# Dimethyl Fumarate improves cognitive impairment by enhancing hippocampal brain-derived neurotrophic factor levels in hypothyroid rats

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**Research Article**

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# Abstract

**Background:**

Dimethyl fumarate (DMF) is an effective drug for multiple sclerosis, and can improve the cognitive dysfunction caused by streptozotocin, but the effect cognitive dysfunction caused by hypothyroidism is unclear.

**Methods:**

After hypothyroidism rat model induced by propylthiouracil, we gave rats 25mg/kg DMF by gavage. The body weight during model building and administration was recorded. The levels of T4 and T3 in serum were detected by an automatic biochemical analyzer. Morris water maze test was used to detect the effect of DMF on the cognitive learning ability. The effect of DMF on Nissl bodies in the brain tissue was evaluated by Nissl staining. The mRNA and protein levels of BDNF in brain tissue were detected by qRT-PCR and western blot.

**Results:**

After DMF treatment, the body weight of hypothyroid rats has recovered, and the levels of T3 and T4 in the serum was ameliorated. DMF also reduced the escape latency and swimming distance, and increased the swimming speed. The number of Nissl bodies and expression of BDNF in the brain tissue were increased after DMF treatment.

**Conclusion:**

DMF improved the cognitive dysfunction of hypothyroid rats by increasing the level of BDNF in the brain tissue of hypothyroid rats.


# Background

Hypothyroidism is a syndrome caused by insufficient thyroid hormone secretion[1]. Thyroid hormones have a vital impact on the development and normal work of the brain throughout life[2]. At the same time, thyroid hormones play an important role in cognition[3]. Clinical psychology studies have shown that the attention, mobility, memory and spatial ability of patients with hypothyroidism are significantly reduced, while the index of depression and anxiety is increased[4]. In addition, animal behavior studies have shown that the spatial memory ability of hypothyroid animals is impaired[5]. Although various degrees of cognitive impairment caused by hypothyroidism have been recognized by academia, the specific mechanism is still unclear.

Studies have suggested that cognitive dysfunction caused by hypothyroidism may be related to the down-regulation of Brain-derived Neurotrophic Factor (BDNF)[6, 7]. BDNF is a member of the neurotrophin family, and it has a high content in the hippocampus, prefrontal cortex. BDNF can not only promote the

growth and differentiation of neurons, but also participate in the regulation of synaptic transmission and synaptic plasticity[8]. Animal experiments have shown that deleting BDNF in the broad forebrain regions of rats will cause damage to the hippocampus-dependent learning ability of rats[9]. And artificially increasing the level of BDNF can improve the spatial memory ability of rats[10, 11]. BDNF is also important for human brain function. Michael F Egan et al. found that BDNF val66met polymorphism is associated with human hippocampal-dependent memory impairment[12].

Fumaric acid esters (FAEs) are compounds that have antioxidant and anti-inflammatory effects in a variety of tissues and cells, and dimethyl fumarate (DMF) is the most biologically active compound in FAE[13]. DMF was used in the treatment of psoriasis[14]. In addition, DMF can also be used to treat multiple sclerosis[15]. DMF was first applied to the study of the nervous system. Isabel Lastres-Becker et al. reported the improvement of DMF on dyskinesia of neurodegenerative disease Parkinson's mice[16]. Ludwig Kappos and his colleagues found that DMF inhibits the oxidative damage of nerve cells by activating the Nrf2 pathway, and maintains the integrity of nerve cell myelin[17]. It is worth noting that studies have shown that DMF reduces the secondary degeneration after spinal cord injury by increasing the expression of BDNF[18]. In addition, DMF also has a certain alleviating effect on the spatial memory impairment of Alzheimer’s rats [19]. However, there is currently a lack of studies evaluating DMF on cognitive function after hypothyroidism. Considering the role of BDNF in hypothyroidism, we speculate that DMF may play a role in hypothyroidism by affecting the expression of BDNF.

Therefore, we constructed a rat model of hypothyroidism induced by propylthiouracil (PTU) to observe the effects of DMF on the behavior of hypothyroidism rats in the Morris water maze, serum thyroid hormone levels and BDNF expression in the hippocampus, in order to clarify the effect of DMF on the learning and memory ability of hypothyroid rats and explore its possible mechanism.

# Methods

## Animals

30 male Sprague–Dawley rats (8 weeks old, 215 ± 20 g) were purchased from Guangdong Medical Experimental Animal Center (http://www.gdmlac.com.cn/). All rats were kept in an environment where the ambient temperature is maintained at 22–23°C, the relative humidity is 45%–50%, and the light cycle is 12/12 hours. All rats were fed adaptively for one week, and they were free to eat and drink. Those with normal drinking and eating were included in the experiment.

