

Original Article

Vitamin C inhibits oxidative stress and apoptosis through activating the Nrf2/HO-1/NQO1 pathway to ameliorate myocardial injury in coronary heart disease

Hongbo Li¹, Wei Zhong², Gulizhaba Keremu³, Hanliang Dan⁴, Yanhui Hu⁵

¹Huankui Academy, Nanchang University, Nanchang 330031, Jiangxi, China; ²The First Clinical Medical College, Nanchang University, Nanchang 330006, Jiangxi, China; ³Department of Cardiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430021, Hubei, China; ⁴Department of Cardiology, Wuhan Dongxihu District People's Hospital Affiliated to Wuhan University of Science and Technology, Wuhan 430000, Hubei, China; ⁵Cardiovascular Clinical Medical Center, Liyuan Hospital Affiliated to Tongji Medical College of Huazhong University of Science and Technology, Wuhan 430077, Hubei, China

Received June 14, 2026; Accepted July 29, 2026; Epub September 15, 2026; Published September 30, 2026

Abstract: Background: Oxidative stress and apoptosis caused by myocardial ischemia and hypoxia are the core mechanisms of myocardial damage in coronary heart disease (CHD). The myocardial protective effect and related molecular mechanisms of vitamin C (Vit C) have not been fully elucidated. Aims: To investigate whether Vit C activates the Nrf2/HO-1/NQO1 axis to inhibit oxidative stress and apoptosis, thereby alleviating CHD myocardial injury. Methods: A CHD rat model was constructed, and the cardiac function was detected by echocardiography after 6 weeks of Vit C intervention. The effects of Vit C intervention on myocardial injury and myocardial fibrosis in rats were observed by histologic staining. The hypoxia-reoxygenation (H/R) model of H9c2 cells was established. Cell viability, cell survival rate, and apoptosis were evaluated by CCK-8 assay, Calcein-AM/Hoechst staining, and flow cytometry. The levels of cardiac troponin I (cTnI), lactate dehydrogenase (LDH), Caspase-3 activity, and inflammatory factors in rat serum and H9c2 cells were measured using biochemical kits; oxidative stress-related indicators of rat myocardial tissue and H9c2 cells were also detected. Western blot was conducted to detect protein expression levels associated with myocardial injury, apoptosis, and the Nrf2/HO-1/NQO1 pathway. Results: Vit C dose-dependently improved cardiac function, and reduced the pathologic damage to myocardial tissue and myocardial fibrosis in CHD rats. Vit C intervention effectively reduced serum cTnI levels and inflammatory factor levels in CHD rats, and inhibited oxidative stress. *In vitro*, Vit C alleviated H/R-induced H9c2 cell injury, reduced LDH release and Caspase-3 activity, and inhibited oxidative stress and apoptosis. In addition, Vit C intervention promoted the nuclear translocation of Nrf2 in myocardial tissue of CHD rats and H9c2 cells, and up-regulated HO-1 and NQO1 proteins. Intraperitoneal injection of Nrf2 inhibitor ML385 in rats or knockdown of Nrf2 in H9c2 cells weakened the protective effect of Vit C. Conclusion: Vit C inhibits oxidative stress and apoptosis in CHD rats and H/R cardiomyocytes by activating the Nrf2/HO-1/NQO1 pathway, affording myocardial protection.

Keywords: Vitamin C, coronary heart disease, oxidative stress, myocardial damage, Nrf2/HO-1/NQO1 pathway

Introduction

As a common cardiovascular disorder, coronary heart disease (CHD) is pathologically characterized by coronary atherosclerosis, which causes myocardial ischemia, hypoxia, and even necrosis resulting from coronary stenosis or occlusion [1, 2]. In China, CHD-related deaths account for 20% of the total deaths [3], and the burden of CHD disease in low- and middle-income countries worldwide continues to rise

[4]. The initiation and progression of CHD involve synergistic effects of multiple pathogenic factors. Hypertension, obesity, diabetes, and high-fat diet are the main risk factors, which can accelerate the progress of CHD by damaging vascular endothelial function, promoting lipid deposition and inflammatory response [5-7]. At present, the treatment strategies of CHD are mainly drug intervention, interventional therapy and surgery [8]. Although these treatment schemes reduce the mortality of CHD, it is

difficult to reverse established atherosclerotic plaques, and long-term drug intervention may be accompanied by adverse reactions such as liver and kidney function damage [9, 10]. Therefore, it is of great significance to find natural active substances with myocardial protective effects and explore the molecular mechanism of their improvement in CHD to optimize treatment.

As a naturally occurring water-soluble antioxidant, vitamin C (Vit C) widely participates in maintaining redox homeostasis and modulating lipid metabolism [11]. Research evidence has validated its protective effect in cardiovascular diseases [12-14]. Vit C can eliminate accumulated reactive oxygen species (ROS) in cardiac fibroblasts and effectively regulate the synthesis of fibrocollagen, thereby alleviating myocardial fibrosis [15]. In a rat model of septic cardiomyopathy, Vit C pre-treatment reduced lipopolysaccharide-induced myocardial cell injury, increased antioxidant enzyme activity, reduced myocardial tissue pathologic damage, and reduced inflammatory factor levels [16]. In addition, Vit C deficiency impaired the vasomotor function in a high fat diet-induced guinea pig model of coronary atherosclerosis, suggesting that Vit C deficiency had adverse effects on coronary artery function [17]. Although the cardiovascular protective effect of Vit C has been widely confirmed, its specific regulatory effect and molecular mechanism on CHD myocardial injury were still unclear.

The Nuclear factor E2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1)/NAD (P) H:quinone oxidoreductase 1 (NQO1) signaling axis is the core endogenous pathway regulating oxidative stress and apoptosis [18, 19]. HO-1 can specifically recognize and bind to heme molecules, and then catalyze the decomposition of heme. Its metabolites can effectively reduce oxidative stress injury in the body [20]. NQO1 is a cytosolic antioxidant flavin that increases intracellular NAD⁺ levels and reduces the production of strong oxidizing semiquinone radicals, thereby protecting cells from oxidative damage [21]. Under oxidative stress, Nrf2 undergoes nuclear translocation and interacts with antioxidant response elements, and then up-regulates the downstream target genes such as HO-1 and NQO1, thereby eliminating ROS and maintaining redox homeostasis [22, 23]. A previous

study demonstrated that activating the Nrf2/HO-1/NQO1 signaling axis reduced myocardial pathological damage, inhibited myocardial oxidative stress and apoptosis, and improved blood lipid levels in mice with CHD [24]. Notably, Vit C can up-regulate Nrf2 and its downstream antioxidant genes, which in turn alleviated myocardial cell damage caused by cadmium exposure [25]. However, whether Vit C inhibits oxidative stress and thus reduces myocardial injury by stimulating the Nrf2/HO-1/NQO1 axis in CHD models was still unclear.

Therefore, this study constructed a CHD rat model, along with an H9c2 cell injury model caused by hypoxia/reoxygenation (H/R), and systematically explored the effects of Vit C on cardiac function, oxidative stress, and cell injury in CHD rats. It clarified the mediating function of Nrf2/HO-1/NQO1 signaling axis. This study aimed to provide new insight into the molecular mechanism of Vit C in improving myocardial injury, and to offer a theoretical foundation and experimental evidence for the development of new adjuvant drugs for CHD.

Materials and methods

Construction of CHD rat model

Six-week-old male Sprague-Dawley rats (210-225 g) were supplied by Changsheng Biotechnology Co., Ltd. (Liaoning, China), and housed at 22 ± 2°C, 50% ± 20% humidity, under a 12-h light/dark cycle with *ad libitum* access to food and water. After a one-week adaptation period, they were randomly divided into Control, CHD, 50 mg/kg Vit C, 100 mg/kg Vit C, 200 mg/kg Vit C, and 200 mg/kg Vit C + ML385 group (n=6).

CHD was induced by intragastric administration of a high-fat diet (87.3% basal diet, 2% cholesterol, 10% lard, 0.2% propylthiouracil, and 0.5% sodium cholate), once a day (10 mL/kg/d) for 6 weeks [26]. The rats in 50 mg/kg Vit C group, 100 mg/kg Vit C group, and 200 mg/kg Vit C group were given corresponding doses of Vit C (60374ES60, Yeasen Biotechnology, Shanghai, China) once daily for 6 weeks, starting 1 h after high-fat diet feeding [27]. The rats in the 200 mg/kg Vit C + ML385 group received an intraperitoneal injection of ML385 (30 mg/kg, HY-100523, MedChemExpress, NJ, USA) after feeding high-fat diet for 30 min, and then were

orally administered Vit C (200 mg/kg/d) 30 min after injection for 6 weeks [28]. The control group of rats received a basic feed along with daily administrations of same amount of normal saline. Upon completion of the intervention period, the ejection fraction, stroke volume, Posterior Wall Thickness in systole (PWTs), fractional shortening, and Left Ventricular End-Diastolic Diameter (LVDd) were recorded by a Sigma Vet ultrasound imaging system (Yuyan Scientific Instruments Co., Ltd., Shanghai, China), to evaluate cardiac function in rats. This research was approved by the Animal Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (Approval number: 2024-4354).

Tissues and Hematoxylin and Eosin (H&E) staining

After the assessment of cardiac function was completed, all rats were anesthetized via inhalation of 2% isoflurane. Abdominal aortic blood was collected from the rats, and serum was separated for subsequent detection. In addition, the rats were euthanized through intraperitoneal administration of sodium pentobarbital (100 mg/kg), and the rat heart tissues were carefully separated with sterile tissue scissors and gently rinsed with precooled PBS to remove surface blood stains. The heart tissues were exposed to 4% paraformaldehyde (60536ES60, Yeasen Biotechnology) for 24 h, and then trimmed into a tissue block with a thickness of about 2 mm, embedded in paraffin, and preparation of 5 µm thickness of continuous sections. After drying, the slices were dewaxed with xylene (X821391, Macklin, Shanghai, China) and then hydrated in gradient ethanol. Tissue slices were immersed in hematoxylin solution (HY-K0315, MedChemExpress) for 5 min, followed by a 30-second treatment with differentiation solution. After rinsing with PBS, the sections were stained with eosin solution for 2 min. Then they were dehydrated in 95% ethanol and absolute ethanol for 5 min each, subsequently cleared in xylene for 30 min, and mounted with neutral gum (36313ES60, Yeasen Biotechnology). Finally, the pathologic changes to myocardial tissue in rats were observed with DM IL LED microscope (Leica, Heidelberg, Germany).

Masson staining

Paraffin-embedded sections of rat myocardial tissue were dewaxed, followed by rinsing with

sterile distilled water. The nuclei were stained with hematoxylin solution in Masson trichrome staining kit (C0189S, Beyotime, Shanghai, China) for 5 min, fully washed, and differentiated for 30 s. The sections were incubated in Ponceau-acid fuchsin staining solution for 10 min, then immersed in phosphomolybdic acid solution for 2 min. Next, bright green staining reagent was applied to the sections for 1 min, followed by differentiation for 1 min. Then they underwent dehydration through graded ethanol, were exposed to xylene for clearing, and finally were viewed and photographed with a microscope.

Dihydroethidium (DHE) staining

The fresh heart tissue of rats was cut into tissue blocks with a thickness of about 2 mm and immersed in OCT embedding agent (C0171A, Beyotime). The embedded tissue blocks were quickly frozen at -80°C. After the tissues were completely frozen, they were sliced into continuous sections (5 µm). The sections were rewarmed for 5 min at room temperature, then the cleaning solution was applied to evenly cover the surface and allowed to stand for 5 min. After the washing solution was removed, the tissue sections were treated with DHE fluorescent probe (10 µM, HY-D0079, MedChem-Express) and kept in the dark for 30 min. After rinsing twice with PBS, the tissue sections were observed by DM3000 fluorescence microscope (Leica, Heidelberg, Germany), and ROS level in myocardial tissue was quantified by ImageJ 1.54 h software (National Institute of Mental Health, USA).

