Original Article

Asian Pacific Journal of Tropical Biomedicine

apjtb.org

doi: 10.4103/2221−1691.385569

Impact Factor® 1.7

Beta−glucan protects against isoproterenol−induced cardiac remodeling by regulating the ACE−AT_1R axis and attenuates cardiac inflammation and apoptosis

Anitha Roy[1], Vasantha Mallenahalli Neelakantappa[2], Jayashree Ganesan[1], Balakrishnan Ramajayam Asokan[3], Srinivasan Kulandaivel[4], V. V. Sathibabu Uddandrao[5], Sengottuvelu Singaravel[6]✉

[1]*Center for Transdisciplinary Research, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India− 600077*

[2]*Department of Pharmacology, Kasturba Medical College, Mangolore, Manipal Academy of Higher Education, Manipal, India−575001*

[3]*Department of Pharmacology, Aarupadai Veedu Medical College & Hospital, Vinayaka Mission Research Foundation, Puducherry, India−607403*

[4]*Department of Pharmaceutical Chemistry, Nandha College of Pharmacy, Erode, Tamilnadu, India−638052*

[5]*Department of Biochemistry, K.S. Rangasamy College of Arts and Science (Autonomous), Tiruchengode, Namakkal District, Tamilnadu, India−637215*

[6]*Department of Pharmacology, Nandha College of Pharmacy, Erode, Tamil Nadu, India− 638052*

ABSTRACT

Objective: To investigate the cardioprotective effect of beta-glucan against isoproterenol-induced cardiotoxicity in rats, and elucidate the underlying mechanism.

Methods: Rats were orally pretreated with beta-glucan (40 mg/kg body weight) for 30 d, and isoproterenol (20 mg/100 g body weight) was administered on days 31 and 32. The effects of beta-glucan on markers of cardiac injury, hemodynamic changes, production of proinflammatory cytokines, and the corresponding mRNA expressions were evaluated. In addition, histological analysis was performed.

Results: Pretreatment with beta-glucan prevented isoproterenol-induced cardiac injury by preserving the structural and functional integrity of the plasma membrane and attenuating the production of proinflammatory cytokines (NF-κB, TNF-α, IL-6, IL-1β, and IFN-γ) in the heart. Moreover, beta-glucan significantly downregulated the mRNA expression of *ACE*, *AT_1R*, *TNF−α*, *IL−6*, *NF−κB*, *caspase−3*, *TLR−4*, and *Bax*, and upregulated *Bcl−2* in the heart. At the same time, pretreatment with beta-glucan alleviated myocardial damage as reflected in a reduction in myonecrosis, edema, and erythrocyte extravasation with almost imperceptible inflammation.

Conclusions: Beta-glucan can protect against isoproterenol-induced cardiotoxicity by attenuating cardiac inflammation and apoptosis and regulating the ACE-AT_1R axis, thereby preventing cardiac remodeling.

KEYWORDS: Beta-glucan; Isoproterenol; Cardiac inflammation; Cardiac apoptosis; Cardiovascular diseases; Heart failure; Myocardial infarction

1. Introduction

Acute myocardial infarction (MI) is a major form of ischemic heart disease and a leading cause of death and morbidity worldwide. A pathological condition called MI is caused by induction of necrosis, which occurs as a result of an imbalance between the demand and supply of blood to the heart[1]. Because of coronary occlusion-induced blood loss, MI is characterized by irreversible damage to the myocardium. Patients who survive MI often exhibit cardiac

Significance

Beta-glucan is one of the active compounds widely found in mushrooms and other sources such as yeast, cereals, and grass with various medicinal properties. However, there is no scientific data on its cardioprotective effect. The present study revealed that beta-glucan attenuated isoproterenol-induced cardiotoxicity by regulating the ACE-AT_1R axis and alleviating cardiac inflammation and apoptosis, thereby protecting against myocardial infarction.

✉To whom correspondence may be addressed. E-mail: sengottuvelu@nandhapharmacy.org

How to cite this article: Roy A, Neelakantappa VM, Ganesan J, Ramajayam Asokan B, Kulandaivel S, Uddandrao VVS, et al. Beta-glucan protects against isoproterenol-induced cardiac remodeling by regulating the ACE-AT_1R axis and attenuates cardiac inflammation and apoptosis. Asian Pac J Trop Biomed 2023; 13(9): 384-392.

Article history: Received 2 June 2023; Revision 25 June 2023; Accepted 27 July 2023; Available online 21 September 2023

remodeling, compensatory mechanisms fueled by oxidative stress and inflammation. Cardiac remodeling is critical for the repair, replacement, and removal of nonviable cardiomyocytes in infarcted areas. In contrast, adverse cardiac remodeling can lead to ventricular dilatation and cardiac dysfunction, which accelerate the progression of chronic heart failure and sudden mortality in individuals after MI if left unchecked[2].

Reactive oxygen species (ROS), the renin-angiotensin system, inflammatory cells, and numerous cell death pathways in the heart are involved in the convoluted process of adverse cardiac remodeling[3]. Pharmacological treatments that target ROS or elements of the renin-angiotensin system, such as angiotensin-converting enzyme (ACE), angiotensin Ⅱ (Ang Ⅱ), and Ang Ⅱ type Ⅰ receptor (AT_1R), have been shown to prevent adverse cardiac remodeling after MI by regulating these mechanisms[4]. Moreover, the development of MI depends on the inflammatory response. Previous studies have shown that proinflammatory cytokines damage the myocardial tissue. Moreover, an increase in proinflammatory cytokines leads to tissue infiltration by inflammatory cells and deteriorates the consequences of MI[5]. In this context, apoptosis plays a crucial role in the pathophysiology of MI[6]. Inhibition of apoptosis can reduce the extent of myocardial damage and mitigate MI-related morbidity. Hypoxia-induced mitochondrial damage decreases the level of B-cell lymphoma-2 (Bcl-2) and increases the mitochondrial permeability transition pore[7].

Beta-glucan (BG) is a non-starchy soluble polysaccharide widely present in yeast, mushrooms, bacteria, algae, barley, and oats. BG is considered a functional food ingredient due to its various health benefits and consists of multi-branched β-(1-3)-*d*-glucans or β-(1-6)-*d*-glucose side chains. A number of pharmacological effects of BG, including antibacterial, antiviral, radioprotective, antioxidant, and antidiabetic effects, have been described in previous studies[8]. In addition, Zhang *et al*.[9] reported that salecan, a novel water-soluble BG produced by *Agrobacterium* sp. reduces fat absorption and increases glucose tolerance in diet-induced obesity. Eraniappan *et al*.[10] found that BG can alleviate dyslipidemia and regulate the expression of obesity-related markers in high-fat diet-induced obese rats. However, there is no published research on the cardioprotective efficacy of BG. The current study aimed to determine whether BG can prevent isoproterenol (ISO)-induced MI.

2. Materials and methods

2.1. Chemicals

BG ($C_{18}H_{32}O_{16}$; 98.5% purity) was commercially purchased from Mitushi Biopharma in Ahmedabad, Gujarat, India, whereas the other chemicals used in this study were of analytical grade.

