Huang-Pu-Tong-Qiao Formula Ameliorates the Hippocampus Apoptosis in Diabetic Cognitive Dysfunction Mice by Activating CREB/BDNF/TrkB Signaling Pathway

Shu Ye,1,2,3 Dao-Jun Xie,4 Peng Zhou,1,2,5 Hua-Wu Gao,1,2 Meng-Ting Zhang,1,2 Da-Bao Chen,1,3 Yun-Peng Qin,1,3 Xin Lei,1,3 Xin-Quan Li,1,3 Juan Liu,1,3 Ya-Xun Cheng,1,3 Yong-Chuan Yao,4 Biao Cai,1,2,5 and Guo-Ming Shen1,2,3

1School of Integrated Chinese and Western Medicine, Anhui University of Chinese Medicine, Hefei 230012, Anhui Province, China
2Institute of Integrated Chinese and Western Medicine, Anhui Academy of Chinese Medicine, Hefei 230012, Anhui Province, China
3Graduate School of Anhui, Anhui University of Chinese Medicine, Hefei 230012, Anhui, China
4The First Affiliated Hospital of Anhui University of Traditional Chinese Medicine, Hefei 230031, Anhui Province, China
5Anhui Province Key Laboratory of Chinese Medicinal Formula, Hefei 230012, Anhui Province, China

Correspondence should be addressed to Biao Cai; caibiao@ahtcm.edu.cn and Guo-Ming Shen; shengm_66@163.com

Received 27 January 2021; Revised 2 April 2021; Accepted 3 May 2021; Published 10 June 2021

1. Introduction

Diabetic cognitive dysfunction (DCD), a diabetic central nervous system (CNS) complication, is characterized by cognitive deficits, neurochemical electrophysiological neurostructural abnormalities [1–3]. Without control of glycemic imbalance in CNS, it is considered to be the initiating factor in the development of it [4]. The hippocampus is an important part of the CNS, participates in the regulation of various nervous systems, and plays a fundamental
role in memory and learning [5]. It is sensitive to fluctuations in glucose levels, some studies showed that glucose administration could improve hippocampal function and cognitive ability [6], but chronic hyperglycemia might increase the impact of dementia [7]. In addition, the hippocampus involved in the pathophysiology of streptozocin (STZ) induced cognitive dysfunction [8, 9]. The loss of glucose levels in the hippocampus may lead to mitochondrial dysfunction, initiate apoptosis, and eventually lead to hippocampal neuron damage. The pathogenesis of DCD is complex, including insulin signaling disorders, autonomic dysfunction, and neuroinflammatory pathway abnormalities, advanced glycation end products deposition, mitochondrial metabolism disorders, etc. [10]. Currently, the underlying neuroprotective mechanism of this disorder has not been elucidated. The prolonged occurrence of DCD may have a negative impact on the patient’s social life.

Huang-Pu-Tong-Qiao (HPTQ) is a hospital formula from the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine, which has been used in the clinical treatment of dementia for many years. It is composed of six herbal medicines, including seven chemical compositions, mainly Stilbene glycoside, ferulic acid, ginsenoside Rg1, aloe-emodin, β-asarone, emodin, and chrysophanol [11]. Pharmacological studies have shown that the main extract components of HPTQ, such as ginsenoside Rg1 and Tetrahydroxystilbene glucoside, might improve cognitive impairment of Alzheimer’s disease (AD) animal models in multiple pathways [12, 13]. Our previous studies have demonstrated that HPTQ could improve cognitive impairments of AD rats by inhibiting multiple pathways, including EGFR-PLCγ, CaM-CaMKIV, oxidative stress, and mitochondrial apoptotic signaling pathways [11, 14]. The HPTQ could also improve the apoptosis of primary hippocampal neurons induced by Aβ25-35 [15]. However, due to the complex composition of HPTQ, the potential efficacy and mechanism for treating DCD have not been elucidated.

In this study, we first established the DCD mice model and investigated the neuroprotective effects and potential mechanism of HPTQ based on molecular docking.

2. Materials and Methods

2.1. Animals. Male C57BL/6 mice (10 weeks old, weighing 20–22 g) were purchased from Qinglongshan Animal Breeding Farm, Nanjing, China. Certificate no. SCXK (Su) 2017–0001. All experiments animals were housed at room temperature (25 ± 3°C) and humidity (55–70%) under a light/dark cycle of 12 h/12 h, with free access to water and standard diets. The study protocol was approved by the Animal Management Center of the Anhui University of Chinese Medicine, China.

