Journal of Medicinal Plants Research Vol. 7(4), pp. 140-147, 25 January, 2013
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.361
ISSN 1996-0875 ©2013 Academic Journals

Full Length Research Paper

Protective effect of polysaccharide from the root of Rhizoma Corydalis on focal ischemic cerebral infarct induced by middle cerebral artery occlusion in rats

Yi-wen Tao[1]*, Yun Wang[2], Bao-long Lai[3], Geng-yuan Tian[4], Jian-ping Feng[1] and Jian-xin Su[1]

[1]Department of Pharmacy, School of Pharmaceutical Sciences, Guangzhou Medical University, Guangzhou 510182, P. R. China.
[2]Guangdong Institute for Food and Drug Control, Guangzhou 510180, P. R. China.
[3]Department of Pharmacy, the First Affiliated Hospital of SunYat-Sen University, Guangzhou 510080, P. R. China.
[4]Shanghai Institute of Organic Chemistry, Chinese Academy of Science, Shanghai 200032, P. R. China.

Accepted 11 October, 2012

To investigate the effect of polysaccharide from the root of Rhizoma Corydalis (YhPS-1) on focal cerebral infarct in ischemia-reperfusion injured rats, sixty Wister rats were randomly divided into six groups (n = 10): sham group, control group, YhPS-1-treatment groups (20, 40, and 80 mg/kg), and positive medicine breviscapine table group, respectively. The models of focal cerebral infarct and carotid thrombosis were established, and neurobehavioral scores, the area of cerebral infarct, water content of brain, clotting time and thrombosis time were used to evaluate the effect of YhPS-1. Experiments indicated that YhPS-1-treated groups significantly improved neurological deficits, reduced the area of cerebral infarct and brain water content, and prolonged clotting time and thrombosis time in comparison with control group. These findings suggested that YhPS-1 played a crucial protective role in cerebral ischemia-reperfusion injury.

Key words: Polysaccharide of Rhizoma Corydalis, cerebral infarct, blood clotting time, thrombosis time, free radicals.

INTRODUCTION

Ischemic stroke is a common cerebrovascular disease, and accounted for about 88%of all strokes. Free radicals played an important role in the cerebral damage induced by the cerebral ischemia-reperfusion injury, which destroyed the organizational structure and aggravated tissue damage (Sharp et al., 2000). In recent years, the remarkable results that traditional Chinese medicines were used to treat ischemic stroke were achieved (Hu and Zhu, 2008). Some drugs for promoting blood and strengthening gas had a protective effect on ischemic tissue (Shen et al., 2006; Liu et al., 2004). Rhizoma Corydalis, as a well-known traditional Chinese herbal medicine, is rich in Jiangsu province and Zhejiang province in China.

The root of Rhizoma Corydalis have the efficacies of promoting blood circulation to eliminate blood stasis and promoting circulation of qi to relieve pain, which had been reported to have the activities of analgesic, hypnotic sedative, antihypertensive, anti-cerebral ischemia, myocardial ischemia protection, and protection of gastric ulcer (He et al., 2007; Hsieh et al., 2001). However, no reports have been issued on the protective effect of polysaccharide from Rhizoma Corydalis on focal cerebral infarct. Recently, polysaccharides have been isolated from the root of Rhizoma Corydalis (YhPS-1) and their physicochemical properties were determined (Tao and Tian, 2006). Preliminary experimental results showed that YhPS-1 possessed stronger capacity for scavenging free radicals. The aim of this study was to investigate the effect of polysaccharide YhPS-1 on focal cerebral infarct induced by middle cerebral artery occlusion in rats as well

*Corresponding author. E-mail: yywentao@yahoo.com.cn.m. Tel: 86-20-81340993. Fax: 86-20-81340163.

as on carotid thrombosis. Breviscapine table was used as a positive control (Lin et al., 2007). In addition, the antioxidant activity of YhPS-1 *in vitro* was also assessed by measuring the scavenging effect on 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals.

MATERIALS AND METHODS

2,3,5-triphenyltetrazolium chloride (TTC) and DPPH were purchased from Sigma Chemical Co. (USA). Breviscapine table was produced by Shanghai Leiyunshang Pharmacy Co., Ltd. Other routine laboratory reagents were obtained from commercial sources of analytical grade.

Animals

Adult male and female Wister rats (half and half), weighing 190 to 220 g, were housed in iron cages and maintained on a 12 h light-dark cycle at 25°C. Animal treatment and maintenance were undertaken in accordance with the Principles of Laboratory Animal Care and with the Animal Use.

