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Protective effect and mechanism of different proportions of "*Danggui-Kushen*" herb pair on ischemic heart disease

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ABSTRACT

This study aims to investigate the protective effect and mechanism of *"Danggui-Kushen"* herb pair (DKHP) on ischemic heart disease (IHD). The rat model of myocardial reperfusion injury (MIRI) was established by ligation of the left anterior descending coronary artery. Rats were randomly divided into seven groups and administered orally for 7 days: control group, IHD group, DKHP1:1 group, DKHP1:2 group, DKHP2:1 group, DKHP1:3 group, DKHP3:1 group, the dosage was 2.7 g/kg. Measure electrocardiogram (ECG), myocardial infarction and injury assessment, Hematoxylin and eosin (HE) staining to evaluate myocardial injury and the protective effect of DKHP. Lactate dehydrogenase (LDH), Reactive oxygen species (ROS), IL-1β and IL-6 kit detection, immunohistochemical analysis, establishment of H9c2 cardiomyocyte hypoxia (Hypoxia) model, DKHP pretreatment for 3 h, MTT method to detect cell survival rate, cell immunofluorescence to observe NF- The expression of TLR-4, NF-κB, *p*-NF-κB, IKβα, *p*-IKβα, HIF-1α, VEGF and other genes and proteins were detected by κB nuclear translocation, mitochondrial membrane potential measurement, Western blot and Polymerase Chain Reaction (PCR). Compared with the model group, DKHP can reduce the size of myocardial infarction, reduce the levels of factors such as LDH, ROS, IL-1β and IL-6, and improve the cell survival rate; Compared with the model group, DKHP can inhibit the nuclear transfer of NF-κB and reduce mitochondrial damage; the results of immunohistochemical analysis, PCR and Western blot showed that compared with the model group, DKHP can reduce TLR-4, *p*-NF-κB, Expression levels of *p*-IKβα, HIF-1α, VEGF and other proteins. Reveal that DKHP may play a protective role in ischemic heart disease by reducing inflammation and oxidative stress damage. DKHP may have protective effect on ischemic heart disease, and its mechanism may be through reducing inflammatory response and oxidative stress damage to achieve this protective effect.

1. Introduction

Cardiovascular disease (CVD) is one of the diseases that seriously endanger human health [1], and its morbidity and mortality rank first in the world and are on the rise [2]. The mortality rate of ischemic heart disease remains high [3]. Ischemic heart disease (IHD) is

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mainly due to the lack of blood supply of coronary arteries for a long time and the inability to supply blood oxygen and nutrients in time. It leads to serious problems such as myocardial tissue atrophy, coronary artery blockage, branch stenosis, and myocardial metabolic disorders, mainly manifested as arrhythmia and heart failure, which seriously threaten the health and life of patients [4,5]. IHD can lead to cell death and cardiac insufficiency, and its underlying mechanisms involve oxidative stress, inflammation, neutrophil infiltration, and cytokine release, etc. [6]. Although clinical practice strategies have evolved over the past few years to optimize the prevention and treatment of ischemic heart disease (IHD), the consequences of this condition pose a significant burden on human health in terms of mortality and morbidity [7]. At present, the drugs commonly used in clinical treatment of IHD are mainly thrombolytic drugs [8]. Thrombolytic therapy can make blood reperfusion and blood flow unimpeded. However, restoration of blood flow may lead to myocardial cell death and increase infarct size, a process known as myocardial reperfusion injury (MIRI) [9]. Research on alleviating IHD has been receiving much attention. Therefore, it is necessary to explore new therapeutic targets for IHD.

The inflammatory cascade is an important therapeutic target for the treatment of acute ischemic injury and myocardial repair [10,11]. It is associated with the massive production of a range of mediators, including chemokines, proteases, TNF-α [12] and cytokines such as interleukins (IL) [13]. Inflammation has been noted in IHD and used as a parameter to assess cardiac injury after IHD [14]; this implies that a greater excessive inflammatory response leads to more severe myocardial injury after IHD. Furthermore, crosstalk between HIF and NF-κB has been demonstrated in many diseases, especially in IHD where hypoxia and inflammation coexist [15]. NF-κB has been shown to induce HIF and HIF also regulates NF-κB, suggesting that the crosstalk between HIF and NF-κB is bidirectional [16]. Inflammation and oxidative stress are important pathological mechanisms in the development of cardiovascular diseases [17]. Therefore, studying the role and alteration of inflammatory factors and oxidative stress in IHD injury is crucial for our understanding of this process and the resulting clinical treatment.

Traditional Chinese medicine (TCM) has the concept of holistic treatment and has the advantages of multi-target, multi-link, and multi-channel [18]. Traditional Chinese medicine has good curative effect and broad application prospect in alleviating IHD. The herb pair (combination of two drugs) is the most basic constituent unit of a traditional Chinese medicine compound, and it still has therapeutic characteristics and clinical significance in Chinese herbal medicine (CHM) [19]. Angelica and Sophora flavescens are traditional Chinese medicines widely used in the treatment of IHD [20]. The main component of angelica is ligustilide, which has the functions of anti-inflammation, dilating blood vessels, improving local microcirculation, etc., and is widely used in the research of cardiovascular diseases. The main components of Sophora flavescens are matrine and oxymatrine, which have anti-inflammatory and anti-tumor effects. The combination of the two has been used as a traditional drug pair so far. This study mainly explores the protective effect and mechanism of DKHP on IHD.

2. Materials and methods

2.1. Animal model

The 70 Sprague-Dawley (SD) rats (200 ± 20 g) used in the experiment were purchased from the Animal Safety Evaluation Center of Heilongjiang University of Traditional Chinese Medicine (certificate number of experimental animals, SCXK2018-0001), half male and half female. During the experiment, the animals had free access to water and food. Rats were anesthetized and fixed. After stabilization, an incision was made in the skin at the location of the heart, exposing the underlying ribs by blunt dissection. Separate the ribs using curved hemostats. The ribs are separated and fixed to ensure easy manipulation on the heart. Peel off the pericardium using a cotton applicator. The left anterior descending branch is located between the lower edge of the left atrial appendage and the pulmonary pyramid. Then, a 6–0 silk ligature was threaded approximately 3 mm through the left coronary artery, and myocardial ischemia was initiated by complete ligation of the left ascending (LAD) coronary artery. The chest cavity cannot be ligated until a strong and regular heart rhythm is restored. The retractor is then disconnected until normal spontaneous breathing is established. And the skin is closed. Finally, the limb lead electrocardiogram 20 min after ischemia was recorded by connecting a multi-channel physiological recorder, and compared with the electrocardiogram before modeling. ST-segment elevation was considered a sign of successful modeling. The rats were randomly divided into seven groups (8 rats in each group): control group, model group, DKHP (1:1) group, DKHP (1:2) group, DKHP (2:1) group and DKHP (1:3) group, DKHP (3:1) group. Oral administration (refer to clinical dosage), and the rest of the rats were given the same amount of normal saline. Blood samples were obtained from the abdominal aorta and centrifuged at 3500 g for 15 min, then the supernatant was collected and placed at −80 °C for further analysis. Then, the rats were sacrificed, and the hearts were harvested for Triphenyl tetrazolium chloride (TTC) staining and pathological analysis.