The construction of the PTU rat model referred to the previous literature [20]. Rats were randomly divided into control group (n = 10) and model group (n = 20). The rats in the control group drank water normally, while the rats in the PTU group drank tap water containing 0.05% PTU (IP0420, Solarbio, China). After 28 days, the rats in the model group were randomly divided into PTU group (n = 10) and PTU + DMF25 group (n = 10). The rats in the control group and the PTU group were given saline once a day, while the rats in the PTU + DMF25 group were given 25 mg/kg DMF (ID0320, Solarbio, China) once a day for 14 days. The body weight of each group of rats was measured weekly.

### Morris water maze test

The learning and memory abilities of rats are tested through the Morris water maze test[21]. The diameter of the maze was 1.6 m and the height was 50 cm. The pool water was dyed black with food coloring. The water depth was 30 cm, and the water temperature was 22–23℃. The experiment was divided into two parts: positioning navigation and space search. In the positioning navigation experiment, the rats received 4 days of training, 4 times a day. The specific training was as follows: each time before entering the water, put the rat on the underwater platform to adapt for 30 seconds, record the swimming distance of the rat from the four quadrants and different entry points to the platform within 60 seconds, and the average of the 4 results was the final grade. On the fifth day, the space search experiment was carried out, the platform was removed, and the rats were placed into the water from the entry point in the opposite quadrant of the platform, facing the wall of the pool, and the swimming trajectory of the rats within 60 seconds was recorded and analyzed.

### Specimen collection and preparation

After the water maze experiment, the rats were fasted for 12 hours but were allowed to drink water freely. After the rats were anesthetized, blood was taken from the abdominal aorta, and the collected blood was centrifuged to obtain serum. The content of T3 and T4 in serum was measured by an automatic biochemical analyzer (C1600, Abbott, USA) within 48 hours. The rats were euthanized with an overdose of sodium pentobarbital, the brain tissues of the rats were separated, a part of the brain tissue was fixed in 4% paraformaldehyde, paraffin embedded and sectioned, and the rest was stored in a refrigerator at -80 ℃ for Western blot and quantitative reverse transcription polymerase chain reaction (qRT-PCR).

### Nissl staining

After the paraffin sections were conventionally deparaffinized and hydrated, they were reacted with Nissl staining solution (G1036, Wuhan Google Biotechnology Co., Ltd., China) in an oven at 60°C for 20 min. After washing the sections with distilled water, the sections were dried in an oven at 60°C. Finally, the sections were routinely dehydrated, transparent and sealed.

## QRT-PCR

Total RNA from rat brain tissue was extracted by total RNA extraction kit (R1200, Solarbio, China). Total RNA was reverse transcribed into cDNA with the help of reverse transcription kit (CW2569, cwbiotech, China). Then the SYBR Green qPCR kit (CW2601, cwbiotech, China) was used for qPCR. β-actin was employed as an internal control. The primers were listed as follows: BDNF, forward: 5’-GGCAGGCTTTGATGAGACCG-3’ and reverse: 5’-TCACCTGGTGGAACTCAGGGT-3’; β-actin, forward: 5’-AACCTTCTTGCAGCTCCTCC-3’ and reverse: 5’-TACCCACCATCACACCCTGG-3’. Relative expressions of BDNF were analyzed by Real-Time PCR Detection system (CFX96, Bio-rad, USA) with $2^{-\Delta\Delta Ct}$ method[22].

### Western blot

Western blot was performed as previously described[23]. The protein sample was collected from rat brain tissue by RIPA lysate (P0013D, Beyotime, China), PMSF (ST506, Beyotime, China) and protease inhibitors (60237, Beyotime, China). Then the protein was sequentially quantified by BCA kit (pc0020, Solarbio, China). The protein was separated with 10% separating gel, and then the protein was transferred to the PVDF membrane (10600023, GE Healthcare Life, USA). Before incubating the primary antibody, the membrane needed to be blocked with 5% skimmed milk. Primary antibodies include anti-BDNF antibody (ab108319, Abcam, UK) and anti-β-actin antibody (ab8226, Abcam, UK). After incubating with the primary antibody overnight at 4°C, the membrane reacted with the secondary antibody goat anti rabbit (ab205718, Abcam, UK) or goat anti mouse (ab6789, Abcam, UK) at room temperature for 2 hours. The membrane was developed on a chemiluminescence instrument (610020-9Q, Qinxiang, China) with ECL luminescence reagent (C510045, Sangon, China). β-actin was used as internal control.

