Neuroprotective effect of *Trichosanthes kirilowii cassia twig* on cerebral ischemia-reperfusion injury in rats

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Abstract: In this study, in-depth observation and investigation of blood-brain barrier permeability and neuroprotective effect of *Trichosanthes kirilowii cassia twig* particles on rats with cerebral ischemia-reperfusion injury were performed. Focal cerebral ischemia-reperfusion injury model was established by middle cerebral artery occlusion method, reperfusion was implemented 2 hours after ischemia; qualitative analysis and investigation of trichosanthes kirilowii cassia twig particles in plasma, brain tissue and cerebrospinal fluid in normal and middle cerebral artery occlusion (MCAO) rats were done by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS); changes in neurological deficits, cerebral infarction stereometry, blood-brain barrier permeability and histopathological changes of MCAO model rats were observed. Qualitative analysis by HPLC-MS/MS results showed that ingredients, paeoniflorin, albiflorin, liquiritin in *Trichosanthes kirilowii cassia twig* particles can reach the brain through the blood-brain barrier. In the model group, glycyrrhizin and glycyrrhizic acid can be detected in brain tissue or cerebrospinal fluid. In addition, *Trichosanthes kirilowii cassia twig* particles can significantly lower neurological deficits of rats in middle cerebral artery occlusion model, reduce the Evans blue penetration, contract infarct size, and reduce pathological tissue injury of cerebral ischemia reperfusion. The ingredients of *Trichosanthes kirilowii cassia twig* particles can reach the brain tissue through the blood-brain barrier and play a role in neuroprotection of rats with cerebral ischemia-reperfusion injury, which has important research significance and brings scientific experimental, theoretical basis for clinical drug use.

Keywords: *Trichosanthes kirilowii cassia twig* particles, rats with cerebral ischemia-reperfusion injury, blood-brain barrier permeability, neuroprotection, analysis and exploration.

INTRODUCTION

The cerebral ischemia-reperfusion injury is complicated in pathological process as a kind of brain central nervous system disease. In the central nervous system, the blood-brain barrier is its unique structure. Efficacy of drugs for central nervous system diseases needs to show via the peripheral channels on the one hand. On the other hand, it has a close correlation with effect of blood concentration through blood-brain barrier from recirculation to central nervous system extra cellular fluid (Zhang *et al*., 2015, Farid *et al*., 2018). Therefore, ability to pass the blood-brain barrier is a key factor of whether a drug can effectively treat central nervous system diseases (Esim *et al*., 2018, Naeem *et al*., 2017).

Trichosanthes kirilowii cassia twig particles enjoy a wide range of applications in treatment of cerebrovascular disease. This study aims to observe and explore its various ingredients ability to pass the blood-brain barrier, then explain mode of action, material basis of neuroprotective effect of the drug, so as to provide a solid experimental basis for scientific rational drug use in future clinical research.

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MATERIALS AND METHODS

Instrument used: The instrument used in this experiment includes Agilent 1100 high performance liquid chromatograph, Agilent 6410 triple quadrupole mass spectrometer, KQ2200B ultrasonic cleaner, 5417R small desktop high-speed refrigerated centrifuge, SHB-Ó circulating water multi-purpose vacuum pump, RE-52AA rotary evaporator, DRT-TW thermoregulation electrothermal set, PB-10 glass membrane electrode pH meter and RM2125 slicer and Nikon DS- biological digital microscope (Wroblewska 2015, Shareef *et al*., 2017).

Chemical and reagent used: The reagents used in this experiment include acetonitrile, ultra-pure water, formic acid; citrulline, gallic acid, glycyrrhizic acid; albiflorin, liquiritin apioside, glycyrrhizin, isoliquiritigenin, isoliquiritin apioside, liquiritin and isoliquiritin; *Trichosanthes kirilowii cassia twig* particles (production batch number: 20120809), Nimodipine (production batch number: 130652).