2.2. Preparation of drug extract

The decoction pieces of Angelica sinensis and Sophora flavescens were purchased from the pharmacy of Heilongjiang University of Chinese Medicine (Harbin, China), and were identified as conforming to the 2020 edition of Chinese Pharmacopoeia. Accurately weigh "Danggui-Kushen" herb pair (1:1, 1:2, 2:1, 1:3, 3:1), add 10 times the amount of 70% ethanol to soak for 1 h, heat and condense and reflux for 1.5 h. Add 8 times the amount of 70% ethanol to the residue again, heat, condense and reflux for 1 h, combine the two filtrates, concentrate to obtain 0.5 g of crude drug per ml of liquid medicine, and set aside. Take part of the concentrated solution and add appropriate amount of water to make freeze-dried powder for later use.

2.3. Main reagents and kits

Enzyme-linked immunosorbent assay (ELISA) kits for IL-6, IL-1β and TNF-α were purchased from Nanjing Kaiji Biotechnology Co., Ltd.; lactate dehydrogenase (LDH) activity detection kit, ROS detection kit; 2% TTC dye solution was purchased from Beijing Suolaibao Technology Co., Ltd., 20200927; hematoxylin was purchased from Tianjin Alpha Biotechnology Co., Ltd., A1280; xylene was purchased from Tianjin Fuyu Fine Chemical Co., Ltd.

2.4. ECG detection

ECG was recorded using an ECG Recording and Analysis System (BL-420F, Chengdu TME Technology Company, Chengdu, China).

2.5. HE staining of rat myocardial tissue

Myocardial tissues were fixed in 10% neutral buffered formalin for 2 days, followed by dehydration, permeabilization and paraffin embedding processes. Paraffin sections were cut to a thickness of 5 mm and placed on glass slides. Sectioned specimens were stained with hematoxylin-eosin kit (HE), and then observed under a microscope.

2.6. Determination of myocardial infarction size

The rats after blood collection were sacrificed, and their hearts were taken out and washed with PBS. The obtained ventricular tissue was sliced to the same thickness. The slices were added to 2% TTC (Sigma Co., St Louis, Mo) solution at 37 °C. The infarcted part is pale, and the normal tissue is brick red. Images were collected by a digital camera, and the myocardial infarction area and total ventricular area were calculated using an image analysis system (Image Pro Plus 6.0; Media Cybernetics, LP, USA) to obtain the myocardial infarction rate (%).

2.7. Immunohistochemical staining

Heart tissue samples were fixed with 4% paraformaldehyde for 24 h and embedded in paraffin. About 4 μm paraffin sections were deparaffinized and quenched in 3% H_2O_2 for 10 min at room temperature. Incubate overnight at 4 °C with HIF-1α (1:200), NF-κB (1:200) and TLR4 (1:200) antibodies. Slides were incubated with secondary antibodies for 1.5 h and then developed with avidin peroxidase and DAB and counterstained with hematoxylin. Dehydrated slides were mounted with neutral resin. Positive cells appear as brown areas. Three regions of each sample in the experiment were randomly captured using a camera (Leica, Germany) and analyzed with Image J.

2.8. Cell culture

H9c2 rat cardiomyocytes were purchased from Shanghai Cell Institute, Chinese Academy of Sciences. The cells were cultured with DMEM complete culture solution containing 10% fetal bovine serum and 1% penicillin-streptomycin double antibody in an incubator with 95% air and 5% CO_2 at a constant temperature of 37 °C.

2.9. Cell hypoxia injury model and grouping

The H9c2 cardiomyocytes were pretreated with "*Danggui-Kushen*" herb pair (DKHP) for 3 h, the model group and the control group were cultured normally, and then the drug-containing medium was replaced by sugar-free and serum-free DMEM medium at 95% CO_2 and 5% O_2 Incubate at a constant temperature of 37 °C for 8 h in an incubator, and culture in the control group normally.

Divide H9c2 into 7 groups: control group, Hypoxia group, DKHP 50 μg/mL (1:1) + Hypoxia group, DKHP 50 μg/mL (1:2) + Hypoxia group, DKHP 50 μg/mL (2:1) + Hypoxia group, DKHP 50 μg/mL (1:3) + Hypoxia group, DKHP 50 μg/mL (3:1) + Hypoxia group.

2.10. Cell viability assay

The H9c2 cardiomyocytes in the logarithmic growth phase were seeded in a 96-well plate, and the cell density was adjusted to 2×10^4 cells/mL. After cell administration and modeling, 5 mg/mL of MTT (Solarbio, M8180, Beijing, China) solution 10 μL, incubate in a 37 °C incubator for 4 h, remove the medium, add 100 μL DMSO to each well, shake on an air shaker for 10 min to fully dissolve the purple crystals, use a microplate reader at 490 nm Measure the absorbance.

2.11. Immunofluorescent staining of cells

Immunofluorescence staining H9c2 cells were fixed with 4% paraformaldehyde for 15 min, blocked with 0.3% Triton X-100, incubated with anti–NF–κB p65 antibody (1:100, Abcam, UK) overnight at 4 °C, and then treated with a Cy3 secondary antibody was incubated for 2 h at room temperature in the dark. After incubation, cells were washed 3 times in PBS and stained with 4′,6-diamidino-

2-phenylindole (DAPI, Beyotime, China) for nuclei identification. Images were captured using a fluorescence microscope (Leica DMi8, Germany). Data shown represent at least three replicates. The relative fluorescence intensity in the nucleus (NF-κB) was analyzed by ImageJ software.

2.12. Determination of mitochondrial transmembrane potential ($\Delta\Psi m$)

After treatment, H9c2 cells (5×10^5 cells/well) were incubated with 2 μM JC-1 for 30 min at 37 °C in the dark. Green fluorescent cells (reflecting hyperpolarized and hypopolarized mitochondria) were quantified under a laser scanning confocal microscope (Leica, TCS SP5, Germany). Fluorescence images of cells were thoroughly checked and photographed. Analyze the acquired signal using ImageJ software, analyzing at least ten fields of view and quantifying the mean fluorescence intensity of each field.

