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Protective effect of chlorogenic acid on the focal cerebral ischemia reperfusion rat models



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ABSTRACT

Objective: The aim of the study was to investigate the protective characteristic of chlorogenic acid, a natural glucosyl xanthone found in *Lonicera Japonica* on the cerebral ischemia reperfusion injury and the underlying mechanism.

Methods: Focal cerebral ischemia reperfusion model was built by blocking the left middle cerebral artery in rats by using the suture-occluded method. Before operation, the corresponding drugs were given for each group once a day for 7 days. After 1 h of final administration, the model was built, after operation, reperfusion was conducted for 22 h. Before the reperfusion 10 min tail vein injection of large, medium and small dose of chlorogenic acid and then mortality was calculated, and Neurological deficit score (NDS) was conducted, and serum was collected to measure the NSE level; a 2 mm thick brain slice located at the intersection of optic nerves was collected for TTC staining, and the percentage of cerebral infarction area was calculated; brain homogenate was collected to measure the ICAM-1, VCAM-1, EPO and HIF-1 α levels in brain tissue of cerebral ischemia reperfusion rat models; NGF was detected using immunohistochemical method; the morphological changes in brain tissue was observed with HE staining.

Results: All focal cerebral ischemia reperfusion rat models were duplicated successfully. Every chlorogenic acid group with different dosage can significantly reduce the mortality, NDS and cerebral infarction area of rats, and significantly increase the EPO, HIF-1 α and NGF levels in brain tissue; significantly improve the pathological lesions of hippocampus and cortex in brain tissue.

Conclusion: The results showed that chlorogenic acid could protect the focal cerebral ischemia reperfusion injury rat models by adjusting the inflammatory factor, hypoxia factor and nerve growth factor.

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1. Introduction

Cerebral ischemia is a common disease in the elderly, with high mortality and morbidity, severely threatening human health. Cerebral ischemia reperfusion injury refers to the phenomenon that after blood supply is restored after a certain time of cerebral ischemia, its function not only fails to restore, but more serious brain dysfunction appear (Zhu and Wang, 2010; Thomas et al., 2013). The effective ingredients of Chinese traditional medicine and single Chinese traditional medicine can prevent and treat

various diseases through acting on the multiple links and multiple targets by using multiple ways, and have achieved some success in reducing the cerebral ischemia reperfusion injury, can reduce the injury caused by the reperfusion, showing the advantages of traditional Chinese medicine for prevention and treatment of cerebral ischemia reperfusion injury (Jean et al., 2016; Abbas et al., 2017). “Activating blood to resolve stasis” is a common method for clinical prevention and treatment of cerebral ischemic injury, with remarkable effect, has been widely recognized, “Clearing away heat and removing toxin” is a new viewpoint of traditional Chinese medicine for prevention and treatment of cerebral ischemia injury, in microcirculation and clinical research fields, traditional Chinese medicine and modern medicine recognize each other in brain ischemia injury, blood-activating and stasis-resolving medicine focuses on improving brain circulation, protecting nerve cells, and scavenging free radical. Heat-clearing and toxicity-removing medicine focuses on reducing inflammatory response, activating the self-protection mechanism of brain cell, and reducing the secondary reaction of cerebral ischemia. The application of “Activating

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blood to resolve stasis” combined with “Clearing away heat and removing toxin” methods for prevention and treatment of cerebral ischemic reperfusion injury allows two theories of traditional Chinese medicine and modern medicine to find the best combination point in clinical practice (Cheng et al., 2012; Ghafar et al., 2017) Preliminary study (Cao et al., 2016; Fang et al., 2016a,b; Miao et al., 2013) finds traditional Chinese medicines with heat-clearing and toxicity-removing and blood-activating and stasis-resolving effects, such as Turmeric, rhabdosia rubescens, Maodongqing, trumpet creeper, beggarticks and motherwort, all of which have good effect on resisting cerebral ischemia injury.

Honeysuckle is a commonly used heat-clearing and toxicity-removing medicine, with effects of clearing away heat and removing toxin, quickening the blood and dispersing swelling, anti-inflammation, and tonifying deficiency and treating wind, having obvious effect on the treatment of fullness and ventral disease, warm disease and fever, heat-toxin and large carbuncle, tumor and other disorders. Chlorogenic acid is a kind of phenylpropanoid compound, and the main active ingredient of honeysuckle (Song et al., 2015), having a wide range of biological activities, such as antibacterial, anti-inflammation, antiviral, scavenging the free radicals, reducing blood fat, lowering blood pressure, protecting the liver and gall bladder, anti-tumor.

