

Protective effect of ginkgo proanthocyanidins against cerebral ischemia/reperfusion injury associated with its antioxidant effects

Wang-li Cao¹, Hai-bo Huang², Ling Fang^{2,3}, Jiang-ning Hu^{1,3}, Zhu-ming Jin², Ru-wei Wang^{1,2,3,*}

1 Zhejiang Modern Chinese Medicine and Natural Drug Research Academy Co., Ltd., Hangzhou, Zhejiang Province, China

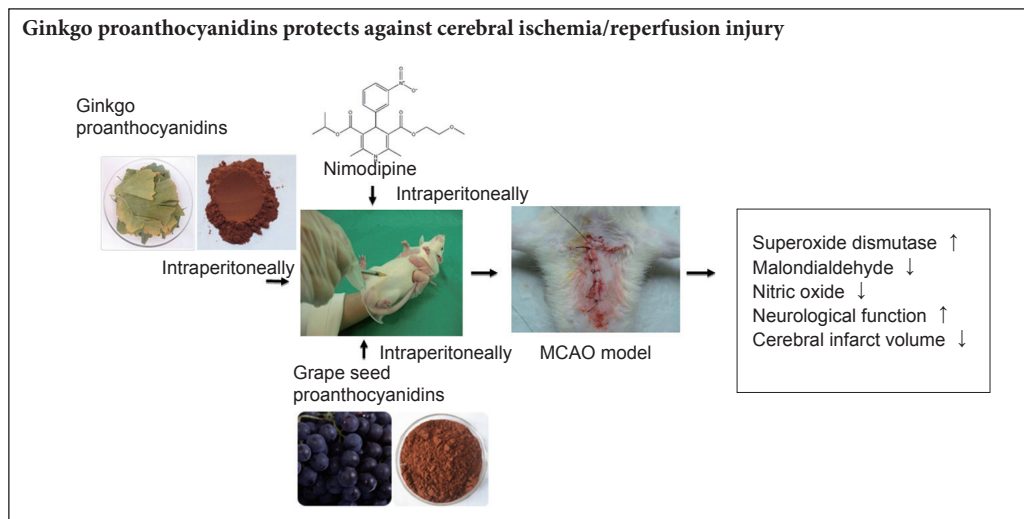
2 Zhejiang Conba Pharmaceutical Co., Ltd., Hangzhou, Zhejiang Province, China

3 Zhejiang Key Laboratory of Traditional Chinese Medicine Pharmaceutical Technology, Hangzhou, Zhejiang Province, China

How to cite this article: Cao WL, Huang HB, Fang L, Hu JN, Jin ZM, Wang RW (2016) Protective effect of ginkgo proanthocyanidins against cerebral ischemia/reperfusion injury associated with its antioxidant effects. *Neural Regen Res* 11(11):1779-1783.

Open access statement: This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

Graphical Abstract



*Correspondence to:

Ru-wei Wang, Ph.D.,
wangrw@conbagroup.com.

orcid:

0000-0002-4354-250X
(Ru-wei Wang)

doi: 10.4103/1673-5374.194722

Accepted: 2016-08-24

Abstract

Proanthocyanidins have been shown to effectively protect ischemic neurons, but its mechanism remains poorly understood. Ginkgo proanthocyanidins (20, 40, 80 mg/kg) were intraperitoneally administered 1, 24, 48 and 72 hours before reperfusion. Results showed that ginkgo proanthocyanidins could effectively mitigate neurological disorders, shorten infarct volume, increase superoxide dismutase activity, and decrease malondialdehyde and nitric oxide contents. Simultaneously, the study on grape seed proanthocyanidins (40 mg/kg) confirmed that different sources of proanthocyanidins have a similar effect. The neurological outcomes of ginkgo proanthocyanidins were similar to that of nimodipine in the treatment of cerebral ischemia/reperfusion injury. Our results suggest that ginkgo proanthocyanidins can effectively lessen cerebral ischemia/reperfusion injury and protect ischemic brain tissue and these effects are associated with antioxidant properties.

