Folic Acid Attenuated Learning and Memory Impairment via Inhibition of Oxidative Damage and Acetylcholinesterase Activity in Hypothyroid Rats

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Abstract

Hypothyroidism has been associated with cognitive decline. Considering the role that has been suggested for folic acid (FA) in cognitive performance, the present study was designed to investigate the effects of FA against hypothyroidism-induced cognitive impairment, oxidative damage and acetylcholinesterase (AChE) activity alterations in propylthiouracil (PTU)-induced hypothyroid rats.

In this study, PTU (0.05% in drinking water) and FA (5, 10, and 15 mg/kg, oral gavage) were administered to the rats for a period of 7 weeks. Then, behavioral performance was tested using Morris water maze (MWM) and passive avoidance (PA) tasks. Finally, oxidative stress indicators and AChE activity were assayed in the brain tissues.

The impairing effect of hypothyroidism on cognitive performance was markedly alleviated by FA especially at the higher doses. In the MWM test, FA reduced escape latency and travelled distance, compared to the non-treated hypothyroid group. In the PA test, the latency to enter the dark chamber was significantly enhanced by FA as compared to the non-treated hypothyroid group ($p < 0.05$-$p < 0.001$). Besides, FA attenuated AChE activity and malondialdehyde level but increased superoxidase dismutase enzyme activity and total thiol content ($p < 0.05$-$p < 0.001$).

In conclusion, FA could improve learning and memory ability in hypothyroid rats. The observed protective effects may be mediated through regulation of oxidative stress and AChE activity.

Introduction

Thyroid hormones (THs) including thyroxine (T4) and triiodothyronine (T3) are of great importance for neuronal developmental stages and play a key role in neurons maturation, migration, differentiation, and signaling. THs-related disorders during development and adulthood can lead to serious clinical problems including cognitive dysfunction (Bernal 2007). Since concentrations of THs decrease with increasing age, hypothyroidism is common among elderlies and can lead to symptoms of severe cognitive impairment, including deterioration of learning and memory, perception, reasoning, problem solving, decision making and language by affecting energy consumption processes such as glucose metabolism, which is necessary for neurotransmission (Bégin et al. 2008; Annerbo and Lökk 2013). THs receptors are abundantly found in the hippocampus. Therefore, THs deficiency can lead to structural and physiological changes as well as a reduction in hippocampal cells growth and their numbers (Cooke et al. 2014). In addition, THs deficiency may lead to an imbalance between oxidants and antioxidants, which can result in an increased level of reactive oxygen species (ROS) (Masullo et al. 2018). Besides increased production of free radicals, hypothyroidism may lead to a decrease in the capacity of antioxidant defense system (Chakrabarti et al. 2016). Studies show that there is a strong association between THs activity and the function of neurotransmitter systems especially the cholinergic system (Fu et al. 2014; Wang et al. 2015). Clinical and experimental reports suggest that THs replacement therapy can partially reverse negative effects of hypothyroidism on learning and memory, but a full recovery of T3 content in target

tissues, especially in the brain, is far from reach and therefore, more appropriate treatments are required (Chaalal et al. 2019). It has been reported that antioxidant agents are able to decrease the side effects of hypothyroidism on learning and memory (Asiaei et al. 2017; Beheshti et al. 2017; Baghcheghi et al. 2018a; Khordad et al. 2018; Baghcheghi et al. 2020). In addition, some of the vitamins have been shown to have beneficial effects on hypothyroidism-related cognitive dysfunctions (Beheshti et al. 2017; Baghcheghi et al. 2018a; Baghcheghi et al. 2020).

Folic acid (FA) is a member of vitamin B family that plays an important role in cognitive activity by increasing the level of vitamins B12 and B6. Deficiency in folate and vitamin B12 may increase the risk of dementia and memory impairment (Ma et al. 2016). Clinical evidence showed that approximately two-thirds of patients with anemia or folate and vitamin B12 deficiency have cognitive impairment (Reynolds 2002). Homocysteine is a sulfur amino acid and its blood level controlled by FA, and vitamins B12 and B6 (Modaghegh et al. 2016). However, a direct relationship exists between increased homocysteine levels and cognitive impairment (Garcia and Zanibbi 2004). FA deficiency increases homocysteine levels, resulting in DNA damage and apoptosis in the hippocampal neurons. Therefore, FA administration can improve cognitive function by controlling homocysteine levels and acting as an effective antioxidant (Singh et al. 2011). Moreover, studies show that FA improves both short-term and long-term memory (Shooshtari et al. 2012). Administration of FA in hypothyroid rats improved oxidative stress and hypothalamic monoamines (Ibrahim et al. 2012). In addition, folate is metabolically bound to choline and is involved in the synthesis and release of acetylcholine (Crivello et al. 2010). Folate deprivation genetically or in the diet, reduces acetylcholine levels and can affect cognitive activity (Chan et al. 2008).

Given that hypothyroidism is one of the most common diseases that leads to cognitive impairment and since folate deficiency is one of the most important dietary health problems worldwide, this study evaluated the effect of FA on learning and memory, oxidative stress indicators and acetylcholine esterase (AChE) activity in hypothyroid rats.

Materials And Methods

Chemicals

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT), acetylthiocholine iodide and propylthiouracil (PTU) were purchased from Sigma (St. Louis, USA).

5,5´-Dithiobis-2-nitrobenzoic acid (DTNB), 2-thiobarbituric acid (TBA), hydrochloric acid (HCl), trichloroacetic acid (TCA), ethylenediamine tetra acetic acid disodium salt (Na2EDTA), tris (hydroxymethyl) aminomethane (Trizma base), phosphate-buffered saline (PBS), and dimethyl sulfoxide (DMSO) were purchased from Merck (Darmstadt, Germany).

Animalsand experimental design

In the present study, 50 male juvenile (21-22 days old) Wistar rats weighing 50-55 g were used. Animals were kept and treated under standard conditions (with 12h light: dark cycle at 24 ± 2 °C) and they had free access to food and water. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by Ethics Committee on Animal Research of Mashhad University of Medical Sciences (Approval No. IR.MUMS.MEDICAL.REC.1399.639).

The animals were randomly divided into 5 groups including: 1) Control group in which the rats received normal drinking water and vehicle instead of FA, 2) Hypothyroid group in which PTU was added to the drinking water and the rats received vehicle instead of FA. Groups 3, 4 and 5 included Hypothyroid - FA 5, Hypothyroid - FA 10 and Hypothyroid - FA 15 groups which received PTU in their drinking water and were respectively treated with 5, 10 or 15 mg/kg FA, by gavage (Singh et al. 2011; Shooshtari et al. 2012). PTU was daily added to the drinking water and treatment by different doses of FA was carried out orally once a day for 7 weeks. Then, behavioral tests were then done and finally, the blood samples were collected and the animals' brains were removed under deep anesthesia induced using ketamine and xylazine. The serum samples separated from the blood, and hippocampus and cortex samples separated from the brains were kept at -80 ° C until biochemical tests.

Morris water maze test (MWM)

A water maze test was used to assess spatial learning and memory. The test uses a circular black pool (136 cm in diameter, 60 cm high and 30 cm deep) filled with water (22–24°C) that has an escape platform (10 cm in diameter and 28 cm high). The pool was divided into the following four zones/quadrants: north, south, east and west. The platform was located in the center of the southwest quadrant. To help the animals’ navigation, visual cues were placed around the apparatus. The experiments were performed on 5 consecutive days, each day four trials. In each trial, the animal was randomly located into the water tank and allowed to find the platform. After finding the platform, it was allowed to stay on the platform for 15 seconds. If the animal was not able to find the platform during 60 seconds, it was located on the platform by the experimenter and allowed to stay on it for 15 seconds. The animals rested outside the apparatus for 20 seconds between each trail. The time latency to find the platform and the length of the swimming path were recorded by a video tracking system. On the sixth day, the probe test was performed in such a way that the platform was removed and the rat was allowed to swim for 60 seconds. The time spent and distance traveled in the target quadrant were recorded (Beheshti et al. 2017; Baghcheghi et al. 2018a; Baghcheghi et al. 2018b).

Passive avoidance test

The passive avoidance (PA) test helps to study non-spatial memory. In this test, an apparatus containing two chambers, one dark and one light, which are separated by a small guillotine door, was used. The floor of the dark chamber is covered with steel bars at a distance of 1 cm. An electric shock is applied to these bars by a stimulator. The experiment was performed in three phases: 1) habituation phase where the

o consecutive days (each day for 5 minutes) and allowed to

move freely between the two chambers; 2) training phase where the animals were placed in the light chamber and 20 seconds later, the guillotine door was opened. As soon as the animal entered the dark room, the door was closed and an electric shock (2 mA for two seconds) was applied to the animal's feet; and 3) retention phase, done 3, 24, 48 and 72 hours after the training phase, where the animals were placed in the light chamber, the guillotine door was opened, and the time latency in entering the dark compartment, the time spent in the light and dark compartments and the frequency of entering into the dark compartment were recorded (Beheshti et al. 2017; Baghcheghi et al. 2018a; Baghcheghi et al. 2018b).