Studies have shown that chlorogenic acid can achieve anti-inflammatory effect so as to play a role in anti-inflammatory effect by inhibiting the activation of inflammatory factors such as TNF- α and IL-6, affecting the metabolism of AA (Feng et al., 2016), or reducing the level of NF- κ B p65 (Guo et al., 2015; Song et al., 2015). Chlorogenic acid, as a kind of phenolic antioxidant, has a certain number of R-OH radicals, which can form the hydrogen free radical with antioxidant activity, and eliminate the activity of free radicals such as superoxide anion, thereby protecting tissue cells from oxidative damage (Zhang et al., 2010). Therefore, chlorogenic acid can create a protective effect on the red blood cell hemolysis and DNA oxidation, so as to work as an antioxidant by capturing free radical cations ABTS⁺ and DPPH, stabilizing cell membrane and other mechanisms (Wang et al., 2011). In addition, chlorogenic acid has a good protective effect on the cardiovascular system, and preventive and therapeutic effects on the atherosclerosis, thromboembolic disease, hypertension and other diseases (Lee et al., 2012).

In summary, it can be inferred that chlorogenic acid may have certain effects on the protection of brain tissues and alleviation of cerebral ischemia reperfusion injury. By observing the intervention effect of chlorogenic acid on the focal cerebral ischemia reperfusion rat models, conducting an in-depth study of its pharmacological effect on the cerebral ischemia reperfusion injury, this paper defined the intervention effect and mechanism, laying the foundation for the follow-up study with a view to developing a new drug which could alleviate the cerebral ischemia reperfusion injury.

2. Material and methods

2.1. Animals, drugs and reagents

96 Wistar rats, SPF, male, weight of 230–250 g, provided by Shandong Lukang Pharmaceutical Co., Ltd., certificate number: 0016029; laboratory certificate number: SYXK (Yu) 2010-001.

Chlorogenic Acid, Chengdu Mansite Pharmaceutical Co., Ltd., content of 99.39%, NO. MUST-13031401; Nimodipine Tablets, Shanxi Yabao Pharmaceutical Group Co., Ltd., NO. 130150; ELISA Kit for the detection of ICAM-1, R&D Systems, NO. 20131202B; ELISA Kit for the detection of VCAM-1, R&D Systems, NO. 20131202B; ELISA Kit for the detection of EPO, R&D Systems, NO.

20131202B; ELISA Kit for the detection of HIF-1 α , R&D Systems, NO. 20131202B; ELISA Kit for the detection of NSE, R&D Systems, NO. 20131202B.

2.2. Model building and administration

96 Wistar rats, SPF, male, weight of 230–250 g, were normally fed for 3 days. Then they were randomly divided into 6 groups, i.e. sham-operated group, model group, Nimodipine group, low, medium and high dosage of chlorogenic acid groups according to body weight, 16 Wistar rats per group. Intra-gastric administration of 1 ml/100 g Nimodipine suspension was carried out for the Nimodipine group (positive control drug, dosage of 20 mg/kg, equivalent to 10 times the clinical dosage, drug with a concentration of 2 mg/ml was made with 0.5% CMC before use); intra-gastric administration of chlorogenic acid suspension was carried out for the low, medium and high dosage of chlorogenic acid groups (dosage of 60 mg/kg, 30 mg/kg and 15 mg/kg, drugs with a concentration of 6 mg/ml, 3 mg/ml and 1.5 mg/ml were made with 0.5% CMC before use); intra-gastric administration of the same volume of 0.5% CMC was carried out for the sham-operated group and model group, administered once a day for 7 days.

At 8 p.m. of the 6th day, fasting without water deprivation in batches, at 8 a.m. of the 7th day, they were weighted in batches, after 1 h of administration, intraperitoneal injection of 10% chloral hydrate (0.3 ml/100 g) was carried out for anesthetized rats, they were incised in the left of center of the neck, separated layer by layer to expose left CCA, ECA and ICA, CCA and ECA were ligated, ICA was blocked with artery clip, a small 0.2 mm wide opening was sheared at the place where CCA was 5 mm from bifurcation, the suture was inserted, which entered into ICA through CCA bifurcation, and penetrated upward 18–20 mm above CCA bifurcation until there was resistance, namely, the entrance of middle cerebral artery was blocked, the ICA incision and suture were ligated, after 2 h, the suture was taken out lightly, reperfusion was realized, and MCAO reperfusion model was built, for the sham-operated group, only the left-side blood vessels were exposed, no suture inserting treatment.

2.3. Detection index

After 22 h of reperfusion for all rats was conducted, NDS was conducted for rat models: scoring was performed using the Longa standard. Scoring standard: 0 score: no NDS, normal activity; 1 score: not fully extending the forepaws; 2 scores: turning around on the hemiplegic side appear when crawling; 3 scores: body dumping to the hemiplegia side when walking; 4 scores: unable to walk spontaneously, loss of consciousness; 5 scores: death. The rats scoring 0 and 5 were excluded; eyeballs were removed and blood was collected, after standing for half an hour, it was centrifuged for 10 min at 3500 r/min, serum was collected, and NSE level was measured according to the kit instructions; after the rats were decapitated and killed, their brain tissues were taken off rapidly, their cerebellum, olfactory bulb and the remainder of the lower brain stem were removed, a 2 mm thick brain slice at the intersection of optic nerves was removed rapidly, and placed in 1% TTC dyeing solution made from phosphate buffer solution with pH = 7.2 for incubation for 10 min using 37 °C water bath without light. After being taken out, it was placed in 10% formalin for preservation away from light for 24 h. After dyeing, non-ischemic area was rosy and infarction area was white. The pictures were taken using a digital camera, the total area of the brain slice and the area of the infarction area were calculated by the image analysis software respectively, and the percentage of infarction area to total area was calculated. The front left side brain at the intersection of optic nerves was used to make 10% brain homogenates,

