MEDICAL SCIENCE MONITOR

ANIMAL STUDY

e-ISSN 1643-3750
© Med Sci Monit, 2020; 26: e918772
DOI: 10.12659/MSM.918772

Received: 2019.07.16
Accepted: 2019.08.12
Published: 2020.01.04

Protective Effects of Naringin in Cerebral Infarction and Its Molecular Mechanism

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Source of support: Departmental sources

Background: Cerebral infarction is a cardiovascular disease with high morbidity and mortality. At present, many studies have reported the treatment of cerebral infarction by traditional Chinese medicine. Naringin, a flavonoid, is a major traditional Chinese medicine. However, the effect and mechanism of naringin on cerebral infarction is unclear.

Material/Methods: In our study, we established a rat model of cerebral infarction through middle cerebral artery occlusion (MCAO) to study the influence of naringin on cerebral infarction *in vivo*. After treatment with naringin, brain water content was detected to assess brain edema. Cerebral infarction volume and neurological deficits were also measured. Production of the inflammatory factors tumor necrosis factor (TNF)-α and interleukin (IL)-6) was measured using enzyme-linked immunosorbent assay (ELISA). Besides, the effect of naringin on cerebral infarction was investigated *in vitro* by establishing an oxygen-glucose deprivation (OGD) model in neuronal cells. Cell apoptosis and cell viability was determined using flow cytometry and MTT assay.

Results: We found that naringin pretreatment significantly decreased the brain water content, cerebral infarction volume, and neurological deficit scores of MCAO subjected rats. And naringin treatment reduced apoptosis of nerve cells in rat hippocampus and the secretion of inflammatory factor such as TNF-α and IL-6. Besides, we found that naringin increased cell viability and inhibited apoptosis in OGD induced neuronal cells. Finally, we found that naringin promoted the expression of p-AKT protein in a concentration-dependent manner and activated the PI3K/AKT pathway in OGD induced neurons.

Conclusions: Naringin played a protective role in cerebral infarction via suppressing neuronal apoptosis and inflammation.

MeSH Keywords: Cerebral Infarction • Infarction, Middle Cerebral Artery • Phosphatidylinositol 3-Kinases • Proto-Oncogene Proteins c-akt

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/918772

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Background

Cerebral infarction, a cardiovascular disease, is caused by brain blood supply disorders [1]. Cerebral infarction patients are usually in the population of people who are: aged 50 to 60 years old, usually have atherosclerosis, hypertension, rheumatic heart disease, coronary heart disease or diabetes, as well as patients with bad health habits such as smoking and drinking. At present, cerebral infarction has a high morbidity and mortality, which seriously affects people's lives [2]. Currently, treatments for cerebral infarction have 2 treatment methods including drug therapy and thrombectomy [3,4]. However, there are many reports on the treatment of cerebral infarction by traditional Chinese medicine. Previous studies showed that Danshen, the dried root of Salvia miltiorrhiza, which promotes blood flow, is a traditional Chinese medicine for treating blood vessels [5]. Chen et al. showed that Huaihua could reduce the size and neurological deficit of cerebral infarction [6].

Naringin, a foremost flavonoids compound, is mainly found in the grapefruit fruit of the Rutaceae family [7]; in the peel and pulp of grapefruit and orange. At present, naringin has anti-inflammatory, anti-viral, anti-cancer, anti-ulcer, analgesic, and antihypertensive activities. In addition, naringin can lower blood cholesterol, reduce the formation of blood clots and improve local microcirculation and nutrient supply. And naringin can be used for prevention cardiovascular and cerebrovascular diseases. Naringin is currently available in the market for use in certain projects and is safe for humans [8–10]. It has been reported that naringin has many biological activities [11]. Geng et al. indicated that naringin promoted cell proliferation and osteogenic differentiation in human bone marrow mesenchymal stem cells, which played an important role in the proliferation of osteoblasts [12]. Zhang et al. showed that naringin has anti-osteoporosis effect and enhances bone consistency by promoting the proliferation and osteogenic differentiation of human bone marrow mesenchymal stem cells [13]. However, it is not clear about the role and function of naringin in cerebral infarction.

The purpose of this study was to explore whether naringin plays a certain role in cerebral infarction *in vitro* and *in vivo,* and further to study specific molecular mechanisms. Our study may provide some theoretical knowledge and new target for the treatment of cerebral infarction.