TUNEL staining

Paraffin-embedded sections of rat myocardial tissue were first dewaxed in xylene and rehydrated through graded ethanol. Protease K (20 µg/mL, without DNase, HY-108717A, MedChem-Express) was added to the surface of the sections and reacted for 15 min at 37°C. The sections were rinsed thoroughly with PBS, followed by dropping the TUNEL assay solution (C1086, Beyotime, Shanghai, China) to ensure uniform coverage of the entire tissue section, and incubated for 60 min in a dark environment at 37°C. Subsequently, DAPI staining reagent (C1006, Beyotime) was applied to stain cell nuclei for 10 min. The myocardial tissue was observed with fluorescence microscope, and the percentage of TUNEL positive cells was quantified by ImageJ software.

Vitamin C reduces myocardial damage in coronary heart disease

Construction and transfection of H/R cell models

H9c2 rat cardiomyocytes (CL-0089) were sourced from Wuhan Pricella Biotechnology (Hubei, China) and maintained in complete DMEM medium (CM-0089, Wuhan Pricella Biotechnology) at 37°C with 5% CO₂ (v/v). After normal culture for 24 h, H9c2 cells were cultured in hypoxic incubator with 94% N₂, 5% CO₂, and 1% O₂ for 24 h, and then in a normoxic incubator with 5% CO₂ and 95% O₂ for another 24 h to establish the H/R cardiomyocyte model [29, 30].

To explore the protective impact of Vit C on the H/R myocardial cell model, H9c2 cells were cultured in a hypoxic incubator for 24 h, and then the media was replaced with complete media containing Vit C in normoxic incubator for 24 h. Small interfering RNA for Nrf2 (si Nrf2, positive-sense strand: CUGAUCAGGCUCAGUCACU; anti-sense strand: AGUGACUGAGCCUGAUCAG) and its control (si NC) were synthesized and constructed by Sangon Biotech Co., Ltd. (Shanghai, China). For 200 µM Vit C + si NC and 200 µM Vit C + si Nrf2 groups, si Nrf2 or si NC was transfected into H9c2 cells for 24 h according to Lipofectamine 3000 (L3000001, Invitrogen, CA, USA) [31]. After transfection, cells were placed in a hypoxic incubator for 24 h, and finally placed into a complete medium containing 200 µM Vit C, and put in a normoxic incubator for another 24 h [32].

Cell counting Kit-8 (CCK-8) assay

H9c2 cells were inoculated into 96-well plates (2 × 10⁴/well). Once the cells were observed to be completely adherent (about 12-16 h), the fresh medium containing Vit C (25, 50, 100, 200, and 400 µM) was replaced and cultured for 24 h [33]. Subsequently, 10% CCK-8 reagent (E606335, Sangon Biotech Co., Ltd.) was introduced, fully mixed, and incubated for 2 h. Microplate reader (SpectraMax iD3s, Molecular Devices, Shanghai, China) was used to measure OD₄₅₀ values to evaluate cell viability.

Assessment of cardiac injury markers

Lactate dehydrogenase (LDH) assay kit (A020-1-2), Caspase-3 activity assay kit (G015-1-3), and Cardiac troponin I (cTnI) assay kit (H149-2-2) were all obtained from Jiancheng Biotech-

nology Research Institute (Nanjing, China). Following the kit instructions, LDH release, Caspase-3 activity, and cTnI level in H9c2 cell culture supernatant and serum of rats in different treatment groups were detected to evaluate myocardial injury.

Assessment of oxidative stress markers

Superoxide dismutase (SOD) assay kit (S0101S), malondialdehyde (MDA) assay kit (S0131S) and catalase (CAT) assay kit (S0051) were purchased from Beyotime. The heart tissues of rats in different treatment groups were washed with pre-cooled PBS and cut into pieces of about 1 mm³, and incubated with lysis buffer (P0013, Beyotime), and the supernatant was collected after homogenization. In addition, the H9c2 cells were fully lysed with the above lysis buffer, and the supernatant was collected. The levels of oxidative stress markers in heart tissue and H9c2 cell lysate supernatant were measured using the above kits.

ELISA

The levels of inflammatory factors in H9c2 cell culture supernatant and rat serum were evaluated by rat interleukin (IL)-1β ELISA kit (E-EL-R0012), IL-6 ELISA kit (E-EL-R0015), and tumor necrosis factor-α ELISA kit (TNF-α, E-EL-R2856). These ELIAS kits were obtained from Elabscience (Wuhan, China). Serum samples and cell culture supernatants were added to the corresponding wells of the ELISA plate, and the corresponding antibodies were added at 37°C for 90 min. After discarding the liquid from all wells, HRP-labeled Streptavidin was introduced and reacted for 20 min in a dark environment. The wells were repeatedly washed with washing solution, and the color developing agent A solution and B solution were added and incubated in dark for 15 min. Termination solution was added and gently blended, the OD450 values were determined, and the specific concentration of inflammatory factors was calculated according to the kit instructions.

Calcein-AM/Hoechst staining

H9c2 cells were inoculated into 12-well plates, and when the cells had grown to a confluence of 60% to 70%, 1 mL Calcein-AM staining solution (5 µM, 40719ES50, Yeasen Biotechnology) was introduced and kept at 37°C in the dark for

Vitamin C reduces myocardial damage in coronary heart disease

20 min. Then Hoechst 32258 staining reagent (5 µg/mL, 40729ES76, Yeasen Biotechnology) was added and incubated for 5 min for nuclear staining [34]. After rinsing twice with PBS, the staining effect was visualized through a fluorescence microscope, with the proportion of Calcein-AM positive (surviving cells) was calculated by ImageJ software.

Flow cytometry

Following various interventions, H9c2 cells were gathered through centrifugation, and then resuspended in Binding Buffer (500 µL, AP107, Multi Sciences, Hangzhou, China). Subsequently, Annexin-V-APC (5 µL) and propidium iodide staining solution (10 µL) were added to the cell suspension, gently blown and mixed, and incubated at 25°C in a dark environment for 5 min. The apoptosis rate was determined using flow cytometry (BD FACSCalibur™, BD Biosciences, San Jose, CA, USA).

2',7'-dichlorofluorescein (DCFH) staining

DCFH-DA working solution (10 µM, 50101ES01, Yeasen Biotechnology) was added to ensure full cover adherent H9c2 cells, followed by a 30-min light-protected incubation at 37°C. Cells were rinsed thoroughly with PBS, the staining was visualized using a fluorescence microscope, and the fluorescence intensity was quantified with ImageJ software to evaluate the ROS level in the cells.

Immunofluorescence

The myocardial tissue paraffin sections were dewaxed and hydrated, and then placed into a citric acid buffer (pH 6.0) and subjected to microwave heating for 15 min for antigen retrieval. The tissue sections were immersed in 0.3% Triton X-100 (T824275, Macklin) for 10 min, followed by rinse with PBS. 5% bovine serum albumin (BSA, A801320, Macklin) was dropped onto the surface of the sections and blocked for 30 min. The sections were incubated at 4°C with the primary antibody 8-oxoG (1:100, sc-130914, Santa Cruz Biotechnology, Inc., TX, USA) overnight. Sections were rinsed thoroughly with PBS afterwards, and exposed to secondary antibody IgG (1:100, F-2765, Invitrogen) for 90 min in dark. The sections were covered with DAPI staining reagent for 10 min, mounted with AntiFade mounting medium

(E675011, Sangon Biotech Co., Ltd.), and then visualized by fluorescence microscope.

H9c2 cells were fixed with 4% paraformaldehyde for half an hour after different treatments, and then incubated with 0.3% Triton X-100 for 10 min to increase cell membrane permeability. After blocking with 5% BSA, cells were incubated overnight with primary antibody 8-oxoG at 4°C. The subsequent operation is consistent with the fluorescent staining steps of myocardial tissue sections.

Western blot

H9c2 cells in different treatment groups were treated with RIPA lysate (P0013B, Beyotime), and the protein supernatant was collected after centrifugation. At the same time, the rat heart tissue was cut into pieces of about 1 mm³, and the supernatant was collected after the lysate was fully homogenized. The supernatant was mixed with SDS-PAGE loading buffer for gel electrophoresis. After protein separation, the target protein was moved onto PVDF membrane (Invitrogen) and then blocked using 5% BSA for 2 h. The membrane was placed at 4°C with primary antibody matrix metalloproteinase (MMP)-2 (1:500, AF5330, Affinity Biosciences, OH, USA), vascular endothelial growth factor (VEGFA, 1:1000, ab46154, Abcam, MA, USA), Nrf2 (1:500, AF7006, Affinity Biosciences), HO-1 (1:500, AF5393, Affinity Biosciences), Bcl-2 (1:2000, AF0769, Affinity Biosciences), NQO1 (1:10000, ab80588, Abcam), Bax (1:500, AF0120, Affinity Biosciences), c-Caspase-3 (1:500, ab32042, Abcam), or caspase-3 (1:2000, ab184787, Abcam) overnight. After washing with TBST buffer for three times, the membranes were treated with the secondary antibody IgG (1:10000, 31460, Invitrogen) for 2 h. The ECL luminescence reagent (C520045, Sangon Biotech Co., Ltd.) was uniformly covered on the membrane surface, and visualized by JP-2880 gel imaging system (Jinpeng Analysis Instrument Co., Ltd., Shanghai, China). Using GAPDH (1:3000, T0004, Affinity Biosciences) as an internal reference, the gray values of the protein bands were quantitatively analyzed through ImageJ software. Additionally, Nuclear and Cytoplasmic Protein Extraction Kit (P0028, Beyotime) was used to separate the nucleus and cytoplasm of rat myocardial tissue and H9c2 cells, and Nrf2 protein expression in these two components was detected respectively.

Statistical analysis

All experiments were independently repeated at least three times, and all data were expressed as mean $\pm$ standard deviation. Statistical analyses were conducted using SPSS 26.0 software (IBM SPSS Statistics 26). Normality and homogeneity of variance were verified by the Shapiro-Wilk test and Levene's test, respectively. All data met the assumptions of normal distribution and equal variance. Comparisons between two groups were performed using Student's t-test, while comparisons among multiple groups were performed using one-way ANOVA followed by Bonferroni post-hoc test. $P < 0.05$ was considered significant. The data graph was drawn using Prism software (Graphpad 9.0), and the Mechanistic diagram was drawn using BioRender (<https://www.biorender.com/>).

Results

Vit C reduces cardiac dysfunction and myocardial damage in CHD rats

A rat model of CHD was established to systematically evaluate the effects of Vit C on cardiac dysfunction and myocardial damage. The ejection fraction and fractional shortening of CHD rats were notably decreased, and the stroke volume, PWTs and LVDd were markedly increased, suggesting that CHD rats had severe cardiac dysfunction. Vit C intervention improved the above cardiac function indexes in a dose-dependent manner and effectively reverse CHD-induced cardiac dysfunction (**Figure 1A-E**). H&E staining showed disordered arrangement of myocardial fibers accompanied by inflammatory cell infiltration in CHD rats. However, Vit C treatment effectively alleviated inflammatory cell infiltration and normalized the structure and arrangement of myocardial fibers (**Figure 1F**). Masson staining showed extensive degeneration and necrosis of myocardial fibers in CHD rats, accompanied by marked fibrosis; Vit C intervention attenuated myocardial fibrosis (**Figure 1G**). In addition, the serum levels of cTnI and LDH in CHD rats were significantly increased, suggesting severe myocardial injury. Vit C intervention dose-dependently reduced serum cTnI and LDH levels and alleviated CHD-induced myocardial injury (**Figure 1H, 1I**). Western blot indicated a notable elevation in MMP-2 and VEGFA protein levels in CHD rats; Vit C interven-

tion effectively reduced their expression (**Figure 1J, 1K**). Moreover, the serum levels of TNF- α , IL-1 β , and IL-6 in CHD rats were markedly increased, whereas Vit C intervention reduced these inflammatory factor levels (**Figure 1L**). These results indicate that Vit C effectively improves cardiac dysfunction in CHD rats and reduces myocardial pathological damage, fibrosis, and inflammation.