# Statistical analysis

Data were analyzed by SPSS 16.0 (SPSS, Chicago, USA) and represented as mean ± standard deviation. One-way analysis of variance was used for measurement data among multiple groups, and SNK analysis was used for comparison between groups. Kruskal-Wallis H test was used for results of uneven variance. $P < 0.05$ was accepted to be statistically significant.

# Results

### Effect of DMF treatment on body weight and thyroid level in rats with hypothyroidism

After the SD rats were experimentally fed for one week, the rats were divided into groups. At this time, there was no significant difference in the body weight of rats in each group (Fig. 1). During the four weeks when the rats were treated with 0.05% PTU, the weight of the rats was significantly lower than that of the control rats (Fig. 1, $P < 0.01$). However, after DMF treatment, the trend of weight loss in PTU group rats was reversed (Fig. 1, $P < 0.05$). In addition, the levels of T3 and T4 in the serum of rats with PTU-induced hypothyroidism were also significantly lower than those in the control group (Fig. 2, $P < 0.01$). However, the treatment of DMF restored the levels of T3 and T4 in the serum of rats in the PTU group to normal (Fig. 2, $P < 0.01$).

### Effect of DMF on the learning ability of hypothyroid rats

We evaluated the effect of DMF on the learning and memory abilities of hypothyroid rats through a water maze experiment. As shown in Fig. 3, the escape latency of rats in the PTU group was much higher than that of the control group, while DMF treatment can effectively reduce the escape latency of hypothyroid rats to a normal level ($P < 0.01$). Not only that, compared with the control group, rats in the PTU group swam longer and had a slower swimming speed, but these problems can be improved by DMF treatment ($P < 0.01$).

### DMF increased the number of Nissl bodies in hippocampal CA1 area of hypothyroidism rats

Nissl body is a small triangular or elliptical mass distributed in the cytoplasm of nerve cells, which can be stained blue-purple by Nissl staining solution. The disappearance of Nissl body is an important indicator of nerve cell damage. From Fig. 4, we can see that the Nissl body in the control group is very complete, while the number of Nissl bodies in the PTU group is significantly less than that in the control group ($P < 0.01$). Compared with the PTU group, the number of Nissl bodies in the PTU + DMF25 group was increased ($P < 0.01$).

### DMF increased the mRNA and protein levels of BDNF in hippocampal CA1 area of hypothyroid rats

By detecting the expression of BDNF mRNA and protein in the brain tissue of each group of rats (Fig. 5, 6, $P < 0.01$), we found that PTU-induced hypothyroidism rats express less BDNF in the brain tissue ($P < 0.01$). But the decrease of BDNF expression in rat brain tissue caused by PTU can be reversed by DMF treatment ($P < 0.05$, $P < 0.01$).

## Discussion

Thyroid hormones are essential for the normal development of the mammalian brain[24]. Mounting studies have shown that thyroid hormone deficiency during brain development can lead to abnormal brain structure and dysfunction, which seriously affects learning and memory functions[25, 26]. At present, the treatment of hypothyroidism mostly uses thyroid hormone replacement therapy[27]. Thyroid hormones mainly include T3, T4 and TSH. Clinically, hypothyroidism is usually diagnosed by detecting the levels of T3 and T4. However, when the levels of thyroid-stimulating hormone and thyroid hormone return to normal, whether the impairment of cognitive function can be completely restored, there is no sufficient research to prove[28].

DMF was approved by the U.S. Food and Drug Administration in 2013 for the treatment of multiple sclerosis[29]. The mechanism involved is related to reducing inflammation and activating the Nrf2 antioxidant pathway. Graciela Freitas Zarbato et al. found that the anti-inflammatory and antioxidant effects of DMF were exerted in experimental sepsis rats. In addition, they also found that DMF can improve cognitive impairment after bacterial sepsis[30]. Coincidentally, Sofia P das Neves and others also found that DMF can enhance the cognitive ability of mice with experimental autoimmune encephalomyelitis[31]. Our study used the classic Morris water maze experiment to evaluate the cognitive function of rats in each group, and found that DMF has the same alleviating effect on the cognitive impairment of PTU-induced hypothyroid rats.

At present, the research on the regulation of cognitive function by DMF has mostly focused on the Nrf2 pathway and other oxidative stress pathways[30, 32, 33]. This is because neurons are extremely sensitive to the damage of reactive oxygen species. When reactive oxygen species are generated excessively, the oxidative stress response is enhanced, which can damage the nucleic acids, proteins, and lipids on the inner membrane of neuronal mitochondria, causing cell damage or death, causing cognition disfunction[34, 35]. We found through Nissl staining that DMF can significantly improve the pathological morphology of neurons in the CA3 region of the hippocampus of hypothyroid rats. Similar to our

conclusion, Xiaowen Hou et al. also found that DMF has a repairing effect on neuronal damage in the hippocampus CA1 area of rats with middle cerebral artery occlusion[36].

