

## Protective effect of phillyrin against cerebral ischemia/reperfusion injury in rats and oxidative stress-induced cell apoptosis and autophagy in neurons

Shu Chen<sup>a,#</sup>, Shan Zhang<sup>b,#</sup>, Honggang Wu<sup>a</sup>, Daobao Zhang<sup>a</sup>, Guoliang You<sup>a</sup>, Jing You<sup>a</sup>, and Niandong Zheng<sup>a</sup>

<sup>a</sup>Department of Cerebrovascular Disease, The People's Hospital of Leshan, Leshan, Sichuan, China; <sup>b</sup>Department of Neurology, The People's Hospital of Leshan, Leshan, Sichuan, China

### ABSTRACT

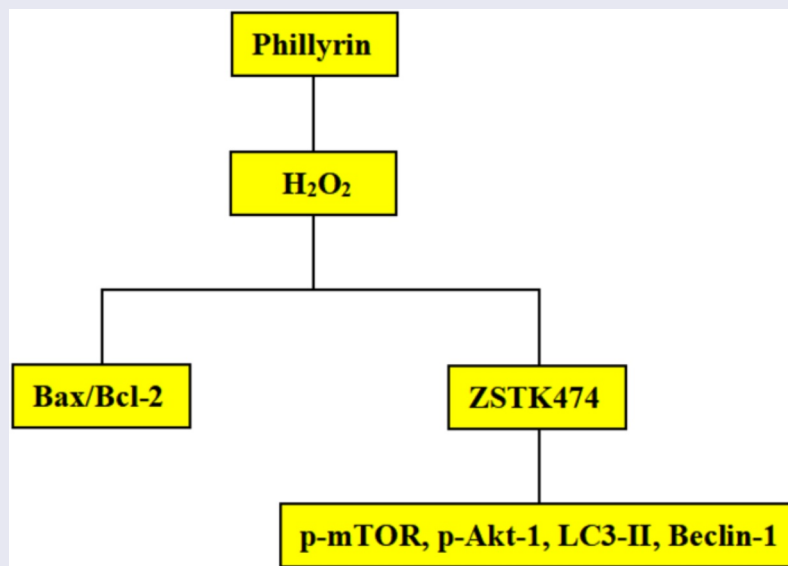
This study explored the role and potential molecular mechanism of phillyrin in cerebral ischemia/reperfusion (I/R) injury. The rat middle cerebral artery occlusion (MCAO)/R model was constructed, and cerebral infarction volume, brain water content, and neurological score were measured. Neuron morphological structures in brain tissues and primary neuron apoptosis were detected using hematoxylin and eosin (H&E) staining and Hoechst 33258 staining, respectively. In MCAO/R rats, phillyrin markedly reduced cerebral infarction volume, neurological score, and brain water content and inhibited neuron apoptosis. *In vitro* experiments showed that phillyrin remarkably increased viability and decreased lactate dehydrogenase (LDH) release of H<sub>2</sub>O<sub>2</sub>-injured neurons. Moreover, phillyrin remarkably downregulated the proportion of apoptosis-related protein B-associated X (Bax)/B-cell lymphoma protein 2 (Bcl-2) and reduced procaspase-3, phospho-Akt (p-Akt-1), and phosphorylation-mammalian target of rapamycin (p-mTOR) levels in H<sub>2</sub>O<sub>2</sub>-injured neurons. Furthermore, phosphatidylinositol-3 kinase (PI3K) inhibitor ZSTK474 weakened the effects of phillyrin on p-mTOR, p-Akt-1, characteristic proteins of autophagy 3-II (LC3-II) and beclin-1 levels, and H<sub>2</sub>O<sub>2</sub>-induced neuronal apoptosis and autophagy. Taken together, phillyrin alleviates I/R injury by inhibiting neuronal cell apoptosis and autophagy pathway, which may provide a new treatment strategy for cerebral I/R injury.



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**CONTACT** Niandong Zheng  [niandongzheng@aliyun.com](mailto:niandongzheng@aliyun.com)  Department of Cerebrovascular Disease, The People's Hospital of Leshan, No. 238, Baita Street, Central District, Leshan, Sichuan 614000, China

<sup>#</sup>Shu Chen and Shan Zhang contributed equally to this work.

 Supplemental data for this article can be accessed [here](#).

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## Background

Cerebral ischemia reperfusion (I/R) injury is a disease caused by restoring blood flow after cerebral ischemia for a certain period of time, leading to the aggravation of ischemic injury of brain tissues, with a high incidence and disability rate [1,2]. Oxidative stress is a major complication of cerebral I/R injury, which could result in the maladjustment of the body's antioxidant defense system, toxicity, cell damage, and apoptosis [3]. Therefore, many researchers have suggested that oxidative stress is one of the primary mechanisms that aggravates injury in the cerebral ischemia pathological process [4,5]. The main pathological features of cerebral I/R injury are neuron injury and apoptosis, with the latter being the major modality of neuron damage after cerebral I/R injury [6,7]. Neuronal apoptosis is a dynamic process. Apoptotic neuron number increases with the reperfusion time extending, further promoting nerve injury occurrence and development [8]. Autophagy is a complex physiological process in which damaged intracellular proteins and organelles are degraded into cell proliferating materials [9]. Moderate autophagy has a protective effect on cell growth, while excessive autophagy will lead to apoptosis [10]. Studies have shown that oxidative stress induces apoptosis and activates autophagy [11,12]. Thus, the relationship between apoptosis and autophagy is complex, involving multiple signal transduction pathways in cells [13].