Key Words: nerve regeneration; cerebral ischemia/reperfusion injury; proanthocyanidins; nimodipine; superoxide dismutase; malondialdehyde; nitric oxide; neural regeneration

Introduction

The incidence of ischemic cerebrovascular disease, which is one of the main diseases threatening the rapidly aging human population, has been increasing over the years. Reperfusion is beneficial in reducing ischemic brain injury and in recovering some reversible damage. However, recent studies found that blood reperfusion for ischemic tissue could induce further damage and dysfunction in some cases. There-

fore consideration is always taken of how to inhibit reperfusion injury during the treatment of ischemic stroke (Prakash and Kumar, 2013).

Cerebral ischemia is caused by free radical damage (Floyd and Carney, 1992). Proanthocyanidins (PC), which are a form of condensed tannins or oligomeric flavonoids, and have been known to be extremely strong antioxidants (Aras et al., 2015). Many studies have proved that PC has strong

anti-inflammatory activity, and this effect was associated with reduced activation of nuclear factor-kappa B p65 pathway (Li et al., 2001; La et al., 2010; Bak et al., 2013). Growing evidence has suggested the effect of PC on improving blood circulation, visual protection and edema elimination (Pons et al., 2014; Yang et al., 2016). Whether PC can be used to treat cerebral ischemia/reperfusion (I/R) injury remains unclear. The ginkgo leaf has an important medicinal value and the content of PC in its extracts (7%) is higher than in many other plants (Kan et al., 2013).

In this study, we investigated the protective effects of ginkgo PC (GPC) on the rat with cerebral I/R injury, and found some clues on the underlying mechanism of protection. We also included grape seed PC (GSPC) to compare the PC effects from another source, because PC can be extracted from different plant species.

Nimodipine is commonly used for the effective treatment of cerebral ischemia caused by cerebral vasospasm after subarachnoid hemorrhage. It regulates the intracellular calcium level effectively and helps maintain the normal physiological function, especially the cerebrovascular system (Honig, 1991). In our experiment, we used nimodipine as a positive control.

Materials and Methods

Ethics statement

The animal studies were approved by the Experimental Animal Ethics Committee of Zhejiang Key Laboratory of Traditional Chinese Medicine Pharmaceutical Technology of China (animal license No. 2015-0003) and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Precautions were taken to minimize suffering and the number of animals used in each experiment.

Experimental animals

Fifty-six adult male Sprague-Dawley rats, weighing 250–320 g and aged 8–10 weeks, were purchased from Zhejiang Experimental Animals Center in China (license No. 2008-0016). The rats were equally and randomly divided into seven groups: I/R, sham operation, nimodipine (I/R + 2 mg/kg nimodipine), GPC low-, moderate- and high-dose (GPCL, GPCM, GPCH; I/R injury + 20, 40, 80 mg/kg GPC), and GSPC (I/R injury + 40 mg/kg GSPC). Before the experiment, the rats were acclimatized in individual cages for one week at 20–25°C, with standard chow and tap water.

PC pre-treatment

At 1, 24, 48, 72 hours before operation, the rats in the nimodipine, GPCL, GPCM, GPCH, and GSPC groups were intraperitoneally injected with either 2 mg/kg nimodipine (Jiangsu Jiquan Pharmaceutical Co., Ltd., China; calculated by body surface areas), 20, 40, 80 mg/kg GPC (0.2 g/L, dissolved in normal saline, stored in cool dry place and protect from light; Zhejiang Conba Pharmaceutical Co., Ltd., China) or 40 mg/kg GSPC (dissolved in normal saline, stored in cool dry place and protect from light; Zhejiang Conba Pharmaceutical Co., Ltd.), respectively. The rats in the I/R and

sham operation groups were intraperitoneally injected with normal saline (10 mL) at the same time points.

Model establishment

Models of cerebral I/R injury were established in the rats based on the methods described by Longa et al. (1989). Briefly, rats were intraperitoneally anesthetized with 10% chloral hydrate (300 mg/kg; Shanghai Baihe Chemical Company, China). The right common carotid artery, external carotid artery, and internal carotid artery were exposed. A 4–0 suture (Ningbo Medicinal Suture Needle Co., Ltd., Ningbo, China; diameter 0.26 mm) with a blunted tip coated with poly-L-lysine was gently advanced into the internal carotid artery through the external carotid artery. The suture was advanced 18–20 mm (reaching the origin of the right middle cerebral artery) beyond the carotid artery bifurcation. The suture was slowly removed after 2 hours of middle cerebral artery occlusion and followed by reperfusion.