In the central nervous system, BDNF is mainly synthesized in neurons[37]. A large number of studies have shown that BDNF plays an important role in the survival, differentiation, migration of neurons, the development of axons and dendrites of new neurons, and the formation of synapses[8, 38]. When simulating learning-related signals, the expression of BDNF in the pre- and post-synaptic membranes will increase, which means that BDNF is one of the key proteins in learning and memory[39]. In the study of Mohammad Saied Salehi and others, DMF can promote the expression of BDNF in epidermal neural crest stem cells[40]. The regulatory effect of DMF on BDNF has also been confirmed to improve the depressive behavior of rats and the secondary degeneration after spinal cord injury[18, 41]. In our study, DMF treatment can increase the level of BDNF in the brain tissue of hypothyroid rats, which indicates that the regulation of DMF on BDNF can also improve the cognitive dysfunction of hypothyroid rats.

# Conclusion

In general, we constructed a hypothyroid rat model, which proved that DMF can improve the cognitive behavior of hypothyroid rats to a certain extent. However, we have only initially studied the effect of DMF on the expression of BDNF, and the regulation of its specific signaling pathway has not yet been clarified.

# List Of Abbreviations

BDNF
Brain-derived Neurotrophic Factor
FAEs
Fumaric acid esters
DMF
dimethyl fumarate
PTU
propylthiouracil

# Declarations

### Ethics approval

All animal procedures were in accordance with the National Instaitutes of Health Guide for the Care and Use of Laboratory Animals. The animal experiments were in accordance with the guidelines of laboratory animal care and were approved by the Animal Experimentation Ethics Committee of Hangzhou Eyong Biotechnological Co., Ltd. Animal Experiment Center (SYXK, (Zhe)2020-0024). ALL methods are reported in accordance with ARRIVE guidelines.

### Consent for publication

Not applicable.

### Data availability

All data generated or analyzed during this study are included in this article.

### Competing interests

The authors declare that there are no conflicts of interest.


### Funding

Not applicable.


### Authors' contributions

Haiyan Pan acquired data and drafted the manuscript; Yanbo Wang analyzed and interpreted the data; Xiaowei Wang Statistical analyzed these data; Ci Yan designed this study and revised the manuscript.