and ICAM-1, VCAM-1, EPO and HIF-1 α were measured according to the kit instructions; the rear left side brain at the intersection of optic nerves was fixed with 10% formaldehyde solution, NGF was detected using the immunohistochemical method, 5 non-overlapping fields in the infarction area were randomly selected, and the number of immune positive expression cells in 1 mm \times 1 mm area in every field was counted, and their average was taken; the morphological changes in brain tissue was observed with HE staining.

2.4. Statistical processing method

For data analysis, the statistical processing of data was conducted using the SPSS 17 Statistical Software Package, measurement data were represented by the average \pm standard deviation ($\pm s$), and the comparison among groups was conducted using the one-factor analysis of variance, groups showing homogeneity of variances through the variance test adopted the LSD method for testing, while groups showing non-homogeneity of variances through the variance test adopted the Games-Howell method for testing, and ranked ordinal data adopted the *Radit* for testing.

3. Results

3.1. Effect on the mortality and NDS of focal cerebral ischemia reperfusion rat models

From the data in Table 1, the mortality of rats in the model group was the highest, while the mortality of rats in the Nimodipine group and low, medium and high dosage of chlorogenic acid groups was reduced, showing that all medication administration groups could reduce the mortality of focal cerebral ischemia reperfusion rat models in different degrees, reduce the brain tissue damage, and protect the brain tissues. Compared with the sham-operated group, the NDS of rats in the model group significantly increased ($P < 0.01$), suggesting the success of model building; compared with the model group, the NDS of rats in the Nimodipine

group and high dosage of chlorogenic acid group significantly decreased ($P < 0.01$), and the NDS of rats in the low and medium dosage of chlorogenic acid groups obviously decreased ($P < 0.05$), suggesting that all medication administration groups could improve the cranial nerve function of focal cerebral ischemia reperfusion rat models in different degrees.

3.2. Effect on the NSE level in serum and percentage of cerebral infarction area of focal cerebral ischemia reperfusion rat models

See Tables 2 and 3.

3.3. Effect on the ICAM-1 and VCAM-1 levels of focal cerebral ischemia reperfusion rat models

From the data in Table 4, compared with the sham-operated group, the ICAM-1 and VCAM-1 levels of rats in the model group significantly increased ($P < 0.01$), suggesting the success of model building; compared with the model group, the ICAM-1 and VCAM-1 levels of rats in the Nimodipine group and medium and high dosage of chlorogenic acid groups significantly decreased ($P < 0.01$), and the ICAM-1 and VCAM-1 levels of rats in the low dosage of chlorogenic acid group obviously decreased ($P < 0.05$), suggesting that all medication administration groups could reduce the ICAM-1 and VCAM-1 levels in brain tissue of focal cerebral ischemia reperfusion rat models in different degrees.

3.4. Effect on the EPO and HIF-1 α levels of focal cerebral ischemia reperfusion rat models

From the data in Table 5, compared with the model group, the EPO level in brain tissue of rats in the Nimodipine group and medium and high dosage of chlorogenic acid groups significantly increased ($P < 0.01$), and the EPO level in brain tissue of rats in the low dosage of chlorogenic acid group showed an increasing trend ($P > 0.05$), and the HIF-1 α level in brain tissue of rats in the Nimodipine group and medium and high dosage of chlorogenic acid groups significantly increased ($P < 0.01$), and the HIF-1 α level

Table 1
Effect on the mortality and NDS of focal cerebral ischemia reperfusion rat models ($\bar{x} \pm s$).

Group	Dosage (mg/kg)	Number of animals (pcs)		Mortality (%)	NDS
		Before model building	After model building		
Sham-operated group	–	16	16	0	0.0 \pm 0.0 ^{**}
Model group	–	16	9	43.75	3.1 \pm 0.6
Nimodipine group	20	16	11	31.25	1.2 \pm 0.4 ^{**}
High dosage of chlorogenic acid group	60	16	12	25.00	1.6 \pm 0.5 ^{**}
Medium dosage of chlorogenic acid group	30	16	11	31.25	1.9 \pm 0.5 [*]
Low dosage of chlorogenic acid group	15	16	11	31.25	2.0 \pm 0.6 [*]

Notes: compared with the model group.

^{**} $P < 0.01$.

^{*} $P < 0.05$.

Table 2
Effect on the NSE level in serum and percentage of cerebral infarction area of focal cerebral ischemia reperfusion rat models ($\bar{x} \pm s$).

