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Research Article

Cerebroprotective Effects of Yizhitongmai Granule and Decomposed Recipes on Vascular Dementia Rats Via the Nod-like Receptor Protein 3 Inflammasome Pathway

Min PENG [1,a] Guomin SI [1,b] Hongbo MA [1,c] Yuan LIU [1,d] Qianqian LIU [1,e] Hongyan MENG [1,f (*)]

[1] Department of Traditional Chinese Medicine, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan 250021, Shandong Province, CHINA
ORCIDs: [a] 0000-0002-2238-5701; [b] 0000-0002-9281-1489; [c] 0000-0002-7631-9996; [d] 0000-0003-3537-2667; [e] 0000-0001-5227-8846
[f] 0000-0003-4122-3887

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Abstract: We aimed to evaluate the cerebroprotective effects of Yizhitongmai Granule and its decomposed recipes on vascular dementia (VD) rats via the Nod-like receptor protein 3 (NLRP3) inflammasome pathway. Sixty rats were randomly divided into Sham, VD Model, Yizhitongmai Recipe, Bushen Recipe, Tongluo Recipe and positive control groups (n=10). From 12 d after operation, Yizhitongmai Recipe, Bushen Recipe and Tongluo Recipe groups were gavaged with corresponding drug liquid. The drugs were administered at 2 mL once a day for 28 consecutive days. The reactive oxygen species (ROS), superoxide dismutase (SOD), total antioxidant capacity (T-AOC) and lactate dehydrogenase (LDH) in hippocampal tissues were detected using biochemical methods. Tumor necrosis factor-α (TNF-α), interleukin-18 (IL-18) and IL-1β were detected by enzyme-linked immunosorbent assay. Western blotting was performed to detect the expression levels of neuronal growth-associated protein-43 (GAP43), synaptophysin (SYN), aquaporin 4 (AQP4), NLRP3 and Caspase-1. Compared with the Model group, the number of apoptotic cells, levels of ROS, LDH, TNF-α, IL-18, IL-1β, NLRP3 and Caspase-1 decreased, and the levels of SOD, T-AOC, GAP43, SYN and AQP4 increased in the Yizhitongmai Recipe, Bushen Recipe and Tongluo Recipe groups ($P<0.05$). However, the Bushen Recipe and Tongluo Recipe groups had similar indices ($P>0.05$). Compared with the Bushen Recipe and Tongluo Recipe groups, the Yizhitongmai Recipe group had fewer apoptotic cells, decreased levels of ROS, LDH, TNF-α, IL-18, IL-1β, NLRP3 and Caspase-1, and increased levels of SOD, T-AOC, GAP43, SYN and AQP4 ($P<0.05$). Yizhitongmai Granule and its decomposed recipes can protect hippocampal neurons, relieve oxidative stress and inflammatory response caused by hypoperfusion brain injury.

Keywords: *Brain protection, Inflammasome, Nod-like receptor protein 3, Oxidative stress, Vascular dementia*

Yizhitongmai Granülü ve Dekompoze Tanımlarının Nod-benzeri Reseptör Protein 3 İnflamasom Yoluyla Vasküler Demans Sıçanlarındaki Serebroprotektif Etkileri

Öz: Yizhitongmai granülü ve dekompoze tariflerinin, vasküler demans (VD) sıçanları üzerindeki serebroprotektif etkilerinin Nod-like reseptör protein 3 (NLRP3) inflamatuar yolağı üzerinden değerlendirilmesini amaçladık. Altmış sıçan rastgele Sham, VD Model, Yizhitongmai grubu, Bushen grubu, Tongluo grubu ve pozitif kontrol gruplarına ayrıldı (n=10). Deneyden 12 gün sonra, Yizhitongmai grubu, Bushen grubu ve Tongluo grubuna karşılık gelen formülasyonlar verildi. İlaçlar, 28 gün boyunca hergün ve günde bir kez olmak üzere 2 mL şeklinde uygulandı. Hipokampal dokulardaki reaktif oksijen türleri (ROS), süperoksit dismutaz (SOD), toplam antioksidan kapasite (T-AOC) ve laktat dehidrojenaz (LDH) biyokimyasal yöntemlerle tespit edildi. Tümör nekrozis faktör-α (TNF-α), interlökin-18 (IL-18) ve IL-1β, ELISA ile tespit edildi. Nöronal growth-associated protein-43 (GAP43), sinaptofizin (SYN), aquaporin 4 (AQP4), NLRP3 ve Kaspaz-1'in ekspresyon seviyelerini saptamak için Western blot uygulandı. Model grubu ile karşılaştırıldığında, Yizhitongmai, Bushen ve Tongluo gruplarında apoptotik hücre sayısı, ROS, LDH, TNF-α, IL-18, IL-1β, NLRP3 ve Kaspaz-1 seviyeleri azalmış, SOD, T-AOC, GAP43, SYN ve AQP4 seviyeleri artmıştı ($P<0.05$). Ancak, Bushen grubu ile Tongluo grubu benzer indekslere sahipti ($P>0.05$). Bushen ve Tongluo gruplarıyla karşılaştırıldığında, Yizhitongmai grubunda daha az apoptotik hücre, ROS, LDH, TNF-α, IL-18, IL-1β, NLRP3 ve Kaspaz-1 seviyelerinde azalma ve SOD, T-AOC, GAP43, SYN ve AQP4 seviyelerinde artış saptandı ($P<0.05$). Yizhitongmai granülü ve dekompoze tarifleri, hipokampal nöronları koruyabilir, oksidatif stresi ve hipoperfüzyon beyin hasarının neden olduğu inflamatuar yanıtı hafifletebilir.

