Article

Discovery Medicine 2025; 37(196): 914–922
https://doi.org/10.24976/Discov.Med.202537196.81

Investigation of Berberine's Cardioprotective Effects and Its Association With the Notch Signaling Pathway in Rat Myocardial Ischemia-Reperfusion Injury

Daxing Jiang[1,†], Xin Tang[2,†], Haifan Yang[2], Hong Cheng[2,*]

[1]Department of Cardiovascular Medicine, Zhangjiagang Gangcheng Rehabilitation Hospital, 215699 Zhangjiagang, Jiangsu, China
[2]Yangzhou University Medical College, Jiangsu Key Laboratory of Experimental & Translational Non-coding RNA Research, Institute of Translational Medicine, Yangzhou University, 225012 Yangzhou, Jiangsu, China
*Correspondence: Hongcheng322@outlook.com (Hong Cheng)
[†]These authors contributed equally.
Published: 20 May 2025

Background: Ischemia-reperfusion injury poses a significant challenge in cardiac pathology, leading to myocardial cell damage and dysfunction. Berberine, a natural compound, has shown potential cardioprotective effects. This study aims to investigate the protective role of berberine in myocardial cells post-ischemia-reperfusion injury, focusing on its modulation of the Neurogenic locus notch homolog protein (Notch) signaling pathway.

Methods: Male rats were subjected to ischemia-reperfusion injury and treated with berberine. Triphenyltetrazolium chloride (TTC) and hematoxylin and eosin (HE) staining were used to assess myocardial tissue damage and structure. Gene expression of Neurogenic locus notch homolog protein 1 (*Notch1*), Hairy and enhancer of split-1 (*Hes1*), B-cell lymphoma 2 (*Bcl-2*), and Bcl-2-associated X protein (*Bax*) was analyzed using Quantitative Polymerase Chain Reaction (qPCR). Apoptosis rate in myocardial cells was evaluated using Terminal deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) staining. Protein expression of Bcl-2 and Bax was examined. Molecular docking and Microscale Thermophoresis (MST) validation were performed to investigate the interaction between berberine and Notch1.

Results: Berberine treatment was associated with effective protection of myocardial tissue post-ischemia-reperfusion, indicated by reduced infarct area ($p < 0.05$) and improvements in tissue structure. Coinciding with these observations, berberine also influenced the expression of Notch1, Hes1, Bcl-2, and Bax in myocardial tissue, alongside modulation of Bcl-2 and Bax protein levels ($p < 0.05$). Additionally, molecular studies revealed that berberine binds to Notch1, suggesting a potential interaction.

Conclusion: The study suggests an association between berberine's cardioprotective effects and Notch signaling pathway modulation in the context of myocardial ischemia-reperfusion injury. While berberine was found to bind Notch1 and affect gene and protein expression related to apoptosis, further research is necessary to determine whether these effects directly contribute to reduced apoptosis and enhanced cell survival.

Keywords: berberine; ischemia-reperfusion injury; Notch signaling pathway; apoptosis; myocardial cells

Introduction

Ischemia-reperfusion injury remains a significant concern in the field of cardiovascular research, characterized by the restoration of blood flow to previously ischemic tissues, which paradoxically exacerbates tissue damage [1]. This pathological process occurs in various cardiovascular conditions, including myocardial infarction and cardiac surgery, leading to significant morbidity and mortality rates worldwide [2,3].

Berberine, a bioactive alkaloid derived from plants such as Berberis vulgaris, has gained attention for its potential therapeutic properties in cardiovascular diseases. Studies have highlighted its anti-inflammatory, antioxidant, and anti-apoptotic effects in different cell types and tissues, suggesting a promising cardioprotective role [4,5]. However, the precise mechanisms underlying the cardioprotective benefits of berberine, particularly in the context of ischemia-reperfusion injury, remain incompletely understood.

The Neurogenic locus notch homolog protein (Notch) signaling pathway, a highly conserved pathway crucial for cell fate determination and tissue homeostasis, has emerged as a significant regulator of cardiovascular development and disease [6,7]. Notch signaling influences various cellular processes, including proliferation, differentiation, and apoptosis, with implications for myocardial function and response to injury [8,9].

Given the potential of berberine in cardiovascular protection and the critical involvement of the Notch signaling pathway in cardiac pathophysiology, this study aims to elu-

cidate the protective mechanisms of berberine in myocardial cells following ischemia-reperfusion injury, with a specific focus on its modulation of Notch signaling. By clarifying the interplay between berberine and the Notch pathway, this research seeks to provide insights into novel therapeutic strategies for mitigating ischemia-reperfusion injury and preserving myocardial function.

Materials and Methods

Construction of the Rat Myocardial Ischemia-Reperfusion Model

Twenty healthy male Sprague-Dawley rats (Enswell, Chongqing, China), aged 6 months and weighing 220–260 g, were fasted for 12 hours prior to the surgery but had free access to water. General anesthesia was induced with sodium pentobarbital (1%, 0.4 mL/100 g, 803-21-1, Sigma-Aldrich, St. Louis, MO, USA). An incision was made along the left fourth or fifth intercostal space to expose the heart and surrounding structures. A surgical ligature was looped around the origin of the left coronary artery and temporarily occluded with a microvascular clamp to induce myocardial ischemia for 30 minutes. Blood flow was later restored to the left coronary artery by releasing the clamp, thus achieving myocardial reperfusion. The wounds were carefully sutured closed. Post-surgery, the rats received an intramuscular injection of 150,000 units of penicillin sodium (SP303201, Sinopharm Chemical Reagent Beijing Co., Ltd., Beijing, China) to prevent infection and were provided with appropriate analgesic treatment. Seventy-two hours post-operation, cervical dislocation was performed for euthanasia after administering with sodium pentobarbital (1%, 200 mg/kg, 803-21-1, Sigma-Aldrich, St. Louis, MO, USA) for heart tissue sample collection [10,11].