Animals used: The 128 SPF (SD) male rats of 3 months old were selected, with an average weight at (250±22) g; standard feeding conditions were provided for the

experimental animals, including standard diet, free access to sterile pure water, room temperature maintained at 25°C, regular replacement of padding at dry state, plus 12 hours of light (Abdel, 2016; Ali, *et al*., 2017). This study is approved by the Research Committee. Informed consent, approval paper and the like are signed.

Experimental methods

(1) Preparation of mixed control series of solutions and drugs. Take 20g reference substance, including paeoniflorin, citrulline, isoliquiritin apioside, glycyrrhizin, albiflorin, liquiritin, gallic acid, isoliquiritin, isoliquiritigenin, glycyrrhizic acid and liquiritin apioside were taken and placed them in 5mL volumetric flask, shaken up after dissolution and diluted with pure methyl alcohol, saved as stock solution; Weigh 20g of *Trichosanthes kirilowii cassia twig* particles, ground to fine powder. Transferred it into double distilled water to prepare suspension (Altun *et al*., 2017, Barkat and Mahmood 2018).

(2) Establishment of middle cerebral artery occlusion (MOCA) model of rat. The left middle cerebral artery occlusion operation was performed with previous modeling method. The left middle cerebral artery was used as the infarct side. The anesthetized rats were fixed on the operating table in a supine position. The incision was made along the left side of the neck, and the muscle and fascia were separated along the inner edge of sternocleidomastoid. Meanwhile, the left common carotid artery and external carotid artery were separated and ligated (Hazra 2015, Khan *et al*., 2017). The internal carotid artery was isolated and fish tape was inserted, the distal artery clamp of internal carotid was removed, the fish tape was advanced to the proximal end of intracranial cerebral anterior artery. After insertion, internal carotid artery and fish tape were ligated. After disinfection suture, 10mm fish tape was left outside the skin, to be pulled out after 2 hours so that the ball end removed from the left carotid artery for the sake of reperfusion. For the sham operation, only the skin was cut, the left common carotid artery and external carotid artery and internal carotid artery were separated without ligation and threading, followed by disinfection suture (Ofori-Kwakye 2016).

(3) Neuroprotective effect of kirilowii cassia twig particles on rats with cerebral ischemia-reperfusion injury. The animals were divided into sham operation group, middle cerebral artery occlusion model group, administration group (different dosage) and positive group, each with 18 rats. The sham operation group and middle cerebral artery occlusion group were treated with 9.0g /L of 0.9% sodium chloride solution, the low dose group was treated with 1.8g• kg-1• d-1 and the middle dose group was treated with 3.6g• kg-1• d-1, high dose group was treated with 7.2g• kg-1• d-1, the positive group was treated with 12mg• kg-1• d-1 of nimodipine (Ibrahim 2016). Medication was given 2 hours after modeling, with continuous medication of 8 days.

The neurological function of the MOCA model was scored by Zealonga 5-point assessment method; The blood-brain barrier permeability was quantitatively measured by Evans blue seepage; The infarct size was measured by TTC (Triphenyltetrazolium chloride). After the experiment ended, histopathological changes in the brain were closely observed under light microscope.

(4) Detection of ingredients of *Trichosanthes kirilowii cassia twig* particles into the blood and brain with HPLC-MS method. The rats were divided into sham operation group, middle cerebral artery occlusion model group and sham operation administration group, each with 14 rats. Rats in the sham operation administration group and middle cerebral artery occlusion group were administered with3.6g• kg-1• d-1 particles, the sham operation group was administered with 0.9g/L of 0.9% sodium chloride solution. At 15min, 30min, 60min after administration, cerebrospinal fluid, blood sample and brain tissue were removed and save in the refrigerator (Alnaim and Almaz 2017). The biological sample pretreatment was then performed and HPLC-MS analysis was done (Kuscu *et al*., 2017).

