Original Article

Asian Pacific Journal of Tropical Biomedicine

apjtb.org

doi: 10.4103/2221-1691.387750

Impact Factor® 1.7

Naringenin suppresses NLRP3 inflammasome activation *via* the mRNA-208a signaling pathway in isoproterenol-induced myocardial infarction

Ayman Eldourghamy[1], Toka Hossam[2], Mohammed Abdalla Hussein[3]✉, Amal Abdel-Aziz[4], Samir A. El-masry[4]

[1]*Environmental Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, Sadat University, Egypt*

[2]*Department of Medical Labs, Faculty of Applied Medical Sciences Technology, October 6 University, Egypt*

[3]*Department of Biotechnology, Faculty of Applied Health Sciences Technology, October 6 University, Egypt*

[4]*Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute, Sadat University, Egypt*

ABSTRACT

Objective: To investigate the cardioprotective effect of naringenin against isoproterenol (ISO)-induced cardiotoxicity in rats.

Methods: Rats were divided into five groups: the normal group, the ISO group (85 mg/kg b.w.); the ISO+naringenin (50 mg/kg b.w.) group, the ISO+naringenin (100 mg/kg b.w.) group and the ISO+propranolol (10 mg/kg b.w.) group. Plasma creatine kinase-MB (CK-MB), cardiac troponin T, lactate dehydrogenase, brain natriuretic peptide (BNP), and IL-10, as well as cardiac transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF) and malondialdehyde (MDA) were examined. In addition, *NLRP3* and *mRNA-208a* expressions were evaluated by RT-PCR analysis. Histopathological examination was also performed to assess cardiac damages.

Results: Naringenin treatment significantly decreased plasma lactate dehydrogenase, CK-MB, cardiac troponin T, BNP, and IL-10, as well as cardiac TGF-β1, VEGF, and MDA while increasing p-Akt and superoxide dismutase in ISO-administered rats. It also reduced *NLRP3* and *mRNA-208a* gene expression levels. Furthermore, naringenin improved ISO-induced cardiac damage.

Conclusions: Naringenin attenuates myocardial dysfunction in ISO-treated rats by decreasing oxidative stress and increasing cardiac endogenous antioxidant system, which may be modulated partly by improvement of *NLRP3* and *mRNA-208a* gene expression.

KEYWORDS: Naringenin; Isoproterenol; Myocardial infarction; Antioxidants; NLRP3; mRNA-208a

1. Introduction

Nearly three million individuals worldwide suffer from myocardial infarctions, one of the major causes of mortality in the developed world[1]. Atherosclerosis, or the buildup of fatty deposits in the arteries, and a higher risk of blood clots are typically connected with it[2]. It may also be linked to artery damage in several organs, including the kidneys, eyes, heart, brain, and heart[3]. Isoproterenol (ISO) is a drug used to treat bradycardia conditions[4]. The drug has a structural resemblance to epinephrine[5]. In addition to treating heart

Significance

Naringenin is a flavonoid that exhibits cardioprotective activity due to its anti-inflammatory and antioxidant effects. The current work demonstrated that treatment of isoproterenol-injected rats with naringenin attenuates myocardial dysfunction by regulating cardiac inflammatory mediators and oxidative stress biomarkers and improving the expression of *NLRP3* and *mRNA-208a* genes. Naringenin may be used as a cardioprotective agent which needs further investigation.

✉To whom correspondence may be addressed. E-mail: prof.husseinma@o6u.edu.eg

How to cite this article: Eldourghamy A, Hossam T, Hussein MA, Abdel-Aziz A, El-masry SA. Naringenin suppresses NLRP3 inflammasome activation *via* the mRNA-208a signaling pathway in isoproterenol-induced myocardial infarction. Asian Pac J Trop Biomed 2023; 13(10): 443-450.

Article history: Received 29 May 2023; Revision 9 July 2023; Accepted 6 September 2023; Available online 20 October 2023

block and Adams-Stokes syndrome episodes that are not brought on by ventricular tachycardia or fibrillation, ISO is also used to treat bronchospasm during anaesthesia, hypovolemic shock, septic shock, low cardiac output (hypoperfusion) states, congestive heart failure, and cardiogenic shock as an adjunctive therapy[6–8].

ISO also rapidly undergoes auto-oxidation, and it has been suggested that the oxidative products of catecholamines are responsible for changes in the myocardium[9]. According to reports, experimental animals exposed to high concentrations of ISO develop necrotic lesions in the heart that lead to myocardial infarction[8–10].

Naringenin, one of the most significant naturally occurring flavonoids, is primarily present in several edible fruits, such as tomato and citrus species[11–13]. Naringenin has significant biological impacts on human health[14]. It enhances glucose metabolism, boosts antioxidant defenses, scavenges reactive oxygen species, modifies immune system activity, and has anti-atherogenic and anti-inflammatory properties. This includes a reduction in lipid peroxidation biomarkers and protein carbonylation[15–19]. Additionally, it has been shown to have a strong capacity to control signaling pathways involved in fatty acid metabolism, favoring fatty acid oxidation and inhibiting lipid buildup in the liver to avoid fatty liver[20], along with effectively limiting the buildup of plasma lipids and lipoproteins[21].

Propranolol is a non-selective beta receptor antagonist that blocks both beta-1 or beta-2 receptors[22]. Inhibiting sympathetic activation of the heart, it competes with sympathomimetic neurotransmitters for binding to receptors[23]. Numerous investigations have documented the cardioprotective effects of propranolol against myocardial necrosis[24,25]. Considering the abovementioned benefits of naringenin, the current study aimed to investigate the effects of naringenin on ISO-induced cardiotoxicity.