Material and Methods

Establishment of animal models

We selected 50 healthy Sprague-Dawley male rats, each weighing approximately 250 to 280 g. All experimental procedures were received prior approval of the Institutional Animal Care and Use Committee of Taicang City Hospital of Traditional Chinese Medicine and followed the guidelines by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Rats were kept for a period of time and given plenty of water and food to adapt to the environment. Subsequently, we randomly assigned rats to 5 groups of 10 rats in each group: control group; sham group; model group; model+vehicle (normal saline) group; and model+naringin group. Rats in the model+naringin group were intraperitoneally injected with naringin (5 mg/kg) for 7 consecutive days prior to middle cerebral artery occlusion (MCAO) surgery. Rats in model+vehicle group were injected with normal saline of 10 mL/kg per rat weigh for 7 consecutive days, and then MCAO surgery was performed. Briefly, we first anesthetized the rats by intraperitoneal injection with 5% chloral hydrate and then cut them along the midline of the neck. The exposed external carotid artery is then closed with a sterile suture, and the internal carotid artery is clamped using a vascular clamp to prevent major bleeding. Then, the carotid artery was incised and ligated using silicone-coated sutures (4-0). After 2 hours, the rats were reperfused. When the rats developed hemiplegia on the left side, the contralateral forelimb sag, standing instability and other symptoms, indicating that the cerebral infarction model was successfully constructed.

Cell culture and treatment

Primary nerve cells were acquired from newborn rats. In brief, we used ether to anesthetize the rats and then rats were killed by cervical dislocation. We collected hippocampus that was digested with 0.05% trypsin (Gibco) and centrifuged hippocampus. After a while, the deposits were resuspended in Dulbecco's Modified Eagle Medium (DMEM) medium (Gibco, Grand Island, NY, USA) containing 5% serum (Gibco). The cells were treated with different ways and divided into 3 groups: control group, oxygen-glucose deprivation (OGD) group, and OGD+naringin treatment group. The cells were cultured with normal medium at 37°C in a 5% CO_2 incubator in the control group. The cells were pretreated with OGD (95% N_2 and 5% CO_2) for 24 hours, then we added 6, 12, or 25 μg/mL naringin and continued culturing (95% N_2 and 5% CO_2) for 48 hours.

Brain edema assessment

To assess the brain edema, we detected the brain water content in rat brain tissues. Briefly, after reperfusion for 24 hours, we used 5% chloral hydrate to anesthetize the rats (400 mg/kg) and the rats were killed by cervical dislocation. Then, the rat was immediately dissected, and the brain part was divided into 2 parts along the midline, and the cerebellum was removed. Immediately, we weighed the brain samples to get a wet weight, and then we placed the brain samples to a dry

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gravity oven at 100°C for 24 hours to get a dry weight. Finally, brain water content was calculated as: brain water content (%)=(wet weight–dry weight)/wet weight×100%.

Cerebral infarction volume measurement

After 24 hours of reperfusion, we used 5% chloral hydrate to anesthetize the rats and the brain of the rats was quickly cut. The forebrain was then cut into 6 coronal (2 mm thick) sections, and we stained each section for 5 minutes using 2% 2,3,5-triphenyltetrazolium chloride (TTC) (Sigma, St. Louis, MO, USA). Finally, we performed ImageJ software to analyze the volume of cerebral infarction in every section.

Neurological evaluation

After 24-hour reperfusion, neurological deficits were assessed according to previous methods [14]. The score was rated as 0: no neurological deficit; 1: not fully extended left forepaw; 2: left forepaw was circled; 3: fell to left forepaw; 4: could not be released and had depression.

MTT assay

Neuronal cells viability was determined by MTT assay. In brief, cells were plated in 96-well plate at a density of 5×10^3 cells/well. Neuronal cells were pretreated with OGD for 24 hours and then added with 6, 12, or 25 μg/mL naringin and cultured for another 48 hours, then 20 μL MTT reagent (Solarbio, Beijing, China) was added into each well for another 4 hours of incubation at 37°C. Then 150 μL DMSO was added into each well and shaken for 15 minutes. The optical density (OD) values were read at 490 nm using a micro-plate reader.

Apoptosis detection

To analyze cell apoptosis, flow cytometry (BD Accuri™ Flow Cytometer, Franklin Lakes, NJ, USA) was performed. Neuronal cells were pretreated with OGD for 24 hours and then 6, 12, or 25 μg/mL of naringin was added and cells cultured for another 48 hours. Then, the cell apoptosis was determined by using the Annexin V-FITC/PI apoptosis detection kit (BD Biosciences, USA) following the manufacturer's instructions. FlowJo 7.6 software (TreeStar Inc., USA) was used to analyze the cell apoptosis rate.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was collected with TRIzol from neuronal cells (Takara, Nanjing, Jiangsu, China) according to the experimental procedure. And all processes were carried out on ice. After extracting the RNA, the concentration of each sample was measured using an ultraviolet spectrophotometer. Then, cDNA was synthesized by RNA using a reverse transcription kit (Vazyme, Nanjing, Jiangsu) according to the instructions. The reaction conditions were: 70°C for 5 minutes, 37°C for 5 minutes, and 42°C for 60 minutes. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed by SYBR kit (Vazyme). The qPCR transcription was performed using the following parameters: 95°C for 3 minutes, 40 cycles of 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds. GAPDH served as an internal control for normalization.

