared to the hypothyroid group (P<0.05 and P<0.001; Fig. 3B). Treatment of hypothyroid rats with 150 mg/kg AC showed significant difference *versus* hypo-AC50 group (P<0.01 and P<0.001; Fig. 3A).

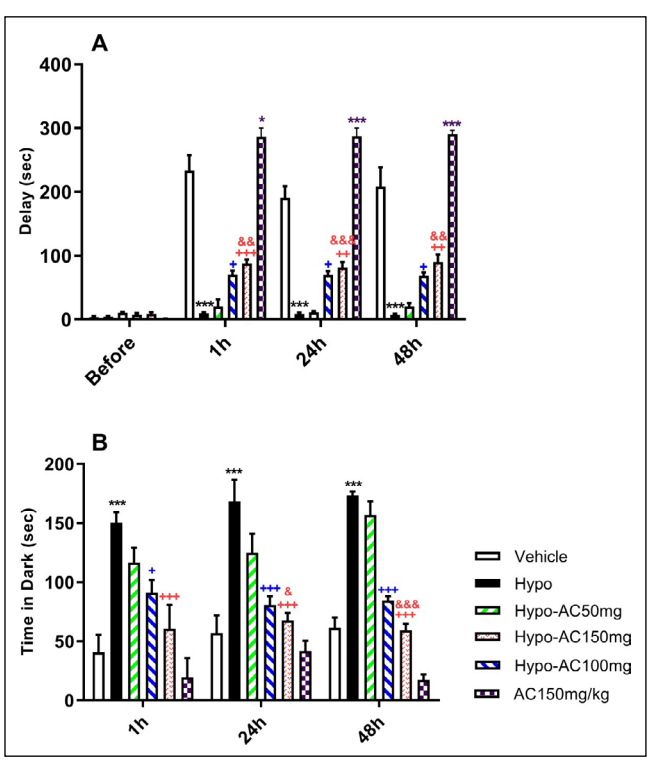

Fig. 3. The latency time to enter the darkroom (A) and the time spent in the darkroom (B) in the passive avoidance test (n=10 rats/group). ***P<0.001 *vs.* Vehicle group, $^{+}$P<0.05 and $^{+++}$P<0.001 *vs.* Hypothyroid group, $^{\&}$P<0.05, $^{\&\&}$P<0.01 and $^{\&\&\&}$P<0.001 *vs.* Hypothyroid-AC50 group.

Biochemical findings

The effects of AC on hippocampal oxidative stress indicators and nitrite

Based on the results, both MDA and nitrite levels in the hippocampus of the hypothyroid rats were higher than vehicle ($F_{(5,54)}$=42.82, P<0.001; Fig. 4A). Although, the hippocampal concentrations of MDA in hypo-AC groups 150 mg/kg were lower than that in the Hypo group (P<0.001; Fig. 4A). Treatment by AC150 mg/kg decreased MDA concentration of hippocampal tissues of hypothyroid rats compared to hypo-AC50 group (P<0.01 and P<0.001; Fig. 4A). Nitrite levels in both hypothyroid rats treated with AC (100 and 150 mg/kg) was lower than non-treated hypothyroid group ($F_{(5,54)}$=33.55, P<0.01 and P<0.001; Fig. 4B). So, nitrite levels in hypothyroid rats received 150 mg/kg dose of AC were higher than that of rats in hypo-AC50 group (P<0.001; Fig. 4B).

Hypothyroidism status was also accompanied by decreased levels of thiol concentration, CAT and SOD activities in the hippocampal tissue of the hypothyroid rats compared to vehicle ($F_{(5,54)}$=177.6, P<0.001 for thiol, $F_{(5,54)}$=25.11, P<0.001 for SOD and $F_{(5,54)}$=161.8, P<0.001 for CAT; Fig, 5A-C, respectively). There was significant difference between thiol content of hippocampal tissues of groups only treated with AC at dose of 150 mg/kg compared to hypo group (P<0.001; Fig. 5A). Therefore, even the administration of AC without induction of hypothyroidism could have a beneficial effect on thiol concentration and CAT and SOD activities (P<0.01 and P<0.001; Fig. 5). Total thiol content, CAT and SOD activity in the hippocampal tissues of the Hypothyroid-AC group received 150 mg/kg was significantly higher than that of Hypothyroid, Hypothyroid-AC received at different dose of 50 mg/kg, and 100 mg/kg (P<0.05, P<0.01, P<0.001).