Preparation of YhPS-1

According to the method described by Tao et al. (2006), the polysaccharide of Rhizoma Corydalis named as YhPS-1 was isolated from the root of Rhizoma Corydalis and purified by means of gel-permeation chromatography and ion-exchange chromatography.

Animal model of middle cerebral artery occlusion

The model of middle cerebral artery occlusion (MCAO) was induced as previously described with minor modifications (Ginsberg and Busto, 1989). Briefly, the rats were anesthetized with an intraperitoneal injection (ip) of chloral hydrate (10 mg/kg). The right common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA) were exposed via a median incision in the neck skin. A 3-0 nylon thread, with its tip rounded by heating near a flame, was inserted from the ECA lumen to ICA to block the right middle cerebral artery (MCA). Two hours after MCAO, the thread was removed to allow complete reperfusion. The rectal temperature was maintained at 37 ± 0.5°C throughout the surgery by using a heating lamp.

Animal model of carotid artery thrombosis

The model of carotid thrombosis was induced electrically in rats as previously described with minor modifications (Kawasaki et al., 1993). Briefly, the rats were anesthetized with an ip of napental (40 mg/kg). The rats were placed in a supine position and CCA was exposed through a midline incision in the neck. Electric stimulation was delivered to the artery using an electrode-stimulator device integrated into the signal recorder (BT-87-3modle). The thrombus occlusion time was recorded after stimulating with a direct current of 2.0 mA for 30 s.

Evaluation of neurological status

Instantly, when rats awaked thoroughly and 24 h after surgery, the neurological status of each rat was scored twice by a 5-point scoring system (grading of 0 to 4) as reported (Tatlisumak et al., 1998).

Measurement of infarct area

The area of cerebral infarction was measured by TTC staining. After evaluation of neurological status, rats were decapitated, and their brains were carefully removed with eliminating olfactory bulb, lower brainstem and cerebellum. The brain tissues of the rats were placed at -80°C for 5 min and then were sectioned coronally with a brain slicer at 2-mm thickness pieces. These slices were incubated for 30 min in a 2% solution of TTC at 37°C and then were fixed in 4% formaldehyde solution. After the staining, the white cerebral infarct area and the red-purple normal brain tissue area could be differentiated clearly. The area of infarction (mm^2) and total area of each section were calculated by computerized image analysis system (Image-Pro Plus 3.0.1). The average infarct size of the experimental rats was expressed as a ratio of infarct region to total area.

Measurement of weights and water contents of brain tissue

The brain of each experimental rat was separated carefully, then cut longitudinally, and divided into left and right hemisphere. Their weights were measured accurately by electronic balance. The water contents of the areas of the cerebral infarct were calculated through the weighing method which was established by Rosenberg and Resh (1993). The formula of water contents in brain tissue is as follow:

Water contents of brain tissue = (wet weight - dry weight)/wet weight of brain tissue

Measurement of coagulation time

Coagulation time was measured according to the manufacturer's instructions at pre-administration, surgery and 24 h after surgery, respectively (Lemini et al., 2005).

DPPH radicals scavenging assay

The scavenging effects of YhPS-1 on DPPH radicals were measured according to the procedure described previously. The sample solution (2 ml) with various water concentrations (0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 mg/ml) was added to 2 ml of 0.1 mM DPPH in ethanol. After incubation at 25°C for 30 min, absorbance was measured at 517 nm. The scavenging effects were calculated according to the following equation:

Scavenging effect (DPPH, %) = $[1-(Ai- Aj)/A_0] \times 100$

where Ai, Aj and A_0 are represented as absorbance of 2 ml DPPH solution and 2 ml sample solution, absorbance of 2 ml ethanol and 2 ml sample solution, and absorbance of 2 ml water and 2 ml DPPH solution.

Protocol

Experiment 1 (Effect of YhPS-1 on MACO in rats): This experiment was performed on Wister rats as shown in Figure 1.