2.2. Animals

The Nandha College of Pharmacy in Erode, Tamil Nadu, India provided the male Wistar albino rats (140-160 g and 8-10 weeks old). The rats had one week to acclimate to laboratory conditions with a 12-hour day/night cycle at (22±2)℃, and 45%-64% humidity, and food and water were provided *ad libitum*.

2.3. Experimental design

The rats were divided into four groups of six animals each: Group Ⅰ: normal control; Group Ⅱ: MI control; Group Ⅲ: MI + BG (40 mg/kg body weight)[10] and Group Ⅳ: MI + α-tocopherol (60 mg/kg body weight)[11]. Groups Ⅲ and Ⅳ received oral pretreatment with respective drugs *via* an intragastric tube for 30 d. On days 31 and 32, rats in groups Ⅱ to Ⅳ received a single dose of ISO (20 mg/100 g body weight) subcutaneously[12]. At the end of the experimental period, all rats were decapitated after receiving pentobarbital sodium (40 mg/kg body weight) intraperitoneally to induce anesthesia. Blood was collected by retro-orbital sinus puncture. Hearts were then dissected, weighed, and stored at −80℃ until further analysis. The heart-to-body weight ratio was also determined.

2.4. Evaluation of cardiac injury markers

Commercial kits were used to determine markers of cardiac injury, including cardiac troponin I (cTnI), creatinine kinase-MB (CK-MB), lactate dehydrogenase (LDH), and homocysteine.

2.5. Determination of cardiac function

On the last day of the experiment, animals received a peritoneal injection of pentobarbital sodium (40 mg/kg body weight) to induce anesthesia. Then, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and heart rate (HR) were determined according to the methods of Sathibabu Uddandrao *et al*[13]. Pressure rate index, a parameter used as an indicator of myocardial oxygen demand, was determined as the product of MAP × HR/1 000.

2.6. Measurement of proinflammatory cytokines in the heart

An enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, USA) was used to measure the levels of proinflammatory cytokines such as nuclear factor kappa-light-chain-enhancer of activated B (NF-κB), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin-6 (IL-6) and interleukin-1 beta (IL-1β).

2.7. Determination of apoptosis markers in the heart

The levels of cardiac Toll-like receptor-4 (TLR-4), caspase-3, Bcl-2-associated X protein (Bax), and Bcl-2 were determined using ELISA kits (Elabscience, USA) according to the manufacturer's instructions.

2.8. RT-PCR analysis

Using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and a DNA synthesis kit (RevertAid First Strand cDNA Synthesis Kit, Thermo ScientificTM, India), total RNA was isolated from the heart tissue of control and experimental rats. For semiquantitative PCR, 20 ng of cDNA was extracted and primed with specific primers (Table 1), including *ACE*, *AT_1R*, *TNF-α*, *IL-6*, *NF-κB*, *caspase-3*, *TLR-4*, *Bax* and *Bcl-2*. The corresponding primers were used in 38 cycles of PCR amplification at the following cycle temperatures: 30 s of denaturation at 94℃, 30 s of annealing at 59℃, and 1 min of extension at 72℃.

Table 1. The sequence of the primers used for RT-PCR.

Gene	Primer sequence
ACE	F 5′-TCCATTGGTCTTCTGTCACCCG-3′
	R 5′-AGACCATCCACCTCCACTTCTC-3′
AT_1R	F 5′-CAGCGTCAGTTTCAACCTGTACG-3′
	R 5′-GCAGGTGACTTTGGCTACAAGC-3′
TNF-α	F 5′-ATGTGGAACTGGCAGAGGAG-3′
	R 5′-AGAAGAGGCTGAGGCACAGA-3′
IL-6	F 5′-AGCCAGAGTCATTCAGAGCA-3′
	R 5′-AGAGCATTGGAAGTTGGGGT-3′
NF-κB	F 5′-ACCTTTGCTGGAAACACACC-3′
	R 5′-ATGGCCTCGGAAGTTTCTTT-3′
Caspase-3	F 5′-AGCAAACCTCAGGGAAACATT-3′
	R 5′-CTCAGAAGCACACAAACAAAACT-3′
TLR-4	F 5′-TGGCATCATCTTCATTGTCC-3′
	R 5′-CAGAGCATTGTCCTCCCACT-3′
Bax	F 5′-TCAGGATGCGTCCACCAAGAAG-3′
	R 5′-TGTGTCCACGGCGGCAATCATC-3′
Bcl-2	F 5′-CTGGTGGACAACATCGCTCTG-3′
	R 5′-GGTCTGCTGACCTCACTTGTG-3′
β-actin	F 5′-CCTGCTTGCTGATCCACA-3′
	R 5′-CTGACCGAGCGTGGCTAC-3′

F: forward; R: reverse.

2.9. Histopathological analysis of the heart

Heart tissue was embedded in paraffin and stored in 10% formalin. Subsequently, 5 µm thick dewaxed sections were stained with hematoxylin and eosin (H&E) to reveal morphological changes. Histoarchitectural changes in the prepared sections were assessed under a light microscope (Olympus BX41, Olympus Corporation, Tokyo, Japan). Slides were examined for edema, inflammatory cell infiltration, and myocardial necrosis. Each slide contained at least 10 fields, which were evaluated and graded on a severity scale of severe (>4), moderate (<3), mild (<2), and none or negligible (<1).

2.10. Statistical analysis

Data were expressed as mean ± SD for each group of six animals. All collected data were statistically analyzed using SPSS (28.0.1.1). Least significant difference (LSD) test and one-way analysis of variance (ANOVA) were used to evaluate the data. $P<0.05$ was used as the threshold for statistical significance.

2.11. Ethical statement

The protocol of this study was approved by the Institutional Animal Ethical Committee, Nandha College of Pharmacy, Erode, Tamilnadu, India (approval No: NCP/IAEC/2022-23/16; Date: 08.10.2022), and the experiments were performed according to the guidelines outlined by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

3. Results

3.1. Effect of BG on heart to body weight ratio

The hearts of rats were greatly enlarged after treatment with ISO, as evidenced by a greater heart weight-to-body weight ratio in the MI control animals (Figure 1A). In contrast, pretreatment with BG significantly reduced the toxicity of ISO ($P<0.05$), as shown by the normal heart weight-to-body weight ratio compared with the MI control group.