Group	Number of animals (pcs)	Dosage (mg/kg)	NSE (ng/ml)	Percentage of cerebral infarction area (%)
Sham-operated group	16	–	6.054 \pm 0.639 ^{**}	0.0 \pm 0.0 ^{**}
Model group	9	–	8.055 \pm 0.831	47.667 \pm 6.919
Nimodipine group	11	20	6.341 \pm 0.645 ^{**}	20.105 \pm 12.311 ^{**}
High dosage of chlorogenic acid group	12	60	6.386 \pm 0.586 ^{**}	28.721 \pm 11.202 ^{**}
Medium dosage of chlorogenic acid group	11	30	6.678 \pm 0.595 ^{**}	34.393 \pm 13.003 ^{**}
Low dosage of chlorogenic acid group	11	15	6.986 \pm 0.463 ^{**}	37.323 \pm 11.281 [*]

Notes: compared with the model group.

^{**} $P < 0.01$.

^{*} $P < 0.05$.

Table 3
Pictures of percentage of cerebral infarction area of focal cerebral ischemia reperfusion rat models.

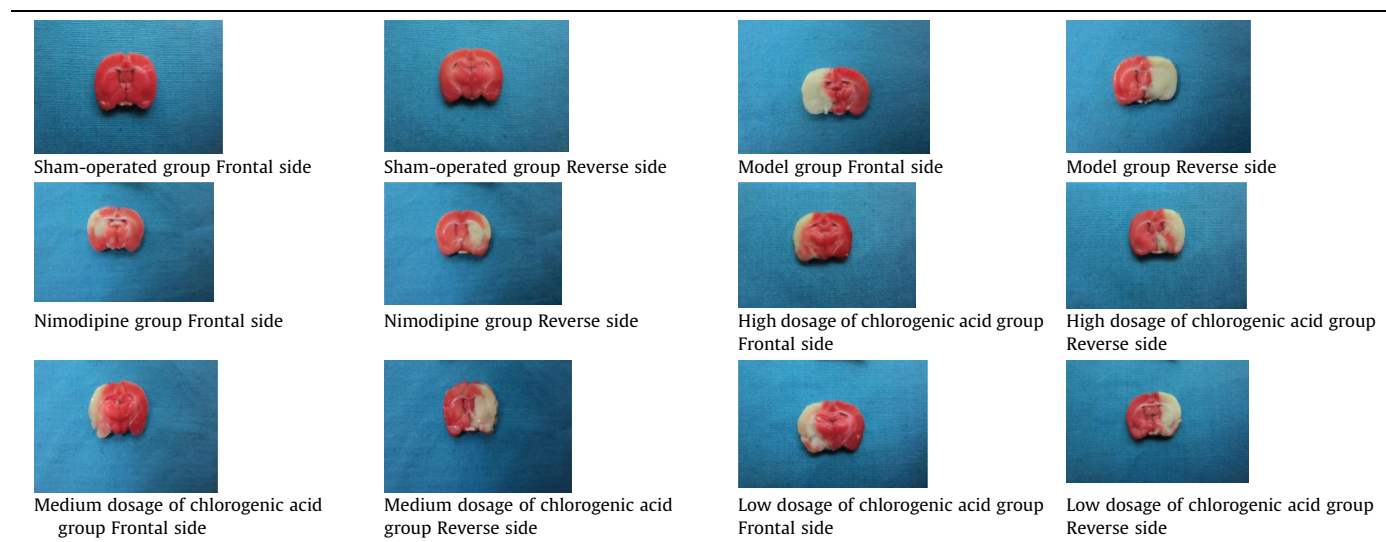


Table 4
Effect on the ICAM-1 and VCAM-1 levels of focal cerebral ischemia reperfusion rat models ($\bar{x} \pm s$).

Group	Number of animals (pcs)	Dosage (mg/kg)	ICAM-1 (ng/ml)	VCAM-1 (ng/ml)
Sham-operated group	16	–	20.193 ± 4.426**	39.161 ± 10.934**
Model group	9	–	30.845 ± 5.482	56.284 ± 7.423
Nimodipine group	11	20	22.506 ± 5.224**	41.935 ± 8.704**
High dosage of chlorogenic acid group	12	60	23.702 ± 5.288**	42.581 ± 9.878**
Medium dosage of chlorogenic acid group	11	30	24.811 ± 5.356**	43.619 ± 7.834**
Low dosage of chlorogenic acid group	11	15	25.316 ± 5.436*	47.761 ± 8.025*

Notes: compared with the model group.

** $P < 0.01$.

* $P < 0.05$.

Table 5
Effect on the EPO and HIF-1 α levels of focal cerebral ischemia reperfusion rat models ($\bar{x} \pm s$).

Group	Number of animals (pcs)	Dosage (mg/kg)	EPO (IU/L)	HIF-1 α (pg/ml)
Sham-operated group	16	–	6.769 ± 1.058	11.465 ± 2.949
Model group	9	–	7.048 ± 1.05	12.073 ± 4.000
Nimodipine group	11	20	8.337 ± 0.892**	16.859 ± 3.825**
High dosage of chlorogenic acid group	12	60	8.286 ± 0.581**	17.143 ± 3.672**
Medium dosage of chlorogenic acid group	11	30	7.998 ± 0.623**	16.497 ± 3.120**
Low dosage of chlorogenic acid group	11	15	7.587 ± 0.373	15.803 ± 3.835*

Notes: compared with the model group.