Phillyrin is an important active ingredient in *Forsythia suspensa*, a traditional Chinese medicine [14]. Recent studies have found that phillyrin has anti-viral, anti-inflammation, anti-oxidation, and many other physiological functions. Phillyrin plays a protective role against  $H_2O_2$  in PC12 cells, a human brain neuroblastoma cell line by activating mitochondria-dependent apoptosis and reducing Bax/Bcl-2 ratio [15]. In addition, phillyrin could inhibit LPS-induced pulmonary inflammation in acute lung injury mice via suppressing mitogen-activated protein kinase (MAPK) and nuclear factor  $\kappa B$  (NF- $\kappa B$ ) activation [16]. Nevertheless, how phillyrin involves in cerebral I/R injury, neuronal cell apoptosis, and autophagy induced by oxidative stress remains unclear. Therefore, to explore the role and potential

molecular mechanism of phillyrin in cerebral ischemia/reperfusion (I/R) injury, we established a rat MCAO/R model using an improved MCAO method and an oxidative stress injury cell model induced by  $H_2O_2$  and further investigated whether and how phillyrin involves in cerebral I/R injury.

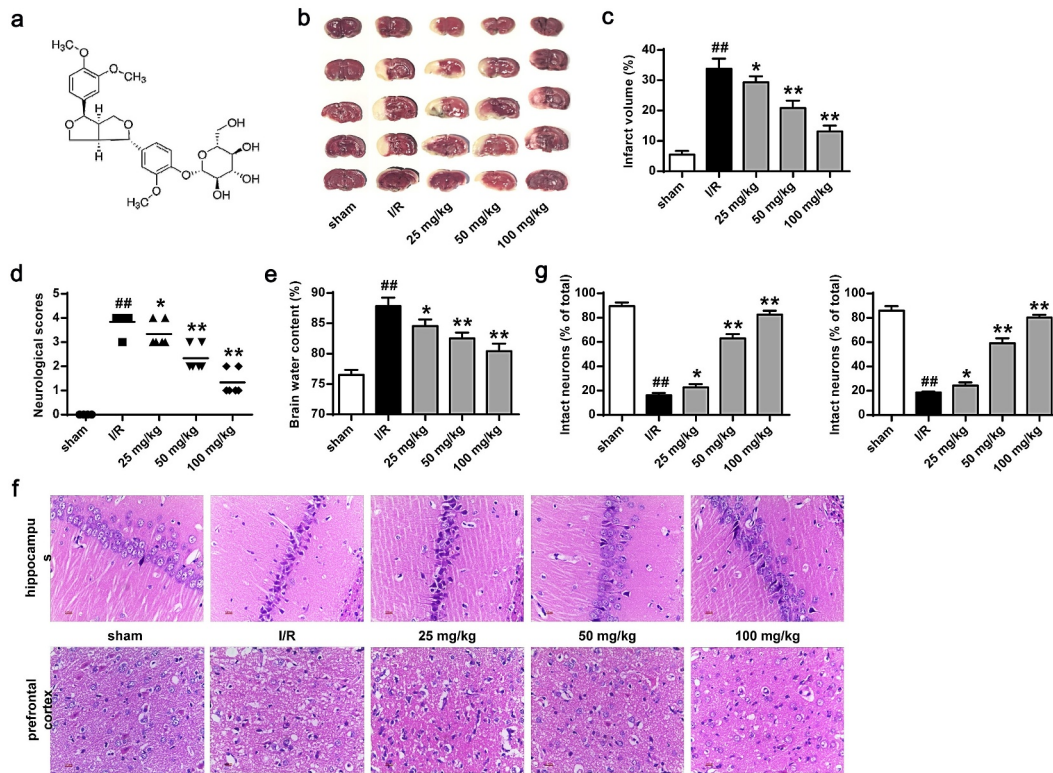
## Methods

### Materials

From the Experimental Animal Center of Leshan People's Hospital, male Sprague Dawley (SD) rats (220–250 g weight, 5–7 weeks old) were purchased and used to establish MCAO/R model. Primary cortical neurons prepared from SD rats (2 days old) were obtained from the Experimental Animal Center of Leshan People's Hospital. The Animal Research Committee of Leshan People's Hospital in China approved this study (No. LS753), which was conducted strictly following the guidelines of the National Institutes of Health for the care and use of experimental animals. Phillyrin with purity greater than 98% (molecular weight: 576.85) was obtained from Yuanye Biotechnology Company, Limited (Shanghai, China), and its chemical structure is shown in Figure 1(a). Using Neurobasal medium (Invitrogen, Carlsbad, CA, USA), phillyrin was dissolved as 10 mM DMSO and diluted to the required concentration as indicated.