Vit C inhibits oxidative stress and cell apoptosis in CHD rats

Next, we investigated the effects of Vit C intervention on oxidative stress and apoptosis in myocardial tissue of CHD rats. DHE staining revealed that ROS level in myocardial tissue of CHD rats was markedly elevated, and Vit C intervention could reduce ROS level (**Figure 2A, 2B**). Moreover, the content of MDA in myocardial tissue of CHD rats increased significantly, and CAT and SOD activity decreased significantly. Vit C intervention reduced MDA level, increased CAT and SOD activity, and effectively reduced CHD-induced myocardial oxidative stress injury (**Figure 2C-E**). Immunofluorescence showed that the level of oxidative damage marker 8-oxoG in CHD rats was notably increased, and Vit C treatment markedly reduced the expression of 8-oxoG, further confirming the attenuation of myocardial oxidative damage (**Figure 2F, 2G**). The proportion of TUNEL positive cells in myocardial tissue of CHD rats was increased, while Vit C treatment significantly reduced the proportion of TUNEL-positive cells (**Figure 2H, 2I**). Caspase-3 activity in myocardial tissue of CHD rats was markedly increased, and Vit C treatment effectively reduced Caspase-3 activity (**Figure 2J**). At the same time, Bax and c-Caspase-3/Caspase-3 levels in CHD rats were notably increased, while Bcl-2 expression was down-regulated. Vit C intervention reversed the expression trend of the above apoptosis-associated proteins (**Figure 2K, 2L**). These results together indicated that Vit C inhibits the oxidative stress and apoptosis of myocardial tissue in CHD rats, thereby exerting a cardioprotective effect.

Vit C inhibits oxidative stress and apoptosis by activating the Nrf2/HO-1/NQO1 axis in CHD rats

To clarify the specific molecular mechanism by which Vit C inhibits oxidative stress and apoptosis in myocardial tissue of CHD rats, we focused

Vitamin C reduces myocardial damage in coronary heart disease

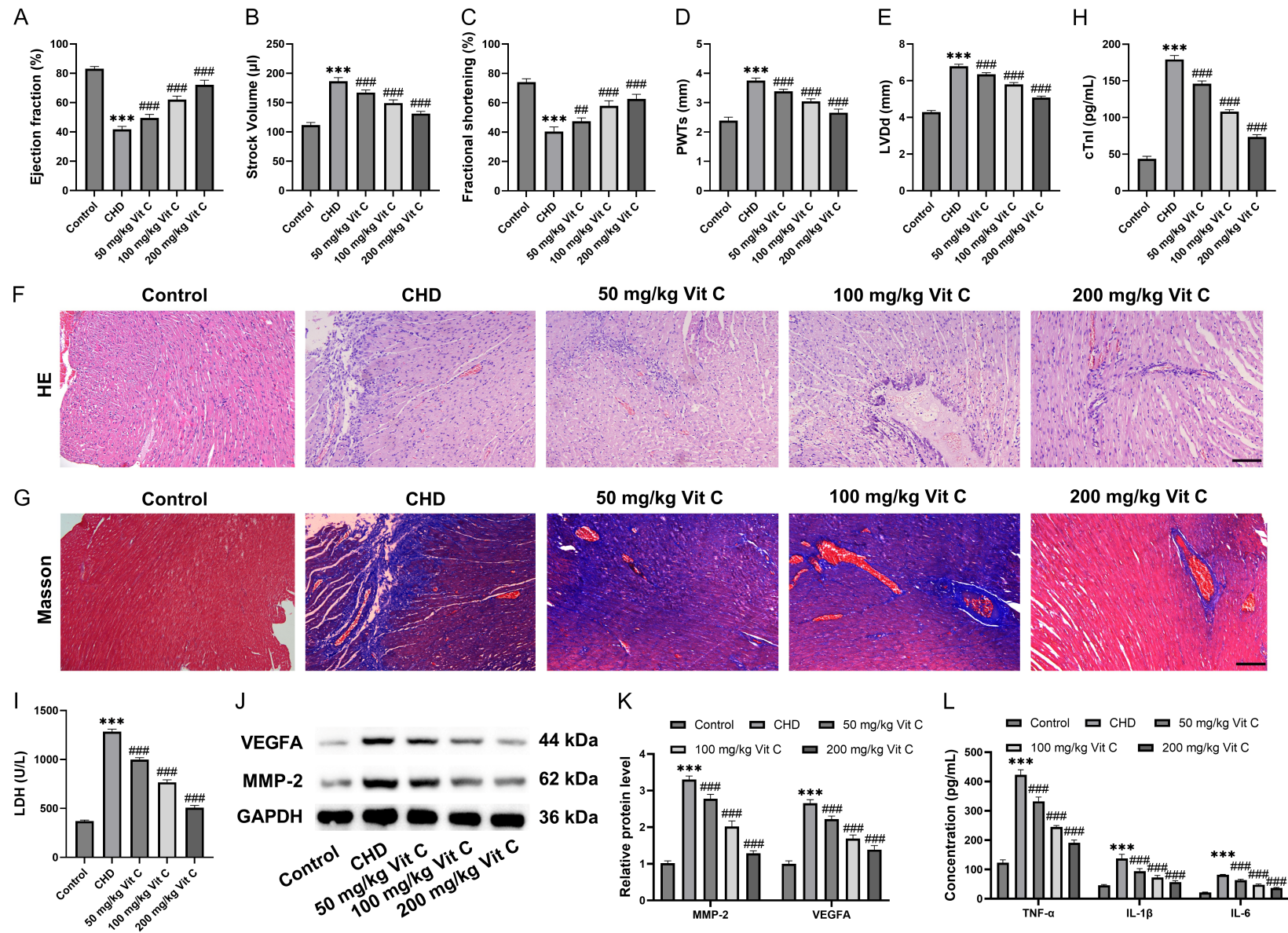
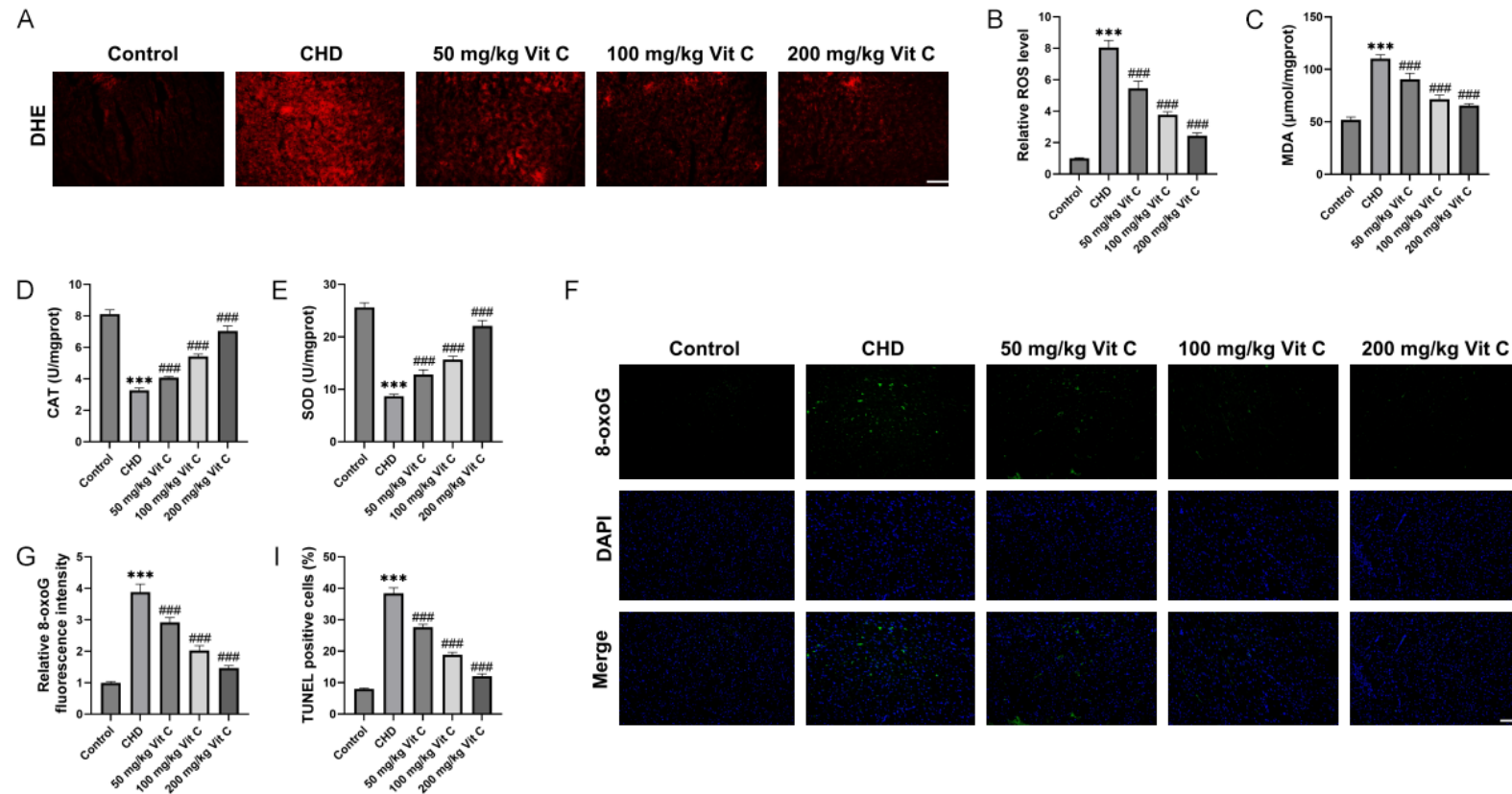


Figure 1. Vit C can reduce cardiac dysfunction and myocardial injury in CHD rats. (A-E) The ejection fraction (A), stroke volume (B), fractional shortening (C), PWTs (D), and LVDD (E) in CHD rats were detected by echocardiography. (F) The extent of myocardial damage in rats following vitamin C intervention was assessed using

Vitamin C reduces myocardial damage in coronary heart disease

H&E staining (20×, 100 μm). (G) Masson staining detected the effect of vitamin C on myocardial fibrosis in CHD rats (20×, 100 μm). (H, I) The cTnI and LDH levels in the serum of rats with CHD following vitamin C intervention were measured using different assay kits. (J, K) Western blot detected MMP-2 and VEGFA protein levels in myocardial tissue from rats with CHD. (L) Serum TNF-α, IL-1β, and IL-6 levels in CHD rats were measured by ELISA kits. n=6, *** P <0.001 vs. control; ## P <0.01, ### P <0.001 vs. CHD.