For experimental purposes, a total of 20 rats were used, with 6 in the sham operation group (control group), 7 in the model group receiving phosphate-buffered saline (PBS) (C0221A, Beyotime, Shanghai, China) as a control solvent by gastric lavage, and 7 in the berberine treatment group. For the berberine treatment group, berberine (141433-60-5, Sigma-Aldrich, St. Louis, MO, USA) was administered at a dose of 200 mg/kg [12] by gastric lavage daily for 2 weeks before surgery, at a volume of 1.5 mL/day. At the end of the study, 3 rats from each of the three groups were randomly selected for further index testing. All procedures were approved by the Ethics Committee of Yangzhou University Medical College (No.: YXYLL-2023-088).

Triphenyltetrazolium Chloride (TTC) Staining

The heart was rinsed in cold saline to remove blood. The heart was then sectioned transversely into slices approximately 1–2 mm thick. The slices were incubated in 1% TTC solution (T8877, Sigma-Aldrich, St. Louis, MO, USA) in PBS at 37 °C for 4 hours. Following incubation, the slices were fixed in 4% formaldehyde solution (252549, Sigma-Aldrich, St. Louis, MO, USA) for 24 hours. Photographs of the heart slices were taken to document the staining results. Infarcted myocardial areas were quantified using the ImageJ image analysis software (version 1.3.8, National Institutes of Health, Bethesda, MD, USA).

Hematoxylin and Eosin (HE) Staining

HE staining was carried out according to the reagent kit (C0105S, Beyotime, Shanghai, China). The heart sections were fixed in 10% neutral buffered formalin and then dehydrated through an ascending series of ethanol concentrations (70%, 80%, 95%, and 100%). After dehydration, tissues were cleared in xylene. Hematoxylin was then applied to stain the cell nuclei for 5 minutes, followed by rinsing in tap water, facilitating the 'blueing' process in a weak alkaline solution. Subsequently, sections were briefly dipped in 95% ethanol to remove excess water before eosin was applied for 1 minute to stain the cytoplasm and extracellular matrix. Tissues were dehydrated again in ethanol and cleared in xylene before being mounted with a resinous medium for microscopy analysis. The stained slides were evaluated under a microscope (MF53, Mshot, Guangzhou, China) to determine the histological details.

Quantitative Polymerase Chain Reaction (qPCR) Detection of Gene Expression

RNA was extracted from myocardial tissue, followed by reverse transcription to detect gene expression levels. qPCR was performed for the relative quantification of data using the $2^{-\Delta\Delta CT}$ method; amplification was done separately with primers for the target genes and internal reference gene, which were designed based on the rat (*Rattus norvegicus*) gene sequences. The primers used were as follows: *Notch1*-F, 5′-TGTGACAGCCAGTGCAACTC-3′; *Notch1*-R, 5′-TGGCACTCTGGAAGCACTGC-3′; Hairy and enhancer of split-1 (*Hes1*)-F, 5′-GCCAGTGTCAACACGACACCGG-3′; *Hes1*-R, 5′-TCACCTCGTTCATGCACTCG-3′; B-cell lymphoma 2 (*Bcl-2*)-F, 5′-AAACGTCCAGAGTGCTAC-3′; *Bcl-2*-R, 5′-CAGCCAGATTTAGGTTCA-3′; Bcl-2-associated X protein (*Bax*)-F, 5′-GGCGATGAACTGGACAAC-3′; *Bax*-R, 5′-GTGAGTGAGGCAGTGAGGA-3′; *β-actin*-F, 5′-GAGGGAAATCGTGCGTGAC-3′; *β-actin*-R, 5′-CTGGAAGGTGGACAGTGAG-3′.

Myocardial Cell Ischemia-Reperfusion Model Experiment

Third-generation rat myocardial cells H9c2 (CL-0089, Procell, Wuhan, China) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (12430054, Gibco, Waltham, MA, USA) containing 10% Fetal Bovine Serum (FBS) (SH30084.03, HyClone, Marlborough, MA, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin (15140122, Gibco, Waltham, MA, USA) at 37 °C in a 5% Carbon Dioxide (CO_2) incubator, with the culture medium being re-

placed every 2 days. Cells were shipped by the supplier after being qualified by Short Tandem Repeat (STR) and mycoplasma identification.

To construct the ischemia-reperfusion model, cells were grown to 80% confluence, washed with PBS, then supplemented with serum-free low-glucose DMEM before being placed in an anaerobic incubator at 37 °C with 85% Nitrogen (N_2), 10% Hydrogen (H_2), and 5% CO_2 at 37% humidity. After a 6-hour hypoxia culture, fresh DMEM was added, and cells were placed in a 5% CO_2 incubator to continue reoxygenation culture for 2 hours. The control group was cultured in a 37 °C, 5% CO_2 incubator. The model group received the control reagent PBS, and the berberine group's medium contained 50 µM berberine [13] for continuous treatment.

Fluorescence Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) Staining

The fixed myocardial cell slides were washed three times with PBS, 5 minutes each time. 0.3% Triton X-100 permeabilization solution (P0096, Beyotime, Shanghai, China) was added to the slides, which were then incubated at room temperature for 5 minutes. Three PBS washes followed this. 50 µL of Equilibration Buffer (C1090, Beyotime, Shanghai, China) was added to each sample to cover the entire area of interest, incubated at room temperature for 10 minutes, followed by three PBS washes. The nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) (C1002, Beyotime, Shanghai, China) for 5 minutes. The slides were then sealed with an anti-fade mounting medium. Images of the sections were captured using a Mshot MF53 inverted microscope (MF53, Mingmei, Guangzhou, China).