After the 2-hour period of ischemia, without anesthesia or cutting the skin, the nylon thread was gently removed to achieve middle cerebral artery reperfusion. If Homer's sign occurred in the right and left anterior limbs, the models of ischemia were considered successful. The sham operation group followed the same steps above without the insertion of a surgical line. The mortality rate in each group was recorded.

Neurobehavioral evaluation

Neurological function scores were calculated according to Zea Longa's 5-point scale (Roof et al., 2001), at 4, 8, and 24 hours after reperfusion. A higher score means a higher level of behavior disorder.

2,3,5-Triphenyltetrazolium chloride (TTC) staining

After neurobehavioral evaluation and 24 hours after reperfusion, the rats were decapitated. The olfactory bulb, cerebellum and lower brain stem were removed. Five slices of brain tissue were placed into 0.25% TTC (Sigma, Munich, German) solution at 37°C for 30 minutes in the dark, and then fixed with 10% formalin for 4 hours (Chen et al., 2010). We calculated and compared the infarct volume with a morphology analysis system (Jiangsu JEDA Science and Technology Development Co., Ltd., Jiangsu Province, China).

Superoxide dismutase (SOD) activity, malondialdehyde (MDA) and nitric oxide (NO) contents in brain tissue of rats with cerebral I/R injury

At 24 hours after reperfusion, the brain was stripped quickly and washed with normal saline. Brain homogenates in cold normal saline (10% w/v) were made using a glass homogenizer. They were then centrifuged and the supernatant was used for biochemical determination. SOD activity was detected by xanthine oxidation method with a SOD Detection Kit (Jiangsu Jiancheng Bioengineering Institute, China). MDA contents were measured by thiobarbituric acid colorimetric method with an MDA Detection Kit (Jiangsu Jiancheng Bioengineering Institute). NO contents were measured by nitric reductase with an NO Detection Kit

(Jiangsu Jiancheng Bioengineering Institute). The contents of tissue proteins were determined with Coomassie Brilliant Blue (Bennett and Scott, 1971). The methods used followed the specific instructions of each kit.

Statistical analysis

Data, expressed as the mean \pm SD, were analyzed by SPSS 17.0 software (SPSS, Chicago, IL, USA). The comparisons among groups were made by one-way analysis of variance followed by Bonferroni *post-hoc* test. A value of $P < 0.05$ was considered statistically significant.

Results

GPC decreased mortality rate of rats with cerebral I/R injury

The mortality rate of cerebral I/R model was high 24 hours after reperfusion (Figure 1). The most important cause of death was asphyxia induced by epilepsy after brain injury, followed by subarachnoid hemorrhage. There was no mortality in the nimodipine group. GPC significantly reduced the mortality rate of rats in a dose-dependent manner and GSPC also resulted in a decreased mortality.

GPC improved neurological function of rats with cerebral I/R injury

Rats in each group had obvious symptoms of neurological impairment, including convulsion, contralateral dumping, and rotary motion 4 hours after reperfusion. Rats in all groups had similar severity. Neural injury was mitigated 8 and 24 hours after reperfusion, especially in the nimodipine, GPCM and GPCH groups (Table 1).

Effect of GPC on cerebral infarct volume in rats with focal cerebral ischemia

Cerebral infarct volume is the intuitive and accurate indicator to evaluate cerebral ischemia (Zhang et al., 2014b). The results suggested that no significant difference in the infarct volume was determined between the GPCL or the GSPC group and the I/R group ($P > 0.05$). The infarct volumes were significantly smaller in the GPCM and GPCH group compared with the I/R group ($P < 0.01$). The infarct volume was similar between the GPCH and nimodipine groups ($P > 0.05$; Figure 2).

