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Cardioprotective Effect of Naringenin against Myocardial Ischemia-Reperfusion Injury via Alteration of Apoptotic Signaling Pathway

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Myocardial ischemia, I/R injury, Naringenin, Anti-oxidant, Anti-inflammatory

ABSTRACT

Ischemic heart disease (IHD) is a common multiple cardiovascular disease (CVD) in clinical settings. It is a major contributor to mortality and morbidity worldwide and causes a serious threat to human life and health. Naringenin, a flavonoid possesses the potent antioxidant potential and it is proposed to be useful in the treatment of CVD. In this experimental study, we aimed to scrutinize the cardio-protective effect of naringenin against the I/R induced myocardial injury and elucidate the possible mechanism of action. *In vitro* studies, the H9c2 cardiomyocytes cells were treated with naringenin or without naringenin and then subjected to I/R, respectively. At end of the experimental study, the rats were anesthetized and blood samples were collected to scrutinize the various parameters such as creatine kinase myocardial band (CK-MB), *high-density lipoprotein* (HDL), lactate dehydrogenase (LDH), creatine, troponin-T (TRT), cholesterol, C - reactive protein (CRP), and concentration of mitochondrial enzymes viz., Ca^{2+}, Na^{+} and K^{+} ions were estimated in blood. Heart tissue was also isolated for caspase-3 activity. Our result showed that naringenin pretreatment significantly increased cardiac dysfunction via scavenged free radicals and a reduction of inflammatory reaction. Dose-dependent treatment of naringenin significantly altered the CK-MB, HDL, LDL, creatinine, cholesterol, CRP, Ca^{2+}, Na^{+} and K^{+}, respectively. A significant alleviating change in these biochemical parameters along with caspase-3 activity was noticed. Thus, in our study, we determined that I/R induced cardiac remodeling can be successfully mitigated by naringenin.

INTRODUCTION

Myocardial ischemic heart disease is a common cardiovascular disease (CVD) and it is having a higher incidence of mortality and morbidity worldwide. It is well documented that acute myocardial ischemia having a higher rate of death. Due to sudden death, myocardial ischemia heart disease leads the numerous clinical dysfunctions including cardiac myocyte hypertrophy (Seki and Fishbein, 2014; Müller-Nordhorn and Willich, 2016). Myocardial ischemia-reperfusion is definite as tissue damage that arises when early and fast coronary flow returns to the heart tissue after the ischemia, which commonly augments the myocardial damage. During the myocardial ischemia, the organ showed a lack of nutrients and oxygen, which cause oxidative stress, apoptotic cell death and inflammatory reaction, and these circumstances will additionally deteriorate during the reperfusion (Hausenloy and Yellon, 2013; Hashmi and Al-Salam, 2015). During the I/R, it induces the structural injury and dysfunction or

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metabolic disturbance. These alterations are induced via the retrieval of blood flow to the ischemic myocardium. I/R induced ischemic myocardium is very complicated, and inflammation, ERS and oxidative stress are the main targets for the current research. Previous research suggests that the excessive generation of ROS was observed during the myocardial ischemic, which further induce oxidative stress and cause harmful changes (Kalogeris *et al*., 2012; Raedschelders *et al*., 2012). Various researches have been explored to scrutinize the cardioprotective drug. It is well known that myocardial ischemia-reperfusion is an effectual treatment for acute myocardial ischemia, which can decrease the size of myocardial infarction and release the symptoms of heart disease such as heart failure (Sanada *et al*., 2011; Hausenloy and Yellon, 2013). Various signaling pathway dysregulationhas been induced the myocardial ischemia-reperfusion. Signaling dysregulation leading the differentiation, cell proliferation, apoptosis and autophagy under various pathological and physiological conditions may be closely related to cardiac injury (Burke and Virmani, 2007; Kalogeris *et al*., 2012; Ibáñez *et al*., 2015).

It is well proofed that flavonoids are the major phytoconstituents in various Chinese medicine and used to treat various disease especially CVD (Zeng *et al*., 2017). Epidemiological investigations have demonstrated that a regular intake of flavonoids can decrease the risk of myocardial infarction and decrease mortality due to CVD (Zeng *et al*., 2017; Zhu *et al*., 2018).