2.2. Experimental Induction of DCD Model and Treatments Schedule. After excluding animals with outlying results by the Morris water maze (MWM) test, the mice were randomly divided into two groups: the control group (CTRL, n = 20) and the diabetes mellitus group (DM, n = 40). DM (or DCD) group was induced by a mix of high-fat diet (HFD) and low dose streptozocin (STZ, St. Louis, MO, USA). DM (or DCD) group were fed with HFD (formula: 15% lard, 10% sucrose, 3% cholesterol, 3% sodium chloride, 69% standard rat feed) for 4 weeks and were administrated with streptozocin (STZ) by intraperitoneal (i. p.) at 55 mg/kg (dissolved in 100 mM sodium citrate buffer pH = 4.5) for 5 consecutive days [16]. The CTRL group was administrated with an equivalent volume of citrate buffer. Seven days after the STZ was administrated, the fasting blood glucose (FBG) levels more than 16.7 mmol/L were considered as diabetic mice and the blood was collected from the tail vein. Body weight was measured weekly and FBG every two weeks. The MWM test was used to detect the changes of learning and memory ability at the 0, 4th, 8th, 12th, and 16th week and the brain-derived neurotrophic factor (BDNF) was detected at the same time.

When the MWM test screened diabetic mice for cognitive impairment, the mice were randomly divided into three groups (Four mice did not make the DCD model, NDCD): Control group (CTRL, n = 11), diabetic cognitive dysfunction (DCD) group (n = 13), and Huang-Pu Tong-Qiao formula intervention group (HPTQ group n = 14). The HPTQ group mice were treated with HPTQ (1.974 g/kg) for 4 weeks. After 4 weeks of administration by gavage, MWM and novel object recognition test were performed to assess the cognitive functions, and then the next experiment was carried out. The schedule for this test was shown in Figure 1.

2.3. Behavioral Experiments

2.3.1. Morris Water Maze Test. The spatial learning and memory ability of mice was detected with MWM. The MWM was a circle pool (diameter: 120 cm; height: 55 cm), filled with a depth of 30 cm water mixed with milk powder. The pool was divided into four equal spaced quadrants and then a hidden platform (diameter: 8 cm) was placed 1 cm below the water in one of the quadrants. Spatial training was conducted for 5 consecutive days. The mice were put into the water from each quadrant in turn and the time is taken to find the platform within 90 s was recorded. If the mice did not find the platform within 90 s, they were placed on the platform and left there for 15 s. On the fifth day, without guidance, the duration for finding the platform was recorded as escape latency. On the sixth day, the platform was removed, allowing each mouse to swim freely for 90 seconds inside the pool. The number of times that the mice crossed the area (where the platform was removed) was recorded by a video camera.

2.3.2. Novel Object Recognition Test. The novel object recognition (NOR) test was performed for assessing cognition, based on the principle that animals have the instinct to explore new objects [17]. The test consists of three phases: habituation phase, training phase, and test phase. The test...
The apparatus was composed of a rectangular white box (25 × 25 × 40 cm) and three objects named A, B, and C, of which A was the same as B, while the object of C was completely different in shape and colour from the A and B. The test is based on previous research with some modifications [18]. The first day was the habituation phase, and mice were acclimated to the box for 5 min in the absence of objects, then returned to the mouse cage. The next day was the training phase; mice were exposed to A and B (placed in opposite corners of box 3 cm away from the walls) and each mouse was allowed to explore for 5 min. 24 hours later, B was replaced with C, and mice were also placed back in the box to explore freely for 5 minutes. Exploration was defined as the nose to the object at a distance within 2 cm or touching it with the nose. The box and objects were cleaned with 70% ethanol between trials to avoid residual odors. The relative exploration time of the novel object (TN) and the familiar object (TF) was recorded. The preference index was calculated as 

\[
\text{Preference Index} = \frac{(TN - TF)}{(TN + TF)}
\]

2.4. Preparation of Tissue Samples. After the behavioral test, the mice were euthanized with 1% sodium pentobarbital (40 mg/kg). The hippocampus tissues were quickly separated on ice and stored at −80°C for western blot experiments.

2.5. Determination of Insulin Level in Hippocampus. The frozen hippocampus tissue samples were cleaned by precooling phosphate buffer saline (PBS) and then put into a glass homogenizer to add the corresponding volume of PBS for full grinding. After centrifugation for 10 minutes at 3000 r/min, the supernatant was detected by an insulin ELISA kit provided by Elabscience Biotechnology Co., Ltd (Elabscience, Wuhan, China).

2.6. Hematoxylin-Eosin Staining (HE). The mice were sacrificed by anesthesia with an intraperitoneal injection of 1% sodium pentobarbital (40 mg/kg). The brain samples were fixed overnight with 4% formaldehyde and then embedded in paraffin. The 5 mm thick coronal sections of brain samples were dewaxed and dyed with HE. The hippocampal neurons in the CA1 region were observed with an Olympus X51 microscope.

2.7. Molecular Docking. CB-Dock predicts cavities of the protein and calculates the centers and sizes of the top N (n = 5 by default) cavities. PDB formats of caspase-3 (PDB Code: 1GFW) [19], Bax (PDB Code: 4S0O) [20], Bcl-2 (PDB Code: 2W3L) [21], and ligand files of in SDF formats of the main chemicals in HPTQ were input to CB-Dock to elevate the binding activities [22, 23].