Biochemical tests

The blood samples were centrifuged at 500 *g* for 10 min and the obtained serums were used for measuring the levels of thyroxin. Serum thyroxin level was measured using a radioimmunoassay method in Navid Medical laboratory, Mashhad, Iran. The homogenates of cerebral cortex and hippocampus (10 % w/v) were prepared in ice-cold PBS (0.1 M, pH 7.4). The homogenates were then centrifuged at 4 °C, at 10000×g to separate the supernatants for estimation of malondialdehyde (MDA) and total thiol concentration as well as superoxide dismutase (SOD) and AChE activities.

Measurement of MDA and total thiol concentration

As a marker of lipid peroxidation, MDA was measured in the hippocampus and cortex. Briefly, one milliliter of each sample was added to 2 ml of TBA/TCA/HCl reagent and the reaction mixtures were incubated in a boiling bath for 45 min. After cooling, the whole solutions were centrifuged at 1000 g for 10 min. Finally, the supernatants were collected and the absorbance of the pink chromogen was measured at 535 nm using a spectrophotometer (Beheshti et al. 2017; Baghcheghi et al. 2018a; Baghcheghi et al. 2018b). The MDA concentration was calculated by the following equation:

$$C\ (M) = \text{Absorbance}/\ (1.56 \times 10^5)$$

To measure thiol content, DTNB was used. In this assay, 50 µl of the homogenates was added to 1 ml of tris-EDTA buffer (pH 8.6) and the first absorbance (A1) was recorded at 412 nm using a spectrophotometer. Afterwards, 20 µl DTNB solution (10 mM in methanol) was added to each sample and the second absorbance (A2) was recorded at the same wavelength (Beheshti et al. 2017; Baghcheghi et al. 2018a; Baghcheghi et al. 2018b). Total thiol concentration (mM) was calculated by the following equation:

$$C\ (mM) = (A2\text{-}A1\text{-}B) \times 1.07/0.05 \times 13.6$$

Estimation of SOD Activity

SOD activity in the cerebral cortex and hippocampus was assessed based on the ability of the enzyme to inhibit autoxidation of pyrogallol (Madesh and Balasubramanian 1998). Based on the method of Madesh

homogenate) was mixed with MTT and pyrogallol solution

and then incubated at room temperature. After 5 min, DMSO was added to solubilize the resultant color. The optical absorbance was measured at 570 nm and the activity of SOD was expressed as unit per gram of tissue (Madesh and Balasubramanian 1998).

Estimation of AChE Activity

The AChE activity in the supernatants was determined by the method of Ellman using acetylthiocholine iodide as a substrate. Briefly, each sample (50 µl) was added into a solution (containing PBS (pH 8), 0.1 ml DTNB (10 mM), and 0.02 ml acetylthiocholine (75 mM). The changes in absorbance of the samples were spectrophotometrically recorded at 412 nm within 10 min and AChE activity was estimated as µmol/g tissue/min (Ellman et al. 1961).

Statistical Analysis

Statistical analysis was performed using the SPSS 11.5 software and normality of the data was checked by the Kolmogorov–Smirnov test. Data about learning phase in MWM test was analyzed by repeated measures analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Other data were analyzed by one-way ANOVA followed by Tukey's *post hoc* test. All data are expressed as means ± SEM and a $p<0.05$ was considered statistically significant.

Results

Morris water maze

The time latency and the distance traveled to reach the platform during the 5-day training in the MWM test in hypothyroid group were significantly increased compared to the control group ($P<0.001$ for the latency and $P<0.05$-$P<0.001$ for the distance). The time latency was significantly reduced in all FA-treated groups compared to the hypothyroid group ($P<0.05$ to $P<0.001$) (Fig. 1a). Moreover, the distance traveled to reach the platform in hypothyroid rats with different doses of FA was lower than the hypothyroid group ($P<0.05$ to $P<0.01$) (Fig. 1b).

The animals in hypothyroid group showed significant decreases in the time spent and distance traveled in target quadrant on the probe day compared to the control group ($P<0.001$ for both). The hypothyroid animals treated with FA (5, 10 or 15 mg) better remembered the location of the platform and spent longer time and traveled longer distance in the target area of MWM than the hypothyroid group ($P<0.001$ for all). The animals of the hypothyroid-FA 15 group spent longer time and traveled longer distance in the target area of MWM than the hypothyroid-FA 10 group ($P<0.05$ and $P<0.01$, respectively) and traveled a longer distance in the target area than the hypothyroid-FA 5 group ($P<0.05$). The traveling time in the target area of MWM showed no significant difference between FA-treated hypothyroid rats and the control group. The distance traveled in the target area in the hypothyroid-FA 5 and hypothyroid-FA 10 groups was shorter than that of the control group ($P<0.01$ for both) but there was no significant difference between hypothyroid-FA 15 and the control group in this regard (Fig. 2).

Passive avoidance

Based on the results presented in Fig. 3 and 4, in the hypothyroid group, the latency to enter and the time spent in the light chamber were decreased while the time spent and frequency of entry to the dark chamber were significantly increased compared with the control group, 3, 24, 48 and 72 h after receiving the electric shock ($P<0.01$ to $P<0.001$). The latency to enter the dark chamber in all FA-treated hypothyroid groups was higher than that of the control group, 3 h after the delivery of the electric shock ($P<0.01$, $P<0.001$ and $P<0.001$ for FA 5, 10 and 15, respectively). The latency to enter the dark chamber in the hypothyroid-FA 10 and hypothyroid-FA 15 groups was higher than the hypothyroid group 24, 48 and 72 h after the shock ($P<0.05$ to $P<0.001$) but there was no significant difference between the hypothyroid-FA 5 and the hypothyroid groups at these time-points ($P>0.05$). The latency to enter the dark chamber in the hypothyroid-FA 15 group was significantly higher compared to the hypothyroid-FA 5 group, 3, 24, 48 and 72 h post-shock ($P<0.01$, $P<0.001$, $P<0.001$ and $P<0.05$, respectively). As shown in Fig. 3a, the latency in the hypothyroid-FA 15 group was higher compared to the hypothyroid-FA 10 group, 24 h after the shock ($P<0.05$).

All FA-treated hypothyroid rats spent shorter times in the dark chamber than the hypothyroid group, 3 h post shock time ($P<0.001$ for the three groups) but there was no significant difference in this parameter among the three FA-treated groups. The rats of the hypothyroid-FA 10 and hypothyroid-FA 15 groups also spent shorter times in the dark chamber than the hypothyroid group, 24, 48 and 72 h post-shock ($P<0.01$ to $P<0.001$) but there was no significant difference between the hypothyroid-FA 5 and the hypothyroid group at these time-points (Fig. 3b). The rats of hypothyroid-FA 15 group spent shorter time in the dark chamber than the hypothyroid-FA 5 group, 24, 48 and 72 h post-shock ($P<0.05$ to $P<0.001$). In addition, the rats of the hypothyroid-FA 10 group spent shorter time in the dark chamber than the hypothyroid-FA 5 group, 24 and 72 h after the shock ($P<0.05$ for both times).

The results also showed that the FA-treated hypothyroid animals spent longer times in the light chamber than the hypothyroid group 3 h post-shock ($P<0.001$ for all groups) but there was no significant difference among FA-treated hypothyroid groups in this regard. Moreover, the rats of hypothyroid-FA 10 and hypothyroid-FA 15 groups spent longer times in the light chamber than the hypothyroid group, 24, 48 and 72 h post-shock ($P<0.01$ to $P<0.001$) but there was no significant difference between hypothyroid-FA 5 and hypothyroid groups (Fig. 4a). The time spent in the dark chamber in the hypothyroid-FA 10 and hypothyroid-FA 15 groups was longer than the hypothyroid-FA 5 group, 24, 48 and 72 h post-shock ($P<0.05$ to $P<0.001$).

The results of the number of dark chamber entries (Fig. 4b) showed that the frequency of entries in the hypothyroid-FA 5 group was higher than the control group ($P<0.01$ to $P<0.001$) but there was no significant difference between the hypothyroid-FA 5 and control groups. The rats of the hypothyroid-FA 10 and hypothyroid-FA 15 groups had lower number of entries into the dark chamber than the hypothyroid group at all time-points after the shock ($P<0.05$ to $P<0.001$). The number of entries in the hypothyroid-FA 10 and hypothyroid-FA 15 groups was lower than the hypothyroid-FA group ($P<0.05$ to $P<0.001$).

