Protective effect of Huangpu Tongqiao capsule against Alzheimer's disease through inhibiting the apoptosis pathway mediated by endoplasmic reticulum stress in vitro and in vivo

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Abstract

Objectives: Huangpu Tongqiao Capsule (HTPQC) is a traditional Chinese medicine (TCM) that has been used to treat Alzheimer's disease (AD). This study was to explore the pharmacological action and molecular mechanism of HTPQC in the treatment of AD.

Methods: The possible targets of HTPQC were predicted by the molecular docking technique. Intraperitoneal injection of D-galactose and bilateral injection of Ab25-35 in hippocampus induced AD rat model. Morris water maze was used to observe learning and memory function. The primary hippocampal neurons were induced by Ab25-35. Moreover, the apoptosis rate of hippocampal nerve cells was detected through AnnexinV/PI double standard staining. The mRNA and protein levels of GRP78, CHOP, Caspase 12, Caspase 9, and Caspase 3 were detected by PCR and western blot.

Results: The prediction results suggest that HTPQC may act on GRP78. HTPQC significantly improved the learning and memory function, and decreased neuronal apoptosis in vivo and in vitro. In addition, HTPQC could decrease the mRNA and protein expression levels of GRP78, CHOP, Caspase 12, Caspase 9, and Caspase 3, and the effect trend was consistent with the specific inhibitor of GRP78.

Conclusions: HTPQC has a neuroprotective effect against AD by inhibiting the apoptosis pathway mediated by endoplasmic reticulum stress.

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1. Introduction

Alzheimer's disease (AD) is a type of neurodegenerative disease, its main feature is the progressive deterioration of memory and cognitive function (Fassler et al., 2021). Currently, the pathological features of AD mainly include β-amyloid (Aβ) deposition, neurofibrillary tangles formed by hyperphosphorylation of tau protein, and massive loss of hippocampal neurons (Nanjundaiah et al., 2021). However, the current treatment for AD is mostly limited to palliative drugs, which can only relieve the symptoms of cognitive dysfunction in AD, but cannot significantly change the progression of AD due to the multifactor and complexity (Abdi et al., 2022). Therefore, it is still very necessary to develop successful treatment and preventive intervention drugs based on the clear mechanism of action, to provide a new approach for effective prevention and treatment of AD. According to the amyloid cascade hypothesis, the accumulation of Aβ is one of the important reasons for the occurrence and development of AD (Yang et al., 2022). Therefore, reducing the excessive accumulation of Aβ can be an important means to effectively prevent and treat AD.

Clearance of Aβ defects is involved in the accumulation and aggregation of Aβ, interfering with a variety of cellular processes, triggering endoplasmic reticulum stress (ERS), resulting in a complex multi-step cascade that ultimately leads to neuronal death and the development of AD (Lai et al., 2022). ERS-mediated apop-
tosis is also closely related to the pathophysiological development of AD (Luo et al., 2020). Previous studies have suggested that the activation of ERS pathway induced by Aβ lead to apoptosis and may be involved in the pathogenesis of AD (Promyo et al., 2020). The endoplasmic reticulum (ER) is involved in the synthesis of membrane proteins, secretory proteins, glycogen, and lipids, as well as the spatial folding and glycosylation of proteins. Under ERS, the 78-kDa glucose-regulated protein (GRP78) sensor may activate the unfolded protein response (UPR) branch, restoring the homeostasis of the ER (Li et al., 2022). When intracellular homeostasis is disrupted, large amounts of unfolded proteins accumulate in the ER accumulate, triggering to ER lead to pro-apoptotic signals by activating the transcription factor C/EBP homologous protein (CHOP), which promotes the activation of pro-apoptotic proteins and leads to cell apoptosis (Spreenke et al., 2017, Wang et al., 2021). It is necessary to develop potential therapeutic strategies against ERS for the treatment of AD.

