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Role of SIRT1-HMGB1-NLRP3 inflammasome axis in the protective effects of trans-chalcone on myocardial ischemia and reperfusion injury

Xiuming Zhou[1], Juan Sun[1], Shouming Ren[1], Qifeng Shen[1], Qinghua Chi[1] and Haijun Pang[1]

[1] *Department of Cardiology, Qianwei Hospital of Jilin Province, Changchun Jilin, China*

Abstract. Myocardial ischemia and reperfusion (MIR) injury, a major cause of cardiovascular morbidity, involves oxidative stress, inflammation, and cell death. This study examines the protective effects of trans-chalcone, a natural flavonoid, on MIR-induced myocardial damage *via* the Sirtuin 1 (SIRT1)-High Mobility Group Box 1 (HMGB1)-inflammasome-pyroptosis axis. Young adult male Sprague-Dawley rats were subjected to MIR injury and treated with trans-chalcone (100 mg/kg) or the SIRT1 inhibitor EX-527 intraperitoneally for seven days prior to MIR induction. Cardiac function, infarct size, mitochondrial function, oxidative stress, inflammasome and pyroptosis markers were assessed, alongside protein expression analysis of SIRT1, HMGB1, caspase-1, gasdermin D N-terminal fragment, Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2), and Nuclear Factor Kappa B-subunit 65 (NF-κB-p65). Trans-chalcone treatment significantly improved left ventricular pressures, infarct size, and mitochondrial function compared to untreated MIR rats. Oxidative stress was reduced, as shown by decreased malondialdehyde and increased glutathione levels. Western blot analysis confirmed upregulation of SIRT1 and Nrf2 and downregulation of HMGB1, NOD-like receptor protein 3 (NLRP3), cleaved-caspase-1, gasdermin D, and NF-κB-p65. SIRT1 inhibition by EX-527 diminished these protective effects, emphasizing SIRT1's role in trans-chalcone-mediated cardioprotection. These results indicate that trans-chalcone mitigates myocardial MIR injury by targeting SIRT1 to suppress HMGB1, enhance mitochondrial function, and reduce oxidative stress, inflammasome, and pyroptotic markers, positioning trans-chalcone as a promising therapeutic option for ischemic heart disease.

Key words: Ischemia — Cardioprotection — Inflammasome — Pyroptosis — Trans-chalcone

Highlights:

- Trans-chalcone protects against myocardial ischemia/reperfusion (MIR) injury by modulating the SIRT1-HMGB1 axis.
- Inhibition of inflammasome and pyroptosis pathways underlies trans-chalcone's cardioprotective effects.
- SIRT1 activation by trans-chalcone enhances mitochondrial function and reduces oxidative stress in MIR hearts.

Introduction

Myocardial ischemia and reperfusion (MIR) injury is a critical clinical challenge associated with acute myocardial infarction and other cardiovascular disorders (Neri et al. 2017). During ischemia, the heart muscle is deprived of oxygen and nutrients, leading to cellular damage and apoptosis. Reperfusion, although essential to restore blood flow, paradoxically exacerbates myocardial injury

Correspondence to: Haijun Pang, Department of Cardiology, Qianwei Hospital of Jilin Province, Changchun Jilin, 130012, China
E-mail: HaijunPang2000@outlook.com

through the generation of reactive oxygen species (ROS), inflammatory responses, oxidative stress, cellular dysfunction and apoptosis (Buja 2023). This process contributes significantly to the morbidity and mortality associated with cardiovascular diseases, particularly in the management of acute myocardial infarction and revascularization procedures.

A key molecular mechanism underlying MIR injury is the activation of inflammatory pathways and pyroptosis, a form of programmed cell death characterized by inflammasome activation and caspase-1-mediated processing of pro-inflammatory cytokines (Zheng et al. 2022). High Mobility Group Box 1 (HMGB1) is a pivotal mediator of inflammasome and pyroptosis and has been implicated in the pathogenesis of MIR injury (Ding et al. 2013; Chen et al. 2022). Concurrently, sirtuin 1 (SIRT1), a nicotinamide adenine dinucleotide $(NAD)^+$-dependent deacetylase, has emerged as a crucial regulator of mitochondrial function, oxidative stress, and inflammation in several cellular physiological and pathophysiological conditions (Wu et al. 2022). Through deacetylation and sequestration of HMGB1, SIRT1 indirectly regulates the NLRP3 (NOD-like receptor protein 3) inflammasome and, consequently, pyroptosis and mitigates the pro-death effects of HMGB1, particularly in the context of cardiac stress (Chen et al. 2023). The SIRT1-NLRP3 inflammasome pathway plays a pivotal role in the regulation of oxidative stress and inflammation by modulating key mediators such as nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor-κB (NF-κB), thereby attenuating NLRP3-inflammasome activation (Mohamed et al. 2021; Gao et al. 2023). Dysregulation of the inflammasome and pyroptosis during reperfusion injury leads to extensive tissue damage and adverse cardiac outcomes (Liu et al. 2022). Given the critical role of SIRT1 in inhibiting HMGB1 and inflammasome activation and enhancing cardiac protection, exploring therapeutic strategies that target this pathway offers a promising approach to mitigate MIR injury.

Trans-chalcone, a naturally occurring flavonoid, has been studied for its diverse pharmacological properties, including anti-inflammatory, antioxidant, hepatoprotective, and neuroprotective effects (Annapurna et al. 2012; Singh et al. 2016; Martinez et al. 2017; Chen et al. 2020). It has shown promise in reducing tissue damage in experimental models of oxidative stress and inflammation, primarily attributed to its ability to modulate the cardiac phosphoinositide 3-kinase (PI3K)/Akt signaling pathway (Wang et al. 2024). However, the cardioprotective effects of trans-chalcone have not been comprehensively investigated. Despite promising findings, the role of the SIRT1-HMGB1 axis – a key pathway regulating inflammasome-mediated cell death – remains inadequately explored as a mechanism by which trans-chalcone may exert its cardioprotective effects, particularly in MIR injury. By evaluating the effects of trans-chalcone on myocardial function, oxidative stress and inflammasome-pyroptosis mediators, mitochondrial function, and SIRT1/HMGB1 activity, this study aims to provide a deeper understanding of the mechanisms through which trans-chalcone exerts its protective effects and to identify potential therapeutic targets for cardiovascular diseases.

Materials and Methods

Chemicals and reagents

Trans-chalcone (purity >98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). EX-527, a selective inhibitor of SIRT1, was obtained from Selleck Chemicals (Houston, TX, USA). Primary antibodies against SIRT1, HMGB1, caspase-1, gasdermin D-N, Nrf2, NF-κB-p65, and GAPDH were sourced from Cell Signaling Technology (Danvers, MA, USA). Secondary antibodies were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). ELISA kits for measuring tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and NLRP3 levels and other relevant biomarkers, as well as kits for assessing oxidative stress markers such as malondialdehyde (MDA) and reduced glutathione (GSH), were obtained from Abcam (Cambridge, UK) and Thermo Fisher Scientific (Waltham, MA, USA).