MDA and thiol concentrations in cortical and hippocampal tissues

Hypothyroidism induced by PTU increased MDA but decreased total thiol levels in both cortex and hippocampus ($P<0.001$ for all) (Fig. 5 and 6). FA at all doses reduced the MDA concentration while increased thiol content in the hippocampus ($P<0.05$ to $P<0.001$). Also, FA 10 and 15 mg/kg decreased MDA while increased thiol content in the cortex of hypothyroid rats compared to the hypothyroid group ($P<0.05$ to $P<0.001$) but there was no significant difference between hypothyroid-FA 5 and hypothyroid groups in the cortical levels of MDA and thiol. There was no significant difference among the three FA-treated groups in the hippocampal levels of MDA and thiol but in the cortex of the hypothyroid-FA 15 group, MDA concentration was lower ($P<0.01$) while thiol content was higher ($P<0.05$) than the hypothyroid-FA 5 group (Fig. 5 and 6).

SOD activity in hippocampal and cortical tissues

Figure 7 shows that hippocampal and cortical SOD activity significantly decreased following hypothyroidism induced by PTU administration ($P<0.001$ for both tissues). SOD activity in hypothyroid-FA 10 and hypothyroid-FA 15 groups significantly increased compared to the hypothyroid group ($P<0.01$ and $P<0.001$) and it was higher in the hypothyroid-FA 15 group than the hypothyroid-FA 5 group ($P<0.001$ in the hippocampus and $P<0.01$ in the cortex). There was no significant difference between the hypothyroid-FA 5 and the hypothyroid group in both hippocampus and cortex SOD activity (Figure 7). Both hippocampal and cortical SOD activity in all FA-treated hypothyroid groups were still lower than that of the control group ($P<0.05$ to $P<0.001$).

AChE activity in hippocampal and cortical tissues

Fig. 8 shows that hippocampal and cortical AChE activity significantly increased following hypothyroidism induced by PTU ($P<0.001$ for both cortex and hippocampus tissues). Administration of 10 and 15 mg/kg of FA reduced AChE activity in the hippocampus and cortex compared with the hypothyroid group ($P<0.05$ to $P<0.001$) but there was no significant difference between the hypothyroid-FA 5 and the hypothyroid group. AChE activity in the cortex of both hypothyroid-FA 10 and hypothyroid-FA 15 groups was lower than that of the hypothyroid-FA 5 group ($P<0.01$ for both) but there was no significant difference in the hippocampal AChE activity among FA-treated hypothyroid groups. The results also showed that AChE activity in the hippocampus of all FA-treated hypothyroid groups and in the cortex of hypothyroid-FA 5 group was higher than that of the control group ($P<0.05$ to $P<0.001$).

Thyroxin level in the serum

The results showed that serum thyroxin level in the hypothyroid group was significantly lower than the control group ($P<0.001$). The results also showed that FA was not able to reverse serum thyroxin level as there was no significant difference between FA-treated hypothyroid groups and the hypothyroid group. Serum thyroxin level in all FA-treated hypothyroid groups was lower than the control group ($P<0.001$ for all cases)

Discussion

In the present study, PTU administration induced a hypothyroidism state as reflected by a low serum level of thyroxin in the hypothyroid group compared to the control group. PTU as a well-known drug used for hyperthyroidism treatment, has been frequently used to produce hypothyroidism in rodents (Asiaei et al. 2017; Beheshti et al. 2017; Baghcheghi et al. 2018a; Baghcheghi et al. 2018b; Baghcheghi et al. 2020). Hypothyroidism, especially during developmental and growth periods has been shown to negatively affect the central nervous system (Asiaei et al. 2017; Beheshti et al. 2017; Baghcheghi et al. 2018a, Baghcheghi et al. 2018b; Baghcheghi et al. 2020). Studies in humans and animals have shown that hypothyroidism during developmental period impairs cognitive functions such as attention, learning and memory (Hosseini et al. 2010; Beheshti et al. 2017; Baghcheghi et al. 2018a; Baghcheghi et al. 2018b).

The results of the current study showed that PTU-induced hypothyroidism was accompanied with learning and memory impairment as confirmed by both MWM and PA tests. Also, we found that the rats of the hypothyroid group spent longer time to reach the platform during the five-day learning period in the MWM test. The rats of the hypothyroid group also traveled longer distance to reach the hidden platform than the control group. Interestingly, the rats of the hypothyroid group could not remember the location of the platform and spent less time and traveled shorter distance in the target area in the probe trial in the MWM. The results of PA test also showed that the rats of the hypothyroid group had a shorter delay but a higher frequency in entering the dark compartment, and spent longer time there, than the control group. These results are consistent with the previous studies which showed that hypothyroidism during lactation, infancy or developmental periods causes cognitive impairments such as learning and memory dysfunction (Hosseini et al. 2010; Beydoun et al. 2013; Farrokhi et al. 2014). Thyroid hormones are known to be important for non-hippocampal and hippocampal-related learning and memory, synaptic flexibility, and neurogenesis (Cooke et al. 2014; Asiaei et al. 2017; Baghcheghi et al. 2020).

The exact mechanism(s) responsible for adverse effects of hypothyroidism on learning and memory have not been well elucidated. It is suggested that hypothyroidism causes cerebral atrophy and cerebral neuro-inflammation as it is also accompanied with amyloid beta (Aβ) production, tau hyperphosphorylation, and impairment of signaling pathways responsible for hippocampal-dependent spatial memory (Beydoun et al. 2013; Chaalal et al. 2019). An imbalance between the production of peroxidants and antioxidants and production of high levels of ROS and RNS (reactive nitrogen species) are also suggested to have a role in negative impacts of hyperthyroidism on learning and memory (Venditti and Di 2006).

The results of the current study also showed that hypothyroidism-associated learning and memory was accompanied with a decrease in thiol content and SOD activity but an increase in MDA level in both hippocampus and cortex tissues. These findings confirmed the occurrence of an oxidative stress state in the brain of hypothyroid rats which may have a role in learning and memory impairing effects of hypothyroidism seen in the present study. The results of previous researches have shown that MDA levels are reduced in patients with controlled hypothyroidism due to antioxidant mechanisms mediated by

thyroid hormones (Villanueva et al. 2013). It has also been previously reported that hypothyroidism is accompanied with a decrease in thiol content and SOD and CAT activities in the brain.

Interestingly, AChE activity in the hippocampus and cortex of hypothyroid rats was significantly decreased compared to the control ones. Acetylcholine is one of the major neurotransmitters involved in cognitive function. It has been previously reported that hypothyroidism is associated with cholinergic system dysfunction (Smith et al. 2002). It was also shown that thyroxin increased choline acetyltransferase activity and acetylcholine level in the brain and consequently, improved learning and memory (Fu et al. 2014). Considering the results of the present study and the mentioned evidence, negative effects of hypothyroidism may at least in part be due to its effects on the cholinergic system.

Supplementation with THs is widely done to treat hypothyroidism. Levothyroxine has been reported to reduce hippocampal cognitive impairment in hypothyroid mice (Smith et al. 2002; Fu et al. 2014). Recently, vitamins C and E and anti-oxidant natural products were suggested to reduce the adverse effects of hypothyroidism on brain functions including learning and memory (Beheshti et al. 2017; Baghcheghi et al. 2018a; Baghcheghi et al. 2018b; Baghcheghi et al. 2020). In the current research, treatment by three doses of FA (i.e. 5, 10 and 15 mg/kg) improved learning and memory of the hypothyroid rats. The results showed that the hypothyroid rats treated with different doses of FA spent less time and traveled shorter distances to reach the platform during the 5-day learning period in the MWM. They also better remembered the location of the platform and spent more time and traveled longer distances in the target area of the MWM in the probe trial. The results of PA test also showed that treatment by different doses of FA prolonged the latency to enter the dark compartment and increased the total time spent in the light compartment while decreased the time in the dark chamber and reduced the number of entries to the dark compartment. To the best of our knowledge, the effects of FA on hypothyroidism-associated learning and memory impairment were not previously reported. Previous studies have shown that administration of vitamin B improves cognitive function in people with low FA levels by decreasing homocysteine (Shooshtari et al. 2012). FA deficiency has been reported to be followed by increases in homocysteine and cognition and learning and memory impairments (Dam et al. 2017).

Our results also showed that FA attenuated MDA while increased thiol and SOD in both hippocampus and cortex tissues. It has been previously reported that FA improves the level of glutathione (GSH), but causes a significant reduction in brain MDA levels, indicating suppression of lipid peroxidation (Singh et al. 2011). FA administration in patients with Alzheimer's disease could improve cognition and reduce inflammatory factors (Chen et al. 2016; Calderón et al. 2020). FA deficiency increases the risk of other neurological disorders including stroke, through induction of oxidative DNA damage associated with morphological damage and increased cell autophagy function (Zhao et al. 2016). Short-term administration of FA for 7 weeks in patients with metformin-treated type 2 diabetes was able to significantly decrease serum MDA levels (Aghamohammadi et al. 2011). Moreover, FA with or without vitamin B12 given for 30 days could prevent mitochondrial dysfunction and DNA damage caused by rats (Majumdar et al. 2009). FA treatment in stressed rats

was able to reduce depressive-like behaviors and brain oxidative damage, and ameliorate hippocampal antioxidant imbalance (Budni et al. 2013; Réus et al. 2018; Menegas et al. 2020).