** $P < 0.01$.

* $P < 0.05$.

Table 6
Effect on the number of NGF immune positive expression cells in the cortex in brain tissue of focal cerebral ischemia reperfusion rat models ($\bar{x} \pm s$).

Group	Number of animals (pcs)	Dosage (mg/kg)	Number of NGF immune positive expression cells
Sham-operated group	16	–	22.486 ± 8.265**
Model group	9	–	32.667 ± 11.031
Nimodipine group	11	20	46.545 ± 10.825**
High dosage of chlorogenic acid group	12	60	45.867 ± 14.318**
Medium dosage of chlorogenic acid group	11	30	41.945 ± 15.616**
Low dosage of chlorogenic acid group	11	15	41.818 ± 12.873**

Notes: compared with the model group.

* $P < 0.05$.

** $P < 0.01$.

in brain tissue of rats in the low dosage of chlorogenic acid group obviously increased ($P < 0.05$), suggesting that all medication administration groups could activate the self-protection mechanism of brain cell of focal cerebral ischemia reperfusion rat models, and mitigate the cerebral ischemia reperfusion injury.

3.5. Effect on the number of NGF immune positive expression cells in the cortex in brain tissue of focal cerebral ischemia reperfusion rat models

From the immunohistochemical detection, after 2 h of cerebral ischemia and 22 h of reperfusion, the NGF expression in the cortex, hippocampus and other parts in brain tissue of rats in each group was observed, mostly in the cortex around the infarction (ischemic penumbra), and rarely in the central part of infarction; its positive

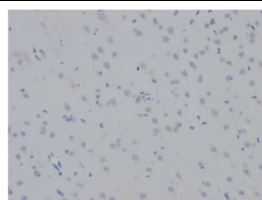
Table 7The number of NGF immune positive expression cells in the cortex in brain tissue of focal cerebral ischemia reperfusion rat models ($\times 400$).

Fig. 1 Sham-operated group

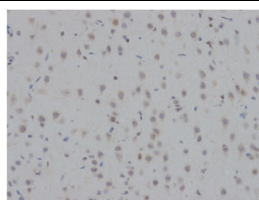


Fig. 2 Model group

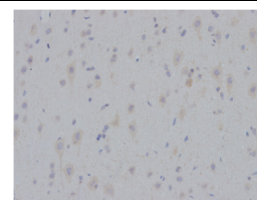


Fig. 3 Nimodipine group

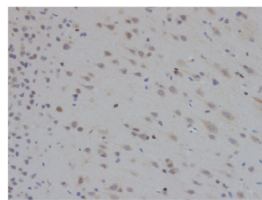


Fig. 4 High dosage of chlorogenic acid group

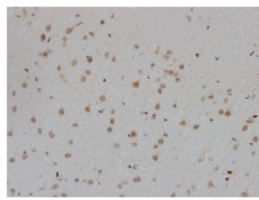


Fig. 5 Medium dosage of chlorogenic acid group

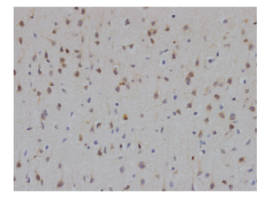


Fig. 6 Low dosage of chlorogenic acid group

Table 8

Effect on the pathological changes of the cortex in brain tissue of focal cerebral ischemia reperfusion rat models.

Group	Number of animals (pcs)	Dosage (mg/kg)	–	+	++	+++	P
Sham-operated group	16	–	16	0	0	0	**
Model group	9	–	0	0	4	5	**
Nimodipine group	11	30	3	5	2	1	**
High dosage of chlorogenic acid group	12	100	2	6	3	1	**
Medium dosage of chlorogenic acid group	11	50	1	5	4	1	**
Low dosage of chlorogenic acid group	11	25	1	3	5	2	*

Notes: compared with the model group.

** $P < 0.01$.* $P < 0.05$.

expression occurred mainly in neurons, and can be found in the cell membrane, cytoplasm, nuclear membrane, cytoplasm and axon of neurons, the cytoplasmic staining was dark brown and uniform, and the cell body was similar in form of pyramid, and the main dendrite staining was light, followed by cytoplasm of glial cells. The results are shown in Table 7.

In Table 6, compared with the sham-operated group, the number of NGF immune positive expression cells in brain tissue of rats in the model group significantly increased, suggesting the success of model building; compared with the model group, the number of NGF immune positive expression cells in brain tissue of rats in the Nimodipine group and low, medium and high dosage of chlorogenic acid groups significantly increased ($P < 0.01$), suggesting that all medication administration groups could promote the NGF expression of brain cells of rats, prevent the degeneration or death of brain cells of rats, promote the survival and regeneration of neurons, maintain the normal function of cranial nerves, and mitigate the cerebral ischemia reperfusion injury.