2. Materials and methods

2.1. Chemicals

Naringenin, propranolol, and ISO (99%) were purchased from Sigma-Aldrich. In addition, only analytical-grade chemicals were employed in this investigation.

2.2. Rats

Adult rats weighing around (185 ± 7) g were bought from Cairo University's National Cancer Institute, Egypt. Each rat was kept in a cage at (22 ± 2)℃, 60% relative humidity, and an 8:00 to 20:00 light cycle. Each animal was fed on a consistent diet *ad libitum* during the acclimation phase.

2.3. Experimental approach

Rats were randomly divided into 5 groups with 6 rats in each group: Group Ⅰ served as the normal group and fed a regular diet; group Ⅱ was given ISO (85 mg/kg b.w.) subcutaneously on the 29th and 30th day[26]; group Ⅲ was treated with naringenin (50 mg/kg b.w.) orally for 28 d[27] and then ISO subcutaneously on the 29th and 30th day; group Ⅳ was treated with naringenin (100 mg/kg b.w.) orally for 28 d and then ISO subcutaneously on the 29th and 30th day; group Ⅴ was treated with propranolol (10 mg/kg b.w.) orally for 30 d and then ISO subcutaneously on the 29th and 30th day.

2.4. Biochemical tests

On day 31, rats were sacrificed by cervical decapitation, and blood samples were obtained. Heparinized blood samples were centrifuged at 2 500 ×*g* for 15 min. Separated plasma was used to estimate plasma levels of lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), cardiac troponin T (cTnT), brain natriuretic peptide (BNP), and interleukin-10 (IL-10). Plasma LDH was determined using a test reagent kit (Cayman, USA). In addition, plasma CK-MB activity was determined (Abcam, USA) as well as cTnT, BNP, and IL-10 were estimated using ELISA technique (Elbascience™, USA) and (RAF028R, BioVendor, Czech Republic), respectively.

Hearts were dissected out and then cardiac samples were homogenized in a glass homogenizer (Universal Lab. Aid MPW-309, mechanikaprecyzyjna, Poland). The first portion was homogenized with ice-cold saline to make a 25% *w/v* homogenate. Three separate aliquots of the homogenate were produced. Using ELISA methods, the first aliquot was utilized to assess cardiac transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), and p-Akt (MyBioSource, San Diego, CA; Boster biological technology, CA; Mybioscience™ USA, respectively). The second aliquot was deproteinized with 12 % (ice-cold) trichloroacetic acid and centrifuged at 1 000 ×*g*, and the supernatant produced was used to quantify the amount of malondialdehyde (MDA) (Mybioscience, USA). The cytosolic fraction of the heart was obtained by centrifuging the third aliquot of homogenate at 10 500 ×*g* for 15 min at 4℃ in a cooling ultra-centrifuge, and the diagnostic kit technique was used to evaluate the activity of superoxide dismutase (SOD) in the clear supernatant based on the approach of Kakkar and Das[28].

2.5. qRT–PCR assays

Total RNA was collected from the second part of heart specimens using real-time quantitative PCR, and sections (10-15 mg) of recovered RNA were submitted to real-time PCR analysis using Sepasol-RNA1Super according to the manufacturer's instructions. RT-PCR was performed with 50 ng of RNA template per reaction using the SensiFAST™ SYBR® Hi-ROX One-Step Kit (Meridian Bioscience Inc./Bioline; Memphis, TN, USA) in a 25 μL reaction

volume containing 70 nM of specific primers in the Applied Biosystems 7500 Fast Real-Time PCR System (Foster City, CA, USA). Sequences of the primers were as follows: The primers for *mRNA–208a* are F: 5'-TGTTCACTGTTCCTAATC-3' and R: 5'-CTGAAACACTGGCTTAAA-3'; and for *NLRP3* F: 5'-GTCATCTAGAAAGCTTGATGC AGGAAAGAGCTTTGG-3' and R: 5'-TGACAGATCTCAGCTGACATCCTCTAGGCT GGGGTT -3'. *β–actin* mRNA (F: 5'-GAACTTTGGGGGATGCTCGC-3' and R: 5'-CGGGAAATCGTGCGTGACAT-3') was employed as a housekeeping gene.

2.6. Histological assessment

The third section of cardiac specimens was dried in graded alcohol and embedded in paraffin after being fixed in a 10% neutral formalin solution. The fine slices (5 µm thickness) were stained with hematoxylin & eosin (H&E) and mounted on glass slides for light microscopic inspection using Bancroft and Steven's approach[29].

2.7. Statistical analysis

Using the SPSS/20 programme, all data were statistically assessed. The data were presented as mean ± SD (n=6) and analyzed by one-way analysis of variance (ANOVA). P values less than 0.05 were considered statistically significant.

2.8. Ethical statement

The experiment was approved by the Ethics Committee of Faculty of Applied Health Science Technology (No. 20221025). All animal research procedures were carried out in accordance with the standards set forth in the eighth edition of Guide for the Care and Use of Laboratory Animals (published by the National Academy of Sciences, The National Academies Press, Washington, D.C.).