Immunofluorescence Detection in Cells

The fixed myocardial cell slides were washed three times with PBS, each for 5 minutes. An appropriate amount of 0.3% Triton X-100 permeabilization solution was added to the slides, followed by a 5-minute incubation at room temperature. The slides were then blocked with goat serum at room temperature for 30 minutes. This was followed by overnight incubation with the primary antibody (Notch1, A7636, abclonal, Wuhan, China, diluted at 1:200; Bcl-2, A0208, abclonal, Wuhan, China, diluted at 1:500; Bax, A0207, abclonal, Wuhan, China, diluted at 1:500) at 4 °C. The specimens were then washed three times with PBS, each for 5 minutes. The secondary antibody (FITC-conjugated Goat anti-Rabbit IgG, AS011, abclonal, Wuhan, China, diluted at 1:2000) was applied to the specimens and incubated in the dark at room temperature for 1.5 hours. DAPI was added for nuclear staining and incubated in the dark for 5 minutes, followed by three 5-minute washes with PBS to remove excess DAPI. Images of the sections were captured using a microscope (MF53, Mingmei, Guangzhou, China).

Molecular Docking

The 3D structure of berberine was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and converted from sdf to mol2 format using OpenBabel (http://openbabel.org/index.html). This file was imported into AutoDock Tools (version 1.5.7, Scripps Research Institute, La Jolla, CA, USA) and saved in pdbqt format. The molecular structure of Notch1 was obtained from the RCSB PDB database (https://www.rcsb.org/). Using PYMOL software (version 2.5.2, Schrodinger, New York, NY, USA), water molecules initially present in the structure and any other ligands contained in the protein structure name were removed. The protein structure was prepared with hydrogenation and charge addition, and the results were saved in pdbqt format. Molecular docking was performed utilizing a semi-flexible docking approach. The top 10 conformations with the highest scores were selected based on binding affinity calculated by the scoring function, choosing the conformation with the lowest binding energy for docking.

Microscale Thermophoresis (MST) Binding Assay

The protocol was followed according to the Monolith Red-N-Hydroxysuccinimide (RED-NHS) second-generation protein labeling kit (MO-L011, NanoTemper Technologies GmbH, Munich, Germany). 10 µL of the gradient-diluted non-labeled molecule (berberine) (141433-60-5, Sigma-Aldrich, St. Louis, MO, USA) and 10 µL of a fixed concentration of labeled molecule (Notch1) (ab276893, Abcam, Cambridge, MA, USA) were mixed and allowed to react for 5 minutes at rest. The mixed samples were loaded into glass capillaries, and the analysis was performed using the MST-NT.115 (NT.115, Nanotemper Technologies, San Francisco, CA, USA). Firstly, a concentration gradient dilution of the small molecule was prepared by diluting the stock solution of the small molecule 50 times. Then, 100% Dimethyl Sulfoxide (DMSO) (BP231-100, Fisher Scientific, Tempe, AZ, USA) was used to prepare an assay buffer with a 2% DMSO content. More than 10 µL of this buffer was transferred into 15 PCR tubes. An empty PCR tube was taken, and 20 µL of the diluted small molecule solution was drawn, from which 10 µL was transferred to the second tube. This operation was repeated until the dilution was completed sequentially in the 16 tubes, discarding 10 µL of solution in the last PCR tube. After dilution, 10 µL of the diluted 40 nM protein solution was added to each solution tube, gently mixed several times with a pipette, then drawn into a capillary and analyzed with the equipment.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics (version 22, IBM Corp., Armonk, NY, USA), and the data were presented as mean ± standard deviation. Multiple group comparisons were conducted using Analysis of

