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Protective Effect of Paeoniflorin on Hypoxia Reoxygenation Cardiomyocytes and its Mechanism Based on MAPK Signal Pathway

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Key words
Paeoniflorin, Hypoxia reoxygenation myocardial cells, Protective effect, MAPK signal pathway

ABSTRACT

Mitogen-activated protein kinases (MAPK) is an important signal pathway involved in cardiomyocyte injury. To investigate the protective effect of paeoniflorin (PF) on hypoxia reoxygenation (H/R) injury and its effect on MAPK signal pathway to reveal the mechanism of PF against myocardial ischemia-reperfusion injury, in this study, the H/R model of H9C2 cells was established by hypoxia for 3 h and reoxygenation for 3 h. H9C2 cells were divided into 4 groups, the control, PF + control group, H/R group and H/R + PF group. The activities of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were measured by colorimetry; Apoptosis rate and reactive oxygen species (ROS) content were measured by flow cytometry; the expression of Bcl-2, Caspase-3, p38 MAPK, ERK1/2 and JNK proteins were determined by WB technique. The results showed that, compared with control group, LDH, AST content, ROS level, apoptosis rate, Caspase-3, p38 MAPK, ERK1/2, JNK protein expression in H/R group were significantly increased, while cell survival rate and Bcl-2 protein expression were significantly decreased (P <0.05). Moreover, compared with H/R group, H/R+PF group could reduce the levels of AST, LDH, ROS (P <0.05), reduce the apoptosis rate and the expression of p38 MAPK, ERK1/2, JNK proteins (P <0.05), and increase the cell survival rate and the expression of Bcl-2 protein (P <0.05). In conclusion, PF can protect H9C2 cells from H/R injury, which may be related to regulating the expression of MAPK pathway.

INTRODUCTION

Ischemic heart disease (IHD) is a heart disease characterized by myocardial ischemia, hypoxia and even necrosis (Severino *et al*., 2018). With the acceleration of the pace of life and the increase of life pressure, its incidence rate is increasing year by year (Khan *et al*., 2020). Unblocking the coronary arteries and restoring blood supply is the main treatment modality for IHD. However, myocardial injury often increases after blood reperfusion, which is called myocardial ischemia reperfusion injury (MIRI). The pathological mechanisms underlying the development of MIRI are complex, which are related to a variety of factors including oxidative stress, calcium overload, mitochondrial dysfunction, inflammatory response, and apoptosis (Shen *et al*., 2019; Luo *et al*., 2020). Among them, Mitogen-activated protein kinases (MAPK) is an important signal pathway involved in cardiomyocyte injury (Yu *et al*., 2015) and can be activated by cytokines, cellular stress and other stimuli to mediate different cell biological responses. MAPK signal pathway mainly includes three main pathways: Extracellular signal-regulated protein kinase (ERK), C-Jun N-terminal protein kinase (JNK) and p38 mitogen-activated protein kinase (p38 MAPK) (Guo *et al*., 2018). It was found that activation of JNK and p38MAPK pathways drives apoptosis during MIRI, while activation of ERK1/2 inhibits apoptosis (Degirmenci *et al*., 2020; Xu *et al*., 2015).

Paeoniflorin (PF), a monoterpene glucoside isolated from the dried root of *Paeonia lactiflora* Pall., is an effective component of Chinese herbal Radix Paeoniae. It has multiple bioactivities, such as antioxidant, anti-tumor, hepatoprotection, neuroprotective, hypolipidemic, hypoglycemic, antithrombotic, and regulation of immune function (Li *et al*., 2018; Ngo *et al*., 2019; Zhang *et al*., 2017, 2018; Zhao *et al*., 2016; Zhu *et al*., 2016). In recent years, it has been used in the pharmacological research of prevention and treatment of MIRI and IHD. Liu and Wei (2009) investigated the cardioprotective effects of PF on ischemic reperfused rat heart *in vitro*, and found that it could

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significantly reduce myocardial ultrastructural damage and kept the morphology and structure of myofibrils and mitochondria basically normal. Gao *et al*. (2015) concluded that PF pretreatment could exert myocardial protection on MIRI rats by inhibiting NF-κB signal pathway. Wang *et al*. (2016) demonstrated that PF could significantly reduce the myocardial infarction area of rats with MIRI injury, inhibit myocardial oxidative damage and inflammatory reaction. Yousif *et al*. (2020) found that the administration of PF attenuated chemokine and cytokines through up-regulation of Notch-1 and Jagged-1 activation signaling pathway leading to improved left ventricular function of MIRI mice. Wu *et al*. (2020) studied the myocardial benefit effect and mechanism of PF on MIRI in rats, and found that its protective effect on MIRI may related to reducing oxidative stress and apoptosis by inhibiting the expression of apoptosis-related signaling pathway. However, the deeper mechanism of PF in MIRI is still unclear.