2.8. Western Blot. The total protein was extracted from hippocampus tissue using a radio-immunoprecipitation assay (RIPA) buffer containing protease inhibitors (Abmole biotechnology, Shanghai, China). The 20 µg extracted proteins were separated by 10% SDS-PAGE (Bio-Rad) and transferred onto polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The membrane was blocked with 5% nonfat dry milk for 2 h and incubated overnight at 4°C with the relevant primary antibodies. The primary antibodies were anti-BDNF (1:1000, novus biologicals, Beijing, China), anti-caspase-3 (1:1000, Affinity, Liyang, China), anti-Bax (1:1000, Abcam, Cambridge, UK), anti-Bcl-2 (1:1000, Abcam, Cambridge, UK), anti-p-CREB (1:1000, Cell Signaling Technology, MA, USA), anti- TrkB (1:1000, Cell Signaling Technology, MA, USA), and anti-p-Akt (1:2000, Cell Signaling Technology, MA, USA). The membrane was incubated with the horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G (IgG) (1:20,000, Abbkine, Wuhan, China). The membrane was visualized by the proteins by ECL reagent and analyzed by ImageJ software. Rabbit anti-rat β-actin (1:2000, Abbkine, Wuhan, China) was used as an internal control.

2.9. Statistical Analysis. The results were expressed as means ± standard deviation (SD). Data were analyzed by a one-way ANOVA test and followed by Tukey’s multiple
3. Results

3.1. The Establishment of DCD Animal Model. To establish the mice model of DCD, we measured the body weight, the fasting blood glucose, the learning and memory, and the level of BDNF at different stages.

The body weight of mice with HFD was significantly increased, but the level of blood glucose changed not obviously; two weeks after the STZ administrated, the mice exhibited severe hyperglycemia in the DM group, blood glucose assessment identified a total of 40 mice developed DM, the body weight of in different groups mice increased continuously, but the difference was not statistically significant (Figures 2(a) and 2(b)).

The MWM test was used to investigate the learning and memory of mice in different time periods. From the first week to the eighth week, there were no significant changes in escape latency and times of crossing platform tests in different groups. In the 12th week, the escape latency of the DM group was signally prolonged and the number of times the DM group crossed the platform was signally decreased compared with CTRL (Figures 2(c)–2(d)). The level of BDNF in the hippocampus was determined with western blot. Compared with the CTRL group, the level of BDNF was significantly lower in 12 weeks (Figures 2(e)–2(f)).

The MWM and the level of BDNF assessment identified a total 27 out of the 31 HFD/STZ mice developed DCD. The results have shown that 12 weeks of HFD/STZ intervention, the DCD model was successfully established in C57BL/6 mice. The remaining mice were then excluded from the further study, which was divided into two groups: diabetic cognitive dysfunction (DCD group) and Huang-Pu-Tong-Qiao formula intervention group (HPTQ).

3.2. Effect of HPTQ on Weight, Blood Glucose, and Insulin Levels of DCD Model. To investigate the metabolic changes of HPTQ in DCD, the weight, blood glucose, and insulin levels in the hippocampus were detected. The body weight of mice in the CTRL and DCD group has been a tendency toward rising, but there is no statistical significance; after HPTQ intervention, the weight loss trend of mice in the HPTQ group was slower than that in the DCD group, but there was no significant difference between two groups (Figure 3(a)). The fasting blood glucose and insulin levels of DCD group mice were elevated continuously, compared with the CTRL group; after intragastric administration of HPTQ for 28 days, the fasting blood glucose and insulin levels of HPTQ group mice decreased significantly, compared with the DCD group (Figures 3(b)–3(c)).

3.3. HPTQ Improved Learning and Memory in DCD Mice. To investigate whether HPTQ could relieve memory and learning impairments, we performed the tests of Morris water maze and novel object recognition. In the MWM test, the time for DCD group mice to find target platform was signally increased and the number of times crossed the platform was signally decreased compared with the CTRL group (Figure 4(a)); after being treated with HPTQ, the time of mice to find target platform was signally decreased and the number of times it crossed the platform was signally increased compared with the DCD group (Figure 4(b)). As an alternative method to confirm whether HPTQ ameliorates diabetes-induced memory dysfunction, we used the NOR test, which is based on the principle that animals have the instinct to explore new objects. In the NOR test, the preference index of the DCD group mice was significantly lower than that of the CTRL group. However, the preference index in HPTQ group mice improved compared to the DCD group (Figures 4(c)–4(d)).

3.4. Effects of HPTQ on Hippocampal Neurons in CA1 Region of DCD Mice. HE staining was used to observe the damage of hippocampal neurons in the CA1 region. As shown in Figure 5, CTRL group neuron’s structure was round closely arranged and pale-stained nuclei (Figure 5(a)); DCD group neuron’s structure was disordered notably shrunken, atrophied, and irregularly arranged, indicating that injury induced significantly neurons deteriorated and damaged compared with CTRL group in hippocampal CA1 area (Figure 5(b)). However, the neuronal damage was significantly improved after treatment with HPTQ in DCD, indicating that HPTQ might have a neuroprotective effect (Figure 5(c)).

