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Original Article

P-Coumaric Acid Improves Pyroptosis Induced by Myocardial Ischemia-Reperfusion Injury via the SIRT1/NLRP3/NF-κB-p65 Pathways

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ABSTRACT

Background: Myocardial Ischemia-Reperfusion Damage (MI/RI) has been identified as a major risk factor for cardiovascular disease. Reducing oxidative stress, inflammation, and apoptosis has emerged as a key therapeutic strategy for MI/RI. According to reports, p-Coumaric Acid (PCA) is a potent antioxidant with cardioprotective, anti-inflammatory, and anti-apoptotic properties. The potential cardioprotective mechanisms of PCA against MI/RI are still unknown, nevertheless. **Materials and Methods:** Therefore, the anti-inflammatory and anti-apoptotic properties of PCA against MI/RI-induced Sprague-Dawley rats have been the main focus of this investigation. The four groups of rats were MI/R+PCA (10 mg/kg b.w), MI/R+PCA (20 mg/kg bw), MI/R-induced, and sham. To evaluate ECG, toxicity enzymes, oxidative stress, cytokines, histopathology, apoptosis, and pyroptosis, MI/RI was created. The ST segment of the ECG, cardiotoxicity enzymes, oxidative stress, cytokines, apoptosis, pyroptosis, and histopathological changes in MI/RI rats are all reduced dose-dependently by PCA. **Results:** By decreasing p53 and cleaved Caspase-3 levels and increasing the Bcl-2/Bax proportion, PCA has anti-apoptotic effects. PCA suppresses the production of NLRP3, Caspase-1, ASC, p-P65, and P65 proteins, which may attenuate pyroptosis, while activating SIRT1 to reduce cardiomyocyte apoptosis. **Conclusion:** These findings recognized that PCA exhibited anti-apoptotic, anti-inflammatory, and anti-pyroptosis activity correlated to the modulation of the Sirt1/NLRP3/NF-κB signaling.

Keywords: P-Coumaric acid, Myocardial Ischemia-reperfusion Injury, Oxidative Stress, Inflammation, Apoptosis, Pyroptosis, SIRT1/NLRP3/NF-κB-p65 Pathway.

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INTRODUCTION

Myocardial Infarction (MI) is a prominent Cardiovascular Disease (CVD) globally.[1,2] Drug thrombolysis or interventional surgery for appropriate refurbishment of blood perfusion is crucial to saving the MI patient's life.[2,3] Myocardial Ischemia-Reperfusion Injury (MI/RI) blocks the myocardial bloodstream over a slight era, and coronary artery recanalization occurs within an assured time. Though the usual blood source is reinstated after reperfusion, the tissue hurts serious pathological injury.[4] MI/RI is a complex pathophysiological development and its pathogenesis remains obscure. Accumulating reports have unveiled that when blood reperfusion occurs, frequently released Reactive Oxygen Species (ROS) trigger myocardial cell apoptosis and autophagy, hence severely affecting the diagnosis of MI patients.[5,6] MI/RI is a nonspecific impairment produced by ischemia, ROS, and pro-inflammatory cytokines throughout reperfusion, which ultimately leads to cardiomyocyte apoptosis and adverse cardiovascular effects. Moreover, extreme Oxidative Stress (OS) can trigger cardiomyocyte apoptosis after RI, with irretrievable consequences, which results in cardiac dysfunction and structural mutilation.[5-7] Therefore, the latent mechanisms of MI/RI require being promoted and decrypted to regulate possible beneficial approaches.

Sirtuin 1 (SIRT1), involves a crucial action in numerous cellular pathways, including aging, stress, metabolism, inflammation, and apoptosis.[8,9] Silent information regulator Sirtuin 1 (SIRT1) exerts a probable anti-oxidative stress action in vascular endothelial cells.[10] The preceding document has exposed that SIRT1 is not only a vital regulator in diabetic patients and MI/RI, but is also extremely sensitive to the cell's redox state.[11] Furthermore, both quercetin and vitamin-D can inverse ROS-activated cellular injury by triggering SIRT1. Presently, few studies investigated that SIRT1 involves a significant controlling part in CVD by the stimulation of Forkhead box O (FOXO) to reduce the apoptotic molecules and OS for cardioprotection.[12] Among them, tumor protein p53 (p53) is a transcription factor controlled by SIRT1,

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which can intensely affect cell survival and death. Acetylated p53 can stimulate ROS-prompted apoptosis, while SIRT1 can reduce deacetylation of the acetylated p53, hence shifting the process of cell apoptosis.[13] Thus, screening effective medications that can enhance SIRT1 which attenuates myocardial apoptosis after MI/RI displays key medical implications in MI treatment.

A newly identified programmed inflammatory cell death process is known as pyroptosis, whose features are plasma membrane pore formation and discharge of pro-inflammatory extracellular mediators. The NLR is a type of configuration identified receptor, and its family associates mostly contribute to the formation of the inflammasome, concerning them NOD-Like Receptor protein 3 (NLRP3) NLRP3 is the utmost investigated. Previous findings have recognized that inflammasome NLRP3, a vital signal sensor stage comprising NLRP3, Caspase-1, apoptosis-associated speck-like protein containing a CARD (ASC), and p65, which are necessary for pyroptosis.[14,15] Former research has pointed out the central part of pyroptosis in CVD and established that extreme pyroptosis might hurt cardiomyocytes.[16-18] Outcomes from animal trials confirmed that repression of p65, NLRP3, Caspase-1, and ASC may perhaps avert myocardial damage.[18,19] Moreover, it was described that MI/R-induced NLRP3 inflammasome was mostly conveyed in non-cardiomyocytes, consisting of fibroblasts, permeated leukocytes within the myocardium, and endothelial cells.[19,20] Thus, involvement in NLRP3-facilitated pyroptosis can diminish inflammatory complements and auxiliary employ myocardial defense. However, no effective treatment for averting reperfusion damage is accessible to date;[21] hence, there is an urgent necessity to explore tactics to alleviate MI/RI.

p-Coumaric Acid (PCA) is a phenolic acid, also known as 4-hydroxycinnamic acid; plentifully existing in many fruits, cereals, and vegetables.[22] With immunomodulatory, antioxidant, anti-cancer, anti-inflammatory, anti-mutagenic, anti-diabetic, anti-hyperlipidemic, anti-microbial, and antiviral qualities,[22] PCA exhibits significant therapeutic benefits.[23-25] According to a previous study, PCA reduced inflammation in Raw 264.7 cells by modifying the NF-κB/MAPK pathway.[26] It has previously been demonstrated that PCA treatment reduced hepatic fibrosis by inhibiting TLR4/NF-κB signaling, oxidative damage, and ER stress.[27] Also, PCA decreases doxorubicin-activated OS.[28] prevents cerebral damage triggered by cerebral ischemia-reperfusion,[29] and controls the expression of Indoleamine 2,3-Dioxygenase (IDO) in immune mouse cells.[30] Hyperexpression of IDO stimulates the divergence of dTHP-1 cells into M2 macrophages. Thus, PCA switches the polarity of macrophages by controlling the IDO manifestation, thus alleviating MI/RI mutilation produced by macrophage-facilitated inflammation.[31] PCA exhibited anti-lipid peroxidative, antioxidant, and anti-apoptotic effects in isoproterenol-induced MI rats by inhibiting OS.[32] However, the action of PCA on MI/RI in attenuating OS, inflammation, and apoptosis has not garnered much consideration. Hence, we intended to determine the potential cardioprotective effects of PCA against OS, inflammation, and apoptosis on MI/RI-induced rat model for the first time and to elucidate the underlying anti-apoptotic and anti-inflammatory actions through the regulation of SIRT1/NLRP3/NF-κB signaling pathway.