3.2. Effect of BG on markers of cardiac damage

The effects of BG on markers of myocardial damage such as cTnI (Figure 1B), CK-MB (Figure 1C), LDH (Figure 1D), and

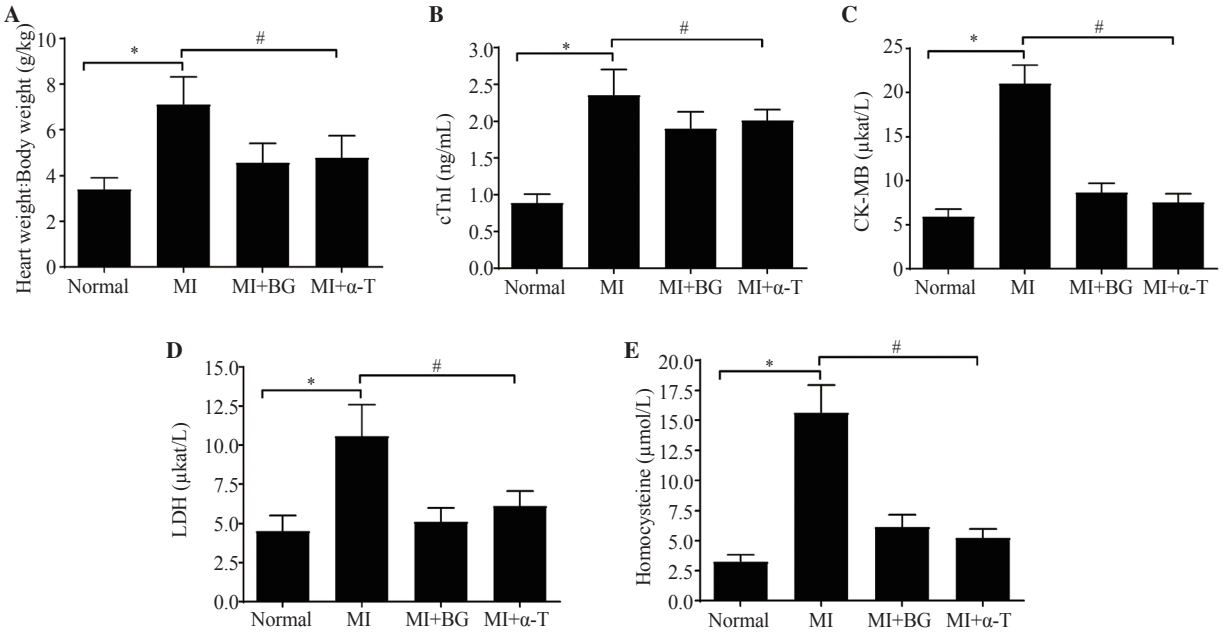

Figure 1. Effect of beta-glucan (BG) on (A) heart weight to body weight ratio, (B) cardiac troponin I (cTnI), (C) CK-MB, (D) lactate dehydrogenase (LDH), and (E) homocysteine in isoproterenol-induced myocardial infarction (MI) rats. Values are expressed as mean±SD (n=6) and significantly different at $^{*}P<0.05$ *vs.* control and $^{\#}P<0.05$ *vs.* MI control. α-T: α-tocopherol.

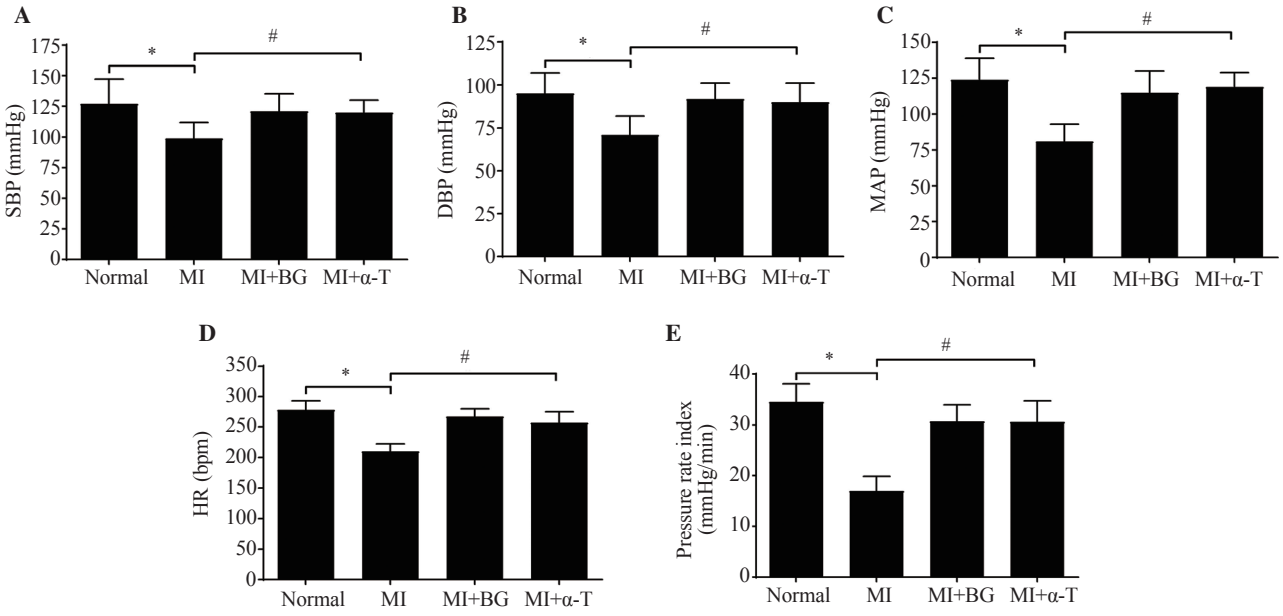

Figure 2. BG improves (A) systolic blood pressure (SBP), (B) diastolic blood pressure (DBP), (C) mean arterial pressure (MAP), (D) heart rate (HR), and (E) pressure rate index in isoproterenol-induced MI rats. Values are expressed as mean±SD (n=6) and significantly different at $^{*}P<0.05$ *vs.* control and $^{\#}P<0.05$ *vs.* MI control.

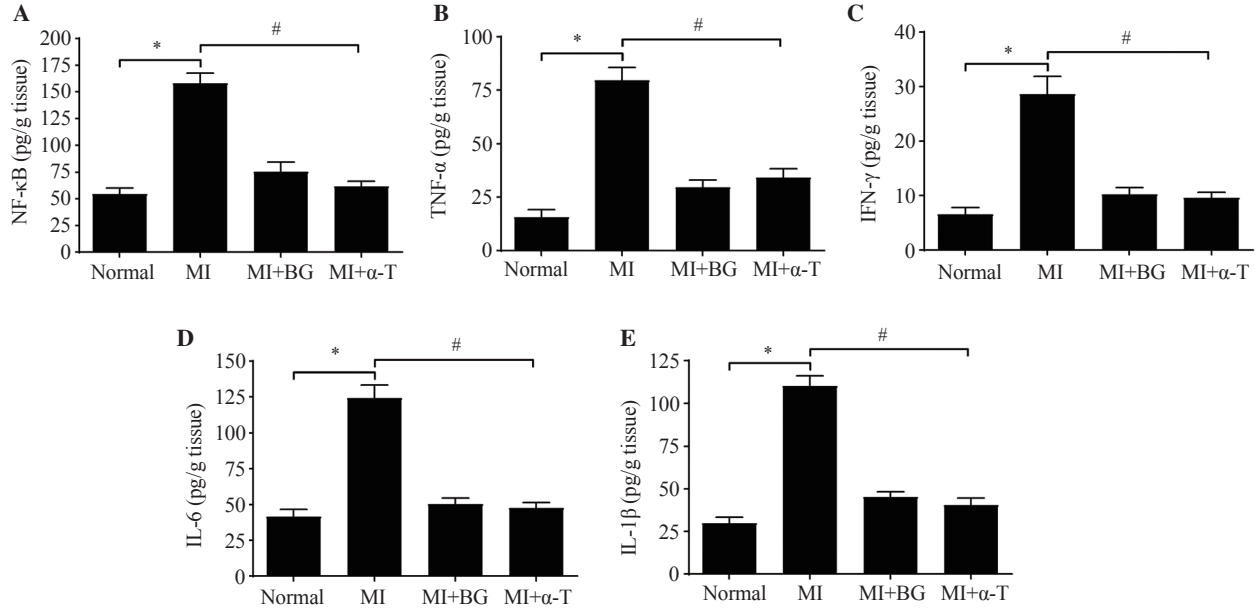

Figure 3. BG suppresses the production of (A) NF-κB, (B) TNF-α, (C) IFN-γ, (D) IL-6, and (E) IL-1β in isoproterenol-induced MI rats. Values are expressed as mean±SD (n=6) and significantly different at $^{*}P<0.05$ *vs.* control and $^{\#}P<0.05$ *vs.* MI control.