Western blot assay

Total proteins were acquired by using radioimmunoprecipitation assay (RIPA) lysis buffer (Solarbio, Beijing) containing protease inhibitor from neuronal cells. Cells were incubated with lysis buffer for 30 minutes on ice and then the cells were resuspended with lysate. Cells were centrifuged at 12 000 rpm for 10 minutes at 4°C to collect the proteins and then the supernatants were stored at -20°C. The protein concentration was detected with BCA assay kit. 30 μg of protein was subjected to SDS-PAGE electrophoresis and then transferred to PVDF membrane (Millipore, Billerica, MA, USA). It was blocked at room temperature with 5% fat-free powdered milk that was dissolved in TBS containing 0.1% Tween for 1.5 hours. The membrane was incubated with primary antibodies at 4°C overnight. Then it was incubated with HRP-conjugated secondary antibody at 4°C overnight. The protein band was visualized by enhanced chemiluminescence method (ECL, Millipore, Billerica, MA, USA). GAPDH served as loading control for normalization.

Primary antibodies: anti-Bcl-2, anti-Bax, anti-p-AKT, and anti-AKT were purchased from Cell Signaling Technology. Secondary antibodies: goat anti-rabbit antibody was purchased from Cell Signaling Technology.

Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) was performed to examine the expression of tumor necrosis factor (TNF)-α and interleukin (IL)-6 in the brain tissues of rats from different groups. Specific ELISA kits were used to detect related markers expression according to the manufacturer's instructions.

Statistical analysis

These data were presented as the mean ± standard deviation (SD) from at least 3 independent experiments. SPSS 18.0 (SPSS, Inc, Chicago, IL, USA) was used for statistical analysis. The differences between groups were analyzed by Student's *t*-test. $P<0.05$ indicated statistically significant difference.

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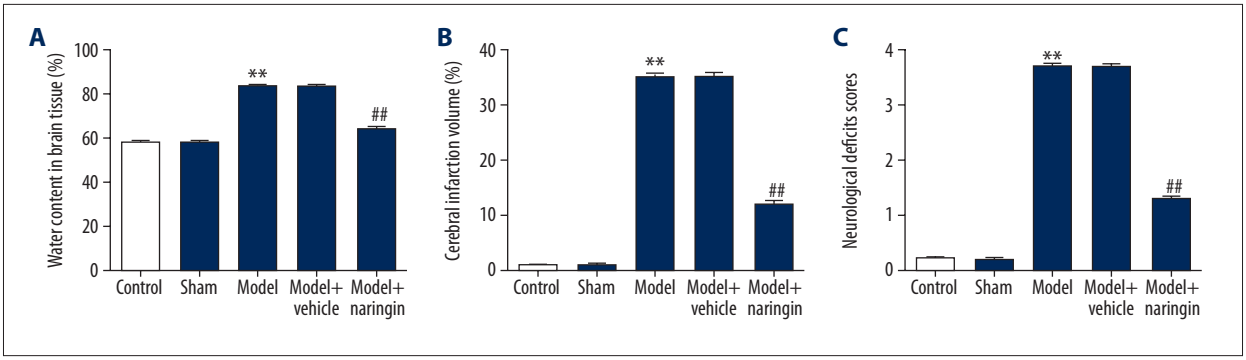

Figure 1. Naringin relieved cerebral infarction in the rat model. (**A**) Brain edema assessment that the brain water content between control group and rat model group. (**B**) Cerebral infarction volume was analyzed by ImageJ between control group and rat model group. (**C**) Neurological deficit scores were counted between control group and rat model group. Data were presented as mean±standard deviations. ** *P*<0.01 versus Control; ## *P*<0.01 versus Model.