3. Results

3.1. Effect of naringenin on plasma LDH, CK–MB, cTnT, and BNP in ISO–administered rats

Plasma levels of LDH, CK-MB, cTnT, and BNP in different groups are shown in Table 1. Plasma levels of LDH, CK-MB, cTnT, and BNP were increased significantly by 73.97%, 95.17%, 158.93% and 52.59%, respectively in ISO-administered rats as compared with the normal group (P<0.05). Naringenin (50 and 100 mg/kg b.w.) treatment significantly reduced plasma levels of LDH, CK-MB, cTnT, and BNP (P<0.05) in ISO-administered rats (P<0.05), with a better efficacy observed at the higher dose.

3.2. Effect of naringenin on plasma IL–10 as well as cardiac TGF–β1, VEGF, and p–Akt in ISO–administered rats

Compared to the control group, subcutaneous injection of ISO (85 mg/kg b.w.) resulted in substantial increases in plasma IL-10 by 164.98% and cardiac TGF-β1 and VEGF by 169.17% and 196.91%, respectively, as well as a significant decrease in cardiac p-Akt level by 75.29%, indicating acute heart toxicity (P<0.05). When compared to the ISO control group, treatment with naringenin (50 mg/kg b.w.) significantly reduced plasma IL-10 and cardiac TGF-β1 and VEGF,

Table 1. Effect of naringenin on plasma lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), cardiac troponin T (cTnT) and brain natriuretic peptide (BNP) in isoproterenol (ISO)-administered rats.

Parameters	Normal	ISO (85 mg/kg b.w.)			
		-	Naringenin (50 mg/kg b.w.)	Naringenin (100 mg/kg b.w.)	Propranolol (10 mg/kg b.w.)
LDH (U/L)	740.14±28.32^{a}	1287.66±56.84^{e}	907.04±47.63^{d}	782.61±21.69^{b}	856.33±19.76^{c}
CK-MB (U/L)	225.09±12.23^{a}	439.31±21.90^{e}	363.52±18.55^{d}	244.72±14.37^{b}	268.74±9.09^{c}
cTnT (pg/mL)	112.08±10.91^{a}	290.21±26.70^{e}	223.58±10.12^{d}	131.74±11.43^{b}	163.57±12.46^{c}
BNP (pg/mL)	132.81±6.27^{a}	202.66±11.75^{d}	174.36±7.30^{c}	134.12±9.79^{a}	159.25±7.06^{b}

Data are presented as mean ± SD (n=6) and analyzed by one-way analysis of variance (ANOVA). Different superscripts along the same line indicate a significant difference (P < 0.05).

Table 2. Effect of naringenin on plasma interleukin-10 (IL-10) as well as cardiac transforming growth factor (TGF)-β1, vascular endothelial growth factor (VEGF) and phosphorylated alpha-serine/threonine kinase (p-Akt) in ISO-administered rats.

Parameters	Normal	ISO (85 mg/kg b.w.)			
		-	Naringenin (50 mg/kg b.w.)	Naringenin (100 mg/kg b.w.)	Propranolol (10 mg/kg b.w.)
IL-10 (pg/mL)	12.25±1.90^{a}	32.46±3.84^{e}	25.70±1.77^{d}	16.45±2.22^{b}	21.40±4.52^{c}
TGF- β1 (pg/mg protein)	55.46±6.68^{a}	149.28±10.66^{d}	83.56±7.92^{b}	70.40±6.49^{b}	99.07±8.41^{c}
VEGF (pg/mg protein)	28.13±3.57^{a}	83.52±6.25^{e}	50.24±3.27^{d}	34.69±3.85^{b}	43.15±4.72^{c}
p-Akt (ng/g tissue)	7.81±0.54^{d}	1.93±0.25^{a}	3.43±0.20^{b}	6.98±0.58^{c}	4.72±0.42^{b}

Data are presented as mean ± SD (n=6) and analyzed by ANOVA. Different superscripts along the same line indicate a significant difference (P < 0.05).

as well as increased p-Akt levels (P<0.05) (Table 2). Moreover, the higher dose of naringenin (100 mg/kg b.w.) induced a more pronounced effect than the lower dose.

3.3. Effect of naringenin on cardiac SOD and MDA in ISO-administered rats

Cardiac levels of SOD and MDA in different groups are shown in Table 3. Cardiac levels of SOD were decreased significantly by 65.66%, while cardiac MDA was dramatically elevated by 229.11% in ISO-treated rats as compared with the normal group. Treatment with naringenin at 50 and 100 mg/kg b.w. significantly increased cardiac SOD level and decreased cardiac MDA level (P<0.05).

3.4. Effect of naringenin on cardiac mRNA-208a and NLRP3 expression in ISO-administered rats

Figure 1 shows cardiac *NLRP3* and *mRNA-208a* gene expressions were significantly increased in rats treated with ISO compared to the normal group (P<0.05). Administration of naringenin at both doses markedly reduced *NLRP3* and *mRNA-208a* gene expressions, with 100 mg/kg b.w. showing a more significant effect (P<0.05).

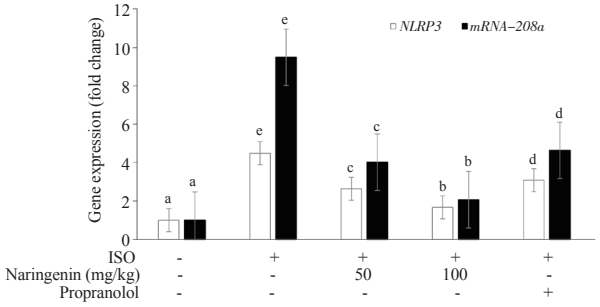

Figure 1. Effect of naringenin on cardiac *NLRP3* and *mRNA-208a* gene expression in ISO-administered rats. Data are presented as mean ± SD of three independent experiments and analyzed by one-way analysis of variance (ANOVA). Different superscripts indicate a significant difference (P < 0.05).