2.13. Extraction of total RNA and real-time fluorescent quantitative PCR

Total cell RNA was extracted according to the instructions of TRIzol kit (R401-01, Vazyme), then reverse transcription kit (R312-

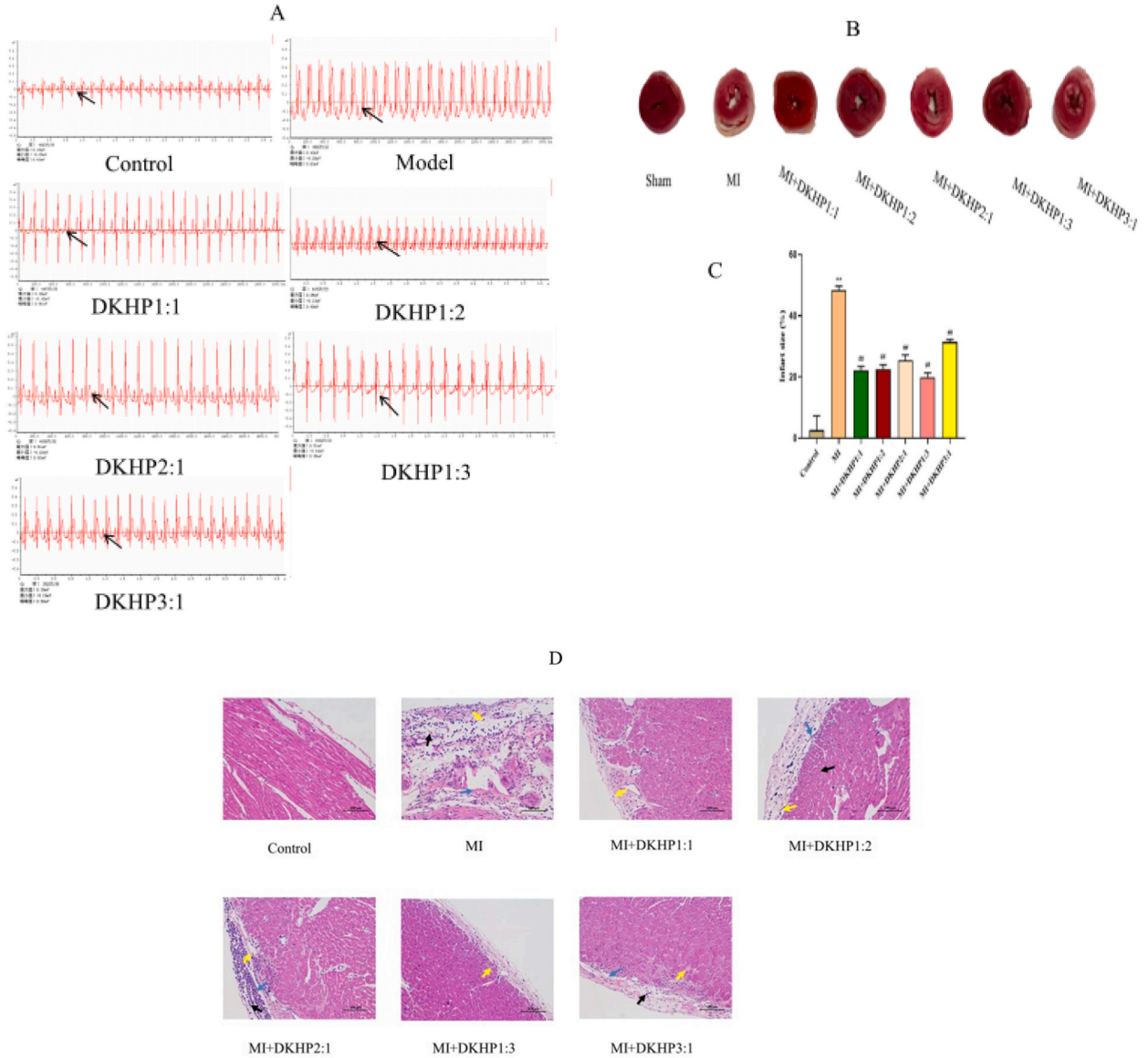

Fig. 1. DKHP is protective against ischemic heart disease. (A) Electrocardiogram. (B) TTC-stained image of myocardial infarct size. Normal tissue is brick red and infarcted tissue is white. (C) Infarct size as a percentage of total area. (D) HE staining of rat myocardium. Mean ± SEM (n = 3 in each group), compared with the normal group, **P < 0.01; compared with the model group, #P < 0.05; compared with the model group, ##P < 0.01.

02, HiScriptIII1st Strand cDNA Synthesis Kit Vazyme) was used to synthesize cDNA according to the kit instructions, and then ChamQ Universal SYBR Qpcr Master was used Mix (Q711-02, L/N 7E561F1, Vazyme) for PCR reaction. Finally, the $2^{-\Delta\Delta CT}$ method was used to determine the mRNA expression level of the target gene. The primers used in this experiment are as follows:HIF1α(forward): GTCGGACAGCCTCACCAAACAG,(reverse):TAGGTAGTGAGCCACCAGTGTCC;TLR4(forward):ATGGCATGGCTTACACCACC,(reverse): GAGGCCAATTTTGTCTCCACA;IKβα(forward):TACGCCCCAGCATCTCCACTCCG,(reverse):CTCCACGATGCCCAGGTAGCCAT;NFκB (forward):CCCTACGGAACTGGGCAAAT,(reverse):CCTGGCGGATGATCTCCTTC;VEGF(forward):CGAGACGCAGCGACAAGGCA, (reverse):ACCTCTCCAAACCGTTGGCACG;GAPDH(forward):AACGACCCCTTCATTGAC,(reverse):TCCACGACATACTCAGCA.

2.14. Western blot

Cells were lysed using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China), and intracellular proteins were extracted. After the extracted protein was processed, it was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, then transferred to PVDF membrane, and then incubated with QuickBlock™ Western blocking solution (P0252, Beyotime, China) at room temperature for 1.5 h. To block non-specific binding sites, incubate the blocked PVDF membrane with the primary antibody overnight at 4 °C, then incubate with the secondary antibody at room temperature for 1 h, and the membrane was then washed 3 times with TBST for 1 min each. The protein bands were detected with an Odyssey color infrared laser scanning imager (LI-COR, USA), and the images were analyzed using Odyssey Application Software 3.0. The antibodies used are: TLR-4 (1:1000, Abcam, UK), NF-κB p65 (1:1000, Abcam, UK), NF-κB p-P65 (1:1000, Abcam, UK), Iκβ-α (1:1000, Abcam, UK), *p*-Iκβ-a(1:1000, Abcam, UK), HIF-1α(1:1000, Abcam, UK), VEGF(1:1000, Abcam, UK). β -actin (ab8226, 1:1000; Abcam, UK).