The current investigations' scope included examining the mechanism by which p-coumaric acid induces apoptosis, triggering inflammatory marker proteins through SIRT1-induced MLRP3 and NF-kB responses in cerebral ischemia by pyroptosis.

MATERIALS AND METHODS

Chemicals

p-Coumaric acid (PCA, purity>98%) was acquired from Solarbio (Beijing, China). Sodium Pentobarbital, Dimethyl Sulfoxides (DMSO), Triphenyl Tetrazolium Chloride (TTC), Evans blue dye, and Sodium Dodecyl Sulfate (SDS) were attained by Ruicong Ltd (Shanghai, China). Biochemical assay kits were delivered by Ese-Bio (Shanghai, China). ELISA assay kits were procured from Elabscience Biotechnology (Wuhan, China). The antibodies (p53, Bcl-2, Bax, Cleaved caspase 3, Sirt 1, NLRP3, Caspase 1, ASC, p-P65, P65, and β-actin) and secondary antibody for western blot assay were obtained from ZSGB-bio (Beijing, China). Biochemicals and solvents analytical grades were used.

Investigational animals

Adult male *Sprague Dawley* (SD) rats having 200-220 g were used for the trials. These were conserved in sterile polypropylene coops under specific research environments. Rats were fed a fixed pellet diet with ad libitum and an unceasing 12 hr light/dark phase. Animals have adapted one week before the tests. All treatment protocols were conducted as per the Institutional animal ethics guidelines Pengzhou People's Hospital Medical Ethics Committee 2022 Kollen Trial No. (01).

Experimental plan

Rats were separated into 4 sets containing 6 animals each. Group 1-Sham; Group 2- MI/R rats; Group 3- MI/RI+PCA (10 mg/kg b.w); and Group 4- MI/RI+PCA (20 mg/kg bw). PCA was dissolved with DMSO to form the essential dose and intragastrically administered to the 3rd and 4th groups for four weeks. The MI/R surgery was performed when the last PCA gastric intubation. The sham and MI/R-developed groups were administered a similar quantity of DMSO.

MI/RI induction in rats

The MI/RI rat model was performed based on a formerly described approach.[33] Intraperitoneal administration of sodium pentobarbital (60 mg/kg b.w) anesthetized into the rats was conducted and ventilation was sustained with a 15 mL/kg tidal volume per air ambient and 70 times/min respiratory rate. The left coronary artery ligation was accomplished with a 6-0 suture

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above a tube, 3-4 mm width, and around 1.5-2 mm deepness. The effective implementation of the typical MI/RI was established by the myocardial pallor at the distant end of the knotted point and an incessant enhancement of the segment ST. After ischemia, the left coronary artery ligation was maintained for 30 min. ST-segment promotion on the ECG indicated a successful ligation. Reperfusion persisted for a day after the ligature was left undisturbed. The ischemia myocardium's transformation from a pale to a reddish condition and the segment ST's depletion served as indicators of reperfusion. With the exception of the left coronary artery ligation, the same procedures worked for the Sham group. MI/RI was the same as the other operating processes.

Estimation of ST

The previously described method[34] with an Oscilloscope was used to measure the ST segment, after the J point 0.04~0.08s a horizontal line was prepared, and then the additional line of the segment TP as the standard or the line linking the initial points of QRS complexes of two nearby heartbeats as the standard to decide whether there was segment ST alteration. When the segment ST is higher, it is determined from the superior edge of the standard to the superior edge of the ST segment, and once it is depressed, it is determined from the inferior edge of the baseline to the inferior superiority of the ST segment.

Preparation of serum

Following the ECG analysis, 6 mL of blood were drawn from the rat's stomach aorta, which had been left at 25°C for an hour. It was then separated into an Eppendorf tube at -80°C for further biochemical examination after being centrifuged for 10 min at 3500 rpm/min.

Determination of toxicity biomarkers in serum

The kits supplied by Ese-Bio (Shanghai, China) were used to measure the activity of marker enzymes such Lactate Dehydrogenase (LDH), Creatine Kinase (CK), and aspartate Aminotransferase (AST) in the serum in accordance with their protocols.

Analysis of OS markers and antioxidants in serum

Nitric Oxide (NO), Glutathione (GSH), Malondialdehyde (MDA), and Superoxide Dismutase (SOD) concentrations were determined using commercial kits (Ese-Bio, Shanghai, China) in accordance with their protocols.

Estimation of inflammatory mediators

The intensities of pro-inflammatory cytokines, particularly Interleukin-1 beta (IL-1β), Interleukin-18 (IL-18), Interleukin-6 (IL-6), and Tumor Necrosis Factor alpha (TNF-α) in serum and heart tissue extracts were estimated by an Enzyme-Linked Immunosorbent Assay (ELISA) kit provided by Elabscience Biotechnology (Wuhan, China), as per their instructions.

TTC staining of cardiac tissue

Toward the end of the trial, the left coronary artery was ligated and then infused with the Evans blue dye through the femoral vein to notice the risk areas. Immediately, the rat's heart was isolated and the blood stains were removed with normal cold saline, unrelated cells beyond the ligature were detached with a shaver blade, and the heart was at -20°C freezer for half an hour and then eliminated from the heart apex. The tissue was cut into a 1-mm-thick section, which is preserved in a TTC (1%) solution. This is permitted to stand for 15 min in a water zone under dark conditions at 37°C, and it is reversed every 5 min. The myocardial damage was determined by computer software to characterize the comparative size of the myocardial infarct zone.[34]

Western blotting analysis

The proteins were isolated from rat's heart tissues with an ice-cold solution of lysis buffer and the protein quantity was estimated by employing an assay kit (Ese-Bio, Shanghai, China). When SDS-PAGE electrophoresis was accomplished, the gel was relocated to a PVDF film. The PVDF film was wrapped with skimmed milk (5%) and the diluent (1:1000) of the primary antibody (p53, Bcl-2, Bax, Cleaved caspase 3, Sirt1, Caspase 1, NLRP3, ASC, p-P65, P65, and β-actin) and kept at 4°C for one day. Then, the diluent (1:5000) of the HRP-conjugated secondary antibody with skimmed milk powder (5%) and treated to the PVDF film, and then incubated. Lastly, an ECL chemiluminescence device was employed for the membrane development. Quantified the protein bands through densitometry with software Image J and homogeneous to β-actin expression.