To better understand the responsible mechanism(s), AChE activity was also evaluated in the brain. The results showed that FA decreases AChE activity in the hippocampus and cortex. Considering these results, it seems that learning and memory improving effects of FA seen in the present study are at least in part due to its attenuating effect on AChE activity. It has also been reported that FA deficiency is associated with cholinergic system dysfunction and increased AChE activity (Crivello et al. 2010).

Nevertheless, FA was not able to restore the thyroxin concentration to normal levels. Thus, it seems that FA was not able to protect the thyroid grain from damaging effects of PTU. To better understand the effects of FA on serum thyroxin level, further investigations using other animal models including thyroidectomy model need to be done.

Conclusion

FA could improve learning and memory ability in hypothyroid rats. The observed protective effects may have been achieved by suppression of oxidative stress and regulation of AChE activity.

Declarations

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

All procedures performed in this studies including animals were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by Ethical Committee of the Animal Research of Mashhad University of Medical Sciences (Ethical code: IR.MUMS.MEDICAL.REC.1399.639).

Data availability statement

The authors confirm that all data generated or analyzed during this study are included in this published article.

Author contributions Mahmoud Hosseini conducted conception and design of the project, Sabiheh Amirahmadi, Somaieh Ahmadabady, Mahsa Akbarain, Kataneh Abrari, Arezoo Rajbian, and Farzaneh Vafaee performed the experiments. Mahmoud Hosseini, Arezoo Rajbian, and Farzaneh Vafaee prepared a draft of the manuscript. Mahmoud Hosseini, Arezoo Rajbian, Farzaneh Vafaee performed statistical analysis. Mahmoud Hosseini, Farzaneh Vafaee, and Arezoo Rajbian provided final revision of the manuscript. All authors contributed to manuscript preparation and approved the submitted version.

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Figures

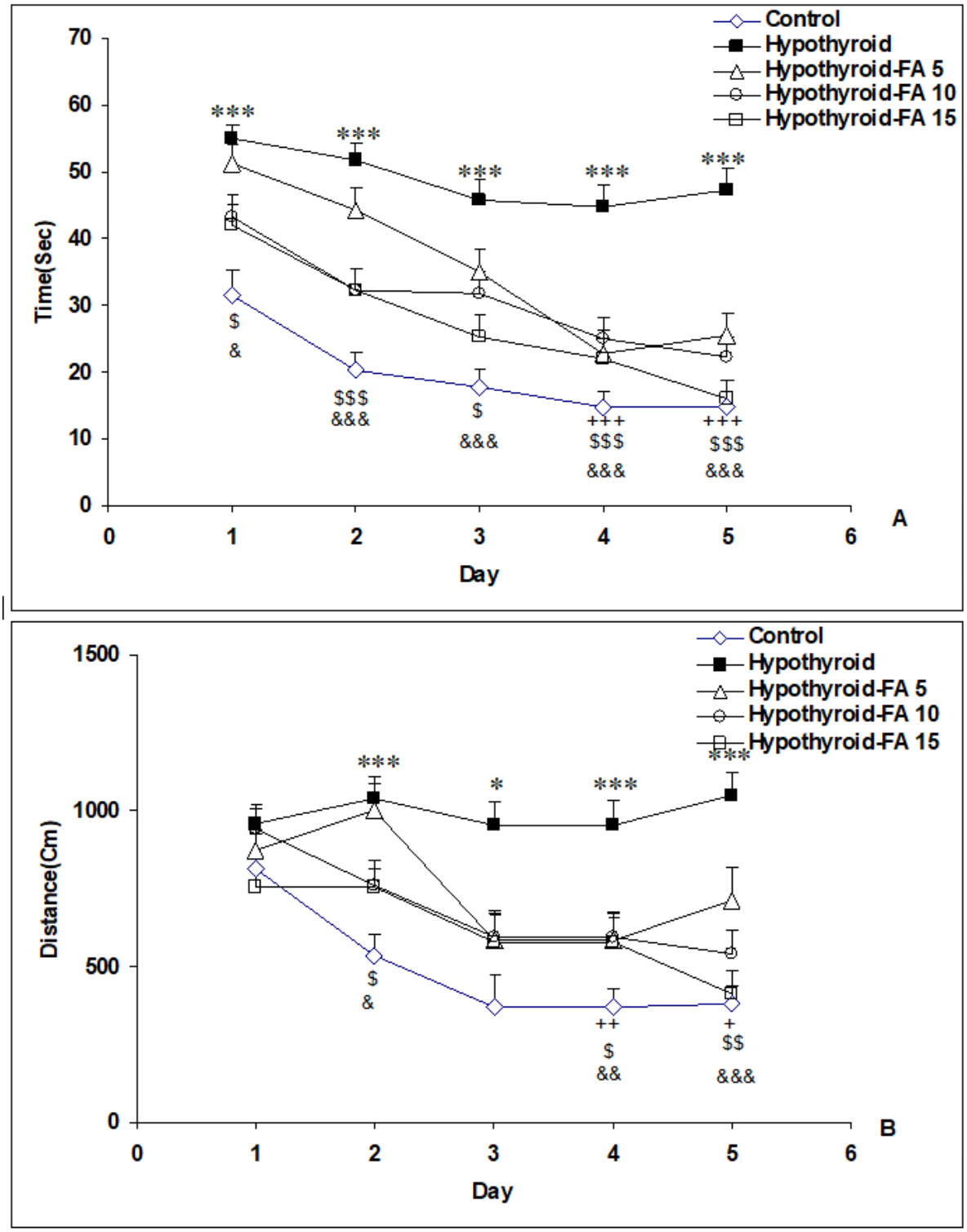

Figure 1

The results of traveling time and distance during 5 days learning in Morris water maze. The data were expressed as mean ± SEM (n = 10). *P<0.05, ***P<0.001 compared to the control group. +P<0.05, ++P<0.01, +++P<0.001 comparison between Hypothyroid FA 5 and Hypothyroid groups, $P<0.05,

$$P < 0.01,$$

$P<0.001 comparison between Hypothyroid FA 10 and Hypothyroid groups, &P<0.05, &&P<0.01, &&&P<0.001 comparison between Hypothyroid FA 15 and Hypothyroid groups FA: Folic acid.