GPC improved SOD activity, MDA and NO contents in the brain of rats with cerebral I/R injury

SOD activity of the rats was significantly lower in the I/R group than in the sham operation group ($P < 0.01$). SOD activity was significantly higher in the GPCL, GPCM and GPCH groups than in the I/R group ($P < 0.05$ or $P < 0.01$). No significant difference in SOD activity was detected between the nimodipine or GSPC groups and the I/R group ($P > 0.05$; Figure 3A).

The MDA content was significantly higher in the I/R group than in the sham operation group ($P < 0.01$). MDA contents were significantly lower in the GPCM and GPCH groups than in the I/R group ($P < 0.01$). The nimodipine and GSPC groups had lower mean MDA content than the I/R group, but were

not significantly different ($P > 0.05$; Figure 2B).

NO content was significantly higher in the I/R group than in the sham operation group ($P < 0.01$). NO content decreased significantly in the GPCL, GPCM, GPCH and nimodipine groups compared with the I/R group ($P < 0.01$). No significant difference in NO content was detectable between the GSPC and I/R groups ($P > 0.05$; Figure 2C).

Discussion

Middle cerebral artery is where stroke always happens and the infarction in the middle cerebral artery area accounts for 82.2% in patients with cerebral infarction. The occlusion model of focal cerebral ischemia in the middle cerebral artery is considered to be an ideal model (Morin and Simon, 2006; Wu et al., 2009; Wang et al., 2011). Reperfusion injury, a complex pathological process involving a variety of cytokines and signaling pathways, seriously affects the treatment and prognosis of patients with cerebral ischemia (Imai et al., 2007; Li et al., 2011). At present, there are no established methods and drugs for the treatment of cerebral I/R injury because there is a lack of clarity in the mechanism of function, safety, and ethics. Our data show that treatment with GPC could not only improve neurological function after focal cerebral ischemia caused by artery occlusion, but also significantly reduce infarct area and mortality rate, especially at a high dose. The underlying mechanism of its protective effects may be related to anti-oxygen radical and anti-inflammatory activity. The purpose of this study was to investigate the function of PCs, but only one dose of grape seed (40 mg/kg) was studied. Grape seed had a weaker influence on improving cerebral ischemia in rats compared with the same dose of GPC. We noted that GPC in each group could inhibit epilepsy caused by brain injury but GSPC did not. The anti-epilepsy mechanism of GPC needs further investigation.

Our data suggest that there was less nerve dysfunction in rats 8 hours after I/R operation in the GPCM, GPCH and nimodipine groups compared with the untreated I/R group. Nerve dysfunction in rats improved further 24 hours after operation, but was not obvious in the GSPC group. Our results show that compared with the I/R group, the infarct area was significantly reduced in the GPCM, GPCH and nimodipine groups, and suggested that GPC has protective effect against cerebral ischemia.

When acute cerebral infarction occurs, oxygen free radicals are produced through enzymatic and non-enzymatic systems; the latter can attack polyunsaturated fatty acids, which are abundant in vascular endothelial cells and brain cells (Weaver and Liu, 2015). Lipid peroxidation is initiated by free radicals and result in products such as aldehyde (MDA), keto, hydroxy, hydroperoxy carbonyl or inner peroxy radicals (Candelario-Jalil, 2009; Friedman, 2013; Zhang et al., 2014a). Free radical damage is one of the most important mechanisms of cerebral I/R injury and drugs that target free radicals are considered candidates to treat cerebral I/R injury (Viuda-Martos et al., 2014). MDA content is a measure of the degree of lipid peroxidation and indirectly indicates cell injury in the brain. Because of ischemia and free radical oxidation, SOD activity is decreased in the ischemic

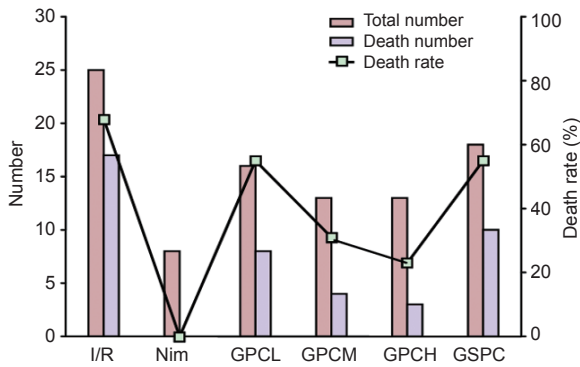


Figure 1 Mortality rate in rats of each group 24 hours after focal cerebral I/R injury.