Therefore, this research intends to study the protective action of PF on H9C2 cells and its effect on MAPK signal pathway by constructing the model of hypoxic/reoxygenation (H/R) damage in H9C2 cells, to investigate the protective effect and its possible mechanism of PF on myocardium, so as to provide evidence for drug development of IHD.

MATERIALS AND METHODS

Drugs, reagents and main instruments

The PF (purity ≥98%) was provided by Spring and Autumn Bioengineering Co., Ltd. (Nanjing, China). Lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) test kits were provided by Jiancheng Inc. (Nanjing, China). Antibodies against Caspase-3, Tubulin, P38 MAPK, ERK1/2 were provided by Proteintech Group Inc. (Chicago, IL, USA). Antibodies against Bcl-2 was provided by ImmunoWay Biotechnology (Plano, TX, USA). Antibodies against JNK (Anti-Rabbit Antibody) was provided by Affinity Biosciences LTD (Cincinnati, OH, USA). Antibodies against β-actin and Horseradish peroxidase-labeled goat anti-rabbit IgG were provided by Zhongshan Jinqiao Biotechnology Co., Ltd. (Beijing, China). H9C2 cells were provided by Bena Biology (Beijing, China). BCA kit was purchased from Jiangsu Cowin Biotech Co., Ltd. (Jiangsu, China).

Epoch™ enzyme-linked immunosorbent assay reader (BioTek, Instruments, Inc., Winosky, VT, USA), Mini-PROTEAN Tetra Cell Protein Electrophoresis Transfer System (Bio-Rad Laboratories Inc, Hercules, CA, USA); IX51 Inverted fluorescence microscope (Olympus Corporation, Tokyo, Japan); ChemiScope 6100 Fluorescent chemiluminescence imaging system (Qinxiang Scientific Instrument Co. Ltd., Shanghai, China).

H9C2 cell culture and preparation of hypoxia/reoxygenation (H/R) model

H9C2 cells commonly used as an alternative for cardiomyocytes were cultured in a complete DMEM containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C in an incubator with 5% CO_2.

The preparation of H/R models referred to the method described by Su *et al*. (2021). H9C2 cells were centrifuged and resuspended for counting, inoculated into 96-well plates, phosphate buffered saline (PBS) was added and incubated in a triple-gas incubator containing 5% CO_2 and 95% N_2 for 3 h. Then the medium was discarded, injected with fresh complete medium and incubated in a 5% CO_2 incubator for 3 h to construct an H/R model.

Cell counting Kit-8 (CCK-8) assay for cell viability

To determine the cytotoxicity of paeoniflorin, H9C2 cells were separated by centrifugation, resuspended and counted. Then the cells were added to the complete DMEM medium and cultured for 24 h under normal oxygen conditions. After that, the medium was discard and PF solutions of different concentrations (0, 25, 50, 100, 200 and 300 μmol/L) were added, then cultured for 24 h. The cell survival rate was determined by CCK-8 method (Xu *et al*., 2022).

To screen the concentration of paeoniflorin, H9C2 cells were cultured under conventional conditions for 24 h, and then used 0, 50, 100 and 200 μmol/L PF for 24 h. The H/R model was prepared according to method 2.2, and the cell survival rate after hypoxia reoxygenation was determined by CCK-8 method.

Experimental grouping and dose

H9C2 cells were randomly divided into four groups: Control group, PF + Control group, H/R group and H/R + PF group. The Control group was incubated using complete DMEM medium in an incubator at 37°C with 5% CO_2. PF + Control group was first pretreated with PF (200 μmol/L) for 24 h, and then replaced with complete DMEM medium and incubated for 6 h under normoxic conditions. H/R group was constructed as described in section 2.2. The H/R + PF group was pretreated with PF (200 μmol/L) for 24 h and the remaining treatment was the same as H/R group.

Measurement of AST and LDH levels

After treatments, the cell supernatant was collected and the AST and LDH levels were measured after myocardial H/R injury according to the instructions of AST and LDH kit.