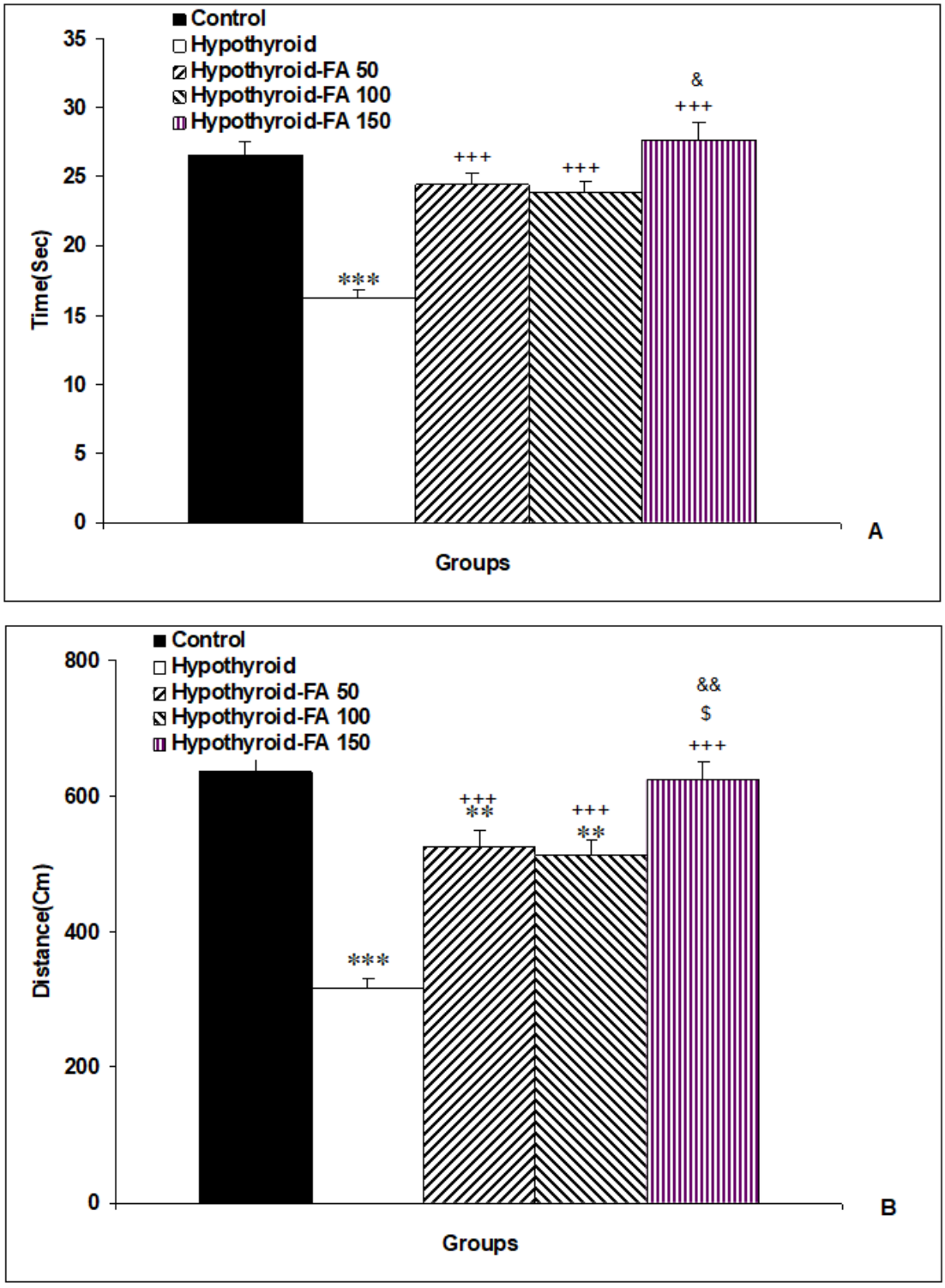

Figure 2

The results of traveling time and distance in the target quadrant during probe test in Morris water maze. The data were expressed as mean ± SEM (n = 10). **P<0.01 and ***P<0.001 compared to the control

group. +++P<0.001 compared to Hypothyroid group, $P<0.05 compared to Hypothyroid FA 5 group, &P<0.05 and &&P<0.01compared to Hypothyroid FA 10 group. FA: Folic acid

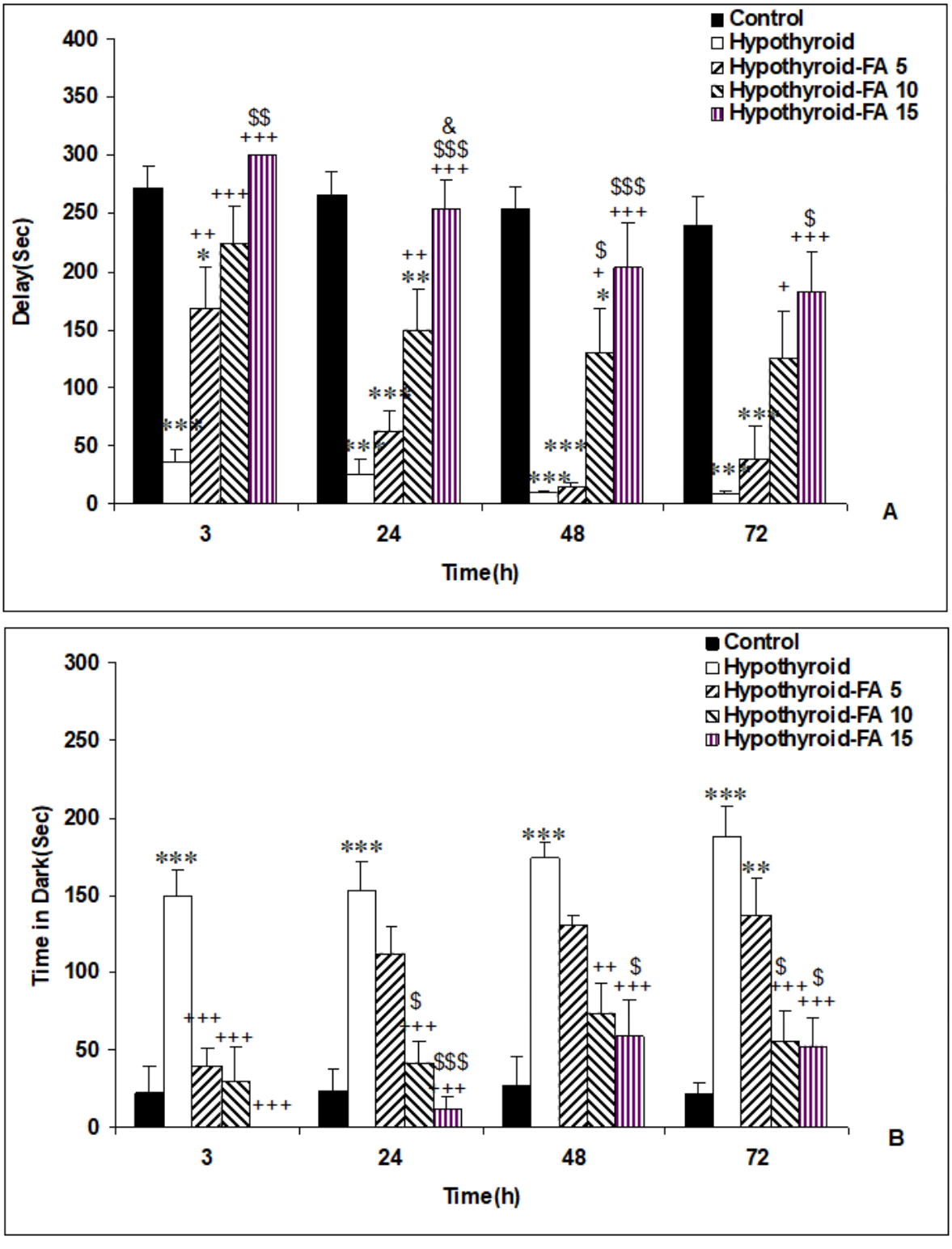

Figure 3

The results of delay time for entering the dark and the total time spent in the dark in passive avoidance test. The data were expressed as mean ± SEM (n = 10). *P<0.05, **P<0.01 and ***P<0.001 compared to

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+P<0.001 compared to Hypothyroid group, $P<0.05,

$P < 0.01 and$

$P<0.001 compared to Hypothyroid FA 5 group, &P<0.05 compared to Hypothyroid FA 10 group. FA: Folic acid