Experiment 2 (Effect of YhPS-1 on carotid artery thrombosis in rats): This experiment was also performed on Wister rats as shown

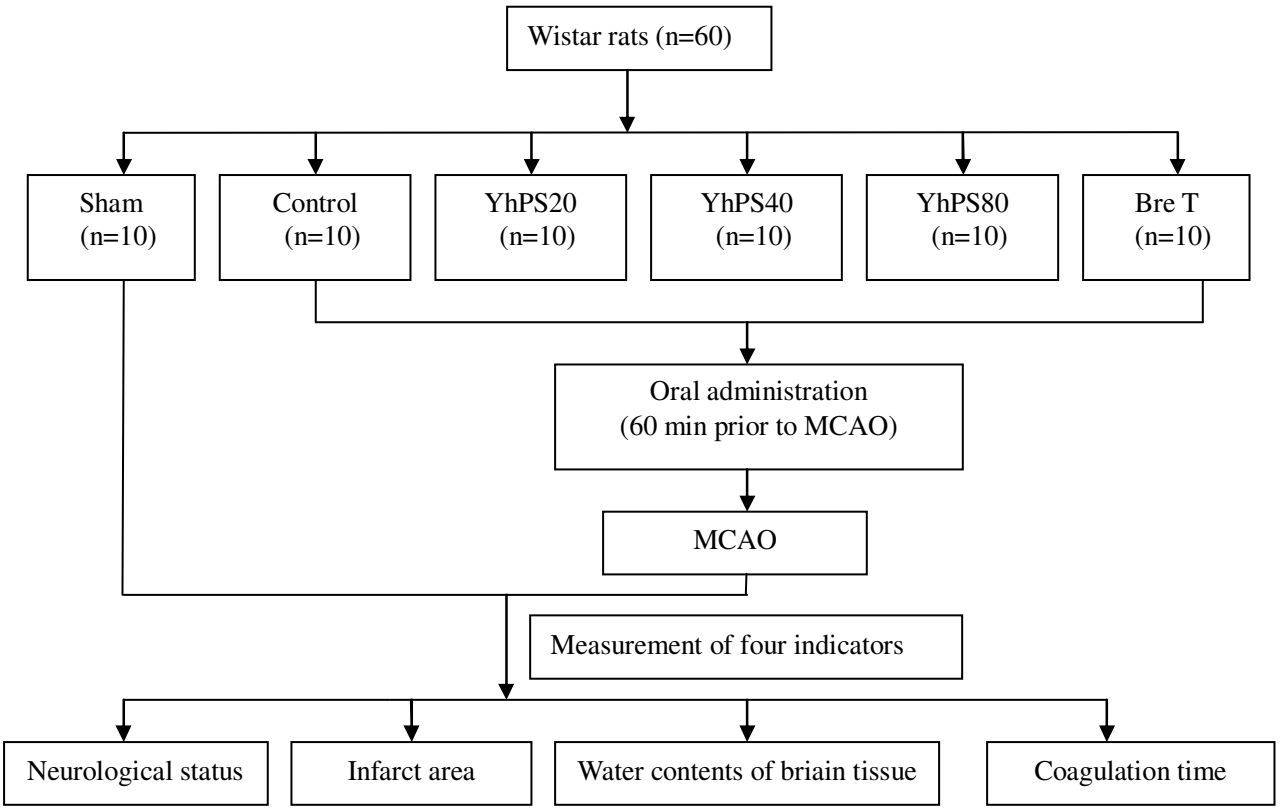

Figure 1. Effect of YhPS-1 on MACO in rats. Control: rats without any drug treatment; YhPS20: rats with YhPS-1 20 mg/kg treatment; YhPS40: rats with YhPS-1 40 mg/kg treatment; YhPS80: rats with YhPS-1 80 mg/kg treatment; Bre T: rats with breviscapine table 10.8 mg/kg treatment.

in Figure 2.

Experiment 3 (Antioxidant effect of YhPS-1 *in vitro*): The antioxidant effect of YhPS-1 *in vitro* was evaluated on the basis of the free radicals scavenging ability of the stable DPPH free radicals with aforementioned method.

Statistical analysis

The data are presented as mean ± standard error of mean (SEM). The neurological grading was carried out on the Ridit analysis, and the other data was processed by one-way analysis of variance (ANOVA) with Statistical Package for Social Sciences (SPSS) 13.0 software (SPSS Inc., USA). Differences were considered significant when $P < 0.05$.

RESULTS

Effects of YhPS-1 on neurological scores of MCAO rats

The results of neurological grades in Table 1 showed that rats of MCAO showed behavioral obstacles to various certain extents and had very significant differences when compared with sham group ($P < 0.001$) at awake promptly and 24 h after surgery by the Ridit analysis. Compared with the control group, there were significant differences ($P < 0.01$) in neurological status of three YhPS-1 treatment groups and Bre T group at 24 h after surgery. In addition, a similar finding about neurological integrals is as shown in Figure 3.