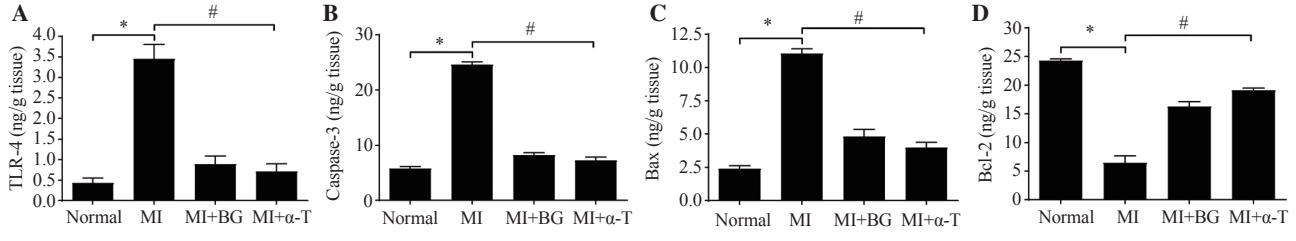

Figure 4. Effect of BG on cardiac apoptotic markers (A) TLR-4, (B) caspase-3, (C) Bax, and (D) Bcl-2 in MI rats. Values are expressed as mean±SD (n=6) and significantly different at $^{*}P<0.05$ *vs.* control and $^{\#}P<0.05$ *vs.* MI control.

homocysteine (Figure 1E) in control and experimental MI rats are shown in Figure 1. Compared with the normal control group, the levels of cTnI, CK-MB, LDH, and homocysteine were significantly raised in the MI control (P<0.05). In contrast to the MI control, pretreatment with BG reduced these abnormalities, as evidenced by lower cTnI, CK-MB, LDH, and homocysteine levels.

3.3. BG improves ISO-induced hemodynamic abnormalities

Administration of ISO to rats resulted in a marked (P<0.05) decline in SBP (Figure 2A), DBP (Figure 2B), MAP (Figure 2C), HR (Figure 2D), and pressure rate index (Figure 2E). However, pretreatment with BG significantly restored these changes to near normal (P<0.05).

3.4. BG attenuates the production of proinflammatory cytokines in the heart

NF-κB (Figure 3A), TNF-α (Figure 3B), IFN-γ (Figure 3C), IL-6 (Figure 3D), and IL-1β levels (Figure 3E) in the heart were significantly higher in the MI control group (P<0.05). BG treatment protected the rats from ISO-mediated proinflammatory cytokine production (P<0.05).

3.5. BG reduces ISO-induced cardiac apoptosis

Compared with normal control, administration of ISO resulted in increased levels of TLR-4, caspase-3, Bax, and a concomitant decrease in Bcl-2 in MI control animals (P<0.05) (Figure 4). In addition, rats pretreated with BG showed a significant (P<0.05) reduction in the levels of TLR-4, caspase-3, Bax, and simultaneously increased Bcl-2 (P<0.05) (Figure 4).

3.6. BG regulates the ACE-AT_1R axis and downregulates inflammatory and apoptosis genes in the heart of MI rats

In MI control rats, exposure to ISO resulted in upregulation of *ACE*, *AT_1R*, *TNF-α*, *IL-6*, *NF-κB*, *caspase-3*, *TLR-4*, *Bax*, and downregulation of *Bcl-2* (Figures 5&6). Nevertheless, pretreatment with BG in ISO-induced MI rats resulted in significant downregulation of mRNA expressions of *ACE*, *AT_1R*, *TNF-α*, *IL-6*, *NF-κB*, *caspase-3*, *TLR-4*, *Bax*, and concomitant upregulation of *Bcl-2* (P<0.05), highlighting that BG can protect against ISO-induced cardiac remodeling, inflammation, and apoptosis in rats.

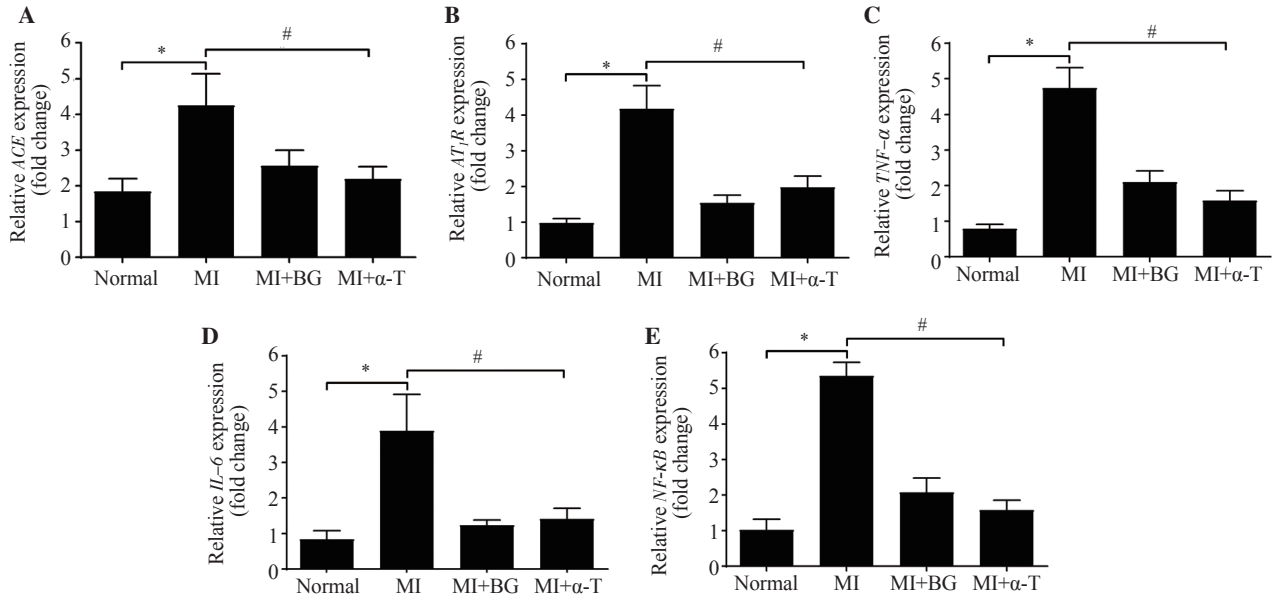

Figure 5. BG regulates the mRNA expressions of (A&B) the ACE-AT_1R axis and reduces the mRNA expressions of (C) *TNF-α*, (D) *IL-6*, and (E) *NF-κB* in isoproterenol-induced MI rats. Values are expressed as mean±SD (n=6) and significantly different at $^*P$<0.05 *vs.* control and $^\#P$<0.05 *vs.* MI control.