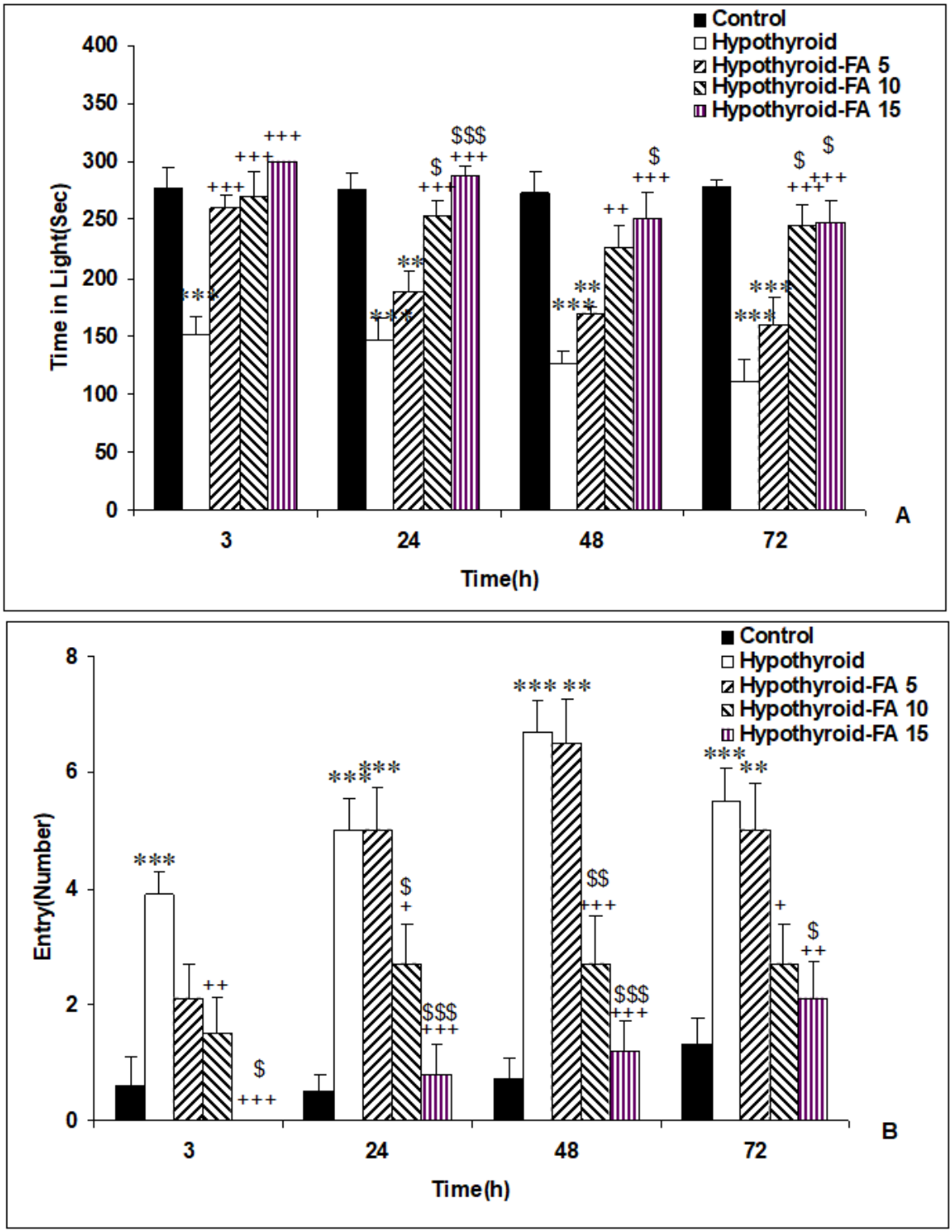
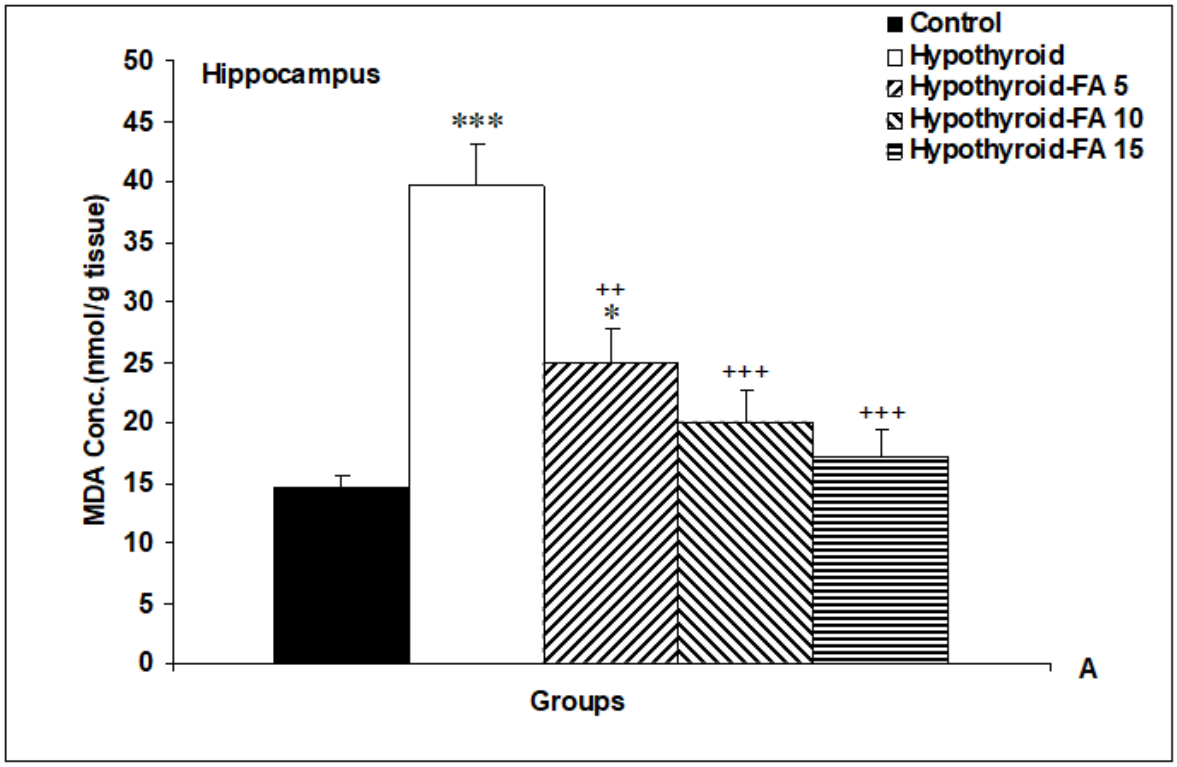


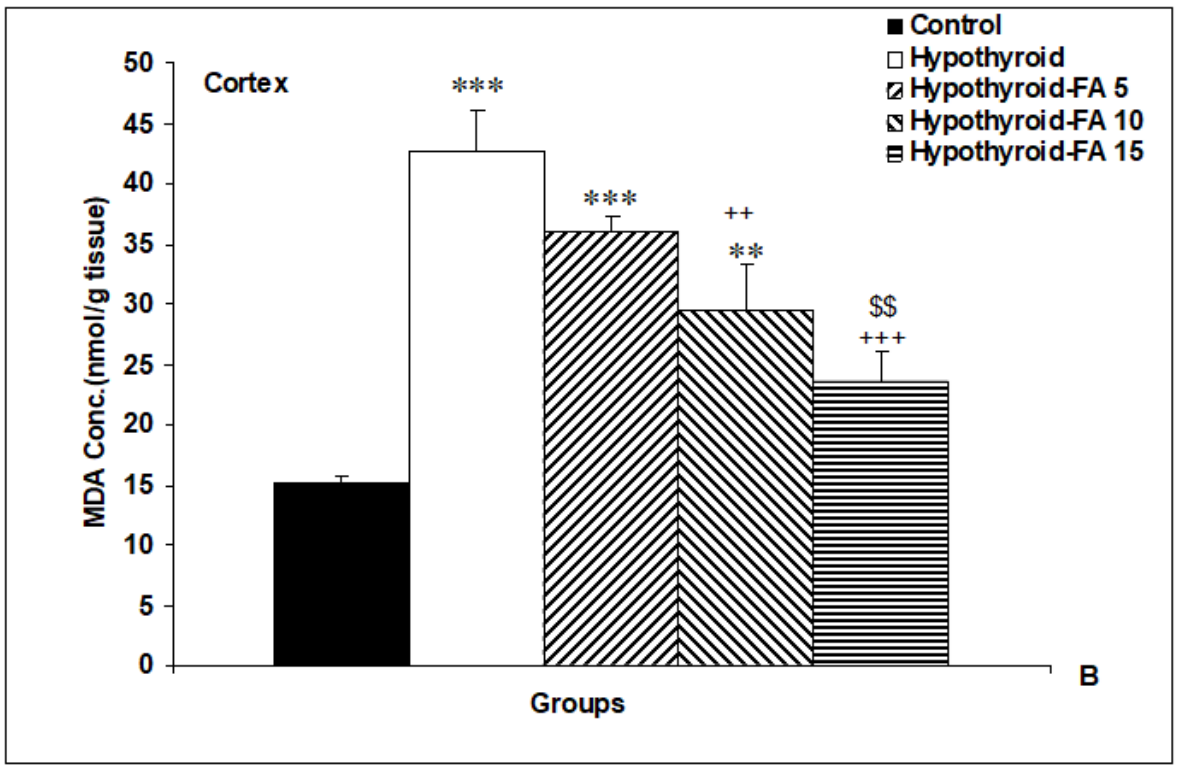
Figure 4

The results of total time spent in the light and the number of entering into the dark in passive avoidance test. The data were expressed as mean ± SEM (n = 10). **P<0.01 and ***P<0.001 compared to the control group. +P<0.05, ++P<0.01 and +++P<0.001 compared to Hypothyroid group, $P<0.05,

$$P < 0.01 and$$

$P<0.001 compared to Hypothyroid FA 5 group. FA: Folic acid





The results of MDA in the hippocampus (a) and cortex (b). The data were expressed as mean ± SEM (n = 10).*P<0.05, **P<0.01 and ***P<0.001 compared to the control group. ++P<0.01 and +++P<0.001 compared to Hypothyroid group, $$P<0.01 compared to Hypothyroid FA 5 group. FA: Folic acid