3.5. Histological examination of cardiac tissues of ISO rats after treatment with naringenin

The cardiac myocytes in the normal group had branching and anastomosing longitudinal muscle fibers, acidophilic sarcoplasm, and central oval vesicular nuclei (Table 4 and Figure 2A).

In the ISO-treated group, the large distances between cardiomyocytes increased noticeably. The expanded and congested blood vessels, inflammatory cellular infiltration, fragmented necrotic cardiac muscle fibers were observed. Cardiomyocytes lost their sarcoplasmic striations, resulting in areas of discontinuity. Furthermore, apoptotic muscle fibers could be seen, with hyperacidophilic cytoplasm and pyknotic nuclei in some cases (Table 4 and Figure 2B).

The morphology of cardiomyocytes in rats treated with ISO and naringenin (50 mg/kg b.w.) was intact, with central oval vesicular nuclei. No cytoplasmic vacuoles were observed with some clogged dilated blood vessels and mild cellular infiltration (Table 4 and Figure 2C).

In addition, rats treated with ISO and naringenin (100 mg/kg b.w.) showed a normal histological structure without vacuoles found among cardiac muscle fibers, leucocytes and lymphocytes. In addition, there was no spaces detected between muscle fiber (Table 4 and Figure 2D). Treatment with propranolol (10 mg/kg b.w.) improved ISO-induced cardiac damage and showed a normal histological structure (Table 4 and Figure 2E).

4. Discussion

Several cardiovascular disorders have been linked, at least in part, to an increase in the generation of reactive oxygen species (ROS)[30]. Excessive oxidative stress induced by either increased ROS generation or insufficient antioxidant defenses has been shown to cause heart lesions[31].

Table 3. Effect of naringenin on cardiac superoxide dismutase (SOD) and malondialdehyde (MDA) in rats.

Parameters	Normal	ISO (85 mg/kg b.w.)			
		-	Naringenin (50 mg/kg b.w.)	Naringenin (100 mg/kg b.w.)	Propranolol (10 mg/kg b.w.)
SOD (U/mg protein)	13.63±1.17^{d}	4.68±0.45^{a}	6.27±0.39^{b}	11.72±0.88^{d}	9.39±0.72^{c}
MDA (nmol/mg protein)	10.41±1.89^{a}	34.26±2.69^{e}	23.54±3.61^{d}	12.63±1.73^{b}	17.95±2.22^{c}

Data are presented as mean ± SD (n=6) and analyzed by ANOVA. Different superscripts along the same line indicate a significant difference (P < 0.05).

Table 4. Effect of naringenin on cardiac histopathological changes in ISO-administered rats.

Changes	Normal	ISO (85 mg/kg b.w.)			
		-	Naringenin (50 mg/kg b.w.)	Naringenin (100 mg/kg b.w.)	Propranolol (10 mg/kg b.w.)
Disorganization	0	++	+	+	+
Lymphatic infiltration	0	++	+	+	0
Vacuoles	0	++	0	+	0
Congestion	0	++	++	+	+
Atrophy in focal areas	0	++	+	+	0

No changes (0), mild congestion (+) and moderate congestion (++).

The current study shows that naringenin and propranolol have a cardioprotective impact on ISO-induced cardiotoxicity, as indicated by increased antioxidant defense, inhibition of lipid peroxidation, and avoidance of leakage of myocyte damage marker enzyme from the heart. ISO is a drug that is commonly used in toxicological investigations to produce cardiac muscle damage *via* an exaggerated pharmacological action[32]. ISO injection is a well-established animal model of acute myocardial infarction[33].

ISO is a sympathomimetic medication that primarily operates on beta-adrenergic receptors and is used to treat allergy crises, bronchial asthma, ventricular bradycardia, cardiac arrest, and glaucoma[34]. ISO, on the other hand, can cause side effects and adverse responses, including myocardial infarction at high dosages[35]. In the current investigation, ISO injection caused substantially higher plasma levels of LDH, CK-MB, cTnT, and BNP, confirming the results of various reports that proved the elevation of LDH, CK-MB, cTnT, and BNP in ISO-injected rats[36].

The increased levels of LDH, CK-MB, cTnT, and BNP may be explained by the increased generation of free radicals during ISO metabolism. These free radicals might be boosted by calcium-mediated proteases, which could break cellular proteins and release cytosolic enzymes into the plasma. Also, the levels of LDH, CK-MB, cTnT, and BNP in plasma of ISO-exposed rats were depleted by administration of naringenin at 50 and 100 mg/kg b.w.

Several papers proved the relationship between consumption of naringenin and depletion of cardiovascular risk[37]. Naringin has been employed in the vast majority of clinical trials (naringenin glycoside)[38].

Flavonoids also have anti-cancer, anti-bacterial, and anti-inflammatory effects. Citrus flavonoids naringin and naringenin have therapeutic properties, most notably antioxidant, anti-free radical, anti-inflammatory, and decreasing blood lipid levels[36]. Furthermore, naringin and naringenin are strong anticancer medicines that are used to treat a variety of cancers.

Furthermore, beta-blockers are commonly utilized to treat both cardiovascular and non-cardiovascular illnesses[39]. Nonetheless, their mechanism of action is unknown and differs greatly from that of other drugs in this class. Nonselective β-blockers such as propanolol, metoprolol, or carvedilol have adrenoceptor-independent actions such as free radical scavenging, resulting in regulated cellular redox state and, as a result, functional recovery in organs such as the heart[40].