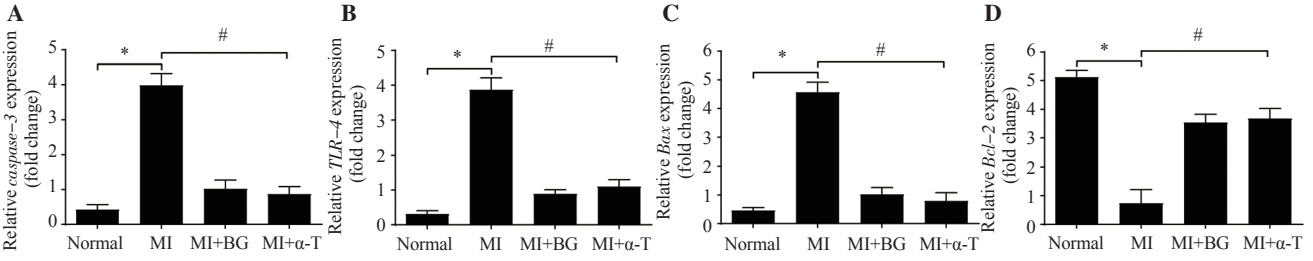

Figure 6. BG attenuates cardiac apoptosis by suppressing the mRNA expressions of (A) *caspase-3*, (B) *TLR-4*, (C) *Bax*, and upregulating (D) *Bcl-2* expression in isoproterenol-induced MI rats. Values are expressed as mean±SD (n=6) and significantly different at $^*P$<0.05 *vs.* control and $^\#P$<0.05 *vs.* MI control.

3.7. Histopathological analysis of the heart of MI rats

Figure 7 shows the histological architecture of the heart of control and MI rats. The hearts from the normal control group showed no necrosis, edema, or inflammation but exhibited characteristic myofibrillar striations, a branched appearance, and continuity with adjacent myofibrils (Figure 7A). The heart of the MI control group showed confluent central necrosis of muscle fibers with cell infiltration and a higher degree of inflammatory cell infiltration, damaged myocardial fibers, infiltration of neutrophilic granulocytes, and interstitial edema (Figure 7B). However, pretreatment with BG (Figure 7C) and α-tocopherol (Figure 7D), followed by administration of ISO, alleviated myocardial damage, as evidenced by a reduction in myonecrosis, edema, and erythrocyte extravasation with almost imperceptible inflammation. The severity of inflammatory cell infiltration (Figure 7E), myocardial necrosis (Figure 7F), and edema (Figure 7G) was significantly high in MI control rats, whereas a decrease in the severity of inflammatory cell infiltration, myocardial necrosis, and edema was observed in rats pretreated with BG.

4. Discussion

Preclinical studies suggest that certain plant-based therapeutics can be used to treat MI[14,15]. Because of their efficacy and safety, plant-based drugs are widely used to treat cardiac injuries and have potent anti-inflammatory and antioxidant properties[16,17]. In this experimental study, we investigated the anti-inflammatory and anti-apoptotic effects of BG on ISO-induced MI, as well as its effect on cardiac remodeling and histological changes.

Although catecholamines are responsible for controlling cardiac function, their high levels can lead to heart failure, cardiac hypertrophy, and ischemic heart diseases such as MI. These occur due to abnormal β-adrenergic activity[18]. As high doses of ISO (up to 100 mg/kg) induce necrosis, hypoxia, and pathological changes during MI and the abnormalities of cardiac structure and function in rats after ISO administration are comparable to those in humans MI, ISO has been used as a trigger to build models to evaluate the cardioprotective effects of various drugs[11,14].

During myocardial injury, LDH and CK-MB levels increase significantly as these enzymes are released into the bloodstream due to rupture of the cell membrane. The degree of damage is closely correlated with serum levels of these enzymes[19]. Therefore, other methods of stabilizing the cell membrane, such as the use of natural products and phytochemicals, could lower these indicators[14,20,21]. The results of the current study showed that ISO increased the serum levels of LDH and CK-MB, while pretreatment with BG pretreatment effectively decreased the levels of these cardiac markers. Furthermore, the contractile protein cTnI has been described as a particular marker of cardiac cell damage, which is present in serum as a result of cardiomyocyte damage[22]. In addition, the non-essential 4-carbon α-amino acid homocysteine is formed during the metabolism of methionine by demethylation of methionine. It contains thiols and may be cytotoxic. An independent risk factor for acute MI is a chronic increase in homocysteine[23]. Homocysteine has been shown to cause atherosclerosis by either reducing the efficacy of coronary microvascular dilators or promoting smooth muscle growth, platelet activation, and thrombogenesis. It is also a potent trigger of inflammatory processes[24].

In the current study, MI control rats had significantly higher cTnI and homocysteine levels compared with normal control rats. This is