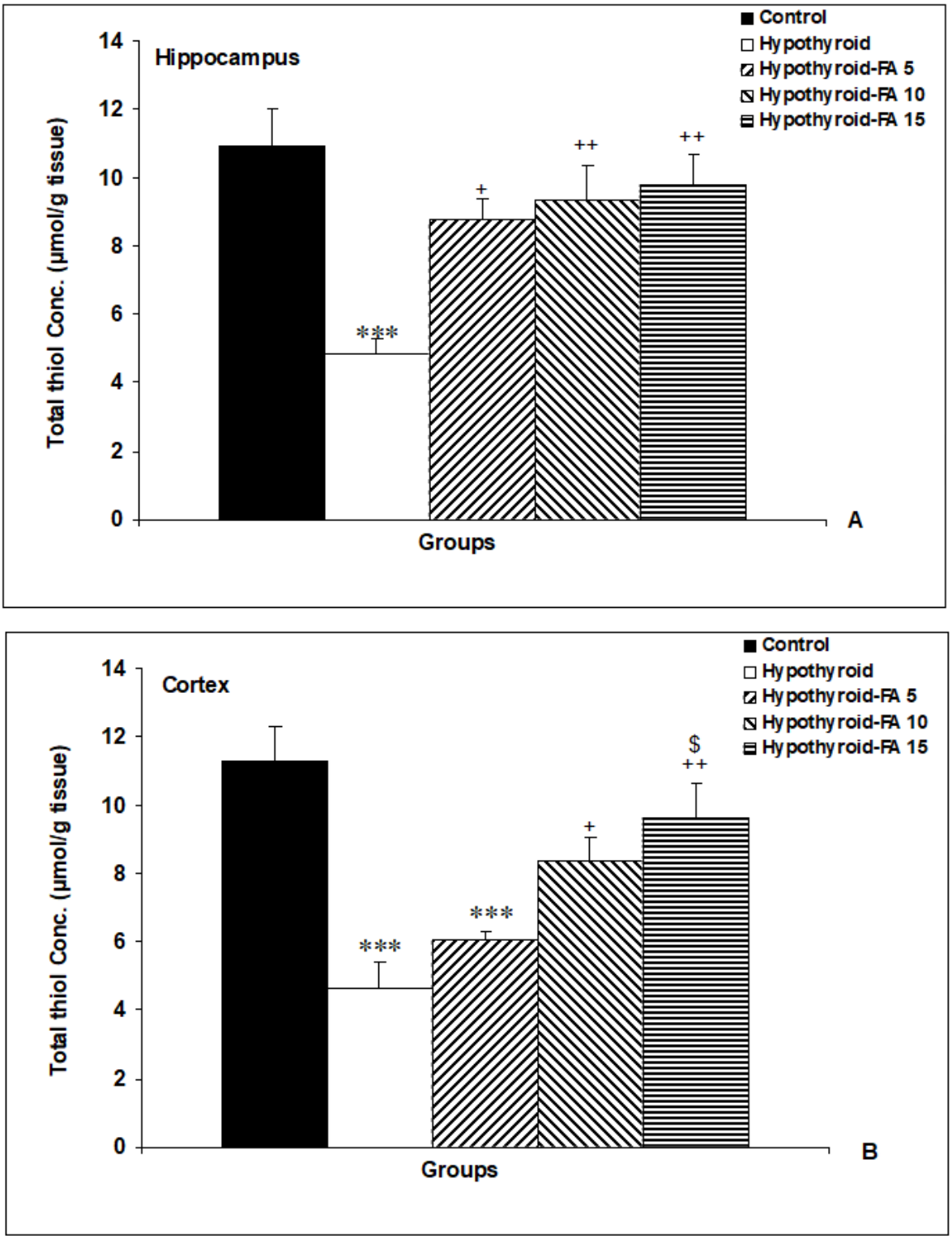


Figure 6

The results of thiol in the hippocampus (a) and cortex (b). The data were expressed as mean ± SEM (n =

up. +P<0.05 and ++P<0.01 compared to Hypothyroid group,

$P<0.05 compared to Hypothyroid FA 5 group. FA: Folic acid

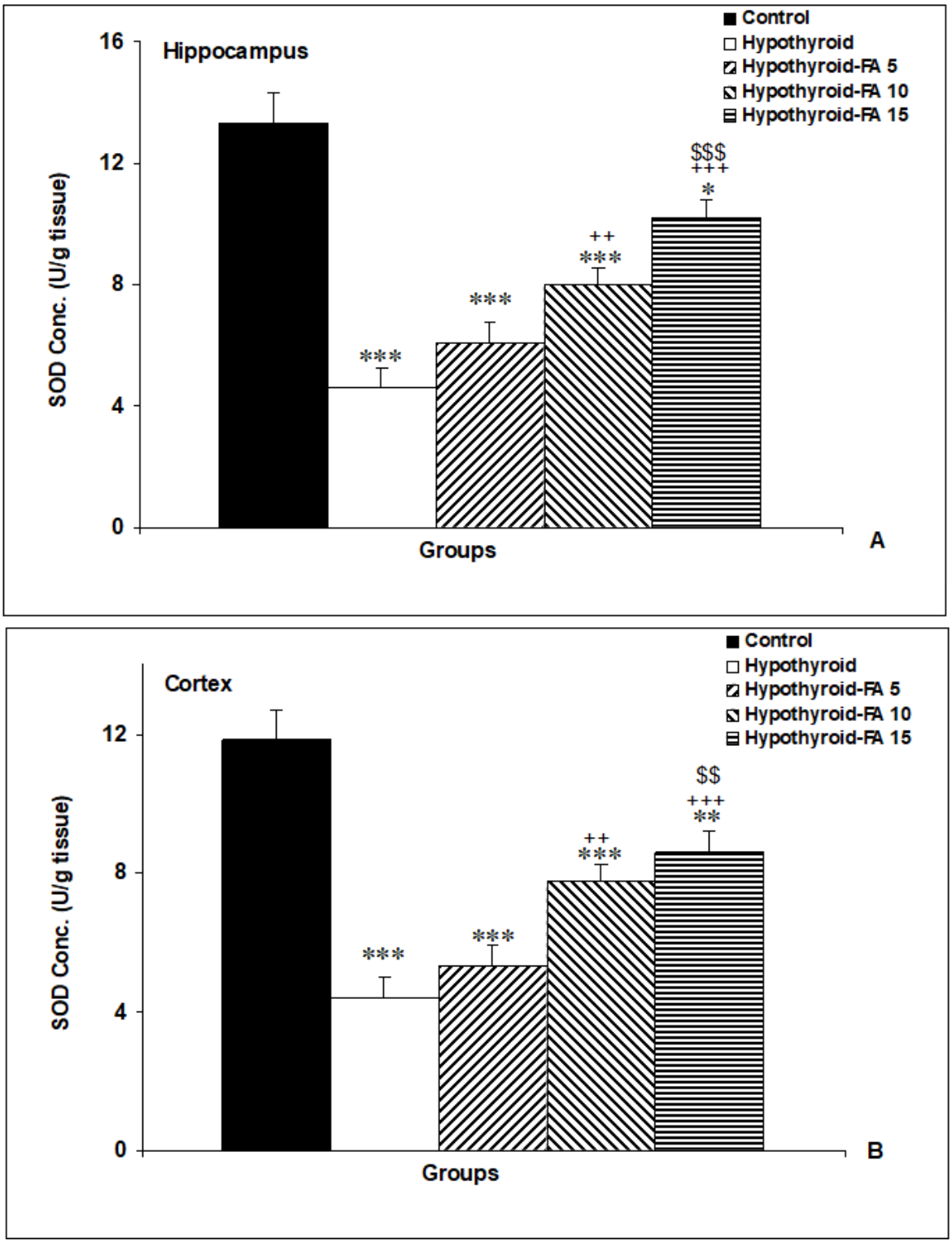
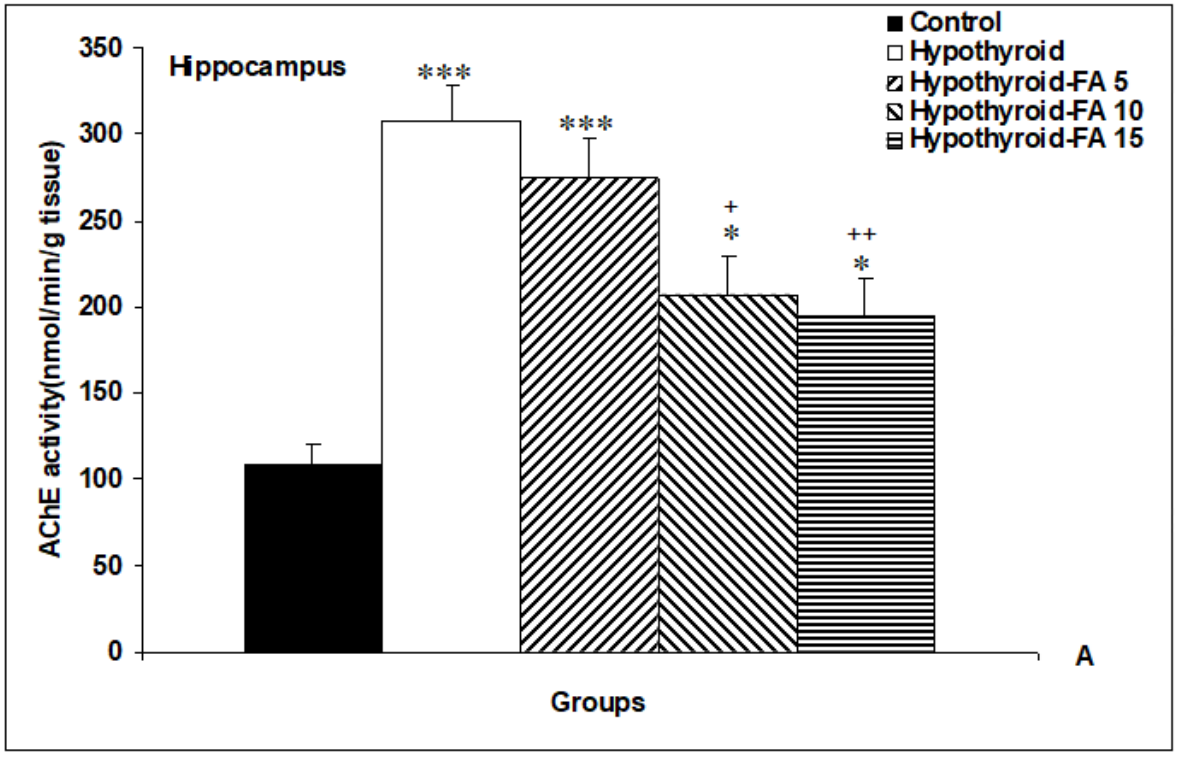