Anahtar sözcükler: *Beynin korunması, Inflamasom, Nod-like reseptör protein 3, Oksidatif stres, Vasküler demans*

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(*) Corresponding Author

Tel: +86-531-68776357 (H. Meng)
E-mail: menghysph@shu-edu.cn (H. Meng)

Introduction

Vascular dementia (VD) defined as an acquired cognitive impairment syndrome caused by hypoperfusion brain injury is the most common dementia disease following Alzheimer's disease [1,2]. According to a meta-analysis, the morbidity rate of VD is about 0.96% in China, and it frequently occurs in the elderly [3]. VD is primarily caused by ischemic stroke, hemorrhagic stroke, and acute/chronic hypoxic cerebrovascular disease, making it one of the important diseases seriously affecting the quality of life of the elderly [4,5]. The specific pathogenesis of VD remains unclear, and relevant research suggests that it is related to the cholinergic system, oxidative stress, inflammatory response and neuronal apoptosis, among which inflammatory response plays a key role [6]. Hypoperfusion brain injury can lead to inflammatory response and neurovascular unit injury, the latter of which is considered the major cause of cognitive impairment in VD in many studies [7,8]. The Nod-like receptor protein 3 (NLRP3) inflammasome pathway is the key for neurovascular unit cell pyroptosis, which is a crucial player in various brain diseases [9]. VD occurs secondary to cerebrovascular events. Traditional Chinese medicine suggests that blood stasis is an important pathological factor leading to the onset and progression of VD [10]. Meanwhile, the kidney can promote blood circulation, indicating that kidney deficiency and blood stasis are mutually causal [11]. Various pathological factors, such as phlegm and blood stasis, invade the brain and eventually damage to the collaterals. Therefore, brain collateral stasis is an inevitable result of VD which is treated by Chinese herbal medicine through nourishing the kidney and dredging brain collaterals [12].

Yizhitongmai Granule is an empirical traditional Chinese medicine prescription for the clinical treatment of VD, with effects of tonifying kidney and dredging collaterals, promoting blood circulation and removing blood stasis, and eliminating phlegm and inducing resuscitation, which can effectively raise the patients' cognitive ability and improve the activity of daily living [13]. In this study, the effects of Yizhitongmai Granule and its decomposed recipes on the cognitive ability, hippocampal tissue morphology, and levels of NLRP3 pathway-related molecules in VD model rats were compared, the cerebroprotective effect of Yizhitongmai Granule on VD rats was explored, and its target and possible molecular mechanism were investigated, thereby providing some references for the selection of prescriptions and drugs in the clinical treatment of VD.

Material and Methods

Ethical Approval

This study has been approved by the animal ethic committee of Shandong Provincial Hospital Affiliated to Shandong First Medical University (Approval No. 2021120082), and all experiments were carried out as per related guidelines.

Laboratory Animals

Sixty SPF male SD rats (15 months old, 280-320 g) were purchased from Shandong Laboratory Animal Center [animal certificate No. SCXK (Shandong) 2017-007]. They were fed adaptively in the SPF room for 1 week before experiments. In the feeding period, the rats had free access to food and water.