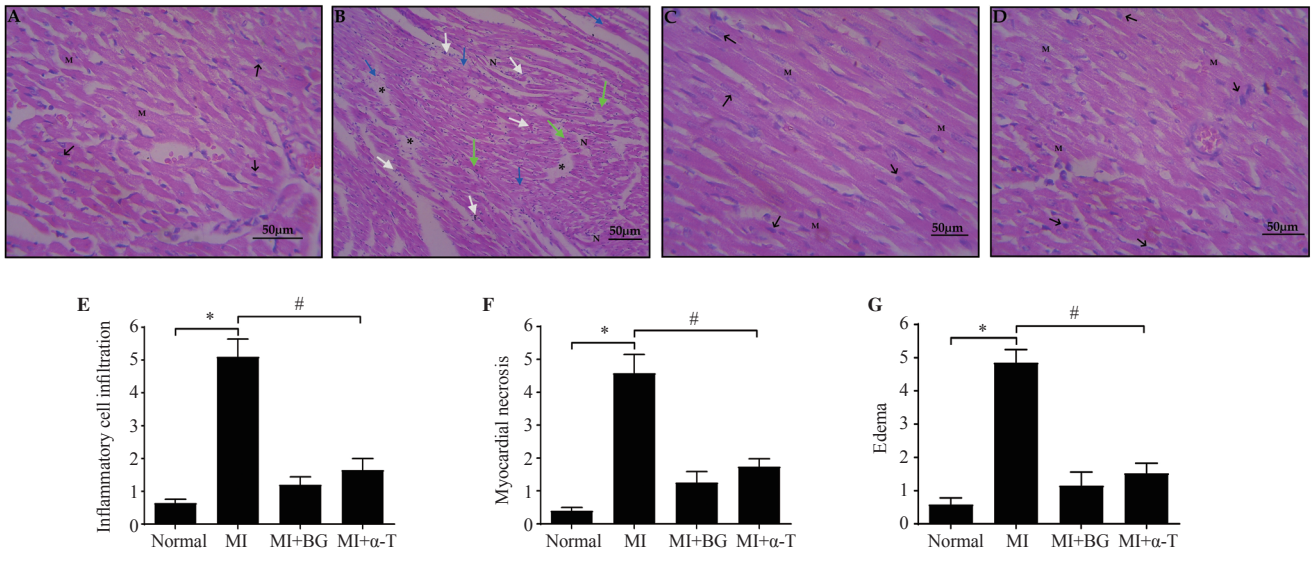

Figure 7. Histoarchitecture of the heart in MI and BG-treated rats (magnification: ×40). (A) Normal control, (B) MI control, (C) MI + BG, and (D) MI + α-tocopherol. The grades of severity of (E) inflammatory cell infiltration, (F) myocardial necrosis, and (G) edema. Black arrow: central nucleus; Star: separated cardiac muscle fibers; Green arrow: edema; White arrow: inflammation; Blue arrow: interstitial fibrosis; M: myocardial fibers; N: necrosis. Values are expressed as mean±SD (n=6) and significantly different at $^{*}P<0.05$ *vs.* control and $^{\#}P<0.05$ *vs.* MI control.

in agreement with previously published studies by Sangeethadevi *et al*.[14] and Jansy *et al*[17]. However, in rats with ISO-induced MI, pretreatment with BG significantly lowers serum cTnI. It has been shown that BG can limit cTnI leakage from the myocardium by preserving the structural and functional integrity and permeability of the cardiac membrane. These results suggest that BG protects the heart by preventing leakage of cardiac enzymes into the circulation and preserving the structural and functional integrity of the myocyte plasma membrane and contractile mechanism. In addition, pretreatment with BG reduced hyperhomocysteinemia in rats administered ISO, suggesting that BG may protect against atherosclerosis, thrombogenesis, and inflammatory processes.

When administered subcutaneously, ISO immediately increases blood pressure because it is a β-adrenergic receptor agonist. However, over time, it may lead to an increase in oxygen demand, hypoxia, and myocardial ischemia, whereupon arterial pressure decreases[25]. Necrosis and cardiovascular damage lead to a decrease in MAP, HR, and cardiac contractility. ISO-induced decreases in SBP, DBP, MAP, and HR raise the possibility of heart failure associated with MI. As blood pressure and heart rate drop, there is a greater requirement for oxygen. As a result, hypoxia and ischemic heart disease eventually develop[26]. This was also confirmed by a decrease in the pressure rate index. It is because the balance between the supply of coronary blood and the demand imposed on the heart is losing strength. According to the results of the current study, pretreatment with BG ameliorated MI, as evidenced by improved hemodynamic parameters.

The study shows that ISO increases the release of various chemokines and proinflammatory cytokines that trigger NF-κB. The inflammatory response is of critical importance in the pathogenesis of MI[14,27]. Inflammatory cytokines are regulated by NF-κB, which also increases the transcription of target genes (IL-6 and IL-1β), thereby enhancing the inflammatory response triggered by the activation of proinflammatory cytokines[28]. IL-1β can increase neutrophil chemotaxis and inflammatory mediator production, leading to tissue damage and inflammatory responses. Neutrophil maturation and recruitment are two other proinflammatory cytokines, such as IL-6, involved in numerous inflammatory processes[29]. TNF-α, IL-1β, and IL-6 are examples of proinflammatory cytokines that are not constitutively produced in healthy hearts[16]. The upregulation and production of these cytokines represent an intrinsic or inherent stress response to prevent cardiac injury. According to previous studies[30,31], from the initial few hours to a day after MI in rodent models, a significant increase in the mRNA expression of intramyocardial cytokines such as TNF-α, IL-1β, and IL-6 in both the infarcted region (up to 50-fold) and noninfarcted myocardium (up to 15-fold) was observed. On the other hand, if the infraction is minor, this strong upregulation may revert to baseline levels[14].

Nevertheless, either a sustained upregulation of cytokines or a second wave of cytokine upregulation corresponding to the chronic remodeling phase may occur if the infarction is substantial or the host inflammatory response is strong. The non-infarct zone may also be affected by the second wave, which would mediate significant myocardial remodeling activities[32]. The current study showed that administration of ISO significantly increased the production of proinflammatory cytokines such as NF-κB, TNF-α, IFN-γ, IL-6, and IL-1β in the heart and upregulated the mRNA expressions of *TNF–α*, *IL–6*, and *NF–κB*, which is consistent with previous findings[14,32]. On the other hand, our results highlighted that administration of BG significantly reduced the production of inflammatory mediators and proinflammatory cytokines. This may be explained by the ability of BG to prevent phosphorylation of proteins involved in signal transduction, such as those responsible for NF-κB control. BG may inhibit the activation of NF-κB, thereby suppressing the production of IL-6, IFN-γ, IL-1β, and TNF-α.

Along with cardiac inflammation, we also examined how BG affects ISO-induced cardiomyocyte apoptosis in rats. It is well known that apoptosis in cardiomyocytes is a crucial mode of cell death. Toll-like receptors (TLRs) are primarily responsible for controlling inflammation in the cardiac muscle; TLR-4 is the well-studied TLR and an important mediator of inflammation[33]. In addition, TLR-4-mediated NF-κB signaling induces cell death and controls inflammatory and immunological responses[34]. When the β1-adrenergic receptor is activated, stronger cardiac contractions lead to cardiomyocyte apoptosis, resulting in hypoxia and ischemia[35]. Caspase activation is a crucial step in the apoptotic signaling cascade. Caspase-3 is the main downstream apoptosis-regulating protein of the caspase family, which is activated by various apoptotic stimuli[36].

Bcl-2 family proteins also play a critical role in regulating the apoptotic process. In addition, excessive formation of ROS activates Bax, which is critical for triggering apoptosis by opening mitochondrial pores through which mitochondrial cytochrome C can flow into the cytosol[37]. Through the formation of apoptosomes, the released cytochrome C activates the executioner caspases[38]. In the present study, it was observed that ISO activates the intrinsic apoptotic pathway by activating TLR-4, caspases, and Bcl-2 family proteins, while the administration of BG protects the myocardium from cardiomyocyte apoptosis by blocking the intrinsic apoptotic pathway in ISO-induced MI rats.

Moreover, it has been suggested that the etiology of cardiac remodeling after MI is related to the deleterious ACE-AT_1R axis of the renin-angiotensin system[39]. Increasing data suggest that ACE levels negatively affect the heart by causing cardiac hypertrophy and decreased contractility, which are caused in part by suppression of ACE2-mediated cardioprotection[40,41]. According to Li *et al*.[42], experimental rats also exhibit increased ACE2 levels and activities in the heart when they receive ACE inhibitors and AT_1R blockers. Moreover, overexpression of ACE2 protects against ACE-mediated cardiac fibrosis and hypertrophy[40]. Thus, feedback inhibition can be used by ACE and ACE2 to control each other. This is supported by the results of our study, which showed that both ACE and AT_1R expressions were significantly upregulated in the MI control group but were successfully reduced by pretreatment with BG. To control cardiac development, repair, and remodeling through activation of NADPH oxidase, prohypertrophic signaling, and fibrosis, loss of cardiomyocytes after MI is thought to cause upregulation of ACE and increased Ang Ⅱ production[43]. The AT_1R plays an important role in mediating these effects, and the use of pharmaceutical antagonists such as losartan and irbesartan targeting these receptors

significantly reduced myocardial oxidative stress, inflammation, hypertrophy, and fibrosis in several animal models[39,44]. In the present work, BG dramatically downregulated the expression of the ACE-AT_1R axis and may influence cardiac remodeling.