3.5. Molecular Docking. Molecular docking results showed that the main components of HPTQ have a good binding ability with Bax, Bcl-2, and caspase-3. In our docking results, emodin has the strongest binding ability with Bax, followed by chrysophanol and aloe-emodin (Table 1, Figures 6(a) and 6(f)); ginsenoside Rg1 has the strongest binding ability to Bcl-2, followed by aloe-emodin and chrysophanol (Table 2, Figures 7(a)–7(f)); Stilbene glucoside has the strongest binding ability to caspase-3, followed by ginsenoside Rg1 and chrysophanol (Table 3, Figures 8(a)–8(f)).

3.6. Effect of HPTQ on Apoptosis-Related Proteins in DCD Model. Based on previous research study and molecular docking results, the expression of Bax, Bcl-2, and caspase-3 was determined by western blot to further validate underlying the protective effect of HPTQ in the DCD model. Compared with the CTRL group, the protein expression of Bax/Bcl-2 and caspase-3 in the DCD group was higher significantly. When treated with HPTQ, the Bax/Bcl-2 and caspase-3 level in the HPTQ group were lower significantly than those in the DCD group (Figures 9(a) and 9(b)).

3.7. Effect of HPTQ on Antiapoptosis Related Proteins in DCD Model. In order to further validate the mechanism of HPTQ in DCD. The protein levels of p-CREB, BDNF, TrkB, and p-Akt in hippocampal neurons were detected by WB. The result showed that compared with the CTRL group, the level
of p-CREB, BDNF, TrkB, and p-Akt were significantly decreased in the DCD group. However, compared with the DCD group, the protein expression of p-CREB, BDNF, TrkB, and p-Akt were significantly increased (Figure 10) in the HPTQ group.

4. Discussion

A large number of epidemiological studies showed that diabetes mellitus is prone to cognitive decline and dementia [24, 25], whether it is type 1 diabetes or type 2 diabetes. In recent years, there are more and more researches on DCD, but the exact mechanism is not clear.

Previous studies have shown that a high-fat diet combined with streptozotocin (HFD/STZ) could successfully establish a DCD animal model, but the dosage and use time of STZ are different [16, 18]. Based on this, a high-fat diet combined with low dose STZ was used to replicate the natural history and metabolic characteristics of human type 2 diabetes mellitus. In order to establish a stable DCD model, the time points of cognition impairment after HFD/STZ modeling were screened out. We used the MWM to detect
the cognitive changes once every 4 weeks and the expression of BDNF in the hippocampus at the same time point. The MWM results showed that the escape latency increased, and the times of crossing the platform decreased significantly in the 12th week. The WB results showed that the level of BDNF decreased significantly in the 12th week. However, we did not find any significant difference of morphological in the hippocampal region between the different groups in the first 12 weeks. It confirmed that C57BL/6 mice suffered cognitive impairment in 12 weeks from behavioral and molecular biology. Hippocampus is vulnerable to hyperglycemic, prolonged high glucose induced neuronal damage [26]. BDNF level was measured as a brain-derived neurotrophic function marker in DCD mice hippocampus. It is an essential modulator in CNS, supporting neuronal survival and promoting the growth and differentiation of new neurons [27].

Currently, the treatments for diabetes have failed to prevent the decline in cognitive function, so alternative treatment strategies are urgently needed. Traditional Chinese medicine (TCM) compound has the advantages of multicomponent and multitarget, so it has gradually become a new research hotspot. HPTQ is mainly composed of six natural herbal medicines, including a variety of chemical components, showing multicomponent and multitarget. Our previous study documented the positive effects of HPTQ in cognitive impairment [11]. 28 days after HPTQ administration, it abolished the enhanced insulin level in the hippocampus of mice with DCD, indicating that HPTQ could facilitate the efficiency of hippocampal insulin in DCD mice. Notably, HPTQ decreased the escape latency considerably, increased the times of crossing platform, and improved the preference index of DCD mice, implying that HPTQ could improve hippocampal-mediated learning and memory abilities of DCD mice. In addition, it significantly improved the damage of hippocampal neurons in DCD mice and upregulated the protein expression of BDNF, which may be an important reason for HPTQ to enhance memory.

To further investigate the preventive effect of HPTQ on the progression of DCD, molecular docking was performed. The results of molecular docking showed that the main chemical components of HPTQ could be well combined with the targets of Bax, Bcl-2, and caspase-3, indicating that HPTQ may act on the targets of Bax, Bcl-2, and caspase-3. Bax, Bcl-2, and caspase-3 are all mitochondria-related apoptosis proteins. As reported, mitochondria are central regulators of neurons, which are damaged as key factors underlying cognitive defects in neurodegenerative diseases [28]. Bax is a proapoptotic factor and Bcl-2 is an anti-apoptotic factor, which localizes on the mitochondrial surface, play an important role in the process of apoptosis [29, 30]. Following overexpression of Bax, it moves from the cytosol to the outer mitochondrial membrane and forms homo-oligomeric complexes with Bax, resulting in the
changes in the permeability of the mitochondrial outer membrane. Disorders of mitochondrial metabolism can result in the release of a variety of proapoptotic factors, activate caspase 3 or caspase 7, and initiate apoptosis [31, 32]. The hippocampus plays an important role in the learning and memory process. Therefore, targeting hippocampal neurotroph may be a potential strategy to reduce the risk of DCD and improve DCD outcomes. To further

**Figure 4**: HPTQ improved learning and memory in DCD mice. The escape in the hidden platform (a), the crossing times of target platform (b), the trajectory of mice in NOR (c), and the preference index in DCD mice (d), the preference index (time spent at the novel object-time spent at the familiar object)/(time spent at the novel object + time spent at the familiar object)]. Values are means ± SD. *P < 0.05, **P < 0.01 vs. CTRL; #P < 0.05, ##P < 0.01.