Statistical study

The statistical examination was conducted by the software version 9.0 Graphpad Prism and expressed as mean±SD. It was working to perform an analysis of variance (ANOVA) and subsequently Duncan's assessment. Student T-test has been executed and $p<0.05$ was deliberated as statistically significant.

RESULTS

Impact of PCA on cardiotoxicity marker enzymes in serum

To explore the cardioprotective efficiency of PCA on MI/RI-induced rats, consider the results of PCA on ST segment and cardiotoxicity marker enzymes in serum including CK, LDH, and AST. The serum ECG-ST segment, CK, LDH, and AST were markedly elevated ($p<0.05$) in the serum of the MI/R rats versus the sham group. Treatment with PCA (10 and 20 mg/kg b.w) considerably decreased ($p<0.05$) the ST segment, CK, LDH, and AST in a quantity-related mode (Figure 1).

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PCA alleviates MI/R-stimulated OS in the serum

To inspect the antioxidant action of PCA on MI/R-induced rats, study the outcomes of PCA on OS markers such as MDA, NO, SOD, and GSH in serum. MI/RI-induced rats revealed a higher (p<0.05) level of NO and MDA, whereas considerably diminished (p<0.05) antioxidant status such as SOD activity and GSH level against the sham group. Administration of PCA (10 and 20 mg/kg b.w) in MI/RI rats uncovered a substantial decrease (p<0.05) in NO and MDA levels, while enhanced (p<0.05) the SOD activity and GSH level in an amount-related way (Figure 2). These data proposed that PCA could remarkably attenuate MI/RI and ameliorate antioxidant function.

PCA diminishes MI/R-stimulated inflammatory mediators

Inflammatory cytokines comprising IL-18, IL-6, IL-1β, and TNF-α in the serum and heart were also determined. MI/R-induced rats exhibited considerably elevated (p<0.05) concentrations of IL-18, IL-6, IL-1β, and TNF-α levels when related to the sham. In contrast, PCA (10 and 20 mg/kg b.w) administered MI/RI-induced rats markedly reduced (p<0.05) the quantity of IL-18, IL-6, IL-1β, and TNF-α levels in a dosage-related manner (Figures 3 and 4). These findings unveiled the anti-inflammatory activity of PCA against MI/RI in rat models.

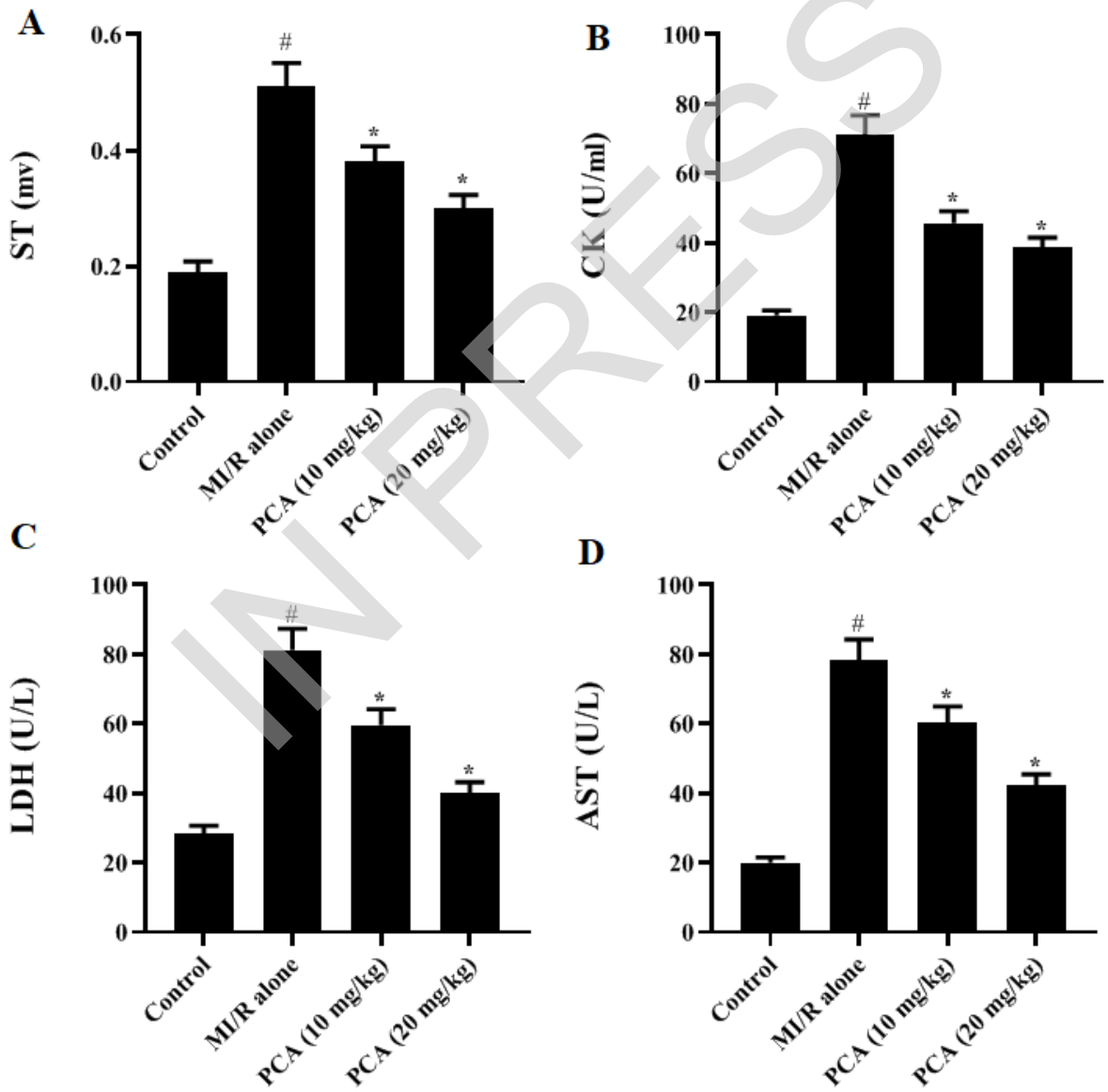


Figure 1: Effect of PCA on MI/R-induced ECG variations and cardiotoxicity marker enzymes in serum. (A) ST (B) CK (C) LDH (D) AST levels. The findings were exhibited as mean±SD of 6 observations and the statistical significance is considered as $^{\#,*}p$<0.05 against the sham group.