Treatment with 50 mg/kg, and 100 mg/kg doses of AC were not able to improve thiol content and SOD activity. However, cortical CAT activity in the hypothyroid-AC groups treated with 50 mg/kg and 100 mg/kg revealed significant difference compared to hypothyroid group (P<0.05, P<0.001 respectively).

The effects of AC on oxidative stress indicators and nitrite in the cortex

In the hypothyroid group compared to vehicle, a considerable increase in the hippocampal MDA and nitrite metabolite content was observed ($F_{(5,54)}$=46.33, P<0.001 for MDA, $F_{(5,54)}$=9.441, P<0.001 for nitrite; Fig. 6A, 6B). Significant reduction in MDA and nitrite concentrations were resulted in cortex of rats treated with 150 mg/kg dose of AC compared to hypothyroid group (P<0.001; Fig. 6A), while there was no significant difference in groups received 50 mg/kg dose of AC for both MDA and nitrite metabolite. Also, hypothyroid-AC at dose of 100 mg/kg showed significant decrease in ni-

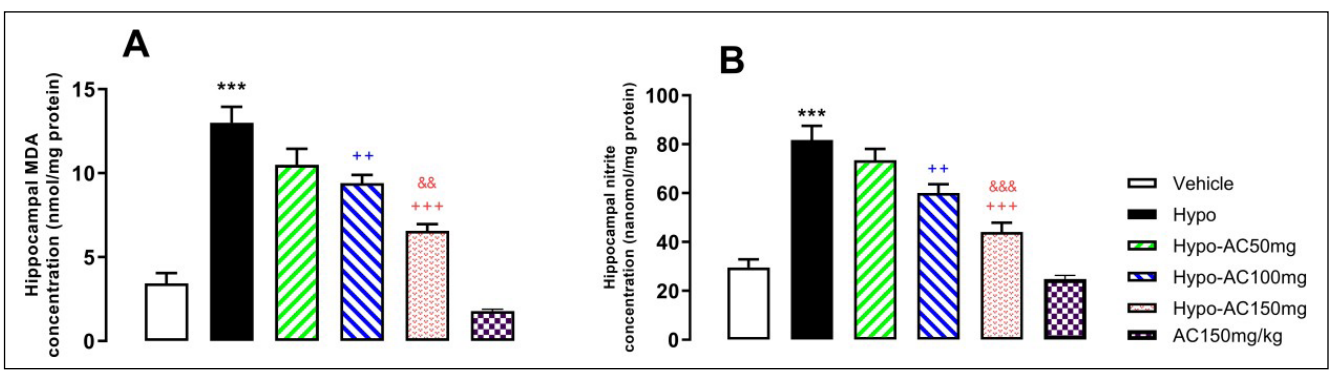

Fig. 4. The MDA concentration (A) and nitrite metabolites concentration (B) in the hippocampus (*n*=10 rats/group). **P<0.01 and ***P<0.001 *vs.* Vehicle group, $^{++}$P<0.01 and $^{+++}$P<0.001 *vs.* Hypothyroid group, $^{\&}$P<0.05 and $^{\&\&\&}$P<0.001 *vs.* Hypothyroid-AC50 group.