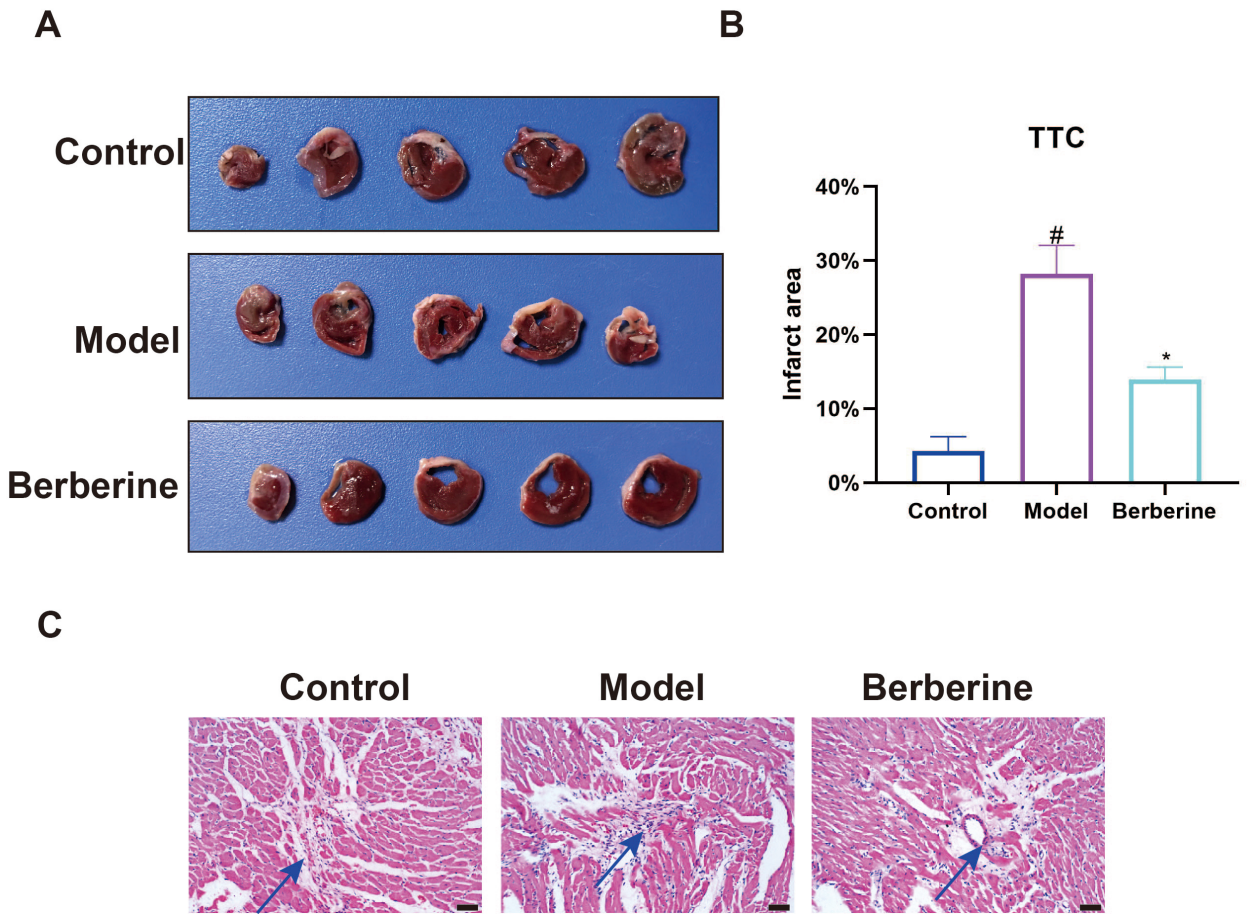

Fig. 1. Berberine effectively protects myocardial tissue after ischemia-reperfusion. (A) Observation of myocardial tissue via triphenyltetrazolium chloride (TTC) staining. (B) Statistical results of infarct size. (C) Observation of myocardial tissue structure through hematoxylin and eosin (HE) staining. The blue arrows indicate areas of myocardial injury. Scale bar = 50 µm. "#" denotes comparison with the control group, $p < 0.05$; "*" denotes comparison with the model group, $p < 0.05$. N = 3.

Variance (ANOVA) and Tukey's post hoc test for pairwise comparisons. Statistical significance was considered at a p-value < 0.05.

Results

Berberine Effectively Protects Myocardial Tissue Post-Ischemia-Reperfusion

Observations from TTC staining indicate that berberine treatment significantly protected the myocardial tissue. Statistical analysis of the infarct area revealed that the infarct size in the berberine-treated group was significantly reduced compared to the model group ($p < 0.05$) (Fig. 1A,B). HE staining showed that berberine treatment improved myocardial tissue structure and showed a significant reduction in the damaged areas, with significant differences compared to the control and model groups (Fig. 1C).

qPCR analysis revealed changes in the expression of *Notch1*, *Hes1*, *Bcl-2*, and *Bax* in myocardial tissue post-berberine treatment. Compared to the model group, the differences were significant ($p < 0.05$) (Fig. 2).

Berberine Protects Myocardial Cells and Reduces Apoptosis Rate

Further experimental results have revealed that berberine treatment significantly decreased the apoptosis rate of myocardial cells. Fluorescence TUNEL staining showed a significant reduction in the apoptosis rate of myocardial cells in the berberine-treated group compared to the model group ($p < 0.05$) (Fig. 3). Additionally, berberine regulated the expression of Bcl-2 and Bax proteins. It was found that compared to the model group, berberine could enhance the expression level of Bcl-2 protein while reducing the content of Bax protein ($p < 0.05$) (Fig. 4).

Berberine Targets and Binds Notch1 to Exert Regulatory Functions

Lastly, our molecular docking predictions indicated that berberine may interact with the Notch1 receptor, with a predicted binding energy of –10.43 kcal/mol, underscoring a strong potential interaction (Fig. 5A). Complementary to this, our MST assay data revealed a dissociation constant (Kd) of 2.35×10^{-6} M for the berberine-Notch1 interac-

