The effect of Huanglian Jiedu Decoction on inflammatory factors and oxidative stress in patients with acute ischemic stroke

Zhen-ying Niu¹, Meng-xue Jin*²

¹Department of Geriatrics, The Third Affiliated Hospital of Yunnan University of Chinese Medicine, Kunming 650599, China.
²Correspondence to: Meng-xue Jin, Department of Geriatrics, The Third Affiliated Hospital of Yunnan University of Chinese Medicine, No. 2686, Xiang yuan Street, Kunming 650599, China. E-mail: 49310352@qq.com.

Author contributions
Zhen-ying Niu carried out the experiments and manuscript writing. Meng-xue Jin provided idea and technical guidance for the whole work.

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
AIS: acute ischemic stroke; NIHSS, the National Institute of Health stroke scale; ADL, activities of daily living; IL-4, interleukin-4; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; TGF-β, transforming growth factor β; T-AOC, total antioxidative capacity; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase.

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Abstract
Objective: To study the clinical efficacy of Huanglian Jiedu decoction in treating acute ischemic stroke (AIS) and its effects on inflammatory factors and oxidative stress. Method: A total of 53 patients with AIS were recruited as the study subjects and randomly divided into a control group and a treatment group using a random number table method. The control group consisted of 26 patients and the treatment group consisted of 27 patients. The control group received conventional Western medicine treatment. The control group received routine Western medicine treatment, while the treatment group received Huanglian Jiedu decoction based on the control group, with 14 days as a course of treatment. The effects of Huanglian Jiedu decoction on neurological function and activities of daily living were evaluated using the National Institute of Health stroke scale (NIHSS) and activities of daily living (ADL) scores. The effects of Huanglian Jiedu decoction on inflammatory reactions and oxidative stress were evaluated by detecting interleukin-4 (IL-4), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), transforming growth factor β (TGF-β), total antioxidative capacity(T-AOC), malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) levels. Results: After treatment with Huanglian Jiedu Decoction, the ALD scores of AIS patients in both groups increased, while the NIHSS scores decreased, suggesting that Huanglian Jiedu Decoction has therapeutic effects on AIS patients. It also reduces the levels of serum IL-6, TNF-α, MDA in AIS patients and increases the levels of IL-4, TGF-β, CAT, SOD, T-AOC, suggesting that Huanglian Jiedu decoction can improve the anti-inflammatory and antioxidant abilities of AIS patients. Conclusion: Huanglian Jiedu decoction can help AIS patients recover their neurological function, increase their capacity for self-care in daily life, and strengthen the body's anti-inflammatory and antioxidant defenses.

Keywords: Huanglian Jiedu decoction, acute ischemic stroke, inflammatory factors, oxidative stress
**Introduction**

According to the China Stroke Report 2019 (Chinese Version), roughly 70% of stroke patients in China have an incidence of AIS, with high rates of disability and mortality, posing a burden on both patients and society [1, 2]. Cerebrovascular recanalization is an important early intervention for AIS, including intravenous thrombolysis, interventional thrombectomy, and angioplasty, among which intravenous thrombolysis is the most commonly used [3]. However, an international survey indicates [4] that only 2%-4% of stroke patients can receive thrombolytic therapy within 3 hours, thus the short treatment time window largely limits the application of intravenous thrombolysis. Therefore, it is urgent to seek more effective alternative treatments for AIS patients who miss the thrombolytic therapy. To address this issue, this study adopted Huanglian Jiedu decoction for the treatment of AIS patients, and the results were satisfactory.