Animal handling and ethical approval

Sixty male Sprague-Dawley rats, initially aged 6–8 weeks, were included in this study. The rats underwent a 2–3-week acclimation period under controlled conditions (12-hour light/dark cycle) with unrestricted access to food and water. At the time the experiments began, the rats were 8–12 weeks old and weighed between 250–300 grams, corresponding to the young adult stage for male rats. All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996) and approved by the Institutional Animal Ethics Committee (Approval No. XJTU1AF-CRF-2022-036).

Experimental groups

Following the MIR procedure, rats were randomly assigned to one of the following groups for treatment:

1. Control (Control): Rats underwent sham surgery without ischemia or reperfusion.
2. MIR injury (MIR): Rats underwent MIR and received an *intraperitoneal* (*i.p.*) injection of vehicle (1% DMSO) before MIR injury induction.

3. Trans-chalcone (MIR+TC): Rats were treated with trans-chalcone (100 μg/kg/day, *i.p.*) for seven consecutive days before MIR injury induction (Wang et al. 2024).
4. EX-527 (MIR+EX): Rats were treated with a potent SIRT1 inhibitor, EX-527 (5 mg/kg, *i.p.*), for seven consecutive days before MIR injury induction, starting one hour before trans-chalcone administration.
5. Trans-chalcone + EX-527 (MIR+TC+EX): Rats were treated with both trans-chalcone and EX-527 under the same regimen as above.

Myocardial ischemia and reperfusion (MIR) induction

To induce MIR injury, rats were anesthetized with a combination of ketamine (75 mg/kg) and xylazine (10 mg/kg) administered *i.p.*. Anesthesia was maintained throughout the procedure. Following induction, a thoracotomy was performed to expose the heart. The left anterior descending (LAD) coronary artery was occluded using a 6-0 silk suture passed through a small piece of polyethylene tubing, which was placed around the artery. Ischemia was maintained for 30 minutes, after which the suture was removed to allow for reperfusion for 24 hours. The thoracotomy incision was closed, and rats were allowed to recover in a heated chamber. The control (sham-operated) group underwent the same surgical procedure without coronary artery ligation.

Evaluation of cardiac function

Cardiac function was assessed 24 hours' post-reperfusion using a Millar pressure-sensing catheter inserted into the left ventricular chamber, attached to a pressure transducer (Harvards Apparatus, USA). Rats were anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg) during the hemodynamic assessment. Myocardial function parameters, including left ventricular end-diastolic pressure (LVEDP), left ventricular end-systolic pressure (LVESP), and left ventricular developed pressure (LVDevP) were measured and data were analyzed using the built-in software (Harvards Apparatus, USA).

Measurement of cardiac infarct size

Cardiac infarct size was determined after the reperfusion phase by re-ligating the coronary artery and perfusing 2.5% Evans blue dye through the femoral vein. Hearts were then sectioned, stained with triphenyl tetrazolium chloride, and the infarct size was quantified using ImageJ software. This method allows for the visualization and measurement of infarcted versus viable myocardial tissue by differentiating between stained and non-stained areas. The infarct size (necrotic parts in non-stained area by Evans blue dye) was calculated and reported as a percentage of the risk zones in the left ventricle, which were determined by calculating the non-stained areas as a fraction of the total left ventricle area.

Serum creatine kinase-mB

Blood samples were collected from the abdominal aorta at the end of the experiment. Serum was separated by centrifugation at 3000×*g* for 10 minutes at 4°C and stored at −80°C until analysis. Serum levels of cardiac biomarker creatine kinase-mB (CK-mB) were measured using commercially available ELISA kit according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, China). The assays were conducted in duplicate to ensure accuracy.

Measurement of inflammatory and inflammasome-pyroptosis markers

Inflammatory and inflammasome-pyroptosis markers levels including TNF-α, NLRP3 and IL-1β as well as NF-κB-p65, cleaved caspase-1 and gasdermin D were assessed through ELISA and Western blotting techniques, respectively. After 24 hours of reperfusion, the hearts' ischemic regions (risk zones) were homogenized in RIPA lysis buffer (Beyotime, China). Commercial ELISA kits (MyBioSource, Inc., USA) were utilized to measure TNF-α, NLRP3, and IL-1β levels, normalized to the protein concentration of each sample assessed *via* a bicinchoninic acid assay kit (MyBioSource, Inc., USA).

Oxidative stress markers

Heart tissue samples were homogenized in ice-cold RIPA buffer with protease and phosphatase inhibitors (Sigma-Aldrich, USA). The homogenate was centrifuged at 12,000×*g* for 15 minutes at 4°C to obtain the supernatant. Heart tissue homogenates from the ischemic regions were prepared, and protein levels were normalized to total protein concentrations. MDA levels were measured using the MDA assay kit (Thermo Fisher Scientific, USA). Briefly, tissue homogenate was mixed with thiobarbituric acid reagent and heated. The MDA-TBA adduct was quantified spectrophotometrically at 532 nm. GSH levels were also measured using the GSH assay kit (Thermo Fisher Scientific, USA). The assay involved the reaction of GSH with a chromophore to form a colored product, which was measured spectrophotometrically at 405 nm.

Mitochondrial function assessment

Mitochondrial function was assessed by evaluating mitochondrial ROS levels, mitochondrial membrane potential, and ATP production. Mitochondrial ROS levels were measured using the DCFDA assay (Sigma-Aldrich, USA),

where isolated mitochondria from the left ventricular samples were homogenized, double-centrifuged, and incubated with DCFDA reagent, allowing for the detection of ROS through fluorescence, indicating oxidative stress within the mitochondria. The mitochondrial membrane potential was assessed using the JC-10 assay (Sigma-Aldrich, USA), which differentiates between healthy and depolarized mitochondria based on the fluorescence emission shift of the dye, reflecting changes in membrane integrity. Finally, ATP production levels were quantified using a luciferase bioluminescence assay (Sigma-Aldrich, USA), where the emitted light correlates with ATP concentration, following the kit instructions.

Western blot analysis

Protein extraction was performed from heart tissues (from risk zones samples) using RIPA buffer containing protease and phosphatase inhibitors. The homogenate was centrifuged at 12,000×*g* for 15 minutes at 4°C to obtain the supernatant. Protein concentration was determined using the BCA protein assay kit (Pierce, Thermo Fisher Scientific). Equal amounts of protein (approximately 20 µg) were separated by SDS-PAGE on 10% gels and transferred to PVDF membranes (Millipore, Billerica, MA, USA). Membranes were blocked with 5% non-fat milk in Tris-buffered saline (TBS) with 0.1% Tween-20 detergent for 1 hour at room temperature. Incubation with primary antibodies against SIRT1, HMGB1, cleaved caspase-1, gasdermin D-N, Nrf2, NF-κB, and GAPDH (1:1500 dilution, Cell Signaling Technology, USA) was carried out overnight at 4°C. After washing, membranes were incubated with HRP-conjugated secondary antibodies for 1 hour at room temperature. Protein bands were visualized using an ECL detection system (Bio-Rad), and densitometric analysis was performed using ImageJ software.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical comparisons between groups were made using one-way ANOVA followed by Tukey's *post-hoc* test for multiple comparisons. Normality of the data distribution was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Bartlett's test. A *p*-value of <0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism software.