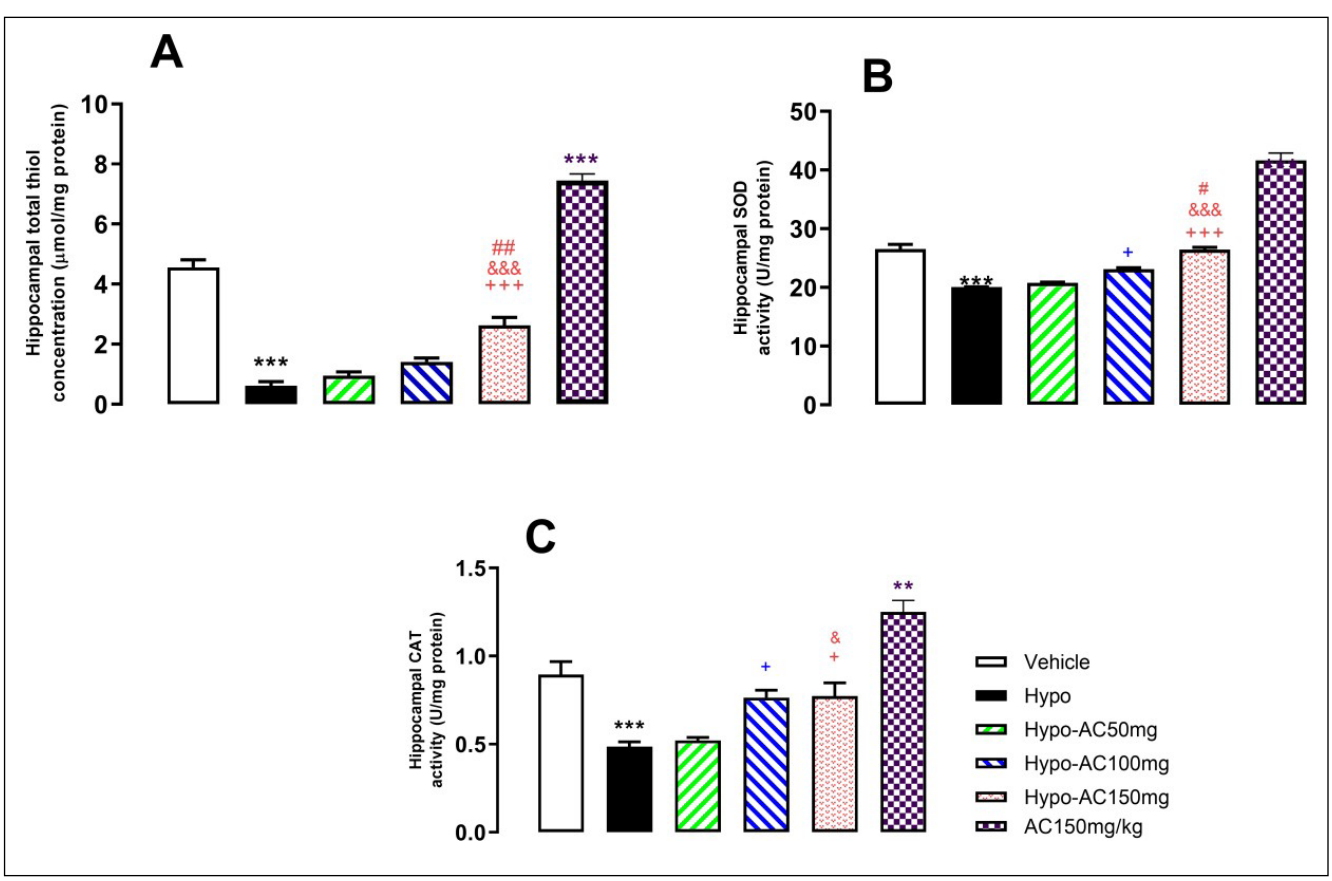

Fig. 5. The thiol content (A) and SOD activity (B), and CAT activity (C) in the hippocampus (*n*=10 rats/group). ***P<0.001 *vs.* Vehicle group, $^{+++}$P<0.001 *vs.* Hypothyroid group, $^{\&\&}$P<0.01 and $^{\&\&\&}$P<0.001 *vs.* Hypothyroid-AC50 group, $^{\#}$P<0.05 and $^{\#\#}$P<0.01 *vs.* Hypothyroid-AC100 group.

trite metabolite (P<0.001; Fig. 6B), whereas there was no effective decrease in MDA content.

As Fig. 7 shows, a decreased levels of cortical thiol content and the activities of SOD and CAT were detected in hypothyroid rats compared to the vehicle ($F_{(5,54)}$=23.68, P<0.001 for thiol, $F_{(5,54)}$=89.76, P<0.001 for SOD and $F_{(5,54)}$=19.61, P<0.001 for CAT; Fig. 7A-C respectively). While increased level of thiol content, SOD and CAT activity were resulted in hypothyroid groups treated with 150 mg/kg of AC (P<0.001, P<0.05, P <0.001 respectively; Fig. 7). Interestingly, the hippocampal thiol content, SOD and CAT activity also increased in groups received only AC compared to vehicle (P<0.001; Fig. 7). Treatment with 50 mg/kg of AC was not able to improve