Reagents

Positive drug Ginkgo biloba hevert tablets (Ginaton®) were purchased from Dr. Willmar Schwabe GmbH & Co. KG (Germany), and prepared with 1% sodium carboxymethyl cellulose into a suspension. Yizhitongmai recipe: 3 g Dragon's blood, 10 g earthworm, 3 g centipede, 10 g ginseng, 10 g Rhizome of rehmannia, 10 g Sharpleaf galangal fruit, 10 g Gastrodia elata, and 6 g Hirudo. Bushen recipe: 10 g Ginseng, 10 g Rhizome of rehmannia, 10 g Sharpleaf galangal fruit, and 10 g Gastrodia elata. Tongluo recipe: 3 g Dragon's blood, 10 g earthworm, 3 g centipede, and 6 g Hirudo. Crude drugs were bought from Anhui Tienho Herbal Source Co., Ltd. (China). Each milliliter of extract was equivalent to 4 g crude drug, and prepared by the Preparation Room of Shandong Provincial Hospital.

Hematoxylin-eosin (HE) staining kit (Cat. No. C0105S) and one-step terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) apoptosis assay kit (Cat. No. C1086) were purchased from Shanghai Beyotime Biotechnology Co., Ltd. Reactive oxygen species (ROS) assay kit (Cat. No. E004-1-1), superoxide dismutase (SOD) assay kit (Cat. No. A001-3-2), total antioxidant capacity (T-AOC) assay kit (Cat. No. C0212-4-2) and lactate dehydrogenase (LDH) assay kit (Cat. No. A020-2-2) were purchased from Nanjing Jiancheng Bioengineering Institute. Enzyme-linked immunosorbent assay (ELISA) kits of tumor necrosis factor-α (TNF-α) (Cat. No. ml002859), interleukin-18 (IL-18) (Cat. No. ml002816) and IL-1β (Cat. No. ml037361) were purchased from Shanghai MLBio Co., Ltd. RIPA reagent (Cat. No. R0278) was bought from Sigma, USA. Pierce BCA protein quantification kit (Cat. No. 23225) and SuperSignal West Pico PLUS chemiluminescent substrate (Cat. No. 34580) were bought from Thermo Fisher, USA. Antibodies of neuronal growth-associated protein-43 (GAP43) (Cat. No. ab75810), synaptophysin (SYN) (Cat. No. ab32127), aquaporin 4 (AQP4) (Cat. No. ab9512), NLRP3 (Cat. No. ab270449) and Caspase-1 (Cat. No. ab207802) were bought from Abcam, UK. Other reagents were of commercially available and analytical grade.

Apparatus

A ZS-Morris water maze (Beijing Zhongshi Dichuang

Technology Development Co., Ltd.), an optical microscope and a fluorescence microscope (Leica, Germany), an automatic biochemical analyzer (Beijing Pulang New Technology Co., Ltd.), an HBS-ScanX full-wavelength microplate reader (Nanjing DeTie Laboratory Equipment Co., Ltd.), a Mini Gel Tank (Thermo Fisher, USA), an eBlot™ L1 rapid wet transfer system (Nanjing GenScript Biotechnology Co., Ltd.), and a contact nondestructive quantitative imager (Shanghai e-BLOT Optoelectronics Technology Co., Ltd.) were used.

Grouping and Modeling

The 60 rats were randomly divided into Sham group, VD Model group, Yizhitongmai Recipe group, Bushen Recipe group, Tongluo Recipe group and positive control group (n=10). The VD model was established in each group except for Sham group as follows [14]: The rats were anesthetized by intraperitoneal injection of 10% chloral hydrate, and fixed in a supine position on the laboratory table. After skin preparation and disinfection, a median incision was made on the neck, the tissue was bluntly separated to expose the bilateral common carotid arteries, the bilateral common carotid arteries were ligated with surgical suture, and the incision was sutured. In Sham group, the bilateral common carotid arteries were only separated without ligation. All rats were injected with penicillin (2000 U) locally at the incision to prevent infection. No rats died after modeling.

Drug Intervention

From 12 d after operation, Yizhitongmai Recipe, Bushen Recipe, Tongluo Recipe and positive control groups were gavaged with corresponding drug liquid, while Sham and Model groups were gavaged with normal saline of the same volume. The drugs were administered at 2 mL once a day for 28 consecutive days.

Detection of Degree of Dementia by Morris Water Maze Test

Morris water maze test was performed in each group at 7 d after operation and after the end of drug administration. Four points were set as entry points in the east, west, south and north directions on the wall of a round pool (diameter: 1.2 m, height: 0.5 m, depth: 0.35 m, water temperature: 25°C). A black platform (diameter: 10 cm, height: 33 cm) was placed in the center of the pool. The rats were put into the pool randomly from the entry point, and the time for rats to swim to the platform was recorded. If the rat failed to find the platform within 120 s, it was guided to the platform by the experimenter, and the latency was recorded as 120 s. After the rats stayed on the platform for 30 s, the test was repeated from a new entry point. The training test lasted for 4 d, during which the external environment of the water maze remained the same. At 5 d, the platform was withdrawn, the rats were put into the water from the pool wall, and the number of times of crossing the original position of platform within 120 s was recorded.