I/R group: Focal cerebral ischemia model only; nimodipine group: I/R injury + nimodipine; GPCL, GPCM, GPCH groups: I/R injury + 20, 40, 80 mg/kg GPC, respectively; GSPC group: I/R injury + 40 mg/kg GSPC. I/R: Ischemia/reperfusion; Nim: nimodipine; GPC: ginkgo proanthocyanidins; GPCL: low-dose GPC; GPCM: moderate-dose GPC; GPCH: high-dose GPC; GSPC: grape seed proanthocyanidins.

Table 1 Effect of GPC on neurological function impairment in rats with focal cerebral I/R injury

Group	Hours after focal cerebral I/R injury		
	4	8	24
I/R	4.19±0.74	3.38±1.04	3.54±1.33
Nimodipine	3.38±0.74 [#]	3.37±0.92	1.71±0.49 ^{##}
GPCH	3.08±0.99 ^{##}	2.45±1.44 ^{##}	1.25±0.46 ^{##}
GPCM	3.44±0.96 ^{##}	2.38±1.04 ^{##}	2.50±1.88 [#]
GPCL	3.67±0.84	3.33±0.97	3.46±0.83
GSPC	3.88±0.68	3.67±0.90	3.25±1.36

The higher score means the higher level of behavior disorder. I/R group: Focal cerebral ischemia model only; nimodipine group: I/R injury + nimodipine; GPCL, GPCM, GPCH groups: I/R injury + 20, 40, 80 mg/kg GPC, respectively; GSPC group: I/R injury + 40 mg/kg GSPC. Data are expressed as the mean ± SD, with eight rats in each group. [#]*P* < 0.05, ^{##}*P* < 0.01, vs. I/R group (one-way analysis of variance followed by Bonferroni *post-hoc* test). I/R: Ischemia/reperfusion; GPC: ginkgo proanthocyanidins; GPCL: low-dose GPC; GPCM: moderate-dose GPC; GPCH: high-dose GPC; GSPC: grape seed proanthocyanidins.

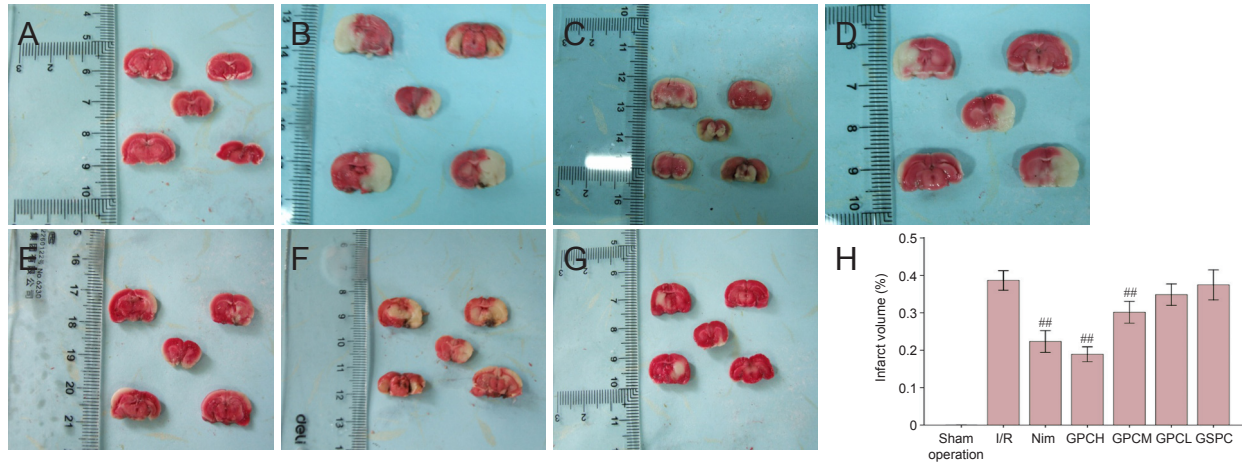


Figure 2 Effect of GPC on infarct volume in rats 24 hours after cerebral I/R injury by TTC staining.