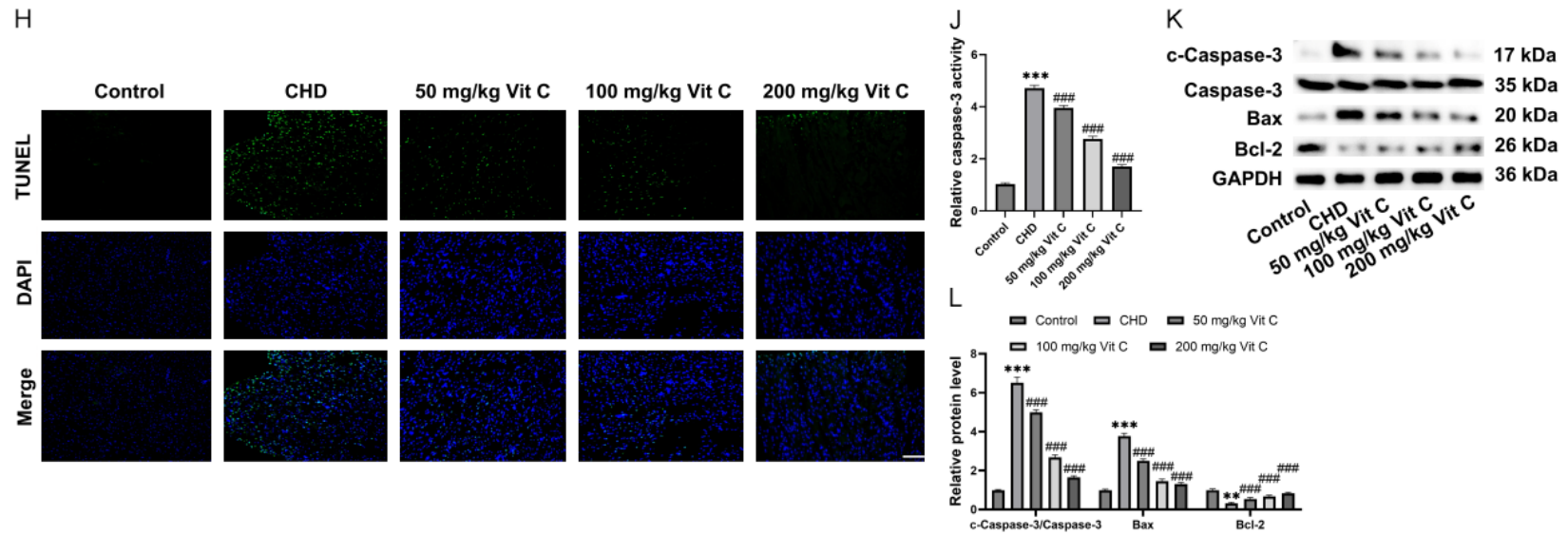


Figure 2. Vitamin C suppress oxidative stress and apoptosis in the hearts of rats with CHD. A, B. ROS levels in myocardial tissue were detected by DHE staining (20 $\times$, 100 μ m). C-E. MDA levels were increased and CAT and SOD activities were decreased in CHD rats, which were reversed by Vit C intervention. F, G. The level of 8-oxoG in myocardial tissue was detected by immunofluorescence (20 $\times$, 100 μ m). H, I. The proportion of TUNEL-positive cells in CHD rats (20 $\times$, 100 μ m). J. Caspase-3 activity was increased in CHD rats, which was reduced by Vit C intervention. K, L. Bax, Bcl-2, and C-caspase-3/Caspase-3 in myocardial tissue of CHD rats were detected through western blot. n=6, ** P <0.01, *** P <0.001 vs. control; ### P <0.001 vs. CHD.

on the Nrf2/HO-1/NQO1 axis. The nuclear Nrf2 level in myocardial tissue of CHD rats was significantly decreased, while cytoplasmic Nrf2 protein level was increased, and HO-1 and NQO1 protein were also significantly downregulated. Vit C treatment promoted the translocation of Nrf2 into the nucleus, and simultaneously up-regulated HO-1 and NQO1, suggesting that Vit C stimulates the Nrf2/HO-1/NQO1 axis (**Figure 3A-D**). Next, we used Nrf2-specific inhibitor ML385 to verify the role of this pathway in Vit C reducing myocardial injury in CHD rats. After ML385 intervention, the nuclear Nrf2 level in rat myocardial tissue decreased, and the level of Nrf2 in the cytoplasm increased, confirming that ML385 could successfully inhibit Nrf2 nuclear translocation (**Figure 3E, 3F**). ML385 intervention attenuated the improvement of Vit C on myocardial injury in CHD rats, leading to the recurrence of disordered fiber arrangement and aggravated pathologic damage in rat myocardial tissue (**Figure 3G**). After ML385 intervention, serum cTnI and LDH levels in CHD rats increased again (**Figure 3H, 3I**), and TNF- α , IL-1 β , and IL-6 levels also increased significantly (**Figure 3J**), suggesting that inhibition of Nrf2 activation aggravated myocardial tissue damage and the inflammatory response. In addition, ML385 reversed the inhibitory effect of Vit C on ROS production in myocardium of CHD rats (**Figure 3K, 3L**), and weakened the regulatory effect of Vit C on oxidative stress indicators, resulting in a further rise in MDA level and a decline in CAT and SOD activities in myocardium (**Figure 3M-O**). Moreover, ML385 intervention led to a significant increase in Bax and c-Caspase-3/Caspase-3 levels in the myocardial tissue of CHD rats, and Bcl-2 expression was notably down-regulated (**Figure 3P, 3Q**). Importantly, ML385 also blocked the activation effect of Vit C on the Nrf2/HO-1/NQO1 axis, causing a decline in nuclear Nrf2 levels and down-regulation of HO-1 and NQO1 expression in myocardial tissue (**Figure 3R-U**). The above results indicate that Vit C inhibits oxidative stress and apoptosis by triggering the Nrf2/HO-1/NQO1 axis, thereby alleviating myocardial damage in CHD rats.

Vit C ameliorates H/R myocardial cell viability damage and apoptosis

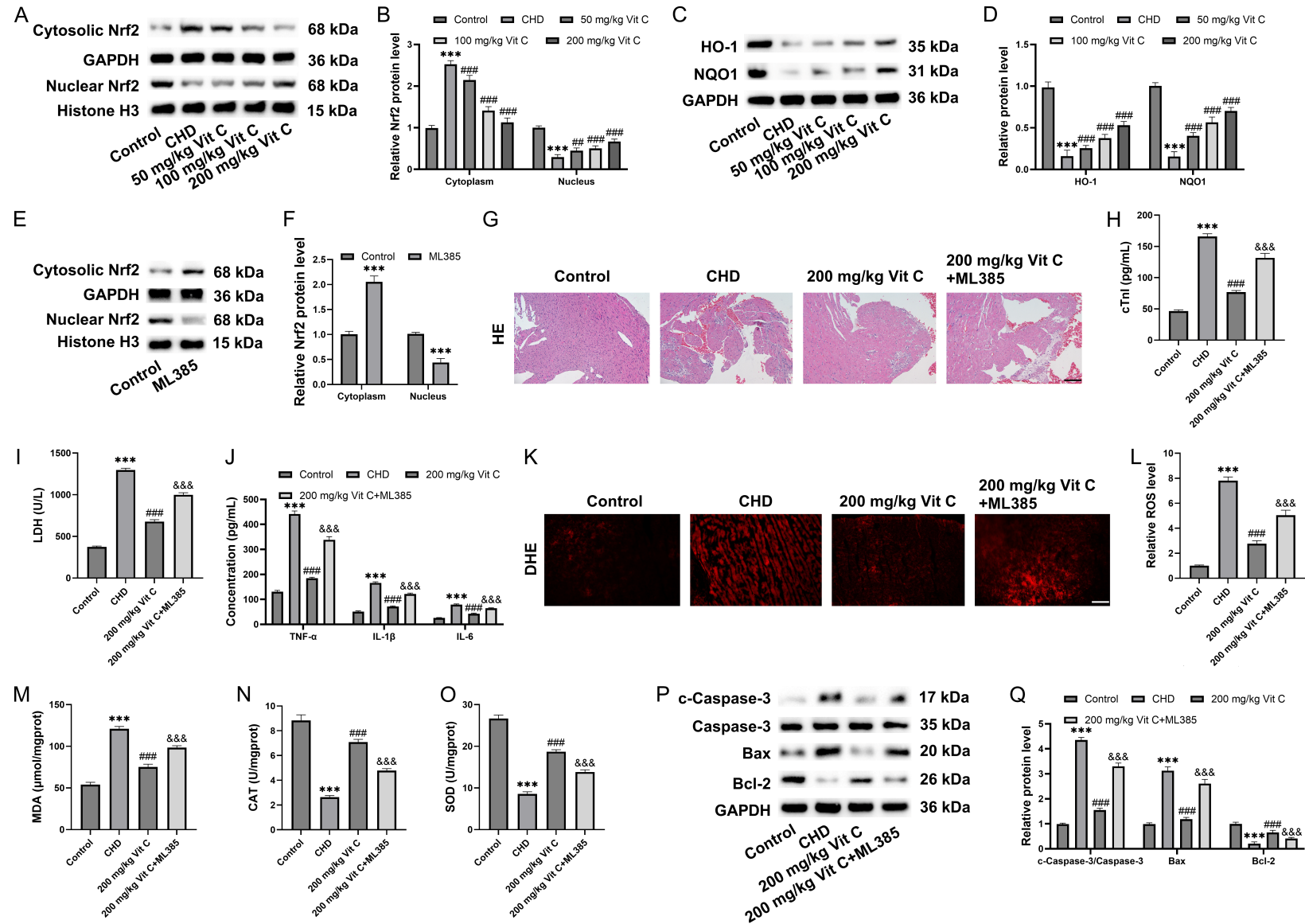
At the cellular level, an H9c2 cardiomyocyte injury model induced by H/R was established to

investigate the cardioprotective effect of Vit C. CCK-8 assay results showed that treatment with 0-400 μ M Vit C for 24 h had no significant toxicity to H9c2 cells, and Vit C effectively improved cell viability at 50-400 μ M (**Figure 4A**). Subsequently, we selected 50-200 μ M Vit C to investigate the protective effects of Vit C in H9c2 cardiomyocytes. H/R treatment caused a marked reduction in H9c2 cell viability, whereas Vit C intervention increased H9c2 cell viability (**Figure 4B**). In addition, LDH release and cTnI levels in the supernatant of H9c2 cardiomyocytes were significantly increased after H/R treatment. Vit C treatment significantly reduced LDH release and cTnI levels, thereby alleviating myocardial cell injury (**Figure 4C, 4D**). Calcein-AM/Hoechst staining showed that H/R treatment caused a notable decline in the survival rate of H9c2 cardiomyocytes, while Vit C treatment significantly increased cell viability (**Figure 4E, 4F**). Flow cytometry demonstrated that H/R treatment led to a significant rise in the apoptosis rate of H9c2 cells, while Vit C treatment reduced the apoptosis rate (**Figure 4G, 4H**). Furthermore, Caspase-3 activity in H/R-treated H9c2 cells was increased, whereas Vit C intervention significantly reduced Caspase-3 activity (**Figure 4I**). Moreover, H/R treatment also increased Bax and c-Caspase-3/Caspase-3 levels in H9c2 cells, and down-regulated Bcl-2 expression. These changes were effectively reversed by Vit C intervention (**Figure 4J, 4K**). These findings suggest that Vit C alleviates H/R-induced H9c2 cell injury, effectively enhances cell viability, and inhibits apoptosis.

