

Vol 2 Issue 03 (2026)

<http://fsrma.org.hostajournal.com/>

ONLINE ISSN: 3134-7517

PRINT ISSN: 3134-7509

Buyang Huanwu Decoction inhibits neuronal ferroptosis induced by ischemic stroke in rats

Bin Lan*, Sitian Luo, Shiyu Lin, Haigang Li

School of Traditional Chinese Medicine, Changsha Medical University, Changsha, 410219, China

Correspondence: lanbin@csmu.edu.cn

Abstract.

Objective: To investigate the regulatory mechanism of the ferroptosis signaling pathway in ischemic cerebral neurons and evaluate the intervention effect of Buyang Huanwu Decoction (BYHWD).

Methods: Sixty male Sprague-Dawley rats were randomly assigned to four groups: sham, ischemic stroke (IS), IS+DFP (deferiprone), and IS+BYHWD group. After a 5-day acclimatization period, drug administration commenced. The middle cerebral artery occlusion (MCAO) model was established via the intraluminal suture method. Neurological deficits were assessed using the Longa score at 2 h post-operation. Protein expression levels of TFR1 and DMT1 were semi-quantitatively detected by Western blot. Ferroptosis-related indicators in the ischemic brain were evaluated: brain iron deposition, malondialdehyde (MDA), and glutathione (GSH) levels were measured using assay kits, and reactive oxygen species (ROS) levels were determined by luminol-based chemiluminescence.

Results: Compared with the sham group, the IS group exhibited significantly increased neurological deficit scores ($P < 0.01$) and up-regulated TFR1 and DMT1 protein expression ($P < 0.01$). Brain iron deposition, MDA, and ROS levels were elevated, while GSH content was decreased ($P < 0.05$). Compared with the IS group, the IS+BYHWD group showed significantly alleviated neurological deficits ($P < 0.01$), down-regulated TFR1 and DMT1 expression ($P < 0.01$), reduced brain iron deposition, MDA, and ROS levels, and increased GSH content ($P < 0.05$).

Conclusion: BYHWD confers an anti-ferroptotic effect on acute cerebral ischemic neurons via regulation of the TFR1/DMT1 signaling pathway, resulting in decreased iron deposition, lipid peroxidation, and ROS accumulation in the ischemic brain, and ultimately improving neurological outcomes in IS rats.

Keywords:

Ischemic stroke;
Ferroptosis; Iron
metabolism;
TFR1/DMT1;
Buyang Huanwu
Decoction

Ischemic stroke (IS) is characterized by clinical neurological deficits resulting from the disruption of blood supply to localized brain regions. IS exhibits notoriously high rates of incidence, disability, mortality, and recurrence. Furthermore, its onset is demonstrating a progressively younger demographic trend, thereby imposing a profound socioeconomic and personal burden^[1].

Therefore, further elucidating the underlying pathogenic mechanisms of IS and identifying novel therapeutic targets as well as preventative strategies are of paramount clinical significance. Ferroptosis is a recently defined form of cell death driven by iron accumulation, which is tightly regulated by the TFR1/DMT1 signaling pathway. Intracellular iron overload initiates lipid peroxidation via the Fenton reaction, leading to a massive accumulation of reactive oxygen species (ROS). This overwhelming oxidative stress inflicts severe damage on biomembranes, proteins, and nucleic acids, ultimately executing the process of ferroptosis and subsequently exacerbating ischemic brain tissue injury^[2, 3]. Buyang Huanwu Decoction (BYHWD), a compound Chinese traditional formula, consists of seven herbal medicines: Radix Astragali, Radix Angelicae Sinensis, Radix Paeoniae Rubra, Pheretima, Rhizoma Chuanxiong, Semen Persicae, and Flos Carthami. Traditionally characterized by its efficacy in invigorating Qi, promoting blood circulation, and unblocking meridians, BYHWD has demonstrated favorable clinical efficacy in patients with ischemic stroke^[4, 5]. However, its underlying pharmacological mechanisms and specific molecular targets against ischemic stroke remain to be fully elucidated. By investigating the regulatory mechanism of the TFR1/DMT1 signaling pathway on ferroptosis in ischemic cerebral neurons and evaluating the interventional effects of BYHWD, this work aims to explore novel therapeutic pathways and molecular targets for the prevention and treatment of ischemic brain injury using TCM.