Effects of YhPS-1 on infarct area of MCAO rats

The ratio of cerebral infarct area was significantly reduced in YhPS 40, YhPS 80 and Bre T groups (Figure 4) when compared with the control group.

Effects of YhPS-1 on edema of MCAO rats

The data in Table 2 showed that the weights of whole brain and right brain in YhPS 20, YhPS 40, YhPS 80 and control groups increased significantly when compared with sham group, while the ones of left brain between each groups had insignificant difference ($P > 0.05$). Furthermore, the results of water contents of brain tissue indicated that YhPS 40 and Bre T groups could improve cerebral edema in MCAO rats in comparison with control group ($P < 0.05$ and $P < 0.01$, respectively).

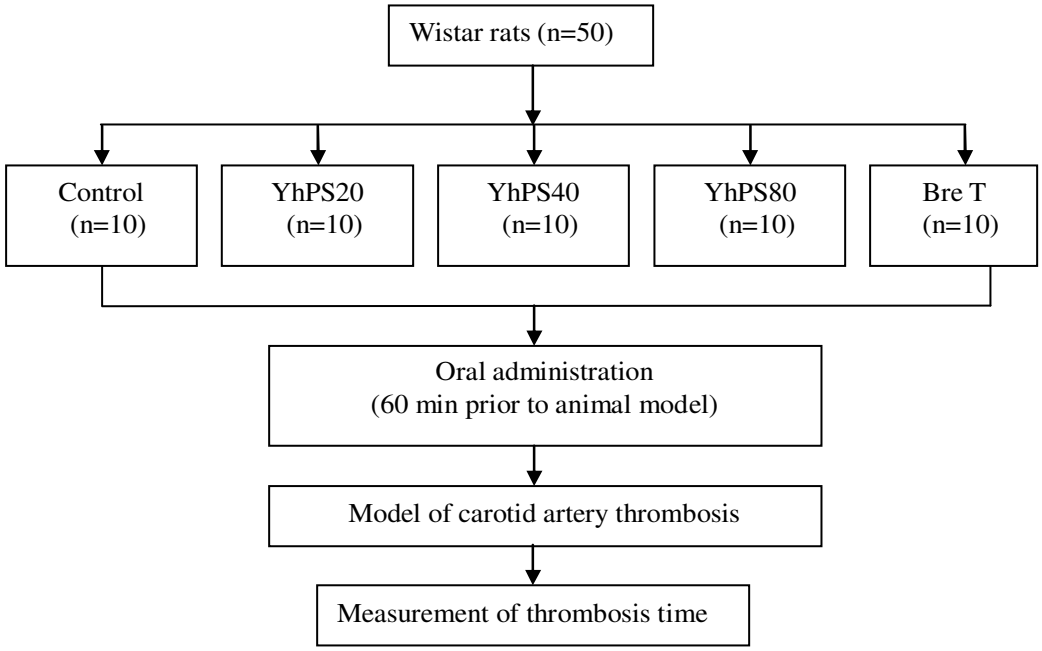

Figure 2. Effect of YhPS-1 on carotid artery thrombosis in rats.