Flow cytometry determination of apoptosis and ROS levels

After treatments, the culture medium was collected referring to the method described by Chen *et al*. (2022) and the apoptosis assay was performed according to the instructions. Cells were trypsin digested, washed, and supernatant removed by centrifugation. 200 μL of membrane binding solution was used to resuspend the cells, and 10 μL of Annenxin V-fluorescein isothiocyanate (FITC) dye and 5 μL of propidium iodide (PI) dye were added, stained for 20 min, and the apoptotic rate was determined by flow cytometry.

After treatment, the intracellular ROS level was determined using the method of Chen *et al*. (2022) and ROS assay instructions. Briefly, cells were digested after trypsin digestion, ROS fluorescent probe was added, and incubated at 30°C for 20 min, and intracellular ROS content was determined by flow cytometry.

Determination of the expression of p38 MAPK, Bcl-2, Caspase-3, ERK1/2 and JNK proteins

After treatment, the culture medium was discarded, and the cell lysate (containing RIPA) was added into each well to lyse the cells and extract the protein. Protein concentration was determined according to the BCA kit. The proteins were denatured, loaded in equal amounts, and SDS-PAGE electrophoresis was performed, followed by transferring at a constant current of 200 mA for 120 min. Primary antibody: p38 MAPK (1:500), Bcl-2 (1:500), Caspase-3 (1:500), ERK1/2 (1:500), JNK (1:500) solution, incubation, overnight at 4°C. Secondary antibody: Horseradish enzyme-labelled goat anti-mouse IgG (1:2000) solution, incubated at room temperature for 2 h. ECL luminescent solution was added dropwise to the film and exposed in the gel imaging system. Bcl-2 and Caspase-3 used Tubulin as an internal reference and p38 MAPK, ERK1/2 and JNK used β-actin as an internal reference. Greyscale value was analyzed using Image J software 1.52a (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

Each experiment was performed at least three times. The experimental data were expressed with $\bar{x}\pm s$. One-way analysis of variance (ANOVA) was used for statistical analysis, P <0.05 was considered as statistical significance difference.

RESULTS

Effect of PF on cell viability rate

The cell viability rate of H9C2 cultured with PF under normal and anoxic reoxygenation conditions was tested (Fig. 1). Under the condition of normal oxygen culture (Fig. 1A), there was no significant change in cell survival rate of the PF concentration groups of 50, 100 and 200 μmol/L (P >0.05), while the cell survival rate of the concentration group of 300 μmol/L decreased significantly (P <0.05). The results showed that PF 300 μmol/L group had certain cytotoxicity to H9C2 cells.

Moreover, the survival rate of H9C2 cells decreased significantly after H/R treatment (Fig. 1B) (P <0.05). Compared with H/R group, the cell viability rate was significantly improved in the PF concentration groups of 50, 100 and 200 μmol/L (P <0.05), with the highest cell viability rate at 200 μmol/L. Therefore, 200 μmol/L was selected as the final dosing concentration.

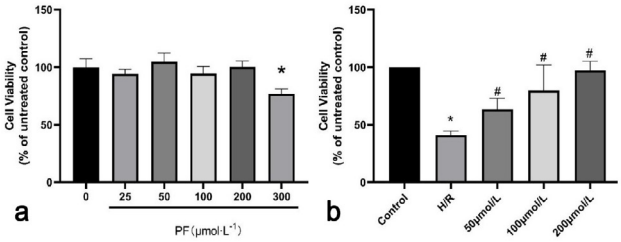

Fig. 1. Effect of different PF concentrations on H9C2 cell viability (a) under normal oxygen culture and (b) with H/RI treatment.
Notes: $\bar{x} \pm s$, n = 3; Compared with 0 μmol/L, * P <0.05; Compared with H/R group, # P <0.05.

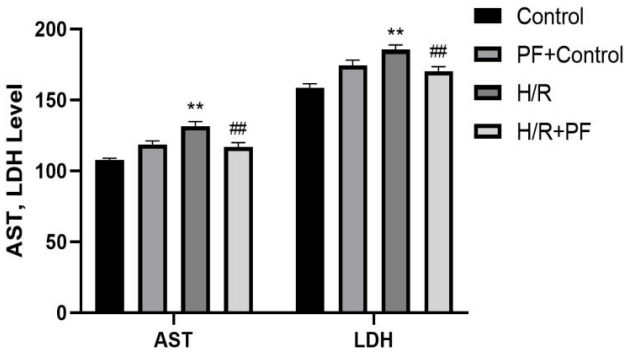

Fig. 2. Effect of PF on AST and LDH levels in H9C2 cells injured by H/R.
Notes: $\bar{x} \pm s$, n = 3; ** P <0.01, compared with control group; ## P <0.01, compared with H/R group.

