Submitted: 03 July, 2024 Accepted: 05 August, 2024 Published: 08 October, 2024 DOI:10.22514/sv.2024.130

Open Access
Signa Vitae

ORIGINAL RESEARCH

Perillaldehyde reduces myocardial ischemia-reperfusion injury in rats by inhibiting MAPK1

Wei Chen[1,†], Juan Huang[1,†], Qinke Li[1], Qi Wu[1,*], Chengwei Zhang[1], Rui Yin[1]

[1]Department of Cardiology, The Second Affiliated Hospital of Chengdu Medical College, Nuclear Industry 416 Hospital, 610051 Chengdu, Sichuan, China

***Correspondence**
wuqi8371157@126.com
(Qi Wu)

[†] These authors contributed equally.

Abstract

Myocardial ischemia-reperfusion (MI/RI) injury is a type of cardiac damage that occurs during the reperfusion of myocardial tissue following a period of ischemia. While perillaldehyde (PAE) has been suggested to have anti-inflammatory properties, its effects on MI/RI remain unclear. This study aimed to evaluate the impact of PAE on MI/RI injury. To simulate MI/RI *in vivo*, an ischemia-reperfusion (I/R) rat model was established. The levels of lactate dehydrogenase (LDH), creatine kinase (CK) and oxidative stress-related factors were measured using commercial assay kits. The myocardial infarct size was assessed through triphenyl tetrazolium chloride (TTC) staining. The expression levels of miR-133a-3p and inflammatory factors were determined using quantitative reverse transcription polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA). Myocardial cell apoptosis was evaluated by terminal-deoxynucleoitidyl transferase mediated nick end labeling (TUNEL) staining, and the protein levels of BCL2 associated X (Bax), BCL2 apoptosis regulator (Bcl-2) and mitogen-activated protein kinase 1 (MAPK1) were analyzed by Western blot. PAE could effectively alleviate MI/RI-induced myocardial injury by reducing the levels of LDH and CK, as well as decreasing infarct size. It also mitigated the myocardial inflammatory response by lowering the levels of proinflammatory factors. Additionally, PAE reduced oxidative stress and apoptosis in myocardial cells. Further experiments showed that these protective effects of PAE were associated with the up-regulation of miR-133a-3p, which in turn decreased MAPK1 levels. In conclusion, PAE attenuated MI/RI-induced myocardial injury, inflammatory response, oxidative stress and apoptosis in rats by inhibiting MAPK1, indicating that PAE may effectively reduce myocardial damage caused by I/R injury.

Keywords

Perillaldehyde; Myocardial ischemia-reperfusion injury; Inflammation; Oxidative stress; Apoptosis; MAPK1

1. Introduction

Acute myocardial infarction (AMI) is a critical condition characterized by myocardial necrosis resulting from inadequate blood supply [1]. This condition is commonly caused by the obstruction of a coronary artery due to a blood clot or atherosclerotic plaque [1]. Myocardial ischemia and hypoxia frequently result from the narrowing or occlusion of coronary arteries [2], and without prompt intervention, AMI can lead to extensive myocardial necrosis, which may progress to heart failure, cardiogenic shock or sudden death. The common complications of AMI include arrhythmias (*i.e.*, ventricular fibrillation and atrial fibrillation), heart failure, cardiogenic shock, cardiac rupture, valvular dysfunction and thrombosis. Patients who survive AMI may face long-term cardiac impairments, chronic heart failure, and recurrent myocardial infarctions [2]. Thus, emergency interventional surgery is the primary treatment during the acute phase to quickly clear arterial lesions and restore blood flow [3]. However, subsequent reperfusion of the ischemic myocardium can exacerbate cardiomyocyte damage, a phenomenon known as myocardial ischemia-reperfusion (MI/RI) injury [4]. MI/RI involves various physiological processes, including oxidative stress, mitochondrial dysfunction, inflammatory responses, intracellular calcium overload and apoptosis [5]. Current pharmacological treatments for MI/RI have shown limited effectiveness, highlighting the urgent need for new therapeutic strategies and a deeper understanding of its underlying mechanisms.

Perillaldehyde (PAE) is a monoterpenoid compound derived from *Perilla* [6]. It has a long history of traditional use as a flavoring ingredient in food and as an essential oil in healthcare [6] and has been shown to exhibit various pharmacological activities, including antioxidant, antifungal, anti-tumor and antidepressant effects [7–9]. Early research indicates that PAE has

protective effects in cardiovascular diseases, such as vasodilation, reduction of blood lipids, improvement of endothelial dysfunction, and anti-atherosclerosis [10, 11]. Additionally, PAE has been found to ameliorate adriamycin-induced cardiotoxicity by modulating Na[+]/H[+] hydrogen exchanger 1 (NHE1) and the phosphatidylinositol 3-kinase/Akt kinase (PI3K/AKT) pathway [12]. MicroRNAs (miRNAs) are non-coding RNA molecules that play a crucial role in regulating protein translation [13]. The MAPK pathway is essential for regulating key biological functions and cellular responses to external stress, including cell growth, apoptosis and immune responses [14–17]. Our previous studies have demonstrated that the knockdown of miR-133a-3p increases MAPK1 levels, which promotes MI/RI injury [18]. Therefore, we hypothesize that PAE may mitigate MI/RI-induced myocardial damage by modulating the miR-133a-3p/MAPK1 axis.