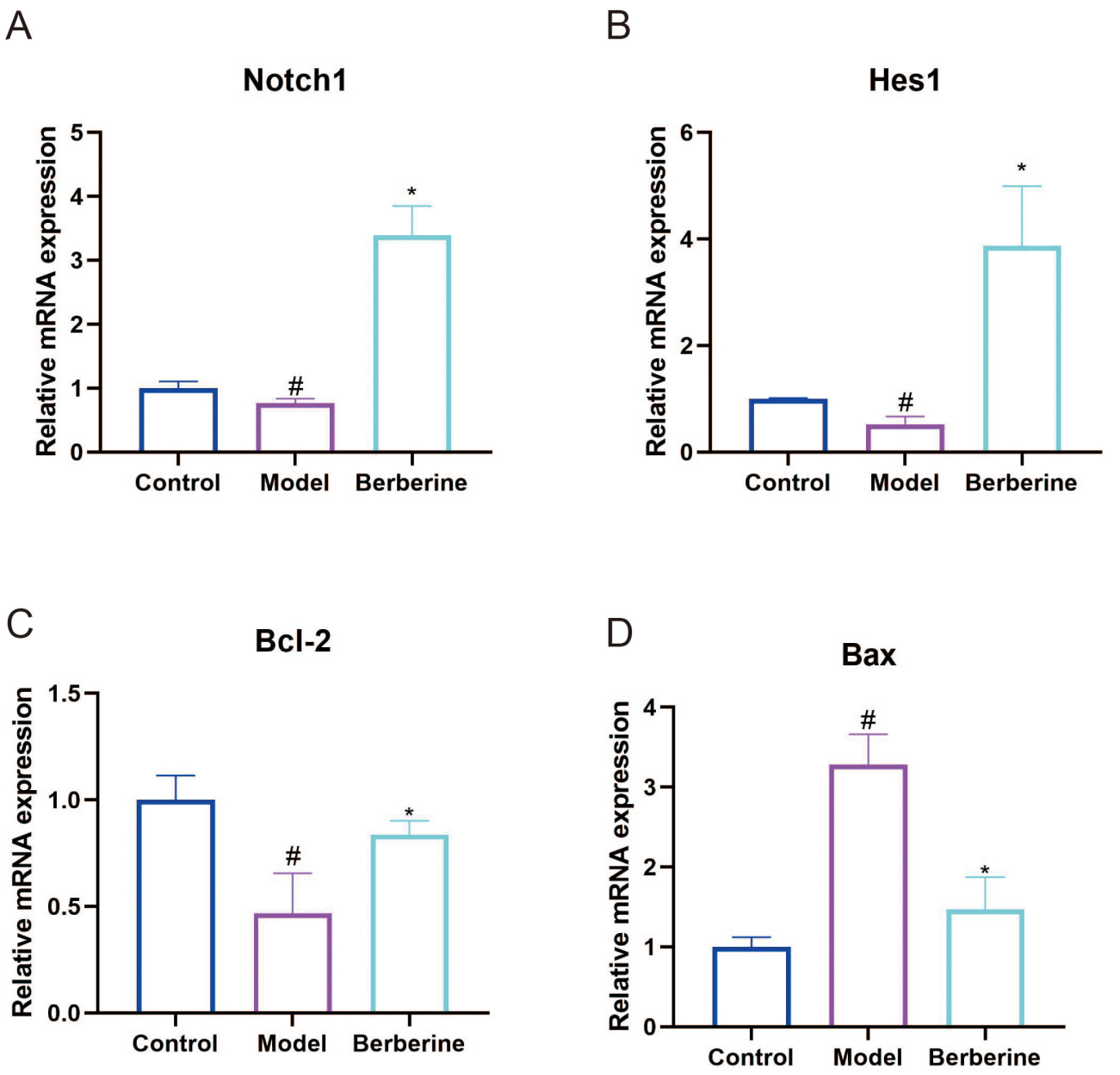

Fig. 2. Quantitative Polymerase Chain Reaction (qPCR) analysis of the expression levels of Neurogenic locus notch homolog protein 1 (*Notch1*) (A), Hairy and enhancer of split-1 (*Hes1*) (B), B-cell lymphoma 2 (*Bcl-2*) (C), and Bcl-2-associated X protein (*Bax*) (D) in myocardial tissue. "#" indicates a significant difference compared to the control group, $p < 0.05$; "*" indicates a significant difference compared to the model group, $p < 0.05$. N = 3.

tion, suggesting a high-affinity binding (Fig. 5B). These molecular studies suggest that berberine has the capacity to interact closely with the Notch1 receptor. Concomitant with this binding data, we observed an increased expression of Notch1 in the presence of berberine (Fig. 5C). However, it is important to note that while these findings illustrate a possible direct interaction between berberine and Notch1, they do not conclusively link this interaction to the observed reductions in myocardial cell apoptosis and need to be interpreted with caution. The biological implications of berberine's potential modulation of the Notch signaling pathway through this interaction warrant further investigation. Notwithstanding, we found that the differences in Notch1 expression were statistically significant when compared with both control and model groups ($p < 0.05$).

Discussion

This study delved into exploring the cardioprotective potential of berberine in the context of ischemia-reperfusion injury, focusing on its modulation of the Notch signaling pathway. The research unveiled promising results regard-

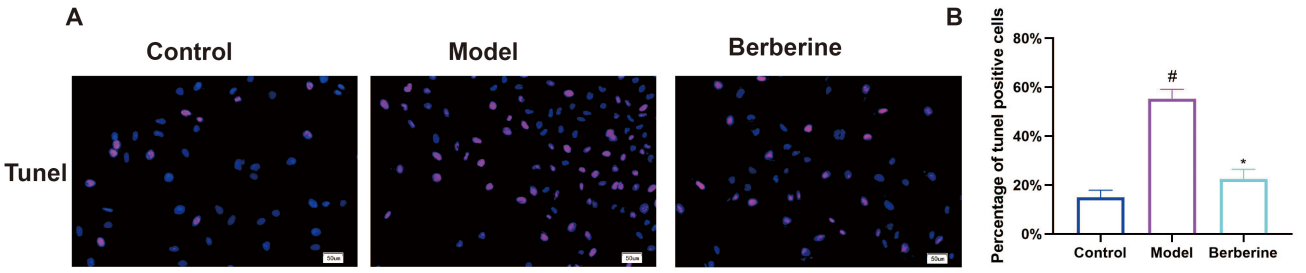

Fig. 3. Berberine protects myocardial cells and reduces apoptosis rate. (A) Fluorescence Terminal deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) staining was utilized to assess the apoptosis rate in myocardial cells. (B) Percentage of tunel positive cells. "#" denotes a significant difference compared to the control group, $p < 0.05$; "*" indicates a significant difference compared to the model group, $p < 0.05$. N = 3. The nuclei are stained blue with 4′,6-diamidino-2-phenylindole (DAPI); the positive TUNEL staining is red.