Huangpu Tongqiao Capsule (HPTQC) is a Chinese Medicine formula developed by the First Affiliated Hospital of Anhui University of Chinese Medicine that has been used in the treatment of vascular dementia for many years (Wang et al., 2015). HPTQC is composed of Acorus tatarinowii, Alpinia oxyphylla Miq, Fallopia multiflora (Thunb.) Harald, Ligusticum chuanxiong hort, Rheum palmatum L. and Panax ginseng C. A. Meyer. Our previous studies have found that HPTQC could inhibit the over-activation of the JNK signal pathway (Jiang et al., 2019), regulate the EGFR-PLC signalling pathway (Wang et al., 2020), reduce oxidative stress and inhibit the mitochondrial apoptosis pathway (Cai et al., 2018a, 2018b) and inhibit the CaM-CaMKIV pathway (Ye et al., 2020) in vitro. Moreover, HPTQC could also decrease the apoptosis of primary hippocampal neurons induced by Aβ25-35, and the mechanism of action was related to its inhibition of the CaM-CaMKIV pathway, thereby reducing tau hyperphosphorylation (Cai et al., 2018a, 2018b; Ye et al., 2020). Our further studies have found that the main active ingredients were 2,3,5,4-tetrahydroxystilbene 2-O-beta-D-glucoside, ferulic acid, ginsenoside Rg1, alo-emodin, beta-asarone, emodin, and chrysophanol (Ye et al., 2021). However, the protective effects of HPTQC prevent AD by inhibiting the apoptosis pathway mediated by ERS still remain unclear.

In this study, we established in vivo rat model to evaluate the therapeutic and anti-apoptotic effects associated with ERS, and in vitro cell model to investigate the anti-apoptosis pathway mediated by ERS for exploring the molecular mechanism of HPTQC. Molecular docking prediction combined with experimental verification may provide new insight into the protective effects and mechanism of HPTQC against AD.

2. Material and methods

2.1. Molecular docking

CB-Dock was used to predict the action mechanism of HPTQC, and the center and size of the cavity could be obtained (Liu et al., 2020). The procedure is as follows: GRP78 (PDB ID: 5EY4) (Hughes et al., 2016) was obtained in PDB format from the RCSB PDB database. PubChem website was used to obtain the chemical structure of VER-155008, emodin, chrysophanol, ginsenoside Rg1, alo-emodin, 2,3,5,4-tetrahydroxystilbene 2-O-beta-D-glucoside, ferulic acid, and beta-asarone (Fig. 1). GRP78 and chemicals were input to CB-Dock for molecular docking.

2.2. Antibodies and reagents

Aβ25-35 was purchased from Sigma-Aldrich (A4559, St Louis, MO, USA). Donepezil Hydrochloride Tablet (Don) was purchased from Eisai, Shanghai, China. HPTQC (Z20080006) was provided by the First Affiliated Hospital of Anhui University of Chinese Medicine. Annexin V-PI kit (Lot.600125a) was purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). Protein lysate (Lot. 70126373), PMSF (Lot. 329-98-6), Tween-20 (Lot. 20CS180) and Tris (Lot. 1130K07) were purchased from Beijing Solarbio Science & Technology Co., Ltd. The following antibodies, Caspase3 (Lot. 89Z0306), Caspase9 (Lot. 74Z1125), Caspase12 (Lot. 76r9498), CHOP (Lot. 37s3359), and GRP78 (Lot.642874), were from Affinity Biosciences (Cincinnati, OH, USA). PDVF membrane (Lot. RR88250) was purchased from Millipore. TRizol kit (Lot. 15596-026) was purchased from Ambion (Carlsbad, CA, USA). PBS (Lot.10099140C) was purchased from Bio-Rad Laboratories.

2.3. Animals

Healthy Sprague-Dawley rats (male, ten-month-old, 250-300 g), and neonatal male and female SD rats (<24 h-old) were from Nanjing Qinglongshan Animal Breeding Farm (SCXK (Zhe) 2019-0001). All rats were fed under suitable temperature and humidity, that is, the temperature was controlled at 22 ± 2 °C, the humidity was controlled at 50 ± 10%, and the 12-hour light-/dark cycle. All procedures were approved by the Center of Scientific Research of Anhui University of Chinese Medicine.

2.4. In vivo studies

2.4.1. Drug treatment

All rats were divided into 4 groups with 20 rats in each group. The grouping was as follows: control group (Control), model group (Model), HPTQC group (i.g. 1.41 g/kg) (HPTQC), and donpezil group (i.g. 1.5 mg/kg) (Don). The control and model groups were given an equal volume of normal saline. All groups were given corresponding interventions for 4 weeks.