Naringenin, a well-known flavonoid, that can exert a wide range of pharmacological effects including anti-cancer, anti-inflammatory, anti-diabetic, anti-viral, free radical scavenging effect, anti-tubercular and other biological activities (Smeriglio *et al*., 2017). Due to its anti-oxidant and anti-inflammatory nature, it contributes to the cardio-protective effect. Previous studies suggest that the naringenin not only reduce the peroxidase activity of cytochrome C with lipid peroxidative or free radical generation and dioleylcardiolipin, but also circulated the oxidative enzymes activities and over-generation of ROS for enhancing the I/R injury (Agouni *et al*., 2011; Smeriglio *et al*., 2017). Moreover, the exact mechanism of action is not fully elucidated. Consequently, in the current experimental study, we aimed to scrutinize the protective effect of naringenin on I/R injury and explore the possible mechanism of action.

MATERIALS AND METHODS

Chemicals

Naringenin(95%) was purchased from the Sigma Aldrich, U.S.A. Radio-immunoprecipitation assay (RIPA) lysis buffer (Beyotime Institute of Biotechnology, Beijing, China), 2,3,5-Triphenyl tetrazolium chloride (TTC) was purchased from the Sigma, Beijing, China. Catalase (CAT), superoxide dismutase (SOD), Malondialdehyde (MDA), glutathione (GSH), serum creatinine kinase MB (CK-MB) assay kits were purchased from the Jiancheng Bioengineering Institute, Nanjing, China. The primary antibodies against the Akt, phosphorylation of Akt (p-Akt), Bax, Bcl-2, cleaved caspase-3, caspase-3, caspase-7, caspase-9, PI3K, phosphorylation of PI3K (p-PI3K), XIAP, HrtA2/Omi, β-actin and GAPDH were purchased from the Cell Signaling Technology, Inc (CST, USA).

In vitro *experimental study*

Cell culture

H9c2 (clonal cell lines of BDIX rat embryonic heart tissue) cells were procured from the Cell Bank of Chinese Academy of Sciences, Shanghai, China. The cell was cultured according to using the previously reported method with minor modification. Briefly, the cells H9c2 were cultured into the high glucose DMEM supplemented with L-glutamine (2 mM), streptomycin/penicillin (1% v/v) and fetal bovine serum (10% v/v) and maintained into the humidified incubator in a CO_2 (5%) atmosphere at 37°C. The cells were plated at a suitable density and propagate for 24-36 h before the experimental study (Shu *et al*., 2019).

The H9c2 cells were divided into the following groups presented in Figure 1. Briefly, high glucose DMEM medium replaced the glucose-free DMEM to ischemia and further cells were incubated for 6h at 37°C in the environment of H_2 (5%), N_2 (5%) and CO_2 (5%) in an anaerobic glove box. After that, the medium replaced with the high glucose medium and kept for 16h to mimic reperfusion. In the treatment group, different doses of naringenin (1.25, 2.5, 5, 10, 20, 40 μM) for 12h before the H/R. Nevertheless, different doses of naringenin pre-treated group, pretreated with the H9c2 cells for 12h (Shu *et al*., 2019).

Cell viability assay

MTT assay was used for the estimation of cell viability via using the previously published method with minor modification (Shu *et al*., 2019). The H9c2 cells were plated in the 96 well plates at a density of 5×10^4 cells per well with DMEM (100 μL) medium. After that MTT solution (20 μL) was mixed in each well and again incubated at 37°C for 4h. The MTT medium was replaced with the DMSO (150 mL each well) and added the formazan crystals and finally estimated the absorbance at 570 nm on a microplate reader (Shu *et al*., 2019).

Antioxidant parameters

ROS detection kit was used for the estimation of

intracellular ROS production via using the manufacture instruction (Shu *et al*., 2019). Briefly, H9c2 cells were cultured into the 6 well plates for 24-36h. After that, the cells were propagated, washed with washing buffer and finally centrifuged at 1g rpm for 5 min at room temperature and finally supernatant was discarded. After that, the cells were re-suspended and incubated with 5-(and-6)-carboxy-20,70-dichlorodihydrofluorescein diacetate (25 µM) at 37°C for 30 min in a dark room and washed with the PBS buffer. After the various treatments, the supernatant was utilized for the estimation of LDH level and then the cells were collected and centrifuged for 5 min at 1 g rpm at 4°C. Further, the supernatant was used for the estimation of SOD, MDA, GSH-Px and CAT level via using the manufacture instruction (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

In vivo *study*

Experimental animal

Sprague-Dawley (SD) rats (7-9 weeks; 250-300 g) were used for the current experimental study. The rats were received from the departmental animal house and kept in the standard experimental condition (22±5°C; 70±2 relative humidity 12/12 h dark/light cycle). The rats have received the standard pellet diet with water *ad libitum*. The current experimental study was approved from the Institutional animal ethical.

Experimental protocol

The rats were divided into 5 groups and each group contains the 10 rats. The group as follows: Group I, sham control received the normal saline (10 mL/kg); Group II, I/R control; Group III, I/R control + naringenin (2.5 mg/kg); Group IV, I/R control + naringenin (5 mg/kg); Group V, I/R control + naringenin (10 mg/kg).