(A–G) Coronal sections of rat brains in the sham operation, I/R, nimodipine, GPCL, GPCM, GPCH, and GSPC groups. Red is normal tissue and white is ischemic tissue. (H) Infarct volume 24 hours after reperfusion. Infarct volume percentage (%) = white tissue volume/total volume × 100%. Sham operation group: Without modeling; I/R group: focal cerebral ischemia model only; nimodipine group: I/R injury + nimodipine; GPCL, GPCM, GPCH groups: I/R injury + 20, 40, 80 mg/kg GPC, respectively; GSPC group: I/R injury + 40 mg/kg GSPC. Data are expressed as the mean ± SD, with eight rats in each group. ^{##}*P* < 0.01, vs. I/R group (one-way analysis of variance followed by Bonferroni *post-hoc* test). I/R: Ischemia/reperfusion; Nim: nimodipine; TTC: 2,3,5-triphenyltetrazolium chloride; GPC: ginkgo proanthocyanidins; GPCL: low-dose GPC; GPCM: moderate-dose GPC; GPCH: high-dose GPC; GSPC: grape seed proanthocyanidins.

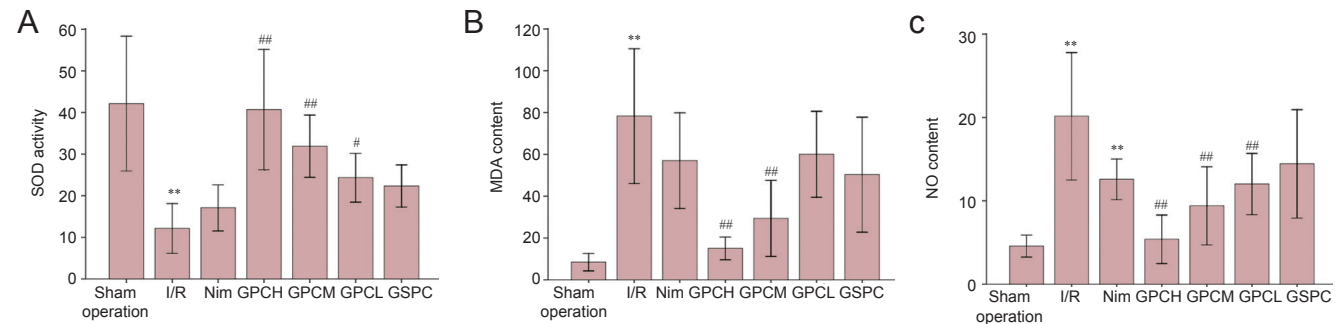


Figure 3 Effect of GPC on SOD activity, MDA and NO contents in the brain of rats 24 hours after cerebral I/R injury.

(A) SOD activity (U/mg); (B) MDA content (nmol/mg); (C) NO content (μmol/g). Sham operation group: Without modeling; I/R group: focal cerebral ischemia model only; nimodipine group: I/R injury + nimodipine; GPCL, GPCM, GPCH groups: I/R injury + 20, 40, 80 mg/kg GPC, respectively; GSPC group: I/R injury + 40 mg/kg GSPC. Data are expressed as the mean ± SD, with eight rats in each group. ^{**}*P* < 0.01, vs. sham operation group; [#]*P* < 0.05, ^{##}*P* < 0.01, vs. I/R group (one-way analysis of variance followed by Bonferroni *post-hoc* test). I/R: Ischemia/reperfusion; Nim: nimodipine; GPC: ginkgo proanthocyanidins; GPCL: low-dose GPC; GPCM: moderate-dose GPC; GPCH: high-dose GPC; GSPC: grape seed proanthocyanidins; SOD: superoxide dismutase; MDA: malondialdehyde; NO: nitric oxide.