Compared to the control group, subcutaneous injection of ISO resulted in substantial increases in plasma IL-10 and cardiac TGF-β1 and VEGF as well as a significant decrease in cardiac p-Akt level, indicating acute heart toxicity. The adverse effects of ISO may be caused by the oxidation of catecholamines' hydroxyl groups, which produce quinones, and the subsequent creation of adrenochromes, which damage cells and impair contractility in the heart. The formation of extremely dangerous oxygen-derived free radicals during this phase harms both extracellular and intracellular enzymes and proteins.

ISO are significant sources of $O_2^{\bullet-}$ that cause vascular oxidative stress that has inhibited NADPH as a major molecular mechanism for treating mouse endothelial impairment and lowering arterial oxidative stress[41]. Numerous studies have demonstrated that endothelial dysfunction, the underlying cause of cardiovascular disease, obesity, and type 2 diabetes mellitus, is exacerbated

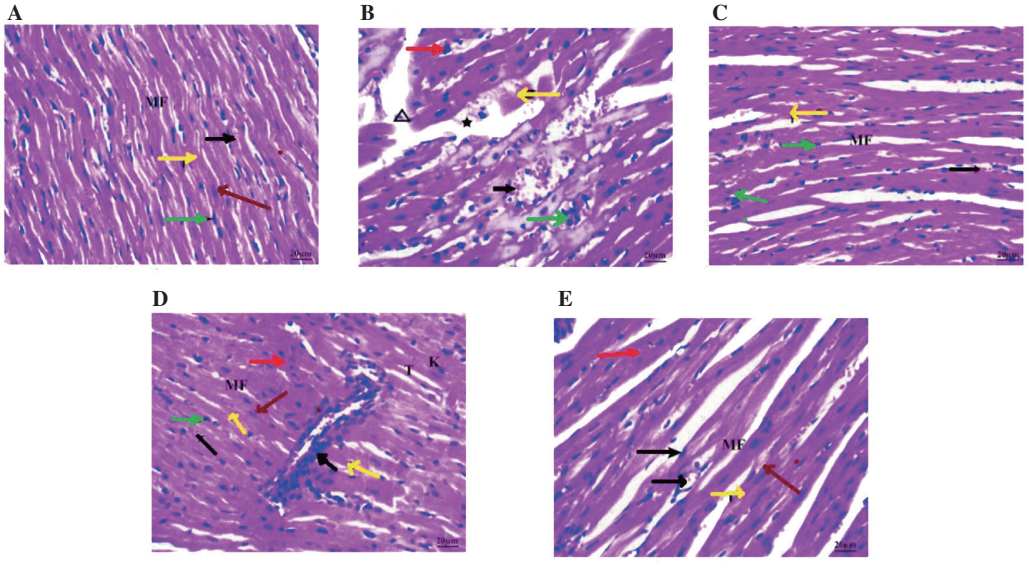

Figure 2. Histological examination of cardiac tissues of rats treated with ISO and naringenin (hematoxylin and eosin; 200 ×) A: Normal control; B: ISO (85 mg/kg b.w.); C: ISO + Naringenin (50 mg/kg b.w.); D: ISO + Naringenin (100 mg/kg b.w.); E: ISO + Propranolol (10 mg/kg b.w.). Acidophilic sarcoplasm (red arrows), vesicular nuclei (brown arrow), blood capillaries (black arrow), interstitial cells with elongated nuclei (green heads), interstitial edema (star), cytolysis of myocytes (yellow arrows), areas of discontinuation (triangle), thick fibers (K), thin fibers (T), and muscle fibers (MF).

by inflammation. Age-related endothelial dysfunction has been linked to increased levels of pro-inflammatory cytokines such as IL-10, TGF-β1, and VEGF as well as lower levels of cardiac p-Akt, mostly through the activation of the NF-κB pathway[42]. The expression of genes involved in cell adhesion, proliferation, inflammation, redox status, and tissue-specific enzymes is regulated by the transcription factor VEGF. It is found in all cell types and plays a crucial part in the development of cardiovascular disease by encouraging the transcription of genes that are associated with inflammation, adhesion, and oxidative stress[42]. Our results proved the anti-inflammatory activity of naringenin *via* depletion of IL-10, TGF-β1, and VEGF as well as induced p-Akt elevation. The present results are in line with several articles that proved the biological properties of flavonoids, including antioxidant, anticancer, and anti-inflammatory[41]. Additionally, persistent low-grade inflammation in and around the affected tissue or organ results in tissue damage and genetic abnormalities that lead to chronic diseases including cancer, diabetes, cardiovascular disease, autoimmune disease, and neurological disorders. Patients demand safer, less harmful, and more affordable therapeutic choices because the effects of current treatments of many chronic disorders can frequently be larger than those of the conditions themselves. For thousands of years, flavonoids and their derivatives have been used to treat a range of human diseases, and their use is still prevalent today.

The development of myocardial damage is influenced by oxidative stress and lipid peroxidation. In the present study, cardiac SOD level was decreased significantly whereas cardiac MDA level was significantly elevated in ISO-treated rats as compared with the normal group. ISO has been proven to enhance cardiac contraction and oxygen demand, which results in electron leakage in the respiratory chain. These liberated electrons combine with molecular oxygen to form ROS, which further oxidizes cell components such as proteins, DNA, and membrane lipids, causing direct damage to the myocardium. Soliman *et al.* also found that antioxidant enzyme levels were considerably lower in ISO-treated rats, but MDA level was higher[41].