Figure 7

The results of SOD in the hippocampus (a) and cortex (b). The data were expressed as mean ± SEM (n = 10). *P<0.05, **P<0.01 and ***P<0.001 compared to the control group. ++P<0.01 and +++P<0.001 compared to Hypothyroid group,

$P < 0.01 and$

$P<0.001 compared to Hypothyroid FA 5 group. FA: Folic acid



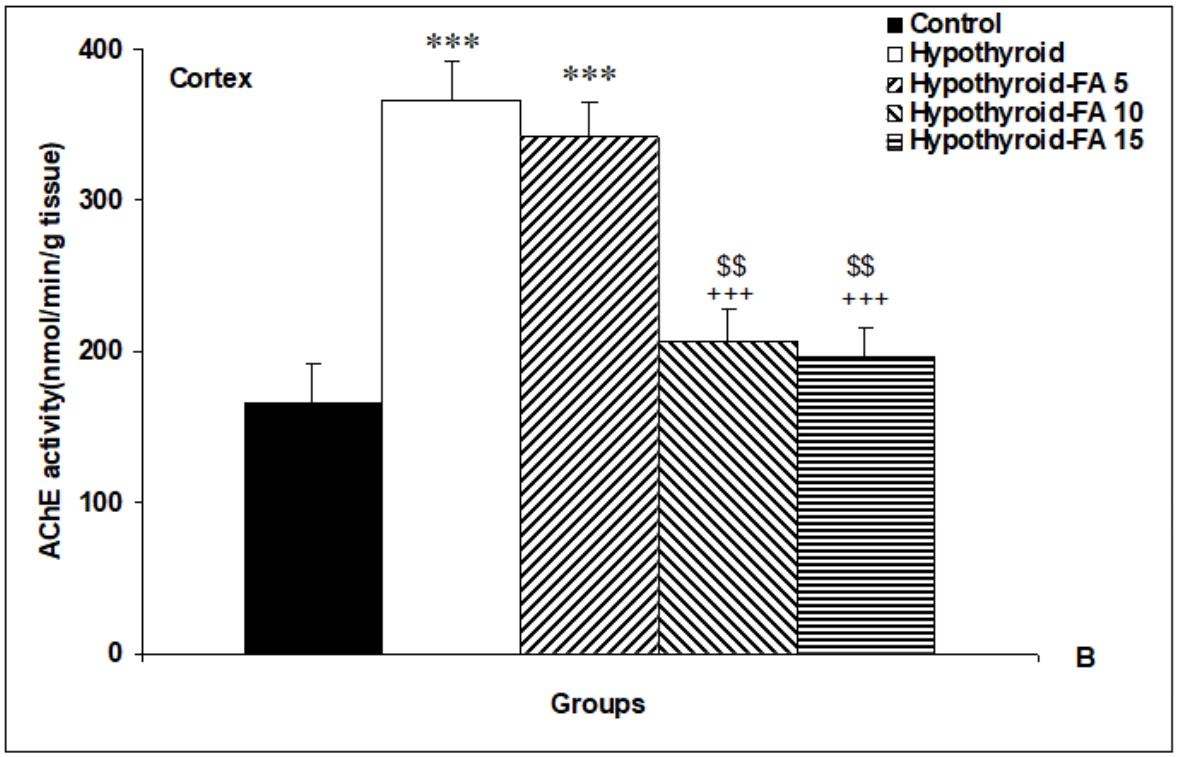


Figure 8

The results of AChE activity in the hippocampus (a) and cortex (b). *P<0.05, and ***P<0.001 compared to the control group. The data were expressed as mean ± SEM (n = 10). +P<0.05, ++P<0.01 and +++P<0.001

compared to Hypothyroid group, $$P<0.01 compared to Hypothyroid FA 5 group. FA: Folic acid

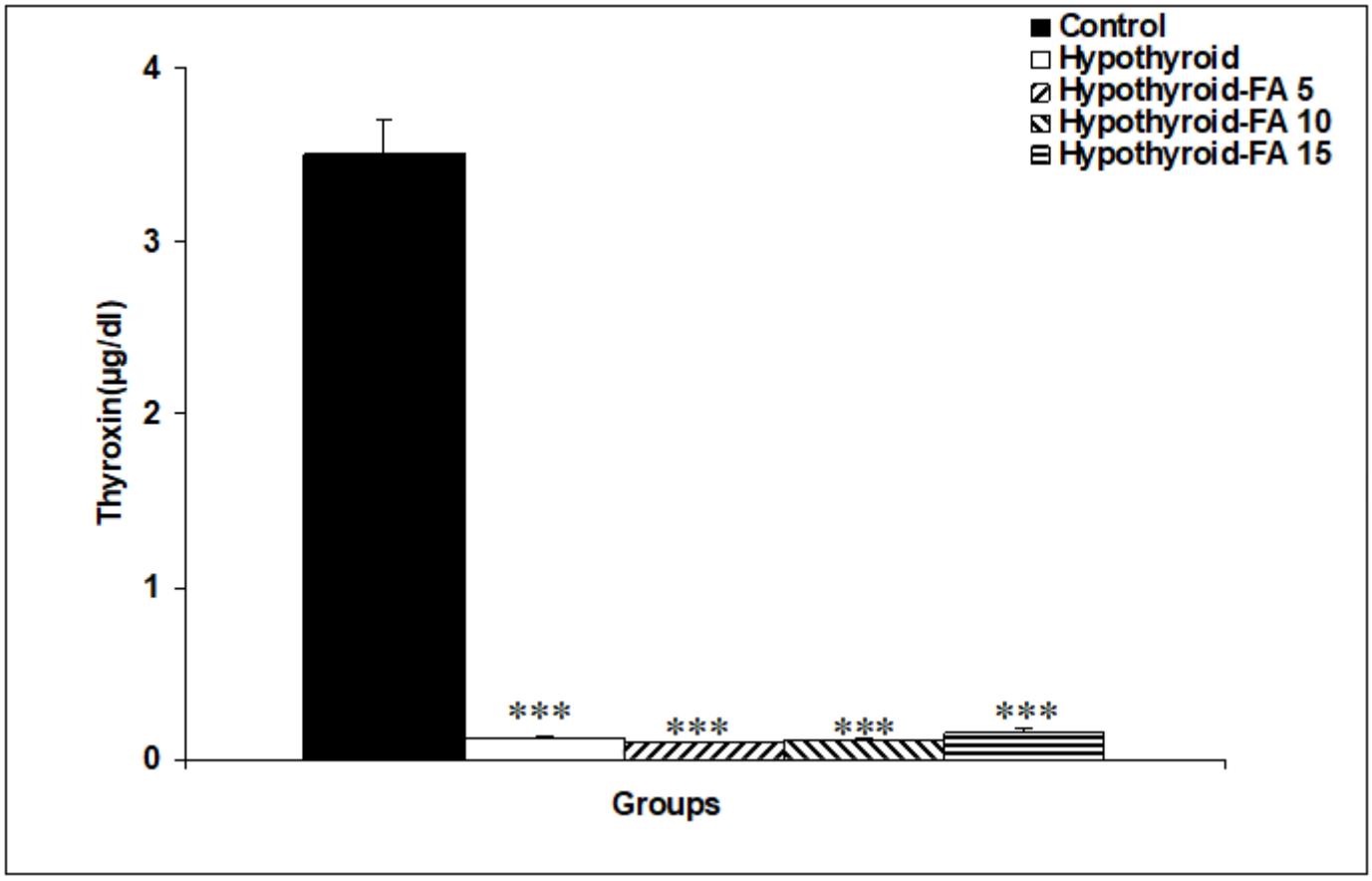


Figure 9

The results of thyroxin level in the serum. ***P<0.001 compared to the control group. The data were expressed as mean ± SEM (n = 10). FA: Folic acid