brain tissue. A large quantity of SOD is consumed during free radical scavenging and the enzyme activity decreases significantly. Therefore, SOD activity indirectly reflects the content of scavenged oxygen free radicals (Andersson et al., 2012; Rakhunde et al., 2014). Our results show that PC effect on cerebral ischemia may contribute to reducing excessive generation of free radicals and improving SOD activity in brain tissue, or act against oxygen free radical damage. GSPC has decreased MDA content, and increased SOD activity, but was not significantly different from the I/R group. The infarct area significantly reduced in the nimodipine group. However, there was no significant difference in MDA content and SOD activity between nimodipine and I/R groups, because the action of nimodipine to improve cerebral ischemia is not by antioxidant effect, but through a scavenged pathway of lipid peroxidation. Nimodipine is a kind of Ca²⁺ channel blocker, effectively prevents Ca²⁺ into the cells, inhibits the smooth muscle contraction, and relieves vasospasm (Yedinak, 1993). We also measured the NO content in brain homogenates; the results demonstrated that GPC in each dose group significantly reduced the NO content in homogenates of ischemic brain tissue. Thus, the antioxidant activity in GPC plays an important role in the reduction of oxidative damage caused by excessive NO.

In conclusion, PC has preventive and protective effects on cerebral I/R injury in rats. The mechanism was associated with the decrease in oxidative injury. PC inhibits inflammatory reaction and activates survival pathways after I/R injury. Our study can provide evidence for relevant PC drugs.

Author contributions: WLC, HBH and RWW conceived and designed the study. WLC, LF, JNH and ZMJ performed the experiments. ZMJ provided data analysis. WLC wrote the paper. LF, HBH and RWW reviewed and edited the paper. All authors approved the final version of the paper.

Conflicts of interest: Zhejiang Conba Pharmaceutical Co., Ltd. provided financial support.

Plagiarism check: This paper was screened twice using CrossCheck to verify originality before publication.

Peer review: This paper was double-blinded and stringently reviewed by international expert reviewers.