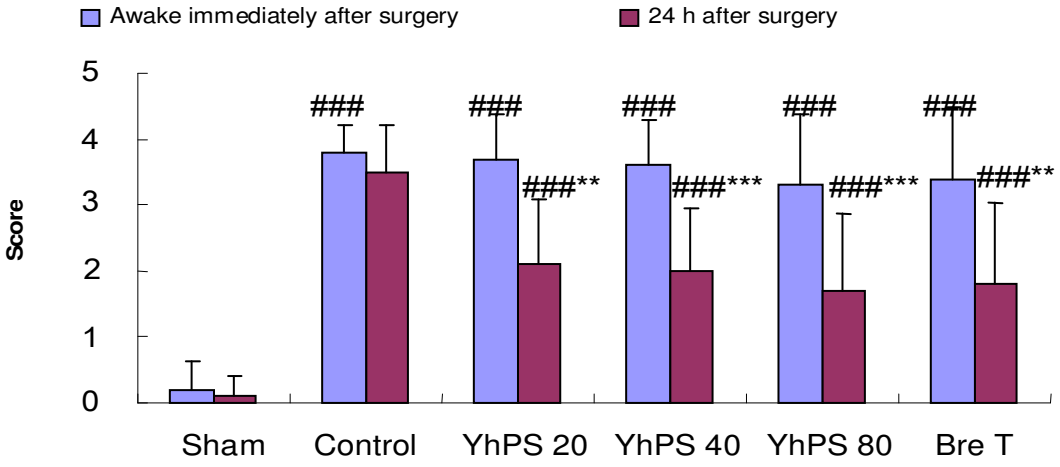

Figure 3. Effects of YhPS-1 on neurological integrals of MCAO rats at different time. YhPS 20 (20 mg/kg), YhPS 40 (40 mg/kg), YhPS 80 (80 mg/kg) and Bre T (10.8 mg/kg) were administered orally once 60 min prior to the surgery of MCAO. The data of neurological integrals at awake immediately and 24 h after surgery represent means ± SD (n = 10). The significant differences of each group were determined by one-way ANOVA. $^{###}P < 0.001$ versus sham group; $^{**}P < 0.01$, $^{***}P < 0.001$ versus control group. SD: Standard deviation.

Effects of YhPS-1 on coagulation time of MCAO rats

Results in Figure 5 showed that the differences had no significance between each group at pre-administration, while blood coagulation time of the control group was significantly reduced during surgery and 24 h after surgery ($P < 0.05$), which indicated that there were no remarkable differences between each group rats and the surgery of MCAO promoted the blood clotting. Furthermore, pretreatment with YhPS 80 and Bre T prolonged the coagulation time at operating and 24 h after surgery when compared with the control group.

Effect of YhPS-1 on carotid artery thrombosis in rats

The occlusion time in the carotid artery thrombosis model was significantly higher in the YhPS 80 and Bre T groups

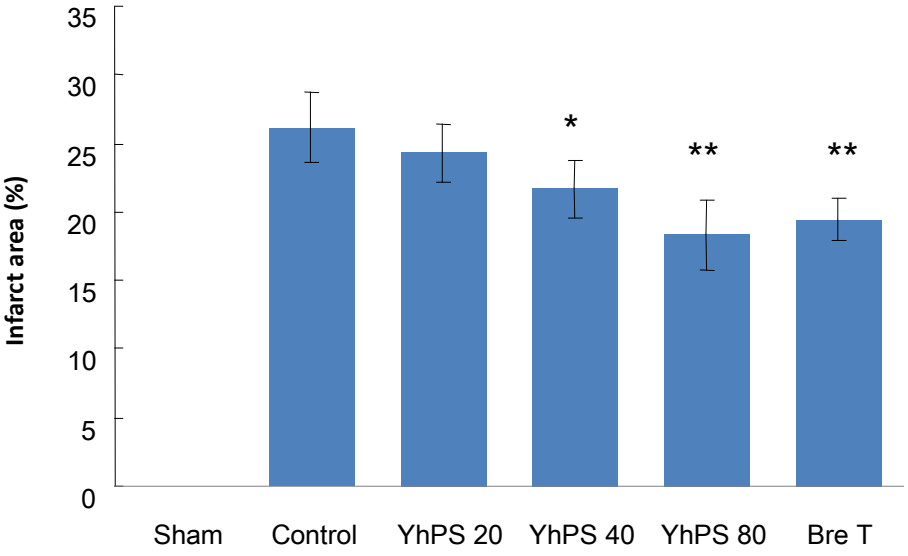

Figure 4. Effects of YhPS-1 on infarct area of MCAO rats. YhPS 20 (20 mg/kg), YhPS 40 (40 mg/kg), YhPS 80 (80 mg/kg), and Bre T (10.8 mg/kg) were administered orally once 60 min prior to the surgery of MCAO. The ratio of cerebral infarct area represent means ± SD (n = 10). Pretreatment with YhPS-1 reduced the infarct rate in a dose-dependent manner. The significant differences of each group were determined by one-way ANOVA. *P < 0.05, **P < 0.01 versus control group.SD: Standard deviation.