Flavanones vary from other flavonoids because they have a chiral carbon at the C2 position and no substitution at the C3 position[35]. Flavanones have high antioxidant action, which is important for free radical scavenging and metal ion chelating actions and is dependent on the amount and structure of functional hydroxyl groups[41]. The 7-OH, 4′-OH, and 5-OH groups, as well as the 4(C=O) carbonyl group on the C ring and the 5-OH group on the A ring[40], are responsible for naringenin's antioxidant action, which is weaker than that of other flavonoids due to the lack of the C2=C3 double bond[43].

Our results also showed a dramatic increase in cardiac *NLRP3* and *mRNA-208a* genes in rats treated with ISO in comparison to normal rats. Circulating levels of the NLRP3 inflammasome may be linked to cardiac function and rehospitalization in individuals with dilated cardiomyopathy. The NLRP3 inflammasome is activated during several cardiac disorders, such as myocardial infarction, aortic valve diseases, myocarditis, ischemia/reperfusion injury and hypertension[44]. Addressing NLRP3 inflammasome activation and concomitant pyroptosis may be a potential therapeutic strategy given the ineffectiveness of dilated cardiomyopathy therapy.

These investigations found that NLRP3 could control the formation of ROS, which matched our findings. Thus, NLRP3 may have a dual function in various cell types, both upstream and downstream of ROS, to modulate cellular signaling processes. In the current study, ROS and NLRP3 are also involved in this circular reaction relationship; overexpression of NLRP3 increased the inflammatory response, which then increased ROS production in response to ISO stimulation; knocking down NLRP3 decreased the inflammatory response, which then decreased ROS production in response to ISO stimulation.

Additionally, the NLRP3 level in ISO-rats treated with naringenin was dramatically decreased. This result was consistent with the result of Kim *et al.*[44], where showed that naringenin improved cardiac function in ISO-induced dilated cardiomyopathy rats.

Importantly, an increase in cardiac *miR-208a* gene expression is found to be associated with the development of cardiac lesions in ISO-treated rats and may be an important biomarker of cardiac damage compared to cTnI.

In a previous study, naringenin was used for the treatment of severe acute pancreatitis. However, the underlying processes by which naringenin performed its pharmacological activities still need further research[45]. In histopathological analysis, ISO caused alterations in cellular structure and architecture with increased neutrophil infiltration and cardiac tissue injury in rats. Our results were consistent with those of Jain *et al.*[45] who observed the same histological alteration. Treatment with naringenin alleviated inflammatory lesions with edema and dramatically reduced neutrophil infiltration, thus mitigating ISO-induced cardiac injury.

In summary, the present study found that naringenin improves ISO-induced cardiac infraction by decreasing cardiac cytokines, MDA levels and *mRNA-208a* gene expression and suppressing NLRP3 inflammasome activation. Therefore, naringenin may be a potential therapeutic agent for cardiac injury. Future research is required to determine the mechanisms of action of naringenin, which will pave the way for therapeutic applications.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Funding

The authors received no extramural funding for the study.

Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

The experimental plan for the study was influenced by all authors. MAH was responsible for experimental design and animal grouping. Biochemical, molecular and histological evaluations were conducted by AE and TH. ELISA and PCR examinations were done by AAA and SAE. The protocol, the first version of the article, research analysis, and literature searches were all performed collaboratively by all authors.

References

[1] Namkhah Z, Naeini F, Mahdi Rezayat S, Mehdi Yaseri, Mansouri S, Javad Hosseinzadeh-Attar M. Does naringenin supplementation improve lipid profile, severity of hepatic steatosis and probability of liver fibrosis in overweight/obese patients with NAFLD? A randomised, double-blind, placebo-controlled, clinical trial. *Int J Clin Pract* 2021; **75**(11): e14852. doi: 10.1111/ijcp.14852.

[2] Naeini F, Namkhah Z, Tutunchi H, Rezayat SM, Mansouri S, Yaseri M, et al. Effects of naringenin supplementation on cardiovascular risk factors in overweight/obese patients with nonalcoholic fatty liver disease: A pilot double-blind, placebo-controlled, randomized clinical trial. *Eur J Gastroenterol Hepatol* 2022; **34**(3): 345-353.

[3] Li Y, He B, Zhang C, He Y, Xia T, Zeng C. Naringenin attenuates isoprenaline-induced cardiac hypertrophy by suppressing oxidative stress through the AMPK/NOX2/MAPK signaling pathway. *Nutrients* 2023; **15**(6): 1340. doi: 10.3390/nu15061340.

[4] Chen X, Wan W, Ran Q, Ye T, Sun Y, Liu Z, et al. Pinocembrin mediates antiarrhythmic effects in rats with isoproterenol-induced cardiac remodeling. *Eur J Pharmacol* 2022; **920**: 174799. doi: 10.1016/j.ejphar.2022.

[5] Hoff R, Koh CK. Isoproterenol induced insulin resistance leading to diabetic ketoacidosis in type 1 diabetes mellitus. *Case Rep Endocrinol* 2018; **2018**. doi: 10.1155/2018/4328954.

[6] Baraka SA, Tolba MF, Elsherbini DA, El-Naga RN, Awad AS, El-Demerdash E. Rosuvastatin and low-dose carvedilol combination protects against isoprenaline-induced myocardial infarction in rats: Role of PI3K/Akt/Nrf2/HO-1 signalling. *Clin Exp Pharmacol Physiol* 2021; **48**(10): 1358-1370.