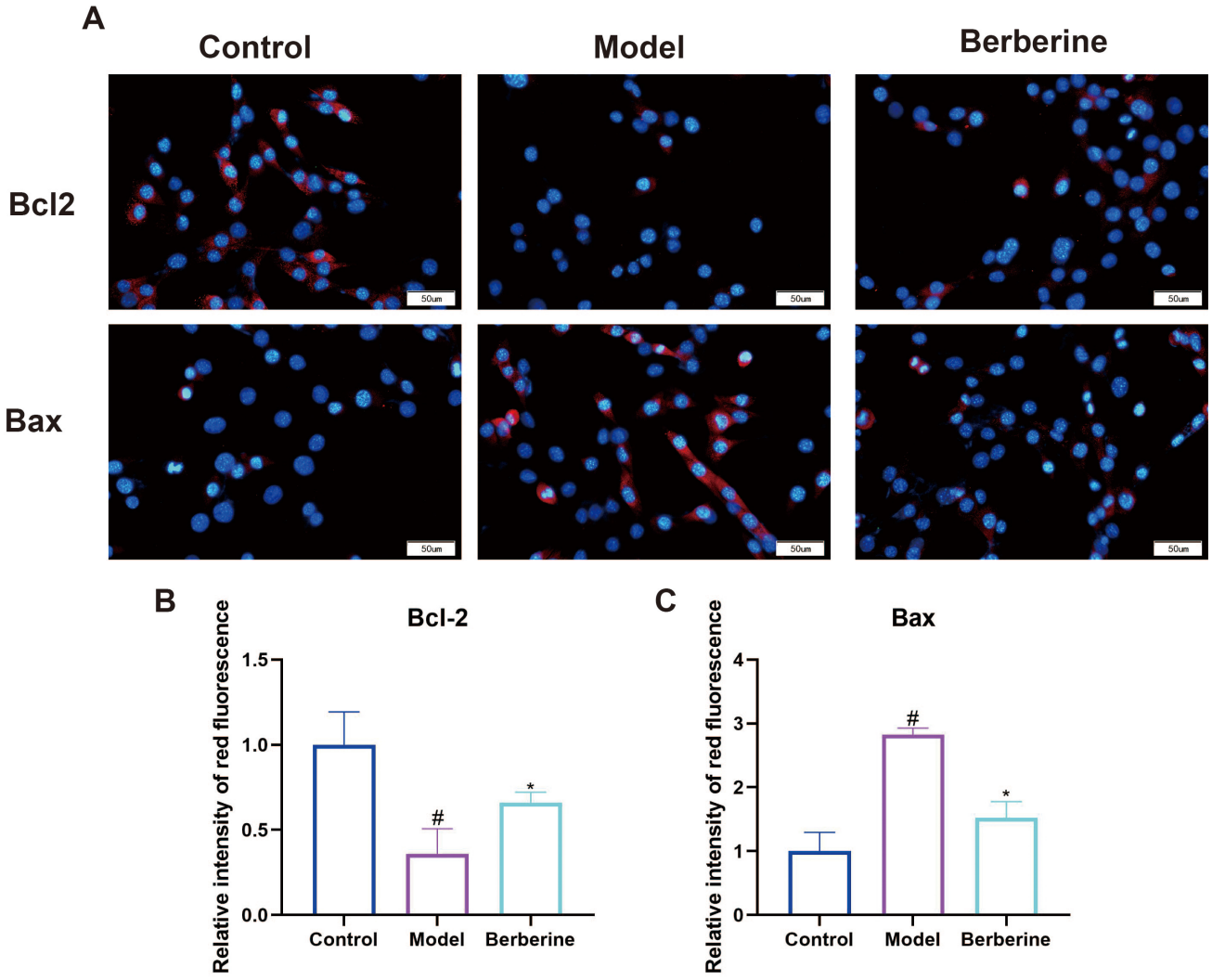

Fig. 4. Berberine regulates Bcl-2 and Bax protein expression. (A) Detection of Bcl-2 and Bax protein expression through immunofluorescence methodology. (B) Evaluation of the relative fluorescence intensity pertinent to Bcl-2 protein. (C) Assessment of the relative fluorescence intensity associated with Bax protein. "#" indicates a significant difference compared to the control group, $p < 0.05$; "*" signifies a significant difference compared to the model group, $p < 0.05$. N = 3. The nuclei are stained blue with DAPI; the positive Bcl-2 or Bax staining is red.