2.4.2. Establishment of the AD rat model

An AD rat model was established by intraperitoneal injection of D-galactose and bilateral hippocampal injection of Aβ25-35 (dissolved in deionized water inducing aggregation) (Ye et al., 2017; Wang et al., 2020). Briefly, rats in the model group, HPTQC, and Don groups were intraperitoneally injected with D-galactose (100 mg/kg), once a day, for consecutive 42 days. After intraperitoneal injection of D-galactose for 21 days, 5 µL (10 µg) Aβ25-35 was slowly injected into the bilateral hippocampus by a microinjection pump. Rats in the control group were injected with the same amount of normal saline in intraperitoneal injection and bilateral hippocampus. After intraperitoneal injection of D-galactose for 14 days, the HPTQC group and Don group were given corresponding interventions respectively. After rats were anesthetized with pentobarbital sodium, blood was taken from the abdominal aorta and centrifuged. The serum and plasma were taken and stored in a refrigerator at −20 °C. The brain was collected on the ice surface, and the hippocampal tissues on both sides were carefully separated, and stored at −80 °C for western blot, RT-PCR, and Annexin V/PI detection.

2.4.3. Morris water maze test

7 days after intracerebral injection of Aβ25-35 in rats, Morris water maze test was used by Shekarian et al. (2020). Briefly, the experiment lasted for 6 days. Day 1 to day 4 was platform training. Each rat was trained once in each quadrant at a fixed time every morning, with an interval of 5 min. The midpoint was selected as the entry point of each quadrant, and the rats were slowly put into the water facing the pool wall and close to the pool wall. Day 5 was an escape incubation period. The platform was fixed in the first quadrant, the midpoint of the third quadrant was selected as the
entry point, and the rats were put into the water to swim. The time when the rats found the platform was recorded as 90 s. Day 6 was space exploration ability experiment. The midpoint of the third quadrant was selected as the entry point, and the rats were placed into the water to swim in 90 s, during which the number of times the rats crossed the platform position was recorded.

2.4.4. Annexin V/PI double standard staining

The hippocampus of each group was collected, digested with 0.25% trypsin, bathed at 37 °C for 30 min, and filtered. After centrifugation at 1400 r/min, the supernatant was discarded and 1 × 10⁶ cell suspension was prepared. Annexin V/PI kit was used for staining for 15 min, and apoptosis was detected by fluorescence microscope.

2.5. In vitro studies

2.5.1. Primary hippocampal neurons culture

The culture method of primary hippocampal neurons accorded to Korkotian et al (Korkotian and Segal, 1997). Neonatal SD rats were sacrificed after being immersed in 75% alcohol for 20 s. The brain tissues were removed. The hippocampus was isolated from the brain and exposed to PBS (ice-cold). After the blood vessels and meninges were separated, the hippocampus was cut into 1 mm³ slice. The tissues were digested with 0.125% trypsin at 37 °C for 20 min, and then added into the DMEM/F12 medium with 10% fetal bovine serum (FBS). The cell suspension was swirled gently and filtered through a 200-mesh sieve, and then separated and collected by centrifugation at 1500 rpm for 5 min. The isolated primary hippocampal neurons were cultured in DMEM/F12 (20% FBS). Subsequently, the neurons were seeded on a PDL plate at a density of 1 × 10⁶/mL under 5% CO₂ and 37 °C. After 24 h, the medium was replaced with neurobasal + 2% B27 and L-glutamate (0.2 mol/L), and half of the medium was changed every 3 days.

The morphological changes of cells were observed under the microscope. The neurons grown on day 7 were used for the follow-up experiment.

2.5.2. Identification of primary hippocampal neurons

Microtubule-associated protein-2 (MAP-2), a neurocytoskeletal specific structural protein, was used to identify primary hippocampal neurons by immunofluorescence. The identification method of primary hippocampal neurons accorded to the previous studies (Cai et al., 2018a, 2018b).