Surgical protocol

For the surgical protocol, all the experimental rats were subjected to endotracheal and tracheotomy intubation after the anesthesia. For the ventilation performed, inserting the polyethylene-50 (PE-50) tube via trachea and link with the animal ventilator with a tidal volume (1.2 L/kg) and maintain the breath rate (70/min) and maintain the body temperature 37°C via using the heating pad. MP150 data acquisition was used for the determination of heart rate and ECG. All these parameters were monitored to the baseline, Ischemia (ischemia for 45 min) and reperfusion (reperfusion for 4 h) (Yin *et al*., 2013).

Myocardial infarction assessment

For the myocardial infarction, Evans Blue dye (2%) was injected into the aorta on experimental rats and all the rats were euthanized via cervical dislocation. After the euthanized, the heart tissues were immediately removed, washed with saline and frozen and finally cut into the approximately 5 cross-sections from the apex junction site. TTC method was used to identify the myocardial infarct area into the left ventricle (Hu *et al*., 2016).

Biochemical parameter

For the estimation of the biochemical parameter, the heart tissues were homogenized with phosphate buffer (pH=7.4) and finally centrifuged for 15 min at 5g rpm. Collect the supernatant for the estimation of the reactive oxygen species (ROS) level. The serum parameters were scrutinized for aspartate aminotransferase (AST), creatine kinase-MB (CK-MB) and lactic acid dehydrogenase (LDH) via using the manufacture instruction's (NJJC Bio, Nanjing, China).

Antioxidant parameter

Antioxidant parameters such as malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) were estimated via using the standard kits following the manufacture instruction (NJJC Bio, Nanjing, China).

Lipid parameter

Lipid parameters such as triglycerides total cholesterol (TC), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were estimated via using the standard kits following the manufacture instruction (NJJC Bio, Nanjing, China).

Na^+, K^+ and Ca^{2+}

Na, K and Ca were estimated via using the previous method with minor modification (Abdalla *et al*., 2016).

Membrane-bound ATPases

Membrane-bound enzymes such as Ca^{++}ATPase, Mg^{++}ATPase and Na^+K^+ATPase were estimated via using the previously reported method with minor modification (Gandhi *et al*., 2013).

Caspase-3 activity

Caspase-3 activity and C - reactive protein (CRP) were determined by using the standard kits following the manufacture instruction (NJJC Bio, Nanjing, China).

Statistical analysis

For the current experimental study, the whole result presented as mean ± standard error (SD). One-way analysis of variance (ANOVA) was used for scrutinizing the statistical analysis, followed via Turkey's test method using the Graph Pad Prism 7 software. Values were considered statistically significant when $P < 0.05$.

RESULTS

Effect of naringenin against H/R induced H9c2 cell injury

MTT assay was performed for the estimation of the potential effect of naringenin on cell viability (Fig. 1). In the current study, we firstly investigated the cell proliferation or general toxicity of naringenin. In the current experimental study, the cells were treated with the naringenin in a dose-dependent manner. Figure 1A exhibited that the cell viability had no significant difference was observed between groups. After that, the capability of naringenin to reduce the H/R induced H9c2 cell injury was scrutinized. Figure 1B exhibited the considerably down-regulation of cell viability and naringenin treatment significantly maintained the cell viability at approximately (85%) and a similar trend was observed in the LDH release (Fig. 2). Moreover, in the current experimental study, we used the 10 µM naringenin for subsequent in-vitro experimental study.

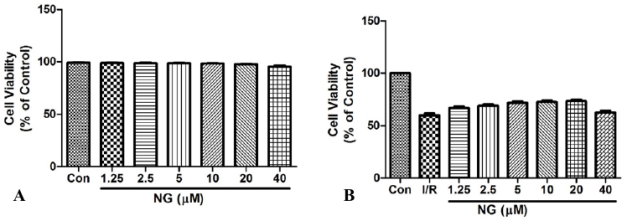

Fig. 1. Effect of naringenin on the cell viability by H/R in H9c2 cardiomyocytes. A) H9c2 cells were treated with various concentration of naringenin for 24 h and MTT assay was used for the estimation of cell viability. B) H9c2 cells were treated with for 12 h with naringenin and then exposed to 6h of hypoxia. The data presented as means±SD from three independent experiments.