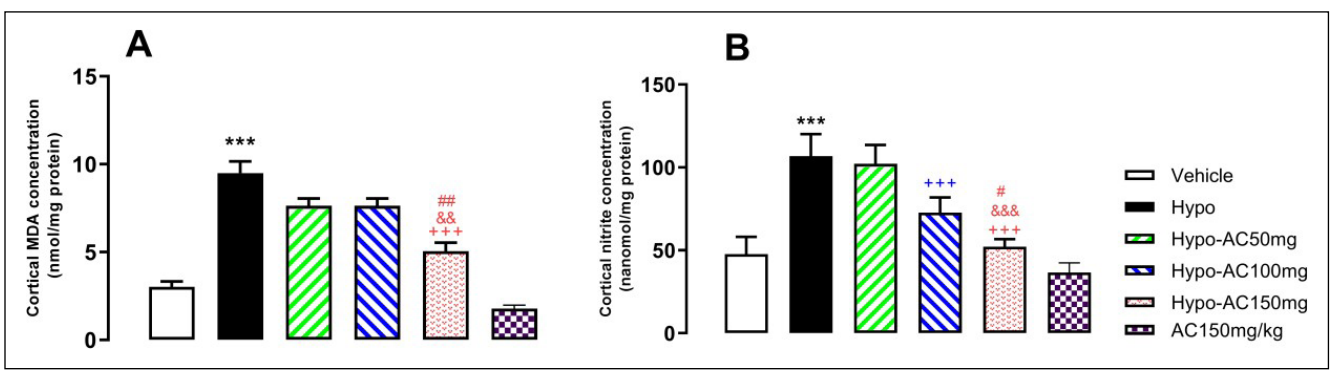

Fig. 6. The MDA concentration (A) and nitrite concentration (B) in the cortex (*n*=10 rats/group). *P<0.05, **P<0.01 and ***P<0.001 *vs.* Vehicle group, $^{+++}$P<0.001 *vs.* Hypothyroid group, $^{\$\$\$}$P<0.001 *vs.* Hypothyroid-Cur 50 group, $^{\&\&\&}$P<0.001 *vs.* Hypothyroid-AC50 group, $^{\#}$P<0.05 and $^{\#\#}$P<0.01 *vs.* Hypothyroid-AC100 group.