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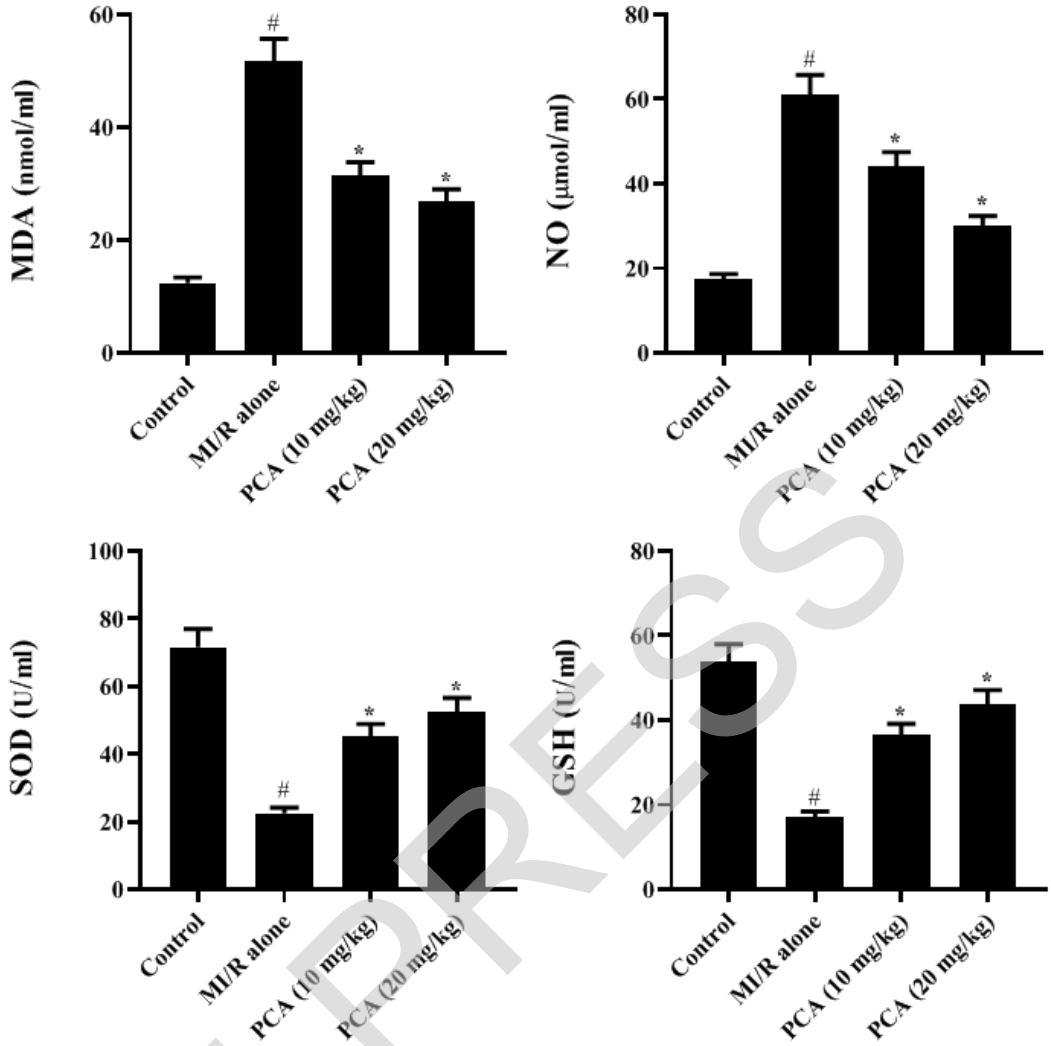


Figure 2: PCA alleviates MI/R-stimulated cardiac OS and anitoxidant in the serum. The findings were exhibited as mean±SD of 6 observations and the statistical significance is considered as #,*p<0.05 against the sham group.

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PCA reduces cardiac histopathological variations and pathology score

Typical myocardial cells observed in the sham group included normal cell morphology, precisely arranged myocardial fibers with clear lines, obviously visible nuclei, and no aberrations in myocardial structure. In MI/RI rats presenting loosened and disordered myocardium, multiple breaks, degeneration, edema, and necrosis, as well as enhanced pathological score. Treatment with PCA (10 and 20 mg/kg b.w) in the MI/RI-developed rats reduced pathological scores, myocardial injuries, disorganized structure, edema, and necrosis against MI/RI model rats in a concentration-related way (Figure 5). These data displayed that PCA could attenuate myocardial damage developed by MI/RI in the experimental rats

PCA suppresses MI/RI-induced cardiac apoptosis

As presented in Figure 6, MI/RI-stimulated rats exhibited the increased protein expression of p53 acetylation, cleaved caspase-3, and Bax however, declined the Bcl-2 in counter to the sham group. Administration of PCA (10 and 20 mg/kg b.w) markedly elevated Bcl-2 expression, while diminishing the protein expression of p53 acetylation, cleaved caspase-3, and Bax in a quantity-related way. The western blot analysis data established that PCA could attenuate cardiac apoptosis in MI/RI-induced rats.

PCA regulates the Sirt1/NLRP3/NF-κB pathway in MI/RI-induced rats

MI/RI-developed rats displayed significantly alleviated (p<0.05) cardiac protein level of Sirt 1, however, upregulated (p<0.05) the NLRP3, ASC, Caspase-1, and NF-κBp65 protein expression versus the sham group. Administration of PCA (10 and 20 mg/kg

b.w) substantially raised ($p<0.05$) the protein expression of Sirt1, while remarkably reducing ($p<0.05$) the NLRP3, ASC, Caspase-1, and NF-κBp65 proteins in a dosage-related manner (Figure 7). These data established that PCA could exert cardiac protection via reducing OS, inflammation, and apoptosis via the modulation of Sirt1/NLRP3/NF-κB signaling in MI/RI-induced rats.

DISCUSSION

Despite significant developments in CVD treatment and medications, this disorder remains the foremost cause of global death, which is mainly caused by coronary heart disease.[1,2] After acute MI, immediately restored the blood flow to the ischemic myocardium, however, this refurbishment may lead to further impediments and the worsening of myocardial injury, known as MI/RI.[4] The MI/RI decreases the cardiomyocyte's action and vascular endothelial cells, which damages cardiac task, and elevates the infarct zone, thus increasing the chance of the patient's death.[5,6] Clinically, statins, ion exchange inhibitors, angiotensin receptor antagonists, and antioxidants are classically used for MI/R remedy. Nevertheless, diverse patients have fluctuating prognostic outcomes because of adverse cardiovascular events and prohibitive costs. Moreover, OS, inflammation, calcium overload, mitochondrial dysfunction, apoptosis, autophagy, and even epigenetic variations may participate in MI/RI.[5-7] Accordingly, numerous studies have been focused on identifying innovative natural antioxidants as drugs that target myocardial survival after MI/RI.[26,27] In this respect, it is imperative to establish an antioxidative, anti-inflammatory, and anti-apoptotic natural compound against the MI/RI animal model with features similar to those of human illness. Therefore, exploring a phenolic acid,