### Acknowledgments

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# Figures

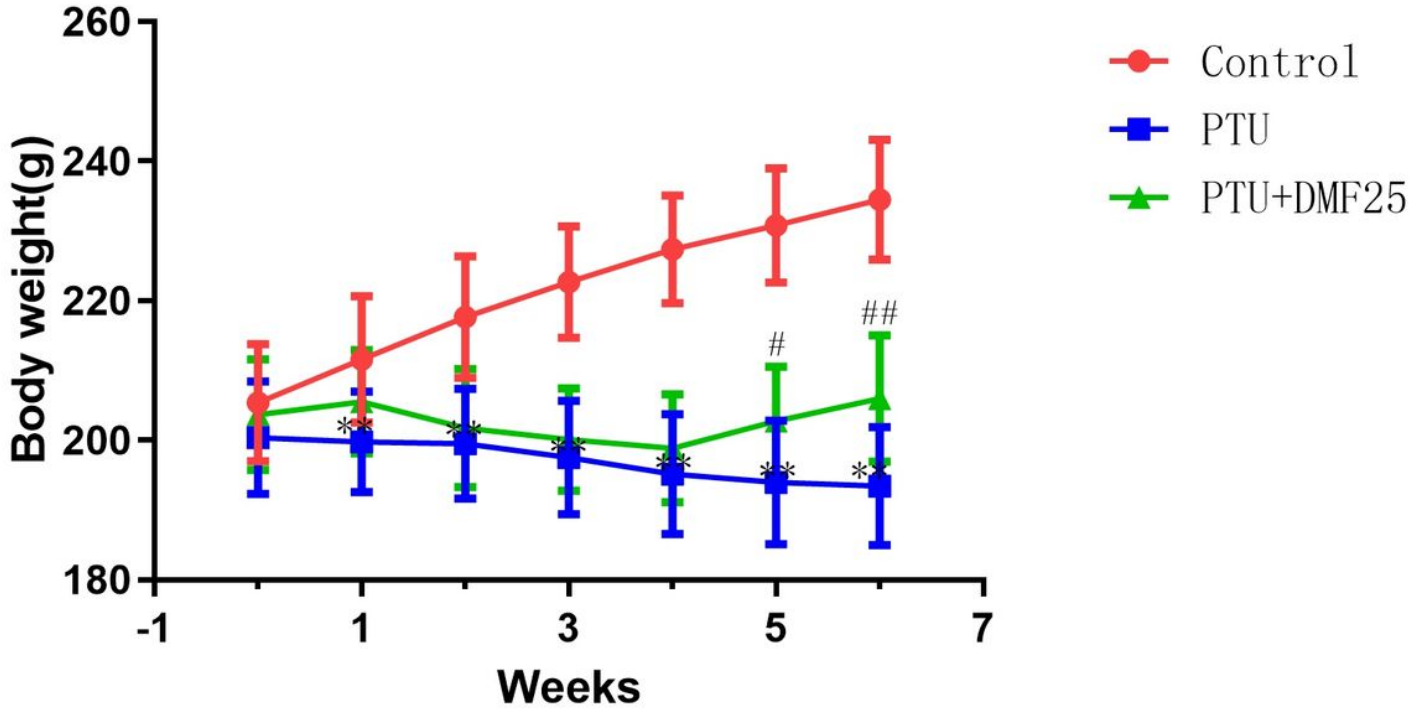
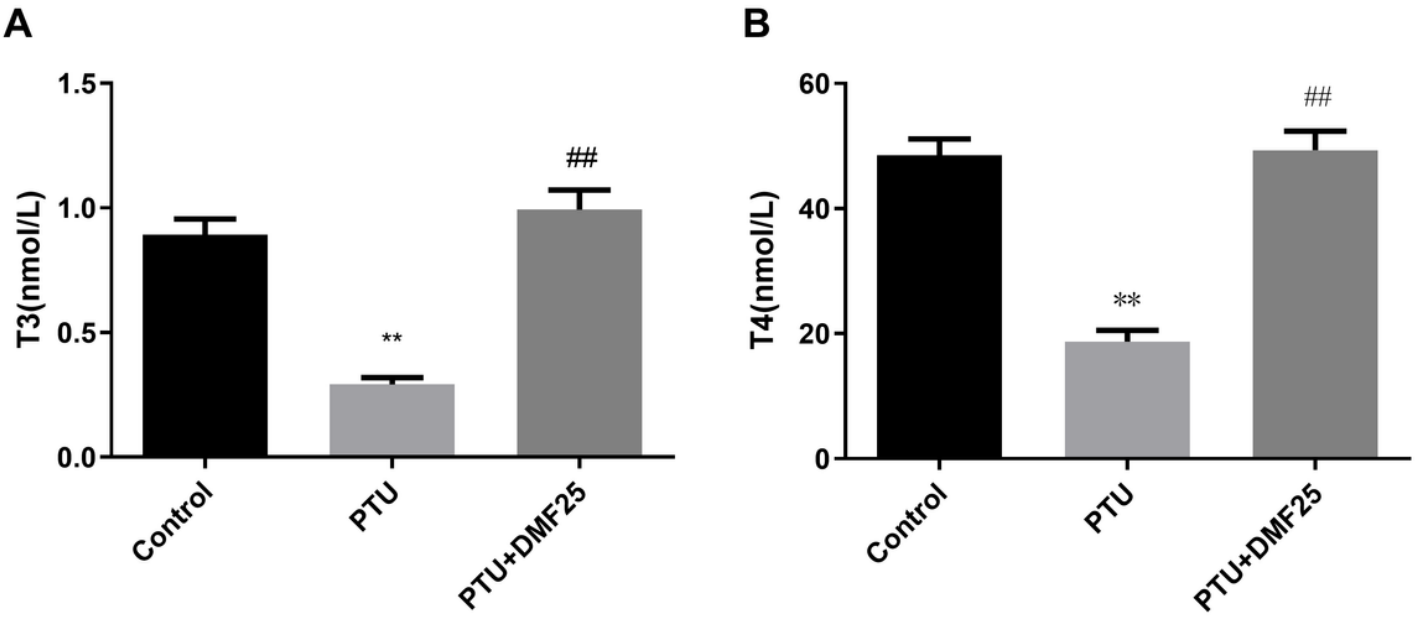