Effect of PF on activity levels of AST and LDH

As shown in Figure 2, compared with the control group, the levels of AST and LDH in PF + Control group had no significant difference (P >0.05), while the levels of AST and LDH in H/R group were significantly increase (P <0.01). Compared with H/R group, AST and LDH levels

in H/R + PF group decreased significantly (P <0.01). The results indicated that PF could reduce the AST and LDH levels of H/R injury, and had a protective benefit on H9C2 cells.

Effect of PF on apoptosis rate of H9C2 cells injured by H/R

The results were shown in Figure 3. Compared with the Control group, the apoptosis rate in the Control + PF group had no significant difference (P >0.05); while the apoptosis rate of H/R group was significantly increased (P <0.01). Compared with H/R group, the apoptosis rate of H/R + PF group was significantly lower (P <0.01). The results indicated that the apoptosis rate was increased in H9C2 cells with H/R injury, while PF reduced the apoptosis rate and inhibited apoptosis.

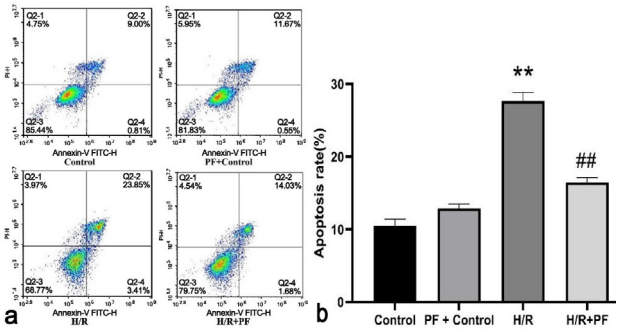

Fig. 3. Effect of PF on apoptosis rate of H9C2 cells injured by H/R.
Notes: $\bar{x} \pm s$, n = 3; ** P <0.01, compared with Control group; ## P <0.01, compared with H/R group.

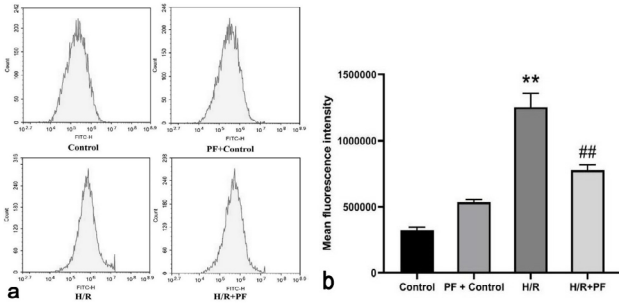

Fig. 4. Effect of PF on ROS level in H9C2 cells injured by H/R.
Notes: $\bar{x} \pm s$, n=3; ** P <0.01, compared with Control group; ## P <0.01, compared with H/R group.

Effect of PF on the ROS content

It can be seen from Figure 4, compared with the Control group, the ROS content in the Control + PF group had no significant difference (P >0.05), while the ROS content in the H/R group was significantly increased (P <0.01). Compared with H/R group, the ROS content in H/R + PF group was significantly decreased (P <0.01). The results showed that PF could reduce the ROS level of H9C2 cells injuried by H/R.

Effect of PF on the expression of p38 MAPK, Bcl-2, Caspase-3, ERK1/2 and JNK proteins in H9C2 cells

It can be seen from Figure 5, compared with the Control group, the expression of Caspase-3 and Bcl-2 protein in the Control + PF group had no significant difference (P >0.05), but the expression of Bcl-2 protein in the H/R group decreased significantly (P <0.01), and the expression of Caspase-3 protein increased significantly (P <0.01). Compared with H/R group, the expression of Bcl-2 protein in H/R+PF group was significantly increased (P <0.01), and the expression of Caspase-3 protein was decreased, but there was no statistical significance (P >0.05). The results showed that PF could inhibit apoptosis induced by H/R injury.