Results

Cardiac function

Hemodynamic analysis revealed significant variations in cardiac function across the experimental groups (Fig. 1). MIR group demonstrated severe impairment in cardiac function, with a marked elevation of LVEDP from 9.45 ± 1.23 mmHg in control group to 20.72 ± 3.51 mmHg in MIR group ($p < 0.001$). Induction of MIR also resulted a significant reduction in LVESP (60.87 ± 8.14 *vs.* 95.45 ± 11.30 mmHg) and LVDevP (40.15 ± 5.30 *vs.* 86.00 ± 6.43 mmHg) as compared with those of control group ($p < 0.001$). Treatment with trans-chalcone resulted in a significant improvement in cardiac function, with LVEDP declining to 13.27 ± 2.3 mmHg as well as LVESP and LVDevP increasing to 86.60 ± 5.15 and 73.84 ± 5.83 mmHg, respectively, in comparison to MIR group ($p < 0.001$). These indicate a protective effect of trans-chalcone against MIR-induced myocardial dysfunction. However, the administration of EX-527, a SIRT1 inhibitor, significantly suppressed the protective effects of trans-chalcone on functional parameters of the heart as compared to those of the MIR+TC group, suggesting that

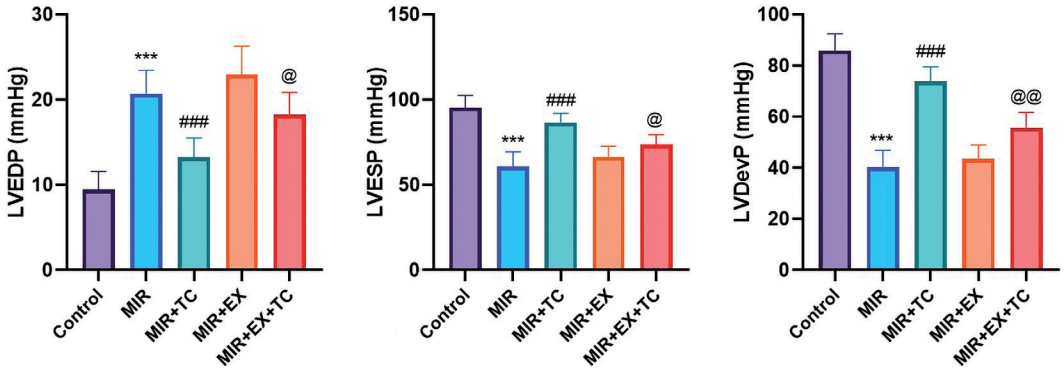

Figure 1. Effects of trans-chalcone (TC) and SIRT1 inhibition on cardiac function. Myocardial function parameters, left ventricular end-diastolic pressure (LVEDP; **A**), left ventricular end-systolic pressure (LVESP; **B**), and left ventricular developed pressure (LVDevP; **C**) were measured. Data are mean ± SD; $n = 6$/group. *** $p < 0.001$ *vs.* Control group; ### $p < 0.001$ *vs.* MIR group; @ $p < 0.05$, @@ $p < 0.01$ *vs.* MIR+TC group. MIR, myocardial ischemia/reperfusion; TC, trans-chalcone; EX, EX527 as a SIRT1 inhibitor.

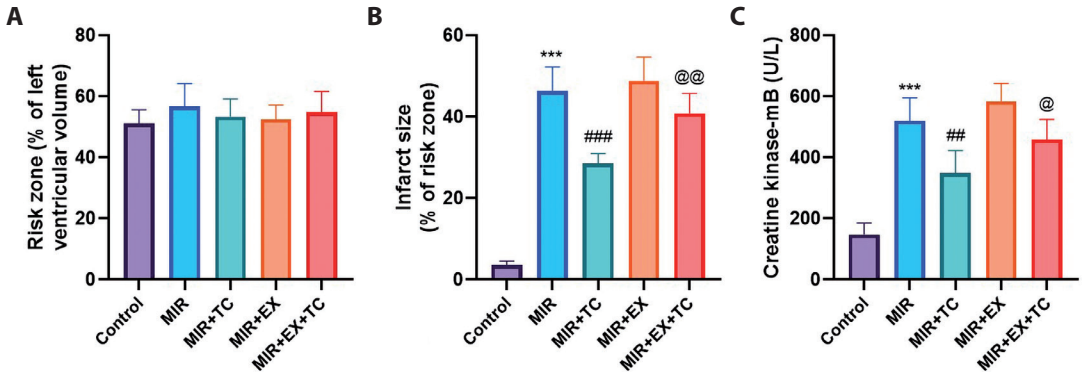

Figure 2. Effects of trans-chalcone and SIRT1 inhibition on cardiac injury**.** Risk zone (**A**) and infarct size (**B**) were determined by dying of 2.5% Evans blue. **C.** Serum levels of cardiac biomarker creatine kinase-mB were measured using ELISA kit. Data are mean ± SD; n = 6/group. *** $p < 0.001$ *vs.* Control group; ## $p < 0.01$ *vs.* MIR group; @ $p < 0.05$, @@ $p < 0.01$ *vs.* MIR+TC group. For abbreviations, see Fig. 1.

the beneficial effects of trans-chalcone is partially mediated by the activation of SIRT1 signaling.

Infarct size and CK-mB levels

To quantify myocardial damage, the infarct size and CK-mB levels were assessed using the TTC staining method and ELISA method, respectively (Fig. 2). Induction of MIR injury resulted in a similar risk zones among the experimental groups (Fig. 2A). In the MIR group, the TTC staining revealed a substantial infarcted area, representing a significant portion of the left ventricular myocardium (Fig. 2B). Quantitative analysis showed a marked increase in infarct size, confirming the extensive myocardial damage induced by MIR injury compared with the control rats ($p < 0.001$). Treatment with trans-chalcone resulted in a notable reduction in infarct size compared to the MIR group ($p < 0.001$). In the MIR+EX+TC group, the infarct size was reduced compared to the MIR group but remained significantly larger than that observed in the MIR+TC group ($p < 0.01$). Similarly, there was a significant increase in CK-mB level in the MIR group compared to controls ($p < 0.001$), and treatment with trans-chalcone in MIR+TC group led to a significant reduction in the level of this injury marker compared to the MIR group ($p < 0.01$) (Fig. 2C). Simultaneous administration of EX-527 to rats receiving TC significantly abolished the effect of TC on CK-mB level ($p < 0.05$). These findings indicate that while trans-chalcone effectively mitigates myocardial injury and offers protection, inhibition of SIRT1 activity substantially hinders its cardioprotective effects.

Inflammatory, inflammasome and pyroptosis components

There was a marked increase in the production and expression levels of pro-inflammatory, inflammasome and pyroptosis-related proteins, including TNF-α, NLRP3, IL-1β, NF-κB-p65, cleaved caspase-1, and gasdermin D-N in MIR group compared to control group ($p < 0.001$) (Fig. 3A–D). These findings indicate an activation of the inflammasome complex and heightened pyroptosis and inflammation in response to MIR injury. Treatment with trans-chalcone resulted in a significant decrease in the levels of those proteins compared to the MIR group ($p < 0.001$). This suggests that trans-chalcone effectively mitigated inflammasome activation and pyroptosis and reduced associated inflammatory responses in the context of myocardial injury. On the other hand, MIR+EX group exhibited elevated levels of NLRP3 ($p < 0.05$), TNF-α, and IL-1β ($p < 0.01$), as well as upregulation of NF-κB-p65 ($p < 0.05$), cleaved caspase-1 ($p < 0.01$) and gasdermin D-N ($p < 0.001$) when compared to the MIR+EX group (Fig. 3). This indicates that inhibiting SIRT1 signaling significantly reduces the production of pro-inflammatory mediators, mitigates inflammasome activation, and restores pyroptotic activity.