Observation of Hippocampal Morphology by HE Staining

After the second Morris water maze test, all rats were sacrificed by decapitation, and the hippocampus tissues were harvested. Part of hippocampus tissues were fixed with 4% paraformaldehyde for 24 h and prepared into paraffin sections. The remaining part was frozen at -80°C. The paraffin sections were stained using HE staining kit, and the hippocampal morphology in each group was observed and photographed under an optical microscope.

Detection of Hippocampal Neuronal Apoptosis by TUNEL Assay

After deparaffinization and hydration, the paraffin sections were incubated with DNase-free proteinase K (20 μg/mL) at 37°C for 20 min, and washed with 1×PBS for 3 times. After drying, the sections were added dropwise with 50 μL of TUNEL reagent on the surface, covered with a cover glass, and incubated at 37°C away from light for 1 h. After washing with 1xPBS for 3 times, the sections were added dropwise with antifade mounting medium, sealed, observed and photographed under a fluorescence microscope away from light. Positive cells were counted in 5 randomly-selected fields in each group, and the average was taken as the apoptosis status of hippocampal tissues.

Detection of Cerebrovascular Endothelial Cell Function

Part of the hippocampal tissues were prepared into homogenate with an appropriate amount of pre-cooled lysis buffer, fully lysed and centrifuged at 10.000 rpm and 4°C for 10 min. The supernatant was harvested for BCA quantification. After sample preprocessing according to the instructions of the biochemical assay kit, the levels of ROS, SOD, T-AOC and LDH in hippocampal tissues were measured in strict accordance with the operation steps.

Detection of Inflammatory Factors in Hippocampal Tissues

The total protein of hippocampal tissues was harvested. After sample preprocessing according to the instructions of the ELISA kit, the levels of TNF-α, IL-18 and IL-1β in hippocampal tissues were measured in strict accordance with the operation steps.

Detection of GAP43, SYN, AQP4, NLRP3 and Caspase-1 Expressions in Hippocampal Tissues by Western Blotting

The total protein of hippocampal tissues was harvested and prepared into samples. Then the sample was separated by gel electrophoresis and transferred onto a membrane,

and the target band was cut and blocked with blocking buffer made of 5% skim milk powder on a shaker at room temperature for 1 h. Later, the sample was incubated with primary antibodies diluted with blocking buffer (1:500) at 4°C overnight. The next day, the membrane was taken out, equilibrated to room temperature, washed and incubated with corresponding secondary antibodies (1:5000) at room temperature for 2 h, followed by washing and reaction with electrochemiluminescence solution away from light for 5 min. The results were collected using a quantitative imager.

Statistical Analysis

According to a previous literature, 8-12 animals were commonly selected for each group [15], so 10 rats were set for each group in this study. SPSS 21.0 software was used for statistical analysis. Measurement data were subjected to the tests of normal distribution and homogeneity of variance. The normal distributed data were expressed as mean ± standard deviation (`X±s), and compared by the independent-samples *t* test between two groups and by one-way analysis of variance among groups. P<0.05 was considered statistically significant.

Results

Dementia Degree

At 7 d after operation (11 d), the escape latency was significantly prolonged and the number of platform-crossing times were reduced in Model group, Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group compared with those in Sham group (P<0.05). After the end of drug administration (44 d), the escape latency was prolonged and the number of platform-crossing times were reduced in Model group, Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group compared with those in Sham group (P<0.05). Compared with Model group, Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group had shortened escape latency and an increased number of platform-crossing times (P<0.05). Escape latency and number of platform-crossing times had no statistically significant differences between Bushen Recipe group and Tongluo Recipe group (P>0.05). Compared with Bushen Recipe group and Tongluo Recipe group, Yizhitongmai Recipe group had shortened escape latency and an increased number of platform-crossing times (P<0.05). There were shorter escape latency and more platform-crossing times at 44 d than those at 11 d in Sham group, Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group (P<0.05) *(Fig. 1)*.

HE Staining Results

It was observed by HE staining that the hippocampal neurons were neatly arranged, and the cells had a regular shape and normal morphology, with clearly visible nucleoli in Sham group. In Model group, the neurons were disorderly arranged, the cells had large intercellular space and abnormal morphology, and the number of cells declined. In Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group, the cells were arranged neatly, the intercellular space was reduced, the cells had good morphology, and the number of cells rose compared with those in Model group, and Yizhitongmai Recipe group exhibited more significant improvement than Bushen Recipe group and Tongluo Recipe group *(Fig. 2)*.