Vit C inhibits oxidative stress in H/R cardiomyocytes

We further verified the suppressive effects of Vit C on H/R-induced oxidative stress in H9c2 cells. The fluorescence intensity of ROS in H/R-treated H9c2 cells increased significantly, while Vit C treatment reduced ROS production (**Figure 5A, 5B**). MDA levels in H/R-treated cardiomyocytes were increased, and SOD and CAT activities were significantly reduced. Vit C treatment reversed these changes, effectively reducing MDA content and increasing CAT and SOD activities (**Figure 5C-E**). In addition, H/R treatment caused a rise in the fluorescence intensity of 8-oxoG in H9c2 cells; Vit C intervention

Vitamin C reduces myocardial damage in coronary heart disease



Vitamin C reduces myocardial damage in coronary heart disease

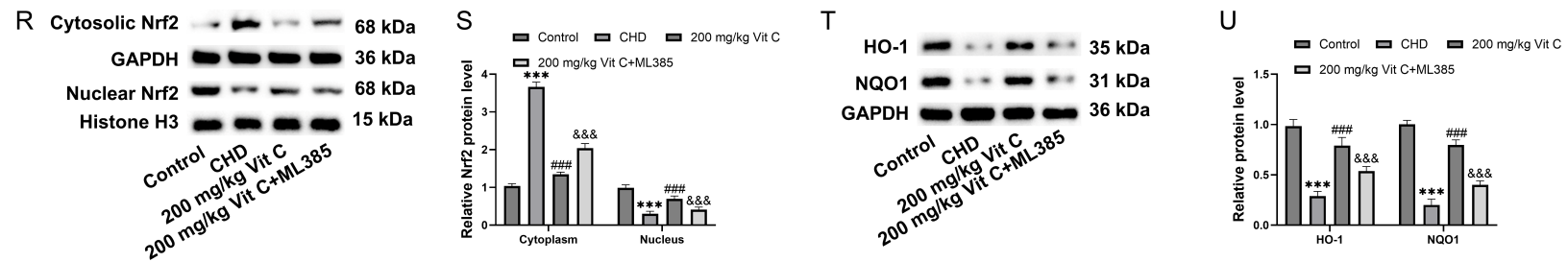


Figure 3. Vit C inhibits oxidative stress and apoptosis by activating the Nrf2/HO-1/NQO1 axis to improve myocardial injury in CHD rats. A-D. Western blot detected Nrf2 levels in the nucleus and cytoplasm of myocardial tissue, and HO-1 and NQO1 levels. E, F. Western blot detected Nrf2 expression in the nucleus and cytoplasm of myocardial tissue after treatment with Nrf2 inhibitor ML385. G. Myocardial pathologic damage in CHD rats following ML385 intervention was viewed using H&E staining (20 $\times$, 100 μ m). H, I. The serum cTnI and LDH levels in rat following ML385 intervention were determined using different assay kits. J. The serum TNF- α , IL-1 β , and IL-6 levels were measured through ELISA kits. K, L. DHE staining revealed that ML385 reduced the inhibitory effect of vitamin C on ROS production in the myocardium of rats with CHD (20 $\times$, 100 μ m). M-O. ML385 attenuated the beneficial effects of vitamin C, leading to a renewed increase in MDA levels and a decline in CAT and SOD activity in the myocardial tissue of rats with CHD. P, Q. Western blot determined Bax, Bcl-2, and c-Caspase-3/Caspase-3 levels in myocardial tissue from rats with CHD. R-U. Western blot detected Nrf2 levels in the nucleus and cytoplasm of myocardial tissue, and HO-1 and NQO1 levels. n=6, *** P <0.001 vs. control; ## P <0.01, ### P <0.001 vs. CHD; &&& P <0.001 vs. 200 mg/kg Vit C.

Vitamin C reduces myocardial damage in coronary heart disease

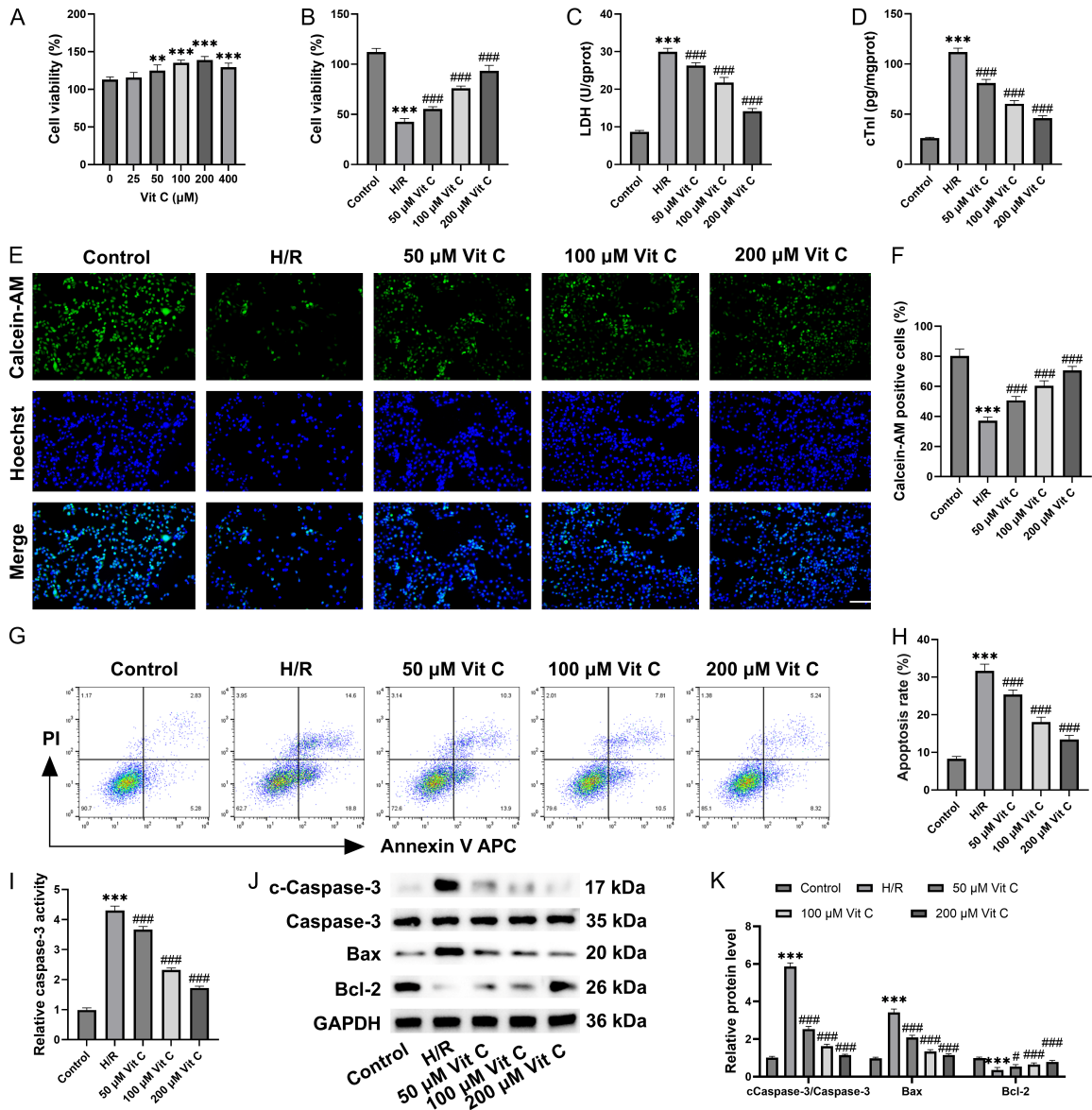


Figure 4. Vit C ameliorates H/R myocardial cell viability damage and apoptosis. A. The CCK-8 assay assessed the effect of 0, 25, 50, 100, 200, and 400 μ M vitamin C treatments on H9c2 cell viability after 24 hours. B. CCK-8 assay showed that H/R reduced the viability of H9c2 cells, while Vit C (50–200 μ M) treatment raised cell viability. C, D. LDH release and cTnI level in the supernatant of H/R cardiomyocytes treated with Vit C were detected by different kits. E, F. Calcein-AM/Hoechst staining showed a decreased viability of H/R cardiomyocytes, which was improved by Vit C treatment (20 $\times$, 100 μ m). G, H. Flow cytometry assessed the apoptosis rate of H/R cardiomyocytes after Vit C treatment. I. Caspase-3 activity in H/R cardiomyocytes after Vit C treatment was assessed using Caspase-3 activity assay kit. J, K. Bax, Bcl-2, and C-caspase-3/Caspase-3 levels in H9c2 cells were detected by western blot. n=6, ** P <0.01, *** P <0.001 vs. control; # P <0.05, ### P <0.001 vs. H/R.

significantly reduced the level of 8-oxoG, suggesting that it reduced H/R-induced oxidative damage in cardiomyocytes (Figure 5F, 5G). The above results indicate that Vit C effectively removes excessive ROS in H9c2 cells and increases the activity of antioxidant enzymes, thereby inhibiting H/R-induced oxidative stress.

Vit C attenuates H/R cardiomyocyte injury through activating the Nrf2/HO-1/NQO1 axis

Finally, we explored whether Vit C alleviated H/R-induced H9c2 cardiomyocyte injury through the Nrf2/HO-1/NQO1 axis. H/R treatment markedly suppressed Nrf2 nuclear translocation

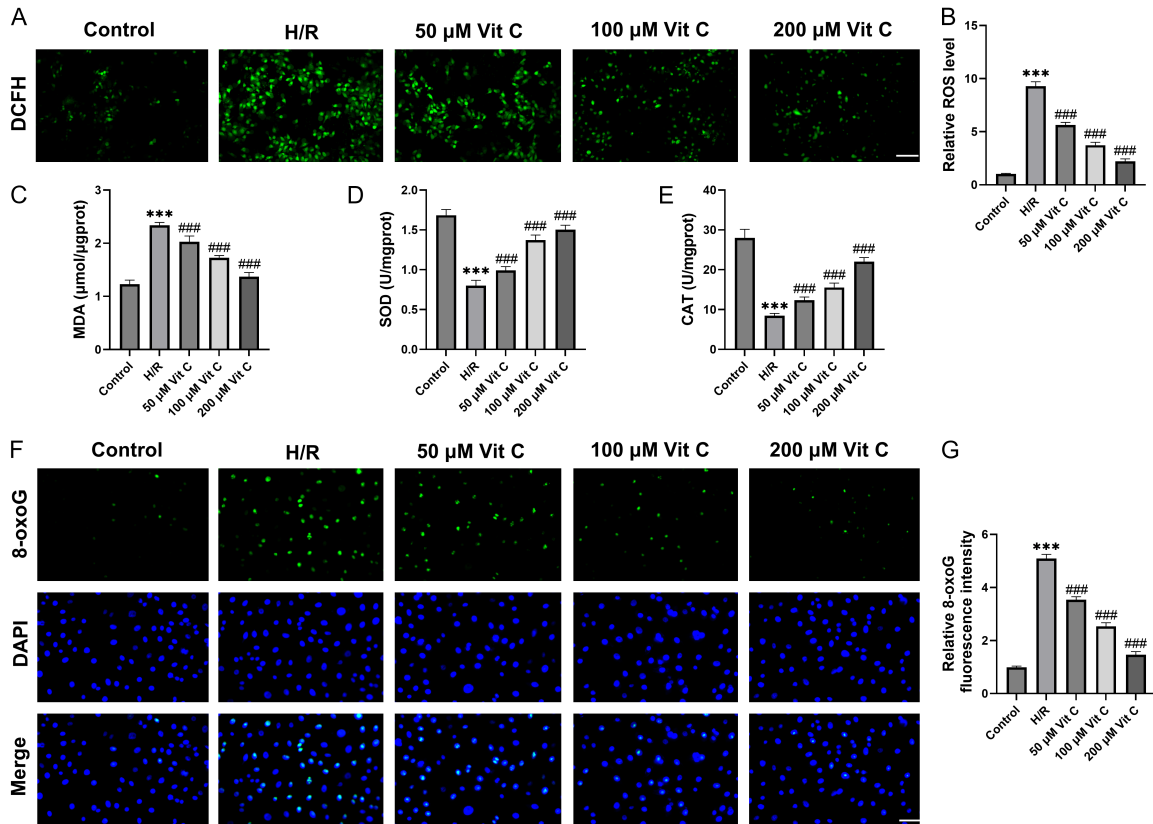


Figure 5. Vit C inhibits oxidative stress in H/R cardiomyocytes. A, B. ROS levels in H/R cardiomyocytes after Vit C treatment were measured by DCFH staining (20 $\times$, 100 μ m). C-E. MDA levels increased and CAT and SOD activities decreased in H/R cardiomyocytes, which were reversed by Vit C treatment. F, G. Immunofluorescence was used to assess the effect of Vit C on 8-oxoG expression, an oxidative damage marker, in H/R cardiomyocytes (40 $\times$, 50 μ m). n=6, *** P <0.001 vs. Control; ### P <0.001 vs. H/R.