2.15. Statistical analysis

The results are presented as the mean ± standard deviation from at least three independent experiments. Statistical comparisons were analyzed by one-way analysis of variance and Tukey's test using GraphPad Prism 5 software (GraphPad Software, Inc.). $P < 0.05$ was considered to indicate a statistically significant difference.

3. Reseult

3.1. The protective effect of different ratios of DKHP on cardiac ischemia and hypoxia injury

3.1.1. Effect of DKHP on ECG of ischemic heart disease

In this experiment, seven days after administration, the electrocardiogram was detected and recorded, and the results are shown in Fig. 1A. Compared with the Control, the ST segment T wave in the model group was significantly inverted, indicating that the IHD model was established successfully. Compared with the model group, DKHP in different ratio groups can significantly reduce ST-segment elevation, and the reduction effect of DKHP in the 1:3 group is the most significant.

3.1.2. Effect of DKHP on myocardial infarct size in ischemic heart disease

The measurement results of myocardial infarction size (TTC) are shown in Fig. 1B and C. Compared with the Control group, the myocardial infarct size in the model group was significantly increased. The 1:3 groups have the strongest effect and the most significant effect.

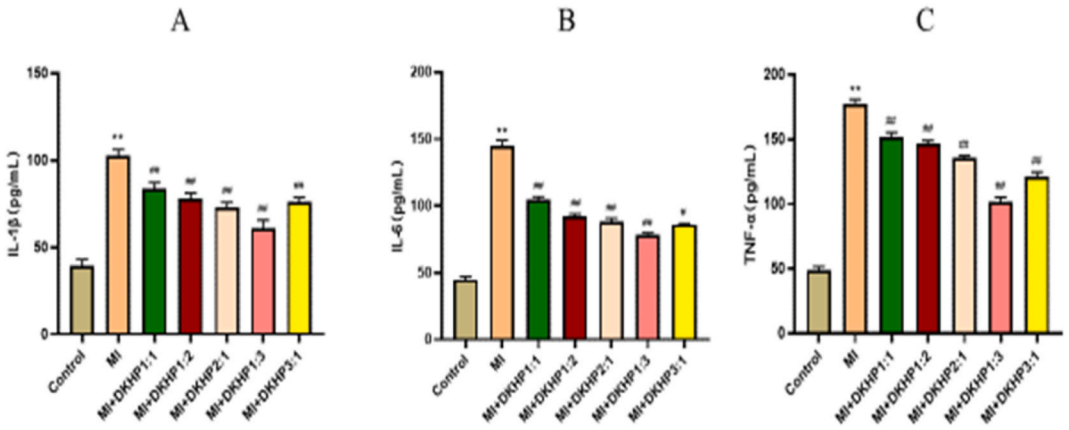

Fig. 2. DKHP inhibits the expression levels of inflammatory factors IL-1β, IL-6 and TNF-α after hypoxia treatment. (A) IL-1β levels in serum. (B) IL-6 levels in serum. (C) Serum TNF-α levels. Mean ± SEM (n = 3 in each group), compared with the normal group, **P < 0.01; compared with the model group, #P < 0.05; compared with the model group, ##P < 0.01.

3.1.3. Effect of DKHP on the morphology of cardiomyocytes in ischemic heart disease

The results of HE staining experiments are shown in Fig. 1D. Compared with the Control group, the myocardium of the model group had myocardial cell swelling, deformation, striations, loss of striations, and cell infiltration. Compared with the model group, DKHP groups with different ratios could significantly reduce this damage.

3.2. DKHP inhibits the inflammatory response induced by hypoxia-induced myocardial injury

3.2.1. DKHP reduces the expression levels of related inflammatory factors

In order to further evaluate and verify the effect of DKHP on ischemia-hypoxia To investigate the protective effect of injury-induced inflammatory response, we measured the levels of IL-1β, IL-6 and TNF-α in serum. The results are shown in Fig. 2A–C: the levels of IL-1β, IL-6 and TNF-α in the model group were significantly increased. It is worth noting that each ratio of DKHP can reduce the secretion level of inflammatory factors in serum, but the inhibitory effect of DKHP1:3 group is the best.

3.2.2. DKHP reduces the positive expression of TLR4 and NF-κB p65 in rat myocardial tissue after hypoxia treatment

The results of immunohistochemical experiments are shown in Fig. 3A and B. Compared with Control. The positive expression of TLR4 and NF-κB p65 in the model group was high. Compared with the model group, the distribution area of TLR4 positive expression in each DKHP ratio group was sparse, and the positive expression decreased, and the DKHP1:3 group had the best effect.

3.2.3. The effect of DKHP on the viability of H9c2 cardiomyocytes induced by hypoxia

The results of the cell viability experiment are shown in Fig. 4: pretreatment with DKHP for 3 h significantly improved the cell survival rate of Hypoxia-induced cardiomyocytes, and the cell survival rate of the 1:3 group was higher than that of other ratio groups.

3.2.4. Effect of DKHP on hypoxia-induced nuclear translocation of NF-κB in H9c2 cardiomyocytes

We performed immunofluorescent staining to visualize nuclear translocation of NF-κB p65. As shown in Fig. 5, compared with the control group, Hypoxia treatment significantly promoted the translocation of p65 to the nucleus, while DKHP administration significantly blocked the nuclear translocation of p65 after Hypoxia.

3.2.5. Effect of DKHP on the expression level of ischemic heart disease-related inflammatory proteins and mRNA

The relevant inflammatory proteins in H9c2 cardiomyocytes were detected by Western Blot experiments (Fig. 6A–D). The results showed that compared with the Control group, TLR-4, p65 and Ikβα in the model group were significantly increased. Compared with the model group, DKHP The ratio group reduced the expression of inflammatory proteins, and the 1:3 group was better than the other ratio groups. The results of PCR experiments (Fig. 6E–G) were consistent with their results.

3.3. DKHP inhibits hypoxia-induced myocardial oxidative stress injury

3.3.1. DKHP reduces the expression levels of LDH and ROS in ischemic heart disease

The results of the LDH and ROS kits showed (Fig. 7A and B), compared with the Control group, the expression levels of LDH and ROS in the model group were significantly increased, and compared with the model group, the DKHP group significantly decreased their expression, while 1:3 group is particularly notable.