1. Materials and Methods

1.1 SD rats

Sixty healthy male Sprague-Dawley (SD) rats, weighing 220–250 g, were provided by the Hunan Slack Jingda Experimental Animal Co., Ltd (Hunan, China), [License No. SCXK (Xiang) 2024-0009]. The rats were housed in the Animal Experiment Center of Changsha Medical University under standard laboratory conditions (temperature: 21–25 °C; humidity: 45%–65%). All animal protocols were conducted in strict accordance with the Guiding Opinions on the Treatment of Laboratory Animals issued by the Ministry of Science and Technology of the People's Republic of China, and the study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Changsha Medical University.

1.2 Drugs Preparation

The BYHWD was prepared by water decoction and alcohol extraction as previously described. The BYHWD was dissolved in saline to achieve a concentration of 2.5 g/mL.

Deferiprone (DFP, Obertek Pharmaceutical Co., Ltd., Canada), an oral clinical medication utilized to alleviate intracellular iron overload, served as the positive control. The DFP was dissolved in saline to achieve a concentration of 500 mg/mL.

1.3 Animal Grouping and Drug Administration

Sixty SD rats were randomly assigned to four groups (n = 15 per group): sham-operated (sham), ischemic stroke (IS), IS+DFP, and IS+BYHWD. After a 5-day acclimatization period, drug administration was initiated. The IS+BYHWD group received BYHWD via intragastric gavage at a dose of 6.25 g/day (2.5 mL). The sham and IS groups received an equal volume of physiological saline. These three groups were treated once daily for 7 consecutive days. The IS+DFP group received DFP via intragastric gavage at a clinically equivalent dose of 12.5 mg/day (2.5 mL), once daily for 7 consecutive days. The MCAO model was established at 2 h after the final administration.

1.4 Rat MCAO model

The middle cerebral artery occlusion (MCAO) model was established using a modified intraluminal suture method originally described by Zea Longa et al^[6, 7]. Rats were anesthetized

with 3% sodium pentobarbital (0.1 mL/100 g). A right paramedian neck incision was made to expose the right CCA, ECA, and pterygopalatine artery. A nylon monofilament (0.26 mm diameter) was inserted into the right CCA via a small incision, with temporary clamping of the pterygopalatine artery to prevent misdirection. The monofilament was advanced 18–20 mm from the CCA bifurcation (adjusted for body weight) to occlude the origin of the right middle cerebral artery. The skin was then sutured, and the distal end of the monofilament was partially fixed to the skin. Successful occlusion was confirmed upon awakening by the presence of contralateral forelimb flexion, circling to the left, and leftward leaning when suspended by the tail. The sham group underwent the same procedures without monofilament insertion.

1.5 Neurobehavioral Score

Neurological function was evaluated at 2 h post-operation according to the 5-point scoring system (0–4) established by Longa et al. : 0, no neurological deficit; 1, failure to fully extend the contralateral forepaw; 2, circling to the contralateral side; 3, falling to the contralateral side; 4, inability to walk spontaneously with impaired consciousness.

1.6 Sample Collection and Tissue Preparation

Brain tissue samples from each group were rapidly collected, briefly washed with ice-cold saline, immediately snap-frozen in liquid nitrogen, and stored for subsequent analyses, including detection of TFR1 and DMT1 protein expression and measurement of brain iron, MDA, GSH, and ROS levels.

2 Measurements and Methods

2.1 Measurement of Iron, MDA, GSH and ROS in the rat Brain

The level of iron in rat brain were measured using an iron assay kit (#MAK025, Sigma). The lipid peroxidation product malonaldehyde (MDA) concentration in rat brain was measured using an MDA assay kit (#A003-2, Nanjing Biotechnology Co. Ltd., China). The level of glutathione GSH in rat brain were measured using GSH assay kit (#A061-1, Nanjing Biotechnology Co. Ltd., China). The amount of reactive oxygen species (ROS) in rat brain were measured using luminol-based chemiluminescent quantitative detection kit (#GMS10112.5 v.A, GENMED SCIENTIFICS INC., USA).

2.2 TFR1 and DMT1 protein expression by Western blotting analysis

Total protein was extracted from the brain tissues of each group and denatured by heating. Equal amounts of protein samples were loaded and separated by electrophoresis at 120 V for 120 min. The proteins were then transferred onto membranes at 200 mA for 2 h. Following transfer, the membranes were blocked with 5% non-fat milk for 1 h. For primary antibody incubation, the membranes were incubated overnight at 4 °C with primary antibodies against TFR1 (#bs-1782R), DMT1 (#bs-3577R), which were purchased from Boosen Biotechnology, China. Subsequently, the membranes were washed three times with TBST buffer (10 min/wash) and incubated with a goat anti-rabbit secondary antibody (1:8000) at 37 °C for 1 h. For visualization, the membranes were washed three times with TBST (10 min/wash) and once with TBS buffer for 10 min. Enhanced chemiluminescence (ECL) reagent was applied, and the cumulative optical density of the relative protein expression was determined by densitometric analysis with ImageJ software (V1.8.0, National Institutes of Health).