tion in H9c2 cells, and down-regulated HO-1 and NQO1 expression, while Vit C intervention effectively reversed this suppression, suggesting that Vit C activated the Nrf2/HO-1/NQO1 axis in H9c2 cells (Figure 6A-D). After transfection of si-Nrf2 in H9c2 cells, Nrf2, HO-1, and NQO1 protein levels were markedly decreased, indicating that Nrf2 was successfully knocked down and could be used for subsequent experiments (Figure 6E, 6F). Transfection of si-Nrf2 caused a marked decrease in cell viability (Figure 6G), and a significant increase in LDH release and cTnl levels (Figure 6H, 6I). In addition, knockdown of Nrf2 increased Bax and c-Caspase-3/Caspase-3 levels, and down-regulated Bcl-2 expression, indicating that knockdown of Nrf2 attenuated the regulatory effect of Vit C on apoptosis-related proteins (Figure 6J, 6K). Transfection of si-Nrf2 weakened the suppressive effect of Vit C on oxidative stress in H9c2 cells, leading to increased ROS and MDA levels and reduced CAT and SOD activities

(Figure 6L-P). Furthermore, transfection of si-Nrf2 also diminished the activating effects of Vit C on the Nrf2/HO-1/NQO1 axis, as evidenced by decreased nuclear Nrf2 levels, increased cytoplasmic Nrf2 levels, and reduced HO-1 and NQO1 expression (Figure 6Q-T). These results suggest that Vit C inhibits apoptosis and oxidative stress by activating the Nrf2/HO-1/NQO1 axis, thereby reducing H/R-induced myocardial cell injury.

Discussion

Coronary heart disease (CHD) has extremely high mortality and disability worldwide [35, 36]. The existing treatment strategies cannot meet all the needs of myocardial protection, and it is urgent to find safe and efficient new intervention drugs. As a natural water-soluble antioxidant, Vit C has demonstrated protective effects in various cardiovascular disease models [14, 37]. However, the cardioprotective role and

Vitamin C reduces myocardial damage in coronary heart disease

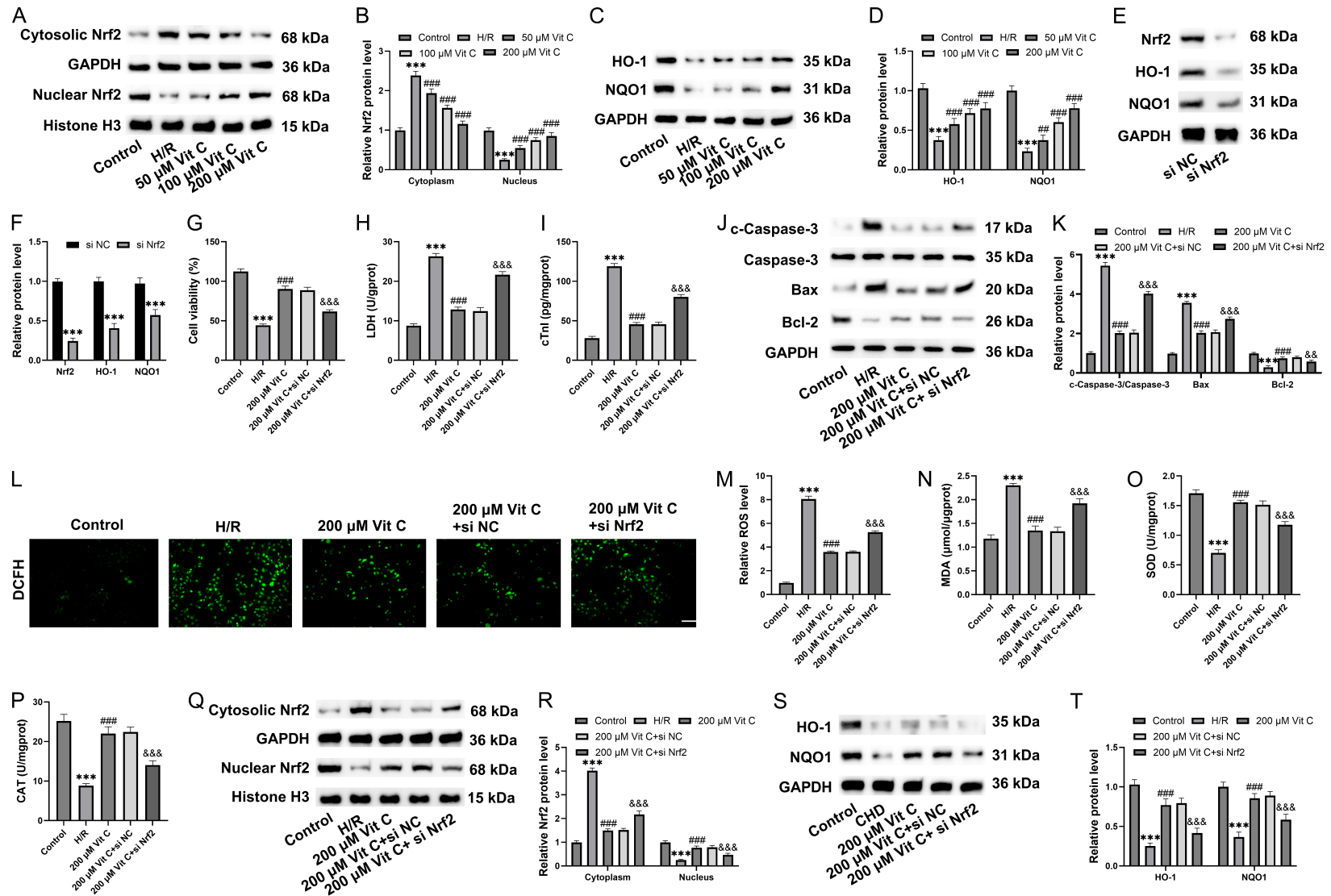


Figure 6. Vit C alleviates H/R cardiomyocyte injury through activating the Nrf2/HO-1/NQO1 axis. A-D. Western blot detected Nrf2 levels in the nucleus and cytoplasm of H/R cardiomyocytes after Vit C treatment, and HO-1 and NQO1 protein levels. E, F. After transfection with siNC or siNrf2, Nrf2, HO-1, and NQO1 levels in H/R cardiomyocytes were determined through western blot. G. CCK-8 assay detected the viability of H9c2 cells. H, I. LDH release and cTnI level in H9c2 cells were detected by kits. J, K. Bax, Bcl-2, and C-caspase-3/Caspase-3 levels in H9c2 cells were determined through western blot. L, M. Knockdown of Nrf2 weakened the suppress-

Vitamin C reduces myocardial damage in coronary heart disease

sive effect of Vit C on intracellular ROS production in H/R cardiomyocytes as measured by DCFH staining (20×, 100 μm). N-P. MDA level, CAT, and SOD activities in H9c2 cells after transfection with si Nrf2 were detected using kits. Q-T. Western blot detected Nrf2 levels in the nucleus and cytoplasm of H/R-treated cardiomyocytes, and HO-1 and NQO1 protein levels. n=6, *** $P<0.001$ vs. control; ## $P<0.01$, ### $P<0.001$ vs. H/R; && $P<0.01$, &&& $P<0.001$ vs. 200 μM Vit C + si NC.

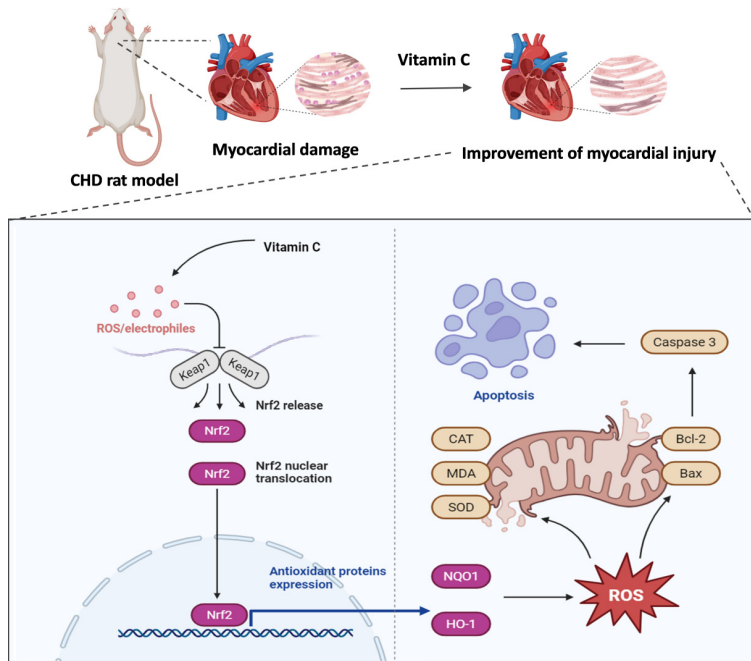


Figure 7. Mechanistic diagram. By promoting Nrf2 nuclear translocation and up-regulating HO-1 and NQO1, Vitamin C inhibits oxidative stress and cardiomyocyte apoptosis, and ultimately improves cardiac function and reduces myocardial pathologic damage in coronary heart disease (CHD) rats.

specific molecular mechanism of Vit C in CHD myocardial damage had not been systematically clarified. In the present work, we established a high-fat diet-induced CHD rat model and a H/R-induced H9c2 cell injury model with reference to the previous CHD model construction methods [26, 29, 30], to systematically explore the cardioprotective role of Vit C on CHD myocardial injury. The 50-200 mg/kg dose of Vit C used in this study was selected based on published studies and pilot experiments. By FDA-recommended body surface area conversion (rat Km=6, human Km=37), this corresponds to a 60-kg adult equivalent of 486-1944 mg/d, which aligns with clinical antioxidant intervention doses (500-2000 mg/d) and remains below the 2000 mg/d tolerable upper intake level [38], supporting its clinical relevance. Moreover, rats synthesize Vit C endogenously, exhibit higher metabolic clearance, and at high doses absorb it mainly through passive diffusion, whereas humans lack the key syn-

these gene and depend on saturable active transport [39-41]. These interspecies pharmacokinetic differences rationalize the higher exogenous doses required in rats. Our findings demonstrate that Vit C could enhance the cardiac function of CHD rats, reduce myocardial pathologic damage and fibrosis, and lower the levels of myocardial injury markers. Additionally, it alleviated H/R-induced reductions in myocardial cell viability, oxidative stress imbalance, and apoptosis. Mechanistic studies have shown that Vit C exerts cardioprotective effects by promoting Nrf2 nuclear translocation and up-regulating HO-1 and NQO1 expression (Figure 7).