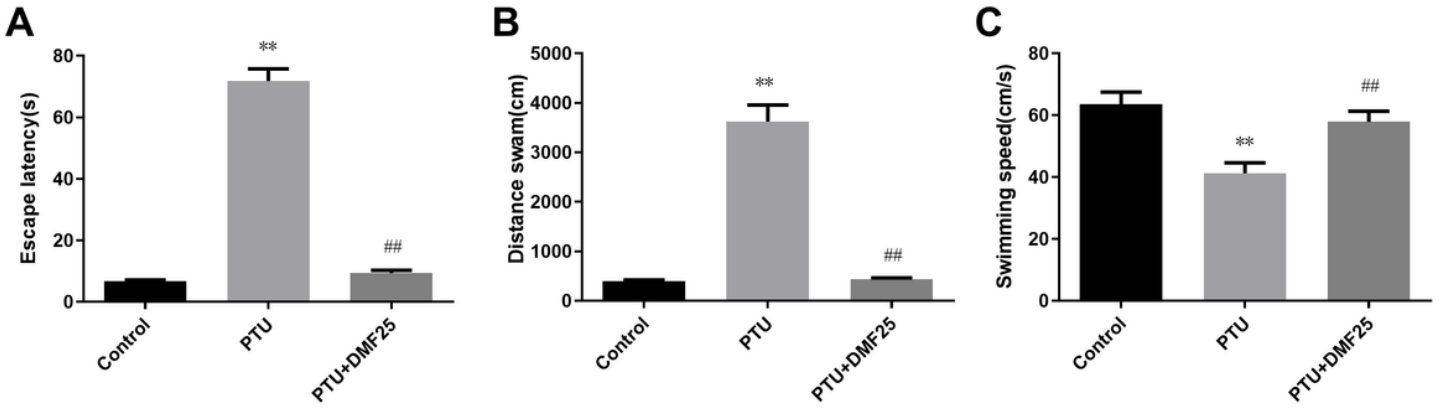

**Figure 1**

comparison of the body weight increment among three groups.Data are presented as mean ± SEM (n = 6 in each group).**p < 0.01 compared with the control group. ##p< 0.01 compared with the PTU group.



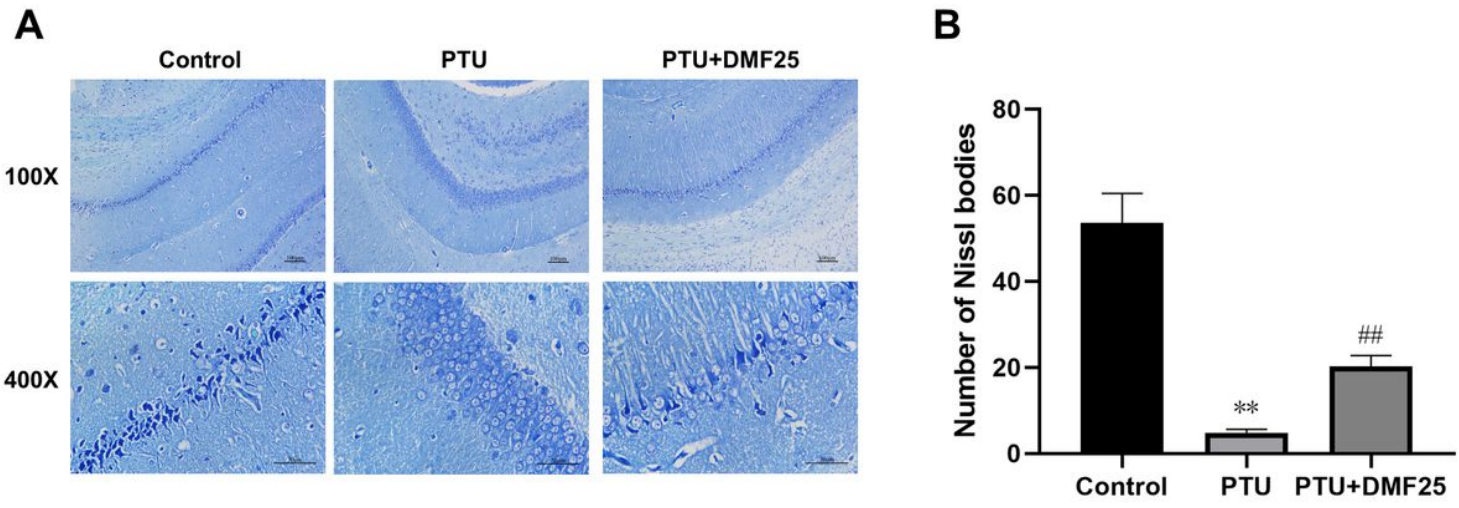
## Figure 2

comparison of serum of thyroid-related hormones levels among three groups. (A) the content of T3. (B) the content of T4. Data are presented as mean ± SEM (n = 6 in each group).**p < 0.01 compared with the control group. ##p< 0.01 compared with the PTU group.