[7] Yue M, Shao L, Cheng J, Fan Y, Cai X, Li H, et al. Prostaglandin E_2 accelerated recovery of chemotherapy-induced intestinal damage by increasing expression of cyclin D. *Exp Cell Res* 2020; **388**(2). doi: 10.1016/j.yexcr.2020.111819.

[8] Hu JN, Yang JY, Jiang S, Zhang J, Liu Z, Hou JG, et al. Panax quinquefolium saponins protect against cisplatin evoked intestinal injury *via* ROS-mediated multiple mechanisms. *Phytomedicine* 2021; **82**. doi: 10.1016/j.phymed.2020.153446.

[9] Fang Z, Liu Z, Tao B, Jiang X. Engeletin mediates antiarrhythmic effects in mice with isoproterenol-induced cardiac remodeling. *Biomed Pharmacother* 2023; **161**. doi: 10.1016/j.biopha.2023.

[10] Shanmugam G, Challa AK, Litovsky SH, Devarajan A, Wang D, Jones DP, et al. Enhanced Keap1-Nrf2 signaling protects the myocardium from isoproterenol-induced pathological remodeling in mice. *Redox Biol* 2019; **27**. doi: 10.1016/j.redox.2019.101212.

[11] Naeini F, Namkhah Z, Tutunchi H, Rezayat SM, Mansouri S, Jazayeri-Tehrani SA, et al. Effects of naringenin supplementation in overweight/obese patients with non-alcoholic fatty liver disease: Study protocol for a randomized double-blind clinical trial. *Trials* 2021; **22**(1): 801. doi: 10.1186/s13063-021-05784-7.

[12] López-Almada G, Domínguez-Avila JA, Mejía-León ME, Robles-Sánchez M, González-Aguilar GA, Salazar-López NJ. Could naringenin participate as a regulator of obesity and satiety? *Molecules* 2023; **28**(3): 1450. doi: 10.3390/molecules28031450.

[13] Li M, Liu Y, Weigmann B. Biodegradable polymeric nanoparticles loaded with flavonoids: A promising therapy for inflammatory bowel disease. *Int J Mol Sci* 2023; **24**(5). doi: 10.3390/ijms24054454.

[14] Joshi R, Laddha AP, Kulkarni YA, Wairkar S. Improved performance of naringenin herbosomes over naringenin in streptozotocin-induced diabetic rats: *In vitro* and *in vivo* evaluation. *Asian Pac J Trop Biomed* 2021; **11**(9): 385-393.

[15] Joshi R, Kulkarni YA, Wairkar S. Pharmacokinetic, pharmacodynamic and formulations aspects of naringenin: An update. *Life Sci* 2018; **215**: 43-56.

[16] Zobeiri M, Belwal T, Parvizi F, Naseri R, Farzaei MH, Nabavi SF, et al. Naringenin and its nano-formulations for fatty liver: Cellular modes of action and clinical perspective. *Curr Pharm Biotechnol* 2018; **19**: 196-205.

[17] Massaro L, Raguzzini A, Aiello P, Valencia DV. The potential role of naringin and naringenin as nutraceuticals against metabolic syndrome. *Endocr Metab Immune Disord Drug Targets* 2023; **23**(4): 428-445.

[18] Memariani Z, Abbas SQ, Ul Hassan SS, Ahmadi A, Chabra A. Naringin and naringenin as anticancer agents and adjuvants in cancer combination therapy: Efficacy and molecular mechanisms of action, a comprehensive narrative review. *Pharmacol Res* 2021; **171**. doi: 10.1016/j.phrs.2020.105264.

[19] Rauf A, Shariati MA, Imran M, Bashir K, Khan SA, Mitra S, et al. Comprehensive review on naringenin and naringin polyphenols as a potent anticancer agent. *Environ Sci Pollut Res Int* 2022; **29**(21): 31025-31041.

[20] Prakash O, Singh R, Singh N, Usmani S, Arif M, Kumar R, et al. Anticancer potential of naringenin, biosynthesis, molecular target, and structural perspectives. *Mini Rev Med Chem* 2022; **22**(5): 758-769.

[21] Duda-Madej A, Stecko J, Sobieraj J, Szymańska N, Kozłowska J. Naringenin and its derivatives-health-promoting phytobiotic against

resistant bacteria and fungi in humans. *Antibiotics (Basel)* 2022; **11**(11): 1628. doi: 10.3390/antibiotics11111628.

[22]Kalam MN, Rasool MF, Rehman AU, Ahmed N. Clinical pharmacokinetics of propranolol hydrochloride: A review. *Curr Drug Metab* 2020; **21**(2): 89-105.

[23]Szpunar M, Vanderloo LM, Bruijns BA, Truelove S, Burke SM, Gilliland J, et al. Children and parents' perspectives of the impact of the COVID-19 pandemic on Ontario children's physical activity, play, and sport behaviours. *BMC Public Health* 2021; **21**: 2271.

[24]Takechi T, Kumokawa T, Kato R, Higuchi T, Kaneko T, Ieiri I. Population pharmacokinetics and pharmacodynamics of oral propranolol in pediatric patients with infantile hemangioma. *J Clin Pharmacol* 2018; **58**(10): 1361-1370.

[25]Del Frari L, Léauté-Labrèze C, Guibaud L, Barbarot S, Lacour JP, Chaumont C, et al. Propranolol pharmacokinetics in infants treated for Infantile Hemangiomas requiring systemic therapy: Modeling and dosing regimen recommendations. *Pharmacol Res Perspect* 2018; **6**(3). doi: 10.1002/prp2.399.