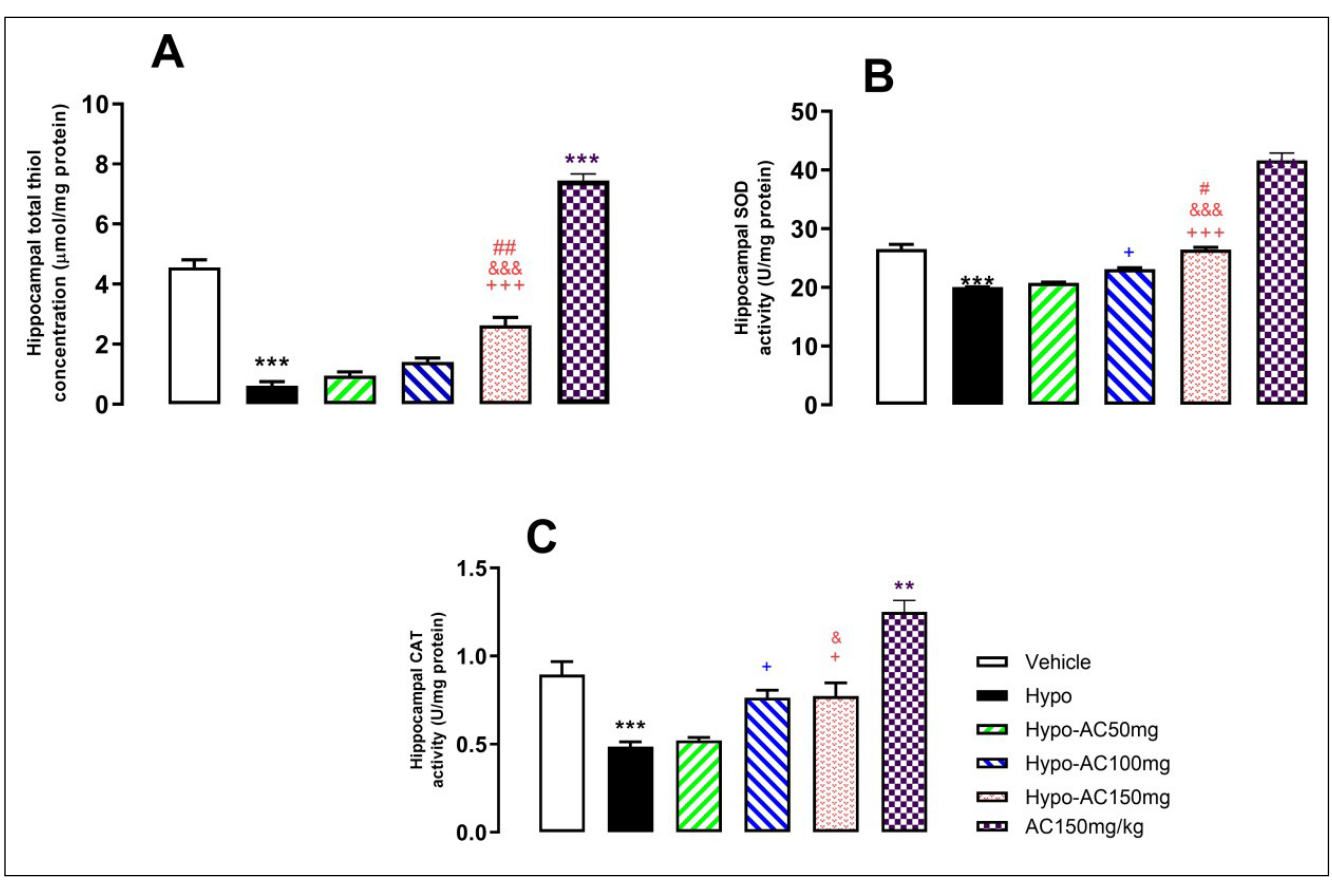

Fig. 7. The thiol content (A) and catalase (CAT) activity (B), and superoxide dismutase (SOD) activity (C) in the cortex (*n*=10 rats/group). **P<0.01 and ***P<0.001 *vs.* Vehicle group, $^{+}$P<0.05 and $^{+++}$P<0.001 *vs.* Hypothyroid group, $^{\&\&\&}$P<0.001 *vs.* Hypothyroid-AC50group, $^{\#\#\#}$P<0.001 *vs.* Hypothyroid-AC100 group.

thiol content and CAT activity. However, cortical CAT activity in the Hypothyroid-AC groups treated with 50 and 100 mg/kg of AC revealed significant difference compared to hypothyroid group (P<0.05 and P<0.0501 respectively; Fig. 7C).

Treatment with AC improved the hippocampal BDNF levels and IL-6

BDNF levels in the hippocampus of the hypothyroid rats were significantly lower and IL-6 was higher than the vehicle group ($F_{(5,54)}$=31.79, P<0.001 for BDNF and $F_{(5,54)}$=15.49, P<0.001 for IL-6; Fig. 8). Treatment by AC 150 mg/kg decreased IL-6 level in hippocampal tissues of animals compared to all another hypo groups (P<0.05 and P<0.001; Fig. 8A). Treatment of the animals with two higher doses of AC (100 and 150 mg/kg) increased BDNF concentration in the hippocampus compared to Hypothyroid and Hypothyroid rats received 50 mg/kg of AC (P<0.001; Fig. 8B).

DISCUSSION

Regarding our findings, the primary null hypothesis seems to be accepted. Based on the results of the current study, it is expected that AC have beneficial effects on memory and is able to improve behavioral abnormalities including learning and memory impairments associated with hypothyroidism in adolescent male rats and interestingly showed positive response in normal groups in which received only AC compared to vehicle. In this study, according to the previous studies that have been done in this field, we conducted the study on male offspring (Baghcheghi et al., 2022; Ahmadabady et al., 2022). In biochemical results, treatment with AC indicated significant reduction in damaging factors production including NO, MDA, and IL-6, while improving thiol content, CAT and SOD activities in both cortex and hippocampus. In addition, administration of AC was accompanied by an improvement in BDNF in the hippocampus.

