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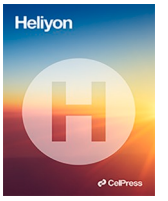

Research article

Qingzhuan dark tea Theabrownin alleviates hippocampal injury in HFD-induced obese mice through the MARK4/NLRP3 pathway

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ABSTRACT

Background: Feeding on a high-fat diet (HFD) results in obesity and chronic inflammation, which may have long-term effects on neuroinflammation and hippocampal injury. Theabrownin, a biologically active compound derived from the microbial fermentation of Qingzhuan dark tea, exhibits anti-inflammatory properties and lipid-lowering effects. Nevertheless, its potential in neuroprotection has yet to be investigated. Consequently, this study aims to investigate the neuroprotective effects of Theabrownin extracted from Qingzhuan dark tea, as well as its potential therapeutic mechanisms.

Methods: Male C57 mice were subjected to an 8-week HFD to induce obesity, followed by oral administration of Theabrownin from Qingzhuan dark tea. Lipid levels were detected by Elisa kit, hippocampal morphological damage was evaluated by HE and Nissl staining, and the expression levels of GFAP, IBA1, NLRP3, MARK4, and BAX in the hippocampus were detected by immunofluorescence (IF), and protein expression levels of NLRP3, MARK4, PSD95, SYN1, SYP, and Bcl-2 were detected by Western Blot (WB).

Results: Theabrownin treatment from Qingzhuan dark tea prevents alterations in body weight and lipid levels in HFD-fed mice. Furthermore, Theabrownin decreased hippocampal morphological damage and reduced the activation of astrocytes and microglia in HFD-fed mice. Moreover, Theabrownin decreased the expression of MARK4 and NLRP3 in HFD-fed mice. Besides, Theabrownin elevated the expression of PSD95, SYN1, and SYP in HFD-fed obese mice. Finally, Theabrownin prevented neuronal apoptosis, reduced the expression of BAX, and increased the expression of Bcl-2 in HFD-fed obese mice.

Conclusions: In summary, our current study presents the first demonstration of the effective protective effect of Theabrownin from Qingzhuan dark tea against HFD-induced hippocampal damage in obese mice. This protection may result from the regulation of the MARK4/NLRP3 signaling pathway, subsequently inhibiting neuroinflammation, synaptic plasticity, and neuronal apoptosis.

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1. Introduction

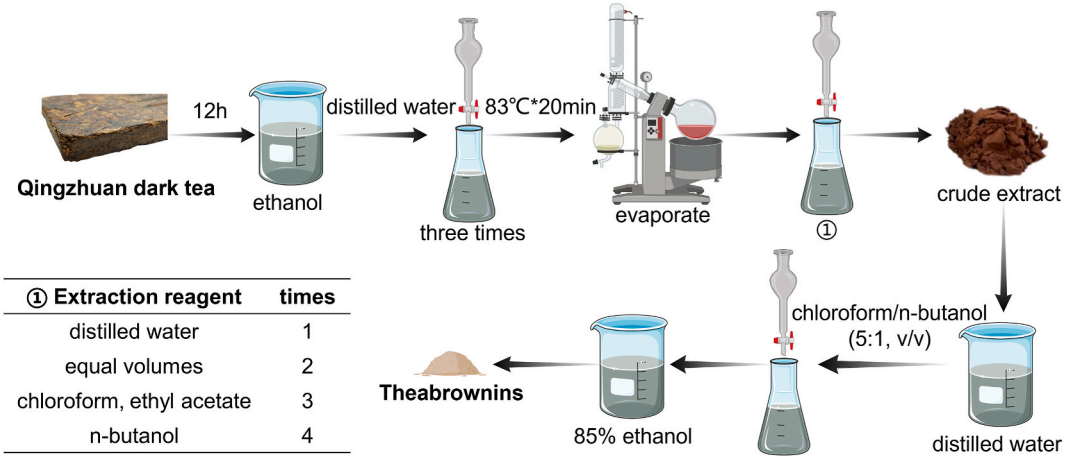
Obesity is a significant global public health concern, with the number of obese individuals increasing annually. This trend is closely linked to people's dietary habits and food intake [1]. Moreover, obesity is associated with several chronic diseases, including type 2 diabetes, coronary heart disease, and certain cancers [2]. Research suggests that excessive fat intake can result in peripheral metabolic disorders, leading to a systemic condition of chronic low-grade inflammation. This perspective is supported by pathological studies in obese patients [3]. Furthermore, some studies propose that adipose tissue may have a pivotal role in triggering inflammation [4]. Interestingly, long-term intake of a high-fat diet (HFD) leads to an elevation in the levels of lipopolysaccharides (LPS) in the intestines [5]. Moreover, HFD feeding induces microglial activation and neuroinflammation within three days [6]. Increasing evidence suggests that HFD -induced obesity has adverse effects on hippocampal structure and function, with the most conspicuous changes being related to hippocampus-dependent learning and memory decline [7]. Collectively, these findings demonstrate that HFD can result in neural damage and neuroinflammation, emphasizing the importance of targeting these aspects for therapeutic interventions.

Several studies have consistently demonstrated that neuroinflammation in the brain accompanies hippocampal damage [8]. NLRP3 inflammasome is a complex of multiple proteins that, upon activation, induces the production of interleukin (IL)-1β and IL-18 mediated by caspase-1 in microglial cells [9]. The generation and activation of NLRP3 are intimately linked to the occurrence of neuroinflammation. Recent research has revealed that Tau protein aggregation can activate NLRP3 [10]. The activation of NLRP3 and Aβ deposition mutually exacerbate each other, resulting in the development of pathological characteristics resembling amyloid-like protein [11]. Several studies have also demonstrated that inhibition of NLRP3 can mitigate neuroinflammation and anxiety cause by obesity [12,13]. These findings indicate that NLRP3 plays a crucial role in the brain, and inhibiting NLRP3 represents a feasible approach to mitigating neuroinflammation.

Microtubule affinity-regulating kinase 4 (MARK4) is a protein that phosphorylates microtubule-associated proteins (MAPs) and Tau proteins, thereby regulating microtubule dynamics. Thus, its role in the brain has been extensively studied. Research indicates that MARK4 can modulate microtubule dynamics, through affecting various biological processes, including cell polarity, cell migration, and adipogenesis [14]. Moreover, it has a significant role in neurodegenerative diseases like Alzheimer's disease (AD) [15]. Recently, it was found that MARK4 can interact with NLRP3 to form an inflammasome complex, and reducing MARK4 levels can relieve the spatial confinement of NLRP3, consequently inhibiting inflammasome activation [16]. Furthermore, in a diabetes model [17], MARK4 triggers NLRP3 activation. These findings collectively imply that MARK4 plays a pivotal role in the regulation of NLRP3 and might serve as a potential therapeutic target for addressing neural damage.

Qingzhuan dark tea is a distinctive dark tea variety specific to the Xianning region in southern China, primarily crafted through microbial fermentation. As the vintage of Qingzhuan dark tea ages, its popularity increases due to flavor changes and a higher content of various metabolites, with the pivotal role played by the formation of Theabrownin [18]. In Pu'er tea, Theabrownin can modulate gut microbiota and bile acid metabolism, effectively mitigating hypercholesterolemia [19]. Interestingly, Theabrownin can influence metabolic syndrome interaction with the gut-liver-brain axis interaction [20]. Furthermore, research has revealed that Theabrownin induces apoptosis and cell cycle arrest in neuroblastoma and astrocytoma [21]. These studies collectively indicate that Theabrownin might possess potential neuroprotective effects. Nevertheless, the precise roles and mechanisms it exerts in the brain remain incompletely elucidated.