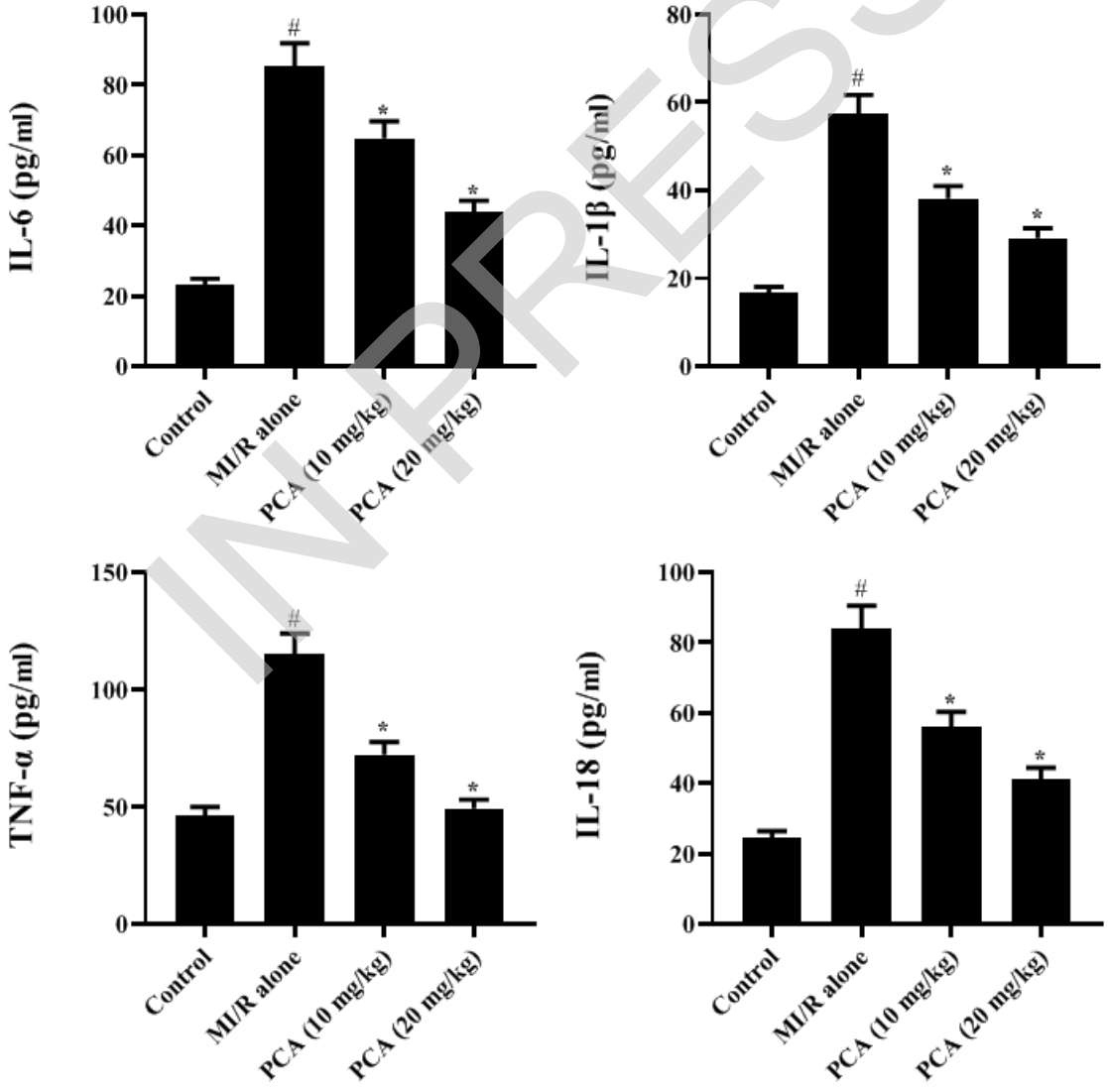


Figure 3: PCA attenuates MI/R-stimulated serum inflammatory mediators. The inflammatory mediators including IL-6, IL-1β, TNF-α, and IL-18 were measured by ELISA kit. The findings were exhibited as mean±SD of 6 observations and the statistical significance is considered as $^{\#,*}p<0.05$ against the sham group.

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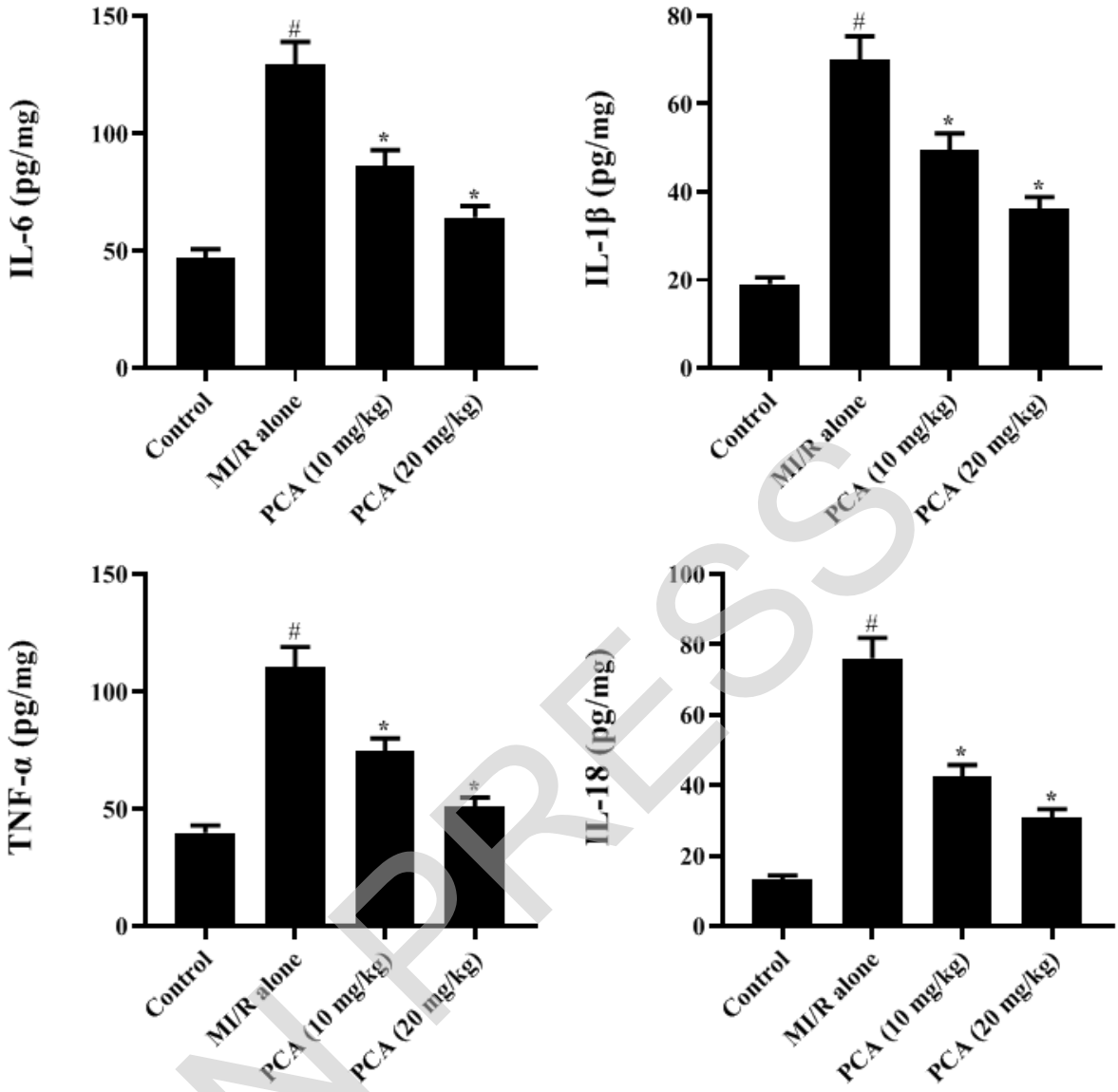