Oxidative stress

Measurement of oxidative stress markers revealed increased levels of MDA and decreased levels of glutathione activity in the MIR group ($p < 0.001$), reflecting heightened oxidative stress (Fig. 4). Treatment with trans-chalcone significantly reduced MDA levels and increased GSH activity compared to the control group ($p < 0.001$), indicating a reduction in oxidative stress. The MIR+EX+TC group showed higher level of MDA and lower level of glutathione in comparison to MIR+TC group.

Mitochondrial function

The assessment of mitochondrial function revealed that induction of MIR in control rats resulted in an increase in

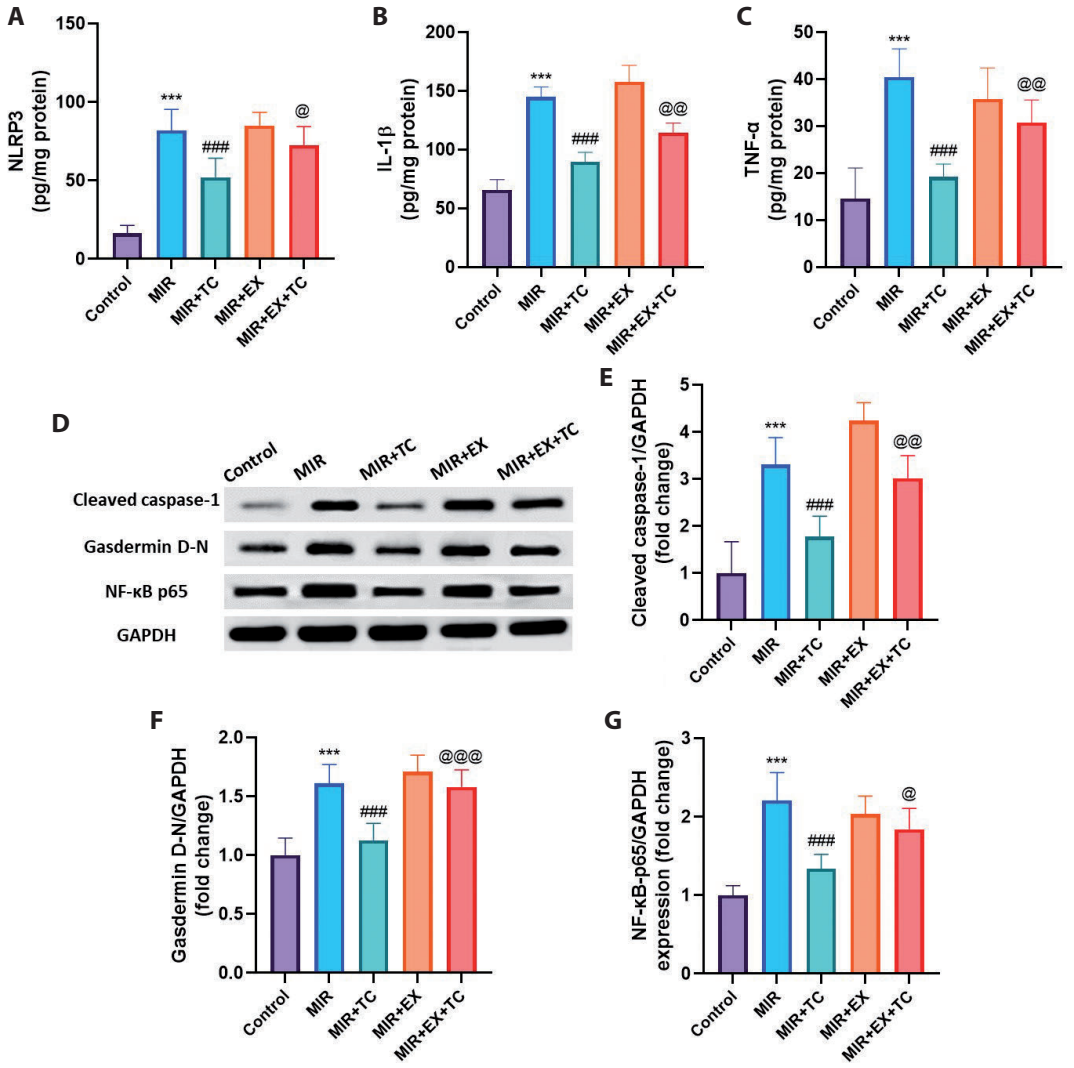

Figure 3. Effects of trans-chalcone and SIRT1 inhibition on cardiac inflammatory, inflammasome, and pyroptosis markers. NOD-like receptor protein 3 (NLRP3; **A**); interleukin 1 beta (IL-1β; **B**) and tumor necrosis factor-alpha (TNF-α; **C**) were assessed through ELISA technique. **D.** Representative Western blot bands and quantitative evaluation cleaved caspase 1, gasdermin D-N and NF-κB p65 levels. Data are mean ± SD; $n = 6$/group. *** $p < 0.001$ *vs.* Control group; $^{\#\#\#}$ $p < 0.001$ *vs.* MIR group; $^{@}$ $p < 0.05$, $^{@@}$ $p < 0.01$, $^{@@@}$ $p < 0.001$ *vs.* MIR+TC group. MIR, myocardial ischemia/reperfusion; TC, trans-chalcone; EX, EX527 as a SIRT1 inhibitor. For more abbreviations, see Fig. 1.

mitochondrial ROS level and reduction in mitochondrial membrane potential and ATP production levels ($p < 0.001$) (Fig. 5). Treatment with trans-chalcone significantly improved mitochondrial parameters in comparison to the MIR group ($p < 0.001$), indicating its important mitoprotective properties. Inhibition of SIRT1 with EX-527 significantly reversed the beneficial effects of trans-chalcone on mitochondrial ROS level ($p < 0.01$) as well as mitochondrial membrane potential and ATP levels ($p < 0.05$) (Fig. 5), indicating the mitoprotective impacts of trans-chalcone in myocardial IR condition is mostly SIRT1-dependent.