In the current research, Theabrownin has been found to possess biological properties such as regulating blood glucose, blood lipid levels, anti-inflammatory effects, and anti-atherosclerotic effects [22]. However, there are a limited number of studies on the neuroprotective properties of Theabrownin. Therefore, we aim to investigate whether Theabrownin demonstrates potential neuroprotective effects in the hippocampus of HFD-induced mice and explore its underlying mechanisms. This study endeavors to furnish more robust evidence for the foundational research on Theabrownin's impact on hippocampal damage.



① Extraction reagent	times
distilled water	1
equal volumes	2
chloroform, ethyl acetate	3
n-butanol	4

Fig. 1. Theabrownin production flowchart.

2. Methods

2.1. Animal experiments and Theabrownin preparation

The preparation of Theabrownin is described earlier [19,23] (Fig. 1). 48 male C57 mice were purchased from Liaoning Changsheng biotechnology co.,LTd (Liaoning, China), Quality Certificate: 210726220101423875, Certificate of Conformity. License No. SCXK (Liaoning) 2020-0001. A one-week adaptation period was provided during which all mice had access to water and standard laboratory chow ad libitum to acclimate to the new environment. HFD was used to establish the mouse model. Subsequently, the mice were randomly assigned into four groups: the control group with a normal diet (Con), the group fed with a high-fat diet (HFD), the group fed with HFD and low-dose Theabrownin (HFD+TBs-L, 180 mg/kg/d) [20], and the group fed with HFD and high-dose Theabrownin (HFD+TBs-H, 360 mg/kg/d). Mice are given Theabrownin by Oral gavage (ig). After 8 weeks of feeding, brain tissues were harvested from these mice. The animal room was maintained under controlled conditions of 25 ± 1 °C temperature, 55 ± 5% humidity, and a 12h light/12h dark cycle. All animal experiments were conducted following the applicable guidelines and regulations approved by the Animal Ethics Committee of Hubei University of Science and Technology (Xianning, China). Lipid levels were measured using mouse serum, which was naturally clotted at room temperature for 1 h and centrifuged at 1000 rpm for 15 min. Serum total cholesterol (TC) and total triglycerides (TG) levels were measured according to the instructions from the manufacturer of the reagents (Nanjing Jiancheng Biotech Co., Ltd., Nanjing, China).

2.2. HE staining

In short, brain tissues were extracted and fixed with paraformaldehyde. Subsequently, they were dehydrated and embedded in paraffin, and the hippocampal tissues were sectioned at 4 μm thickness. After staining with hematoxylin for 1 min, the sections were washed three times in double-distilled water. Then, the sections were incubated in acidic alcohol for 30 s, stained with eosin for 50 s, followed by dehydration in 95% ethanol, 100% ethanol, and finally cleared with xylene and embedded in neutral resin. Images were captured using a microscope (Nikon, Tokyo, Japan).

2.3. Nissl staining

Nissl staining was performed as described earlier [24]. Paraffin sections were incubated with 0.1% cresyl violet at room temperature for 5 min, followed by rinsing with double-distilled water, dehydration in 95% ethanol and 100% ethanol, and clearing in xylene, then covered with neutral resin. Images were captured using a microscope (Nikon, Tokyo, Japan).

2.4. Immunofluorescence (IF)

For brain tissue paraffin sections, after 1 h of dewaxing, antigen retrieval was performed with sodium citrate solution at 90 °C for 15 min, followed by 45 min of blocking with 10% BSA for immunostaining at room temperature, overnight incubation with primary antibodies at 4 °C, and washing three times with PBS, then incubation with appropriate secondary antibodies for 1 h at room temperature. The primary antibodies include rabbit anti-NLRP3 (PAB38738, Bioswamp, 1:200), rabbit anti-MARK4 (AF0693, Affinity, 1:200), rabbit anti-GFAP (bs-0199R, Bioss, 1:200), rabbit anti-Iba1 (A19776, ABconal, 1:200), rabbit anti-BAX (bs-0127R, Bioss, 1:200). The secondary antibody used is donkey anti-rabbit Alexa Fluor568 (A10042, Invitrogen, 1:1000). Images were captured using a fluorescence microscope (Nikon, Tokyo, Japan), and image analysis was performed using Image J.

2.5. Western Blot (WB)

Hippocampal tissues were ground and protein was extracted in RIPA buffer under low-temperature conditions, and the protein level was determined using the supernatant. Proteins (30 μg) were separated by electrophoresis on 10% SDS-PAGE gels using a flat gel apparatus, then transferred onto PVDF (0.45 mm) membranes and blocked with 5% skim milk for 1.5 h. The primary antibodies, mainly including NLRP3 (PAB38738, Bioswamp, 1:1000), MARK4 (AF0693, Affinity, 1:1000), PSD95 (#48638, signalwayantibody, 1:1000), SYN1 (A17362, ABclonal, 1:1000), SYP (#54990, signalwayantibody, 1:1000), Bcl-2 (bs-0032R, Bioss, 1:1000), GAPDH (GB15002, Servicebio, 1:1000)and Beta Actin (#52901, signalwayantibody, 1:10000)were incubated overnight at 4 °C, followed by incubation with appropriate HRP-conjugated secondary antibodies at room temperature for 1 h. The protein bands were visualized using ECL chemiluminescent peroxidase substrate and quantified using the ChemiDoc XRS system (Bio-Rad, USA), and the immunoblot bands were quantitatively analyzed using Image J software.

2.6. Statistical analysis

All experimental numerical data are presented as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used as an appropriate statistical test, and differences between groups were determined using GraphPad Prism 9.0 software to generate bar graphs, with $p < 0.05$ considered statistically significant.

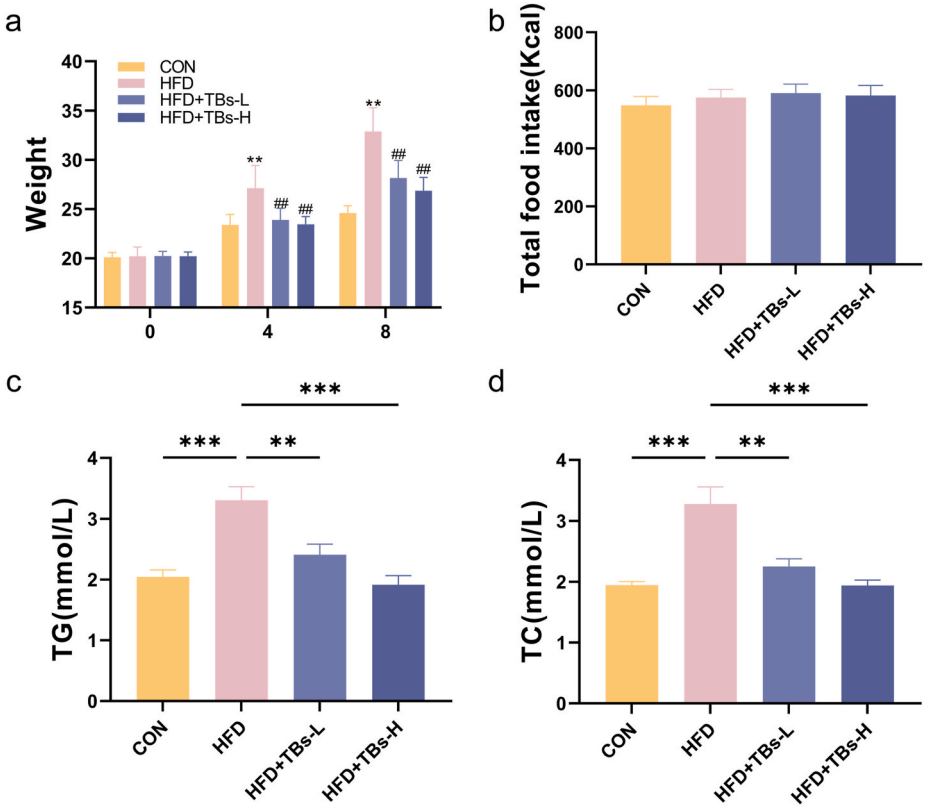


Fig. 2. Theabrownin can improve weight and blood lipid levels in HFD-fed obese mice. (a) Body weights of mice at weeks 0, 4, and 8. (b) Total food intake of mice over 8 weeks (n = 10). (c) TG and (d) TC, mean ± SEM (n = 5). *p < 0.05, **p < 0.01, ***p < 0.001.