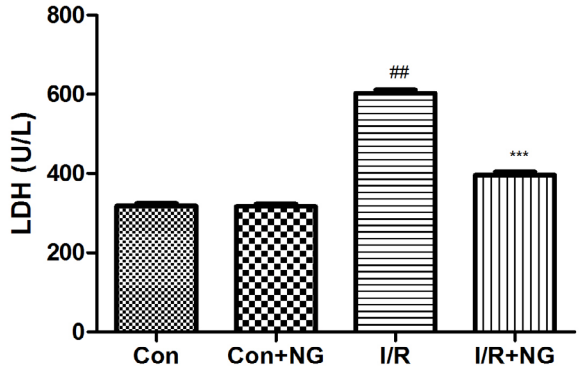

Fig. 2. Effect of naringenin on the LDH release by H/R in H9c2 cardiomyocytes. The data presented as means±SD from three independent experiments. ##P < 0.01 versus control group; *P < 0.05, **P < 0.01 versus H/R group.

Antioxidant activity (in vitro)

It is well documented that oxidative stress plays an important role in the expansion of the cardiac disease. Oxidative stress initiates apoptosis or injury. Due to this point, we have investigated the antioxidant effect of naringenin against the H/R induced oxidative damage during the cardiac disease. H/R induced oxidative stress in the H9c2 cells exhibited the reduce level of CAT (Fig. 3A), GSH-Px (Fig. 3B), SOD (Fig. 3C) and increase the level of MDA (Fig. 3D) and naringenin treatment significantly ameliorated these effects. H/R injured H9c2 cells showed the deposition of ROS and pre-treatment of naringenin significantly reduced the accumulation of ROS (Fig. 4). The result exhibited that the naringenin could be more effectively alleviate the H/R induced oxidative stress injury in H9c2 cells.

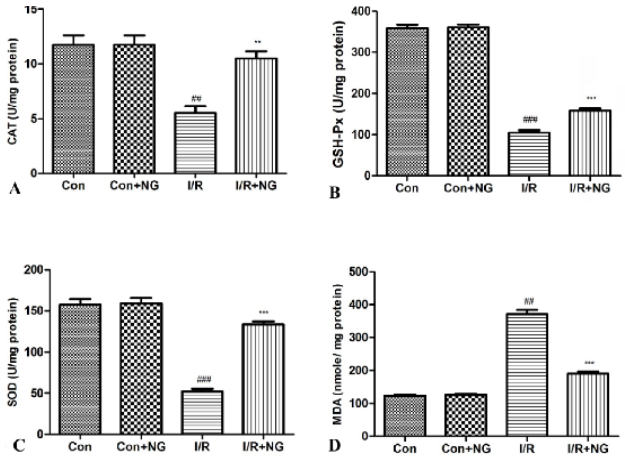

Fig. 3. Effect of naringenin on antioxidant parameter of H/R in H9c2 cardiomyocytes cells. A) CAT, B) GSH-Px, C) SOD and D) MDA. The data presented as means±SD from three independent experiments. ##P < 0.01 versus control group; *P < 0.05, **P < 0.01 versus H/R group.

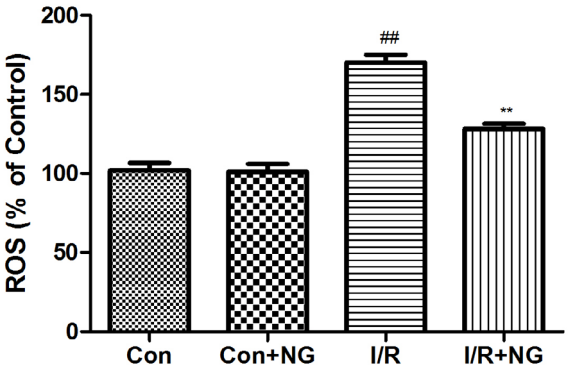

Fig. 4. Effect of naringenin on ROS concentration of H/R in H9c2 cardiomyocytes cells. The data presented as means±SD from three independent experiments. ##P < 0.01 versus control group; *P < 0.05, **P < 0.01 versus H/R group.

Naringenin avert I/R induced myocardial injury

The myocardial enzymes such as CK-MB, LDH and AST were significantly boosted during the I/R induced myocardial injury. I/R induced myocardial damage rodent exhibited the increased level of CK-MB (Fig. 5A), LDH (Fig. 5B) and AST (Fig. 5C) and dose-dependently treatment of naringenin significantly (P<0.001) ameliorated these parameters.