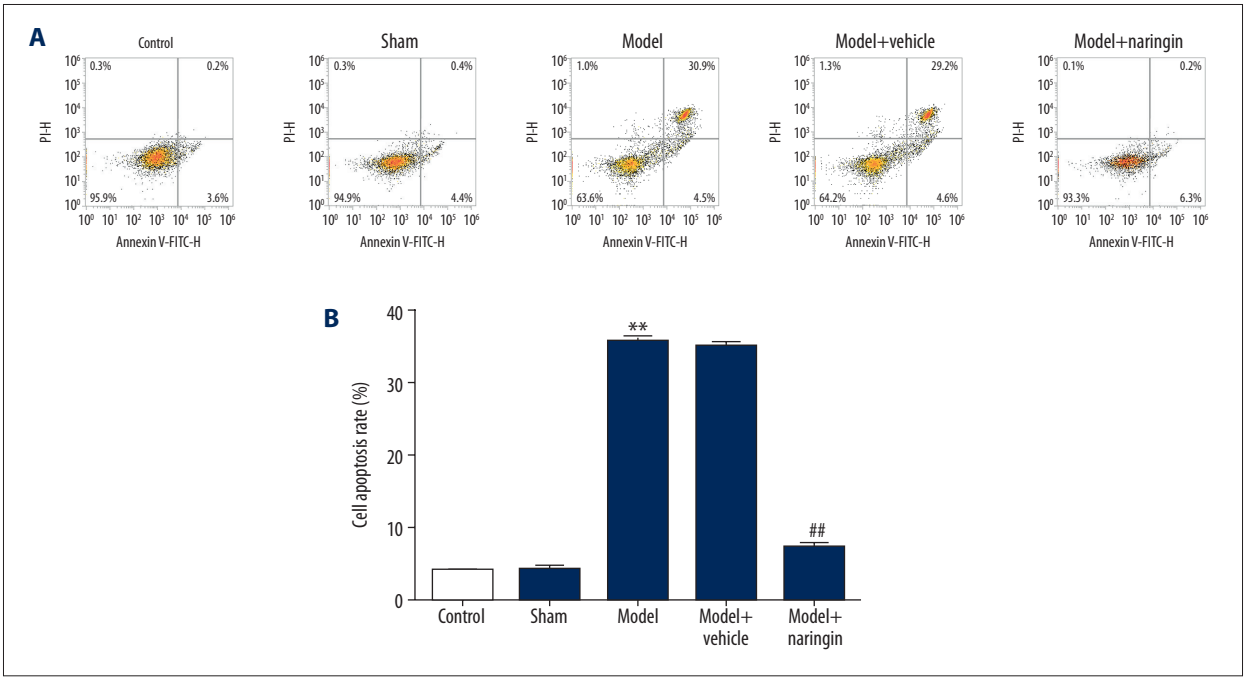

Figure 2. Naringin inhibited apoptosis of hippocampal neurons in cerebral infarction rats. (**A**) Flow cytometry analysis of the apoptosis of nerve cells in the brain tissues of rats in different groups. (**B**) cell apoptosis rate was calculated and presented. Data were presented as mean±standard deviation. ** *P*<0.01 versus Control; ## *P*<0.01 versus Model.

Results

Naringin relieved cerebral infarction in the body

To explore the effects of naringin on the rat model of cerebral infarction, we measured brain water content to evaluate brain edema, and cerebral infarction volume and neurological deficits were also measured. These results demonstrated that in comparison with the control group, there were significant increases in water content in brain tissue, cerebral infarct volume, and neurological deficit scores in the model group. And naringin pretreatment significantly reduced brain water content (Figure 1A), cerebral infarction volume (Figure 1B), and neurological deficit scores (Figure 1C) compared with model group. These results suggested that naringin relieved cerebral infarction in a rat model.

Naringin inhibited apoptosis of hippocampal neurons in cerebral infarction rats

Then, to determine the mechanism of the effect of naringin on rats with cerebral infarction, we detected the apoptosis of nerve cells in rat hippocampus by flow cytometry after reperfusion for 24 hours. These results demonstrated that the nerve

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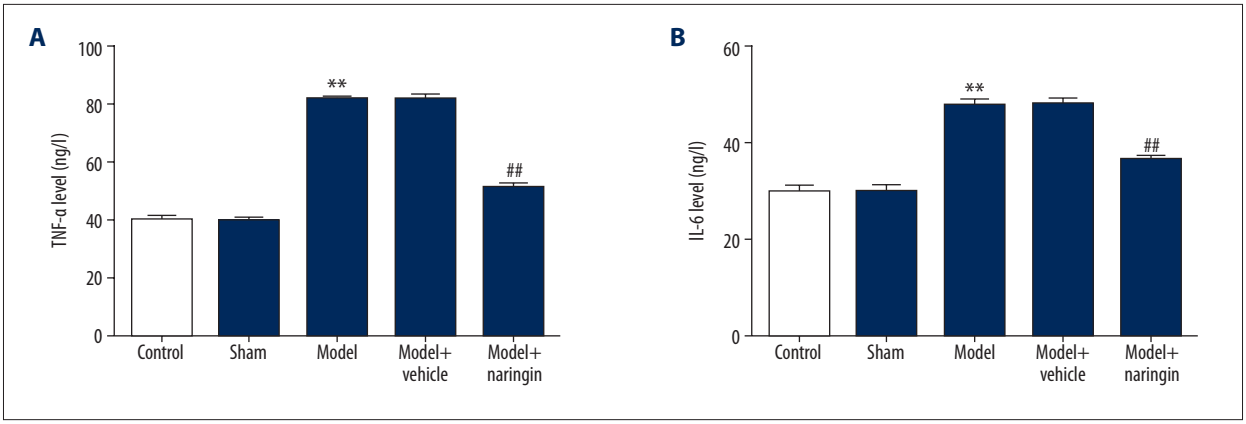

Figure 3. Naringin suppressed the expression of tumor necrosis factor (TNF)-α and interleukin (IL)-6 in the brain tissue of middle cerebral artery occlusion (MCAO) subjected rats. (**A**) Enzyme-linked immunosorbent assay (ELISA) assay was used to detect the expression of TNF-α in the brain tissue of rats from different groups. (**B**) ELISA assay was used to detect the expression of IL-6 in the brain tissue of rats from different groups. Data were presented as mean±standard deviation. ** $P<0.01$ versus Control; ## $P<0.01$ versus Model.

cell apoptosis of the model group was significantly increased (Figure 2A, 2B). However, naringin pretreatment significantly reduced cell apoptosis.