3. Results

3.1. Theabrownin can improve weight and blood lipid levels in HFD-fed obese mice

The study found that the most significant changes in HFD-fed mice were in body weight and blood lipids [25], and we measured the body weight, blood lipids, and blood glucose levels of the mice. The results showed that compared to the CON group, mice given HFD exhibited a significant increase in body weight, reaching twice the original weight; at the same time, TC and TG levels in the HFD-fed mice was higher than in the con group. After administration of Theabrownin, the body weight, TC and TG levels significantly improved ($p < 0.05$) (Fig. 2a–c, d). However, the blood glucose test showed that HDL-fed mice had elevated fasting blood glucose ($p < 0.05$) (Supplementary Fig. 1), but did not reach hyperglycemic levels, and after Theabrownin administration, there was significant decrease in fasting blood glucose either ($p < 0.05$) (Supplementary Fig. 1). However, observations of the total food intake of the mice revealed that there was no significant difference in calorie intake among all groups ($p > 0.05$) (Fig. 2b). These results suggest that Theabrownin can improve body weight and blood lipid levels in HFD-fed mice, but it does not affect their food intake.

3.2. Theabrownin ameliorates hippocampal cell damage in HFD-fed obese mice

The hippocampus is the brain region most susceptible to changes, with the most significant alteration being the morphological changes in hippocampal neurons [26]. Changes in cognitive function due to obesity generally begin with alterations in the hippocampus [27]. HE and Nissl staining can visually assess hippocampal morphological changes, and thus, we used HE and Nissl staining to detect the morphology of mouse hippocampal neurons. The HE results revealed that compared to the CON group, the HFD group showed disordered arrangement and blurred boundaries of hippocampal neurons in the CA3, DG, and cortex areas, with the presence of deeply stained cell nuclei; however, this condition improved after administration of Theabrownin, and the improvement was dose-dependent (Fig. 3). Additionally, Nissl staining results also showed deep staining of hippocampal neurons in the CA3, DG, and cortex areas of the HFD group, which improved after Theabrownin administration (Fig. 4). However, the changes in CA2 area in HE and nissl staining were not significant (Supplementary Fig. 1). These results indicate that Theabrownin can improve the morphological changes of hippocampal neurons in HFD-fed mice.

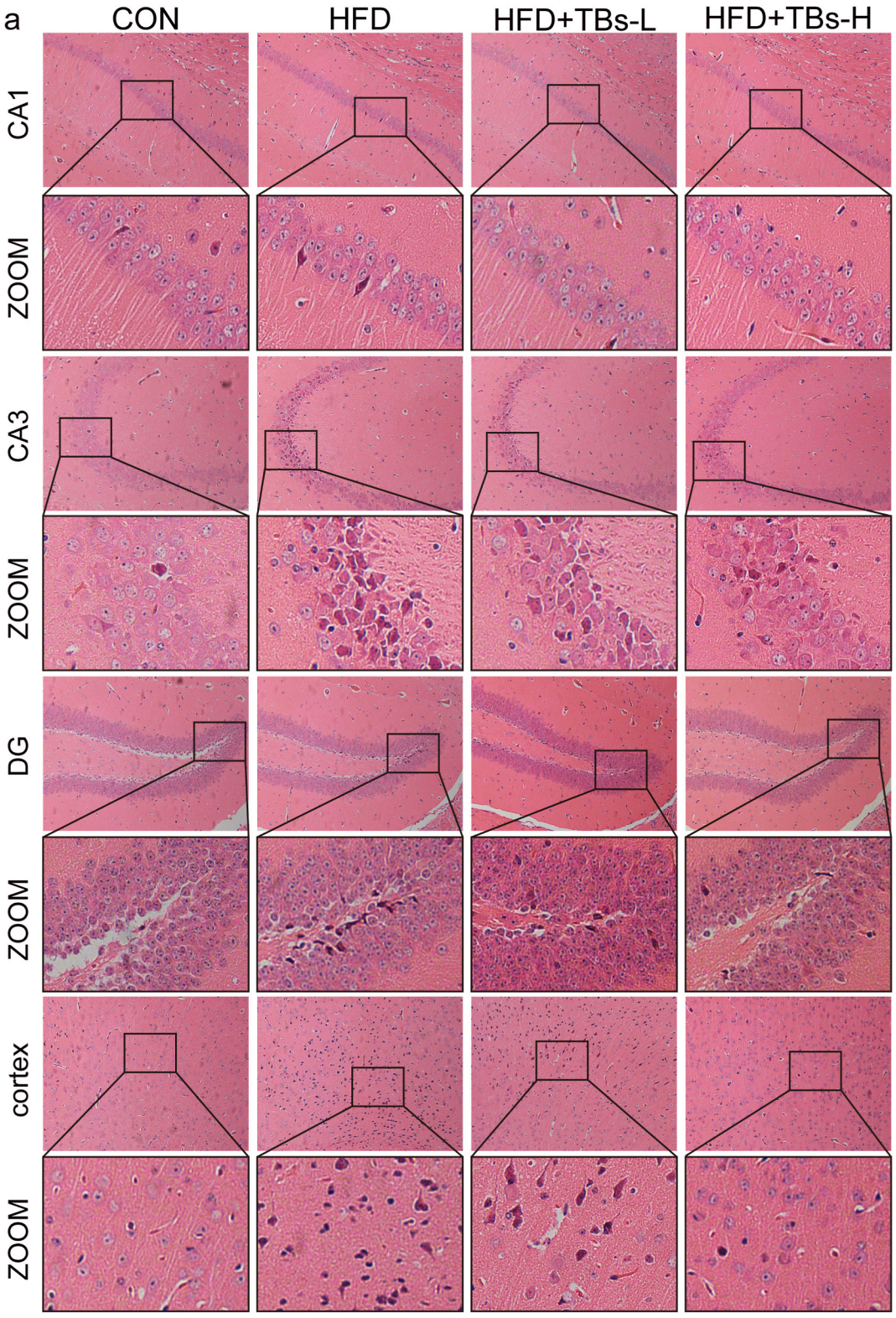


Fig. 3. Theabrownin ameliorates hippocampal cell damage in HFD-fed obese mice. HE staining of hippocampal CA1, CA3, DG area, and cortex for cytomorphologic observation (Scale = 50 μm) and corresponding ZOOM plots.