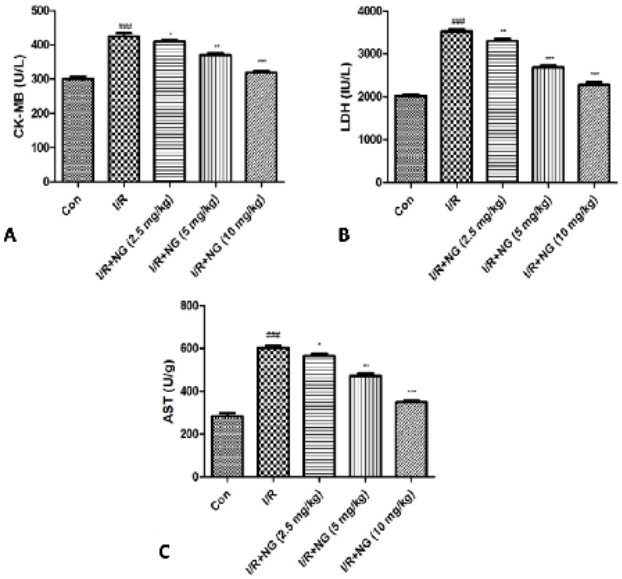

Fig. 5. Effect of naringenin on the biochemical parameter of I/R induced mice. A) CK-MB, B) LDH and C) AST. Dennett's test was used for statistically significance *P<0.05, **P<0.01 and ***P<0.001.

Naringenin avert oxidative damage during the I/R induced myocardial injury

Figure 6 exhibited the effect of naringenin on the antioxidant enzymes. I/R induced myocardial damage rats exhibited the reduce level of MDA (Fig. 6B) and increase the level of SOD (Fig. 6A), CAT (Fig. 6C) and dose-dependently treatment of naringenin significantly (P<0.001) altered the level of antioxidant enzymes.

Naringenin altered lipid parameters

Figure 7 exhibited the effect of naringenin on the lipid parameters of I/R induced myocardial injury. I/R induced myocardial injury rats demonstrated the increased level of total cholesterol, high-density lipoprotein and low-density lipoprotein as compared to normal control. Dose dependently treatment of naringenin significantly (P<0.001) reduced the level of TC, HDL and LDL as compared to the I/R induced myocardial injury rats.

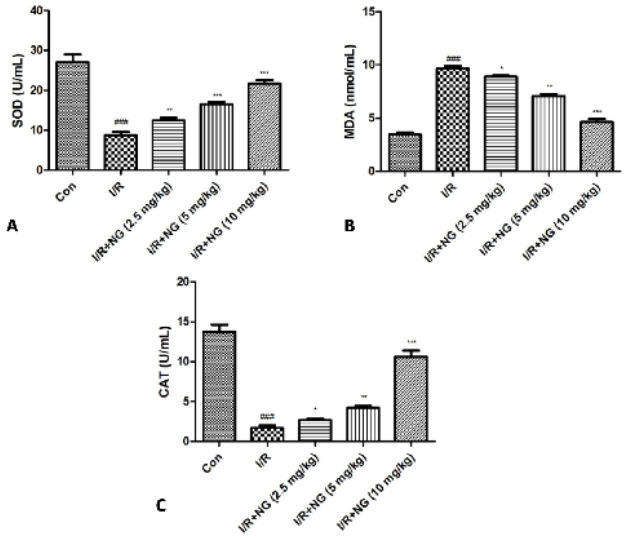

Fig. 6. Effect of naringenin on the antioxidant parameters of I/R induced mice. A) SOD, B) CAT and C) MDA. Dennett's test was used for statistically significance *P<0.05, **P<0.01 and ***P<0.001.

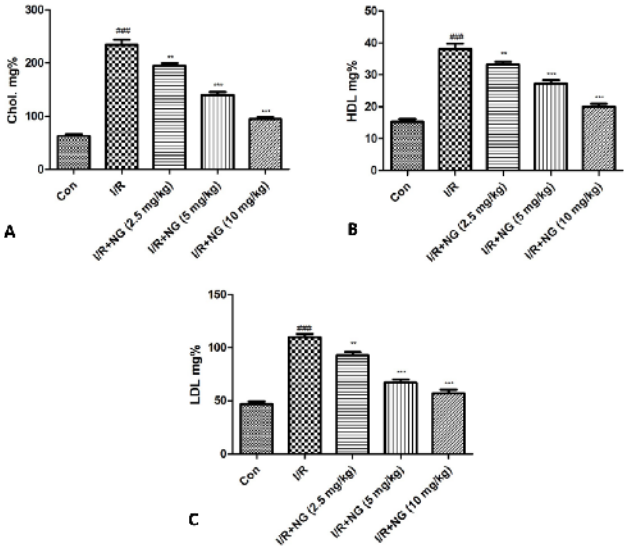

Fig. 7. Effect of naringenin on the lipid parameters of I/R induced mice. A) Cholesterol, B) High density lipoprotein and C) Low density lipoprotein. Dennett's test was used for statistically significance *P<0.05, **P<0.01 and ***P<0.001.