To induce hypothyroidism and thyroid dysfunction in animal model, the administration of 0.05% PTU in drinking water for a period of 6 weeks led to significant decrease in serum T4 levels (Kempers et al., 2006). It has been stated that hypothyroidism can cause Alzheimer's disease (Bavarsad et al., 2019; 2020). In current study we showed that PTU induced reduction in T4 level in Fig. 1. To the best author's knowledge, however, there has been no study in this field, it is suggested that the possible mechanism of increasing the amount of T4 by receiving AC is might be due to the antioxidant effects of this drug on the thyroid gland and modulating the enzymes involved in production of thyroxin, which is in response to the effect of improving the oxidative damage of the thyroid tissue, by which leads to hormone enhancement. Actually, the disrupted performance of hypothyroid rats during the five days of learning trials of the MWM was confirmed the adverse impacts of hypothyroidism. Also, on the probe day of MWM, the hypothyroid rats did not remember the platform's location as indicated by a shorter delay. In PA test, thyroid dysfunction in rats led to increase not only the frequency of entry into the darkroom, but a longer time also spent in the dark chamber. Numerous studies confirmed PTU-induced hypothyroidism led to spatial learning and memory impairment particularly in early developmental periods as seen in the present study (Baghcheghi et al., 2019; Memarpour et al., 2020).

Several studies demonstrated thyroid hormone deficiency negatively affects neurogenesis (Khordad, Alipour 2018, Asiaei et al., 2017, Rastegar-Moghaddam et al., 2022) and synaptic plasticity and impaired learning and memory which is attributed to many reasons including overproduction of NO (Hosseini et

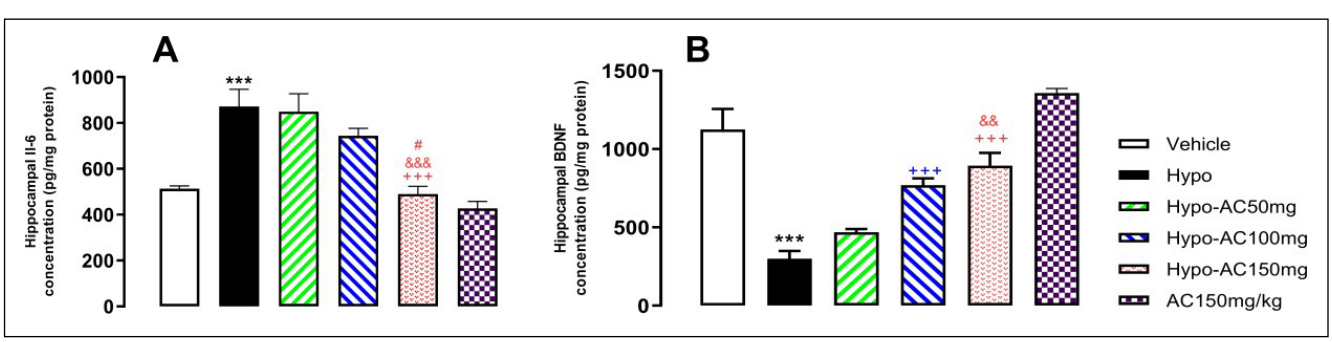

Fig. 8. The IL-6 (A) and BDNF (B) levels (*n*=10 rats/ group). ***P<0.001 *vs.* Vehicle group, $^{+++}$P<0.001 *vs.* Hypothyroid group, $^{\&\&}$P<0.01 and $^{\&\&\&}$P<0.001 *vs.* Hypothyroid-AC50 group, $^{\#}$P<0.05 *vs.* Hypothyroid-AC100 group.

al., 2010), free radicals, IL-6 and reduction of some protective factors such as BDNF (Tanaka et al., 2019). In fact, NO itself or through other pernicious factors such as free radicals, IL-6 might participate in brain damage of which occurred in hypothyroidism. Although the exact mechanism still is unknown. The current results are also in agreement with previous studies, which indicated elevated levels of NO, MDA in cortical and hippocampal, while decreased level of thiol, SOD, CAT activity were reported. Similarly, a significant increase in oxidative stress markers in the brain tissue has been reported in hypothyroid rats (Alikhani et al., 2020). It has also been previously reported that thyroidectomy- induced hypothyroidism increased oxidative stress parameters through elevation of ROS and nitrite production in the brain (Torres-Manzo et al., 2018). Other mechanisms can also be involved that hypothyroidism can cause learning and memory disorders by increasing the activity of 2022, Amirahmadi et al., 2021). In addition, there are many related studies about excessive activity of NOS in hypothyroid rats and this might be one of the probable mechanisms leads to increase in nitrite level in this study (Cano-Europa et al., 2008, Alva-Sánchez et al., 2009, Sánchez-Huerta et al. 2012).