3.3.2. DKHP reduces the positive expression of HIF-1α in myocardial tissue of rats treated with hypoxia

The results of immunohistochemical detection of HIF-1α in myocardium (Fig. 8) showed that compared with the Control group, the

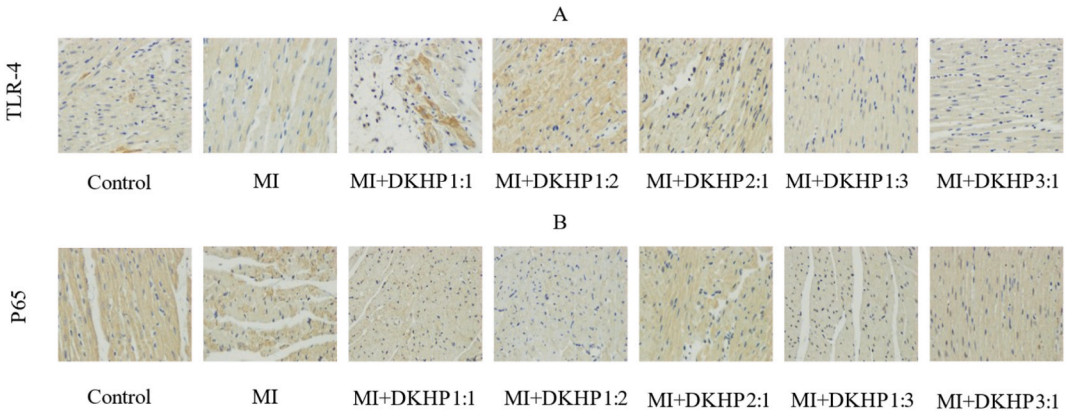

Fig. 3. DKHP reduces the positive expression of TLR4 and NF-κBp65 in myocardial tissue after hypoxia treatment. (A) Expression of TLR-4 in myocardial tissue. (B) Expression of p65 in myocardial tissue.

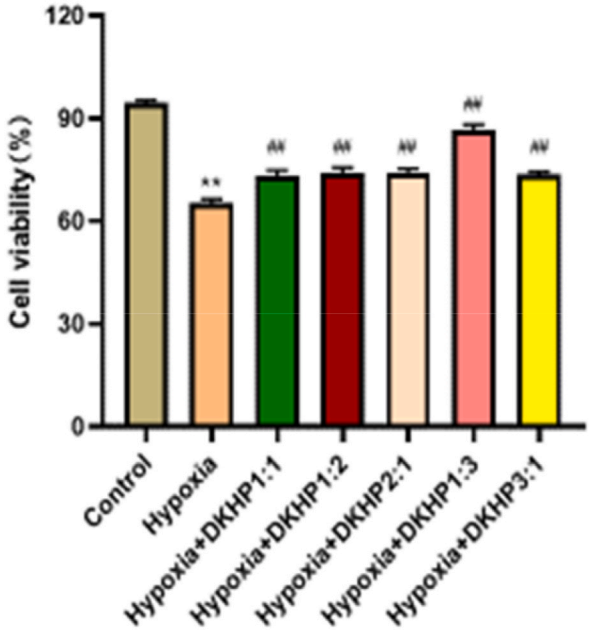

Fig. 4. DKHP increased the survival rate of H9c2 cardiomyocytes treated with hypoxia for 3 h. Mean ± SEM (n = 3 in each group), compared with the normal group, **P < 0.01; compared with the model group, #P < 0.05; compared with the model group, ##P < 0.01.

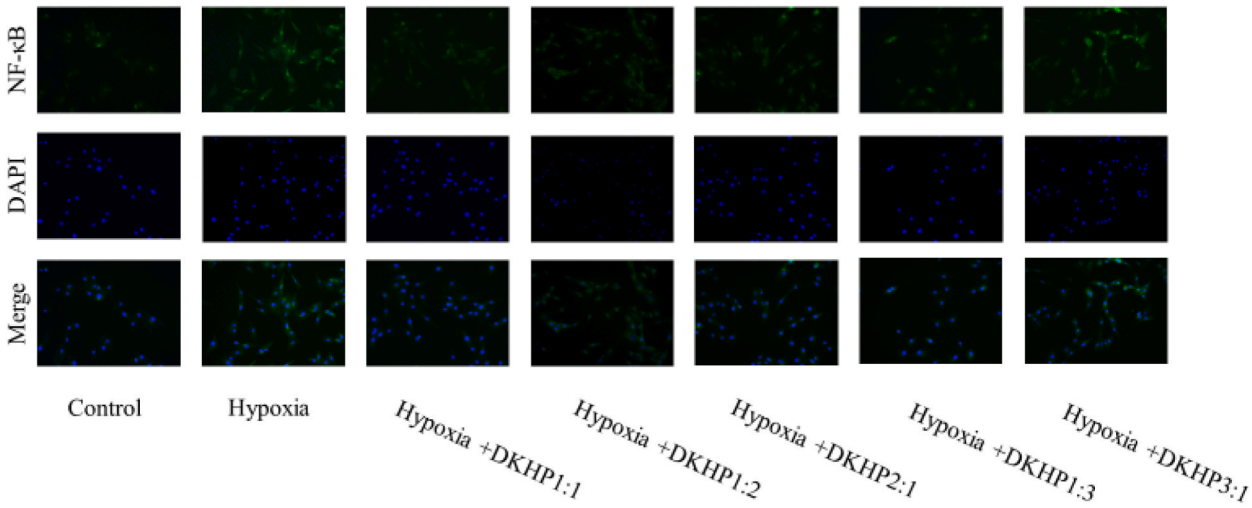

Fig. 5. DKHP inhibits nuclear translocation of NF-κB after hypoxic treatment.

positive expression of HIF-1α in the cardiomyocytes of the model group increased; compared with the model group, the positive expression of HIF-1α in each ratio of DKHP reduce.

3.3.3. Effect of DKHP on mitochondrial membrane potential of H9c2 cardiomyocytes induced by hypoxia

The experimental results of mitochondrial transmembrane potential measurement are shown in Fig. 9, compared with the Control group, the Hypoxia group aggravated cardiomyocyte damage, while the DKHP group attenuated the damage.

3.3.4. DKHP reduced the expression levels of HIF-1α, VEGF and other proteins and their mRNA in cardiomyocytes after hypoxia induction

The results of Western Blot experiments are shown in Fig. 10A–C, compared with the Control group, the protein expression levels of HIF-1α and VEGF in the model group were significantly increased, and compared with the model group In comparison, the DKHP ratio group reduced the expression of oxidative stress-related proteins, and the 1:3 group was superior to other ratio groups. The results of PCR experiments (Fig. 10D and E) were consistent with their results.