Effect of naringenin on Na, Ca and K level

During the myocardial cardiopathy increase the level of Na, Ca and K in the serum and similar results were obtained in the I/R induced myocardial injury rats. I/R induced group rats showed the increased level of Na, Ca and K and naringenin significantly (P<0.001) reduced the

level almost near to normal control group (Fig. 8).

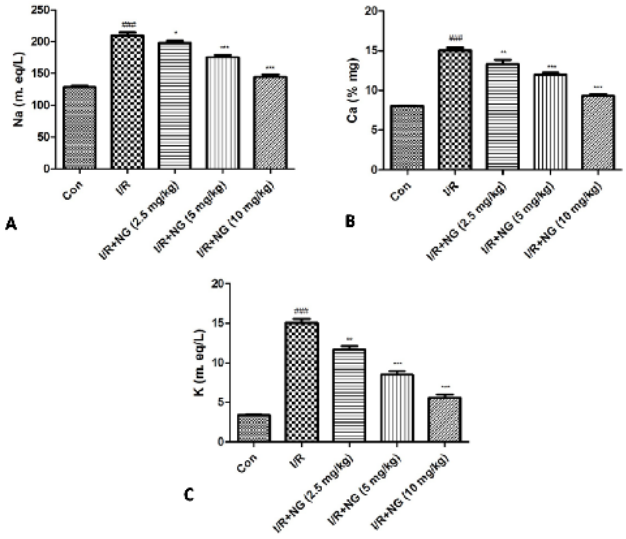

Fig. 8. Effect of naringenin on the Na, Ca and K level of I/R induced mice. A) Na, B) Ca and C) K. Dennett's test was used for statistically significance *P<0.05, **P<0.01 and ***P<0.001.

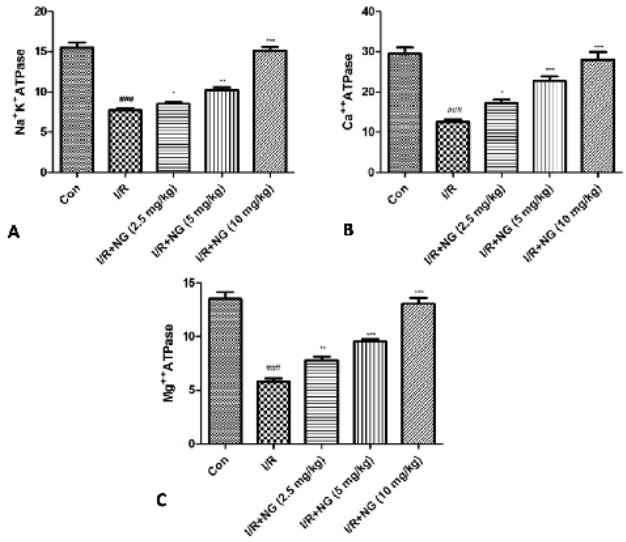

Fig. 9. Effect of naringenin on the membrane-bound enzymes of I/R induced mice. A) Mg++ ATPase, B) Na+K+ ATPase and C) Ca++ ATPase. Dennett's test was used for statistically significance *P<0.05, **P<0.01 and ***P<0.001.

Membrane-bound enzymes

Mg^{++} ATPase, $Na^{+}K^{+}$ ATPase and Ca^{++} ATPase all enzymes are considered as the membrane ATP dependent enzymes. I/R induced group rats exhibited the reduced level of membrane-bound enzymes such as Mg^{++} ATPase, $Na^{+}K^{+}$ ATPase and Ca^{++} ATPase and dose-dependently treatment of naringenin significantly (P<0.001) increased the level of membrane-bound enzymes such as Mg^{++} ATPase, $Na^{+}K^{+}$ ATPase and Ca^{++} ATPase (Fig. 9).

Naringenin avert caspase-3 activity and CRP level

Figure 10 showed the increased level of caspase-3 activity in the I/R induced control group rats. Naringenin significantly (P<0.001) decreased the activity of caspase-3 in a dose-dependent manner.

A similar momentum was observed in the I/R induced myocardial injury rats and concentration-dependent treatment of naringenin significantly (P<0.001) down-regulated the level of CRP (Fig. 11).