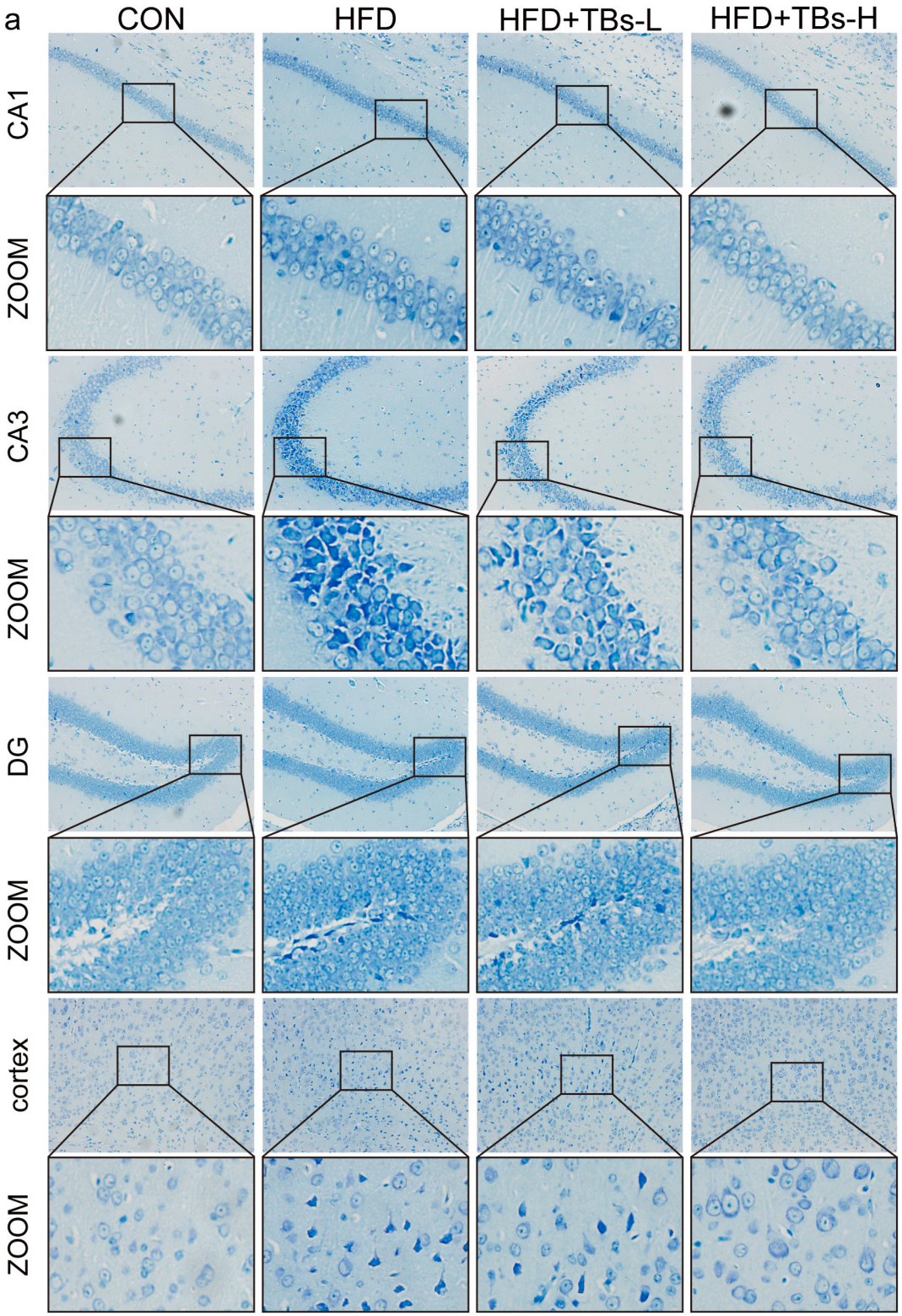


Fig. 4. Theabrownin ameliorates hippocampal cell damage in HFD-fed obese mice. Nissl staining of hippocampal CA1, CA3, DG area, and cortex for cytomorphologic observation (Scale = 50 μm) and corresponding ZOOM plots.

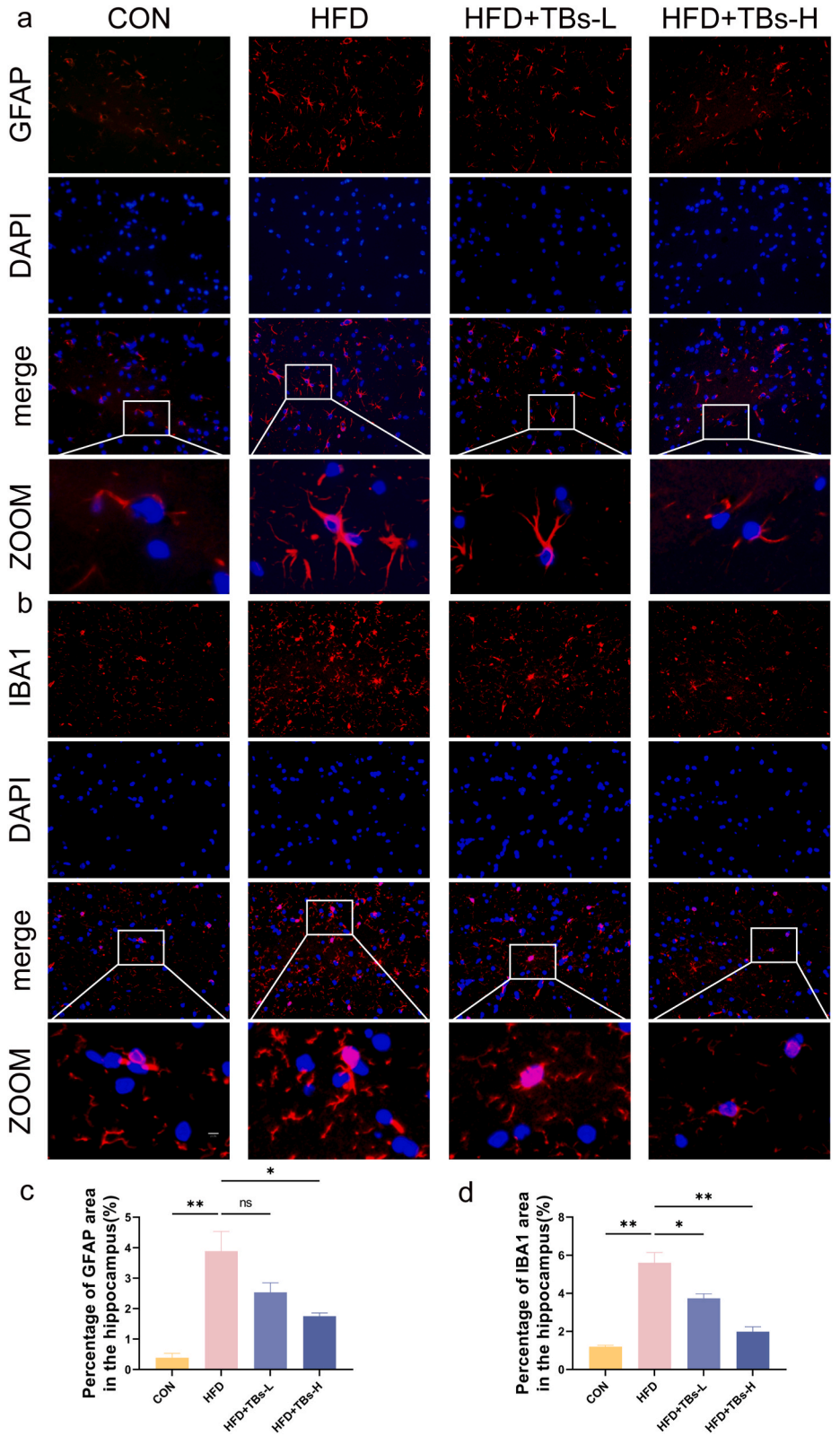


Fig. 5. Theabrownin reduces the activation of astrocyte and microglia in HFD-fed obese mice. Immunofluorescence staining plots of GFAP(a) and IBA1(b) in the hippocampus (Scale = 20 μm) and corresponding ZOOM plots. Immunofluorescence statistical plots of GFAP(c) and IBA1(d) (n = 3). mean ± SEM. *p < 0.05, **p < 0.01.