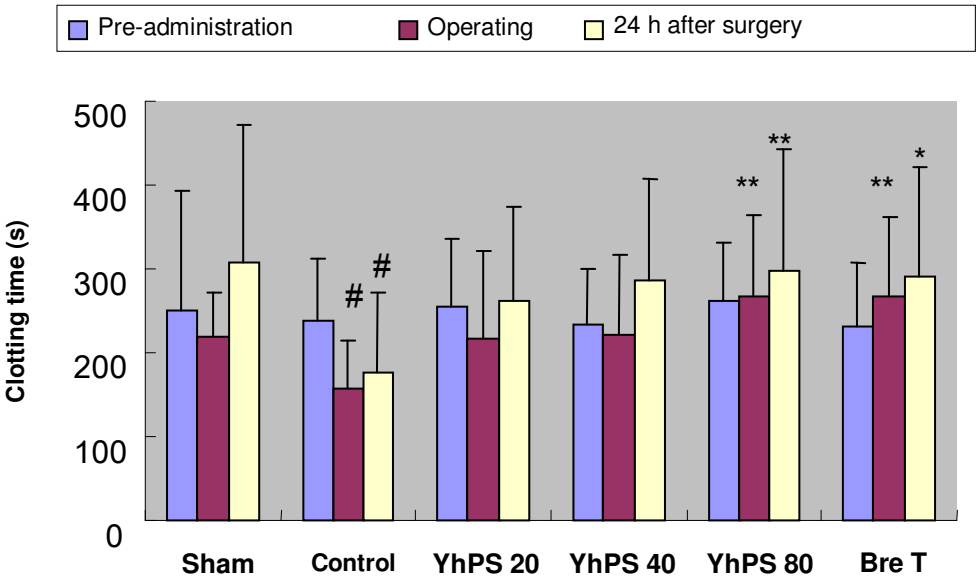

Figure 5. Effects of YhPS-1 on blood clotting time of MCAO rats at different time. YhPS 20 (20 mg/kg), YhPS 40 (40 mg/kg), YhPS 80 (80 mg/kg), and Bre T (10.8 mg/kg) were administered orally once 60 min prior to the surgery of MCAO. The blood clotting time of pre-administration, operating and 24 h after surgery were measured. Data represent means ± SD (n = 10). The significant differences of each group were determined by one-way ANOVA. #P < 0.05 versus sham group; *P < 0.05, **P < 0.01 versus control group. SD: Standard deviation.

than in the control group as shown in Figure 6. Therefore, high dose YhPS-1 as well as Bre T prolonged the thrombosis time by effective anticoagulation.

Antioxidant effect of YhPS-1 *in vitro*

The data showed that YhPS-1 possessed remarkable

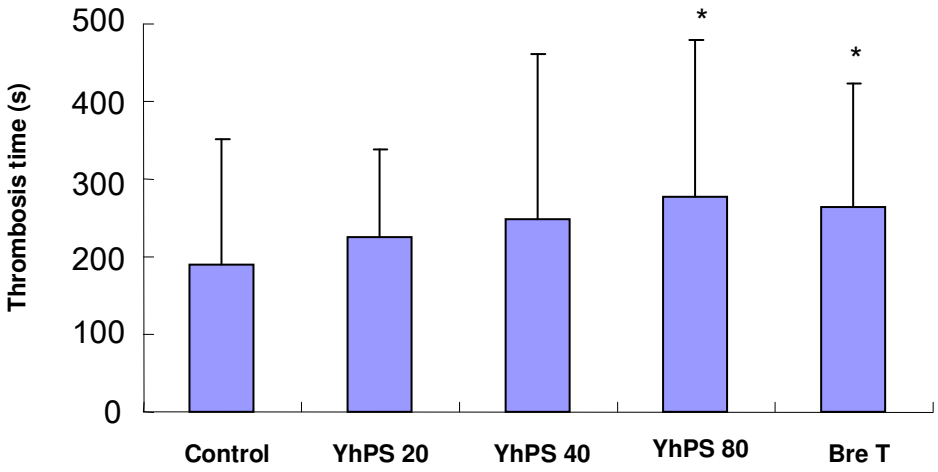

Figure 6. Effect of YhPS-1 on carotid artery thrombosis in rats. YhPS 20 (20 mg/kg), YhPS 40 (40 mg/kg), YhPS 80 (80 mg/kg), and Bre T (10.8 mg/kg) were administered orally once 60 min prior to the surgery of carotid artery thrombosis. Data of thrombosis time represent means ± SD (n = 10). Pretreatment with YhPS 80 and Bre T prolonged the thrombosis time significantly when compared with the control group. The significant differences of each group were determined by one-way ANOVA. *P < 0.05 versus control group. SD: Standard deviation.