### MCAO/R model establishment

MCAO/R model was established as previously reported [17]. The rat core temperature was maintained at 37°C throughout the test. First, 10% (w/v) chloral hydrate (350 mg/kg) was intraperitoneally injected into anesthetized rats. Next, after finding the left common carotid artery (CCA) and separating the external carotid artery (ECA), CCA and ECA were blocked with a hemostat. Subsequently, to avoid damaging the middle cerebral artery, the tip of a heparin-coated nylon monofilament (0.25–0.28 mm in diameter) was inserted into the internal carotid artery (ICA) from CCA. To detect the local cerebral blood flow (rCBF), a laser Doppler flowmeter was used. The criterion for judging the success of arterial occlusion was that the rCBF dropped



**Figure 1.** Phillyrin improved the MCAO/R rat brain injury. (a) Schematic diagram of chemical structure of phillyrin. (b) The cerebral infarction analyzed by TTC staining. (c) Percentage of cerebral infarction volume. (d) Neurological deficits of rats. (e) Brain water content of rats. (f) Surviving neuron number in the prefrontal cortex and hippocampus analyzed by H&E staining. (g) Percentage of infarct neurons in prefrontal cortex and hippocampus of rats. Note:  $n = 6$ ;  $*P < 0.05$  and  $**P < 0.01$  compared with I/R group,  $^{##}P < 0.01$  compared with the sham group.

below 20% of the baseline. After removing Nylon monofilaments 2 h after MCAO, rats were reperfused for 24 h. MCAO was conducted on the sham operation group, but no nylon monofilament was inserted.

The rats were divided into I/R group, sham operation group, and three phillyrin groups (25, 50, and 100 mg/kg). Three days before modeling, the rats in phillyrin treatment groups were given different doses of phillyrin daily via gavage, while rats in sham operation and I/R group were simultaneously given the same volume of saline. The last injection was given 30 min before the operation.

### TTC staining

The cerebral infarction volume in rats was measured using TTC staining [18]. First, the brain tissue was obtained and cut into slices with 1.5 mm in thickness. After fixed in 4% paraformaldehyde, the brain sections were stained with 1% TTC for 30 min. The normal tissues were

stained red, and the infarct tissues were kept white. The infarct volume was quantified using Bio-Rad Quantity One software. The infarct volume/total volume percentage was calculated.

### Neurological score

The effect of phillyrin on nerve injury rats induced by I/R was assessed based on the neurological score [19]. The neurobehavior of rats in each group was scored 24 h after reperfusion based on the specific scoring criteria: 4 for rats lost consciousness, 3 for rats fell to the left side, 2 for rats turned around, 1 for rats unable to fully extend the left foreleg, and 0 for rats with normal performance. Neurological deficit scores were evaluated by an examiner blinded to the experimental groups.

### Brain water content determination

Wet and dry weight of the infarcted cerebral hemisphere in rats 24 h after reperfusion was

measured before and after drying at 105°C as previously reported [20]. The total brain water content = (wet weight - dry weight)/wet weight × 100%.

### **H&E staining**

After fixed overnight with 4% paraformaldehyde, brain tissues in the prefrontal cortex and hippocampus were embedded with paraffin and cut into 6- $\mu$ m-thick coronal sections using a microtome. Sections were selected for H&E staining [21] in each rat, and the neuron survival was analyzed.

### **Culture of primary cortical neurons**

Primary cortical neurons were isolated and cultured as previously reported [22]. In brief, rat brain cortex tissues were digested using 0.125% trypsin in Hank's buffer at 37°C for 10 min. The digested cortical tissues were ground. In Neurobasal medium containing 100 U/mL streptomycin, 0.5 mM glutamine, 2% B27, and 100 U/mL penicillin, the isolated monocytes were cultured for 7 days to obtain mature neurons. The obtained neuron purity examined using MAP2 antibody (Chemicon, CA, USA) was over 98%. The phillyrin with different concentrations was used to treat primary cortical neurons for 2 h and then with H<sub>2</sub>O<sub>2</sub> of different concentrations to induce neuron injury.

### **Cell viability assay**

Cell viability was measured by MTT assay [23]. In brief, cortical neurons with a density of  $5 \times 10^4$  were inoculated in each well of 96-well plates for 7 days. Some cells were treated with different concentrations of H<sub>2</sub>O<sub>2</sub> for 30 min. Some cells were treated with H<sub>2</sub>O<sub>2</sub> (100  $\mu$ M) for different times. Some cells were treated with different concentrations of phillyrin for 2 h and then with H<sub>2</sub>O<sub>2</sub> (100  $\mu$ M) for 30 min. After treatment, the cells in each well were incubated with MTT solution (200  $\mu$ L of 0.5 mg/mL) for 4 h. The formazan in each well was solubilized in 150  $\mu$ L DMSO and determined by optical density at 570 nm on a microplate reader to represent neuron cell viability. The amount of LDH released from neurons

was determined using an LDH kit following the manufacturer's instructions to reflect cell damage degree.