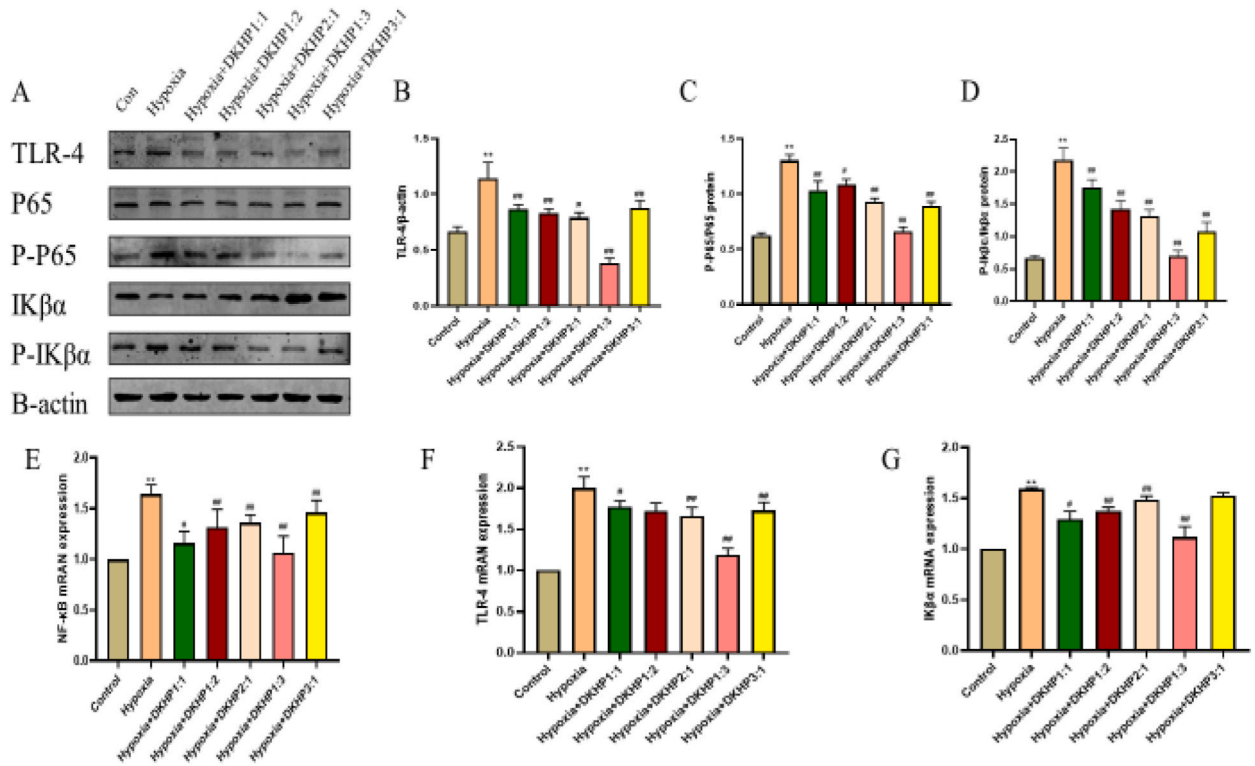

Fig. 6. DKHP reduced the expression levels of TLR-4, p65, p-p65, IKβα, *p*-Ikβα and other related inflammatory proteins and their mRNA in cardiomyocytes after hypoxia induction. (A) The expression of TLR-4, p65, p-p65, IKβα, *p*-Ikβα in H9c2 cells was assessed by Western blot. (B–D) Quantitative analysis diagrams of TLR-4, p-p65/p65, *p*-Ikβα/Ikβα. (E–G) Gene expression levels of NF-κB, TLR-4, IKβα. Mean ± SEM (n = 3 in each group), compared with the normal group, **P < 0.01; compared with the model group, #P < 0.05; compared with the model group, ##P < 0.01.

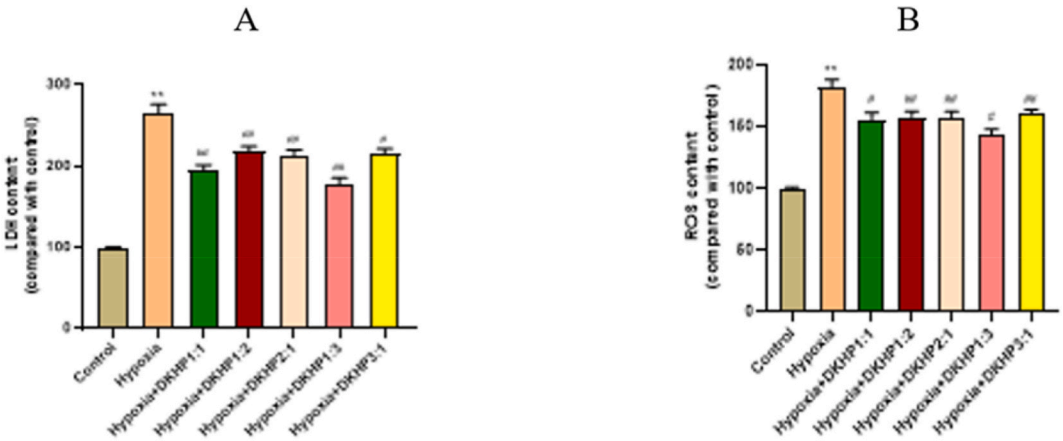

Fig. 7. DKHP reduces the expression levels of LDH and ROS in hypoxia-induced H9c2 cardiomyocytes. (A) LDH levels in cells. (B) ROS levels in cells. Mean ± SEM (n = 3 in each group), compared with the normal group, **P < 0.01; compared with the model group, #P < 0.05; compared with the model group, ##P < 0.01.

4. Discussion

Myocardial ischemic injury is a common pathological feature of ischemic heart disease, which can reduce the activity of cardiomyocytes and vascular endothelial cells [21] and increase the size of myocardial infarction. Previous studies have shown that *"Danggui-Kushen"* herb pair has important functions such as reducing infarct size, protecting blood vessels, promoting vascular reconstruction, and anti-inflammation in ischemic heart disease [21]. This is consistent with the results of our research. TTC results

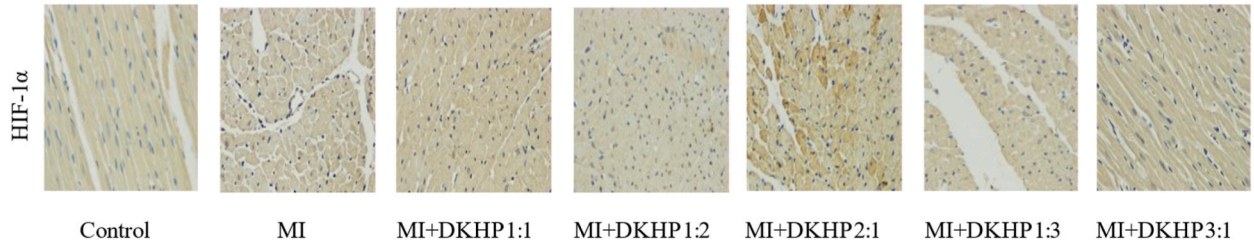

Fig. 8. DKHP decreased the positive expression of HIF-1α in myocardial tissue after hypoxic treatment. This picture shows the expression of HIF-1α in myocardial tissue.