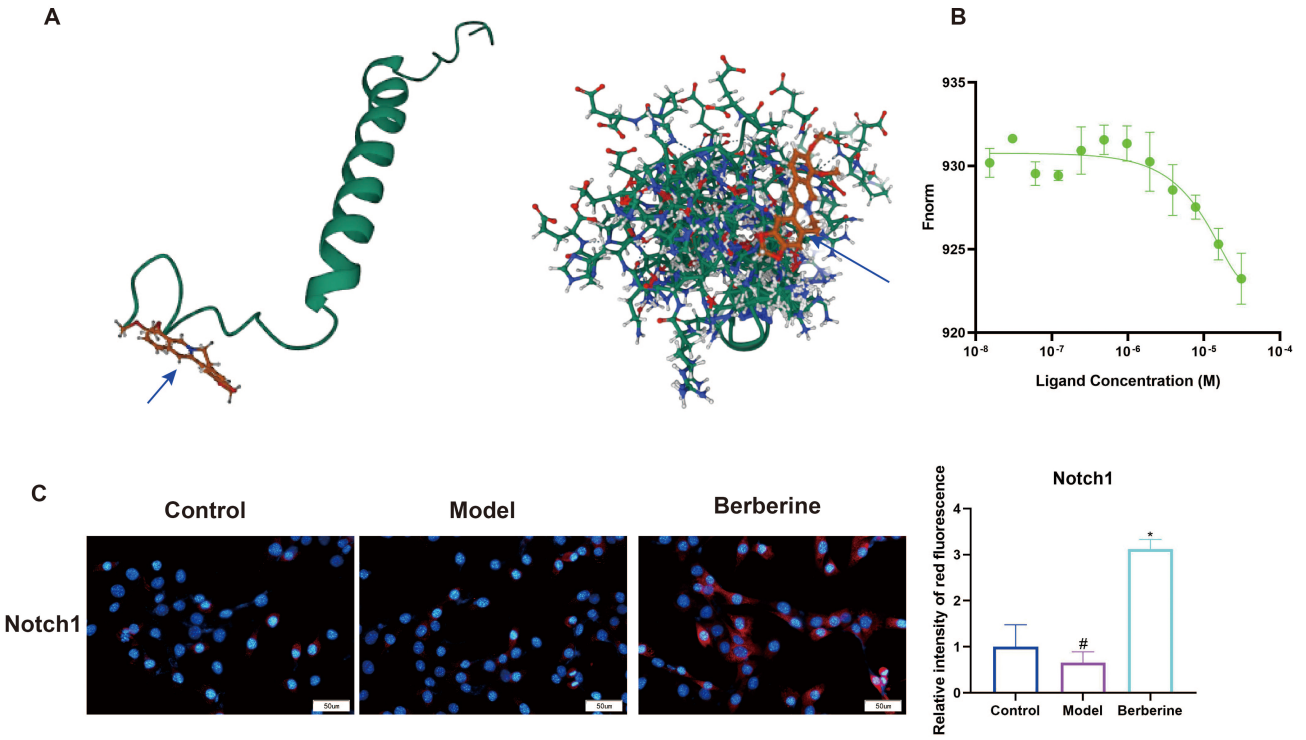

Fig. 5. Berberine targets Notch1 for regulatory function. (A) Molecular docking predicts a binding site between berberine and Notch1. The arrow indicates berberine. (B) Microscale Thermophoresis (MST) assay confirms targeted binding of berberine to Notch1. (C) Berberine treatment significantly increases Notch1 expression in cells. "#" indicates a significant difference compared to the control group, $p < 0.05$; "*" denotes a significant difference compared to the model group, $p < 0.05$. N = 3.

ing the protective effects of berberine on myocardial cells post-injury, demonstrating reductions in infarct size, improvements in tissue structure, and significant alterations in gene and protein expression levels associated with cell survival and apoptosis regulation.

Acute coronary syndrome has become a leading cause of morbidity and mortality from cardiovascular diseases worldwide. Clinically, some patients experience aggravated early ischemic myocardial injury following reperfusion therapy, leading to increased myocardial cell death, enlarged infarct size, and further impairment of heart function, a condition known as myocardial ischemia-reperfusion injury [14,15]. Studies have indicated that excessive apoptosis of myocardial cells plays a significant role in the pathophysiology of myocardial ischemia-reperfusion injury [16,17]. Thus, mitigating myocardial cell apoptosis during reperfusion and preserving cardiac function has been a focal point of both basic and clinical research.

Berberine, an isoquinoline alkaloid extracted from plants like Coptis chinensis or Phellodendron amurense, has historically been used to treat gastrointestinal infections caused by bacteria. Modern pharmacological studies have confirmed that berberine possesses a range of therapeutic effects, including glucose-lowering, anti-tumor, and anti-platelet aggregation effects. Recent studies have demonstrated that berberine can alleviate myocardial ischemia-reperfusion injury and exert myocardial protective effects [18,19]. Our findings align with previous research highlighting berberine's antioxidative, anti-inflammatory, and potential anti-apoptotic properties, and suggest a possible association with the modulation of the Notch pathway. However, the present data stop short of establishing a direct causal relationship between this pathway modulation and apoptosis reduction in myocardial cells post-ischemia-reperfusion injury. Further investigation is warranted to elucidate the exact mechanism by which berberine may exert its observed cardioprotective effects. This experiment revealed that oral administration of berberine to rats for 2 weeks prior to ischemia-reperfusion surgery significantly mitigated myocardial ischemia-reperfusion injury, reduced myocardial cell apoptosis, and improved post-operative cardiac function.

The significance of the Notch signaling pathway in the cardiovascular system's development, physiology, and pathophysiology has been well-documented. In recent years, the role of the Notch1 receptor and its downstream signaling molecule Hes1 in ischemic heart disease has garnered increasing attention [20,21]. The methodologies employed in this study, including molecular analyses, histological assessments, and protein expression studies, align with established protocols in the field. By focusing on the Notch signaling pathway, our research methodology offers

a unique perspective on the cardioprotective mechanisms of berberine, distinguishing our work from broader pharmacological studies.

Limitations of this study include animal models, which may not perfectly replicate human cardiac responses, and the need for additional studies to validate the findings in clinical settings. Addressing these limitations through translational and clinical research would strengthen the validity and applicability of the study results. Future research endeavors could focus on exploring the long-term effects of berberine treatment, investigating potential combinatorial therapies for enhanced cardioprotection, and elucidating the translational potential of berberine-based interventions in clinical settings. Further studies elucidating the specific downstream signaling pathways influenced by berberine could deepen our understanding of its broader therapeutic applications in cardiovascular disease management.

Conclusion

In conclusion, our study suggests that berberine may have cardioprotective effects on myocardial tissue following ischemia-reperfusion injury, which is associated with modulation of the Notch signaling pathway. While berberine was observed to influence the expression of related genes and proteins and, coincided with a reduced infarct size and apoptosis rate, further research is required to determine if these effects are specifically mediated through the Notch signaling and to clarify the precise nature of these interactions. Our findings provide a foundation for future investigations into berberine's therapeutic potential in treating ischemic heart diseases.

Availability of Data and Materials

All experimental data included in this study can be obtained by contacting the corresponding author if needed.