Naringin inhibited the secretion of TNF-α and IL-6 in cerebral infarction rats

Next, we performed ELISA to detect the secretion of inflammatory factors including TNF-α and IL-6 in cerebral infarction rats. ELISA assay indicated that in comparison with the control group, the secretion of TNF-α (Figure 3A) and IL-6 (Figure 3B) significantly increased in the model group. While naringin pretreatment significantly reduced the expression of TNF-α and IL-6.

Naringin increased nerve cell viability

Next, we studied the effect of naringin on neurons *in vitro*, and oxygen-glucose deprivation (OGD) model was established. Primary rat neuronal cells were pretreated with OGD (95% N_2 and 5% CO_2) for 24 hours, then different concentrations of naringin was added to continue culture (95% N_2 and 5% CO_2) for 48 hours. Primary rat neuronal cells of control group were cultured at 37°C with 5% CO_2. MTT assay showed that in comparison with the control group, neuronal cell viability of OGD group was decreased (Figure 4A). And flow cytometry assay demonstrated that cell apoptosis was induced in the OGD group (Figure 4B, 4C). Western blot assay and qRT-PCR assay results showed that Bax was upregulated in the OGD group (Figure 4D, 4E) and Bcl-2 expression was downregulated (Figure 4D, 4F). However, naringin increased neuronal cell viability in a concentration-dependent manner (Figure 4A), inhibited cell apoptosis (Figure 4B, 4C), decreased Bax expression (Figure 4D, 4E) and increased Bcl-2 expression (Figure 4D, 4F).

Naringin activated PI3K/AKT pathway in nerve cells

Finally, we further investigated the effect of naringin on crucial protein expression in the PI3K/AKT pathway. Compared with control group, p-AKT expression was decreased (Figure 5A), and the ratio of p-AKT/AKT was significantly reduced in the OGD group (Figure 5B). Naringin promoted p-AKT expression in a dose-dependent manner at protein level (Figure 5A) and improved the ratio of p-AKT/AKT (Figure 5B). AKT expression was not significantly different between groups at both protein and mRNA levels (Figure 5A, 5C).

Discussion

In our research, firstly, we established a cerebral infarction rat model. We found that compared with the sham group, the brain water content was increased, and the cerebral infarction volume and neurological deficits scores were increased in the rat model group. Naringin pretreatment obviously reduced the brain water content, the volume of cerebral infarction, and the neurological deficit score. Then we found that the apoptosis of nerve cell in rat hippocampus was distinctly increased in the model group, and naringin inhibited cell apoptosis. In addition, naringin pretreatment decreased the secretion of IL-6 and TNF-α. Next, we performed experiments *in vitro*. We established an OGD model. We found that naringin increased neuronal viability, inhibited apoptosis, and activated the expression of p-AKT protein in the PI3K/AKT pathway.

Acute cerebral infarction, also known as ischemic stroke, is the sudden stop of blood circulation in the brain, causing a sudden loss of the corresponding nerve function. Cerebral infarction has a high mortality rate and poses a threat to human health [2].