3.6. Effect on the pathological changes of the cortex in brain tissue of focal cerebral ischemia reperfusion rat models

The pathological changes of the cortex in brain tissue of focal cerebral ischemia reperfusion rat models were observed as follows: for the sham-operated group, there was no edema in the cerebral cortex, and the nerve cells were normal; for the model group, there was edema in the cerebral cortical nerve cells and large fraction of neuronal necrosis, and the infarction area was more than 2/3 of the left cortex area; for the Nimodipine group, there was edema in some cerebral cortical nerve cells and small fraction of neuronal necrosis, and the infarction area was not more than 1/3 of the left cortex area; for the high dosage of chlorogenic

acid group, there was edema in the cerebral cortical nerve cells and small fraction of neuronal necrosis, and the infarction area was not more than 1/3 of the left cortex area; for the medium dosage of chlorogenic acid group, there was edema in the cerebral cortical nerve cells and small fraction of neuronal necrosis, and the infarction area was not more than 1/3–2/3 of the left cortex area; for the low dosage of chlorogenic acid group, there was edema in the cerebral cortical nerve cells and large fraction of neuronal necrosis, and the infarction area was not more than 1/3–2/3 of the left cortex area. The results are shown in Table 9.

“–” there was no edema in the cerebral cortex, and the nerve cells were normal; “+” there was edema in the cerebral cortical nerve cells and small fraction of neuronal necrosis, and the infarction area was not more than 1/3 of the left cortex area; “++” there was edema in the cerebral cortical nerve cells and large fraction of neuronal necrosis, and the infarction area was not more than 1/3–2/3 of the left cortex area; “+++” there was edema in the cerebral cortical nerve cells and large fraction of neuronal necrosis, and the infarction area was more than 2/3 of the left cortex area.

After Redit test, compared with the sham-operated group, there was significant statistical significance in the model group ($P < 0.01$), namely there were significant pathological changes of the cortex in brain tissue of focal cerebral ischemia reperfusion rat models in the model group, suggesting the success of model building; compared with the model group, there was significant statistical significance in the Nimodipine group and medium and high dosage of chlorogenic acid groups ($P < 0.01$), and there was obvious statistical significance in the low dosage of chlorogenic acid group ($P < 0.05$), suggesting that all medication administration groups could reduce the pathological lesion of the cortex in brain tissue of focal cerebral ischemia reperfusion rat models, and protect the brain tissues. The results are shown in Table 8.

Table 9
Pathological changes of the cortex in brain tissue of focal cerebral ischemia reperfusion rat models (HE × 400).

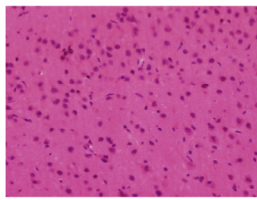


Fig. 1 Sham-operated group

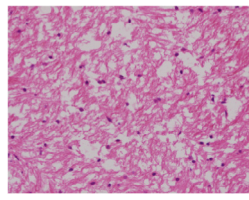


Fig. 2 Model group

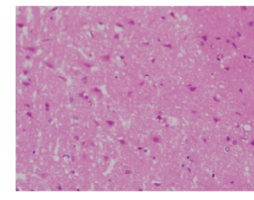


Fig. 3 Nimodipine group

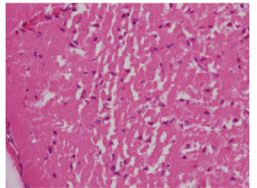


Fig. 4 High dosage of chlorogenic acid group

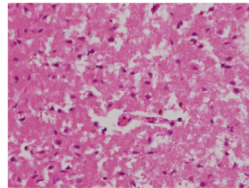


Fig. 5 Medium dosage of chlorogenic acid group

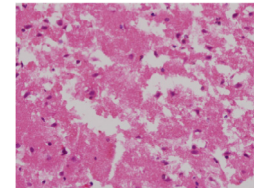


Fig. 6 Low dosage of chlorogenic acid group

Table 10
Effect on the pathological changes of the hippocampus in brain tissue of focal cerebral ischemia reperfusion rat models.

Group	Number of animals (pcs)	Dosage (mg/kg)	–	+	++	+++	P
Sham-operated group	16	–	16	0	0	0	**
Model group	9	–	0	0	5	4	
Nimodipine group	11	30	3	5	3	0	**
High dosage of chlorogenic acid group	12	100	3	6	2	1	**
Medium dosage of chlorogenic acid group	11	50	2	5	3	1	**
Low dosage of chlorogenic acid group	11	25	0	5	4	2	*

Notes: compared with the model group.

** $P < 0.01$.

* $P < 0.05$.

Table 11
Pathological changes of the hippocampus in brain tissue of focal cerebral ischemia reperfusion rat models (HE × 400).