### **Hoechst 33258 staining**

The neuron cell apoptosis was detected by staining the apoptotic nuclei with Hoechst 33258 staining [24] to generate blue fluorescence. Briefly, neuron cells with a density of 600 cells/mL were inoculated into each well. After cultured for 24 h, the cells were incubated with phillyrin for 2 h followed by 30 min treatment with H<sub>2</sub>O<sub>2</sub> (100  $\mu$ M). The cells were then stained with Hoechst 33258 (10  $\mu$ g/mL) and fixed with 4% paraformaldehyde. Cells with bright blue nuclei in 30 random fields of vision were counted under a fluorescence microscope.

### **Western blot**

Western blot was performed as previously reported [25]. In brief, primary neuron cells were incubated with 20, 40, and 80  $\mu$ M phillyrin for 2 h followed by 30 min treatment with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub>. Total proteins were extracted from these cells and transferred onto cellulose acetate membranes after separated by SDS-PAGE. After blocking with PBS solution for 1 h, the membranes were incubated with Bax (1:1000, Chemicon, Temecula, CA, USA), Bcl-2 (1:1000), and procaspase-3 (1:1000) primary antibodies from Chemicon, Temecula, CA, USA; pAkt-1 (1:1000), Akt-1 (1:1000), mTOR (1:1000), and LC3-II (1:1000) from Cell Signaling Technology, Danvers, MA, USA; and beclin-1 (1:1000) and  $\beta$ -actin (1:1000) primary antibodies from Sigma-Aldrich, St. Louis, MO, USA, at 4°C for overnight. Then, secondary antibody was added for 1 h at 25°C. ECL chemiluminescence solution was used to visualize protein bands and analyzed using Image J software.

### **Statistical analysis**

All data are expressed as mean  $\pm$  standard deviation (SD) and analyzed using one-way ANOVA and postmortem LSD tests.  $P < 0.05$  indicated a significant difference.

## Results

In this study, we explored the role and potential molecular mechanism of phillyrin in cerebral ischemia/reperfusion (I/R) injury. The rat MCAO/R model was constructed, and cerebral infarction volume, brain water content, and neurological score were measured. In MCAO/R rats, phillyrin markedly reduced cerebral infarction volume, neurological score, and brain water content and inhibited neuron apoptosis. Phillyrin remarkably increased viability and decreased LDH release of  $H_2O_2$ -injured neurons. Moreover, phillyrin remarkably downregulated the proportion of apoptosis-related protein Bax/Bcl-2 and reduced procaspase-3, p-Akt-1, and p-mTOR levels in  $H_2O_2$ -injured neurons. Furthermore, PI3K inhibitor ZSTK474 weakened the effects of phillyrin on p-mTOR, p-Akt-1, LC3-II, and beclin-1 levels and  $H_2O_2$ -induced neuronal apoptosis and autophagy. Our results suggested that phillyrin protects neurons by inhibiting neuron cell apoptosis and autophagy pathway, which provided new ideas for treating cerebral I/R injury.

### **Phillyrin improves the brain injury of MCAO/R rats**

The rat MCAO/R model was used to measure phillyrin (Figure 1(a)) function in the brain injury of MCAO/R rats (Figure 1(b)). Results (Figure 1(c-e),  $P < 0.05$ ,  $P < 0.01$ ) revealed that the cerebral infarction volume ( $33.77 \pm 2.74\%$ ), neurological score ( $3.83 \pm 0.37\%$ ), and cerebral water content ( $87.80 \pm 1.15\%$ ) in I/R group were remarkably higher than those in sham group but were significantly reduced in groups treated with phillyrin ( $P < 0.01$ ). The mortality rate of rats was about 19% in all ischemic groups (data not shown).

In addition, H&E staining found obvious nuclear pyknosis and disordered arrangement in the neurons in the prefrontal cortex and hippocampus of rats in I/R group (Figure 1(f)). Moreover, intact neuron number was remarkably decreased in the I/R group, but phillyrin treatment increased intact neuron number in rat prefrontal cortex and hippocampus in a dose-dependent manner in the I/R group (Figure 1(g),  $P < 0.05$ ,