**Figure 5**: Effects of HPTQ on hippocampal neurons in the CA1 zone of DCD mice. CTRL group (a), DCD group (b), and HPTQ group treated with dose of HPTQ (1.974 g/kg) (c).
investigate the preventive effect of HPTQ on DCD, with the support of molecular docking technology, the level of mitochondria-related apoptosis proteins Bax, Bcl-2, and caspase-3 in hippocampal tissue were performed. Our results have revealed that the levels of caspase-3 and the ratio of Bax to Bcl-2 were significantly increased in the DCD mice model, confirming that severe apoptosis occurred via the mitochondria-related Bax/Bcl-2 and caspase-3 pathway. However, after treatment with HPTQ, it downregulated levels of caspase-3 and the Bax/Bcl-2.

BDNF plays an important role in the pathophysiology of mitochondrial dysfunction [33]. Similar to BDNF, transmembrane protein receptor tropomyosin receptor kinase B (TrkB) is widely expressed in CNS. BDNF elicits regulatory roles in multiple neuroprotective effects by binding with TrkB that further activates its downstream signaling pathways including/protein kinase B (PI3K/Akt) [34–36]. PI3K/Akt pathway is particularly important in widely mediating survival signals [37]. Phosphorylation of Akt promotes cell survival and differentiation and reduces neuronal apoptosis [38]. Activation of TrkB by BDNF induces the phosphorylation of Akt [39]. cAMP response element-binding protein (CREB), a transcription factor located in the nucleus, is involved in glucose homeostasis [40]. The levels of CREB and the active form of p-CREB were decreased in the rodent models of insulin resistance

| Chemicals     | Vina score | Cavity score | Center (x, y, z) | Size (x, y, z) |
|---------------|------------|--------------|------------------|---------------|
| Emodin        | −8.5       | 316          | 17, −9, 14       | 19, 19, 19    |
| Chrysophanol  | −8.4       | 316          | 17, −9, 14       | 19, 19, 19    |
| Aloe-emodin   | −8.2       | 316          | 17, −9, 14       | 20, 20, 20    |
| Ginsenoside Rg1| −7.7     | 316          | 17, −9, 14       | 27, 27, 27    |
| Stilbene glycoside | −6.9  | 316          | 17, −9, 14       | 20, 20, 20    |
| Ferulic acid  | −6.3       | 316          | 17, −9, 14       | 19, 19, 19    |
| β-asarone     | −5.7       | 316          | 17, −9, 14       | 18, 18, 18    |

Table 1: Docking of main components of HPTQ with Bax.

| Chemicals     | Vina score | Cavity score | Center (x, y, z) | Size (x, y, z) |
|---------------|------------|--------------|------------------|---------------|
| Ginsenoside Rg1| −9.6      | 673          | 38, 35, 2        | 27, 27, 27    |
| Aloe-emodin   | −7.9       | 673          | 38, 35, 2        | 20, 20, 20    |
| Chrysophanol  | −7.9       | 673          | 38, 35, 2        | 19, 19, 19    |
| Emodin        | −7.8       | 673          | 38, 35, 2        | 19, 19, 19    |
| Stilbene glycoside | −7.2   | 673          | 38, 35, 2        | 21, 21, 21    |
| Ferulic acid  | −5.7       | 673          | 38, 35, 2        | 20, 20, 20    |
| β-asarone     | −5.2       | 673          | 38, 35, 2        | 18, 18, 18    |

Table 2: Docking of main components of HPTQ with Bcl-2.
Figure 7: Docking of main components of HPTQ with Bcl-2. Ginsenoside Rg1 docked with Bcl-2 (a, b), aloe-emodin docked with Bcl-2 (c, d), and chrysophanol docked with Bcl-2 (e, f).

| Chemicals       | Vina score | Cavity score | Center (x, y, z) | Size (x, y, z) |
|-----------------|------------|--------------|------------------|---------------|
| Stilbene glycoside | −7.7      | 271          | 37, 35, 32       | 21, 21, 21    |
| Ginsenoside Rg1 | −7.2      | 271          | 37, 35, 32       | 27, 27, 27    |
| Chrysophanol    | −7.1      | 271          | 37, 35, 32       | 19, 19, 19    |
| Emodin          | −6.9      | 271          | 37, 35, 32       | 19, 19, 19    |
| β-asarone       | −6.7      | 271          | 37, 35, 32       | 18, 18, 18    |
| Aloe-emodin     | −6.6      | 271          | 37, 35, 32       | 20, 20, 20    |
| Ferulic acid    | −5.8      | 271          | 37, 35, 32       | 20, 20, 20    |

Figure 8: Docking of main components of HPTQ with caspase-3. Stilbene glycoside docked with Caspase-3 (a, b), Ginsenoside Rg1 docked with Caspase-3 (c, d), and chrysophanol docked with caspase-3 (e, f).
Figure 9: The protein levels of Bax, Bcl-2, and caspase-3 in hippocampal neurons. Bax, Bcl-2, and caspase-3 protein levels in hippocampus neurons were measured by WB (a). The protein loading intensity was calculated with β-actin as internal (b, c). Values are means ± SD. *P < 0.05, **P < 0.01 vs CTRL group; #P < 0.05, ##P < 0.01 vs DCD group.