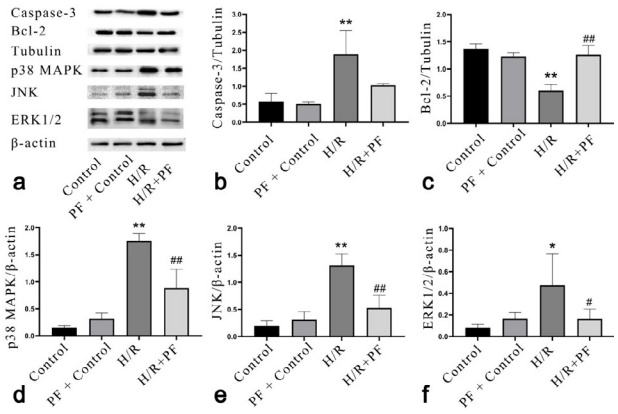

Fig. 5. Representative picture of Caspase-3, Bcl-2, p38 MAPK, ERK1/2, and JNK in H9C2 cell of each group (a), and Effect of PF on the expression of Caspase-3 (b), Bcl-2 proteins (c), p38 MAPK (d), JNK (e), and ERK1/2 (f) proteins in H9C2 cells.
Notes: $\bar{x} \pm s$, n=3; * P <0.05, ** P <0.01, compared with control group; # P <0.05, ## P <0.01, compared with H/R group.

Compared with the Control group, the expression of p38 MAPK, ERK1/2 and JNK protein in the Control + PF group had no significant difference (P >0.05), while the expression of p38 MAPK, ERK1/2 and JNK protein in the H/R group increased significantly (P <0.05). Compared with H/R group, the expression of p38 MAPK, ERK1/2 and JNK protein in H/R + PF group decreased significantly (P <0.05). The results indicated that PF had some regulatory effects on the MAPK signaling pathway.

DISCUSSION

MIRI is a secondary injury resulting from myocardial ischemia-reperfusion, which severely reduces the outcome of ischemic heart disease. PF is an effective component extracted from Chinese herbal paeonia lactiflora, which has a variety of biological activities. It was found that PF has the effects of reducing myocardial fibrosis in rats with heart failure (Liu *et al*., 2020), decreasing the infarct area of MIRI (Chen *et al*., 2018a), and protecting vascular endothelial cells (Wang *et al*., 2019). Chen *et al*. (2015) developed that PF can improve the expression of caspase-3 and caspase-9, inhibit inflammation, and play a protective role in infarcted myocardium, which has certain research value in the treatment of cardiovascular diseases.

LDH and AST are enzymes distributed in myocardial tissue and are routinely tested to reflect myocardial injury (Abdul *et al*., 2015). LDH and AST are released from myocardial tissue into the blood after myocardial damage, resulting in their elevated levels in the serum (Chen *et al*., 2012). The results in this study indicated that H9C2 cardiomyocytes could cause a significant increase in both LDH and AST levels after H/R injury. LDH and AST levels were reduced after administration via PF. It is suggested that PF can protect H9C2 cardiomyocytes from H/R injury.

The occurrence of MIRI is related to several factors, among which oxidative stress is one of the main causes (Cadenas, 2018). It has been found that during MIRI, a large amount of ROS is generated instantaneously, and the excess ROS will damage the cell membrane and induce apoptosis in cardiomyocytes (Chen *et al*., 2018b). Apoptosis is regulated by a variety of genes, among which *Bcl-2* is an anti-apoptotic gene, *Caspase-3* is a pro-apoptotic molecule as well as a marker gene for apoptosis (Lee *et al*., 2007). Xie *et al*. (2001) found that MIRI reduced the expression of Bcl-2 in cardiomyocytes with a pro-apoptotic tendency. Chen *et al*. (2001) found that Bcl-2 overexpressing transgenic mice were able to inhibit apoptosis and attenuate MIRI. Liu (2014) found that interference with Caspase-3 expression reduced apoptosis of cardiomyocytes and improved cardiac function. This study found that after injuried by H/R in H9C2 cells, ROS activity was significantly increased, apoptosis rate was increased, Bcl-2 protein expression level was decreased, while Caspase-3 protein expression was increased, indicating that oxidative stress injury occurred after H/R and induced apoptosis in cells. PF was able to reduce ROS levels, decrease Caspase-3 protein expression, increase Bcl-2 protein expression, and inhibit apoptosis.