RESULTS

Neurological function score

After modeling, score of neurological functional symptoms of MCAO model group rats was higher than that of the sham operation group ($P<0.05$); also higher than that on the seventh day of the positive group, the middle dose group and the high dose group ($P<0.05$), indicating that *Trichosanthes kirilowii cassia twig* particles can protect neurological damage in rats of MCAO model, which is consistent with the relevant domestic research results, as shown in fig. 1.

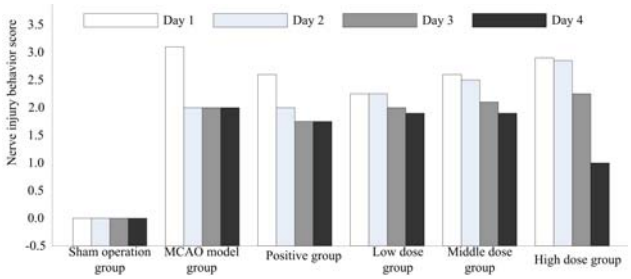

Fig. 1: Histogram of neurological function score at different time points in each group.

Determination of Evans blue seepage volume and cerebral infarction volume

As is shown, compared with sham group, MCAO model group has higher Evans blue content in brain tissue ($P<0.05$), also higher Evans blue content in brain tissue

than the positive group and middle and high dose groups (P<0.05); The brain slices of the sham operation group were dyed into homogeneous red, and there was no infarct. There was significant difference between the MCAO model group and the middle and high dose groups (P<0.05), and between the MCAO model group and the positive group (P<0.05), as shown in fig. 2.

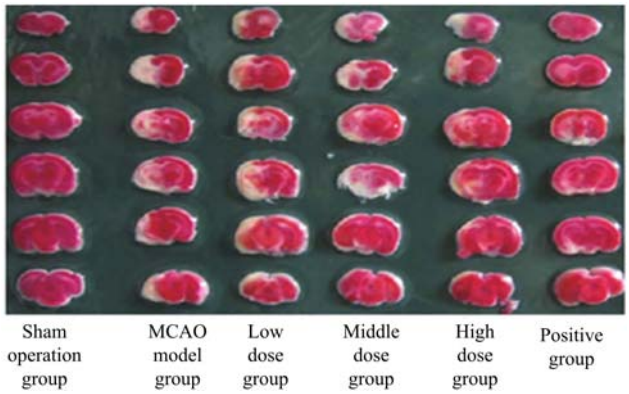

Fig. 2: Cerebral infarction tissue slice.

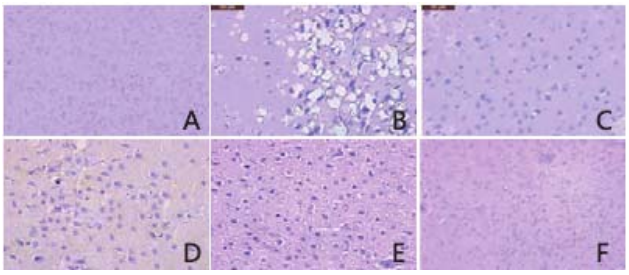

Fig. 3: Pathological picture of the brain tissue of rats in each group.

Histopathological observation of the brain tissue

As shown in fig. 3, cerebral cortex neurons of rats in sham operation group are arranged in order, with clear nucleus and rich cytoplasm; In the model group, there exists disorder of cell arrangement, cellular degeneration, necrosis, obvious interstitial edema and infiltration of inflammatory cells in ischemic cerebral cortex; Compared with the MCAO model group, pathological changes of the positive group and the administration group were obviously relieved.

Establishment of analysis conditions

The 11 major ingredients in *Trichosanthes kirilowii Cassia twig* particles were identified, ingredients of the granules into the blood and brain were determined, the results are shown in table 1 below.