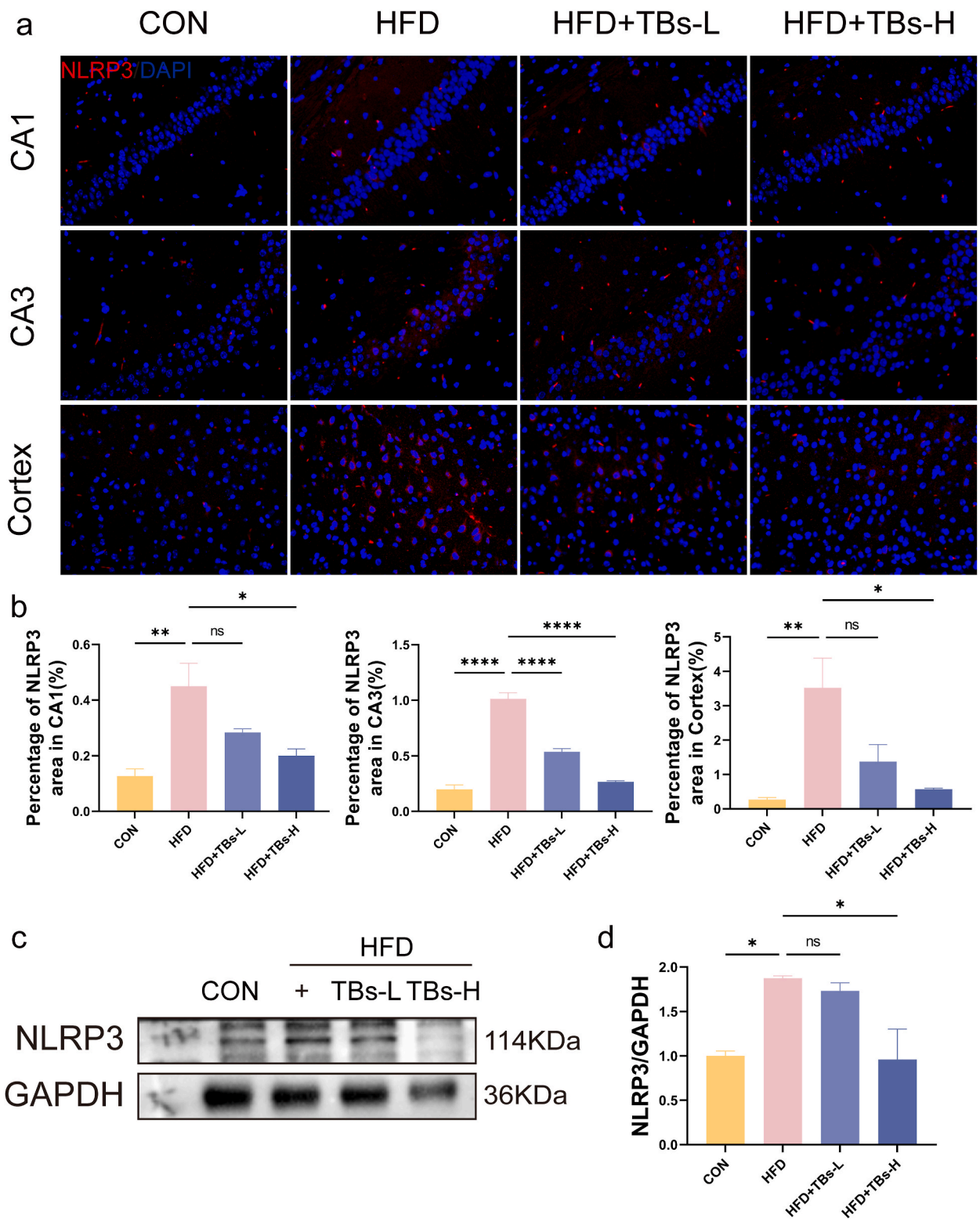


Fig. 6. Theabrownin reduces NLRP3 expression in HFD-fed obese mice. (a) Immunofluorescence of NLRP3 in hippocampal CA1, CA3 regions, and cortex (Scale = 20 μm). (b)Immunofluorescence statistics of NLRP3 in hippocampal CA1, CA3 regions, and cortex (n = 3). (c) Representative Western blot of NLRP3. (d) Histogram representing the quantitative analysis of NLRP3(n = 3). mean ± SEM. *p < 0.05, **p < 0.01.

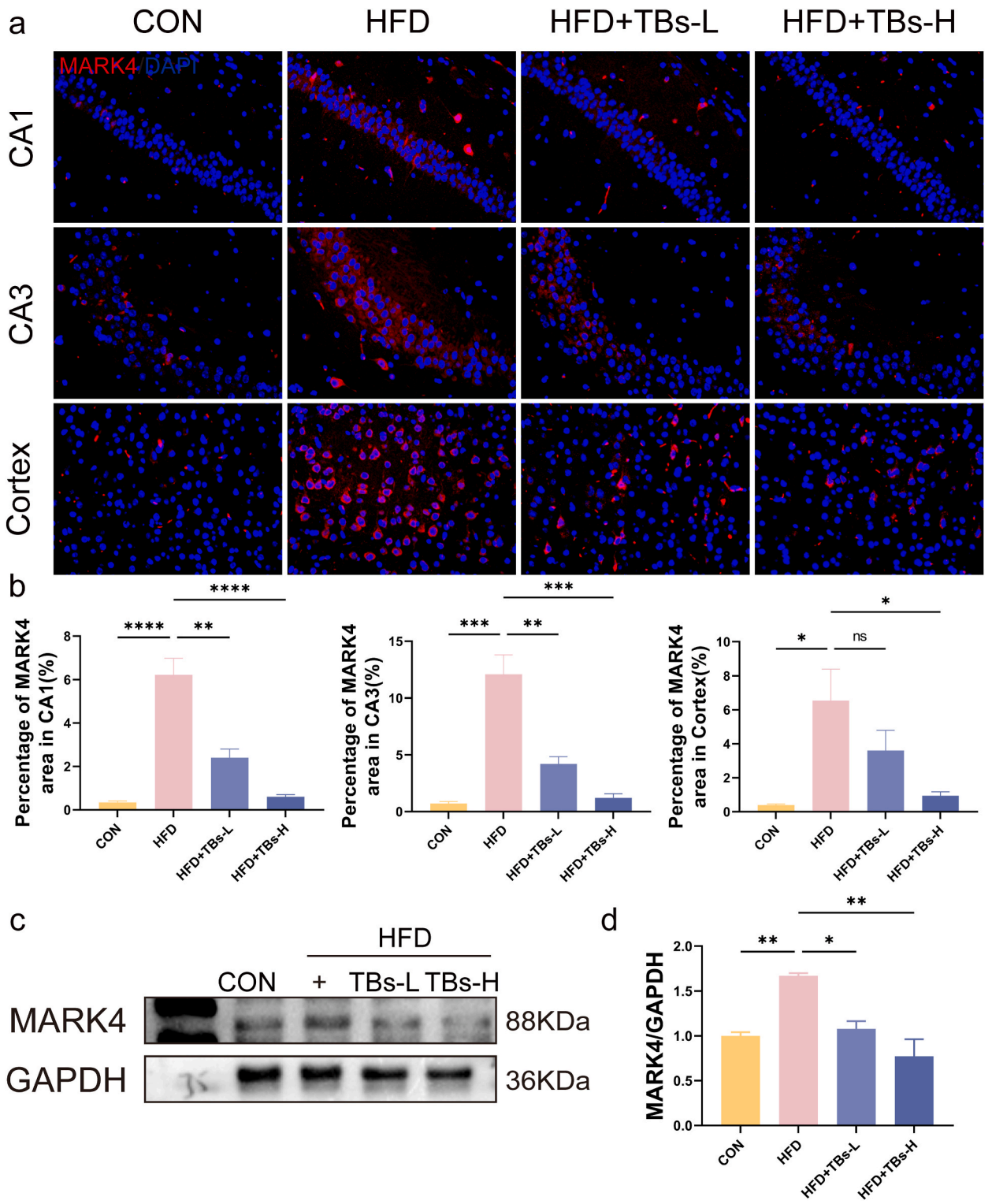


Fig. 7. Theabrownin reduces MARK4 expression in HFD-fed obese mice. (a) Immunofluorescence of MARK4 in hippocampal CA1, CA3 regions, and cortex (Scale = 20 μm). (b)Immunofluorescence statistics of MARK4 in hippocampal CA1, CA3 regions, and cortex (n = 3). (c) Representative Western blot of MARK4. (d) Histogram representing the quantitative analysis of MARK4(n = 3). mean ± SEM. *p < 0.05, **p < 0.01.