SIRT1, HMGB1, and Nrf2 proteins expression

Western blotting analysis revealed a significant decrease in the expression of SIRT1 and Nrf2 and an increase in the expression of HMGB1 in the MIR group compared to the control group ($p < 0.001$) (Fig. 6). Administration of trans-chalcone markedly upregulated the expression of SIRT1 and Nrf2 while downregulating the expression of HMGB1 compared to the untreated MIR group ($p < 0.001$). However, the simultaneous administration of EX-527 significantly reversed the effects of trans-chalcone on SIRT1 ($p < 0.01$)

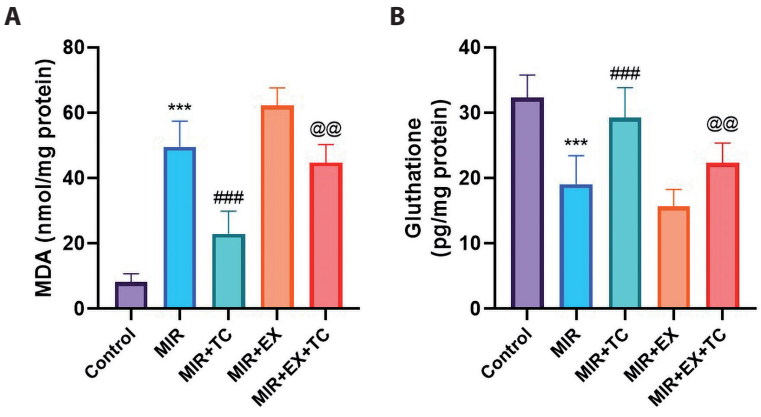

Figure 4. Effects of trans-chalcone and SIRT1 inhibition on cardiac oxidative stress. Oxidative stress markers malondialdehyde (MDA; **A**), and glutathione (**B**) were measured spectrophotometrically at 532 nm, resp. 405 nm. Data are mean ± SD; $n = 6$/group. *** $p < 0.001$ *vs.* Control group; ### $p < 0.001$ *vs.* MIR group; @@ $p < 0.01$ *vs.* MIR+TC group. For abbreviations, see Fig. 1.

and HMGB1 ($p < 0.01$) and partially attenuated its effects on Nrf2 ($p < 0.05$) compared to the MIR+TC group. These findings suggest a critical role for SIRT1 in modulating HMGB1 and Nrf2 activity and highlight that the protective effects of trans-chalcone are significantly diminished upon SIRT1 inhibition.

Discussion

This study aimed to elucidate the cardioprotective effects of trans-chalcone in the context of MIR injury, focusing on its interaction with the SIRT1-HMGB1-inflammosome-pyroptosis pathway. Our results demonstrated that trans-chalcone significantly mitigates myocardial injury, as evidenced by reductions in infarct size, markers of oxidative stress, inflammatory, and inflammasome-pyroptosis responses, and improvements in cardiac hemodynamic and mitochondrial function outcomes. Notably, inhibition of SIRT1 with EX-527 attenuated the protective effects of trans-chalcone, underscoring the critical role of the SIRT1 pathway in mediating these effects (Fig. 7).

Previous studies have demonstrated the tissue-protective effects of trans-chalcone through several mechanisms, including its antioxidant and anti-inflammatory actions across various disease models. For example, research has demonstrated that trans-chalcone effectively reduces intracellular amyloid-beta levels, offering protection against oxidative damage in Alzheimer's disease model (Dhakal et al. 2021). Furthermore, it inhibits inflammatory enzymes such as cyclooxygenase-1 (COX-1) and COX-2, leading to decreased NF-κB-dependent TNF-α release and limiting macrophage activation (Özdemir et al. 2014; Ale-Ebrahim et al. 2022; Jabbar et al. 2024). Additionally, trans-chalcone has also been shown to activate the PI3K/Akt signaling pathway, which helps mitigate cardiac injury (Wang et al. 2024). Though, our study offers new insights by specifically examining the SIRT1-HMGB1-inflammasome-pyroptosis axis, a crucial pathway in the outcomes of MIR injury, which has not been previously investigated in relation to trans-chalcone's cardioprotective effects.

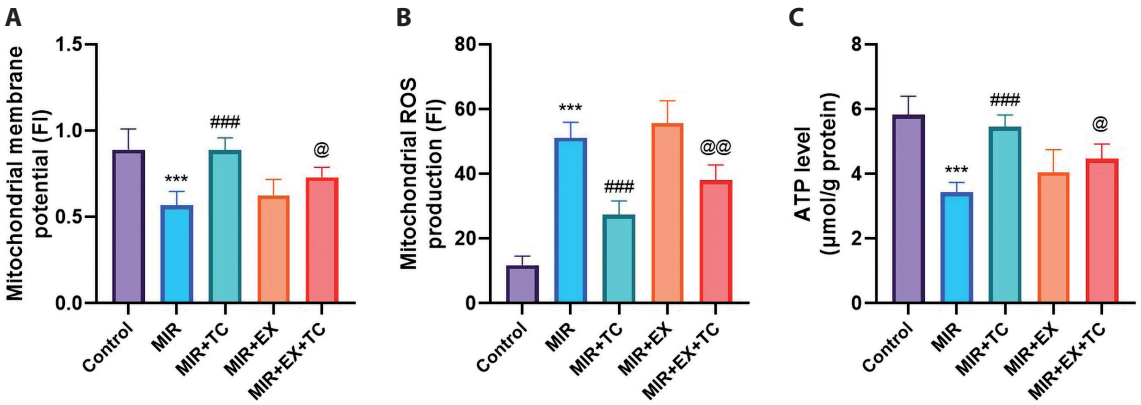

Figure 5. Effects of trans-chalcone and SIRT1 inhibition on cardiac mitochondrial function. **A.** Mitochondrial membrane potential was assessed using the JC-10 assay. **B.** Mitochondrial ROS levels were measured using the DCFDA assay. **C.** ATP levels were quantified using a luciferase bioluminescence assay. Data are mean ± SD; $n = 6$/group. *** $p < 0.001$ *vs.* Control group; ### $p < 0.001$ *vs.* MIR group; @ $p < 0.05$ and @@ $p < 0.01$ *vs.* MIR+TC group. For abbreviations, see Fig. 1.

SIRT1 is widely recognized for its protective role in cellular homeostasis, regulating oxidative stress, inflammation, and mitochondrial function, which are critical in maintaining cardiac tissue viability during stress (Iside et al. 2020; Wu et al. 2022). By deacetylating and activating peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α), SIRT1 promotes mitochondrial biogenesis, which ensures an efficient and healthy mitochondrial network capable of meeting cellular energy demands and reducing dysfunction-associated stress (Qian et al. 2024). It also bolsters antioxidant defenses indirectly by activating transcription factors like Nrf2 and forkhead box protein O3 (FOXO3), leading to increased expression of enzymes such as superoxide dismutase SOD that scavenge ROS, thus minimizing oxidative damage (Gao et al. 2023; Yan et al. 2023). SIRT1 also suppresses the NF-κB pathway by deacetylating the p65 subunit, lowering the production of proinflammatory cytokines (de Gregorio et al. 2020; Wu et al. 2022). Accordingly, we observed that the increase in SIRT1 expression in trans-chalcone-treated animals was associated with enhanced Nrf2 activation and reduced NF-κB p65 activity, further amplifying the antioxidant and anti-inflammatory responses and highlighting a potential mechanism for NLRP3 inhibition. Moreover, by maintaining mitochondrial membrane potential and promoting mitophagy, SIRT1 facilitates the removal of damaged mitochondria, preventing the release of mitochondrial danger signals that could activate inflammatory pathways like the NLRP3 inflammasome (Wu et al. 2022; Chen et al. 2023). In line with this, our findings demonstrated that treatment with trans-chalcone significantly enhanced mitochondrial parameters while also exerting substantial anti-inflammasome and antioxidative effects in MIR hearts. Importantly, inhibiting SIRT1 activity substantially nullified these beneficial effects of trans-chalcone, underscoring the SIRT1-dependency of its actions. Collectively, these findings position SIRT1 as a central regulator of cardiac mitochondrial health, with its beneficial effects on mitochondrial function playing a pivotal role in alleviating the impact of MIR injury.