Furthermore, we showed that hypothyroidism increased the level of IL-6 in hippocampal tissues. In this regard, some studies have been conducted the association between activation of pro inflammatory cytokine including IL-6 and neurodegenerative disease such as Alzheimer and other disorder related to memory and cognition dysfunction which showed the pathological role of IL-6 in the central nervous system (Lyra e Silva et al., 2021; Braida et al., 2004). Considering the importance of the hippocampus in memory, the present study was focused on assessing the effect of AC on BDNF in the hippocampus tissue. The present findings demonstrated decreased level of BDNF which is consistent with numerous research studies have been associated with learning and memory disorders and reduction of BDNF levels (Bortolotto et al., 2021, Madhusudhan et al., 2022).

Regarding this evidence which confirmed the pivotal role of oxidative stress in hypothyroidism-induced learning and memory impairment, it is expected that AC as a well-known exogenous antioxidant agent, may have protective effects. The results of the current study are well illustrated that AC not only in hypothyroid groups, but when it also administers in normal rats improved performance on the MWM. In the rats treated with AC, especially with 100 and 150 mg/kg, the latency of finding the hidden platform was significantly decreased as compared to hypothyroid group. Furthermore, in the probe test, the rats receiving 100 and 150 mg/kg of AC could remember the target quadrant better, in addition to spending more time in the target area in which the platform was previously located. Beside, in the PA test, administration of AC (100, 150 mg/kg) increased the latency of entry while reduced the time spent in the dark-room. The results showed that AC at doses of 100, and 150 mg/kg were more profoundly improved learning and memory compared to AC at dose of 50 mg/kg. Considering the results of PA and MWM tests, it seems that the effect of AC on the performance of the rats on learning and memory was dose-dependent. Similar to the present findings, the best dose of AC for neuroprotective was also reported 100 and 150 mg/kg which is consistent with our results (Rodrigues et al., 2013).

In the current study, AC administration was accompanied by a decrease in both hippocampal and cortical MDA and an increase in both thiol content and activities of CAT and SOD. Interestingly, administration of AC in normal rats also showed better results compared to vehicle. According to known mechanism of AC on reduction of glutathione as endogenous antioxidant, the protective effects of AC on CNS functions, including learning and memory, have been suggested to be related to its protection against brain tissue oxidative damage (Banji et al., 2014; Sevastre-Berghian & Făgărăsan 2017; Kaushal et al., 2020). Thus, it seems that the improving effects of AC on learning and memory are attributed to its antioxidant and anti-inflammatory effects which can be suggested to use AC as neuroprotective agent. Similar to our results, it was previously reported the association between AC which promoted cognitive health and dementia through increasing GSH levels.

Furthermore, IL-6 as a pro-inflammatory cytokine and BDNF levels were assessed as well. AC has been able to balance the oxidative stress mediators over antioxidant indicators along with reduction of inflammation caused by hypothyroidism through reducing IL-6 and more importantly BDNF levels was improved. In line with our results, it has been shown that AC can improve cognitive disorders caused by psychological diseases like schizophrenia by increasing the BDNF levels alongside the inevitable role of that in memory of which the reduced level of that associated with progression of neurodegenerative diseases like Alzheimer's disease (Bühner et al., 2022). In consistent with the present findings, AC was able to reduce inflammation caused by cisplatin as a known chemotherapy treatment by reducing the IL-6 levels (Abdel-Wahab & Moussa 2019). To the best of our knowledge, the effects of AC on hypothyroidism-associated learning and memory impairments have not

been previously reported to compare with the findings of the present research (Rodrigues et al., 2013).

One of the limitations of this study is that it might be better to study in both male and female animals. These issues should be taken into account in future studies in the same condition. Another limitations was associated with lack of considering the other doses of AC (50 and 150 mg/kg) to administer alone in normal rats which must be considered in future researches to assess not only their effect but also compare the results with the highest dose of AC to discuss more confidently.

CONCLUSION

The results of this study showed that AC can have a beneficial effect on the learning and memory impairment caused by hypothyroidism in adolescent rats, probably through the improvement of hippocampal BDNF and reduction of oxidative agents and could be introduced as a new emerging candidate for diseases related to cognitive impairment.

ACKNOWLEDGMENTS

The authors would like to thank the Student Research Committee of Torbat Heydariyeh University of Medical Sciences (No: SRC-1400-112) and the Vice-Chancellery for Research and Education of Torbat Heydariyeh University of Medical Sciences for providing financial support.

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