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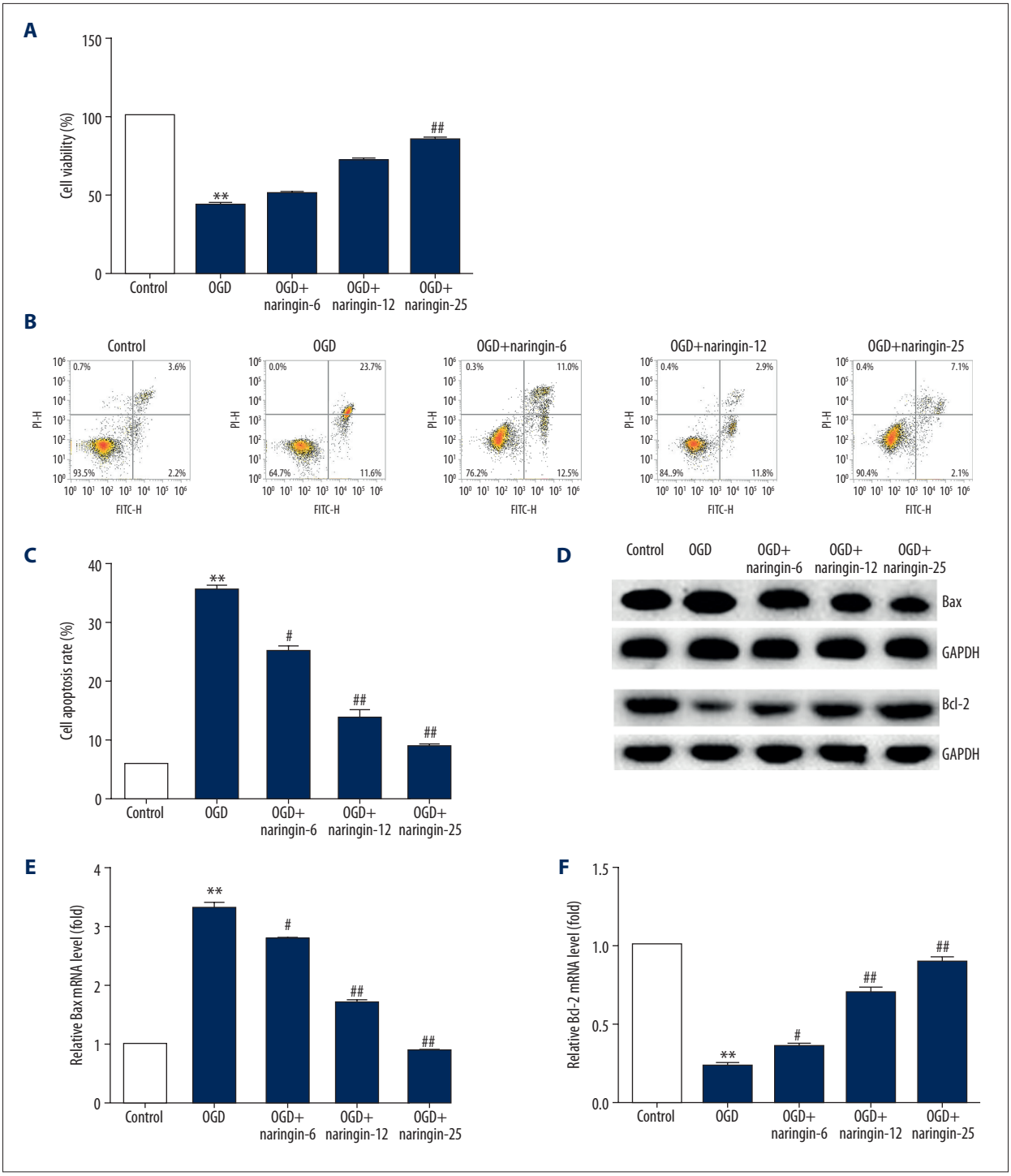

Figure 4. Naringin promoted cell viability and suppressed cell apoptosis in oxygen-glucose deprivation (OGD) induced neurons. (**A**) MTT assay was used to detect cell viability when primary rat neuronal cells were pretreated with OGD (95% N_2 and 5% CO_2) for 24 hour and then treated with 6, 12, or 25 μg/mL naringin for 48 hours. (**B**). Flow cytometry assay was used to detect cell apoptosis. (**C**) The cell apoptotic rate was calculated and presented. (**D**) Western blot analysis of Bax and Bcl-2 expression at protein level. (**E, F**) Quantitative real-time polymerase chain reaction assay was performed to measure the relative expression of Bax and Bcl-2 at mRNA level. Data were presented as mean±standard deviation ** P<0.01 versus Control; #, ## P<0.05, 0.01 versus OGD.

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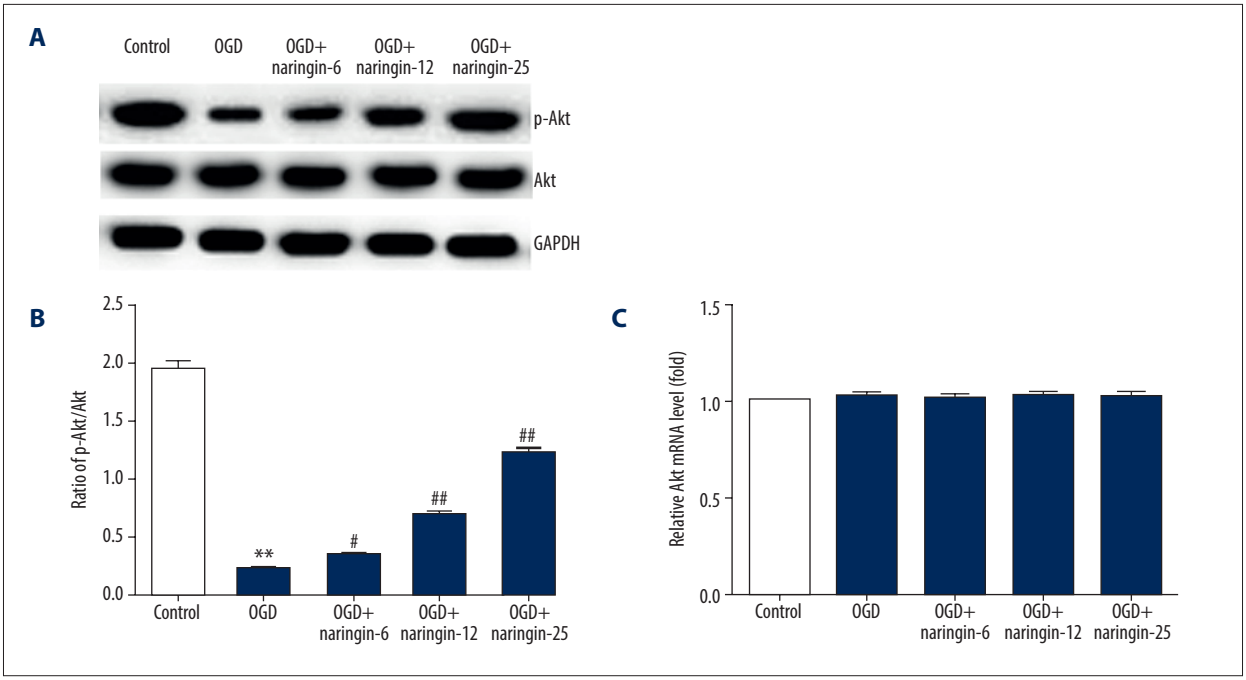