The core pathologic feature of CHD is that coronary atherosclerosis leads to myocardial

ischemia and hypoxia, which drives cardiac dysfunction, myocardial structural damage, and cell death [42, 43]. Accumulation of ROS in ischemic myocardium causes mitochondrial dysfunction, calcium homeostasis imbalance, and the release of inflammatory factors, which ultimately lead to cardiac dysfunction, myocardial fibrosis, and apoptosis [44]. Although current research indicates that natural antioxidants have potential in improving cardiac function and reducing myocardial injury in CHD, their clinical translation still faces challenges [45, 46]. This study found that Vit C intervention improves myocardial systolic and diastolic function in CHD rats, inhibits inflammatory infiltration and collagen deposition in myocardial tissue, and effectively inhibits the accumulation of ROS and the expression of DNA oxidative damage marker 8-oxoG. In line with the findings reported by Fang *et al.*, Vit C reduces ROS production, thereby promoting the reprogramming of human fibroblasts into cardiomyo-

cytes and offering a strategy for treating myocardial infarction (severe clinical phenotype of CHD) [47]. Additionally, Vit C reduced the expression of MMP-2 and VEGFA in myocardial tissue, suggesting that it inhibits extracellular matrix degradation and improves angiogenesis in CHD.

Cardiomyocyte apoptosis is a significant pathologic manifestation of myocardial ischemia and hypoxia damage in CHD, and it is also a key factor leading to irreversible damage to myocardial function and promoting disease progression [48]. In the pathologic state of CHD, excessive ROS accumulation can activate the apoptotic signaling pathway, leading to increased cardiomyocyte apoptosis rate, which further aggravates myocardial structural injury and cardiac dysfunction [49, 50]. Abnormal changes in myocardial injury markers and apoptosis-related indicators can directly reflect the degree of myocardial injury. cTnI is a unique structural protein of cardiomyocytes, and its elevated level is an important marker of cardiomyocyte injury or death [51]. LDH is present mainly in the cytoplasm. When the myocardial cell membrane is damaged, a large amount of LDH is released to the outside of the cell, thus directly reflecting the degree of myocardial cell injury [52, 53]. Previous studies have confirmed that Vit C can alleviate the damage of various tissues or organs by inhibiting oxidative stress and regulating the process of apoptosis [54, 55]. However, in CHD and H/R cardiomyocyte models, its regulatory effect on apoptosis-related indicators remained to be fully elucidated. Our findings demonstrated that Vit C intervention reduced serum cTnI and LDH levels in CHD rats, lowered the proportion of apoptotic cells in myocardial tissue, and reduced Caspase-3 activity. *In vitro*, Vit C also effectively reduced cTnI and LDH levels in H9c2 cell culture medium after H/R, and reversed the aberrant expression of apoptosis-related proteins. Zhang *et al.* found that in the model of lipopolysaccharide-induced cardiomyocyte injury, Vit C inhibits the production of ROS in cardiomyocytes, reduces the level of LDH, and thus alleviates cardiomyocyte pyroptosis [56]. These findings show that Vit C may exert myocardial protective effects by inhibiting more than one type of cell death.

The Nrf2/HO-1/NQO1 signaling axis, as a core pathway of the body's antioxidant defense, is

essential for maintaining redox homeostasis by scavenging ROS, inhibiting lipid peroxidation and apoptosis [57, 58]. Recent studies have shown that Nrf2 pathway activation improves myocardial ischemia-reperfusion injury and reduces myocardial fibrosis [59, 60]. This study found that the nuclear translocation of Nrf2 in myocardial tissue of CHD rats was blocked, and HO-1 and NQO1 expression was down-regulated, while Vit C intervention reversed these changes. Further mechanistic studies revealed that Vit C stimulated Nrf2 nuclear translocation and up-regulated the expression of HO-1 and NQO1, thereby scavenging ROS and inhibiting myocardial oxidative stress injury. In the model of sodium fluoride-induced cardiotoxicity, Labib *et al.* also found that Vit C pretreatment activates the Nrf2 pathway, thereby enhancing cell antioxidant capacity and reducing myocardial tissue damage [61]. In this study, the intervention of Nrf2-specific inhibitor ML385 attenuated the protective effects of Vit C on cardiac function in CHD rats, and aggravated myocardial oxidative stress injury. After transfection of si-Nrf2 in H9c2 cells, the protective effects of Vit C on H/R myocardial cell injury was significantly reduced, suggesting that the Nrf2/HO-1/NQO1 signal axis is the core target of Vit C to exert myocardial protection.

Conclusion

Vit C activates the Nrf2/HO-1/NQO1 pathway, thereby inhibiting oxidative stress and apoptosis, reducing myocardial pathologic damage in CHD rats and alleviating H/R-induced H9c2 cardiomyocyte injury. This study reveals the molecular mechanism of Vit C improving myocardial injury in CHD, and offers a novel theoretical foundation and potential target for the prevention and treatment of CHD. It should be noted that this study verified the protective effect of Vit C only in a CHD rat model and in H9c2 cardiomyocytes, and it was not validated using clinical patient samples. Therefore, the applicability of these findings to clinical translation remains to be validated.

Disclosure of conflict of interest

None.

Address correspondence to: Hanliang Dan, Department of Cardiology, Wuhan Dongxihu District People's Hospital Affiliated to Wuhan University of

Science and Technology, Dongxihu District People's Hospital, Wuhan 430000, Hubei, China. E-mail: danhan1987@163.com; Yanhui Hu, Cardiovascular Clinical Medical Center, Liyuan Hospital Affiliated to Tongji Medical College of Huazhong University of Science and Technology, Internal Medicine Building, Liyuan Hospital, Wuchang District, Wuhan 430077, Hubei, China. E-mail: huyanhui806252@hotmail.com

References

- [1] Stone PH, Libby P and Boden WE. Fundamental pathobiology of coronary atherosclerosis and clinical implications for chronic ischemic heart disease management-the plaque hypothesis: a narrative review. *JAMA Cardiol* 2023; 8: 192-201.
- [2] Volpe M and Gallo G. Hypertension, coronary artery disease and myocardial ischemic syndromes. *Vascul Pharmacol* 2023; 153: 107230.
- [3] Ma Y, Huang Y, Li L, Yu L, Xiao P and Wang Q. Time trends in coronary heart disease mortality attributed to outdoor PM2.5 in China: an age-period-cohort analysis using the global burden of disease study 2019. *Front Public Health* 2025; 13: 1517507.
- [4] Ralapanawa U and Sivakanesan R. Epidemiology and the magnitude of coronary artery disease and acute coronary syndrome: a narrative review. *J Epidemiol Glob Health* 2021; 11: 169-177.
- [5] Tao S, Yu L, Li J, Huang L, Xue T, Yang D, Huang X and Meng C. Multiple triglyceride-derived metabolic indices and incident cardiovascular outcomes in patients with type 2 diabetes and coronary heart disease. *Cardiovasc Diabetol* 2024; 23: 359.
- [6] Freeman AM, Raman SV, Aggarwal M, Maron DJ, Bhatt DL, Parwani P, Osborne J, Earls JP, Min JK, Bax JJ and Shapiro MD. Integrating coronary atherosclerosis burden and progression with coronary artery disease risk factors to guide therapeutic decision making. *Am J Med* 2023; 136: 260-269, e267.
- [7] Majidda Abubakar M. Integrated dynamics of oxidative stress, atherogenic lipids, and vascular inflammation in early-stage hypertensive patients. *Cardiovasc Dis Prev Control* 2026; 1: 13-28.
- [8] Zhong H and Yin X. Efficacy and safety of nicorandil monotherapy and nicorandil-clopidogrel combination therapy on cardiac function in patients with coronary heart disease. *Am J Transl Res* 2023; 15: 3539-3547.
- [9] Chepeleva EV. Cell therapy in the treatment of coronary heart disease. *Int J Mol Sci* 2023; 24: 16844.
- [10] Zhang L, Cao J and Zhou X. Establishment and validation of a risk prediction model for adverse drug reactions in patients with coronary heart disease after taking statins: a retrospective study. *PeerJ* 2025; 13: e19630.
- [11] Cao Q, Hong A, Chen M, Wang C, Zhu M and Liang X. The association between vitamin C and depressive risk based on the national health and nutrition examination survey of 2017-2018 and Mendelian randomization study. *Am J Transl Res* 2024; 16: 5137-5149.
- [12] Xu Y, Zheng H, Slabu I, Liehn EA and Rusu M. Vitamin C in cardiovascular disease: from molecular mechanisms to clinical evidence and therapeutic applications. *Antioxidants (Basel)* 2025; 14: 506.
- [13] Dlodla PV, Nkambule BB, Nyambuya TM, Ziqubu K, Mabhidia SE, Mxinwa V, Mokgalaboni K, Ndevahoma F, Hanser S, Mazibuko-Mbeje SE, Basson AK, Sabbatinelli J and Tiano L. Vitamin C intake potentially lowers total cholesterol to improve endothelial function in diabetic patients at increased risk of cardiovascular disease: a systematic review of randomized controlled trials. *Front Nutr* 2022; 9: 1011002.
- [14] Kumar R and Rizvi SI. Vitamin C improves inflammatory-related redox status in hyperlipidemic rats. *Indian J Clin Biochem* 2023; 38: 512-518.
- [15] Xu Y, Zheng H, Nilcham P, Bucur O, Vogt F, Slabu I, Liehn EA and Rusu M. Vitamin C regulates the profibrotic activity of fibroblasts in in vitro replica settings of myocardial infarction. *Int J Mol Sci* 2023; 24: 8379.
- [16] Shati AA, Zaki MSA, Alqahtani YA, Al-Qahtani SM, Haidara MA, Dawood AF, AlMohanna AM, El-Bidawy MH, Alaa Eldeen M and Eid RA. Antioxidant activity of vitamin C against LPS-induced septic cardiomyopathy by down-regulation of oxidative stress and inflammation. *Curr Issues Mol Biol* 2022; 44: 2387-2400.
- [17] Skovsted GF, Skat-Rørdam J, Frøkiær AP, Jensen HE, Tveden-Nyborg P and Lykkesfeldt J. Vitamin C deficiency exacerbates dysfunction of atherosclerotic coronary arteries in guinea pigs fed a high-fat diet. *Antioxidants (Basel)* 2022; 11: 2226.
- [18] Lu Q, Zhang Y, Zhao C, Zhang H, Pu Y and Yin L. Copper induces oxidative stress and apoptosis of hippocampal neuron via pCREB/BDNF/ and Nrf2/HO-1/NQO1 pathway. *J Appl Toxicol* 2022; 42: 694-705.
- [19] Cai X, Zhang Y, Zhu W and Gu N. Kaempferol inhibits atherosclerotic plaque development via dual-targeting of p53-p21-p16 senescence pathway and Nrf2/HO-1/NQO1 antioxidant mechanism: insights from combined in vivo and in vitro research. *Int Immunopharmacol* 2025; 166: 115587.