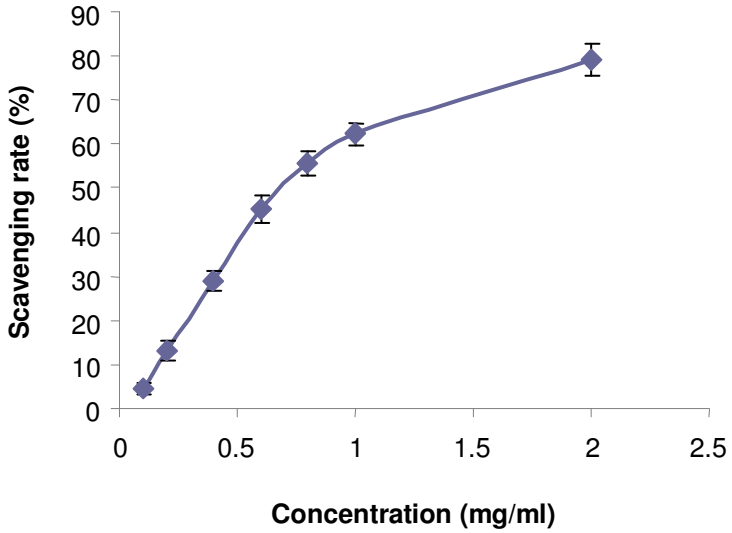

Figure 7. Scavenging effect of YhPS-1 on DPPH radical. The data shown are the means of triplicate measurements. YhPS-1 exhibited strong activity by eliminating DPPH radicals at a concentration of 2 mg/ml.

free radicals-scavenging properties with 79.1% inhibition of DPPH at a concentration of 2 mg/ml (Figure 7).

DISCUSSION

In this study, we investigated whether treatment with a polysaccharide YhPS-1 from the root of Rhizoma Corydalis could protect rat brain from injury induced by MCAO. Firstly, the model of focal cerebral infarct was very successful, because rats of MCAO showed obvious behavioral obstacles in comparison with those of sham group. The findings of neurological status and neurological integrals indicated that YhPS-1treatment groups obviously improved the neurological deficits. Secondly, the histochemical graphs of the ischemic stroke regions showed that YhPS-1 as well as Bre T had a protective effect on focal ischemic cerebral infarct induced by

Table 1. Effects of YhPS-1 on neurological grades of MCAO rats and Ridit analysis (n = 10).

Group	No. of rats in each grade at awake promptly after surgery and Ridit analysis						No. of rats in each grade at 24 h after surgery and Ridit analysis					
	0	1	2	3	4	Ridit analysis	0	1	2	3	4	Ridit analysis
Sham	8	2	0	0	0	-	9	1	0	0	0	-
Control	0	0	0	2	8	###	0	0	1	3	6	###
YhPS 20	0	0	1	1	8	###	0	3	4	2	1	###**
YhPS 40	0	0	1	2	7	###	0	4	2	3	1	###**
YhPS 80	0	1	1	2	6	###	1	4	3	1	1	###**
Bre T	0	1	1	2	6	###	1	4	2	2	1	###**

YhPS 20 (20 mg/kg), YhPS 40 (40 mg/kg), YhPS 80 (80 mg/kg), and Bre T (10.8 mg/kg) were administered orally once 60 min prior to the surgery of MCAO. The significant differences of each group were determined by Ridit analysis. ###P < 0.001 versus sham group**P < 0.01 versus control group.

Table 2. Effects of YhPS-1 on weights of brain tissue and water contents of brain tissue of MCAO rats (means ± SD, n = 10).

Group	Weight of whole brain (g)	Weight of left brain (g)	Weight of right brain (g)	Water contents of brain tissue (g/g brain tissue)
Sham	1.0732 ± 0.1109	0.6282 ± 0.1520	0.5030 ± 0.0802	0.668 ± 0.099
Control	1.2835 ± 0.1329##	0.5651 ± 0.2078	0.7184 ± 0.2634#	0.871 ± 0.084###
YhPS 20	1.2185 ± 0.0414##	0.6171 ± 0.0243	0.6015 ± 0.0544##	0.793 ± 0.088##
YhPS 40	1.1819 ± 0.1035#	0.6119 ± 0.0381	0.5700 ± 0.0568##	0.772 ± 0.098#*
YhPS 80	1.2059 ± 0.0810##	0.6127 ± 0.0486	0.5932 ± 0.0537##	0.791 ± 0.097#
Bre T	1.2081 ± 0.0918##	0.6281 ± 0.0496	0.5800 ± 0.1090	0.757 ± 0.055#**

YhPS 20 (20 mg/kg), YhPS 40 (40 mg/kg), YhPS 80 (80 mg/kg) and Bre T (10.8 mg/kg) were administered orally once 60 min prior to the surgery of MCAO. The significant differences of each group were determined by one-way ANOVA. #P < 0.05, ##P < 0.01, ###P < 0.001 versus sham group; *P < 0.05, **P < 0.01 versus control group. SD: Standard deviation.