Figure 5. The relationship between naringin and PI3K/AKT pathway in oxygen-glucose deprivation (OGD) induced nerve cells. (**A**) Western blot assay that the protein expression level of p-AKT and AKT. (**B**) The ratio of p-AKT/AKT was calculated and expressed. (**C**) Quantitative real-time polymerase chain reaction assay was performed to measure the expression of AKT at mRNA level. Data were presented as mean±standard deviation. ** $P<0.01$ versus Control; #, ## $P<0.05$, 0.01 versus OGD.

At present, the treatment of cerebral infarction is mainly medical treatment. Traditional Chinese medicine is a unique culture in China and can slowly condition the body to achieve good results. Previous research reported that Danshen and Sophora japonica flowers could treat vascular disease. Lao et al. showed that Salvia miltiorrhiza treated cerebral infarction by reducing the volume of infarction [15]. Sophora japonica and Salvia miltiorrhiza have similar mechanisms for the treatment of cerebral infarction. Sophora japonica reduced inflammation by inhibiting the release of the pro-inflammatory factor IL-1β and prevented the death of nerve cells [16,17]. Dong et al. demonstrated that tanshinone has neuroprotective effects on focal cerebral ischemia and the injury of local cerebral ischemia/reperfusion in mice [18]. In our study, we explored the role and specific mechanisms of naringin in the treatment of cerebral infarction.

Naringin is a major component of citrus fruits, especially grapefruit. An increasing evidence showed that naringin and its metabolite naringenin have a variety of biological activities such as anti-hyperglycemia, anti-inflammatory, anti-oxidant, and anti-hyperlipidemic effects [19–23]. In addition, it has been reported that naringin could protect cardiovascular in animal models by preserving endothelial function, enhancing NO bioavailability, and reducing oxidative stress [20,24]. In our study, we established a rat model of cerebral infarction and found that injection of naringin can alleviate cerebral infarction by assessing various indicators related to cerebral infarction such as cerebral edema, cerebral infarction volume, and neurological deficit.

Previous studies have shown that apoptosis and secretion of inflammatory factors are key indicators for assessing cerebral infarction [25]. Our data showed that naringin reduced cell apoptosis and the expression of IL-6 and TNF-α. Bcl-2 and Bax, apoptosis-related proteins, played an important regulatory role in cell apoptosis [26]. It has been reported that Bcl-2/Bax ratios are vital for regulating cell proliferation and death [26,27]. Naringin increased the expression of Bcl-2 and decreased the expression of Bax. Finally, we found that naringin activates PI3K/AKT pathway in nerve cells.

Conclusions

The data indicated that naringin played a protective role in cerebral infarction through suppressing neuronal cell apoptosis and inflammatory response. Naringin is a potential agent for the treatment of cerebral infarction.

Conflict of interest

None.

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References:

1. Sveinsson OA, Kjartansson O, Valdimarsson EM: Cerebral ischemia/infarction-Diagnosis and treatment. Laeknabladid, 2014; 100: 393–401
2. Ma LL, Song L, Yu XD et al: The clinical study on the treatment for acute cerebral infarction by intra-arterial thrombolysis combined with mild hypothermia. Eur Rev Med Pharmacol Sci, 2017; 21: 1999–2006
3. Jeong HS, Song HJ, Kim SB et al: A comparison of stent-assisted mechanical thrombectomy and conventional intra-arterial thrombolysis for acute cerebral infarction. J Clin Neurol, 2013; 9: 91–96
4. Fugate JE, Rabinstein AA: Contraindications to intravenous rtPA for acute stroke: A critical reappraisal. Neurol Clin Pract, 2013; 3: 177–85
5. Lin TH, Hsieh CL: Pharmacological effects of Salvia miltiorrhiza (Danshen) on cerebral infarction. Chin Med, 2010; 5: 22
6. Chen HN, Hsieh CL: Effects of *Sophora japonica*, flowers (Huaihua) on cerebral infarction. Chin Med, 2010; 5: 34
7. Chanet A, Milenkovic D, Deval C et al: Naringin, the major grapefruit flavonoid, specifically affects atherosclerosis development in diet induced hypercholesterolemia in mice. J Nutr Biochem, 2012; 23: 469–77
8. Bailey DG, Arnold JM, Munoz C, Spence JD: Grapefruit juice-felodipine interaction: Mechanism, predictability and effect of naringin. Clin Pharmacol Ther, 1993; 53: 637–42
9. Bailey DG, Arnold JM, Strong HA et al: Effect of grapefruit juice and naringin on nisoldipine pharmacokinetics. Clin Pharmacol Ther, 1993; 54: 589–94
10. Ameer B, Weintraub RA, Johnson JV et al: Flavanone absorption after naringin, hesperidin and citrus ingestion. Clin Pharmacol Ther, 1996; 60: 34–40
11. Kim HJ, Yong HI, Park S et al: Effect of atmospheric pressure dielectric barrier discharge plasma on the biological activity of naringin. Food Chemistry, 2014; 160: 241–45
12. Wang H, Li C, Li J et al: Naringin enhances osteogenic differentiation through the activation of ERK signaling in human bone marrow mesenchymal stem cells. Iranian Journal of Basic Medical Sciences, 2017; 20: 408–14
13. Zhang P, Dai KR, Yan SG et al: Effects of naringin on the proliferation and osteogenic differentiation of human bone mesenchymal stem cell. Eur J Pharmacol, 2009; 607: 1–5
14. Fang L, Gao H, Zhang W et al: Resveratrol alleviates nerve injury after cerebral ischemia and reperfusion in mice by inhibiting inflammation and apoptosis. Int J Clin Exp Med, 2015; 8: 3219–26
15. Lao CJ, Lin JG, Kuo JS et al: Effects of Salvia Miltiorrhiza Bunge on cerebral infarct in ischemia-reperfusion injured rats. Am J Chin Med, 2003; 31: 191–200
16. Lao CJ, Lin JG, Kuo JS et al: Microglia, apoptosis and interleukin-1b expression in the effect of *Sophora japonica* L. on cerebral infarct induced by ischemia-reperfusion in rats. Am J Chin Med (Gard City NY), 2005; 33: 425–38
17. Yamasaki Y, Matsuura N, Shozuhara H et al: Interleukin-1 as a pathogenetic mediator of ischemic brain damage in rats. Stroke, 1995; 26: 676–81
18. Dong K, Xu W, Yang J et al: Neuroprotective effects of Tanshinone IIA on permanent focal cerebral ischemia in mice. Phytother Res, 2009; 23: 608–13
19. Adebiyi OA, Adebiyi OO, Owira PM: Naringin reduces hyperglycemia induced cardiac fbrosis by relieving oxidative stress. PLoS One, 2016; 11: e0149890
20. Alam MA, Kauter K, Brown L: Naringin improves diet induced cardiovascular dysfunction and obesity in high carbohydrate, high fat diet-fed rats. Nutrients, 2013; 5: 637–50
21. Pu P, Gao DM, Mohamed S et al: Naringin ameliorates metabolic syndrome by activating AMP activated protein kinase in mice fed a high-fat diet. Arch Biochem Biophys, 2012; 518: 61–70
22. Sharma AK, Bharti S, Ojha S et al: Up-regulation of PPARγ, heat shock protein-27 and -72 by naringin attenuates insulin resistance, β-cell dysfunction, hepatic steatosis and kidney damage in a rat model of type 2 diabetes. Br J Nutr, 2011; 106: 1713–23
23. Li W, Wang C, Peng J et al: Naringin inhibits TNF-α induced oxidative stress and inflammatory response in HUVECs via Nox4/NF-κ B and PI3K/Akt pathways. Curr Pharm Biotechnol, 2014; 15: 1173–82
24. Ikemura M, Sasaki Y, Giddings JC, Yamamoto J: Preventive effects of hesperidin, glucosyl hesperidin and naringin on hypertension and cerebral thrombosis in stroke-prone spontaneously hypertensive rats. Phytother Res, 2012; 26: 1272–77
25. Zhou L, Zhang J, Wang C, Sun Q: Tanshinone inhibits neuronal cell apoptosis and inflammatory response in cerebral infarction rat model. Int J Immunopathol Pharmacol, 2017; 30: 123–29
26. Vaux D, Cory S, Adams J: Bcl-2 and cell survival. Nature, 1988; 335: 440–42
27. Liang K, Ye Y, Wang Y et al: Formononetin mediates neuroprotection against cerebral ischemia/reperfusion in rats via downregulation of the Bax/Bcl-2 ratio and upregulation PI3K/Akt signaling pathway. J Neurol Sci, 2014; 344: 100–4

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