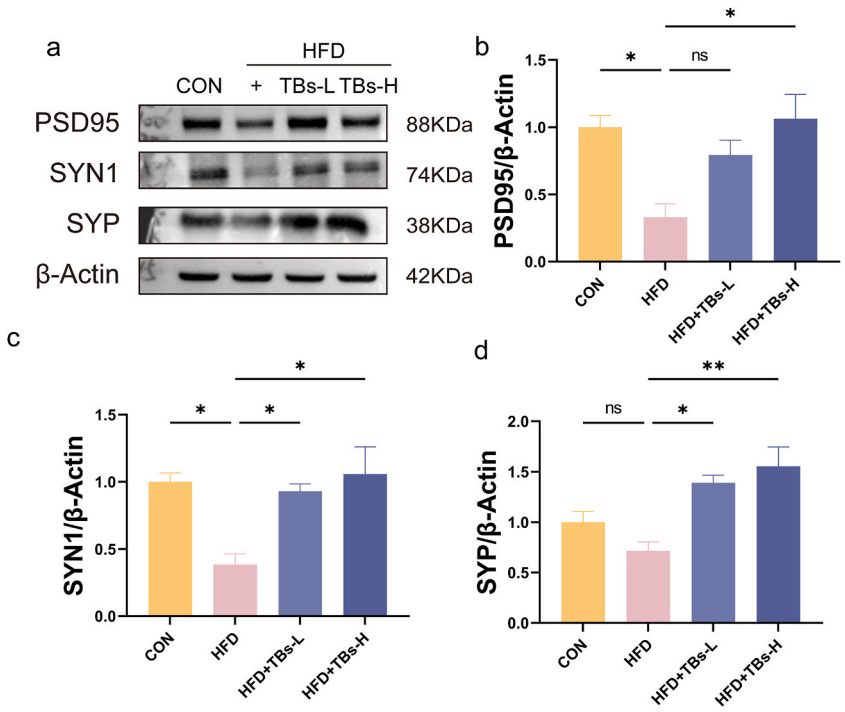


Fig. 8. Theabrownin improves synaptic plasticity in HFD-fed obese mice. (a) Representative Western blot of PSD95, SYN1, and SYP. Histogram representing the quantitative analysis of PSD95(b), SYN1(c), and SYP(d) (n = 3). mean ± SEM. *p < 0.05, **p < 0.01.

3.3. Theabrownin reduces the activation of astrocyte and microglia in HFD-fed obese mice

Microglia and astrocytes are the most abundant glial cells in the brain, and they are activated when neuroinflammation occurs in the brain. To determine the inflammatory status in the brains of HFD-fed obese mice, we used immunofluorescence for detection. The IF results showed that compared to the CON group (Fig. 5a and b), the expression of GFAP and IBA1 was significantly increased in HFD-fed mice ($p < 0.05$), and after administration of Theabrownin, the expression of GFAP and IBA1 was significantly reduced, showing a dose-dependent trend ($p < 0.05$) (Fig. 5c and d). These results indicate that neuroinflammation occurs in the brains of HFD-fed obese mice and Theabrownin can reduce the incidence of neuroinflammation.

3.4. Theabrownin reduces MARK4/NLRP3 expression in HFD-fed obese mice

NLRP3 is the most classical inflammasome and is also upregulated in HFD-fed obese mice [28]. Studies have found that in obesity, visceral adipose NLRP3 can affect cognitive function through IL-1β [29]. Therefore, we analyzed the expression changes of NLRP3 in the HFD-fed obese mouse model using WB and IF. The IF results showed that compared to the CON group, NLRP3 expression was significantly increased in the HFD group, and after administration of Theabrownin, the expression of NLRP3 decreased, especially in the CA1, CA3, and cortex regions ($p < 0.05$) (Fig. 6a and b), while there was no significant change in the CA2 and DG regions($p > 0.05$, Supplementary Fig. 2). Moreover, the results of WB demonstrated that the expression of NLRP3 increased in the HFD group, whereas the expression of NLRP3 declined with the rising dose after teicoplanin treatment ($p < 0.05$, Fig. 6 c,d).

Recently, MARK4 has been identified as an important target in Alzheimer's disease (AD) [30], and it plays a significant role in the assembly and activation of NLRP3 [31]. Therefore, in this study, we used IF to determine the expression of MARK4 in the hippocampus and cortex. Compared to the CON group, the expression of MARK4 was significantly increased in the HFD group, and after administration of black brick Theabrownin, the expression of MARK4 decreased, especially in the CA1, CA3, and cortex regions ($p < 0.05$) (Fig. 7a and b), with an decrease also observed in the CA2 and DG regions ($p < 0.05$), and there was a dose-dependent effect (Supplementary Fig. 3). In addition, the WB results also showed a rise in MARK4 expression in the HFD group, whereas, after teicoplanin treatment, MARK4 expression decreased with increasing dose ($p < 0.05$, Fig. 7c and d). These results also indicate that NLRP3 and MARK4 are upregulated in the hippocampus and cortex of HFD-fed obese mice, and Theabrownin can reduce the expression of NLRP3 and MARK4, thus mediating inflammation.

3.5. Theabrownin improves synaptic plasticity in HFD-fed obese mice

As we all know, synapses are the basis of neural transmission, and changes in synaptic plasticity can affect the transmission of