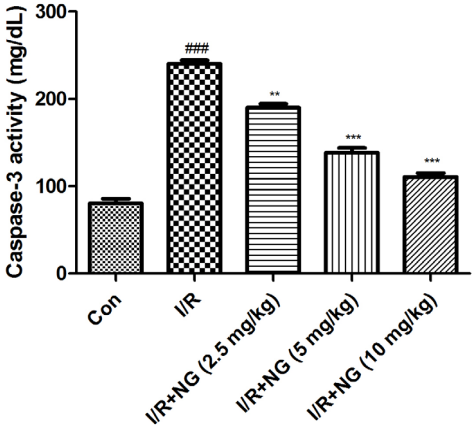

Fig. 10. Effect of naringenin on the caspase-3 marker of I/R induced mice. Dennett's test was used for statistically significance *P<0.05, **P<0.01 and ***P<0.001.

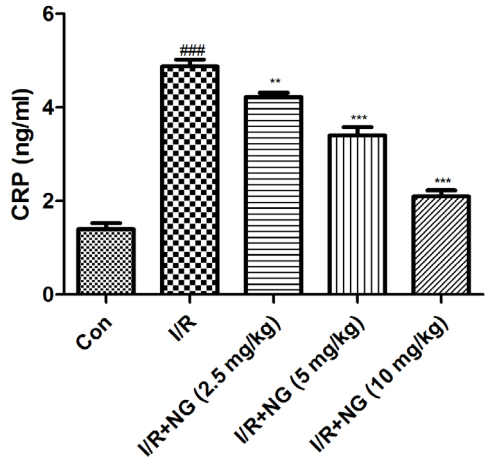

Fig. 11. Effect of naringenin on the CPR level of I/R induced mice. Dennett's test was used for statistically significance *P<0.05, **P<0.01 and ***P<0.001.

DISCUSSION

Ischemic CVD is considered the most common CVD and it predominantly affected human health (Nordlie *et al*., 2005). I/R is a clinical phenomenon that induces the structure injury and metabolic or dysfunction interruption. These alterations are induced via the recovery of blood flow to the ischemic myocardium (Darby *et al*., 2013). The mechanism of I/R induced myocardial injury is not clear, but few of the research suggest that the oxidative stress, inflammatory and apoptosis play an important role during the disease.

It is well documented that flavonoids play a preventive role in the expansion of the CVD. Flavonoids such as naringenin, a well-known Phyto-constituents of various Chinese herbal medicine used in the inflammatory and oxidative stress-induced various diseases via potent anti-apoptotic effects (Zeng *et al*., 2017). Naringenin is a major ingredient of flavonoids and it belongs to the flavanonol subclass. As per the report of the Chinese herbal medicine, various medicinal and edible fruits and vegetables have been reported to contain naringenin (Zygmunt *et al*., 2010). Previous research suggests showed the cardio-protective effect of naringenin against various animal models (Jiang *et al*., 2005; Zygmunt *et al*., 2010). Also, the essential mechanism of naringenin's myocardial protection has still not been fully explained. However, in the current experimental study, we explored the preventive effects of naringenin against the I/R induced myocardial injury and explore the possible mechanism of action.

Previous studies suggest that the reduced level of creatinine assuage the remodelled cardiac cells (Moghimian *et al*., 2013; Anderson *et al*., 2011). Analogously, in the current experimental study, the destruction of the myocardial cell was further controlled via reduce level of creatinine level brought by naringenin. Various researches suggest that the CRP is a significant marker for evaluating the cardiac remodelling and it is well documented that diabetes to be responsible for the cardiac remodeling (Slavíková *et al*., 2007). In the current experimental study, we found that the naringenin prevents cardiac remodelling via down-regulation of the CRP level in a dose-dependent manner. Naringenin also reduced the level of CRP via inhibiting the vascular obstruction in the myocardial cell.

Numerous ions play a significant role in governing the physiology functional nature of cardiac cells (Wright *et al*., 2001). Previous research suggests that basic ions such as Ca^{2+}, Na^{+} and K^{+}directly or indirectly are related to contraction and conduction of assorted phenomenon in myocardiocytes (Shekhawat *et al*., 2009). The alterations in the level of these ions to a significant level have a direct implication on the cardiac structure (Pertoldi *et al*., 2006). Our results demonstrate there is a significant effect of our molecule on K^{+} ion. This molecule decreased the concentration of K^{+} ions in the serum when compared with diseased control. Suggesting its role on K^{+} channels, the release of K^{+}ion from myocardiocytes in the blood serum decreases the cationic concentration of cardiac cells (Wang *et al*., 2012). The decrease in cationic concentration relaxes the myocardial cell giving more relaxation time to cardiac cells and reducing the load of contraction. Another important parameter of evaluating cardiac remodelling is through analyzing the Na^{+} and Ca^{2+} concentrations which may also contribute to cardiac remodelling (Wang *et al*., 2009, 2012). There is no significant alteration in that Ca^{2+} and Na^{+} ions. The channels for these ions are spared by this compound.