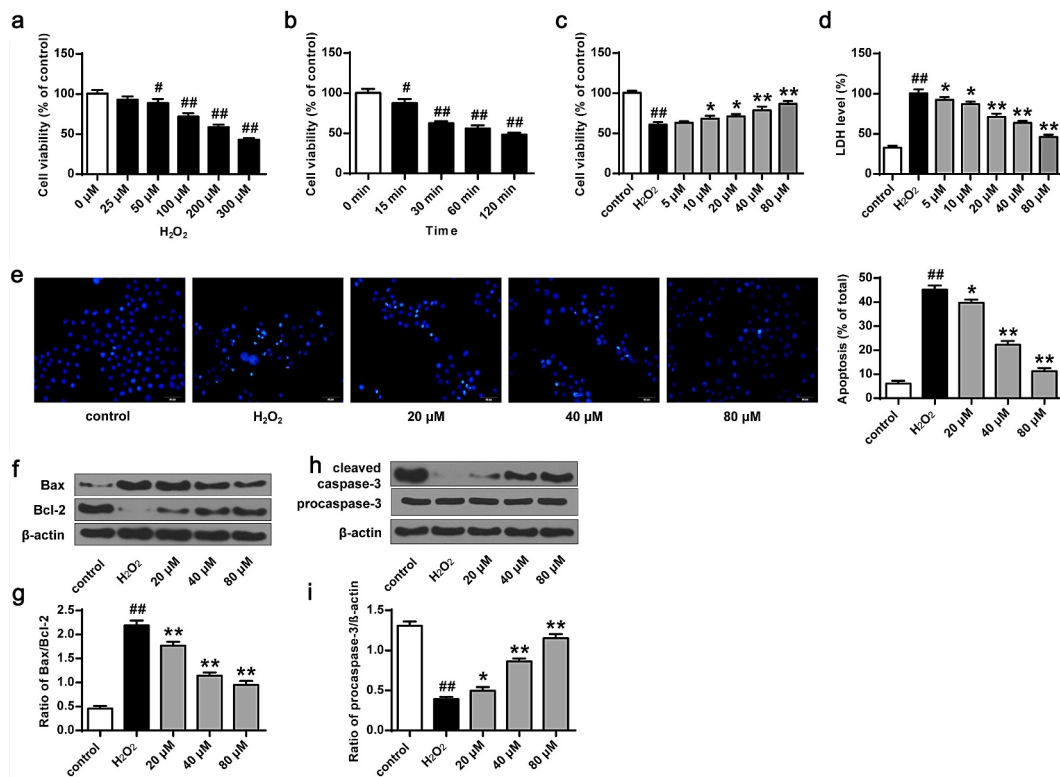
$P < 0.01$ ). These results confirmed that phillyrin could remarkably alleviate rat cerebral I/R injury.

### **Phillyrin protects primary cortical neurons against $H_2O_2$ -induced injuries**

To explore phillyrin function in primary cortical neuron activity damaged by  $H_2O_2$ , we first treated primary cortical neuron cells with  $H_2O_2$  at different doses for different times to establish an oxidative damage model. MTT assay showed that  $H_2O_2$  treatment remarkably reduced the activity of primary cortical neuron cells and increased LDH release ( $P < 0.05$ ,  $P < 0.01$ ) in a dose- (Figure 2(a)) and time-dependent manner (Figure 2(b)). In addition,  $H_2O_2$  treatment also increased Bcl-2 and Bax expression (Figure 2(f),  $P < 0.05$ ) and increased cleaved caspase-3 level (Figure 2(h),  $P < 0.05$ ). To examine the effect of phillyrin treatment, we treated primary cortical neuron cells with  $100 \mu M H_2O_2$  for 30 min. We found that phillyrin treatment, in a dose-dependent manner, improved the viability (Figure 2(c),  $P < 0.05$ ) and reduced apoptosis rate of the  $H_2O_2$ -treated primary cortical neurons (Figure 2(e),  $P < 0.01$ ) and decreased LDH release from the  $H_2O_2$ -treated primary cortical neurons (Figure 2(d),  $P < 0.01$ ). Moreover, phillyrin treatment reduced Bax and Bcl-2 expression (Figure 2(g),  $P < 0.01$ ) and enhanced cleaved caspase-3 expression in the  $H_2O_2$ -induced primary cortical neuron cells in a dose-dependent manner (Figure 2(i),  $P < 0.01$ ). These indicated that phillyrin protected primary cortical neuron cells against  $H_2O_2$ -induced injuries.

### **Phillyrin promotes Akt-1 phosphorylation in primary neurons**

We then determined the effect of phillyrin on Akt-1 phosphorylation in primary neuron cells. First, we examined changes of p-Akt-1 level in primary cortical neuron cells after  $H_2O_2$  treatment. Western blot results showed that p-Akt-1 level was significantly decreased in primary cortical neuron after  $H_2O_2$  exposure for 15 min and 30 min and 60 min (Figure 3(a),  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.01$ ), indicating that  $H_2O_2$  exposure decreased p-Akt-1 in a time-dependent manner.