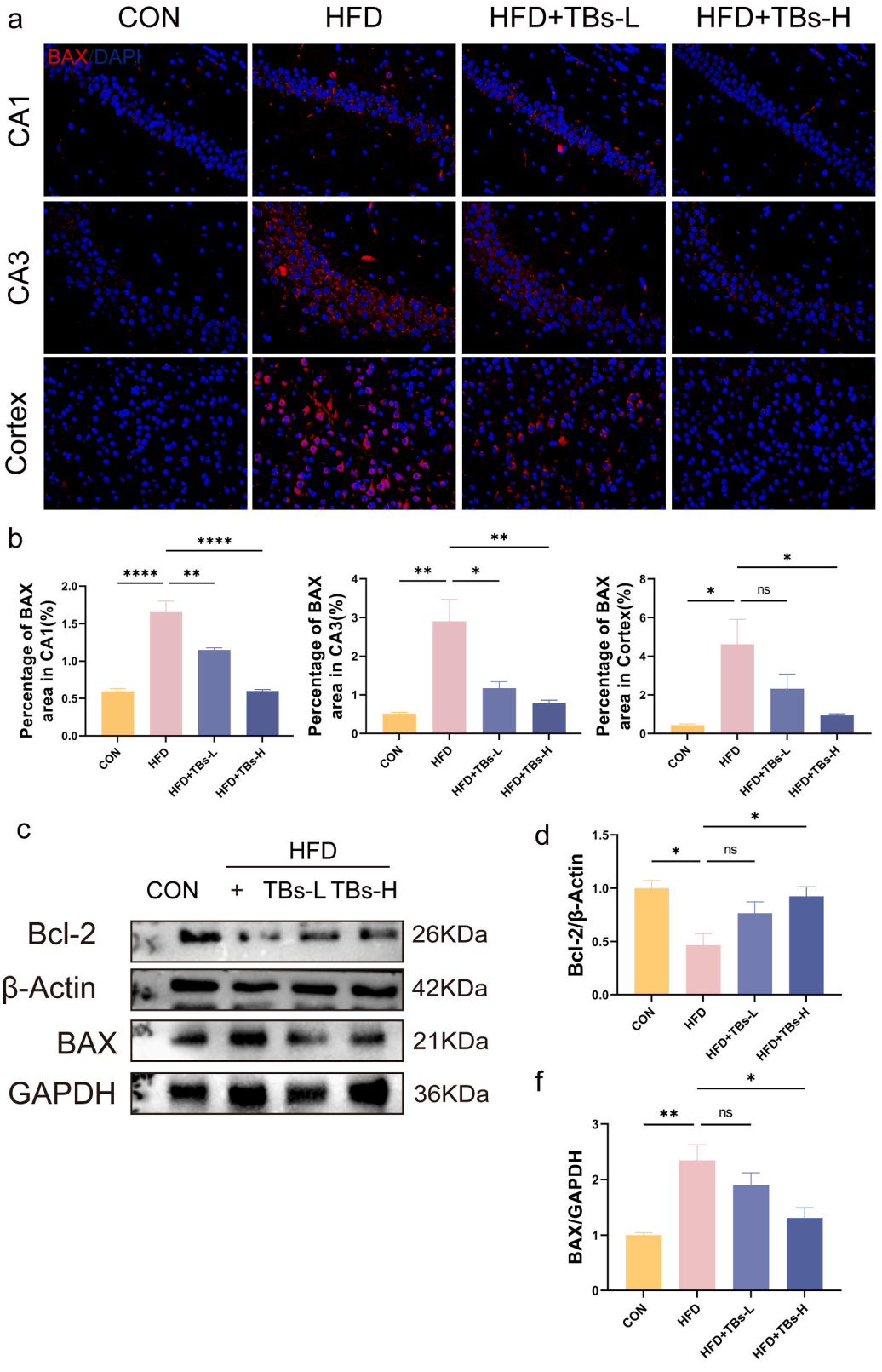


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Fig. 9. Theabrownin reduces hippocampal neuronal apoptosis in HFD-fed obese mice. (a) Immunofluorescence of BAX in hippocampal CA1, CA3 regions, and cortex (Scale = 20 μm). (b) Immunofluorescence statistics of BAX in hippocampal CA1, CA3 regions, and cortex (n = 3). (c) Representative Western blot of Bcl-2. Histogram representing the quantitative analysis of Bcl-2 (d) and BAX (f) (n = 3). mean ± SEM. *p < 0.05, **p < 0.01.

neurotransmitters leading to neuronal death. Next, we detected the expression of PSD95, SYN1, and SYP using WB (Fig. 8a), and the results showed that compared to the CON group, the expression of PSD95 and SYN1 were decreased in the HFD group ($p < 0.05$) (Fig. 8b and c), and SYP also showed a decrease, but without statistical significance ($p > 0.05$) (Fig. 8d); after administration of low-dose Theabrownin, there was an improvement, but without statistical significance ($p > 0.05$), and after administration of high-dose Theabrownin, the expression significantly increased ($p < 0.05$) (Fig. 8). These results indicate that Theabrownin can improve synaptic plasticity in HFD-fed obese mice, and this improvement is concentration-dependent.

3.6. Theabrownin reduces hippocampal neuronal apoptosis in HFD-fed obese mice

Cell apoptosis is necessary for maintaining the normal development of cells, and after apoptosis, the cell nucleus ruptures, apoptotic bodies are formed, and cysteine is activated, ultimately leading to changes in cell morphology [32]. The anti-apoptotic protein Bcl-2 and pro-apoptotic protein BAX are key initiators of this process, Bcl-2 and BAX also plays an important role in neural development. We detected the expression of Bcl-2 and BAX using WB and IF, respectively. The IF results also revealed that compared to the CON group, the expression of BAX increased significantly in the HFD group, especially in the CA1, CA3, and cortex areas ($p < 0.05$) (Fig. 9a and b), which was improved after administration of Theabrownin, and the improvement was dose-dependent. with a decrease also observed in the CA2 and DG regions ($p < 0.05$), and there was a dose-dependent effect (Supplementary Fig. 4). The WB results showed that compared to the CON group, the expression of Bcl-2 was significantly decreased and the expression of BAX was significantly increased in the HFD group, which was improved after administration of Theabrownin, the expression of Bcl-2 increased and the expression of BAX decreased, and the improvement was dose-dependent($p < 0.05$) (Fig. 9c and d). These results suggest that Theabrownin can reduces hippocampal apoptosis in HFD-fed obese mice.

4. Discussion

Theabrownin is a component derived from the fermentation of dark tea from southern China, and it ranks among the most active and prevalent pigments in dark teas, (e.g., Pu'er tea). Qingzhuan dark tea, originating from southern China, is a type of dark tea known for its antioxidant properties and its potential to aid weight loss and enhance metabolism [33–35].Earlier studies have documented that Theabrownin in Pu'er tea exhibits properties such as enhancing metabolism, suppressing obesity, lowering blood lipids, and providing antioxidant effects [19]. Previous studies have found that Theabrownin can improve metabolic syndrome via the gut-liver-brain axis [20]. However, there is no literature report on the role of Qingzhuan dark tea Theabrownin in the hippocampus of HFD-fed mice, so we further investigated the therapeutic and mechanistic effects of Qingzhuan dark tea Theabrownin on hippocampal damage in obese mice.

The hippocampus, a critical brain region for learning and memory in the brain, is susceptible to damage in the early stages of AD [36]. Similar situations can also occur in cognitive impairments induced by obesity and diabetes [37,38]. We evaluated early damage in obesity-induced cognitive impairments, and the results of HE and Nissl staining showed significant nuclear staining, neuronal loss, and morphological changes in the CA3 regions of the hippocampus and cortex in HFD-fed mice; these changes were significantly improved after treatment with Qingzhuan dark tea Theabrownin. Research has shown that HFD accelerates early aging characteristics and induces immune dysregulation (inflammation and aging) [27], both of which are established risk factors for AD. Additionally, research has verified an augmentation in microglia numbers and the emergence of neuroinflammation in HFD-fed mice [39]. Neuroinflammation is frequently linked to the activation of microglia and astrocytes. Subsequently, we conducted immunofluorescence detection for IBA1 (a microglial marker) and GFAP (an astrocytic marker) in the hippocampus. The immunofluorescence results showed increased IBA1 and GFAP expression in the hippocampus of the HFD group, and these changes were significantly improved after treatment with Qingzhuan dark tea Theabrownin, with improvement showing a dose-dependent pattern. Our results provide the first indication of a role for Theabrownin in influencing neuroinflammation, but the exact mechanism of action is unknown.