Author Contributions

DXJ, XT and HC contributed to the design and implementation of the research, DXJ, XT and HFY contributed to the analysis of the results and to the writing of the manuscript. DXJ, XT and HC conceived the original and supervised the project. All authors contributed significantly to editorial changes of important content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All procedures were approved by Ethics Committee of Yangzhou University Medical College (No.: YXYLL-2023-088).

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Prag HA, Aksentijevic D, Dannhorn A, Giles AV, Mulvey JF, Sauchanka O, *et al*. Ischemia-Selective Cardioprotection by Malonate for Ischemia/Reperfusion Injury. Circulation Research. 2022; 131: 528–541.

[2] Mao B, Yuan W, Wu F, Yan Y, Wang B. Autophagy in hepatic ischemia-reperfusion injury. Cell Death Discovery. 2023; 9: 115.

[3] Panconesi R, Widmer J, Carvalho MF, Eden J, Dondossola D, Dutkowski P, *et al*. Mitochondria and ischemia reperfusion injury. Current Opinion in Organ Transplantation. 2022; 27: 434–445.

[4] Tian CX, Li MY, Shuai XX, Jiang F, Dong YL, Gui Y, *et al*. Berberine plays a cardioprotective role by inhibiting macrophage Wnt5a/β-catenin pathway in the myocardium of mice after myocardial infarction. Phytotherapy Research. 2023; 37: 50–61.

[5] Zhang H, Wu X, Tao Y, Lu G. Berberine attenuates sepsis-induced cardiac dysfunction by upregulating the Akt/eNOS pathway in mice. Experimental and Therapeutic Medicine. 2022; 23: 371.

[6] Docshin P, Bairqdar A, Malashicheva A. Interplay between BMP2 and Notch signaling in endothelial-mesenchymal transition: implications for cardiac fibrosis. Stem Cell Investigation. 2023; 10: 18.

[7] Malashicheva A, Kostina A, Kostareva A, Irtyuga O, Gordeev M, Uspensky V. Notch signaling in the pathogenesis of thoracic aortic aneurysms: A bridge between embryonic and adult states. Biochimica et Biophysica Acta. Molecular Basis of Disease. 2020; 1866: 165631.

[8] Blaser MC, Buffolo F, Halu A, Turner ME, Schlotter F, Higashi H, *et al*. Multiomics of Tissue Extracellular Vesicles Identifies Unique Modulators of Atherosclerosis and Calcific Aortic Valve Stenosis. Circulation. 2023; 148: 661–678.

[9] Breikaa RM, Lilly B. The Notch Pathway: A Link Between COVID-19 Pathophysiology and Its Cardiovascular Complications. Frontiers in Cardiovascular Medicine. 2021; 8: 681948.

[10] Wang C, Wang XR, Song DD, Wang JL, Wang Y, Tao TQ, *et al*. The establishment of rat model in myocardial ischemia with psychological stress. Annals of Translational Medicine. 2020; 8: 322.

[11] JianXin C, Xue X, ZhongFeng L, Kuo G, FeiLong Z, ZhiHong L, *et al*. Qishen Yiqi Drop Pill improves cardiac function after myocardial ischemia. Scientific Reports. 2016; 6: 24383.

[12] Liu K, Sun T, Luan Y, Chen Y, Song J, Ling L, *et al*. Berberine ameliorates erectile dysfunction in rats with streptozotocin-induced diabetes mellitus through the attenuation of apoptosis by inhibiting the SPHK1/S1P/S1PR2 and MAPK pathways. Andrology. 2022; 10: 404–418.

[13] Ganjali S, Matbou Riahi M, Mahboobnia K, Iranshahy M, Banach M, Jangjoo A, *et al*. An *in vitro* investigation of the effects

of urolithins A and B on low-density lipoprotein uptake and its regulatory genes. Archives of Medical Science. 2023; 19: 1832–1841.

[14] Schanze N, Hamad MA, Nührenberg TG, Bode C, Duerschmied D. Platelets in Myocardial Ischemia/Reperfusion Injury. Hamostaseologie. 2023; 43: 110–121.

[15] Pizarro G. Ischemia Reperfusion Injury: Harder to Treat Than Cyanide Poisoning. JACC. Basic to Translational Science. 2023; 8: 1295–1297.

[16] Zhang H, Hu H, Zhai C, Jing L, Tian H. Cardioprotective Strategies After Ischemia-Reperfusion Injury. American Journal of Cardiovascular Drugs: Drugs, Devices, and other Interventions. 2024; 24: 5–18.

[17] Felli E, Felli E, Muttillo EM, Urade T, Laracca GG, Giannelli V, *et al*. Liver ischemia-reperfusion injury: From trigger loading to shot firing. Liver Transplantation. 2023; 29: 1226–1233.

[18] Syed Abd Halim SA, Abd Rashid N, Woon CK, Abdul Jalil NA. Natural Products Targeting PI3K/AKT in Myocardial Ischemic Reperfusion Injury: A Scoping Review. Pharmaceuticals. 2023; 16: 739.

[19] An N, Zhang G, Li Y, Yuan C, Yang F, Zhang L, *et al*. Promising Antioxidative Effect of Berberine in Cardiovascular Diseases. Frontiers in Pharmacology. 2022; 13: 865353.

[20] Peng X, Wang S, Chen H, Chen M. Role of the Notch1 signaling pathway in ischemic heart disease (Review). International Journal of Molecular Medicine. 2023; 51: 27.

[21] Karakaya C, van Turnhout MC, Visser VL, Ristori T, Bouten CVC, Sahlgren CM, *et al*. Notch signaling regulates strain-mediated phenotypic switching of vascular smooth muscle cells. Frontiers in Cell and Developmental Biology. 2022; 10: 910503.