2.3 Statistical Analysis

All data are presented as the mean $\pm$ standard error of the mean. SPSS 28.0 software (Chicago, IL, USA) was used for statistical analysis. One-way analysis of variance with multiple comparisons using Student-Newman-Keuls test was used to analyze differences between different groups. In all tests, the difference with $P < 0.05$ was considered statistically significant.

3 Results

3.1 Neurobehavioral Score

Compared with the sham group, the neurological deficit scores of the rats in the IS group were significantly increased ($P < 0.01$). However, compared with the IS group, the scores in the IS+DFP group were significantly reduced ($P < 0.01$; Fig. 1).

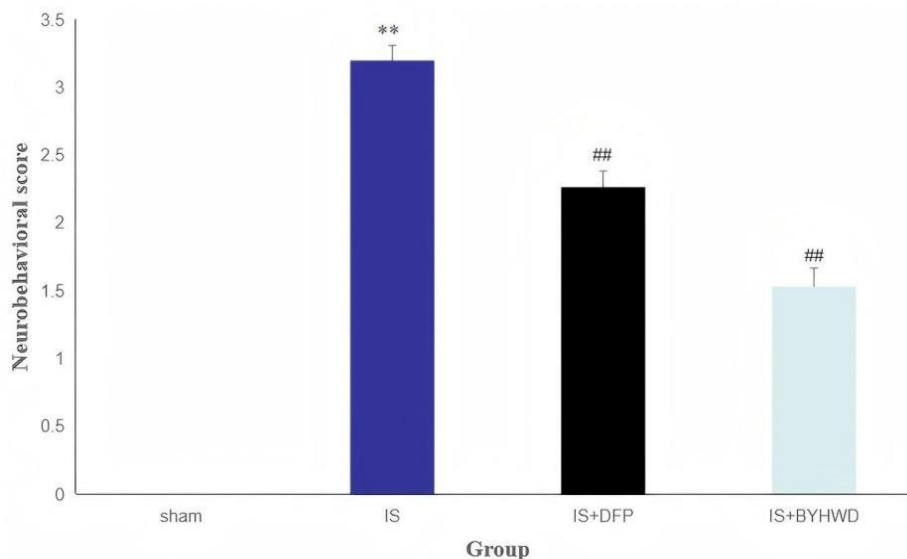


Figure 1 Changes of the neurobehavioral score in the brain tissues of IS rats, which were reversed by BYHWD treatment. The values were presented as mean $\pm$ standard error of mean ($n = 15$). ** $P < 0.01$, vs sham group; ## $P < 0.01$, vs IS group.

3.2 Measurement of MDA, GSH, ROS, and iron levels (mean $\pm$ SD, $n = 5$)

Compared with the sham group, the IS group exhibited significantly decreased GSH content and increased levels of MDA, ROS, and iron in the brain tissue ($P < 0.01$, Fig. 2). Conversely, compared with the IS group, the IS+BYHWD group showed significantly elevated GSH content (Fig. 2B) and markedly reduced levels of MDA (Fig. 2A), ROS (Fig. 2C), and iron (Fig. 2D) ($P < 0.01$).