- [20] O'Rourke SA, Shanley LC and Dunne A. The Nrf2-HO-1 system and inflammaging. *Front Immunol* 2024; 15: 1457010.
- [21] Mohan M, Mannan A, Kakkar C and Singh TG. Nrf2 and ferroptosis: exploring translational avenues for therapeutic approaches to neurological diseases. *Curr Drug Targets* 2025; 26: 33-58.
- [22] Wang Y, Wei J, Deng H, Zheng L, Yang H and Lv X. The role of Nrf2 in pulmonary fibrosis: molecular mechanisms and treatment approaches. *Antioxidants (Basel)* 2022; 11: 1685.
- [23] Sun YY, Zhu HJ, Zhao RY, Zhou SY, Wang MQ, Yang Y and Guo ZN. Remote ischemic conditioning attenuates oxidative stress and inflammation via the Nrf2/HO-1 pathway in MCAO mice. *Redox Biol* 2023; 66: 102852.
- [24] Huang X, Chu Y, Ren H and Pang X. Antioxidation function of EGCG by activating Nrf2/HO-1 pathway in mice with coronary heart disease. *Contrast Media Mol Imaging* 2022; 2022: 8639139.
- [25] Sasikumar S, Yuvraj S, Veilumuthu P, Godwin Christopher JS, Anandkumar P, Nagarajan T, Sureshkumar S and Selvam GS. Ascorbic acid attenuates cadmium-induced myocardial hypertrophy and cardiomyocyte injury through Nrf2 signaling pathways comparable to resveratrol. *3 Biotech* 2023; 13: 108.
- [26] Zhang S, Yu G, Ping M, Du Q and Guo X. Effects of aerobic exercise on myocardial injury, NF-B expression, glucolipid metabolism and inflammatory factors in rats with coronary heart disease. *Clinics (Sao Paulo)* 2024; 79: 100386.
- [27] Patel S, Chaturvedi A, Dubey N, Shrivastava A and Ganeshpurkar A. Ascorbic acid ameliorates isoniazid-rifampicin-induced hepatocellular damage in rats. *ILIVER* 2022; 1: 72-77.
- [28] Lin J, Deng L, Qi A, Jiang H, Xu D, Zheng Y, Zhang Z, Guo X, Hu B and Li P. Catalpol alleviates hypoxia ischemia-induced brain damage by inhibiting ferroptosis through the PI3K/NRF2/system Xc-/GPX4 axis in neonatal rats. *Eur J Pharmacol* 2024; 968: 176406.
- [29] Yang Y, Ding S, Xu G, Chen F and Ding F. MicroRNA-15a inhibition protects against hypoxia/reoxygenation-induced apoptosis of cardiomyocytes by targeting mothers against decapentaplegic homolog 7. *Mol Med Rep* 2017; 15: 3699-3705.
- [30] Yu S, Li J, Wu X, Lu S, Li C, Cheng H, Guo H, Yang P and Wang L. Bioinformatics study of the pharmacological mechanism of sodium-glucose co-transporter 2 inhibitors in type 2 diabetes mellitus and coronary heart disease based on network pharmacology. *Medicine (Baltimore)* 2026; 105: e47306.
- [31] Moon H, Choi JW, Song BW, Kim IK, Lim S, Lee S, Han G, Hwang KC and Kim SW. Brite adipocyte FGF21 attenuates cardiac ischemia/reperfusion injury in rat hearts by modulating NRF2. *Cells* 2022; 11: 567.
- [32] Zhang Z, Jin Q, Zhao H, Liu Y, Wang M, Han Z, Wu J, Lv S, Wang X, Chen W, Wang Q, Bai H and Wang Y. Qinggan Mingshi granules inhibited ferroptosis to treat diabetic retinopathy in mice through NRF2/GPX4 axis. *J Diabetes Res* 2026; 2026: 9978155.
- [33] Sacks B, Onal H, Martorana R, Sehgal A, Harvey A, Wastella C, Ahmad H, Ross E, Pjetergjoka A, Prasad S, Barsotti R, Young LH and Chen Q. Mitochondrial targeted antioxidants, mitoquinone and SKQ1, not vitamin C, mitigate doxorubicin-induced damage in H9c2 myoblast: pretreatment vs. co-treatment. *BMC Pharmacol Toxicol* 2021; 22: 49.
- [34] Xie DP, Gong YX, Lee J, Jeong EM, Ren CX, Guo XY, Han YH, Cui YD, Lee SJ, Kwon T and Sun HN. Peroxiredoxin 5 protects HepG2 cells from ethyl β -carboline-3-carboxylate-induced cell death via ROS-dependent MAPK signalling pathways. *J Cancer* 2022; 13: 3258-3267.
- [35] Drexel H, Mader A, Larcher B, Festa A, Vonbank A, Fraunberger P, Leiberer A and Saely CH. Remnant cholesterol and long-term incidence of death in coronary artery disease patients. *Atherosclerosis* 2025; 401: 119048.
- [36] Sun B, Wang L, Guo W, Chen S, Ma Y and Wang D. New treatment methods for myocardial infarction. *Front Cardiovasc Med* 2023; 10: 1251669.
- [37] Alberts A, Moldoveanu ET, Niculescu AG and Grumezescu AM. Vitamin C: a comprehensive review of its role in health, disease prevention, and therapeutic potential. *Molecules* 2025; 30: 748.
- [38] Institute of Medicine Panel on Dietary A and Related C. Dietary reference intakes for Vitamin C, Vitamin E, selenium, and carotenoids. Washington (DC): national academies press (US); 2000.
- [39] Michels AJ and Frei B. Myths, artifacts, and fatal flaws: identifying limitations and opportunities in vitamin C research. *Nutrients* 2013; 5: 5161-5192.
- [40] Gad AAM, Gora-Sochacka A and Sirko A. Recombinant C-terminal catalytic domain of rat L-gulonolactone oxidase produced in bacterial cells is enzymatically active. *Curr Issues Mol Biol* 2024; 46: 8958-8968.
- [41] Sae-Khow K, Charoensappakit A, Chiewchengchol D and Leelahavanichkul A. High-dose intravenous ascorbate in sepsis, a pro-oxidant enhanced microbicidal activity and the effect on neutrophil functions. *Biomedicine* 2022; 11: 51.
- [42] Fan X, Li A, Yan Z, Geng X, Lian L, Lv H, Gao D and Zhang J. From iron metabolism to ferropto-

- sis: pathologic changes in coronary heart disease. *Oxid Med Cell Longev* 2022; 2022: 6291889.
- [43] Wang J, Xue Z, Lin J, Wang Y, Ying H, Lv Q, Hua C, Wang M, Chen S and Zhou B. Proline improves cardiac remodeling following myocardial infarction and attenuates cardiomyocyte apoptosis via redox regulation. *Biochem Pharmacol* 2020; 178: 114065.
- [44] Schirone L, Forte M, D'Ambrosio L, Valenti V, Vecchio D, Schiavon S, Spinosa G, Sarto G, Petrozza V, Frati G and Sciarretta S. An overview of the molecular mechanisms associated with myocardial ischemic injury: state of the art and translational perspectives. *Cells* 2022; 11: 1165.
- [45] Yan J, Li Z, Liang Y, Yang C, Ou W, Mo H, Tang M, Chen D, Zhong C, Que D, Feng L, Xiao H, Song X and Yang P. Fucoxanthin alleviated myocardial ischemia and reperfusion injury through inhibition of ferroptosis via the NRF2 signaling pathway. *Food Funct* 2023; 14: 10052-10068.
- [46] Liang B, Cai XY and Gu N. Marine natural products and coronary artery disease. *Front Cardiovasc Med* 2021; 8: 739932.
- [47] Fang J, Yang Q, Maas RGC, Buono M, Meijlink B, Lotgerink Bruinenberg D, Benavente ED, Mokry M, van Mil A, Qian L, Goumans MJ, Schiffelers R, Lei Z and Sluijter JPG. Vitamin C facilitates direct cardiac reprogramming by inhibiting reactive oxygen species. *Stem Cell Res Ther* 2024; 15: 19.
- [48] Xiang Q, Yi X, Zhu XH, Wei X and Jiang DS. Regulated cell death in myocardial ischemia-reperfusion injury. *Trends Endocrinol Metab* 2024; 35: 219-234.
- [49] Bi A, Liu R, Xie M, He B, Yan T, Du Y and Jia Y. Semen Ziziphi Spinosae alleviates cardiomyocyte apoptosis in rats with coronary heart disease via the AMPK/SIRT1/PGC-1 α signaling pathway activation. *Phytomedicine* 2025; 142: 156743.
- [50] Rao T, Tong H, Li J, Huang J, Yin Y and Zhang J. Exploring the role and mechanism of hyperoside against cardiomyocyte injury in mice with myocardial infarction based on JAK2/STAT3 signaling pathway. *Phytomedicine* 2024; 128: 155319.
- [51] Ye J, Yang R, Li L, Zhong S, Jiang R and Hu Z. Molecular mechanism of Danxiong Tongmai granules in treatment of coronary heart disease. *Aging (Albany NY)* 2024; 16: 8843-8865.
- [52] Yuan L, Wang D and Wu C. Protective effect of liquiritin on coronary heart disease through regulating the proliferation of human vascular smooth muscle cells via upregulation of sir-tuin1. *Bioengineered* 2022; 13: 2840-2850.
- [53] Liu JW, Liu HT and Chen L. The therapeutic role of Slit2 in anti-fibrosis, anti-inflammation and anti-oxidative stress in rats with coronary heart disease. *Cardiovasc Toxicol* 2021; 21: 973-983.
- [54] Song F, Wu Y, Lin X, Xue D and Wang Y. Vitamin C inhibits apoptosis in THP-1 cells in response to incubation with *Mycobacterium tuberculosis*. *Exp Ther Med* 2022; 24: 717.
- [55] Wu L, Xu W, Li H, Dong B, Geng H, Jin J, Han D, Liu H, Zhu X, Yang Y and Xie S. Vitamin C attenuates oxidative stress, inflammation, and apoptosis induced by acute hypoxia through the Nrf2/Keap1 signaling pathway in gibel carp (*Carassius gibelio*). *Antioxidants (Basel)* 2022; 11: 935.
- [56] Zhang P, Zang M, Sang Z, Wei Y, Yan Y, Bian X and Dong S. Vitamin C alleviates LPS-induced myocardial injury by inhibiting pyroptosis via the ROS-AKT/mTOR signalling pathway. *BMC Cardiovasc Disord* 2022; 22: 561.
- [57] Guo WY, Wu QM, Zeng HF, Chen YL, Xu J, Yu ZY, Shu YK, Yang XN, Zhang CH, He XZ, Mi JN, Chen S, Chen XM, Wu JQ, Yao HQ, Liu L and Pan HD. A sinomenine derivative alleviates bone destruction in collagen-induced arthritis mice by suppressing mitochondrial dysfunction and oxidative stress via the NRF2/HO-1/NQO1 signaling pathway. *Pharmacol Res* 2025; 215: 107686.
- [58] Fang YY, Teng MJ, Peng JC, Zheng XW, Mo YQ, Ho TT, Lin JJ, Luo JJ, Aschner M and Jiang YM. Combined exposure to manganese and iron decreases oxidative stress-induced nerve damage by increasing Nrf2/HO-1/NQO1 expression. *Ecotoxicol Environ Saf* 2024; 270: 115853.
- [59] Li X, Sun YX, Tjahjono AW, Wei Y, Li X, Zheng QH, Qi WC and Liang FR. Acupuncture attenuates myocardial ischemia/reperfusion injury-induced ferroptosis via the Nrf2/HO-1 pathway. *Chin Med* 2025; 20: 61.
- [60] Wei X, Mao Y, Chen Z, Kang L, Xu B and Wang K. Exercise-induced myocardial hypertrophy preconditioning promotes fibroblast senescence and improves myocardial fibrosis through Nrf2 signaling pathway. *Cell Cycle* 2023; 22: 1529-1543.
- [61] Labib H, Badr AM, Abdelgwad M and Abd El-Galil TI. Keap1/Nrf2 pathway in sodium fluoride-induced cardiac toxicity and the prophylactic role of vitamin C versus platelet-rich plasma. *Folia Morphol (Warsz)* 2022; 81: 663-678.