## Figure 3

Comparison of the time latency (A) and traveled distance (B) to reach the platform and the swimming speed (C) between three groups in the Morris water maze test.Data are presented as mean ± SEM (n = 6 in each group).**p < 0.01 compared with the control group. ##p< 0.01 compared with the PTU group.




## Figure 4

Effect of DMF on hippocampal neurons of rats. (A) Representative images showing Nissl bodies in the hippocampal CA1. Original magnification, 100X, Scale bar, 100 mm; 400X , Scale bar, 50 mm. (B) Quantitation of pyramidal cells in the CA1 hippocampal region. The numbers of Nissl bodies were captured in the three fields of the CA1 region of the hippocampus. The data represent the mean± SEM (n = 3). **p < 0.01 compared with the control group. ##p< 0.01 compared with the PTU group.

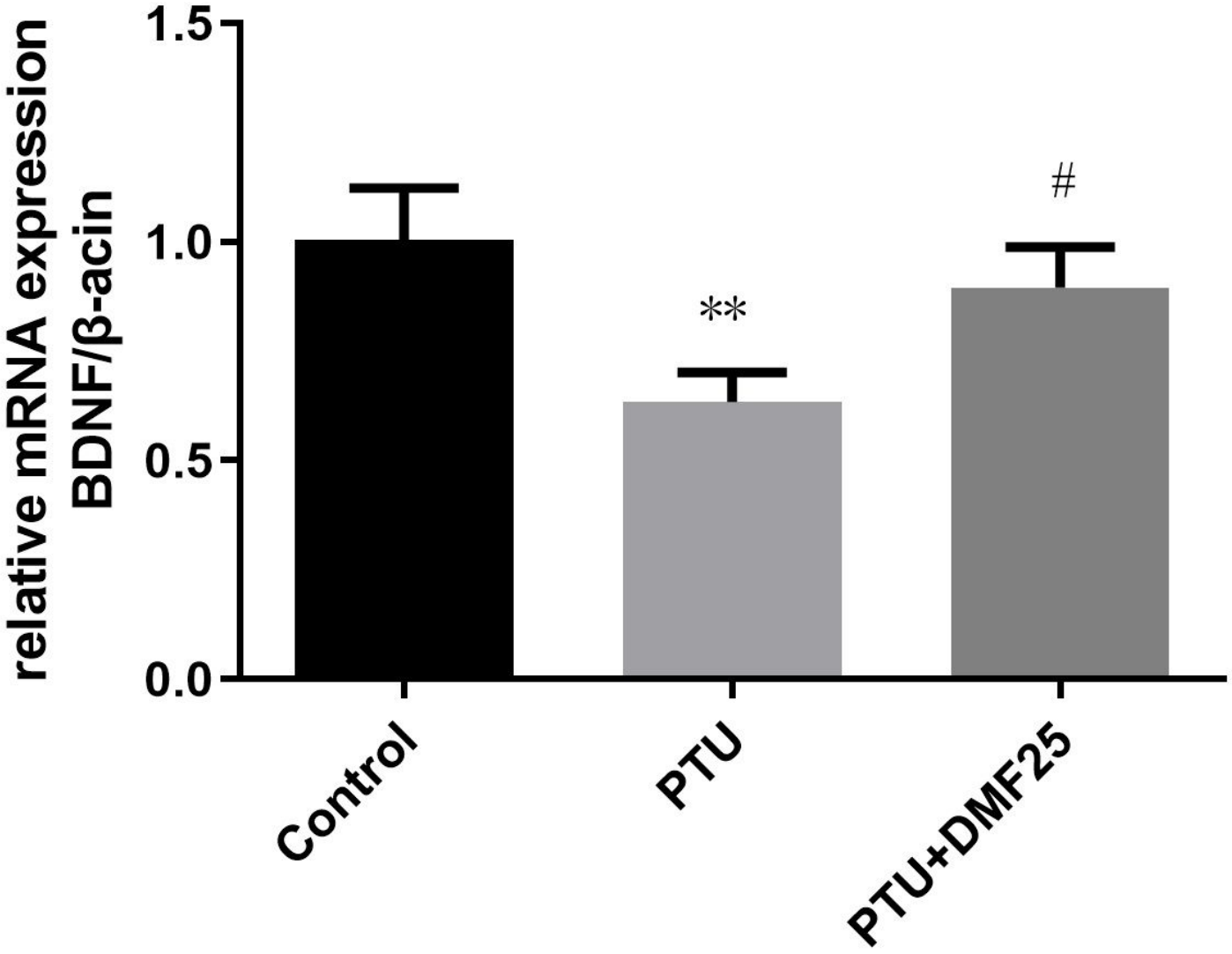



**Figure 5**

Effect of DMF on BDNF mRNA expression in hippocampal CA1 region of hypothyroidism rats. The data represent the mean± SEM (n = 3). **p < 0.01 compared with the control group. ##p< 0.01 compared with the PTU group.