**Materials and methods**

**General information**

Selection of patients: A total of 56 patients with AIS who were admitted to the Department of Geriatrics in the Third Affiliated Hospital of Yunnan University of Chinese Medicine from November 2022 to September 2023 were selected. They were divided into a control group and a treatment group using the random number table method, with 28 patients in each group. However, 2 patients in the control group and 1 patient in the treatment group were lost to follow-up, resulting in 26 patients in the control group (16 males and 10 females, average age: 65.3 ± 5.24 years) and 27 patients in the treatment group (14 males and 13 females, average age: 64.2 ± 5.47 years). In addition, there were 9 patients with hypertension, 8 patients with type 2 diabetes, 6 patients with abnormal blood lipids, and 5 patients with coronary heart disease in the control group, while there were 8 patients with hypertension, 11 patients with type 2 diabetes, 6 patients with abnormal blood lipids, and 4 patients with coronary heart disease in the treatment group. There were no significant differences in the general information between the two groups (P > 0.05), indicating that they were comparable. After understanding the content, purpose, and relevant indicators of this study, the patient or family member voluntarily signed the informed consent form.

**Inclusion criteria**

Diagnostic criteria in accordance with the Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke 2018 [5]: Acute onset; focal neurological deficits (one side of facial or limb weakness or numbness, language disorders, etc.), with a few cases of global neurological deficits; imaging shows responsible lesions or symptoms/signs lasting for more than 24 hours; excluding non-vascular causes; brain CT/MRI excluding cerebral hemorrhage. Inclusion criteria (1) meet the diagnostic criteria for acute cerebral infarction; confirmed by neuroimaging such as CT or MRI; (2) within 2 weeks after the first onset, and ruled out cerebral hemorrhage by transcranial CT or MRI; (3) patients aged 20–80 years old, with a primary school education or above; (4) patients have clear consciousness, no serious mental or intellectual impairment, and can cooperate with the scale evaluation; (5) patients or legal representatives have informed consent and signed the informed consent form. Exclusion criteria: (1) history of recent head trauma; (2) dysfunction of major organs; (3) malignant tumors; (4) allergies to the study medication; (5) development of cerebral hernia or deep coma.

**Study methods**

Both groups received conventional neurology treatment based on the Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke 2018 [6], including correction of water-electrolyte imbalance, neurotroph, antiplatelet aggregation, and proactive prevention and treatment of complications. In addition, the control group received intravenous drip therapy with Edaravone injection (30mg in 100mL normal saline, intravenous drip over 30 min, twice daily) for 14 consecutive days. The treatment group received additional Huanglian Jiedu decoction (formula: Huanglian 9g, Zhizhi 9g, Huangqin 6g, Huangbai 6g) made into a decoction by the hospital's traditional Chinese medicine formula preparation room, 1 dose per day, twice a day. Both groups were treated for 14 days.

**Observation index**

1. Observation of the National Institutes of Health Stroke Scale (NIHSS [7], score range 0–42) in both groups before and after treatment. 2. Activity of daily living (ADL), with a total score of 100. 3. 5mL venous blood samples were collected from both groups before and after treatment, centrifuged at 4°C, and serum was extracted to detect the levels of inflammatory factors (IL-14, IL-6, TNF-α, TGF-β) and the expression levels of oxidative stress (CAT, SOD, T-AOC, MDA).

**Standards for assessing treatment effectiveness**

The efficacy was evaluated based on the NIHSS scores of the two groups before and after treatment. The criteria for assessing therapeutic efficacy were referenced from the Clinical Neurological Function Deficit Scale for Stroke Patients [8]. The outcomes were defined as follows: complete recovery (NIHSS score reduction of 91%–100%), significant improvement (NIHSS score reduction of 46%–90%), improvement (NIHSS score reduction of 18%–45%), and no improvement (NIHSS score reduction of < 18%). The total effective rate was calculated as (number of cases with complete recovery + significant improvement + improvement) / total number of cases × 100%.

**Methodology of statistics**

The data were analyzed statistically using SPSS Statistics 17.0 software. For quantitative data that conformed to a normal distribution, the t-test was used for statistical analysis, and the mean ± standard deviation was used to represent the data. When P < 0.05, it was considered to have statistical significance.