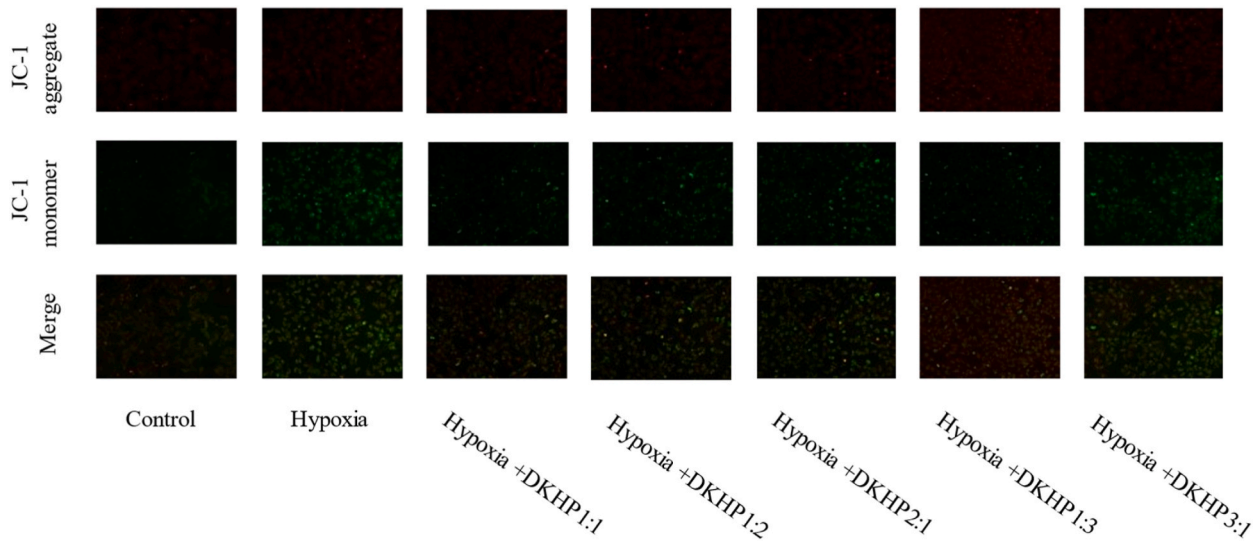

Fig. 9. DKHP increases hypoxia-induced mitochondrial membrane potential in H9c2 cardiomyocytes and attenuates cell damage.

show that DKHP can significantly reduce the myocardial infarct size of rats in the model group, and can reduce the swelling, deformation, appearance, disappearance, and cell infiltration of myocardial cells in the model group. cell damage. However, its role in modulating ischemia-induced inflammation and oxidative stress injury remains unclear. For ischemia-induced injury, the inflammatory cascade and oxidative stress lead to the release of a large number of oxygen free radicals and inflammatory factors, resulting in severe and irreversible myocardial damage [22]. Traditional Chinese medicine has multi-component and multi-target therapeutic effects. In the clinical application of traditional Chinese medicine, the combined use of different traditional Chinese medicines can often produce greater therapeutic effects. This study reveals the protective effect of different proportions of DKHP in ischemic heart disease and its mechanism.

Myocardium is often ischemic due to occlusion of the coronary arteries responsible for myocardial perfusion [23]. Restoration of blood flow induces MI/RI [24], which is characterized by metabolic dysfunction, local inflammatory response [25], and apoptosis [26], leading to cardiac remodeling and dysfunction [27] and exacerbating tissue damage. Studies have shown that, based on the molecular mechanism of hypoxic injury, the key targets of clinical treatment are anti-inflammatory and antioxidative stress [28]. In this study, the HIF-1α/NF-κB signaling pathway was found to play a key role in the protective effect of DKHP on the heart. NF-κB is a key protein of the entire inflammatory family [29], a typical signaling pathway [30] responsible for the process of inflammatory infiltration and inhibition of cell survival. Phosphorylated IKβ can promote the release of NF-κB, which enters the nucleus and activates the expression of inflammatory genes [31]. The degradation of NF-κB leads to the translocation of p65 subunits into the nucleus [32], which in turn triggers specific promoter sequences of target genes [33], such as TNF-α, IL-6, Bcl-2, and Bax, thereby inducing inflammatory damage and apoptosis. In this study, DKHP can inhibit the phosphorylation and degradation of NF-κB inhibitor (IKβα), prevent the nuclear translocation of NF-κBp65, and reduce the levels of inflammatory factors IL-6, IL-1β and tumor necrosis factor TNF-α, inhibiting apoptosis. Inhibition of NF-κB signaling pathway can effectively treat ischemic myocardial injury.

Notably, crosstalk between HIF and NF-κB has been demonstrated in a variety of diseases [34], particularly in IHD where hypoxia and inflammation coexist. In ischemic heart disease, hypoxia inhibits PHD1 activity and accumulates HIF-1α [35], leading to degradation of NF-κB inhibitor (Iκβ) and activation of NF-κB in the nucleus [36]. This is consistent with our findings in the present study, where our experimental results showed that DKHP was able to inhibit the excessive accumulation of HIF-1α in vivo as well as the generation of ROS to exert a protective effect on the myocardium. Under hypoxic conditions, NF-κB activity is regulated by the level of