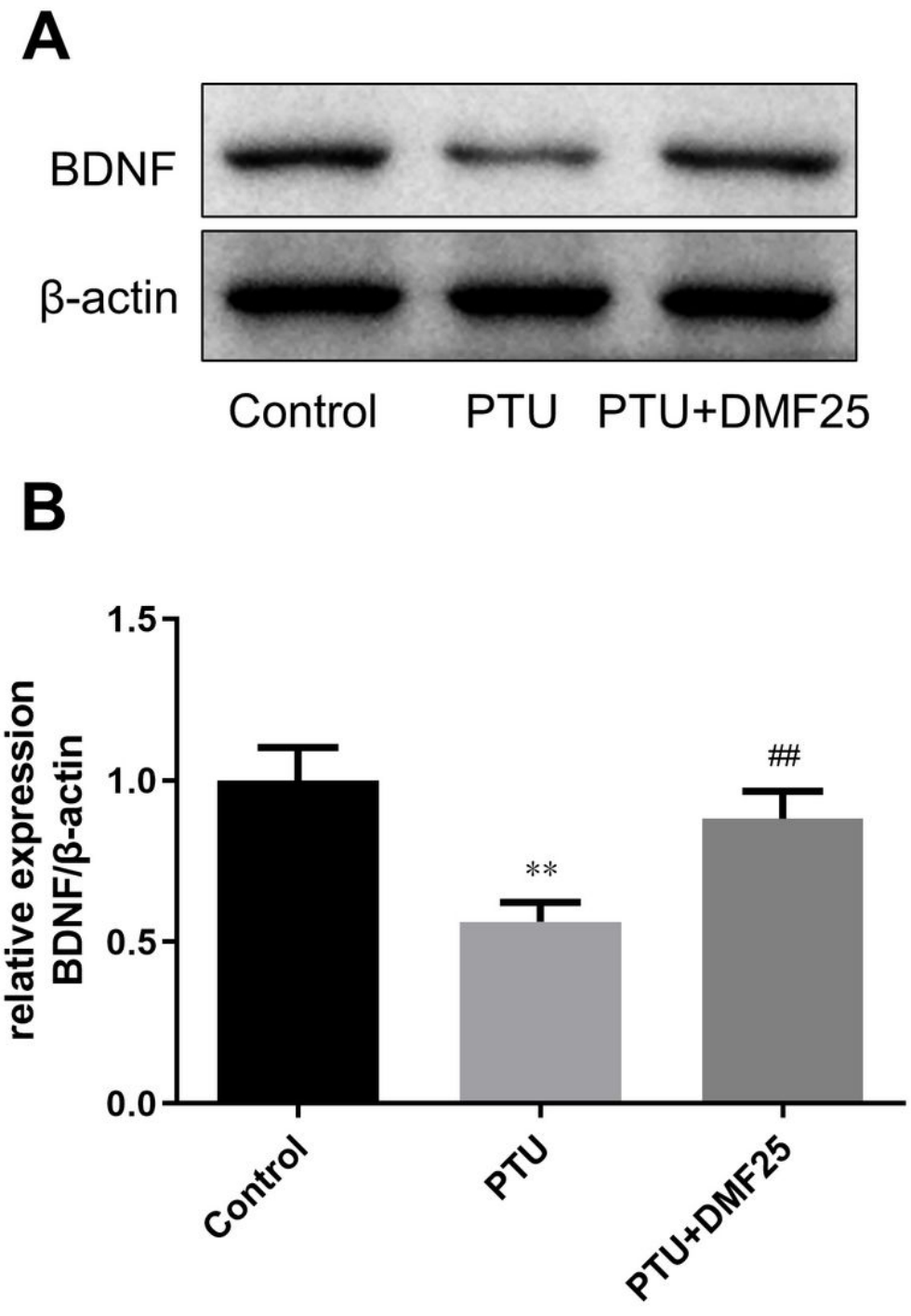



**Figure 6**

Effect of DMF on BDNF protein expression in hippocampal CA1 region of hypothyroidism rats. (A)The protein expression of BDNF . (B) The relative density quantification of BDNF.The data represent the mean± SEM (n = 3). **p < 0.01 compared with the control group. ##p< 0.01 compared with the PTU group.