2.5.3. Drug treatment

The primary hippocampal neurons were divided into six groups: control group (Control), model group (Model), VER-155008 group (Model+), 15% HPTQC serum group (HP), 15% HPTQ serum + VER-155008 group (HP+), and donepezil group (Don). 30 μM of Aβ25-35 was added to all groups except the control group. Model+ group was incubated with 2.6 μM VER-155008 for 2 h before the establishment of the AD cell model. HP and Don groups were given 15% HPTQ serum and 10 μM donepezil solution, respectively, for 24 h after the establishment of the AD cell model. HP+ group was given 15% HPTQ serum for 24 h after being preadministered 2.6 μM VER-155008 and the establishment of the AD cell model.

2.5.4. Morphological observation of neuronal injury

The extracted primary hippocampal neurons were inoculated into 96-well PDL plates at a density of 1 × 10⁶. After 7 days of culture, the fresh conventional medium was changed and divided into 6 groups (n = 6): control group, model group, Model+ group, HP group, HP+ group, and Don group. After the intervention, the growth of the neurons was observed under an inverted microscope.

Fig. 1. The chemical structures of VER-155008 and the main active ingredients of HPTQC. (A) VER-155008, (B) Emodin, (C) Chrysophanol, (D) Ginsenoside Rg1, (E) Aloe-emodin, (F) 2,3,5,4’-tetrahydroxystilbene 2-O-beta-D-glucoside, (G) Ferulic acid, (H) β-asarone.
2.5.5. Cell viability

Primary hippocampal neurons were plated in 6-well plates with a cell density of $1 \times 10^6$ cells/mL and 2 mL for each well. The experiment was carried out after 7 days of growth. The experiment was divided into 4 groups ($n = 6$): control group, model group, HPTQC group, and Don group. Control group was given 2 mL fresh medium, and the other groups were given 2 mL medium containing 30 $\mu$M AP$_{25-35}$. After 24 h, HPTQC group was added to 15% HPTQ medicated serum and 10 $\mu$M donepezil solution, respectively. After continued culture for 24 h, Annexin V-FITC /PI double staining apoptosis detection Kit instructions were used to treat the cells. The apoptosis rate was measured by flow cytometry.

2.6. Mechanism of HPTQC on AD model in vivo and in vitro

2.6.1. Quantitative Real-Time PCR (qRT-PCR)

Trizol kit was used to extract total RNA from hippocampal tissue or treated cells, and then reverse transcribed into cDNA. cDNA was synthesized from total cellular RNA using a RevertAid First Strand cDNA Synthesis Kit. RT-PCR was performed using LightCycler® 96 PCR instrument (Roche, Switzerland). The results were analyzed using 2$^{(-\Delta\Delta C_{t})}$ method to evaluate the mRNA levels of GRP78, CHOP, pro-Caspase12, pro-Caspase9 and pro-Caspase3. The primers used were listed in Table 1.

2.6.2. Western blot assay

The total protein was extracted from hippocampal tissue or cells using a protein extraction kit, and the concentrations were measured using a Bicinchoninic Acid (BCA) Kit. SDS-PAGE separates the same amount of protein and transfers it to the PVDF membrane, and subsequently blocked in 5% non-fat dry milk for 2 h. The blot was incubated overnight at 4°C with anti-Caspase3, anti-Caspase9, anti-Caspase12, anti-CHOP, and anti-GRP78. After washing with TBST 3 times, incubated with HRP-conjugated goat anti-rabbit IgG at room temperature for 1.5 h, and washed with TBST 3 times. Electrogenerated chemiluminescence (ECL) was used to determine its mechanism. Therefore, in vitro and in vivo experiments will be used to study whether HPTQC acts on GRP78. Further experimental verification is needed to determine its mechanism. Therefore, in vitro and in vivo experiments will be used to study whether HPTQC acts on GRP78.

2.7. Statistical analysis

All data are presented as means ± SD and statistical analysis was carried out using SPSS 23.0 software. The mean values of multiple groups obtained in this study were compared and analyzed by one-way analysis of variance (ANOVA), LSD method (homogeneity of variance) or Dunnett’s test (unhomogeneity of variance) was used for the post-hoc test, and $P < 0.05$ was considered statistically significant. Statistical analyses were carried out using GraphPad Prism 8.0.