MCAO in rats. Cerebral edema occurred commonly during the acute phase of a large cerebral injury. According to the reports in the literature, dehydrating hyperosmolar agents such as mannitol had been prescribed to reduce cerebral edema. The findings of YhPS-1 on edema of MCAO rats implied that YhPS-1 might be effective on cerebral edema as a dehydrating agent. In the same time, the results of clotting time (Figure 5) and thrombosis time (Figure 6) showed that YhPS-1 could promote blood circulation, remove blood stasis and improve blood supply to the ischemic brain so as to lessen or improve all kinds of clinical symptoms of brain ischemia injury.

In addition, free radical was involved in the cerebral damage induced by ischemia-reperfusion tissue injury. The generation of free radical played an important role in triggering the ischemic neuronal damages causing delayed neuronal death. So, the capacity of scavenging free radicals was also one of the mechanisms of improving cerebral injury. YhPS-1, as a polysaccharide, contained many alcoholic hydroxyl groups and had the capacity of scavenging free radicals, which might be one of the mechanisms of improving cerebral edema.

Conclusion

Conclusively, this study showed that YhPS-1 had protective effect on cerebral ischemia-reperfusion injury induced by MCAO in rats and its possible mechanisms were related to improve blood circulation to remove blood stasis and possess strong capability of scavenging free radicals.

ACKNOWLEDGEMENTS

This study was supported by the Guangdong Administration Bureau of Traditional Chinese Medicines (No. 20112153).

REFERENCES

Ginsberg MD, Busto R (1989). Rodent models of cerebral ischemia. Stroke 20:1627-1642.

He K, Gao JL, Zhao GS (2007). Advances in studies on chemistry, pharmacology, and quality control of Corydalis yanhusuo. Chin. Tradit. Herbal Drugs 38:1909-1912.

Hsieh CL, Liao ET, Chiang SY, Lao CJ, Tang NY, Hsieh CL, Lin JG

(2001). Effect of Rhizoma Corydalis on focal cerebral infarct in ischemia-reperfusion injured rats. Acta Pharmacol. Sin. 22(12):1143-1148.
Hu GH, Zhu MZ (2008). Academic discussion on promoting blood circulation for removing blood stasis method preventing and treating cerebral ischemic (CI) injury. Chin. J. Tradit. Chin. Med. Pharm. 23(06):513-515.
Kawasaki T, Kawamura S, Katoh S, Takenaka T (1993). Experimental model of carotid artery thrombosis in rats and the thrombolytic activity of YM886, a novel modified tissue-type plasminogen activator. Jpn. J. Pharmacol. 63:135-142.
Lemini C, Franco Y, Estela AM (2005). Contrasting effects of estradiol and 17 β-aminoestrogens on blood clotting time in rats and mice. Eur. J Pharmacol. 510(3):229-233.
Lin LL, Liu AJ, Liu, JG, Yu XH, Qin LP, Su DF (2007). Protective effects of scutellarin and breviscapine on brain and heart ischemia in rats. J. Cardiovasc. Pharmacol. 50(3):327-332.
Liu YH, Lai YZ, Zhang SP, Feng G, Liu SM (2004). Comparison of the effects of huoxue huayu compounds on focal cerebral ischemia damage. Chinese J. Clin. Rehab. 8(19):3831-3833.
Rosenberg DM, Resh VH (1993). Introduction to freshwater biomonitoring and benthic macroinvertebrates. Chapman and hall, New York pp.1-9.
Sharp FR, Lu A, Tang Y (2000). Multiple molecular penumbras after focal cerebral ischemia. J. Cereb. Blood Flow Metab. 20:1011-1032.
Shen SY, Fu XD, Shang W, Dong XL, Zhao LJ, Cai H (2006). Discussion and Analysis of Mechanism on Method of Promoting Blood Circulation and Removing Blood Stasis in Ischemic Stroke. J. Zhejiang Univ. Tradit. Chin. Med. 30(04):322-323.
Tao YW, Tian GY (2006). Studies on the physicochemical properties, structure and antitumor activity of polysaccharide YhPS-1 from the Root of Cordalis yanhusuo Wang. Chin. J. Chem. 24:235-239.
Tatlisumak T, Takano K, Carano RA, Miller LP, Foster AC, Fisher M (1998). Delayed treatment with an adenosine kinase inhibitor, GP683, attenuates infarct size in rats with temporary middle cerebral artery occlusion. Stroke 29(9):1952-1958.