TUNEL Staining Results

The results of TUNEL staining showed that compared with that in Sham group, the number of apoptotic cells rose in Model group, Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group (P<0.05). Compared with that in Model group, the number of apoptotic cells declined in Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group (P<0.05). There was no statistically significant difference in the number of apoptotic cells between Bushen Recipe group and Tongluo Recipe group (P>0.05). Yizhitongmai Recipe group had fewer apoptotic cells than Bushen Recipe group and Tongluo Recipe group (P<0.05) *(Fig. 3)*.

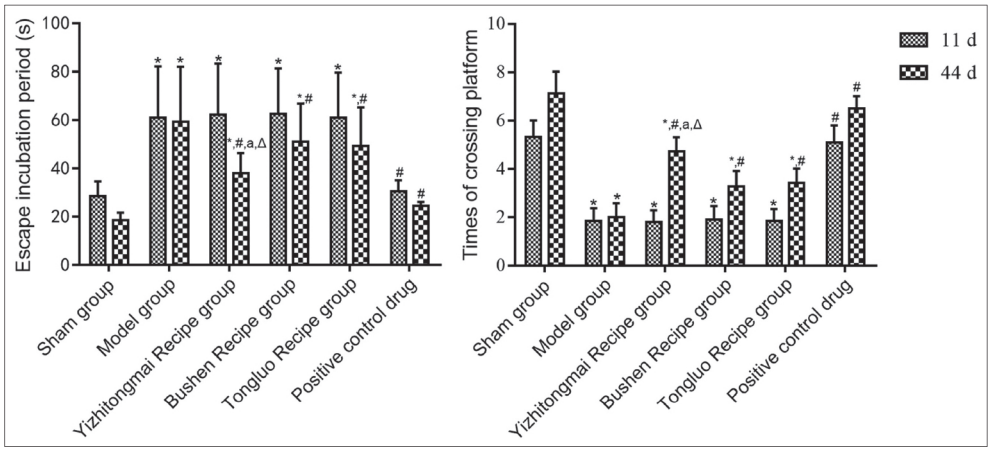


Fig 1. Morris water maze test results. Compared with Sham group, *P<0.05; compared with Model group, #P<0.05; compared with Bushen Recipe group, ΔP<0.05; compared with Tongluo Recipe group, aP<0.05

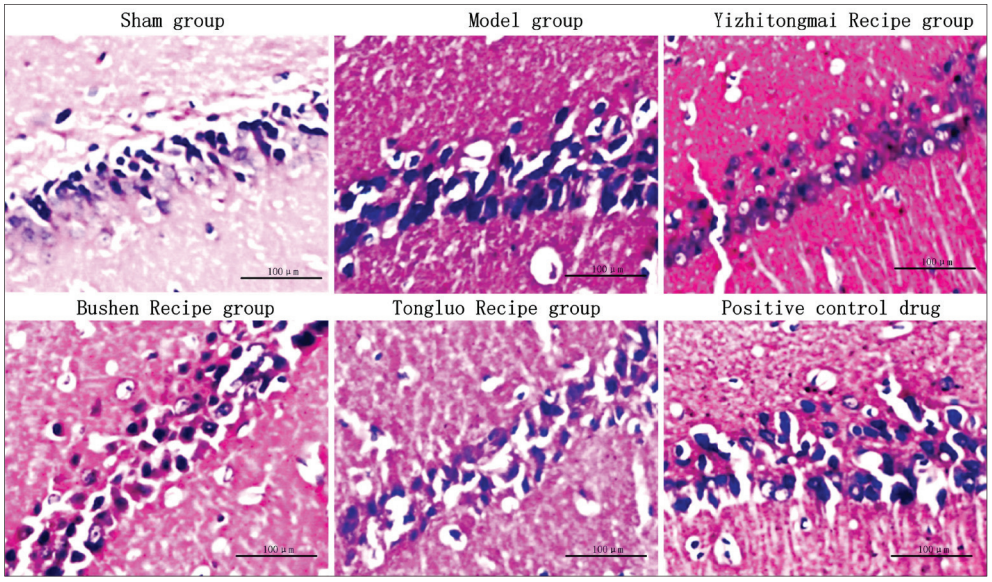


Fig 2. HE staining results (x400). Compared with Sham group, *P<0.05; compared with Model group, #P<0.05; compared with Bushen Recipe group, ΔP<0.05; compared with Tongluo Recipe group, aP<0.05