3. Results

3.1. Molecular docking results

The main chemical components of HPTQC had good binding affinities with GRP78 (Table 2 and Fig. 2). Among them, the binding ability of emodin to GRP78 was similar to VER-155008, which is the GRP78 inhibitor. Further experimental verification is needed to determine its mechanism. Therefore, in vitro and in vivo experiments will be used to study whether HPTQC acts on GRP78.

### Table 1

| Primers | Sequence (5'→3') |
|---------|-----------------|
| β-actin | Forward         |
| GRP78   | Reverse         |
| CHOP    | Reverse         |
| pro-Caspase12 | Reverse |
| pro-Caspase9  | Forward |
| pro-Caspase3   | Forward |

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Table 2
Docking results of the main chemical components of HPTQC with GRP78.

| Chemicals                               | Vina score | Cavity score | Center (x, y, z) | Size (x, y, z) |
|-----------------------------------------|------------|--------------|------------------|---------------|
| VER-155008                              | −9.4       | 8967         | 6, −5, −15       | 33, 24, 35    |
| Emodin                                  | −9.1       | 8967         | 6, −5, −15       | 35, 29, 35    |
| Chrysophanol                             | −8.6       | 8967         | 6, −5, −15       | 35, 29, 35    |
| Ginsenoside Rg1                         | −8.5       | 8967         | 6, −5, −15       | 35, 27, 35    |
| Aloe-emodin                             | −8.4       | 8967         | 6, −5, −15       | 35, 29, 35    |
| 2,3,5,4-tetrahydroxystilbene 2-O-beta-D-glucoside | −7.4       | 8967         | 6, −5, −15       | 35, 29, 35    |
| Ferulic acid                            | −6.2       | 8967         | 6, −5, −15       | 35, 29, 35    |
| β-asarone                               | −6.0       | 8967         | 6, −5, −15       | 35, 29, 35    |

Fig. 2. Binding affinity of the main chemical components of HPTQC with GRP78, (A) VER-155008-GRP78 complex, (B) Emodin-GRP78 complex, (C) Chrysophanol-GRP78 complex, (D) Ginsenoside Rg1-GRP78 complex, (E) Aloe-emodin-GRP78 complex, (F) 2,3,5,4-tetrahydroxystilbene 2-O-beta-D-glucoside-GRP78 complex, (G) Ferulic acid-GRP78 complex, (H) β-asarone-GRP78 complex.
3.3.2. Effect of HPTQC on morphology of neuron injury in AD cell

As shown in Fig. 8, the hippocampal neurons adhered firmly, the cell morphology was complete, and the cell body was round and full in the control group. The hippocampal neurons in the model group were wrinkled, some fell off, and the processes were retracted and broken. In HP, and Don groups, neuronal state was significantly restored, cell body was enlarged, regeneration process was gradually restored, and network structure was gradually restored. These results indicated that HPTQC improved the neuron injury induced by Ab25-35 in the AD cell model.

3.3.3. Effect of HPTQC on apoptosis rate in AD cell

Annexin V/PI double staining was used to detect the effect of HPTQC on the apoptosis rate of AD cells. The results showed a significant increase in neuronal apoptosis compared with the control group (p < 0.01), which was decreased in HPTQC and Don groups (p < 0.01) (Fig. 9). These data indicate that HPTQC can significantly reduce neuronal apoptosis.

3.3.4. Effect of HPTQC on protein expression of ERS-mediated apoptosis pathway in AD cell

Western blot results showed that the protein expressions of GRP78, CHOP, Caspase12, Caspase9 and Caspase3 in the model group were significantly increased (p < 0.01). Model+, HP, HP+, and Don groups could reverse the protein expressions of the ERS-mediated apoptosis pathway (Fig. 10).

3.3.5. Effect of HPTQC on gene expression of ERS-mediated apoptosis pathway in AD cell

Compared with the control group, the mRNA expression levels of GRP78, CHOP, pro-Caspase12, pro-Caspase9 and pro-Caspase3
in the model group were significantly increased ($P < 0.01$). Compared with the model group, mRNA expression levels of GRP78, CHOP, pro-Caspase12, pro-Caspase9 and pro-Caspase3 in Model+, HP, HP+, and Don groups were significantly decreased ($P < 0.01$) (Fig. 11). These data strongly suggest that the neuroprotective effect of HPTQC may be related to the endoplasmic reticulum stress-mediated apoptosis pathway.