Figure 10: The protein levels of p-CREB, BDNF, TrkB, and p-Akt in hippocampal neurons. p-CREB, BDNF, TrkB, and p-Akt protein levels in hippocampus neurons were measured by WB (a). The protein loading intensity was calculated with β-actin as an internal control (b–e). Values are means ± SD. *P < 0.05, **P < 0.01 vs CTRL group; #P < 0.05, ##P < 0.01 vs DCD group.
and insulin deficiency diabetes [41]. Furthermore, a previous study revealed that CREB phosphorylation could upregulate the expression of BDNF [42, 43]. The combination of BDNF and TrkB activates related intracellular signaling pathways, thereby increasing hippocampal neuron synaptic activity, promoting neurogenesis, reducing apoptosis, and improving spatial learning and memory [42, 43]. It has been demonstrated that activation of BDNF/TrkB could inhibit Bax/Bcl-2 ratio and further inhibit caspase-3 [44]. Our study elucidated that the levels of p-CREB, BDNF, TrkB, and p-Akt were decreased in DCD mice and upregulated after HPTQ intervention. Furthermore, our findings elucidated that HPTQ markedly suppressed the enhancement of Bax/Bcl-2 ratio and activation of caspase-3, suggesting that HPTQ could attenuate the injury of a hippocampal neuron, improve the ability of learning and memory in the DCD mice model through activating CREB/BDNF/TrkB signaling pathway and inhibiting mitochondrial apoptosis pathway (Figure 11).

5. Conclusions

In conclusion, our data demonstrated that after 12 weeks of HFD/STZ intervention, the DCD model was successfully established in C57BL6 mice. Simultaneously, chronic HPTQ treatment debilitated diabetes-induced cognitive deficits and regulated hippocampal insulin metabolism activated CREB/BDNF/TrkB signaling pathway and suppressed the mitochondria-related Bax/Bcl-2 and caspase-3 apoptosis pathway. The antiapoptotic effect of HPTQ may be through control the level of insulin and activate CREB/BDNF/TrkB signaling pathway in the DCD mice brain. These findings indicated that HPTQ might be considered as a potential therapeutic agent for diabetic cognitive dysfunction. However, further evidence is still needed to confirm this phenomenon.

Data Availability

The data will be made available upon reasonable request.

Ethical Approval

The study protocol was approved by the Animal Management Center of the Anhui University of Chinese Medicine, China.

Conflicts of Interest

The authors report no declarations of interest.

Authors’ Contributions

Shu Ye, Daojun Xie, and Peng Zhou contributed equally to this work.
Acknowledgments

This work was supported by the Major Research Project of Natural Sciences in Anhui Universities (No. KJ2018ZD029), the Distinguished Young Scholars Project of Natural Science Foundation of Anhui Province in China (No. 1908085J27), National Natural Science Foundation of China (No. 81904095), the Key Project of Overseas Visits for the Excellent Young Talents in Universities of Anhui Province in China (No. gxgfwx2020041), The Key Project Foundation of Natural Science Research in Universities of Anhui Province in China (No. KJ2020A0439), and the Key Project Foundation of National Science Research of Anhui University of Chinese Medicine (No. 2020yfyzc33).

Supplementary Materials

Tables S1–S6: the raw data about the establishment of the DCD model and therapeutic effect of HPTQ on DCD mice.