Apoptosis occurs with the involvement of multiple signaling pathways, of which the MAPK pathway is an important regulatory pathway. p38 MAPK, JNK and ERK play important roles in the process of MIRI (Yu *et al*., 2015). It was found that MIRI causes upregulation of p38 MAPK, JNK, ERK expression and their phosphorylation levels in MAPK pathway, however, reducing the expression of MAPK pathway can help to reduce the damage caused by MIRI (Hsiao *et al*., 2021). This study shows that PF can reduce the expression of p38 MAPK, JNK, and ERK1/2 proteins in the MAPK pathway, indicating that PF may be able to protect H9C2 cells by regulating the MAPK pathway.

CONCLUSION

In summary, H9C2 cardiomyocytes caused peroxidative damage to cardiomyocytes after hypoxic-reoxygenation, release of cardiac enzymes, and induction of apoptosis. PF can reduce LDH and AST levels, resist oxidative stress damage, inhibit apoptosis and protect cardiomyocytes. Its anti-apoptotic effect may be related to the regulation of MAPK pathway.

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IRB approval

This study did not involve human or animals.

Ethical statement

There are no studies on animals or humans, only cultured cells are involved.

REFERENCES

Abdul, K.S.M., Jovanović, S., Du, Q., Sukhodub, A. and Jovanović, A., 2015. Mild hypoxia in vivo regulates cardioprotective SUR2A: A role for Akt and LDH. *BBA-Mol. Basis Dis.*, **1852**: 709-719. https://doi.org/10.1016/j.bbadis.2015.01.001

Cadenas, S., 2018. ROS and redox signaling in myocardial ischemia-reperfusion injury and cardio protection. *Free Radic. Biol. Med.*, **117**: 76-89. https://doi.org/10.1016/j.freeradbiomed.2018.01.024

Chen, Z., Chua, C.C., Ho, Y.S., Hamdy, R.C. and Chua, B.H., 2001. Overexpression of Bcl-2 attenuates apoptosis and protects against myocardial I/R injury in transgenic mice. *Am. J. Physiol. Heart Circ. Physiol.*, **280**: H2313-H2320. https://doi.org/10.1152/ajpheart.2001.280.5.H2313

Chen, X.J., Ni, Z.H. and Li, Y.D., 2012. Effect of ultra-

filtration extract mixture from *Angelica sinensis* and *Radix hedysari* on AST, LDH and LDH1-acute myocardial ischemia rats. *Acta Chin. Med. Pharmacol.*, **40**: 33-35.

Chen, C., Du, P. and Wang, J., 2015. Paeoniflorin ameliorates acute myocardial infarction of rats by inhibiting inflammation and inducible nitric oxide synthase signaling pathways. *Mol. Med. Rep.*, **12**: 3937-3943. https://doi.org/10.3892/mmr.2015.3870

Chen, H.W., Dong, Y., He, X.H., Li, J. and Wang, J., 2018a. Paeoniflorin improves cardiac function and decreases adverse postinfarction left ventricular remodeling in a rat model of acute myocardial infarction. *Drug Des., Dev. Ther.*, **12**: 823-836. https://doi.org/10.2147/DDDT.S163405

Chen, Y.Z., Zhang, L.L., Yu, H.L., Song, K.P., Shi, J.L., Chen, L.L. and Cheng, J., 2018b. Necrostatin-1 improves long-term functional recovery through protecting oligodendrocyte precursor cells after transient focal cerebral ischemia in mice. *Neuroscience*, **371**: 229-241. https://doi.org/10.1016/j.neuroscience.2017.12.007

Chen, Y., Zhang, Y.Z., Wang, Y.Q., Wang, Z.R. and Hu, Y.Y., 2022. miR-195 relieves lipopolysaccharide-induced apoptosis, oxidative stress and inflammatory response in H9c2 cardiomyocytes. *Immunol. J.*, **38**: 132-138.

Degirmenci, U., Wang, M. and Hu, J., 2020. Targeting aberrant RAS/RAF/MEK/ERK signaling for cancer therapy. *Cells*, **9**: 198. https://doi.org/10.3390/cells9010198

Gao, Y.Q., Hao, J.P., Liu, M.L. and Zhao, G.P., 2015. Paeoniflorin pretreatment exerts a protective effect on *in vivo* rat MIRI by interfering with NF-κB signaling pathway. *Shaanxi med. J.*, **44**: 1581-1582.