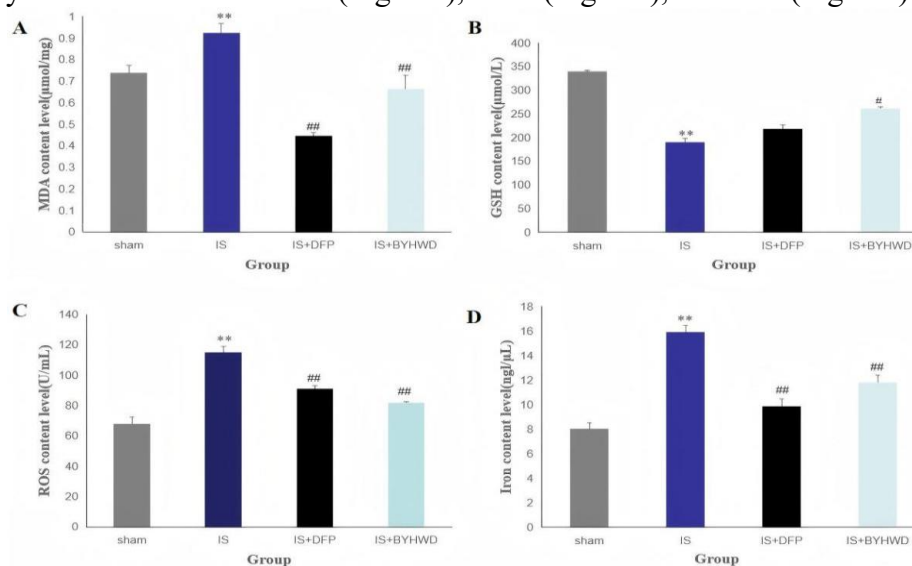


Figure 2 Changes of the major biomarkers of ferroptosis were detected in the brain tissues of IS rats, which were reversed by BYHWD treatment. ** $P < 0.01$, vs sham group; # $P < 0.05$, ## $P < 0.01$, vs IS group.

3.3 BYHWD treatment regulated the expression of ferroptosis-related genes activated in the MCAO rats (mean $\pm$ SD, $n = 5$)

Compared with the sham group, the IS group exhibited significantly upregulated protein expression of TFR1 and DMT1 ($P < 0.01$). Conversely, both the IS+BYHWD and IS+DFP groups showed significantly downregulated expression of TFR1 and DMT1 relative to the IS group ($P < 0.01$; Fig. 3).

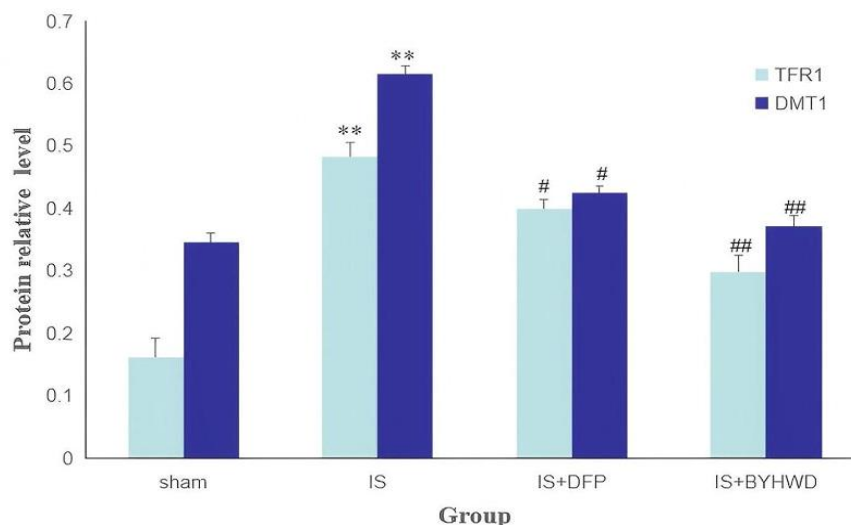


Figure 3 BYHWD treatment regulated the expression of ferroptosis-related genes activated in the IS rats. ** $P < 0.01$, vs sham group; # $P < 0.05$, ## $P < 0.01$, vs IS group.

4 Discussion

Intracellular iron homeostasis is strictly regulated by iron transport signaling pathways. Iron acts as a catalyst; when iron transport in cerebral neurons is imbalanced, the accumulation of excessive (Fe^{2+}) induces the production of a large amount of reactive oxygen species (ROS) via the Fenton reaction, which subsequently oxidizes cell membrane lipids and triggers ferroptosis. The membrane protein transferrin receptor 1 (TFR1) facilitates the entry of ferric iron (Fe^{3+}) into the cell, which is then converted to and transported as ferrous iron (Fe^{2+}) with the involvement of divalent metal transporter 1 (DMT1). As critical transporters in iron metabolism, TFR1 and DMT1 play an essential role in maintaining iron ion stability within cerebral neurons and protecting their normal structure and function^[9, 10]. In the present study, we found that following acute ischemic and hypoxic stimulation in the right cerebral hemisphere of rats, the protein expressions of TFR1 and DMT1 in the cerebral ischemic area were significantly upregulated. Concurrently, the intracellular levels of iron and ROS increased, resulting in neuronal damage. The BYHWD has an important effect on cerebral ischemia in clinical treatments^[11]. In addition, Modern pharmacological studies indicate that *Astragali Radix* contains various active components, including total flavonoids, polysaccharides, and saponins, which exhibit multiple biological functions such as antioxidant activity^[12], reduction of cellular MDA content, and enhancement of superoxide dismutase (SOD) activity^[13, 14]. The principal bioactive constituents of Chuanxiong Rhizoma, such as ligustrazine and chuanxiong phthalide, have been shown to dilate cerebral blood vessels, substantially increase cerebral blood flow, and improve microcirculation, thereby stimulating neuronal recovery following focal cerebral ischemia^[15]. Pheretima contains a range of active constituents, such as lumbrokinase and arachidonic acid, which possess antioxidant, anticoagulant, and vasodilatory properties, thereby contributing to the improvement of microcirculation^[16]. In the present study, both DFP and BYHWD, administered via intragastric