Overall, the results of the current study suggest that pretreatment with BG may prevent the cardiotoxic effects of ISO by reducing the inflammatory response of the heart and apoptosis by controlling the production of proinflammatory cytokines and downregulating genes associated with apoptosis. Moreover, treatment with BG reduces cardiac remodeling by regulating the ACE-AT_1R axis. Therefore, our study suggests that BG may be a promising candidate to attenuate ISO-induced cardiotoxicity, which needs further investigation.

Conflict of interest statement

The authors declare no competing interests.

Acknowledgments

Authors would like to express their sincere thanks to the management of Nandha College of Pharmacy, Erode, and Tamilnadu for providing the Animal House Facility for this study.

Funding

The authors received no extramural funding for the study.

Authors' contributions

AR and VVSU wrote the manuscript; AR, VMN, JG, BRA, SK, and VVSU performed experiments and collected the data; AR, VMN, and SS analyzed the data; AR, VVSU, and SS designed the study; VMN, JG, BRA, SK and SS reviewed the manuscript. All authors read and approved the final manuscript.

References

[1] Michaud K, Basso C, d'Amati G, Giordano C, Kholová I, Preston SD, et al. Diagnosis of myocardial infarction at autopsy: AECVP reappraisal in the light of the current clinical classification. *Virchows Arch* 2020; **476**(2): 179-194.

[2] Bauters C, Dubois E, Porouchani S, Saloux E, Fertin M, de Groote P, et al. Long-term prognostic impact of left ventricular remodeling after a first myocardial infarction in modern clinical practice. *PLoS One* 2017; **12**(11): e0188884.

[3] Mongirdienė A, Skrodenis L, Varoneckaitė L, Mierkytė G, Gerulis J. Reactive oxygen species induced pathways in heart failure pathogenesis and potential therapeutic strategies. *Biomedicines* 2022; **10**(3): 602.

[4] Zainalabidin S, Ramalingam A, Mohamed SF, Ali SS, Latip J, Yap WB. S-allylcysteine therapy reduces adverse cardiac remodelling after myocardial infarction in a rat model. *J Funct Foods* 2020; **66**: 103750.

[5] Viola M, de Jager SCA, Sluijter JPG. Targeting inflammation after myocardial infarction: A therapeutic opportunity for extracellular vesicles? *Int J Mol Sci* 2021; **22**(15): 7831.

[6] Korshunova AY, Blagonravov ML, Neborak EV, Syatkin SP, Sklifasovskaya AP, Semyatov SM, et al. BCL2-regulated apoptotic process in myocardial ischemia-reperfusion injury (Review). *Int J Mol Med* 2021; **47**(1): 23-36.

[7] Chen Y, Peng L, Shi S, Guo G, Wen H. Boeravinone B alleviates gut dysbiosis during myocardial infarction-induced cardiotoxicity in rats. *J Cell Mol Med* 2021; **25**(13): 6403-6416.

[8] Zhu F, Du B, Bian Z, Xu B. Beta-glucans from edible and medicinal mushrooms: Characteristics, physicochemical and biological activities. *J Food Compos Anal* 2015; **41**: 165-173.

[9] Zhang Y, Xia L, Pang W, Wang T, Chen P, Zhu B, et al. A novel soluble β-1,3-*D*-glucan salecan reduces adiposity and improves glucose tolerance in high-fat diet-fed mice. *Br J Nutr* 2013; **109**(2): 254-262.

[10] Eraniappan S, Balasubramaniyan P, Uddandrao VVS, Roy A, Singaravel S. Beta-glucan suppresses high-fat-diet-induced obesity by attenuating dyslipidemia and modulating obesogenic marker expressions in rats. *Fundam Clin Pharmacol* 2023; **37**(3): 629-638.

[11] Pavithra K, Sathibabu Uddandrao VV, Chandrasekaran P, Brahmanaidu P, Sengottuvelu S, Vadivukkarasi S, et al. Phenolic fraction extracted from *Kedrostis foetidissima* leaves ameliorated isoproterenol-induced cardiotoxicity in rats through restoration of cardiac antioxidant status. *J Food Biochem* 2020; **44**(11): e13450.

[12] Govindasami S, Uddandrao VVS, Raveendran N, Sasikumar V. Therapeutic potential of biochanin-A against isoproterenol-induced myocardial infarction in rats. *Cardiovasc Hematol Agents Med Chem* 2020; **18**(1): 31-36.

[13] Sathibabu Uddandrao VV, Sethumathi PP, Parim Brahma Naidu, Vadivukkarasi S, Mustapha Sabana Begum, Saravanan G. Ameliorative potential of biochanin-A against dexamethasone induced hypertension through modulation of relative mRNA and protein expressions in experimental rats. In: Sathibabu Uddandrao VV, Naidu PB (eds.) *Advancements in cardiovascular research and therapeutics: Molecular and nutraceutical perspectives*. Bentham Science Publishers; 2022, p. 156.

[14] Sangeethadevi G, Sathibabu Uddandrao VV, Jansy Isabella RAR, Saravanan G, Ponmurugan P, Chandrasekaran P, et al. Attenuation of lipid metabolic abnormalities, proinflammatory cytokines, and matrix metalloproteinase expression by biochanin-A in isoproterenol-induced myocardial infarction in rats. *Drug Chem Toxicol* 2022; **45**(5): 1951-1962.

[15] Zou G, Wan J, Balupillai A, David E, Ranganathan B, Saravanan K. Geraniol enhances peroxiredoxin-1, and prevents isoproterenol-induced oxidative stress and inflammation associated with myocardial infarction in experimental animal models. *J Biochem Mol Toxicol* 2022; **36**(8): e23098.

[16] Uddandrao VVS, Parim B, Singaravel S, Ponnusamy P, Ponnusamy C, Sasikumar V, et al. Polyherbal formulation ameliorates diabetic cardiomyopathy through attenuation of cardiac inflammation and oxidative stress *via* NF-κB/Nrf-2/HO-1 pathway in diabetic rats. *J Cardiovasc Pharmacol* 2022; **79**(1): e75-e86.

[17] Jansy A, Sathibabu Uddandrao VV, Sangeethadevi G, Saravanan G,

Chandrasekaran P, Sengottuvel S, et al. Biochanin A attenuates obesity cardiomyopathy in rats by inhibiting oxidative stress and inflammation through the Nrf-2 pathway. *Arch Physiol Biochem* 2021; **129**(3): 788-798.