**Results**

NIHSS and ADL scores of the two groups before and after treatment

After treatment, the NIHSS scores decreased and the ADL scores increased in both groups (P < 0.05). Compared between the two groups, the improvement in the treatment group was more significant (P < 0.05) (Table 1).

Comparison of inflammatory factor levels between the two groups before and after treatment

After treatment, the levels of anti-inflammatory factors TGF-β and IL-4 increased in both groups (P < 0.05), with the treatment group being higher than the control group (P < 0.05). Additionally, the levels of pro-inflammatory factors IL-6 and TNF-α decreased in both groups (P < 0.05), with the treatment group being lower than the control group (P < 0.05) (Table 2).

Comparison of oxidative stress levels between the two groups before and after treatment

After treatment, the levels of CAT, SOD, T-AOC increased in both groups (P < 0.05), with the treatment group having higher levels than the control group (P < 0.05). Additionally, the levels of MDA decreased in both groups (P < 0.05), with the treatment group having lower levels than the control group (P < 0.05) (Table 3).

Comparison of total effective rates between the two groups before and after treatment

After treatment, the effective rate of the treatment group was higher than that of the control group (P < 0.05) (Table 4).

**Discussion**

Modern pharmacological researchers have found that Huanglian Jiedu
decoction can exert pharmacological effects such as anti-inflammation and antioxidant activities [9, 10]. It mainly affects antioxidant activity through polyhydroxy flavonoids [11]. In addition, some scholars have suggested that Huanglian Jiedu decoction can antagonize the expression of MDA and SOD in myocardial cells during ischemia-reperfusion, thereby reducing the degree of myocardial cell lipid peroxidation and further enhancing the ability to scavenge oxygen free radicals, resulting in antioxidant activity [12, 13]. Other scholars have studied the effect of Huanglian Jiedu decoction on lipid peroxidation damage in a rat model of multiple cerebral infarction, and found that the effective components of Huanglian Jiedu decoction have a significant protective effect on this injury. The mechanism may be related to reducing nitric oxide (NO) content and anti-oxidative stress [14]. In terms of anti-inflammation, Huanglian Jiedu decoction also showed significant efficacy. Studies have shown that after intervention with Huanglian Jiedu decoction, the levels of inflammatory factors IL-1β and IL-6 induced by lipopolysaccharide were significantly reduced, indicating that Huanglian Jiedu decoction may regulate inflammatory reactions through TLR4/NF-κB signaling pathway [15]. Other studies have shown that some blood-entering components in Huanglian Jiedu decoction, such as berberine, palmatine, and jatrorrhizine, can bind tightly to cysteine protease caspase-3 and matrix metalloproteinase-9 (MMP-9), which can further regulate inflammatory pathways such as IL-17 and TNF pathways, ultimately downregulating serum levels of inflammatory factors IL-1β, IL-6, and TNF-α [16]. These research results demonstrate the important role of Huanglian Jiedu decoction in antioxidant and anti-inflammatory activities.

AIS is a neurological disease caused by local cerebral ischemia resulting in brain tissue necrosis. Clinical manifestations include coma, hemiplegia, aphasia, disturbance of consciousness, sensory disorders, etc. [17]. NIHSS score is a scoring system used to assess the severity of stroke, and its score is positively correlated with the degree of neurological dysfunction. ADL score is an assessment of the prognosis of AIS patients’ activities of daily living, with lower scores indicating poorer daily behavior and self-care ability. This study collected AIS patients as research subjects, and the results showed that both groups could significantly reduce NIHSS scores and improve ADL scores in patients with AIS. Compared with the control group, the treatment group showed more significant improvement, suggesting that the application of Huanglian Jiedu decoction can achieve better results and significantly improve the recovery of limb function and self-care ability of daily life. In addition, no significant adverse reactions were observed in both groups.