Figure 4: PCA alleviates MI/R-induced inflammatory mediators in the heart. The inflammatory mediators including IL-6, IL-1β, TNF-α, and IL-18 were measured by ELISA kit. The findings were exhibited as mean±SD of 6 observations and the statistical significance is considered as $^{\#,*}p<0.05$ against the sham group.

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PCA to reduce MI/RI and associated pathological symptoms may have high therapeutic value.

PCA is abundantly present in numerous fruits, cereals, and vegetables.[22] PCA exhibits several pharmacological activities comprising immunomodulatory, anti-oxidant, anti-cancer, anti-mutagenic, anti-inflammatory, anti-diabetic, anti-hyperlipidemic, anti-microbial, and anti-viral activities.[23-25] In this research, we found that PCA expressively diminished the ST segment of the ECG, myocardial toxicity marker enzymes, cardiac pathological changes, OS markers, and inflammatory cytokines, apoptotic proteins through increased Sirt1 protein level expression by reducing the NLRP3/NF-κB cascade; and lessened myocardial inflammatory and apoptotic reactions, thus effectively improving MI/RI in rats. After myocardial infarction deteriorates myocardial cell membranes, subsequently many cellular enzymes are discharged into the blood, thus increasing the levels of cardiotoxicity enzymes such as AST, LDH, and CK. These isoenzymes are generally employedas enzymatic indicators for diagnosing MI and have more specificity.[35] Herein, PCA-decreased AST, LDH, and CK are considered markers of MI, and once the cardiomyocytes are broken, they are discharged in high amounts into the bloodstream in MI/RI, generating a sequence of following pathological reactions.[36,37] The outcomes of this report presented a substantial increase in serum levels of LDH, AST, and CK in the MI/RI-developed group. Nevertheless, the pre-treatment with PCA repressed the elevated markers of MI, including AST, LDH, and CK. Previously, it has been reported that PCA exerts cardioprotective activity via antioxidative, anti-lipid peroxidative, and anti-apoptotic properties.[31,32] These

outcomes emphasized that the cardioprotective effects of PCA in the MI/RI should be affected by conserving the cardiomyocyte membrane strength and stability through declining the enzymatic biomarker's release to alleviate myocardial damage in MI/R-induced rats.

Oxidative Stress (OS) is also a vital feature associated with MI/RI.[5-7] The concentration of MDA could disclose the lipid peroxidation severity. The free radical-rummaging enzyme SOD turns into the antioxidant defense mechanism by foraging reactive oxygen radicals.[38] Herein, the pre-treatment with PCA (10 and 20 mg/kg bw) could decrease MI/R-stimulated elevation of the NO and MDA levels and enhance the action of SOD and GSH in a quantity-reliant way. These effects mentioned that PCA could defend the cardiomyocytes from oxidative damage by elevating the endogenous antioxidant enzyme level. According to prior investigations, along with our experiments, it is probable that PCA unveiled an anti-oxidative stress activity by controlling the liberation of ROS directly, which sequentially applies the following anti-apoptotic and anti-inflammatory actions.[26,27,32] Histopathology of cardiac tissue assessment of this current study also supported the above findings that MI/RI rats presented loosened and disordered myocardium, multiple breaks, degeneration, edema, and necrosis, as well as enhanced pathological scores. Treatment with PCA (10 and 20 mg/kg b.w) in the MI/RI-developed rats reduced pathological scores, myocardial injuries, disorganized structure, edema, and necrosis against MI/RI model rats in a concentration-related way. MI/RI frequently roots permanent variations in cardiac structural organization and task, in addition to damage and cardiomyocyte demise, which include numerous complicated pathological developments, comprising inflammatory reaction,[39] oxidative stress,[40] intracellular calcium overload, and apoptosis.[39-41]

Our study showed increased cytokines levels, as well as the protein expressions of p53, cleaved Caspase-3, and Bax were considerably elevated, whereas Bcl-2 expressions were pointedly mitigated in MI/RI-induced rats. Administration of PCA (10 and 20 mg/kg bw) unveiled dosage-dependently reduced the inflammatory cytokines, p53, cleaved Caspase-3, and Bax act in the occurrence of cardiomyocyte apoptosis while elevating the anti-apoptotic protein Bcl-2 level. Formerly, several researchers investigated that PCA exhibited anti-inflammatory[26,27] and anti-apoptotic[32] activities. These data mentioned that PCA showed anti-inflammatory and anti-apoptotic effects against MI/RI-stimulated rats. Our study exposed that PCA pre-treatment exerts a cardioprotective effect on the MI/R-developed rats, which is accomplished by triggering the SIRT1 signaling pathway. An earlier document has revealed that deacetylation of p53 by up-regulating SIRT1 expression can guard cardiomyocytes