References

[1] A. I. Vinik, M.-L. Nevoret, C. Casellini, and H. Parson, “Diabetic neuropathy,” Endocrinology and Metabolism Clinics of North America, vol. 42, no. 4, pp. 747–787, 2013.
[2] G. J. Biessels, S. Staeckenborg, E. Brunner, C. Brayne, and P. Scheltens, “Risk of dementia in diabetes mellitus: a systematic review,” The Lancet Neurology, vol. 5, no. 1, pp. 64–74, 2006.
[3] D. R. Whiting, L. Guariguata, C. Weil, and J. Shaw, “IDF Diabetes Atlas: global estimates of the prevalence of diabetes for 2011 and 2030,” Diabetes Research and Clinical Practice, vol. 94, no. 3, pp. 311–321, 2011.
[4] A. D. McNelly, J. R. Gallagher, A. T. Dinkova-Kostova et al., “Nrf2-mediated neuroprotection against recurrent hypoglycemia is insufficient to prevent cognitive impairment in a rodent model of type 1 diabetes,” Diabetes, vol. 65, no. 10, pp. 3151–3160, 2016.
[5] L. R. Squire, “Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans,” Psychological Review, vol. 99, no. 2, pp. 195–231, 1992.
[6] B. Stollery and L. Christian, “Glucose improves object-location binding in visual-spatial working memory,” Psychopharmacology, vol. 233, no. 3, pp. 529–547, 2016.
[7] P. K. Crane, R. Walker, R. A. Hubbard et al., “Glucose levels and risk of dementia,” New England Journal of Medicine, vol. 369, no. 6, pp. 540–548, 2013.
[8] Y. Miao, T. He, Y. Zhu, W. Li, B. Wang, and Y. Zhong, “Activation of hippocampal CREB by rolipram partially recovers balance between TNF-α and IL-10 levels and improves cognitive deficits in diabetic rats,” Cellular and Molecular Neurobiology, vol. 35, no. 8, pp. 1157–1164, 2015.
[9] X. Wang and L. Zhao, “Calycosin ameliorates diabetes-induced cognitive impairments in rats by reducing oxidative stress via the PI3K/Akt/GSK-3β signaling pathway,” Biochemical and Biophysical Research Communications, vol. 473, no. 2, pp. 428–434, 2016.
[10] L. A. Zúñigo, K. Chadrasekaran, J. Y. Kwan et al., “Diabetes and cognitive impairment,” Current Diabetes Reports, vol. 16, no. 9, 2016.
[11] S. Ye, B. Cai, P. Zhou et al., “Huang-Pu-Tong-Qiao formula ameliorates tau phosphorylation by inhibiting the CaMKIV pathway,” Evidence-Based Complementary and Alternative Medicine, vol. 2020, Article ID 8956071, 11 pages, 2020.
[12] Y.-Q. Shi, T.-W. Huang, L.-M. Chen et al., “Ginsenoside Rg1 attenuates amyloid-β content, regulates PKA/CREB activity, and improves cognitive performance in SAMF8 mice,” Journal of Alzheimer's Disease, vol. 19, no. 3, pp. 977–989, 2010.
[13] Y. Zhou, Y. Hou, Q. Yang et al., “Tetrahydroxystilbene glucoside improves the learning and memory of amyloid-β1-42-injected rats and may be connected to synaptic changes in the hippocampus,” Canadian Journal of Physiology and Pharmacology, vol. 90, no. 11, pp. 1446–1455, 2012.
[14] G. Q. Wang, P. Zhou, D. J. Xie et al., “Effects of Huangpu Tongqiao Capsules on EGFR-PLCγ signal pathway of hippocampus in rats with Alzheimer’s disease,” China Journal of Chinese Materia Medica, vol. 45, no. 09, pp. 2165–2171, 2020.
[15] B. Cai, S. Ye, Y. Wang et al., “Effects of Huangpu Tongqiao capsule on apoptosis of Alzheimer’s disease cell model,” China Journal of Chinese Materia Medica, vol. 43, no. 11, pp. 2378–2383, 2018.
[16] Y. Wang, L. Wu, J. Li et al., “Synergistic exacerbation of mitochondrial and synaptic dysfunction and resultant learning and memory deficit in a mouse model of diabetic Alzheimer’s disease,” Journal of Alzheimer’s Disease, vol. 43, no. 2, pp. 451–463, 2014.
[17] Y.-J. Chen, Z.-Z. Tang, L. Du et al., “A novel compound AB-38b improves diabetes-associated cognitive decline in mice via activation of Nrf2/ARE pathway,” Brain Research Bulletin, vol. 150, pp. 160–167, 2019.
[18] Z. J. Yin, H. Y. Yu, S. Chen et al., “Asiatoside alleviates diabetes-induced cognition deficits by regulating P13K/Akt/ NF-κB pathway,” Behavioural Brain Research, vol. 292, pp. 288–299, 2015.
[19] D. Lee, S. A. Long, J. L. Adams et al., “Potent and selective nonpeptide inhibitors of caspases 3 and 7 inhibit apoptosis and maintain cell functionality,” Journal of Biological Chemistry, vol. 275, no. 21, pp. 16007–16014, 2000.
[20] T. P. Garner, D. E. Reyna, A. Priyadarshi et al., “An auto-inhibited dimeric form of BAX regulates the BAX activation pathway,” Molecular Cell, vol. 63, no. 3, pp. 485–497, 2016.
[21] J. Porter, A. Payne, B. de Candole et al., “Tetrahydroisoquinoline amide substituted phenyl pyrazoles as selective Bcl-2 inhibitors,” Bioorganic & Medicinal Chemistry Letters, vol. 19, no. 1, pp. 230–233, 2009.
[22] Y. Liu, M. Grimm, W.-t. Dai, M.-c. Hou, Z.-X. Xiao, and Y. Cao, “CB-Dock: a web server for cavity detection-guided protein-ligand blind docking,” Acta Pharmacologica Sinica, vol. 41, no. 1, pp. 138–144, 2020.
[23] Y. G. Tao, X. F. Huang, J. Y. Wang et al., “Exploring molecular mechanism of Huangqi in treating heart failure using network pharmacology,” Evidence-Based Complementary and Alternative Medicine, vol. 2020, p. 6473745, 2020.
[24] T. Cukierman, H. C. Gerstein, and J. D. Williamson, “Cognitive decline and dementia in diabetes-systematic overview of prospective observational studies,” Diabetologia, vol. 48, no. 12, pp. 2460–2469, 2005.
[25] E. Dybjer, P. M. Nilsson, G. Engström, C. Helmer, and K. Nägga, “Pre-diabetes and diabetes are independently associated with adverse cognitive test results: a cross-sectional, population-based study,” BMC Endocrine Disorders, vol. 18, no. 1, p. 91, 2018.
[26] Q. Xiang, J. Zhang, C.-Y. Li et al., “Insulin resistance-induced hyperglycemia decreased the activation of Akt/CREB in...
hippocampus neurons: molecular evidence for mechanism of diabetes-induced cognitive dysfunction,” *Neuropeptides*, vol. 54, pp. 9–15, 2015.