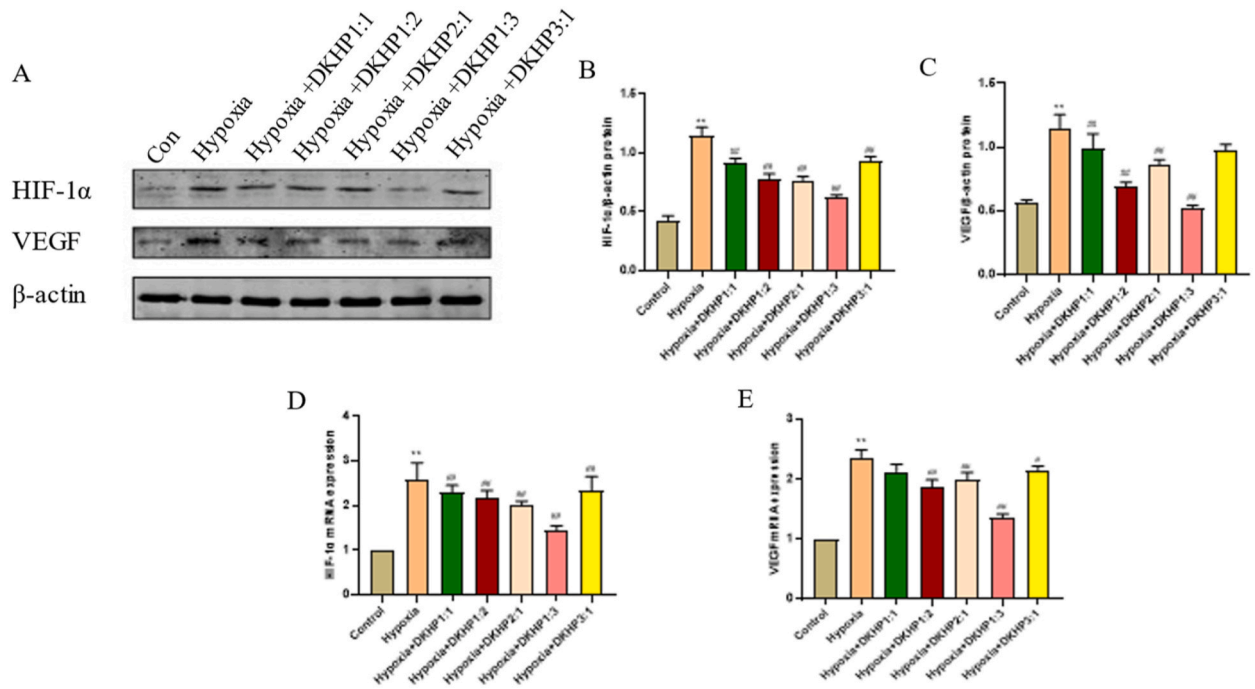

Fig. 10. DKHP reduced the expression levels of HIF-1α, VEGF and other proteins and their mRNA in cardiomyocytes after hypoxia induction. (A) The expression of HIF-1α and VEGF in H9c2 cells was assessed by Western blot. (B–C) Quantitative analysis of HIF-1α and VEGF. (D–E) Gene expression levels of HIF-1α and VEGF. Mean ± SEM (n = 3 in each group), compared with the normal group, **P < 0.01; compared with the model group, #P < 0.05; compared with the model group, ##P < 0.01.

HIF-1α mRNA, and HIF-1α can regulate NF-κB. Through this regulation, intracellular NF-κB is activated by HIF-1α mRNA under ischemic and hypoxic conditions, resulting in an inflammatory response, as shown in Fig. 11. However, myocardial ischemic injury was rarely reported in these studies. In addition, unlike previous studies, our study also found that among the different dosage groups of DKHP, the experimental results of the DKHP 1:3 group were better than the other groups, which may be related to the higher content of alkaloids such as Matrine with better anti-inflammatory effects in the 1:3 group. And we hypothesized that DKHP may alleviate hypoxia-induced myocardial inflammation and oxidative stress through the HIF-1α/NF-κB pathway, thus exerting a protective effect on the myocardium.

Despite these promising results, this study still has some limitations. First of all, we only selected rat and mouse H9c2 cardiomyocytes for simulation research, and all these results need to be verified by in vivo experiments. Second, ischemic heart disease is a complex pathological process. This study only focused on NF-κB and HIF-1α, and the specific mechanism needs further evaluation.

5. Conclusion

Our study demonstrated that DKHP can ameliorate myocardial injury and preliminarily explored its mechanism of action. We concluded that DKHP may protect myocardium from ischemic injury by inhibiting the HIF-1α/NF-κB signaling pathway, and found that DKHP1:3 had the best effect.Our findings provide a rationale for DKHP as a potential drug for cardioprotection, and the DKHP1:3 group works best.

Consent for publication

Not applicable.

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Data availability

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

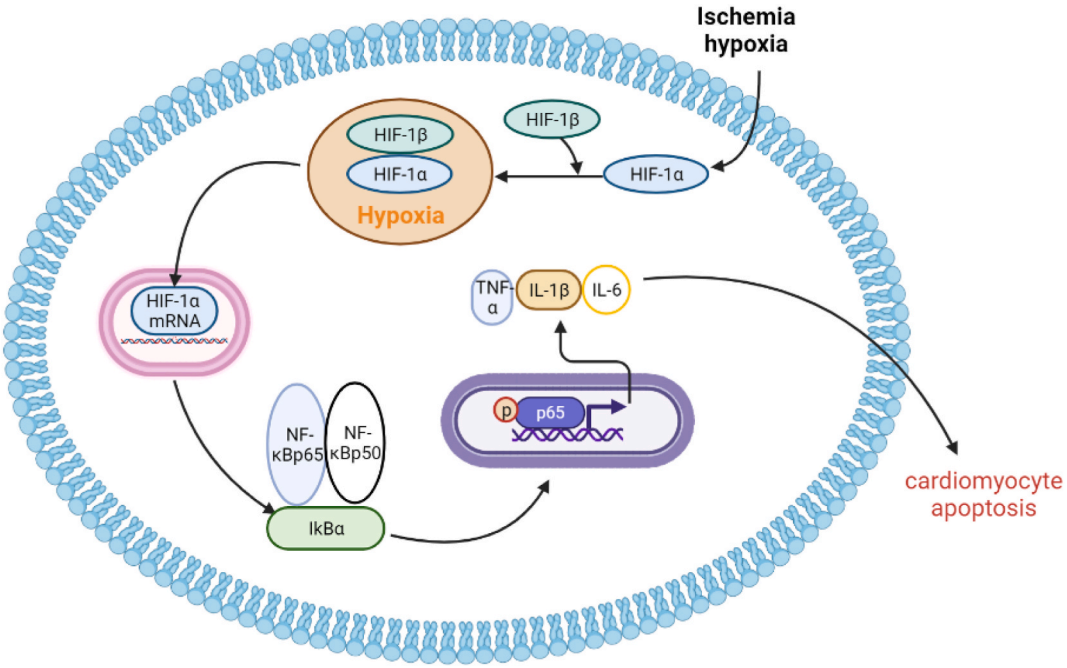

Fig. 11. Schematic diagram of the molecular mechanism of HIF-1α/NF-κB.

Has data associated with your study been deposited into a publicly available repository?
Response: No.
Has data associated with your study been deposited into a publicly available repository?
Response: The data that has been used is confidential.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Heilongjiang University of traditional Chinese Medicine.

Informed consent

All authors have read and approved the publication of this paper.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Xu Yuan: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ke meng Liu:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. **Peiliang Dong:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. **Hua Han:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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