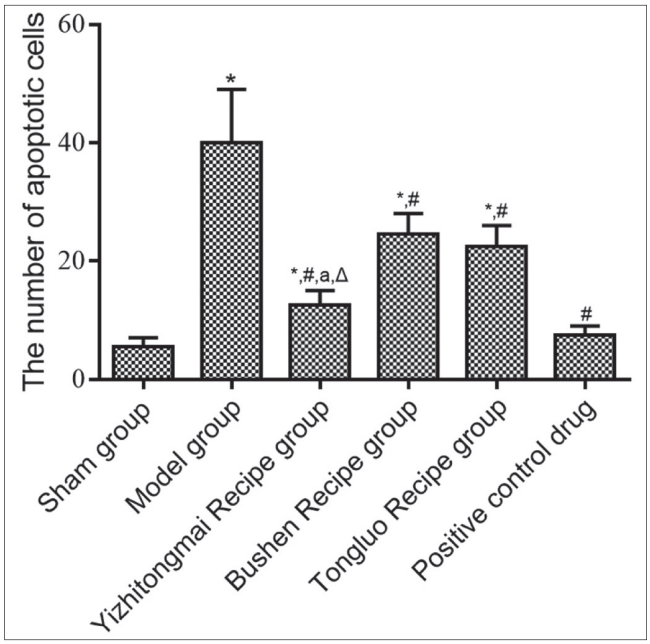


Fig 3. TUNEL staining results. Compared with Sham group, *P<0.05; compared with Model group, #P<0.05; compared with Bushen Recipe group, ΔP<0.05; compared with Tongluo Recipe group, aP<0.05

Cerebrovascular Endothelial Cell Function

Compared with Sham group, the levels of ROS and LDH increased (P<0.05), but the levels of SOD and T-AOC decreased in Model group, Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group (P<0.05). Compared with Model group, the levels of ROS and LDH decreased (P<0.05), whereas the levels of SOD and T-AOC increased in Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group (P<0.05). There were no statistically significant differences in ROS, SOD, T-AOC and LDH levels between Bushen Recipe group and Tongluo Recipe group (P>0.05). Compared with Bushen Recipe group and Tongluo Recipe group, Yizhitongmai Recipe group had decreased levels of ROS and LDH (P<0.05), and increased levels of SOD and T-AOC (P<0.05) *(Fig. 4)*.

Levels of Inflammatory Factors

The levels of TNF-α, IL-18 and IL-1β were higher in Model group, Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group than those in Sham group (P<0.05), while they were lower in Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group than those in Model group (P<0.05). There were no statistically significant differences in the levels of TNF-α, IL-18 and IL-1β between Bushen Recipe group and Tongluo Recipe group (P>0.05). Compared with Bushen Recipe group and Tongluo Recipe group, Yizhitongmai Recipe group had decreased levels of TNF-α, IL-18 and IL-1β (P<0.05) *(Fig. 5)*.

Expressions of GAP43, SYN, AQP4, NLRP3 and Caspase-1

Compared with those in Sham group, the levels of NLRP3 and Caspase-1 were increased (P<0.05), and the levels of GAP43, SYN and AQP4 were decreased in Model group, Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group (P<0.05). Compared with those in Model group, the levels of NLRP3 and Caspase-1 were decreased (P<0.05), and the levels of GAP43, SYN and AQP4 were increased in Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group (P<0.05). There were no statistically significant differences in the above-mentioned indexes between Bushen Recipe group and Tongluo Recipe group (P>0.05). Compared with Bushen Recipe group and Tongluo Recipe group, Yizhitongmai Recipe group had decreased levels of NLRP3 and Caspase-1 (P<0.05), and increased levels of GAP43, SYN and AQP4 (P<0.05) *(Fig. 6)*.