In the Central Nervous System (CNS), neuroinflammation orchestrated by microglia and astrocytes is an innate immune response to counteract stressful and dangerous insults [40]. Currently, NLRP3 is currently the most studied inflammasome and its expression increases significantly in the hippocampus of HFD-fed mice [41,42]. Unregulated NLRP3 inflammasome can play a pivotal role in the pathophysiology of neuroinflammation in neurodegenerative diseases [40,43,44]. The potential mechanism involves the activation of microglia and astrocytes, resulting in the activation of the NLRP3 signaling pathway and the occurrence of neuroinflammation in the hippocampus [45]. Microtubule affinity-regulating kinases (MARKs) regulate tau-microtubule binding and play a crucial role in neurons, where MARK4 is a potential therapeutic target for AD [15,46]. MARK4 is a serine/threonine kinase that phosphorylates Tau protein. Knockout of MARK4 in mice leads to hyperphagia, hyperactivity, and hypermetabolism, which in turn attenuates diet-induced insulin resistance [14]. Furthermore, MARK4 promotes autophagy in adipocytes to inhibit browning of white adipocytes [47]. This evidence illustrates the role of MARK4 in neurological and metabolic diseases. Recent research has revealed that MARK4 co-expresses with NLRP3 [48], and MARK4's function depends on NLRP3 activation. In liver ischemia-reperfusion, downregulation of MARK4 reduced the activation of the NLRP3 inflammasome [49]. Additionally, MARK4 inhibitors can suppress inflammation [50], which is

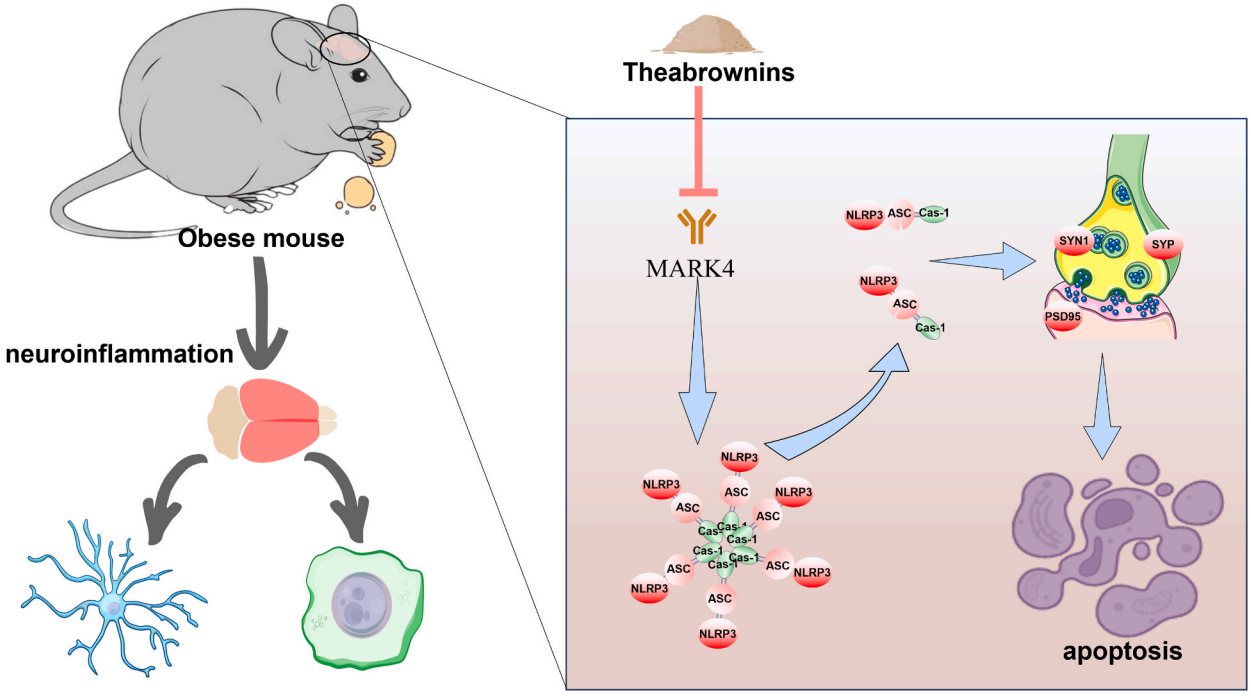

Fig. 10. Mechanism diagram. HFD-induced obesity causes neuroinflammation, triggers activation of microglia and astrocytes, and leads to increased expression of MARK4 and NLRP3, which promotes the release of inflammatory factors, leading to alterations in synaptic plasticity, and ultimately to apoptosis-induced hippocampal injury in neuronal cells. Theabrownin reduces neuroinflammation and nerve injury via MARK4/NLRP3 pathway.

consistent with our results. Our results indicate that HFD leads to elevated expression of MARK4 and NLRP3, which is reversed by treatment with Theabrownin. These findings suggest that Qingzhuan dark tea Theabrownin may reduce MARK4 expression and consequently decrease NLRP3 expression, leading to the reduction of neuroinflammation.

Increasing research indicates that neuroinflammation influences alterations in synaptic plasticity, and postsynaptic density protein 95 (PSD95), synapsin-1 (SYN1), and synaptophysin (SYP) are key proteins in synaptic plasticity [24,51]. PSD95 and SYN1 regulate synaptic strength and plasticity [52]. SYP plays a vital role in synaptic plasticity, synaptic vesicles, and neurotransmitter release [53]. Research has demonstrated that hippocampal damage results in notable alterations in synaptic plasticity, characterized by a reduction in PSD95, SYN1, and SYP [54]. This is supported by our results, which are also the first to find that Qingzhuan dark tea Theabrownin significantly upregulates PSD95, SYN1, and SYP, suggesting that Qingzhuan dark tea Theabrownin ameliorates synaptic plasticity impairments. Lastly, we investigated proteins related to cell apoptosis. Bcl-2, an anti-apoptotic protein, and BAX, a pro-apoptotic protein, can interact to generate apoptotic bodies that trigger cell apoptosis [55]. After cell apoptosis, Bcl-2 expression decreases, while BAX expression increases [56], Our results also validate this view. Additionally, Qingzhuan dark tea Theabrownin can upregulate Bcl-2 expression and downregulate BAX expression. These results demonstrate that Qingzhuan dark tea Theabrownin can improve neuronal apoptosis.

In summary, Qingzhuan dark tea Theabrownin can improve body weight, blood lipid levels, and neuroinflammation in HFD-fed mice. However, our study still has limitations, such as the inability to determine the specific components of tea polyphenols, not using MARK4 inhibitors or siRNA to further validate the results, and the lack of validation at the cellular and patient levels, which are also the directions of our future research. As shown in Fig. 10, HFD-induced obesity induces neuroinflammation, triggers activation of microglia and astrocytes, and leads to increased expression of MARK4 and NLRP3, which promotes the release of inflammatory factors, leading to altered synaptic plasticity, and ultimately to apoptosis of neuronal cells inducing hippocampal injury.

5. Conclusion

In summary, this study demonstrates that Qingzhuan dark tea Theabrownin can improve body weight, blood lipid levels, and neuroinflammation in HFD-induced mice. The main mechanisms involved are as follows: 1. Theabrownin exerts anti-inflammatory effects and inhibits glial cell activation in the hippocampus. 2. Theabrownin downregulates the expression of MARK4, which regulates the expression of NLRP3. 3. Theabrownin improves synaptic plasticity and cell apoptosis. Our results preliminarily illustrate the efficacy and potential mechanisms of Qingzhuan dark tea Theabrownin in mitigating hippocampal damage in HFD-fed obese mice, providing a reference for future use of Qingzhuan dark tea.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Yining Lei: Writing – review & editing, Writing – original draft, Methodology. **Yong Chen:** Methodology. **Shuo Zhang:** Formal analysis. **Wei Wang:** Formal analysis. **Min Zheng:** Supervision. **Ruyi Zhang:** Writing – original draft, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26923.

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