Moreover, a key downstream mediator of SIRT1 pathway is HMGB1 that, when released extracellularly during cellular stress or damage, functions as a proinflammatory alarmin and inflammasome activator (Wei et al. 2022). Our results demonstrated that trans-chalcone treatment significantly reduced HMGB1 levels in myocardial tissues. This modulation is critical in the context of MIR injury, where excessive HMGB1 release contributes to prolonged tissue damage. In our study, the reduction in HMGB1 expression due to trans-chalcone was notably diminished when SIRT1 was inhibited. In the case of myocardial injury, HMGB1 may trigger a cascade of inflammatory responses, primarily through interaction with pattern recognition receptors like Toll-like receptors (TLRs) and the receptor for advanced glycation end

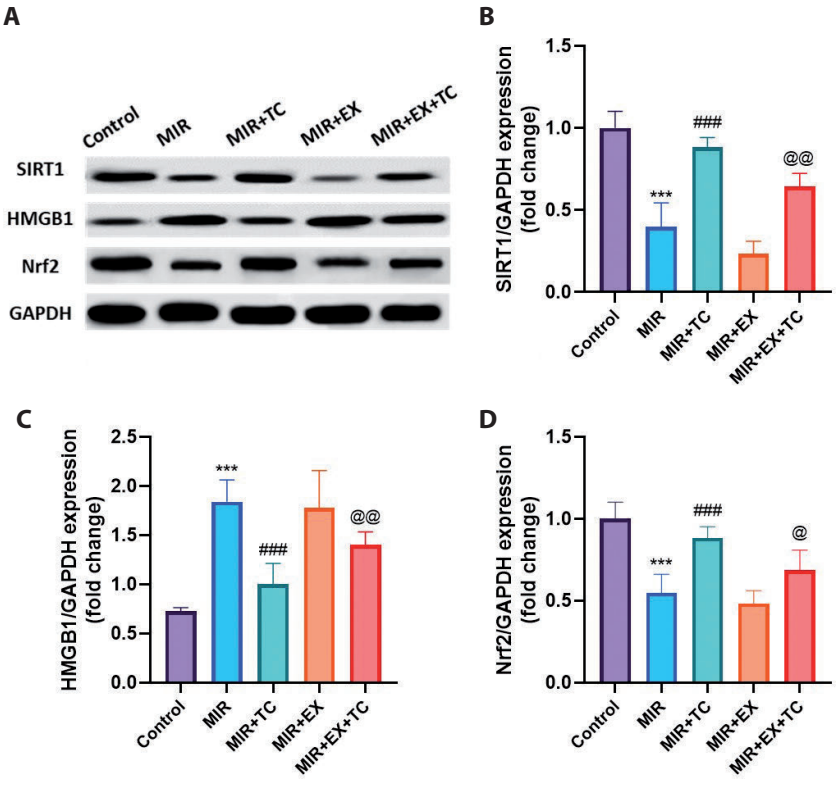

Figure 6. Effects of trans-chalcone and SIRT1 inhibition on cardiac SIRT1-HMGB1 Axis and Nrf2 Activity. Representative Western blot bands (**A**) and quantitative evaluation expression levels of sirtuin 1 (SIRT1, **B**), high-mobility group Box 1 (HMGB1, **C**) and nuclear factor erythroid 2-related factor 2 (Nrf2, **D**). Data are mean ± SD; $n = 6$/group. *** $p < 0.001$ *vs.* Control group; $^{###}$ $p < 0.001$ *vs.* MIR group; $^{@}$ $p < 0.05$ and $^{@@}$ $p < 0.01$ *vs.* MIR+TC group. For abbreviations, see Fig. 1.

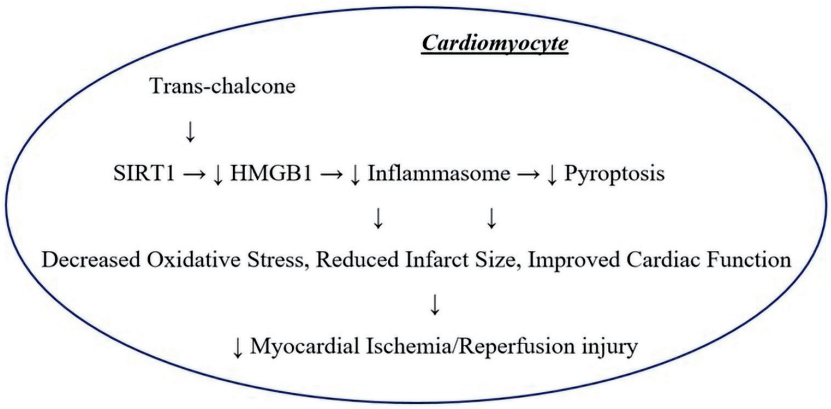

Figure 7. Schematic representation of the proposed cardioprotective mechanism of trans-chalcone in myocardial ischemia/reperfusion injury. Trans-chalcone upregulates SIRT1, which in turn suppresses HMGB1 expression, leading to inhibition of inflammasome activation and reduction in pyroptosis. This cascade results in decreased oxidative stress, reduced infarct size, and improved cardiac function, ultimately mitigating myocardial ischemia/reperfusion injury.

products (RAGE). This interaction promotes the activation of inflammatory pathways, including NF-κB, which leads to the amplification of inflammation in cardiac tissue (Das et al. 2016). However, HMGB1 more directly contributes to the assembly of inflammasome complexes such as NLRP3 (Vande et al. 2011; Reinhart et al. 2022). Thus, we hypothesized that trans-chalcone's modulation of HMGB1 may influence the activation of pro-inflammatory cytokines like TNF-α and IL-1β, potentially through pathways like NF-κB and NLRP3 inflammasome activation. Moreover, HMGB1 can induce mitochondrial dysfunction, leading to the release of mitochondrial DNA and ROS, both of which serve as potent activators of the NLRP3 inflammasome (Pereira et al. 2020). Accordingly, we demonstrated that the reduction in HMGB1 expression following trans-chalcone treatment was linked to lowered mitochondrial ROS levels. Once the inflammasome is activated, it cleaves pro-caspase-1 into active caspase-1, which in turn processes pro-IL-1β into their mature, active forms, further driving inflammation (Zheng et al. 2020). Simultaneously, caspase-1 cleaves gasdermin D, a key executor of pyroptosis, to produce its active N-terminal fragment (gasdermin D-N). This fragment binds to the cell membrane, forming pores that destabilize membrane integrity, leading to pyroptosis, a form of programmed cell death characterized by cell swelling, membrane rupture, and the release of inflammatory contents (Dai et al. 2023). In the context of MIR injury, inhibiting gasdermin D-driven pyroptosis can limit excessive inflammation, preserve cellular integrity, and improve overall cardiac function. As a result, the protective effect of trans-chalcone on reducing infarct size and improving cardiac hemodynamics is largely attributed to its ability to inhibit inflammasome activation and pyroptotic cell death during the initial minutes of reperfusion, by reducing HMGB1 levels. Therefore, HMGB1's role in inflammasome activation not only drives cytokine release but also leads to pyroptosis, amplifying the inflammatory response in MIR hearts.