### 4. Discussion

AD is a common neurodegenerative disease in the elderly, which is characterized by memory and cognitive deterioration. However, the exact etiology and prognosis of AD are not completely clear, and effective treatment drugs and preventive measures are still lacking. Symptomatic treatment of AD with a single target can delay the progression of the disease, but cannot fundamentally treat AD (Calfio et al., 2020). Clinical studies have shown that traditional Chinese medicine (TCM) can improve patients' neuropsychological scale score, TCM symptom score and quality of life, delay Aβ deposition, and tau lesions, and regulate the metabolism of the cholinergic neurotransmitters, etc. (Zhang et al., 2019). Therefore, TCM has a broad application prospect in the prevention and treatment of AD.

The occurrence of AD is directly related to the ERS apoptosis pathway (Zhong et al., 2020). The accumulation of misfolded and unfolded proteins in the endoplasmic reticulum lumen and the imbalance of Ca$^{2+}$ resulted in the occurrence of ERS after cells were affected by injury factors (Szabadkai et al., 2006). The body then responds by activating a stress response system called unfolded protein response (UPR). There are three UPR signaling pathways: RNA dependent protein kinase-like ER kinase (PERK), inositol requiring enzyme 1-a (IRE1-a), and activating transcription factor 6-a (ATF6-a) signal pathways (Kharabi Masouleh et al., 2015). Glucose regulated protein 78 (GRP78) aids in protein folding and regulates the function of ERS transmembrane signaling proteins. Induced GRP78 expression has been frequently employed as a marker of ERS and UPR activation (Gao et al., 2021). When ERS occurs, GRP78 releases PERK, IRE1-a, and ATF6-a to bind to misfolded and unfolded proteins clustered in the ER, and then free PERK, IRE1-a, and ATF6-a undergo dimerization and autophosphorylation. Then, dissociated PERK, IRE1-a, and ATF6-a undergo dimerization and autophosphorylation, inducing a series of downstream signaling events (Guo et al., 2021). In the early stage of ERS, UPR reduces the production of unfolded proteins and promotes the breakdown of misfolded proteins to protect cells through the PERK, IRE1-a, and ATF6-a pathways. However, under long-term stress conditions, the continuous stress exceeds the tolerance of UPR, ER homeostasis...
cannot be restored, and ERS induces cell apoptosis (Jia et al., 2019). C/EBP homologous protein (CHOP), a transcription factor linked with ERS, plays a crucial function in apoptosis (Chen et al., 2018). After activation of the PERK/eIF2α/ATF4 pathway, ATF4 upregulates CHOP expression and induces apoptosis. As a result, preventing the beginning of pro-apoptotic processes by down-regulating GRP78 and CHOP partly protects ERS (Rozpedek et al., 2016). We measured the levels of GRP78 and CHOP proteins in the hippocampus of each group to investigate the influence of HPTQC on the ERS signaling pathway. The mRNA and protein expressions of GRP78 and CHOP were shown to be higher in AD animals and cell models in the current investigation. HPTQC drastically reduced GRP78 and CHOP expression. The protein and gene expressions were down-regulated with the addition of a GRP78 specific inhibitor (VER-155008), and the impact was more pronounced when combined with HPTQC, demonstrating that HPTQC acts on the GRP78 signaling pathway.

Cysteinyl aspartate specific proteinase 12 (Caspase12) is the only molecule of Caspase that locates in the ER and is considered a specific apoptotic mediator in the ERS apoptosis pathway, which is only activated in ERS (Zhang et al., 2016). In ERS state, the increase of intracellular Ca²⁺ level leads to the activation of cytoplasmic calpain, and pro-Caspase12 located on ER membrane is cleaved to activate and release into the cytoplasm (Tian et al., 2021). After Caspase12 is activated, pro-Caspase9 is activated, which in turn activates downstream pro-Caspase3, initiating the classical apoptosis pathway, and ultimately leading to apoptosis (Wootz et al., 2004). To further explore the impact of HPTQC on Caspases, we found that the mRNA and protein expressions of Caspase12, Caspase9, and Caspase3 were higher in vivo and in vitro. HPTQC could significantly down-regulate Caspase12, Caspase9, and Caspase3 expressions.