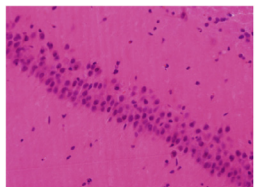


Fig. 1 Sham-operated group

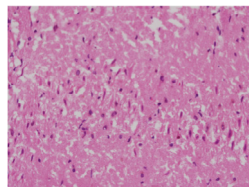


Fig. 2 Model group

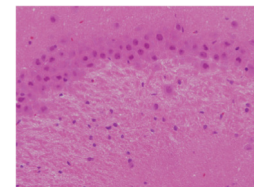


Fig. 3 Nimodipine group

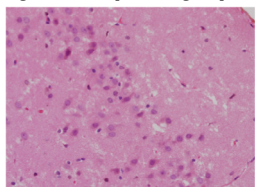


Fig. 4 High dosage of chlorogenic acid group

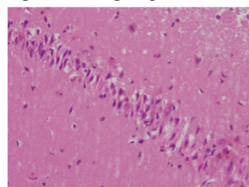


Fig. 5 Medium dosage of chlorogenic acid group

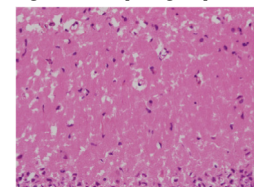


Fig. 6 Low dosage of chlorogenic acid group

3.7. Effect on the pathological changes of the hippocampus in brain tissue of focal cerebral ischemia reperfusion rat models

The pathological changes of the hippocampus in brain tissue of focal cerebral ischemia reperfusion rat models were observed as follows: for the sham-operated group, there was no edema in the cerebral hippocampus, and the nerve cells were normal; for the model group, there was edema in the cerebral hippocampus and large fraction of neuronal necrosis, and the infarction area was focal, and the infarction area was not more than 1/3 of the left hippocampus area; for the Nimodipine group, there was edema in some cerebral hippocampus and small fraction of sporadoneurel

necrosis; for the high dosage of chlorogenic acid group, there was edema in the cerebral hippocampus and small fraction of sporadoneurel necrosis; for the medium dosage of chlorogenic acid group, there was edema in the cerebral hippocampus and small fraction of sporadoneurel necrosis; for the low dosage of chlorogenic acid group, there was edema in the cerebral hippocampus and small fraction of neuronal necrosis, and the infarction area was focal, and the infarction area was not more than 1/3 of the left hippocampus area. The results are shown in Table 11.

“–” there was no edema in the cerebral hippocampus, and the nerve cells were normal; “+” there was edema in the cerebral hippocampus and no neuron infarction; “++” there was edema in the

cerebral hippocampus and small fraction of sporadoneurel necrosis; “+++” there was edema in the cerebral hippocampus and large fraction of neuronal necrosis, and the infarction area was focal, and the infarction area was not more than 1/3 of the left hippocampus area.

After *Ridit test*, compared with the sham-operated group, there was significant statistical significance in the model group ($P < 0.01$), namely there were significant pathological changes of the hippocampus in brain tissue of focal cerebral ischemia reperfusion rat models in the model group, suggesting the success of model building; compared with the model group, there was significant statistical significance in the Nimodipine group and medium and high dosage of chlorogenic acid groups ($P < 0.01$), and there was obvious statistical significance in the low dosage of chlorogenic acid group ($P < 0.05$), suggesting that all medication administration groups could reduce the pathological lesion of the hippocampus in brain tissue of focal cerebral ischemia reperfusion rat models, and protect the brain tissues. The results are shown in [Table 10](#).

4. Discussion

The cerebral ischemia belongs to “Apoplexy” in TCM, in recent years, with the deepening of understanding of the traditional toxin, as well as the development of clinical practice and further exploration of research on modern pathological mechanism, proposing the treatment of apoplexy based on the theory of “toxin” to improve the curative effect of apoplexy has become a new viewpoint and hot point in the study of etiology and therapeutics of apoplexy. Modern medicine attaches great importance to “toxin” in ischemic stroke. For example, Liu Xiaohui discusses the meaning of the pathogenesis of “damage of brain collateral by toxin” of Alzheimer’s disease ([Liu et al., 2017](#); [Ali et al., 2017](#)). Wei Jianglei believes that the “embryology of apoplexy” is distinctive in “heat-toxin”, and proposes the hypothesis of “heat-toxin in apoplexy”. Shao Nianfang believes that the syndrome of heat-toxin is observed not only in the acute phase of apoplexy, but also in the recovery stage, sequela stage and premonitory stage of apoplexy ([An et al., 2012](#)).

The mechanisms of cerebral ischemia reperfusion injury are complex, in light of these mechanisms, the effective ingredients of Chinese traditional medicine and single Chinese traditional medicine have made certain achievements for prevention and treatment of cerebral ischemia reperfusion injury ([Zhang et al., 2016](#); [Yang et al., 2016](#)). At present, most of researches on the prevention and treatment of cerebral ischemia are oriented in the active parts or extraction of traditional Chinese medicine, and there are more researches on compound prescription than on single Chinese traditional medicine ([Xue et al., 2016](#); [Duan et al., 2017](#)) In particular, there is lack of research on single active ingredient, resulting in obscure mechanism for prevention and treatment of cerebral ischemia reperfusion injury by traditional Chinese medicine and low pertinency. Conducting targeted research on active monomer component of blood-activating and stasis-resolving medicine or heat-clearing and toxicity-removing medicine to gradually define the mechanism of its prevention and treatment of cerebral ischemia reperfusion injury and the intervention effect of it on the cerebral ischemia reperfusion injury to provide new approach to the prevention and treatment of cerebral ischemic injury by traditional Chinese medicine has broad research prospect.