It is well proofed that the I/R enhanced all types of blood pressure; mean systolic or diastolic along with heart rate in the pathogenic rodents as compared to normal rats, this trend of I/R induced myocardial injury is well correlated with our experimental study (Plosker and McTavish, 1995). Increase in mean BP and heart rate in diseased rats can be attributed to increase in release of catecholamines and renin, which in turn initiates the formation of Angiotensin II from Angiotensin I (Moghadam *et al*., 2013). CK-MB enzyme is a significant marker for identifying the changes in the remodeled heart, as its normally increased in during the heart disease. In the current experimental study, we observed the increased activity of CK-MB and dose-dependent treatment of naringenin significantly increased the activity of CK-MB. The protective effect may be due to a reduction in myocardial contractility.

It is well documented that HDL is an important parameter of cardiomyopathy and it is directly responsible for the clearance of cholesterol via bile (Villani *et al*., 1991). The clearance of cholesterol significantly decreases the load on cardiomyocytes. In the current experimental study, the level of HDL and cholesterol level considerably increased in I/R induced myocardial injury rats and dose-dependent treatment of naringenin significantly decreased the level of HDL and cholesterol (Villani *et al*., 1991; Sarvari *et al*., 2010). Based on the result, we can say that the HDL and flavonoids correlating.

Previous research suggests that oxidative stress plays an important role in the expansion of various diseases (Malorni *et al*., 1998). During the oxidative stress, observed the imbalance between the free radicals and ROS, which lead to injurious effects on the body. It is well proved that oxidative stress plays an important role in the expansion of I/R injury (Nader *et al*., 2010; Vishnupriya and Padma, 2017). Various research suggests that the ROS are the foremost factors that induce oxidative stress, which in turn

result in cell injury to biological macromolecules, influence the normal physiological state of cells and instigates the oxidative stress reaction. In the current investigation, we observed that the H/R and I/R significantly enhanced ROS levels, induced H9c2 dell damage and pathological changes, and enhanced MDA production (an indicator of LPO) and decreased the level of SOD and CAT (Tang *et al*., 2017; Yu *et al*., 2019). Dose-dependent treatment of naringenin significantly ($P<0.001$) decreased the level of MDA and increased the level of SOD and CAT in the H9c2 and cardiac muscle tissue. Our result revealed that the naringenin considerably reduced the oxidative stress in the H9c2 cells and the cardiac muscle and based on the result; we can conclude that the naringenin reduced the myocardial injury via inhibition of oxidative stress.

Apoptosis of cardiac myocardial injury induced via oxidative stress and inflammation plays an important role in the pathogenesis of CVD, such as ischemic heart disease, heart disease and I/R injury (Zhang *et al*., 1999; Kikuchi *et al*., 2013). Moreover, the preclusion of oxidative stress and inflammation-induced apoptosis may serve as a favorable effect for the treatment of the myocardial injury. It is well documented that apoptosis is regulated via two pathways such as extrinsic and intrinsic cell death pathways, which are correspondingly characterized via caspase and Bcl-2 family (Zhang *et al*., 1999). In this experimental study, we focused on the intrinsic pathway. The intrinsic pathway is arbitrated through the activation of caspase and mitochondrial dysfunction. The last apoptosis pathway is attributed via activation of caspase-12. During the I/R injury, ischemia, particularly combined with the reperfusion, triggers translocation of Bax into the outermost layer of mitochondrial membrane, which is related to the increased level of Bax and decreased level of Bcl-2/Bax ratio (Zhang *et al*., 2017; Xu *et al*., 2019). Among all of the proteins, caspase is the significant endogenous inhibitor of apoptosis. During the apoptosis, XIAP binds with the caspase especially caspase-3, 7 and 9, a process that may be regulated via Smac/Diablo and HtrA2/Omi (both are the inhibitor of XIAP). It has been shown as the increased level of caspase-3 reduces the cardiomyocyte apoptosis and also decreases the myocardial infarct area. In the current experimental study, naringenin significantly reduced the level of caspase-3 and also contribute to its observed anti-apoptotic mode of action.

CONCLUSION

In the current experimental study, we scrutinized the cardio-protective effect of naringenin against the I/R injury and also scrutinized the potential effect against the H9c2 cells (*in-vitro*) via an antioxidant mechanism. Naringenin could be a promising therapeutic drug against myocardial ischemic cardiovascular disease. Moreover, a further detailed investigation is conducted to elucidate the mechanisms involved in the cardioprotective effect of naringenin in detail.

Statement of conflict of interest

The authors have declared no conflict of interest.

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