Guo, W., Liu, X., Li, J., Shen, Y., Zhou, Z., Wang, M., Xie, Y., Feng, X., Wang, L. and Wu, X., 2018. Prdx1 alleviates cardiomyocyte apoptosis through ROS-activated MAPK pathway during myocardial ischemia/reperfusion injury. *Int. J. Biol. Macromol.*, **112**: 608-615. https://doi.org/10.1016/j.ijbiomac.2018.02.009

Hsiao, Y.W., Tsai, Y.N., Huang, Y.T., Liu, S.H., Lin, Y.J., Lo, L.W., Hu, Y.F., Chung, F.P., Lin, S.-F., Chang, S.-L., Higa, S., and Chen, S.A., 2021. *Rhodiola crenulata* reduces ventricular arrhythmia through mitigating the activation of IL-17 and inhibiting the MAPK signaling pathway. *Cardiovasc. Drugs Ther.*, **35**: 889-900. https://doi.org/10.1007/s10557-020-07072-z

Khan, M.A., Hashim, M.J., Mustafa, H., Baniyas, M.Y., Al Suwaidi, S.K.B.M., AlKatheeri, R., Alblooshi, F.M.K., Almatrooshi, M.E.A.H., Alzaabi, M.E.H., Al-Darmaki, R.S. and Lootah, S.N.A.H., 2020. Global epidemiology of ischemic heart disease: Results from the global burden of disease study. *Cureus*, **12**: e9349. https://doi.org/10.7759/cureus.9349

Lee, S., Lim, H.J., Park, J.H., Lee, K.S., Jang, Y. and Park, H.Y., 2007. Berberine-induced LDLR up-regulation involves JNK pathway. *Biochem. biophys. Res. Commun.*, **362**: 853-857. https://doi.org/10.1016/j.bbrc.2007.08.060

Li, Y.C., Qiao, J.Y., Wang, B.Y., Bai, M., Shen, J.D. and Cheng, Y.X., 2018. Paeoniflorin ameliorates fructose-induced insulin resistance and hepatic steatosis by activating LKB1/AMPK and AKT pathways. *Nutrients,* **10**: 1024. https://doi.org/10.3390/nu10081024

Liu, M., Feng, J., Du, Q., Ai, J. and Lv, Z., 2020. Paeoniflorin attenuates myocardial fibrosis in isoprenaline-induced chronic heart failure rats via inhibiting P38 MAPK pathway. *Curr. med. Sci.*, **40**: 307-312. https://doi.org/10.1007/s11596-020-2178-0

Liu, Q., 2014. Lentivirus mediated interference of Caspase-3 expression ameliorates the heart function on rats with acute myocardial infarction. *Eur. Rev. Med. Pharmacol. Sci.*, **18**: 1852-1858.

Liu, X. and Wei, Y., 2009. Cardioprotective effects of Paeoniflorin preconditioning on isolated rat hearts suffering from ischemia and reperfusion injury. *Chin. J. Integr. Med. Cardio-Cerebrovasc. Dis.*, **7**: 1177-1179.

Luo, X., Zhou, J., Wang, Z., He, Y., Yu, L., Ma, S., Wang, S., Wang, X., Yuan, Y., Li, D., Cui, T. and Ding, Y., 2020. An inhibitor role of Nrf2 in the regulation of myocardial senescence and dysfunction after myocardial infarction. *Life Sci.*, **259**: 118199. https://doi.org/10.1016/j.lfs.2020.118199

Ngo, T., Kim, K., Bian, Y., Noh, H., Lim, K.M., Chung, J.H. and Bae, O.N., 2019. Antithrombotic effects of Paeoniflorin from *Paeonia suffruticosa* by selective inhibition on shear stress-induced platelet aggregation. *Int. J. mol. Sci.,* **20**: 5040. https://doi.org/10.3390/ijms20205040

Severino, P., D'Amato, A., Netti, L., Pucci, M., De Marchis, M., Palmirotta, R., Volterrani, M., Mancone, M. and Fedele, F., 2018. Diabetes mellitus and ischemic heart disease: The role of ion channels. *Int. J. mol. Sci.,* **19**: 802. https://doi.org/10.3390/ijms19030802

Shen, Y., Liu, X., Shi, J. and Wu, X., 2019. Involvement

of Nrf2 in myocardial ischemia and reperfusion injury. *Int. J. Biol. Macromol.*, **125**: 496-502. https://doi.org/10.1016/j.ijbiomac.2018.11.190

Su, C.Y., Meng, Y.H. and Wu, M.L., 2021. Effect of SERP1 overexpression on hypoxia/reoxygenation-induced apoptosis of H9c2 cardiomyocytes. *Anhui Med. Pharm. J.*, **25**: 2070-2074.