**Figure 2.** Phillyrin improved the activity of primary neurons damaged by H<sub>2</sub>O<sub>2</sub>. (a) Cell viability treated with different concentrations of H<sub>2</sub>O<sub>2</sub>. (b) Cell viability treated with H<sub>2</sub>O<sub>2</sub> (100 μM) at different time. (c) Effects of phillyrin at different concentrations on H<sub>2</sub>O<sub>2</sub>-treated neuron cell viability. (d) Effects of phillyrin with different concentrations on LDH release in H<sub>2</sub>O<sub>2</sub>-treated neuron cells. (e) Images of Hoechst staining of neurons and apoptosis rate of neurons in each group. (f, g) Effects of phillyrin with different concentrations on Bax and Bcl-2 expression in H<sub>2</sub>O<sub>2</sub>-treated neurons. (h, i) Effects of phillyrin with different concentrations on procaspase-3 expression in H<sub>2</sub>O<sub>2</sub>-treated neurons. Note:  $n = 6$ ; \* $P < 0.05$  and \*\* $P < 0.01$  compared with the H<sub>2</sub>O<sub>2</sub> group; #  $P < 0.05$  and ##  $P < 0.01$  compared with the control group.

The results showed that phillyrin pretreatment increased p-Akt-1 level in primary cortical neuron cells treated with H<sub>2</sub>O<sub>2</sub> for more than 30 min in a time- (Figure 3(b),  $P < 0.01$ ) and dose- (Figure 3(c),  $P < 0.05$ ,  $P < 0.01$ ) dependent manner. These demonstrated that phillyrin promotes p-Akt-1 level in primary neuron cells in a dose- and time-dependent manner.

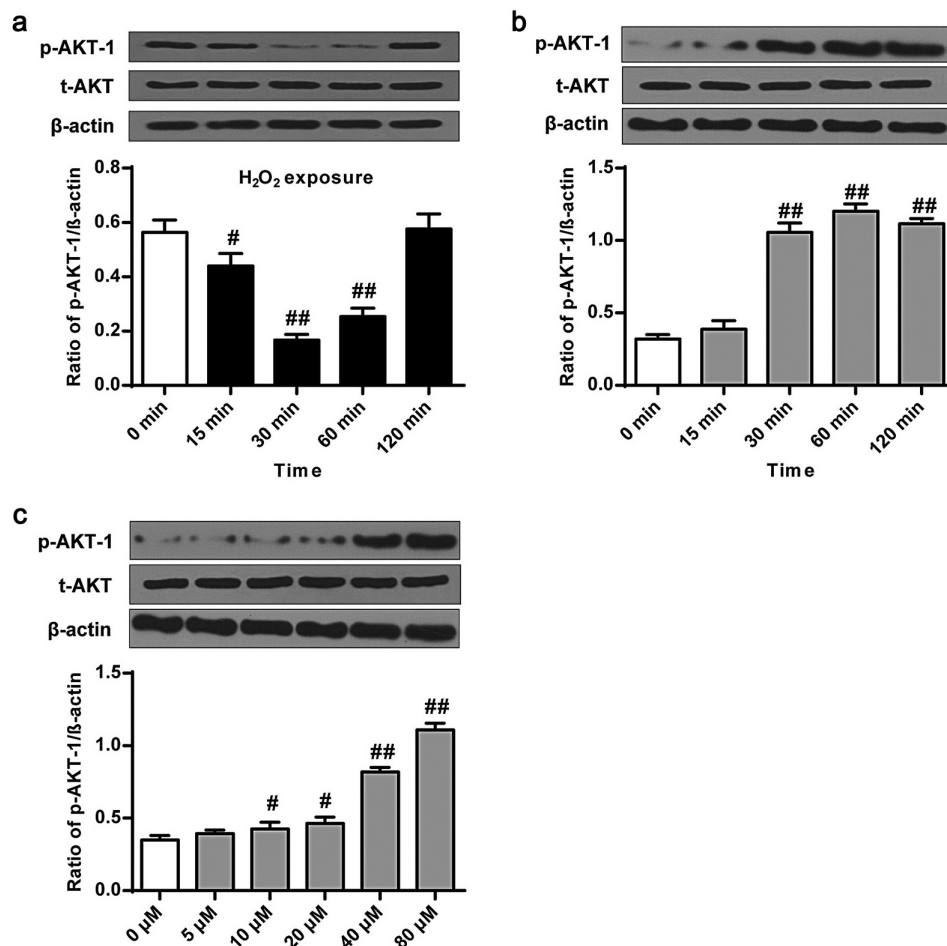
### Phillyrin improves autophagy of H<sub>2</sub>O<sub>2</sub>-treated primary neurons

We further investigated the role of phillyrin in the autophagy of primary neurons after H<sub>2</sub>O<sub>2</sub> exposure by detecting mTOR, LC3-II, and beclin-1 expression. The results showed that phillyrin reversed H<sub>2</sub>O<sub>2</sub>-induced decreases in p-mTOR and p-Akt-1 and increases in beclin-1 and LC3-II in primary cortical neuron cells in a dose-dependent manner (Figure 4(a-c),  $P < 0.05$ ,

$P < 0.01$ ). These effects were blocked by PI3K inhibitor ZSTK474 (Figure 4(d)). Moreover, ZSTK474 treatment also blocked the effects of phillyrin on H<sub>2</sub>O<sub>2</sub>-induced increased LDH release from primary cortical neuron cells (Figure 4(e),  $P < 0.01$ ). It is possible that the dose of ZSTK474 is insufficient to totally block the effects of phillyrin. Altogether, these indicated that phillyrin protects primary cortical neuron cells against oxidative stress-induced injuries via the PI3K/Akt-1/mTOR pathway.