MRM scan analysis of ingredients of Trichosanthes kirilowii cassia twig particles into the blood and brain

The selected ion pairs were extracted from the total ion chromatograms of the granules to obtain the selected ion chromatogram of each ion pair. Judging the ingredients in plasma, brain tissue and cerebrospinal fluid, citrulline, paeoniflorin and albiflorin, liquiritin and liquiritin apioside, isoliquiritin, glycyrrhizin can reach the cerebrospinal fluid through the blood-brain barrier. And in the MCAO model group, isoliquiritigenin, glycyrrhizin can also be detected in the cerebrospinal fluid or brain tissue, which may be because after reperfusion injury in rats, blood-brain barrier is damaged, promoting the transmission of these ingredients (Li *et al.*, 2018).

DISCUSSION

The blood-brain barrier is the barrier between plasma and brain cell formed by the brain capillary wall and neurogliocyte, also the barrier between plasma and cerebrospinal cells formed by choroid plexus, which selectively allows certain substances to enter the brain tissue via the blood. The change of blood-brain barrier function can play an ideal effect in the pathophysiological process of many brain diseases (Peng *et al*., 2016).

In recent year, with ascending attention being paid on brain ischemia/reperfusion injury, a central nervous system disease, piles of relevant researches were focused on exploring effective therapeutic regimens. Of those, drugs that target the blood-brain barrier permeability or exert better nerve-protective effects in models of rats with brain ischemia/reperfusion injury have attracted much of attention, for it could exert better effects on modifying the cerebral pathological changes. In the research of Ofori-Kwakye *et al*., results showed that activation of B2 receptor of endothelial cells could produce a reversible open state of the blood-brain barrier, thus promoting the penetration of Carboplatin and Loperamide. As a material transporter on the blood-brain barrier, P-glycoprotein could promote drug efflux in endothelial cells. Such function could be inhibited by using P-glycoprotein inhibitor, which can also significantly enhance the permeability of colchicine for more than ten times in rat brain.

In the present study, ingredients of *Trichosanthes kirilowii cassia twig* particles in plasma, brain tissue and cerebrospinal fluid of sham operation administration and MCAO model rats were observed and investigated by HPLC-MS/MS. The results showed that its 11 Ingredients can penetrate into the blood, while citrulline, albiflorin, paeoniflorin, liquiritin apioside, isoliquiritin apioside, isoliquiritin can penetrate into brain tissue through the barrier between plasma and brain cells under normal physiological conditions. Glycyrrhizic acid and isoliquiritigenin were detected in the MOCA model rats. Administration of *Trichosanthes kirilowii cassia twig* particles can improve neurological dysfunction and cerebral infarction area caused by cerebral ischemia-reperfusion injury and improve the pathological changes of brain tissue. Furthermore, consider that the sample size of rats is limited in our research, the data advantages

Table 1: Optimization conditions for reference substances of the main ingredients of Trichosanthes kirilowii cassia twig particles

S. No.	Standard substance	MRM	Supply voltage	Fragmentor voltage
1	Citrulline	174→130.9	100	10
2	Gallic acid	169→124.9	100	20
3	Albiflorin	525→120.8	135	20
4	Paeoniflorin	525→120.8	135	20
5	Liquiritin apioside	549→255.1	250	40
6	Liquiritin	417→255.0	135	10
7	Isoliquiritin apioside	549→255.1	250	35
8	Isoliquiritin	417→255.0	135	10
9	Glycyrrhizin	255→119.0	100	30
10	Isoliquiritigenin	255→119.0	100	30
11	Glycyrrhizic acid	821→351.0	100	10

might not be prominent. Therefore, studies with larger sample size are needed to obtain more reliable and generally representative results.

CONCLUSION

In summary, the ingredients of *Trichosanthes kirilowii cassia twig* particles can reach the brain tissue through the blood-brain barrier, and play the role of neuroprotection in rats with brain ischemia-reperfusion injury, which enjoy great research significance and can provide scientific, reasonable experimental theoretical basis for clinical drug use.

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