[26]Khan V, Sharma S, Bhandari U, Ali SM, Haque SE. Raspberry ketone protects against isoproterenol-induced myocardial infarction in rats. *Life Sci* 2018; **194**: 205-212.

[27]Hermenean A, Ardelean A, Stan M, Hadaruga N, Mihali CV, Costache M, et al. Antioxidant and hepatoprotective effects of naringenin and its β-cyclodextrin formulation in mice intoxicated with carbon tetrachloride: A comparative study. *J Med Food* 2014; **17**(6): 670-677.

[28]Kakkar P, Das B, Visvanathan P. A modified spectrophotometric assay of SOD. *Indian J Biochem Biophys* 1984; **21**: 130-132.

[29]Bancroft GD, Steven A. *Theory and practice of histological technique.* 4th ed. Churchill Livingstone, New York; 1983, p. 99-112.

[30]Dhaibar HA, Kamberov L, Carroll NG, Amatya S, Cosic D, Gomez-Torres O, et al. Exposure to stress alters cardiac gene expression and exacerbates myocardial ischemic injury in the female murine heart. *Int J Mol Sci* 2023; **24**(13). doi: 10.3390/ijms241310994.

[31]Elneklawi MS, Mohamed ZN, Hussein MA, Mohamad EA. STEN ameliorates VEGF gene expression by improving XBP1/mRNA-21/mRNA-330 signalling pathways in cisplatin-induced uterus injury in rats. *J Drug Deliv Sci Technol* 2023; **87**. 10.1016/j.jddst.2023.104760.

[32]Mohamad EA, Mohamed ZN, Hussein MA, Elneklawi MS. GANE can improve lung fibrosis by reducing inflammation *via* promoting p38MAPK/TGF-β1/NF-κB signaling pathway downregulation. *ACS Omega* 2022; **7**(3): 3109-3120.

[33]Mosaad YO, Hussein MA, Ateyya H, Mohamed AH, Ali AA, Ramadan Youssuf A, et al. Vanin 1 gene role in modulation of iNOS/MCP-1/TGF-β1 signaling pathway in obese diabetic patients. *J Inflamm Res* 2022; **15**: 6745-6759.

[34]Elgizawy HA, Ali AA, Hussein MA. Resveratrol: Isolation, and its nanostructured lipid carriers, inhibits cell proliferation, induces cell apoptosis in certain human cell lines carcinoma and exerts protective effect against paraquat-induced hepatotoxicity. *J Med Food* 2021; **24**(1): 89-100.

[35]Nagarajan A, Doss VA. *L*-carvone attenuates myocardial injury and dyslipidemia in rats with isoproterenol-induced cardiac hypertrophy. *Asian Pac J Trop Biomed* 2023; **13**(1): 17-25.

[36]Wang XP, Wang PP, Bai JQ, Gao S, Wang YH, Quan LN, et al. Investigating the effects and possible mechanisms of danshen- honghua herb pair on acute myocardial ischemia induced by isoproterenol in rats. *Biomed Pharmacother* 2019; **118**. doi: 10.1016/j.biopha.2019.109268.

[37]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. doi: 10.21037/atm.2020.02.93.

[38]Zhang YF, Meng NN, Li HZ, Wen YJ, Liu JT, Zhang CL, et al. Effect of naringin on oxidative stress and endoplasmic reticulum stress in diabetic cardiomyopathy. *Zhongguo Zhong Yao Za Zhi* 2018; **43**(3): 596-602.

[39]Wang Y, Zhang X, Gao L. Cortistatin exerts antiproliferation and antimigration effects in vascular smooth muscle cells stimulated by Ang Ⅱ through suppressing ERK1/2, p38 MAPK, JNK and ERK5 signaling pathways. *Ann Transl Med* 2019; **7**: 561. doi: 10.21037/atm.2019.09.45.

[40]El Gizawy HA, Abo-Salem HM, Ali AA, Hussein MA. Phenolic profiling and therapeutic potential of certain isolated compounds from *Parkia roxburghii* against AChE activity as well as GABAAα5, GSK-3β, and p38α MAP-kinase genes. *ACS Omega* 2021; **6**(31): 20492-20511.

[41]Soliman SM, Mosallam S, Mamdouh MA, Hussein MA, Abd El-Halim SM. Design and optimization of cranberry extract loaded bile salt augmented liposomes for targeting of MCP-1/STAT3/VEGF signaling pathway in DMN-intoxicated liver in rats. *Drug Deliv* 2022; **29**(1): 427-439.

[42]Hussein MA, Ismail NEM, Mohamed AH, Borik RM, Ali AA, Mosaad YO. Plasma phospholipids: A promising simple biochemical parameter to evaluate COVID-19 infection severity. *Bioinform Biol Insights* 2021; **15**. doi: 10.1177/11779322211055891.

[43]Borik RM, Hussein MA. Synthesis, molecular docking, biological potentials and structure activity relationship of new quinazoline and quinazoline-4-one derivatives. *Asian J Chem* 2021; **33**(2): 423-438.

[44]Kim SR, Lee SG, Kim SH, Kim JH, Choi E, Cho W, et al. SGLT2 inhibition modulates NLRP3 inflammasome activity *via* ketones and insulin in diabetes with cardiovascular disease. *Nat Commun* 2020; **11**: 2127.

[45]Jain PG, Mahajan UB, Shinde SD, Surana SJ. Cardioprotective role of FA against isoproterenol induced cardiac toxicity. *Mol Biol Rep* 2018; **45**(5): 1357-1365.

Publisher's note