Wang, J.S., Huang, Y., Zhang, S.P., Yin, H.J., Zhang, L., Zhang, Y.H., Song, Y.W. and Li, D.D., 2019. A protective role of paeoniflorin in fluctuant hyperglycemia induced vascular endothelial injuries through antioxidative and anti-inflammatory effects and reduction of PKCβ1. *Oxid. Med. Cell. Longevity*, **2019**: 5647219. https://doi.org/10.1155/2019/5647219

Wang, W., Liu, C., Ying, D.S. and Wang, B.M., 2016. The protective effect and mechanism of action of paeoniflorin on myocardial ischemia reperfusion injury in rats. *Pharmacol. Clin. Chin. Mater. Med.,* **32**: 52-56.

Wu, F., Ye, B., Wu, X., Lin, X., Li, Y., Wu, Y. and Tong, L., 2020. Paeoniflorin on rat myocardial ischemia reperfusion injury of protection and mechanism research. *Pharmacology*, **105**: 281-288. https://doi.org/10.1159/000503583

Xie, Z., Koyama, T., Suzuki, J.I., Fujii, Y., Togashi, H., Sawa, H. and Nagashima, K., 2001. Coronary reperfusion following ischemia different expression of Bcl-2 and Bax proteins, and cardiomyocyte apoptosis. *Jpn. Heart J.*, **42**: 759-770. https://doi.org/10.1536/jhj.42.759

Xu, F., Fan, W.J. and Liu, G.B., 2022. Effect of rutin on the ox-LDL-induced vascular endothelial cell injury by regulating NEXN-AS1/miR-410-3p pathway. *Pakistan J. Zool.,* **54**: 2277-2284. https://doi.org/10.17582/journal.pjz/20210209190253

Xu, J., Qin, X., Cai, X., Yang, L., Xing, Y., Li, J., Zhang, L., Tang, Y., Liu, J., Zhang, X. and Gao, F., 2015. Mitochondrial JNK activation triggers autophagy and apoptosis and aggravates myocardial injury following ischemia/reperfusion. *BBA-Mol. Basis Dis.*, **1852**: 262-270. https://doi.org/10.1016/j.bbadis.2014.05.012

Yousif, N.G., Younis, Z., Al-Amran, F.G., Yousif, M.G., Altimim, A. and Hadi, N.R., 2020. Paeoniflorin attenuates myocardial ischemia/reperfusion injury via up-regulation of Notch 1 mediated Jagged1 signaling. *Syst. Rev. Pharm.*, **11**: 363-371.

Yu, D., Li, M., Tian, Y., Liu, J. and Shang, J., 2015. Luteolin inhibits ROS-activated MAPK pathway in myocardial ischemia/reperfusion injury. *Life Sci.*, **122**: 15-25. https://doi.org/10.1016/j.lfs.2014.11.014

Zhang, J., Yu, K., Han, X., Zhen, L., Liu, M., Zhang, X., Ren, Y. and Shi, J., 2018. Paeoniflorin influences breast cancer cell proliferation and invasion via inhibition of the Notch-1 signaling pathway. *Mol. Med. Rep.*, **17**: 1321-1325. https://doi.org/10.3892/mmr.2017.8002

Zhang, Y., Qiao, L., Xu, W., Wang, X., Li, H., Xu, W., Chu, K. and Lin, Y., 2017. Paeoniflorin attenuates cerebral ischemia-induced injury by regulating Ca^{2+}/CaMKII/CREB signaling pathway. *Molecules,* **22**: 359. https://doi.org/10.3390/molecules22030359

Zhao, D.D., Jiang, L.L., Li, H.Y., Yan, P.F. and Zhang, Y.L., 2016. Chemical components and pharmacological activities of terpene natural products from the genus *Paeonia*. *Molecules,* **21**: 1362. https://doi.org/10.3390/molecules21101362

Zhu, Y., Wang, L., Zhao, D., Wang, C., Wu, L. and Zhang, J., 2016. Blood-enriching effects and mechanism of albiflorin and paeoniflorin on immunosuppressional model mice with blood deficiency pattern. *J. Beijing Univ. Tradit. Chin. Med.*, **39**: 204-207.