gavage, significantly downregulated TFR1 and DMT1 protein expression in MCAO rats. The neuroprotective effect of BYHWD is likely mediated by its bioactive constituents, which are capable of crossing the blood–brain barrier and reaching brain tissue. These constituents promote cerebral circulation and neurological functional recovery, indicating substantial therapeutic potential for the treatment of cerebral ischemic injury.

In conclusion, our data indicate that cerebral ischemia triggers neuronal ferroptosis via iron metabolism imbalance and redox disruption. BYHWD inhibits ferroptosis by modulating the TFR1/DMT1 pathway, reducing iron overload and lipid peroxidation in the ischemic brain, and consequently improving neurological outcomes in IS rats.

Acknowledgements

This work was supported by the Scientific Research Foundation of Hunan Provincial Education Department (Grant No. 24C0604) and Natural Science Foundation of Hunan Province (Grant No. 2026JJ81253).

Conflicts of Interest

All authors of this article have no conflicts of interest.

References

- Global, regional, and national burden of stroke and its risk factors, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol*, 2021. 20(10): p. 795–820.
- Xie, Y., et al., Ferroptosis: process and function. *Cell death and differentiation*, 2016. 23(3): p. 369–379.
- Dixon, S.J., et al., Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell*, 2012. 149(5): p. 1060–72.
- Wang, R., et al., Efficacy evaluation of Buyang Huanwu Decoction in the treatment of ischemic stroke in the recovery period: A systematic review of randomized controlled trials. *Front Pharmacol*, 2022. 13: p. 975816.
- Huang, H., et al., Buyang Huanwu Decoction Alleviates Ischemic Stroke Injury by Inhibiting Ferroptosis via the Nrf2/GPX4 Pathway. *Drug Des Devel Ther*, 2025. 19: p. 2285–2305.
- Longa, E.Z., et al., Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke*, 1989. 20(1): p. 84–91.
- Li, H., et al., The pharmacokinetics effects of the MCAO model on senkyunolide I in pseudo germ-free rats after oral co-administration of Chuanxiong and warfarin. *Front Pharmacol*, 2025. 16: p. 1577757.
- Fish, W.W., Rapid colorimetric micromethod for the quantitation of complexed iron in biological samples. *Methods Enzymol*, 1988. 158: p. 357–64.
- Li H , Li F , Zhou Y ,et al.The pharmacokinetics effects of the MCAO model on senkyunolide I in pseudo germ-free rats after oral co-administration of Chuanxiong and warfarin[J].*Frontiers in Pharmacology*, 2025.
- Chen, L., et al., Homeostasis and metabolism of iron and other metal ions in neurodegenerative diseases. *Signal Transduct Target Ther*, 2025. 10(1): p. 31.
- Zhong, D.Y., et al., Systematic evaluation and re-evaluation of buyang huanwu decoction in treating ischemic stroke. *Complement Ther Med*, 2022. 70: p. 102860.
- Zhu, T., et al., Therapeutic targets of neuroprotection and neurorestoration in ischemic stroke: Applications for natural compounds from medicinal herbs. *Biomed Pharmacother*, 2022. 148: p. 112719.

- Yin, Y.Y., F.F. Zhu, and G.C. Wu, [Protective effect of astragalosides on anoxia/reoxygenation injury of hippocampal neuron]. *Zhongguo Zhong Xi Yi Jie He Za Zhi*, 2010. 30(11): p. 1173–7.
- Tian, X.R., et al., Astragaloside IV Pretreatment Alleviates Pulmonary Ischemia-Reperfusion Injury via the TLR4/MyD88/NF- κ B p65 Pathway in Rats. *Phytother Res*, 2025. 39(10): p. 4744–4765.
- Feng, X.F., et al., Tetramethylpyrazine promotes stroke recovery by inducing the restoration of neurovascular unit and transformation of A1/A2 reactive astrocytes. *Front Cell Neurosci*, 2023. 17: p. 1125412.
- Wang, Y.H., et al., Lumbrokinase regulates endoplasmic reticulum stress to improve neurological deficits in ischemic stroke. *Neuropharmacology*, 2022. 221: p. 109277.