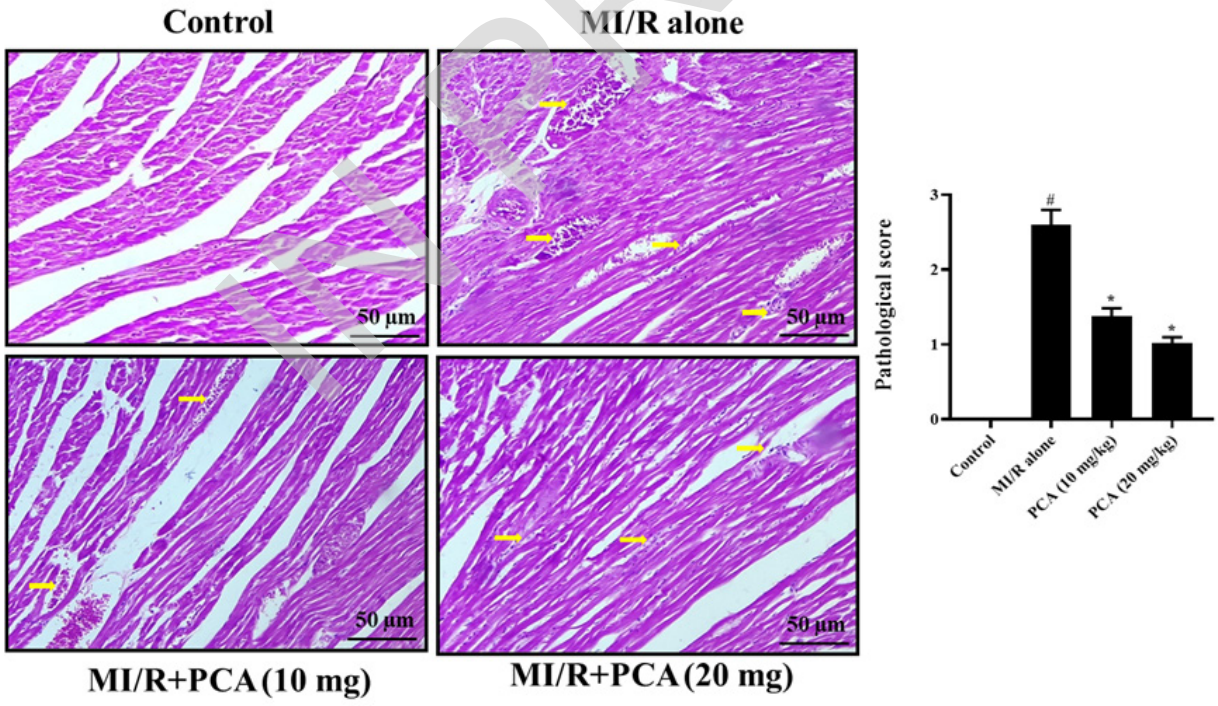


Figure 5: PCA reduces MI/R-induced cardiac histopathological variations and pathology scores. Scale bar = 50 μm. Group-I) Sham rats presenting characteristic myocardial cell morphology, precisely arranged myocardial fibers with clear lines, obviously visible nuclei, and no aberrations in myocardial structure. Group-II) In MI/RI rats displaying loosened and disordered myocardium, multiple breaks, degeneration, edema, and necrosis, as well as enhanced pathological score. Group-III and IV) Treatment with PCA (10 and 20 mg/kg bw) in the MI/R-developed rats reduced pathological scores, myocardial injuries, disorganized structure, edema, and necrosis against MI/R model rats in a quantity-related way. The findings were exhibited as mean±SD of 6 observations and the statistical significance is considered as $^{\#,*}p<0.05$ against the sham group.

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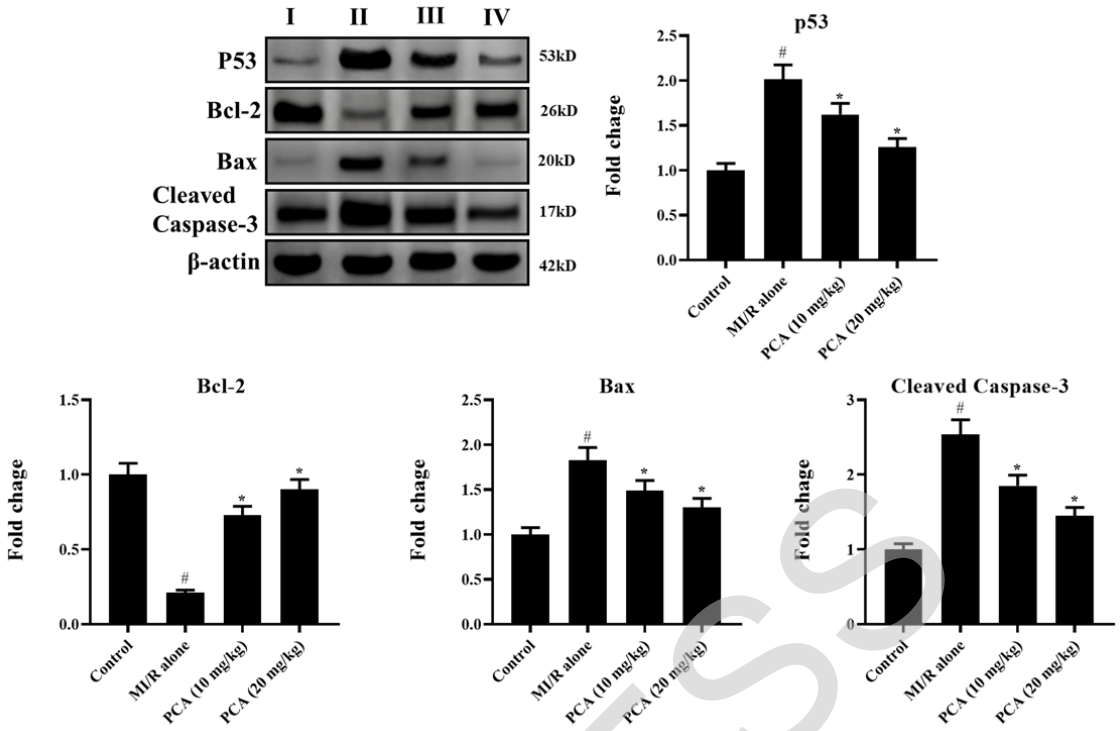


Figure 6: PCA suppresses MI/R-induced cardiac apoptosis in rats. The intensities of p53, Bcl-2, Bax, and cleaved caspase-3 proteins were evaluated by western blot. The findings were exhibited as mean±SD of 6 observations and the statistical significance is considered as $^{\#,*}p<0.05$ against the sham group.