### Discussion

It has been shown that phillyrin could decrease neutrophil infiltration, reduce tissue necrosis, and increase survival rate in the LPS-stimulated zebrafish model [16]. Nevertheless, the mechanism underlying the roles of phillyrin in oxidative stress-induced neuronal cell apoptosis and

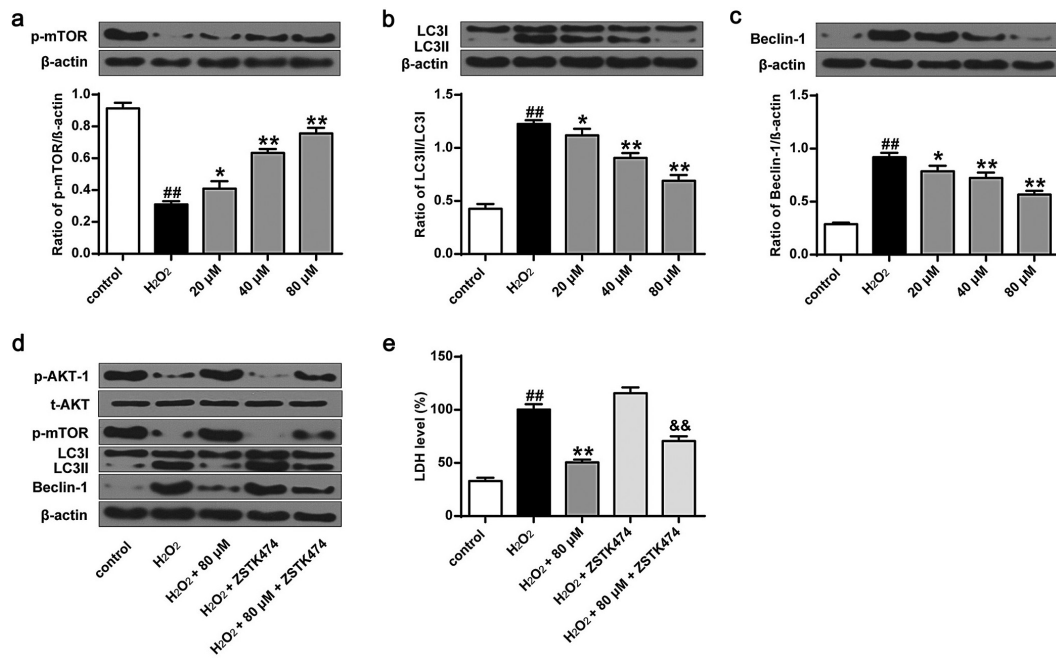


**Figure 3.** Phillyrin promoted Akt-1 phosphorylation in primary neurons. (a) Western blot showing (a) the effects of  $H_2O_2$  (100  $\mu$ M) at different treatment time on Akt and p-Akt-1 levels, (b) the effects of phillyrin at different treatment time on Akt and p-Akt-1 levels in  $H_2O_2$ -treated rat cortical neurons, and (c) the effects of phillyrin at different concentrations on Akt and p-Akt-1 levels in  $H_2O_2$ -treated rat cortical neurons. Note:  $n = 6$ ;  $^{\#}P < 0.05$  and  $^{\#\#}P < 0.01$  compared with control group.

autophagy and cerebral I/R injury remains unclear. Here, we found that phillyrin treatment improved the cerebral water content, neurologic score, and cerebral infarction of rats in the I/R group. Zhang *et al.* reported that dendrobium polysaccharides alleviated focal cerebral I/R injury through decreasing neurological function, brain water content, and cerebral infarction volume in rats [26]. Yang *et al.* reported that ginsenoside Rg1 alleviated cerebral I/R injury via PPAR/HO-1 pathway [25]. Here, we showed that phillyrin mitigated MCAO/R-induced brain injury in rats and had certain neuroprotective effects.

Currently, oxidative stress is considered to be one of the factors causing nerve cell apoptosis in cerebral I/R injury [27].  $H_2O_2$  is a strong oxidant able to produce a large amount of oxygen free

radicals through Haber–Weiss or Fenton reactions in cells, resulting in oxidative damage and apoptosis of cells [28,29]. In this study, we established an oxidative stress injury model by treating rat primary cortical neurons with  $H_2O_2$ . Previous studies have used the ratio of Bax to Bcl-2 to reflect the cell viability [30,31] and revealed that activation of PI3K/Akt signaling pathway inhibits cell apoptosis [32]. Sun *et al.* showed that luteolin markedly decreased myocardial infarction area, myocardial apoptosis rate, and LDH release in rats with myocardial I/R injury by upregulating Bcl-2 and p-Akt, thereby improving cardiac function [33]. In this study, we found that phillyrin increased the activity of  $H_2O_2$ -injured primary neurons and dramatically inhibited  $H_2O_2$ -induced LDH release and the apoptosis of neuron