Under normal physiological conditions, the production and elimination of ROS in the body are in dynamic equilibrium. When the elimination capacity of ROS cannot offset its production, free radicals are generated. ROS has highly reactive chemical properties and can attack cells and tissues of the body, triggering oxidative stress reactions [18]. The increase of ROS induces lipid peroxidation, further generating lipid peroxides. The final product of lipid peroxidation reactions is MDA, which reflects the level of ROS and the degree of oxidative damage in tissues. Therefore, MDA is often used as one of the key indicators to evaluate oxidative stress and cell damage [19]. SOD and GSH-Px belong to antioxidant enzymes that eliminate reactive oxygen species and effectively reduce intracellular peroxidation reactions [20]. Studies have found that during AIS, the imbalance of free radicals in the body is triggered by cerebral blood supply disorders, combined with decreased activity of the free radical defense system enzyme SOD and increased oxidative product MDA. This leads to attacks on neuronal membranes and related microvasculature, initiating lipid peroxidation damage and disrupting the blood-brain barrier, ultimately promoting neuronal damage and driving AIS progression [21]. This study shows that after ischemia occurs, the levels of SOD and GSH-Px in the serum of AIS patients decrease, while the levels of MDA increase, indicating that the steady state of CAT, SOD, T-AOC is disrupted and the body is undergoing oxidative stress damage. After treatment, the levels of these factors change in the opposite direction, and the steady state of CAT, SOD, T-AOC is restored. Compared with the control group, the treatment group showed more significant improvement. It can be seen that Huanglian Jiedu decoction can enhance the body’s antioxidant levels. Inflammation is considered as an important mediator of AIS [22]. An important pathological mechanism of AIS is the inflammation

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<tr>
<th>Table 1 Comparison of NIHSS and ADL between the two groups before and after treatment (scores, T ± S)</th>
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Note: Compared with before treatment, *P < 0.05, **P < 0.01; compared with the control group, *P < 0.05, **P < 0.01.

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<th>Table 2 Comparison of inflammatory factor levels between two groups before and after treatment (ng/mL, T ± S)</th>
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Note: Compared with before treatment, *P < 0.05, **P < 0.01; compared with the control group, *P < 0.05, **P < 0.01.

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<th>Table 3 Comparison of oxidative stress levels between two groups before and after treatment (T ± S) (U/L)</th>
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Note: Compared with before treatment, *P < 0.05, **P < 0.01; compared with the control group, *P < 0.05, **P < 0.01.

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<th>Table 4 Comparison of total effective rates between two groups before and after treatment (T ± S)</th>
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response involving microglia [23]. Under normal physiological conditions, microglia shows a highly branched morphology in a quiescent state and has limited phagocytic and migratory activity [24]. Under pathological conditions, microglia is activated and differentiated into different phenotypes, one of which is the M1 type with pro-inflammatory effects (classic activation), while the other is the M2 type with anti-inflammatory effects (alternative activation) [25]. After AIS occurs, the microglia-dominated inflammatory response is activated, i.e. the M1 type microglia is activated, resulting in increased levels of multiple pro-inflammatory factors such as TNF-α, IL-6, etc.; at the same time, the polarization of anti-inflammatory M2 microglia decreases, such as the reduced levels of IL-4, TGF-β, etc., leading to a decrease in immune regulatory function of the body [26, 27]. This study shows that after ischemia occurs, the levels of IL-4, TGF-β in the serum decrease while, the levels of TNF-α, IL-6 increase, indicating that the body undergoes an inflammatory response that further aggravates brain tissue injury. After treatment, the levels of these factors change in the opposite direction. Compared with the control group, the treatment group shows more significant improvement. It can be seen that Huagnian Jiedu can enhance the body's anti-inflammatory response.

In summary, Huagnian Jiedu decoction has a significant clinical effect on AIS patients, significantly repairing the neurological deficits in patients, improving quality of life and daily living abilities, and enhancing anti-inflammatory and antioxidant abilities of the body. Therefore, it is worth promoting. However, due to the small sample size of this clinical observation, the study has certain limitations and may require multi-center, large-scale clinical research to confirm its effectiveness.

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