The report explained the importance of ERS-mediated apoptosis in AD, which has been proposed as potential targets to treat AD (Xu et al., 2021). As we know, the apoptotic pathway mediated by endoplasmic reticulum stress plays a critical role in the course of AD, and it is used as a therapeutic method for neurodegenerative disease therapy. HPTQC therapy, as predicted, blocked the apoptosis pathway mediated by ERS, reducing neuronal apoptosis and improving neuron damage for AD prevention.

Although 7 active components in HPTQC have been identified in our previous study, and the therapeutic effect and mechanism of chrysophanol were mainly studied, the protective effect of other components of HPTQC on AD needs to be further studied. Moreover, the potential mechanism of HPTQC and its active components will be further studied to reveal the common characteristics of multi-component and multi-target of TCM.
Fig. 8. Effect of HPTQC-mediated serum on a morphological change of hippocampal neurons induced by Aβ25-35 (×400). A: Control group; B: Model group; C: HP group; D: Don group.

Fig. 9. Effect of HPTQC on apoptosis rate in AD cell model. Data are expressed as means ± SD (n = 3). Statistical analysis was carried out using one-way ANOVA followed by LSD method (homogeneity of variance) or Dunnett’s test (unhomogeneity of variance). Compared with the control group, **P < 0.01; Compared with model group, ##P < 0.01.

ANOVA: analysis of variance; CTRL: control; Model: AD; HPTQC: Huangpu Tongqiao capsule; Don: Donepezil.
Fig. 10. Effect of HPTQC on protein expression of ERS-mediated apoptosis pathway in AD cell model. Data are expressed as means ± SD (n = 3). Statistical analysis was carried out using one-way ANOVA followed by LSD method (homogeneity of variance) or Dunnett’s test (unhomogeneity of variance). Compared with the control group, **P < 0.01; Compared with the model group, ##P < 0.01. ANOVA: analysis of variance; GRP78: glucose regulated protein 78; CHOP: C/EBP homologous protein; Caspase 12: cysteinyl aspartate specific proteinase 12; Caspase 9: cysteinyl aspartate specific proteinase 9; Caspase 3: cysteinyl aspartate specific proteinase 3. A: Control group; B: Model group; C: Model+ group; D: HP group; E: HP+ group; F: Don group.

Fig. 11. Effect of HPTQC on gene expression of ERS-mediated apoptosis pathway in AD cell model. Data are expressed as means ± SD (n = 3). Statistical analysis was carried out using one-way ANOVA followed by LSD method (homogeneity of variance) or Dunnett’s test (unhomogeneity of variance). Compared with the control group, **P < 0.01; Compared with the model group, ##P < 0.01. ANOVA: analysis of variance; GRP78: glucose regulated protein 78; CHOP: C/EBP homologous protein; pro-Caspase 12: pro-cysteinyl aspartate specific proteinase 12; pro-Caspase 9: pro-cysteinyl aspartate specific proteinase 9; pro-Caspase 3: pro-cysteinyl aspartate specific proteinase 3. CTRL: control; Model: AD; Model+: VER-155008; HP: Huangpu Tongqiao capsule; HP+: Huangpu Tongqiao capsule + VER-155008; Don: Donepezil.
5. Conclusion

In conclusion, HPTQC improved learning and memory function, and reduced the apoptosis of rat hippocampal neurons in the AD rat model. HPTQC treatment improved the neuron injury and reduced the neuronal apoptosis induced by $\Delta\beta_{25-35}$ in the AD cell model. Moreover, HPTQC inhibited the mRNA and protein overexpression levels of GRP78, CHOP, Caspase12, Caspase9, and Caspase3. These findings indicated that HPTQC has a protective effect against AD in vitro and in vivo by inhibiting the apoptosis pathway mediated by endoplasmic reticulum stress, which may provide new insight into the protective effects and molecular mechanism of HPTQC against AD.

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.

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