**Figure 4.** Phillyrin enhanced autophagy of H<sub>2</sub>O<sub>2</sub>-treated primary neuron cells. Western blot analysis showing the effects of phillyrin with different concentrations on mTOR (a), LC3 (b), and beclin-1 (c) in H<sub>2</sub>O<sub>2</sub>-treated neurons, and (d) the effects of ZSTK474 on the effects of phillyrin in H<sub>2</sub>O<sub>2</sub>-treated neurons. (e) LDH release from H<sub>2</sub>O<sub>2</sub>-treated neurons with different treatments. Note:  $n = 6$ ; \*  $P < 0.05$  compared with the H<sub>2</sub>O<sub>2</sub> group; \*\* $P < 0.01$  compared with the H<sub>2</sub>O<sub>2</sub> group; && $P < 0.01$  compared with the phillyrin group; ## $P < 0.01$  compared with the control group.

cells. Furthermore, phillyrin markedly downregulated the ratio of Bax/Bcl-2 and significantly upregulated pAkt-1 level in H<sub>2</sub>O<sub>2</sub>-injured neurons. These results suggest that phillyrin antagonizes H<sub>2</sub>O<sub>2</sub>-induced neuron cell apoptosis by activating PI3K/Akt signaling pathway and increasing the ratio of Bax and Bcl-2.

Studies have shown that oxidative stress not only induces apoptosis but also activates autophagy [34]. PI3K/AKT/m-TOR signaling pathways are one of the important intracellular pathways widely present in cells and are involved in the regulation of cell growth, migration, differentiation, apoptosis and metabolism, and other biological processes [35]. PI3K is a lipid kinase regulating the activation of numerous intracellular signal cascades and functioning as various inflammatory mediators [36]. In cerebral ischemia/reperfusion injury, PI3K can not only cause the recruitment of a variety of inflammatory cytokines but also regulate the activation of neutrophils, eosinophils, and macrophages [37]. There are a variety of biological molecules downstream of PI3K. AKT is not only a direct target downstream of PI3K but also the most critical target

enzyme responsible for transmitting the biological information of PI3K initiation. m-TOR, a downstream effector molecule of AKT, is a serine-threonine kinase regulating various anabolic and catabolic cellular processes [38]. Studies have reported that the PI3K/AKT/m-TOR signaling pathway is involved in the development of inflammatory responses, such as the onset of temporal lobe mesenchymal epilepsy and the occurrence of inflammatory bowel disease [39]. Here, phillyrin markedly upregulated m-TOR expression and downregulated the levels of beclin-1 and LC3-II in H<sub>2</sub>O<sub>2</sub>-injured neuron cells. Moreover, PI3K inhibitor ZSTK474 significantly inhibited the effects of phillyrin on p-Akt-1, p-mTOR, LC3-II, and beclin-1 levels in H<sub>2</sub>O<sub>2</sub>-injured neuron cells and the effects of phillyrin on H<sub>2</sub>O<sub>2</sub>-induced neuron apoptosis and autophagy. Ye *et al.* reported that brusatol induced liver cancer cell apoptosis by inhibiting autophagy-related PI3K/Akt/mTOR signaling pathway [40]. Yu *et al.* found that tetrandrine activated autophagy of HSC-3 cells by activating the caspases/beclin-1/LC3-I/II signaling pathway and inducing cell apoptosis [41]. Consistently, our results suggested



that phillyrin exerted its protective effects in H<sub>2</sub>O<sub>2</sub>-injured neuron cells by activating PI3K/Akt/m-TOR signaling pathway and inhibiting autophagy via affecting beclin-1 and LC3-II expression. However, this study has some limitations. We established the MCAO/R model through SD rats (weight 220–250 g and 5–7 weeks old) and applied phillyrin to the MCAO/R model rats. In future studies, we hope to include some I/R injury volunteers to further accelerate the clinical process.

## Conclusion

A rat MCAO/R model was constructed to explore the role and potential molecular mechanism of phillyrin in cerebral ischemia/reperfusion (I/R) injury. Cerebral infarction volume, brain water content, and neurological score were measured. H&E staining was used to detect neuron morphological structures in brain tissues. Hoechst 33258 staining was used to determine the apoptosis of primary neurons. Our results as graphical abstract suggested that phillyrin protects neurons by inhibiting neuron cell apoptosis and autophagy pathways, which provided new ideas for treating cerebral I/R injury.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

The authors reported that there is no funding associated with the work featured in this article.

## Availability of data and material

Data are available upon reasonable request.

## Author's contribution

NDZ supervised the whole study and provided the material source. SC, SZ, HGW, DBZ, GLY, and JY performed the experiments, analyzed the data, and prepared the manuscript.

## Ethics approval and consent to participate

This study was approved by the Animal Research Committee of Leshan People's Hospital in China (No. LS753) and conducted strictly following the guidelines of the National Institutes of Health for the care and use of experimental animals.

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