[18]Allawadhi P, Khurana A, Sayed N, Kumari P, Godugu C. Isoproterenol-induced cardiac ischemia and fibrosis: Plant-based approaches for intervention. *Phytother Res* 2018; **32**(10): 1908-1932.

[19]Abdelzaher WY, Ahmed SM, Welson NN, Alsharif KF, Batiha GE, Labib DAA. Dapsone ameliorates isoproterenol-induced myocardial infarction *via* Nrf2/HO-1; TLR4/TNF-α signaling pathways and the suppression of oxidative stress, inflammation, and apoptosis in rats. *Front Pharmacol* 2021; **12**: 669679.

[20]Chen W, Liang J, Fu Y, Jin Y, Yan R, Chi J, et al. Cardioprotection of cortistatin against isoproterenol-induced myocardial injury in rats. *Ann Transl Med* 2020; **8**(6): 309.

[21]Hosseini A, Rajabian A, Sobhanifar MA, Alavi MS, Taghipour Z, Hasanpour M, et al. Attenuation of isoprenaline-induced myocardial infarction by *Rheum turkestanicum*. *Biomed Pharmacother* 2022; **148**: 112775.

[22]Mair J, Lindahl B, Hammarsten O, Müller C, Giannitsis E, Huber K, et al. How is cardiac troponin released from injured myocardium? *Eur Heart J Acute Cardiovasc Care* 2018; **7**(6): 553-560.

[23]Fu Y, Wang X, Kong W. Hyperhomocysteinaemia and vascular injury: Advances in mechanisms and drug targets. *Br J Pharmacol* 2018; **175**(8): 1173-1189.

[24]Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. *Nutr J* 2015; **14**: 6.

[25]Ma S, Ma J, Tu Q, Zheng C, Chen Q, Lv W. Isoproterenol increases left atrial fibrosis and susceptibility to atrial fibrillation by inducing atrial ischemic infarction in rats. *Front Pharmacol* 2020; **11**: 493.

[26]Filipský T, Zatloukalová L, Mladěnka P, Hrdina R. Acute initial haemodynamic changes in a rat isoprenaline model of cardiotoxicity. *Hum Exp Toxicol* 2012; **31**(8): 830-843.

[27]Manjunatha S, Shaik AH, E MP, Al Omar SY, Mohammad A, Kodidhela LD. Combined cardio-protective ability of syringic acid and resveratrol against isoproterenol induced cardio-toxicity in rats *via* attenuating NF-κB and TNF-α pathways. *Sci Rep* 2020; **10**(1): 3426.

[28]Yu H, Lin L, Zhang Z, Zhang H, Hu H. Targeting NF-κB pathway for the therapy of diseases: Mechanism and clinical study. *Signal Transduct Target Ther* 2020; **5**(1): 209.

[29]Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* 2017; **9**(6): 7204-7218.

[30]Hartman MHT, Groot HE, Leach IM, Karper JC, van der Harst P. Translational overview of cytokine inhibition in acute myocardial infarction and chronic heart failure. *Trends Cardiovasc Med* 2018; **28**(6): 369-379.

[31]Bakhta O, Blanchard S, Guihot AL, Tamareille S, Mirebeau-Prunier D, Jeannin P, et al. Cardioprotective role of colchicine against inflammatory injury in a rat model of acute myocardial infarction. *J Cardiovasc Pharmacol Ther* 2018; **23**(5): 446-455.

[32]Bai WW, Wang H, Gao CH, Liu KY, Guo BX, Jiang F, et al. Continuous infusion of angiotensin Ⅳ protects against acute myocardial infarction *via* the inhibition of inflammation and autophagy. *Oxid Med Cell Longev* 2021; **2021**. doi: 10.1155/2021/2860488.

[33]Katare PB, Bagul PK, Dinda AK, Banerjee SK. Toll-like receptor 4 inhibition improves oxidative stress and mitochondrial health in isoproterenol-induced cardiac hypertrophy in rats. *Front Immunol* 2017; **8**: 719.

[34]Meeran MFN, Azimullah S, Adeghate E, Ojha S. Nootkatone attenuates myocardial oxidative damage, inflammation, and apoptosis in isoproterenol-induced myocardial infarction in rats. *Phytomedicine* 2021; **84**: 153405.

[35]Nagoor Meeran MF, Laham F, Azimullah S, Tariq S, Ojha S. α-Bisabolol abrogates isoproterenol-induced myocardial infarction by inhibiting mitochondrial dysfunction and intrinsic pathway of apoptosis in rats. *Mol Cell Biochem* 2019; **453**(1-2): 89-102.

[36]Dai H, Chen L, Gao D, Fei A. Phosphocreatine attenuates isoproterenol-induced cardiac fibrosis and cardiomyocyte apoptosis. *Biomed Res Int* 2019; **2019**. doi: 10.1155/2019/5408289.

[37]Sahu BD, Anubolu H, Koneru M, Kumar JM, Kuncha M, Rachamalla SS, et al. Cardioprotective effect of embelin on isoproterenol-induced myocardial injury in rats: Possible involvement of mitochondrial dysfunction and apoptosis. *Life Sci* 2014; **107**(1-2): 59-67.

[38]Radhiga T, Rajamanickam C, Sundaresan A, Ezhumalai M, Pugalendi KV. Effect of ursolic acid treatment on apoptosis and DNA damage in isoproterenol-induced myocardial infarction. *Biochimie* 2012; **94**(5): 1135-1142.

[39]Gao XM, Tsai A, Al-Sharea A, Su Y, Moore S, Han LP, et al. Inhibition of the renin-angiotensin system post myocardial infarction prevents inflammation-associated acute cardiac rupture. *Cardiovasc Drugs Ther* 2017; **31**(2): 145-156.

[40]Liu Q, Zhang Q, Wang K, Wang S, Lu D, Li Z, et al. Renal denervation findings on cardiac and renal fibrosis in rats with isoproterenol induced cardiomyopathy. *Sci Rep* 2015; **5**: 18582.

[41]Patel BM, Mehta AA. Aldosterone and angiotensin: Role in diabetes and cardiovascular diseases. *Eur J Pharmacol* 2012; **697**(1-3): 1-12.

[42]Li C, Han R, Kang L, Wang J, Gao Y, Li Y, et al. Pirfenidone controls the feedback loop of the AT_1R/p38 MAPK/renin-angiotensin system axis by regulating liver X receptor-α in myocardial infarction-induced cardiac fibrosis. *Sci Rep* 2017; **7**: 40523.

[43]Ma Y, Yuan J, Hu J, Gao W, Zou Y, Ge J. ACE inhibitor suppresses cardiac remodeling after myocardial infarction by regulating dendritic cells and AT_2 receptor-mediated mechanism in mice. *Biomed Pharmacother* 2019; **114**: 108660.

[44]Watanabe R, Suzuki J, Wakayama K, Kumagai H, Ikeda Y, Akazawa H, et al. Angiotensin Ⅱ receptor blocker irbesartan attenuates cardiac dysfunction induced by myocardial infarction in the presence of renal failure. *Hypertens Res* 2016; **39**(4): 237-244.

Publisher's note