Finally, this study's novel focus on the SIRT1-HMGB1 and related mitochondrial-inflammasome pathways could influence the development of adjunct therapies targeting SIRT1-related pathways in cardiac patients, particularly those at risk of reperfusion injuries. However, trans-chalcone may engage various protective signaling pathways beyond the SIRT1-HMGB1 pathway, enhancing its cardioprotective effects in MIR hearts. A key candidate is the AMP-activated protein kinase (AMPK) pathway, which serves as a vital energy sensor (Ale-Ebrahim et al. 2022). Activation of AMPK improves mitochondrial function and promotes mitochondrial biogenesis, ensuring sufficient energy supply during reperfusion (as indicated by elevated levels of ATP production by trans-chalcone). Additionally, AMPK triggers autophagy, facilitating the removal of damaged organelles and proteins to maintain cellular homeostasis and prevent further injury (Ding et al. 2020). Trans-chalcone may also impact the Nrf2 pathway, which reinforces the antioxidant response by inducing the expression of enzymes such as heme oxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase 1 (NQO1), thereby reducing oxidative stress during MIR injury (Funes et al. 2020; Stenvinkel et al. 2020). Moreover, the PI3K/Akt pathway represents another potential target; its activation fosters cell survival and inhibits apoptosis, protecting cardiomyocytes from MIR-induced cell death (Wang et al. 2024).

While our study provides valuable insights, certain limitations should be addressed in future research. Specifically, we primarily evaluated the acute effects of ischemia-reperfusion (MIR) injury, characterized by a short reperfusion time. Long-term studies are essential to determine whether the protective benefits of trans-chalcone are sustained over extended periods and to assess the potential for adverse effects. Additionally, the translational applicability of these findings from animal models to humans remains uncertain, as physiological differences could influence efficacy and safety in clinical settings. Lastly, while our study focused on specific

pathways, other potential mechanisms and mediators might contribute to the cardioprotective effects of trans-chalcone. Future research should explore these additional pathways and interactions to provide a more comprehensive understanding of its mechanisms of action.

Conclusion

This study demonstrated that trans-chalcone provides significant cardioprotective effects against MIR injury through multiple mechanisms. Key to its action is the activation of the SIRT1 pathway, which enhanced mitochondrial function, reduces oxidative stress, and modulates inflammation. Trans-chalcone lowered HMGB1 levels, thereby inhibiting inflammasome activation and pyroptosis. These findings highlight trans-chalcone's potential as a multifaceted therapeutic agent in cardiac protection, warranting further investigation into its comprehensive mechanisms for possible clinical application in cardiovascular diseases.

Conflict of interests. The authors declare no conflicts of interest related to this study.

Author contributions. XZ, QC, and HP performed conceptualization. XZ, JS, and SR performed practical experimentations. JS, SR, QS, QC and HP involved in validation, research, resources, data reviewing, and writing. XZ and HP reviewed and edited the final draft. All authors read and approved the final manuscript.

Ethical approval. All experimental procedures were approved by the Institutional Animal Care and Use Committee under ethical approval number XJTU1AF-CRF-2022-036 and conducted following the National Institutes of Health guidelines for the care and use of laboratory animals.

Data availability. The data can be obtained on request from the corresponding author.

References

Ale-Ebrahim M, Rahmani R, Faryabi K, Mohammadifar N, Mortazavi P, Karkhaneh L (2022): Atheroprotective and hepatoprotective effects of trans-chalcone through modification of eNOS/AMPK/KLF-2 pathway and regulation of COX-2, Ang-II, and PDGF mRNA expression in NMRI mice fed HCD. Mol. Biol. Rep. **49,** 3433-3443 https://doi.org/10.1007/s11033-022-07174-x

Annapurna A, Mudagal MP, Ansari A, Rao AS (2012): Cardioprotective activity of chalcones in ischemia/reperfusion-induced myocardial infarction in albino rats. Exp. Clin. Cardiol. **17,** 110-114

Buja LM (2023): Pathobiology of myocardial ischemia and reperfusion injury: Models, modes, molecular mechanisms, modulation, and clinical applications. Cardiol. Rev. **31,** 252-264 https://doi.org/10.1097/CRD.0000000000000440

Chen H, Deng J, Gao H, Song Y, Zhang Y, Sun J, Zhai J (2023): Involvement of the SIRT1-NLRP3 pathway in the inflammatory response. Cell Commun. Signal. **21,** 185 https://doi.org/10.1186/s12964-023-01177-2

Chen R, Kang R, Tang D (2022): The mechanism of HMGB1 secretion and release. Exp. Mol. Med. **54,** 91-102 https://doi.org/10.1038/s12276-022-00736-w

Chen YF, Wu SN, Gao JM, Liao ZY, Tseng YT, Fülöp F, Chang FR, Lo YC (2020): The antioxidant, anti-inflammatory, and neuroprotective properties of the synthetic chalcone derivative AN07. Molecules **25,** 2907 https://doi.org/10.3390/molecules25122907

Dai Z, Liu WC, Chen XY, Wang X, Li JL, Zhang X (2023): Gasdermin D-mediated pyroptosis: mechanisms, diseases, and inhibitors. Front. Immunol. **14,** 1178662 https://doi.org/10.3389/fimmu.2023.1178662

Das N, Dewan V, Grace PM, Gunn RJ, Tamura R, Tzarum N, Watkins LR, Wilson IA, Yin H (2016): HMGB1 activates proinflammatory signaling via TLR5 leading to allodynia. Cell Rep. **17,** 1128-1140 https://doi.org/10.1016/j.celrep.2016.09.076

de Gregorio E, Colell A, Morales A, Marí M (2020): Relevance of SIRT1-NF-κB axis as therapeutic target to ameliorate inflammation in liver disease. Int. J. Mol. Sci. **21,** 3858 https://doi.org/10.3390/ijms21113858

Dhakal S, Ramsland PA, Adhikari B, Macreadie I (2021): Trans-chalcone plus baicalein synergistically reduce intracellular amyloid beta (Aβ42) and protect from Aβ42 induced oxidative damage in yeast models of Alzheimer's disease. Int. J. Mol. Sci. **22,** 9456 https://doi.org/10.3390/ijms22179456

Ding HS, Yang J, Chen P, Yang J, Bo SQ, Ding JW, Yu QQ (2013): The HMGB1-TLR4 axis contributes to myocardial ischemia/reperfusion injury via regulation of cardiomyocyte apoptosis. Gene **527,** 389-393 https://doi.org/10.1016/j.gene.2013.05.041

Ding R, Wu W, Sun Z, Li Z (2020): AMP-activated protein kinase: An attractive therapeutic target for ischemia-reperfusion injury. Eur. J. Pharmacol. **888,** 173484 https://doi.org/10.1016/j.ejphar.2020.173484