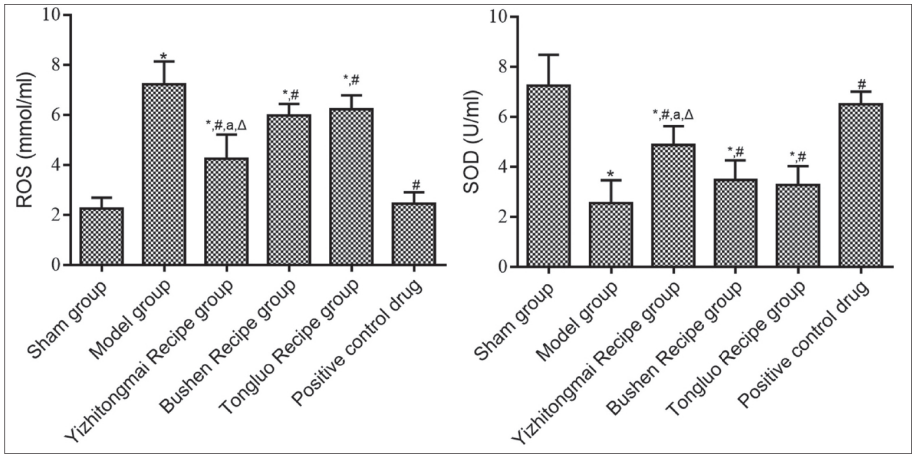
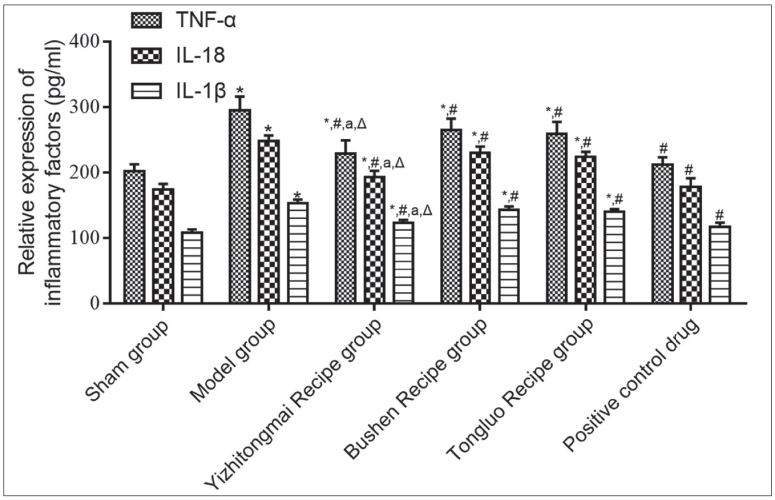


Fig 4. Biochemical detection results. Compared with Sham group, *P<0.05; compared with Model group, #P<0.05; compared with Bushen Recipe group, ΔP<0.05; compared with Tongluo Recipe group, aP<0.05

Fig 5. ELISA results. Compared with Sham group, *P<0.05; compared with Model group, #P<0.05; compared with Bushen Recipe group, ΔP<0.05; compared with Tongluo Recipe group, aP<0.05



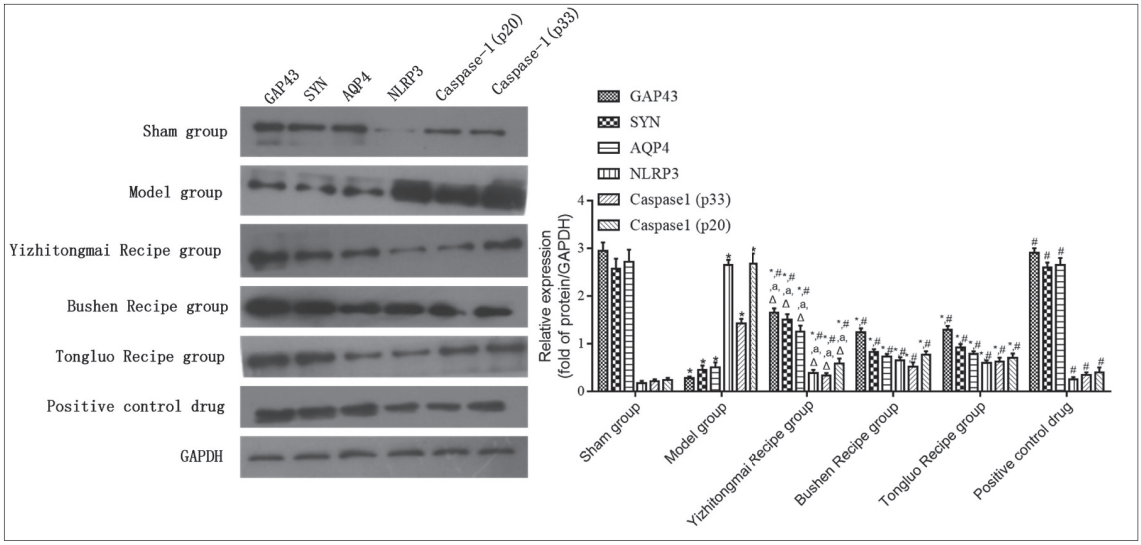


Fig 6. Western blotting results. Compared with Sham group, *P<0.05; compared with Model group, #P<0.05; compared with Bushen Recipe group, ΔP<0.05; compared with Tongluo Recipe group, aP<0.05