Modern medical research suggests that the pathological products produced during the acute phase of cerebral ischemia are directly involved in the process of brain cell injury. These toxic substances include: oxygen free radicals, four arachidonic acid, excitatory neurotoxin, calcium overload, NO and inflammatory factor, which cause irreversible damage or delayed neuronal necrosis in

brain cells and other nerve cells, belonging to “Toxin pathogen” in TCM. Therefore, attaching importance to the role of blood stasis and heat-toxin in the pathogenesis of apoplexy helps the study on the prevention and treatment of apoplexy. In modern medicine, the cerebral ischemia is usually treated with platelet aggregation inhibitor, cerebral vasodilator, calcium channel blocker, thrombolytic agent, free radical scavenger, NOS inhibitor, etc., all of which are inconvenient to use, with serious side effect, high cost or low pertinency, and not suitable for long-term use, so their application is limited ([Chen et al., 2017](#); [Jamal et al., 2017](#)).

In the normal brain tissues, NSEs exist only in the cytoplasm of neurons, after 24 h of cerebral ischemia, the NSEs in the cerebral infarction area are dispersed into the cell gap, and found in some shrinkages in the ischemic area and injured neurons that have not died and blood vessels in the contralateral hemisphere, indicating that the NSEs are released from brain neurons of ischemic injury, which cross the blood-brain barrier and enter into the blood circulation. Therefore, the NSEs in the blood can be used as biochemical markers of neuronal damage after cerebral ischemia ([Barone et al., 1993](#)). The ICAM-1 and VCAM-1 expressions can promote leucocyte to release inflammatory mediators and cytokines, while the inflammatory mediators and cytokines can increase the ICAM-1 and VCAM-1 expressions, the two of them interact, which aggravates the inflammatory response after cerebral ischemia reperfusion, forming a vicious circle. Under anoxic condition of cerebral ischemia, the degradation pathway of HIF-1 α in brain tissue is inhibited, the accumulation of HIF-1 α in cytoplasm increases, which is transferred into the nucleus to bind with HIF-1 β , forming HIF-1 heterodimer, the C end of the transcriptional activation domain of HIF-1 α further binds with hypoxia response element in hypoxia response gene promoter – enhancer, which promotes the transcription of hypoxia responsive genes, and induces the transcription and expression of corresponding downstream target genes. Studies show that HIF-1 has a neuroprotective effect on the cerebral ischemia reperfusion injury ([Si et al., 2014](#); [Hamrick et al., 2005](#); [Wang et al., 2016](#)), and this kind of brain protection is mainly produced by regulating the corresponding gene expression of EPO, VEGF, GLUT-1 and ADM. While the NGF can promote the survival and regeneration of neurons and combat the hypoxia and hypoglycemia injury of neurons, and has some protective and therapeutic effects on the cerebral ischemia reperfusion injury ([Zhan et al., 2014](#); [Sindhu et al., 2017](#)).

Recent studies have shown that chlorogenic acid, an active ingredient extracted from the active parts of honeysuckle, has strong antioxidant and antiapoptotic effects ([Ali et al., 2014](#)). Pharmacological experiments have shown that chlorogenic acid can enhance cell viability, promote cell differentiation and prevent alcohol induced apoptosis. Its mechanism may be related to the up-regulated GAP-43 expression, the apoptotic pathways of mitochondria include the promotion and inhibition of mitochondrial transmembrane potential, up-regulated expression of antiapoptosis gene bcl-2, and down-regulated expression of apoptosis gene caspase-3 ([Fang et al., 2016a,b](#); [Shamsudin et al., 2017](#)).

5. Conclusions

By observing the effect of chlorogenic acid on the mortality, NDS, percentage of cerebral infarction area, NSE level in serum, ICAM-1, VCAM-1, EPO and HIF-1 α levels in brain tissue, NGF expression and pathological changes in brain tissue of focal cerebral ischemia reperfusion rat models, the results indicated that chlorogenic acid could reduce the mortality of animals, NDS, percentage of cerebral infarction area and NSE level in serum, and has a protective effect on the brain tissues and neurons, could reduce the ICAM-1 and VCAM-1 levels in brain tissue, inhibit the

inflammatory response after cerebral ischemia, reduce the inflammatory mediators and cytokines released by a cascade of inflammatory responses, increase the EPO and HIF-1 α levels in brain tissue, increase the expression of NGF in brain tissue, activate the self-protective mechanism of brain cell, reduce the death of nerve cell, promote the regeneration of neuron, reduce the injury to brain tissue by cerebral ischemia reperfusion, protect the brain tissue, and the high dosage of chlorogenic acid group produced the best results.

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