Funes SC, Rios M, Fernández-Fierro A, Covián C, Bueno SM, Riedel CA, Mackern-Oberti JP, Kalergis AM (2020): Naturally derived heme-oxygenase 1 inducers and their therapeutic application to immune-mediated diseases. Front. Immunol. **11,** 1467 https://doi.org/10.3389/fimmu.2020.01467

Gao Z, Zhan H, Zong W, Sun M, Linghu L, Wang G, Meng F, Chen M (2023): Salidroside alleviates acetaminophen-induced hepatotoxicity via Sirt1-mediated activation of Akt/Nrf2 pathway and suppression of NF-κB/NLRP3 inflammasome axis. Life Sci. **327,** 121793 https://doi.org/10.1016/j.lfs.2023.121793

Iside C, Scafuro M, Nebbioso A, Altucci L (2020): SIRT1 Activation by natural phytochemicals: An overview. Front. Pharmacol. **11,** 1225 https://doi.org/10.3389/fphar.2020.01225

Jabbar Z, Irfan HM, Alamgeer, Ullah A, Mahmoud MH, Batiha GE, Ahsan H, Khan A, Kashtoh H (2024): Trans-chalcone

(1-3-diphenyl-2-propen-1-one) as a therapeutic candidate in joint inflammation via reduction of TNF-α, IL-1β, IL-6, and IL-17 in rodents: An in vivo study by RT-PCR and ELISA analysis. ACS Omega **9,** 22123-22135 https://doi.org/10.1021/acsomega.4c00368

Liu Y, Zhang J, Zhang D, Yu P, Zhang J, Yu S (2022): Research progress on the role of pyroptosis in myocardial ischemia-reperfusion injury. Cells **11,** 3271 https://doi.org/10.3390/cells11203271

Martinez RM, Pinho-Ribeiro FA, Vale DL, Steffen VS, Vicentini FTMC, Vignoli JA, Baracat MM, Georgetti SR, Verri WA Jr, Casagrande R (2017): Trans-chalcone added in topical formulation inhibits skin inflammation and oxidative stress in a model of ultraviolet B radiation skin damage in hairless mice. J. Photochem. Photobiol. B **171,** 139-146 https://doi.org/10.1016/j.jphotobiol.2017.05.002

Mohamed GA, Ibrahim SRM, El-Agamy DS, Elsaed WM, Sirwi A, Asfour HZ, Koshak AE, Elhady SS (2021): Terretonin as a new protective agent against sepsis-induced acute lung injury: Impact on SIRT1/Nrf2/NF-κBp65/NLRP3 signaling. Biology (Basel) **10,** 1219 https://doi.org/10.3390/biology10111219

Neri M, Riezzo I, Pascale N, Pomara C, Turillazzi E (2017): Ischemia/reperfusion injury following acute myocardial infarction: A critical issue for clinicians and forensic pathologists. Mediators Inflamm. **2017,** 7018393 https://doi.org/10.1155/2017/7018393

Özdemir A, Altintop MD, Turan-Zitouni G, Çiftçi GA, Ertorun İ, Alataş Ö, Kaplancikli ZA (2014): Synthesis and evaluation of new indole-based chalcones as potential antiinflammatory agents. Eur. J. Med. Chem. **89,** 304-309 https://doi.org/10.1016/j.ejmech.2014.10.056

Pereira CA, Carlos D, Ferreira NS, Silva JF, Zanotto CZ, Zamboni DS, Garcia VD, Ventura DF, Silva JS, Tostes RC (2020): Mitochondrial DNA promotes NLRP3 inflammasome activation and contributes to endothelial dysfunction and inflammation in type 1 diabetes. Front. Physiol. **10,** 1557 https://doi.org/10.3389/fphys.2019.01557

Qian L, Zhu Y, Deng C, Liang Z, Chen J, Chen Y, Wang X, Liu Y, Tian Y, Yang Y (2024): Peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1) family in physiological and pathophysiological process and diseases. Signal Transduct. Target Ther. **9,** 50 https://doi.org/10.1038/s41392-024-01756-w

Reinhart NM, Akinyemi IA, Frey TR, Xu H, Agudelo C, Brathwaite J, Burton EM, Burgula S, McIntosh MT, Bhaduri-McIntosh S (2022): The danger molecule HMGB1 cooperates with the NLRP3 inflammasome to sustain expression of the EBV lytic switch protein in Burkitt lymphoma cells. Virology **566,** 136-142 https://doi.org/10.1016/j.virol.2021.12.002

Singh H, Sidhu S, Chopra K, Khan MU (2016): Hepatoprotective effect of trans-chalcone on experimentally induced hepatic injury in rats: inhibition of hepatic inflammation and fibrosis. Can. J. Physiol. Pharmacol. **94,** 879-887 https://doi.org/10.1139/cjpp-2016-0071

Stenvinkel P, Meyer CJ, Block GA, Chertow GM, Shiels PG (2020): Understanding the role of the cytoprotective transcription factor nuclear factor erythroid 2-related factor 2-lessons from evolution, the animal kingdom and rare progeroid syndromes. Nephrol. Dial. Transplant. **35,** 2036-2045 https://doi.org/10.1093/ndt/gfz120

Vande Walle L, Kanneganti TD, Lamkanfi M (2011): HMGB1 release by inflammasomes. Virulence **2,** 162-165 https://doi.org/10.4161/viru.2.2.15480

Wang J, Hussain SA, Maddu N, Li H (2024): Protective effects of trans-chalcone on myocardial ischemia and reperfusion challenge through targeting phosphoinositide 3-kinase/Akt-inflammosome interaction. J. Physiol. Investig. **67,** 129-138 https://doi.org/10.4103/ejpi.EJPI-D-24-00006

Wei L, Zhang W, Li Y, Zhai J (2022): The SIRT1-HMGB1 axis: Therapeutic potential to ameliorate inflammatory responses and tumor occurrence. Front. Cell Dev. Biol. **10,** 986511 https://doi.org/10.3389/fcell.2022.986511

Wu QJ, Zhang TN, Chen HH, Yu XF, Lv JL, Liu YY, Liu YS, Zheng G, Zhao JQ, Wei YF, et al. (2022): The sirtuin family in health and disease. Signal Transduct. Target Ther. **7,** 402 https://doi.org/10.1038/s41392-022-01257-8

Yan D, Yang Y, Lang J, Wang X, Huang Y, Meng J, Wu J, Zeng X, Li H, Ma H, et al. (2023): SIRT1/FOXO3-mediated autophagy signaling involved in manganese-induced neuroinflammation in microglia. Ecotoxicol. Environ. Saf. **256,** 114872 https://doi.org/10.1016/j.ecoenv.2023.114872

Zheng D, Liwinski T, Elinav E (2020): Inflammasome activation and regulation: toward a better understanding of complex mechanisms. Cell Discov. **6,** 36 https://doi.org/10.1038/s41421-020-0167-x

Zheng Y, Xu X, Chi F, Cong N (2022): Pyroptosis: A newly discovered therapeutic target for ischemia-reperfusion injury. Biomolecules **12,** 1625 https://doi.org/10.3390/biom12111625

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