Discussion

Vascular dementia belongs to the categories of "dementia" and "forgetfulness" in traditional Chinese medicine, and its major pathogenesis is insufficient kidney essence and blood stasis [16]. In Yizhitongmai recipe, dragon's blood, centipede, Gastrodia elata and Hirudo can resist inflammation, remove blood stasis, promote blood circulation and dredge collaterals [17-20]. Additionally, ginseng, Rhizome of rehmannia and Sharpleaf galangal fruit

can protect hippocampal neurons and resist oxidation [21-23]. Moreover, earthworm can repair cerebral ischemia-induced tissue damage and delay thrombosis [24]. As a result, Yizhitongmai recipe, which combines the effects of various medicinal materials, can invigorate the kidneys and replenish the essence, remove blood stasis and dredge collaterals, effectively relieving the symptoms of VD. In this study, the results of Morris water maze test showed that the learning and memory ability of the VD model rats significantly declined compared with that in Sham group at 7 d after operation, suggesting the successful modeling. After drug administration, the learning and memory ability of rats recovered in Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group, especially in Yizhitongmai Recipe group, compared with that in Model group. It can be seen that a better curative effect can be achieved by tonifying kidney in combination with dredging collaterals.

The hippocampus is an important structure responsible for storage and regulation of learning and memory, with unique vascular architecture and densely arranged microglia, and hypoperfusion brain injury can easily cause hippocampal structural damage [25]. The results of HE and TUNEL staining in this study showed that necrotic hippocampal neurons, arranged disorderly and loosely, could be clearly seen, and the number of apoptotic cells was large in Model group. In Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group, the cellular damage was relieved and the number of apoptotic cells declined, suggesting that Yizhitongmai Granule can alleviate hippocampal tissue injury, and protect hippocampal neurons in VD rats, thereby restoring the learning and memory ability of rats.

The neurovascular unit consists of neurons, glial cells and blood vessels, and its injury is closely related to the pathogenesis of VD [26]. Hypoperfusion brain injury-induced oxidative stress and inflammatory response can affect the dynamic balance of neurovascular unit micro-environment. In this study, Model group had significantly higher levels of ROS and LDH but significantly lower levels of SOD, GAP43, SYN and AQP4 in hippocampal tissues than Sham group. An increased level of ROS can inhibit SOD, reduce the body's antioxidant capacity, and worsen oxidative stress injury. LDH is one of the indexes assessing the degree of cellular oxidative stress injury [27]. GAP43, SYN and AQP4 are proteins associated with neuronal synaptic plasticity and synaptic injury repair, and the changes in their levels can affect learning and memory ability [28]. In this study, the oxidative stress indexes and levels of GAP43, SYN and AQP4 in hippocampal tissues were greatly improved in Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group compared with those in Model group, demonstrating that Yizhitongmai Granule can reduce oxidative stress response, repair neuronal injury and enhance synaptic plasticity.

Inflammasomes are implicated in the body's innate immunity, and the activation of NLRP3 inflammasomes is the basis of a series of inflammatory responses. As pointed out in many studies, the expression level of NLRP3 has close correlations with cognitive dysfunction diseases such as VD and Alzheimer's disease [29-31]. Activated NLRP3 can help activate Caspase-1 through apoptosis-related speckle-like protein, and then IL-18 and IL-1β precursors can be made mature and released outside cells by activated Caspase-1, worsening the inflammatory response and resulting in pyroptosis [32]. In this study, the levels of inflammatory factors and NLRP3/Caspase-1 signaling pathway-related proteins in hippocampal tissues were significantly higher in Model group than those in Sham group, while they declined in Yizhitongmai Recipe group, Bushen Recipe group and Tongluo Recipe group, especially in Yizhitongmai Recipe group, compared with those in Model group, suggesting that Yizhitongmai Granule can lower the levels of inflammatory factors through inhibiting the NLRP3/Caspase-1 signaling pathway, thereby alleviating inflammatory injury in hippocampal tissues.

In conclusion, Yizhitongmai Granule and its decomposed recipes can effectively improve the learning and memory ability of VD rats, protect hippocampal neurons, relieve oxidative stress and inflammatory response caused by hypoperfusion brain injury, and inhibit the NLRP3/Caspase-1 signaling pathway, thereby exerting a cerebro-protective effect on VD rats.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author (H. Meng) on reasonable request.

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Ethical Approval

This study has been approved by the animal ethic committee of Shandong Provincial Hospital Affiliated to Shandong First Medical University (Approval No. 2021120082), and all experiments were carried out as per related guidelines.

Competing Interests

There is no conflict of interest.

Authors' Contributions

MP, GS designed this study; HM prepared this manuscript; YL performed this study; QL analyzed experimental data; HM Writing. All authors read and approved the final version of the manuscript.

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