References

- Andersson SEM, Svensson MND, Erlandsson MC, Dehlin M, Andersson KME, Bokarewa MI (2012) Activation of Fms-like tyrosine kinase 3 signaling enhances survivin expression in a mouse model of rheumatoid arthritis. *PLoS One* 7:e47668.
- Aras AB, Guven M, Akman T, Ozkan A, Sen HM, Duz U, Kalkan Y, Silan C, Cosar M (2015) Neuroprotective effects of daidzein on focal cerebral ischemia injury in rats. *Neural Regen Res* 10:146-152.
- Bak MJ, Truong VL, Kang HS, Jun M, Jeong WS (2013) Anti-inflammatory effect of procyanidins from wild grape (*Vitis amurensis*) seeds in LPS-induced RAW 264.7 cells. *Oxid Med Cell Longev* 2013:409321.
- Bennett J, Scott KJ (1971) Quantitative staining of fraction I protein in polyacrylamide gels using Coomassie brilliant blue. *Anal Biochem* 43:173-182.
- Candelario-Jalil E (2009) Injury and repair mechanisms in ischemic stroke: considerations for the development of novel neurotherapeutics. *Curr Opin Investig Drugs* 10:644-654.
- Chen M, Zhao SM, Li H, Kong XY (2010) Effect of pretreatment with scutellaria baicalensis stem-leaf total flavonoid on cerebral infarction volume and lipid peroxidation induced by focal cerebral ischemia reperfusion in rats. *Jieyou Xue Zazhi* 33:495-497.
- Floyd RA, Carney JM (1992) Free radical damage to protein and DNA: mechanisms involved and relevant observations on brain undergoing oxidative stress. *Ann Neurol* 32 Suppl:S22-27.
- Friedman M (2013) Anticarcinogenic, cardioprotective, and other health benefits of tomato compounds lycopene, α-tomatine, and tomatidine in pure form and in fresh and processed tomatoes. *J Agric Food Chem* 61:9534-9550.
- Honig LS (1991) Ischemic brain damage. *Neurology* 41:953.
- Imai F, Suzuki H, Oda J, Ninomiya T, Ono K, Sano H, Sawada M (2007) Neuroprotective effect of exogenous microglia in global brain ischemia. *J Cereb Blood Flow Metab* 27:488-500.
- Kan CY, Petti C, Bracken L, Maritz M, Xu N, O'Brien R, Yang C, Liu T, Yuan J, Lock RB, MacKenzie KL (2013) Up-regulation of survivin during immortalization of human myofibroblasts is linked to repression of tumor suppressor p16(INK4a) protein and confers resistance to oxidative stress. *J Biol Chem* 288:12032-12041.
- La VD, Howell AB, Grenier D (2010) Anti-porphyrinomonas gingivalis and anti-inflammatory activities of A-type cranberry proanthocyanidins. *Antimicrob Agents Chemother* 54:1778-1784.
- Li LX, Cheng YF, Lin HB, Wang C, Xu JP, Zhang HT (2011) Prevention of cerebral ischemia-induced memory deficits by inhibition of phosphodiesterase-4 in rats. *Metab Brain Dis* 26:37-47.
- Li WG, Zhang XY, Wu YJ, Tian X (2001) Anti-inflammatory effect and mechanism of proanthocyanidins from grape seeds. *Acta Pharmacol Sin* 22:1117-1120.
- Longa EZ, Weinstein PR, Carlson S, Cummins R (1989) Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 20:84-91.
- Morin C, Simon N (2006) Mitochondria: a target for neuroprotective interventions in cerebral ischemia-reperfusion. *Curr Pharm Des* 12:739-757.
- Pons Z, Guerrero L, Margalef M, Arola L, Arola-Arnal A, Muguerza B (2014) Effect of low molecular weight grape seed proanthocyanidins on blood pressure and lipid homeostasis in cafeteria diet-fed rats. *J Physiol Biochem* 70:629-637.
- Prakash A, Kumar A (2013) Lycopene protects against memory impairment and mito-oxidative damage induced by colchicine in rats: An evidence of nitric oxide signaling. *Eur J Pharmacol* 721:373-381.
- Rakhunde PB, Saher S, Ali SA (2014) Neuroprotective effect of Feronia limonia on ischemia reperfusion induced brain injury in rats. *Indian J Pharmacol* 46:617-621.
- Roof RL, Schielke GP, Ren X, Hall ED (2001) A comparison of long-term functional outcome after 2 middle cerebral artery occlusion models in rats. *Stroke* 32:2648-2657.
- Viuda-Martos M, Sanchez-Zapata E, Sayas-Barberá E, Sendra E, Pérez-Álvarez JA, Fernández-López J (2014) Tomato and tomato byproducts. Human health benefits of lycopene and its application to meat products: a review. *Crit Rev Food Sci Nutr* 54:1032-1049.
- Wang Y, Li WY, Zheng HX, Dong XJ (2011) The effect of astragaloside IV on neural cell apoptosis and the expression of apoptosis-related genes in hippocampus after cerebral ischemia-reperfusion in rats. *Zhongguo Linchuang Baojian Zazhi* 14:401-403.
- Weaver J, Liu KJ (2015) Does normobaric hyperoxia increase oxidative stress in acute ischemic stroke? A critical review of the literature. *Med Gas Res* 5:11.
- Wu HQ, Guo HN, Wang HQ, Chang MZ, Zhang GL, Zhao YX (2009) Protective effects and mechanism of puerarin on learning-memory disorder after global cerebral ischemia-reperfusion injury in rats. *Chin J Integr Med* 15:54-59.
- Yang LJ, Zhu DN, Dang YL, Zhao X (2016) Treatment of condyloma acuminata in pregnant women with cryotherapy combined with proanthocyanidins: Outcome and safety. *Exp Ther Med* 11:2391-2394.
- Yedinak KC (1993) Use of calcium channel antagonists for cardiovascular disease. *Am Pharm* 33:49-64.
- Zhang WX, Zhang QL, Deng W, Li Y, Xing GQ, Shi XJ, Du YF (2014a) Neuroprotective effect of pretreatment with ganoderma lucidum in cerebral ischemia/reperfusion injury in rat hippocampus. *Neural Regen Res* 9:1446-1452.
- Zhang YM, Xu H, Sun H, Chen SH, Wang FM (2014b) Electroacupuncture treatment improves neurological function associated with regulation of tight junction proteins in rats with cerebral ischemia reperfusion injury. *Evid Based Complement Alternat Med* 2014:989340.