[27] Y. Hu, T. C. Guo, X. Y. Zhang, J Tian, and Y. S Lu, “Paired associative stimulation improves synaptic plasticity and functional outcomes after cerebral ischemia,” *Neural Regeneration Research*, vol. 14, no. 11, pp. 1968–1976, 2019.

[28] M. Khacho, R. Harris, and R. S. Slack, “Mitochondria as central regulators of neural stem cell fate and cognitive function,” *Nature Reviews Neuroscience*, vol. 20, no. 1, pp. 34–48, 2018.

[29] L. C. Santos, R. Vogel, J. E. Chipuk et al., “Mitochondrial origins of fractional control in regulated cell death,” *Nature Communications*, vol. 10, no. 1, p. 1313, 2019.

[30] Y. Zhang, C. Sun, G. Xiao, and Y. Gu, “Host defense peptide Hymenochirin-1B induces lung cancer cell apoptosis and cell cycle arrest through the mitochondrial pathway,” *Biochemical and Biophysical Research Communications*, vol. 512, no. 2, pp. 269–275, 2019.

[31] P. Ghiasi, S. Hosseinkhani, H. Ansari et al., “Reversible permeabilization of the mitochondrial membrane promotes human cardiomyocyte differentiation from embryonic stem cells,” *Journal of Cellular Physiology*, vol. 234, no. 1, pp. 521–536, 2019.

[32] G. Yi, L. Li, M. Luo et al., “Heat stress induces intestinal injury through lysosome- and mitochondria-dependent pathway in vivo and in vitro,” *Oncotarget*, vol. 8, no. 25, pp. 40741–40755, 2017.

[33] HN. Buttenschøn, L. Foldager, B. Elfving et al., “Neurotrophic factors in depression in response to treatment,” *Journal of Affective Disorders*, vol. 183, pp. 287–294, 2015.

[34] C. H. Wu, T. H. Hung, C. C. Chen et al., “Post-injury treatment with 7,8-dihydroxyflavone, a TrkB receptor agonist, protects against experimental traumatic brain injury via PI3K/Akt signaling,” *PloS One*, vol. 9, no. 11, Article ID e113397, 2014.

[35] S. M. Massa, T. Yang, Y. Xie et al., “Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents,” *Journal of Clinical Investigation*, vol. 120, no. 5, pp. 1774–1785, 2010.

[36] D. A. Schmid, T. Yang, M. Ogier et al., “A TrkB small molecule partial agonist rescues TrkB phosphorylation deficits and improves respiratory function in a mouse model of Rett syndrome,” *Journal of Neuroscience*, vol. 32, no. 5, pp. 1803–1810, 2012.

[37] A. Brunet, S. R. Datta, and M. E. Greenberg, “Transcription-dependent and -independent control of neuronal survival by the PI3K-Akt signaling pathway,” *Current Opinion in Neurobiology*, vol. 11, no. 3, pp. 297–305, 2001.

[38] J. Yang, H. Yan, S. Li, and M. Zhang, “Berberine ameliorates MCAO induced cerebral ischemia/reperfusion injury via activation of the BDNF-TrkB-PI3K/Akt signaling pathway,” *Neurochemical Research*, vol. 43, no. 3, pp. 702–710, 2018.

[39] W. Guo, Y. Ji, S. Wang, Y. Sun, and B. Lu, “Neuronal activity alters BDNF-TrkB signaling kinetics and downstream functions,” *Journal of Cell Science*, vol. 127, no. 10, pp. 2249–2260, 2014.

[40] B. M. Mayr, G. Canettieri, and M. R. Montminy, “Distinct effects of cAMP and mitogenic signals on CREB-binding protein recruitment impart specificity to target gene activation via CREB,” *Proceedings of the National Academy of Sciences*, vol. 98, no. 19, pp. 10936–10941, 2001.

[41] P. A. Watson, A. Nesterova, C. F. Burant, D. J. Klemm, and J. E.-B. Reusch, “Diabetes-related changes in cAMP response element-binding protein content enhance smooth muscle cell proliferation and migration,” *Journal of Biological Chemistry*, vol. 276, no. 49, pp. 46142–46150, 2001.