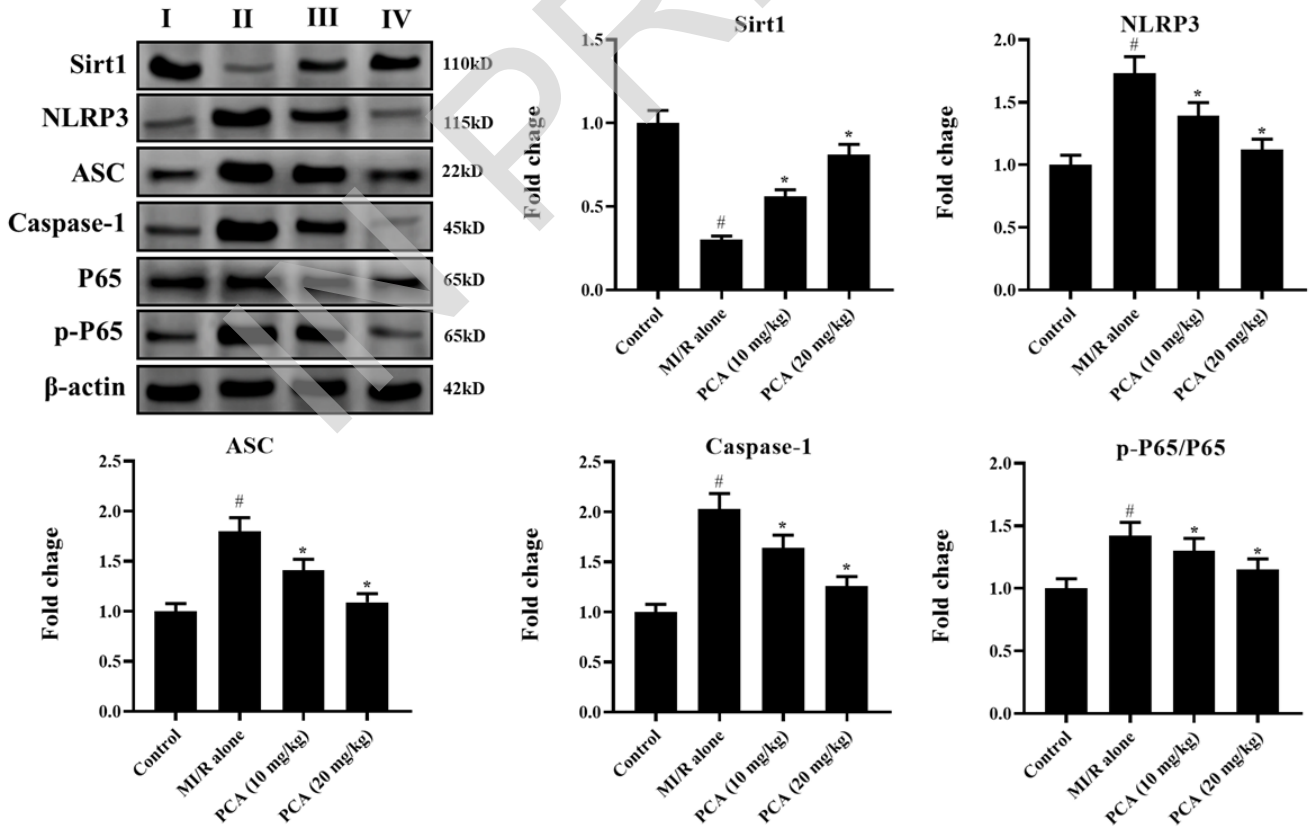


Figure 7: PCA regulates the SIRT1/NLRP3/NF-κB pathway in MI/R-induced rats. The intensities of SIRT1, NLRP3, Caspase-1, ASC, p-P65, and P65 protein expressions were evaluated by Western blot assay. The findings were exhibited as mean±SD of 6 observations and the statistical significance is considered as $^{\#,*}p<0.05$ against the sham group.

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from doxorubicin-stimulated cell apoptosis.[42] In the current work, PCA administration can stimulate SIRT1 and repress the p53 acetylation. In contrast, PCA could increase Bcl-2, while alleviating Bax and cleaved caspase-3 the protein expressions. All these findings point out that PCA can lessen MI/RI-stimulated cardiomyocyte apoptosis through the deacetylation of p53 by activating SIRT1.

To understand the role of the Sirt1/NLRP3/NF-κB signalingin MI/R-induced rat's cardiac tissues, the levels of the Sirt1/NLRP3/NF-κB pathway were studied. SIRT1 controls aging, cell proliferation, differentiation, metabolism, and apoptosis over the deacetylation of histones and non-histones.[8,9] Pyroptosis is one of the extremely pro-inflammatory lytic methods of programmed cell demise, which is characterized by increased plasma membrane permeabilization, cell swelling, and discharge of pro-inflammatory mediators.[14,15] The preceding study has well established that cleaved caspase-1, is efficiently triggered NLRP3 inflammasome, and the latter facilitates cell pyroptosis.[43] Furthermore, cleaved caspase-1 similarly incisions inactive pro-IL-18 and pro-IL-1β to form mature IL-18 and IL-1β. Hence, NLRP3 inflammasome, IL-1β, GSDMD, and IL-18 are all renowned as pyroptosis-correlated molecules.[44,45] In this report, the MI/R-developed rats showed reduced Sirt1 protein levels and augmented protein levels of NLRP3, Casepase-1, ASC, p-P65, and P65 in heart tissues, related to the group sham. While, the Sirt1 expression was elevated and the protein intensities of Casepase-1, NLRP3, ASC, IL-1β, p-p65, and p-65 were alleviated in the cardiac tissue of PCA groups against the model group. An earlier report revealed that PCA exerted an anti-inflammatory activity on Raw 264.7 cells via modulating the NF-κB/MAPK pathway.[26] Previously, it has been established that PCA administration attenuated hepatic fibrosis by suppressing OS, ER stress, and TLR4/NF-κB signalling.[27] PCA displayed a protective effect on MI/R-stimulated rats and the mechanism was connected to the Sirt1/NLRP3/NF-κB signaling, however, its latent mechanism required to be promoted and studied to treat MI/RI clinical research.

CONCLUSION

In conclusion, our current research established that dose-dependently PCA (10 and 20 mg/kg bw) could suppress the ST segment of the ECG, myocardial toxic enzymes, OS, inflammation, and cell apoptosis induced by MI/RI by activating SIRT1, thus alleviating cardiomyocyte apoptosis and protecting against myocardial dysfunction. PCA could down-regulate Caspase-1, NLRP3, ASC, p-P65, and P65 protein expression, through which PCA could attenuate pyroptosis. This research shows that PCA can significantly diminish the inflammatory reaction and myocardial mutilation during reperfusion, and the protection is correlated to the modulation of the Sirt1/NLRP3/NF-κB signaling. Therefore, our study suggests that PCA acts as a possible therapeutic candidate against MI/R injury in rats. We studied only the protein expression of apoptotic and on flammatory but need to work in RNA expression

ACKNOWLEDGEMENT

None.

ABBREVIATIONS

CVD: Cardiovascular disease; **MI/RI:** Myocardial ischemia-reperfusion injury; **ROS:** Reactive oxygen species; **OS:** Oxidative stress; **PCA:** p-Coumaric acid; **IDO:** Indoleamine 2,3-dioxygenase; **DMSO:** Dimethyl Sulfoxides; **TTC:** Triphenyl tetrazolium chloride; **SDS:** Sodium dodecyl sulfate; **ECG:** Electrocardiogram; **LDH:** Lactate dehydrogenase; **CK:** Creatine kinase; **AST:** Aspartate aminotransferase; **NO:** Nitric oxide; **MDA:** Malondialdehyde; **SOD:** Superoxide dismutase; **GSH:** Glutathione; **NF-κB:** Nuclear factor-kappa B; **MAPK:** Mitogen-activated protein kinase.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL STATEMENT

All treatment protocols were conducted as per the Institutional animal Ethics guidelines Pengzhou People's Hospital Medical Ethics Committee 2022 Kollen Trial No. (01).

SUMMARY

In summary, our study demonstrated that PCA (10 and 20 mg/kg bw) could, through the activation of SIRT1, reduce cardiomyocyte apoptosis and prevent myocardial dysfunction by suppressing the ST segment of the ECG, myocardial toxic enzymes, OS, inflammation, and cell apoptosis induced by MI/RI. PCA may reduce pyroptosis by downregulating the expression of the proteins Caspase-1, NLRP3, ASC, p-P65, and P65. According to this study, PCA can considerably reduce myocardial mutilation and the inflammatory response after reperfusion, and this protection is linked to the regulation of Sirt1/NLRP3/NF-κB signaling. Consequently, our research indicates that PCA may be a viable treatment option for rats' MI/RI damage.

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