In this study, we established an *in vivo* rat model to simulate ischemia-reperfusion (I/R) injury, investigated the effects of PAE on MI/RI and explored its underlying molecular mechanisms to provide insights into potentially novel strategies for enhancing the management of MI/RI and our understanding of its molecular pathways.

2. Materials and methods

2.1 I/R rat model establishment

A total of 24 adult male Sprague Dawley (SD) rats, weighing between 260–280 g, were purchased from Shanghai Laboratory Animal Company (Shanghai, China) and housed in a pathogen-free environment with a 12-hour light/dark cycle at a temperature of 23 ± 2 °C, and humidity of 40%–60%. The rats had unrestricted access to standard food and water. The rats were randomly assigned to four groups (n = 6 per group): sham, I/R, I/R + PAE (60 mg/kg), and I/R + PAE (120 mg/kg). For the I/R, I/R + PAE (60 mg/kg), and I/R + PAE (120 mg/kg). To induce anesthesia, mice were exposed to 5% isoflurane for 3 minutes in an induction chamber, followed by maintenance with 1–2% isoflurane during the procedure. A midline chest incision was made, and after thoracotomy, the rats were intubated with a vein puncture needle and connected to a specialized small-animal ventilator. The left anterior descending coronary artery was occluded for 30 minutes, followed by 120 minutes of reperfusion. In the sham group, rats underwent thoracotomy without coronary artery ligation. PAE, purchased from Sigma-Aldrich (W355704; purity >99.9%), was administered intragastrically for 7 consecutive days before the I/R procedure in the I/R + PAE (60 mg/kg) and I/R + PAE (120 mg/kg) groups [19]. After euthanasia, their heart tissues and serum samples were collected for subsequent analysis.

2.2 Detection of lactate dehydrogenase (LDH) and creatine kinase (CK) contents

The serum samples were collected from rats following the different treatments, and the levels of LDH and CK in the serum were measured using the LDH activity assay kit (MAK066; Sigma, St. Louis, MO, USA) and CK activity assay kit (MAK116-1KT; Sigma, St. Louis, MO, USA), according to the manufacturer's instructions.

2.3 Triphenyltetrazolium Chloride (TTC) staining

For this experiment, the rats' myocardial tissues were harvested and sectioned into 2 mm slices, incubated in TTC solution (T8877; 1%; Sigma, St. Louis, MO, USA) at 37 °C for 20 minutes, followed by fixation in formaldehyde (252549; 10%; Sigma, St. Louis, MO, USA) for 6 hours. The slices were then evaluated, and the myocardial infarct size was quantified by measuring the necrotic tissue as a percentage of the total myocardial area.

2.4 Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

RNA was extracted from the myocardial tissues using TRIzol solution (9109; TaKaRa, Dalian, Liaoning, China) and reverse transcribed with either the PrimeScript RT Master Mix (RR036A; TaKaRa, Dalian, Liaoning, China) or the miScript II RT Kit (218160; TaKaRa, Dalian, Liaoning, China). qRT-PCR was performed using the SYBR® Premix Ex Taq™ quantitative kit (RR420A; TaKaRa, Dalian, Liaoning, China) or the TaqMan MicroRNA Assay Kit (4427975; Sigma, St. Louis, MO, USA) on an ABI7500 system. Reduced glyceraldehyde-phosphate dehydrogenase (GAPDH) and U6 were used as the internal controls. The relative expressions of mRNA and miRNA were calculated using the $2^{-\Delta\Delta Ct}$ method. The primers used are listed in Table 1.

TABLE 1. Primers for qRT-PCR.

Name	Primers for PCR (5′-3′)
TNF-α	
Forward	GGCTTTCGGAACTCACTGGA
Reverse	GGCTTTCGGAACTCACTGGA
IL-1β	
Forward	AGCTTCAGGAAGGCAGTGTC
Reverse	TCAGACAGCACGAGGCATTT
IL-6	
Forward	AGAGACTTCCAGCCAGTTGC
Reverse	AGTCTCCTCTCCGGACTTGT
miR-133a-3p	
Forward	GCCGAGTTTGGTCCCCTTCAA
Reverse	TGGTGTCGTGGAGTCGT
U6	
Forward	AGAAGACTGAAACAGCACAGAGA
Reverse	GAACGCCTCATGATTTGCAGG
GAPDH	
Forward	GCATCTTCTTGTGCAGTGCC
Reverse	GATGGTGATGGGTTTCCCGT

TNF-α: tumor necrosis factor-alpha; IL-1β: interleukin-1 beta; IL-6: interleukin-6; GAPDH: reduced glyceraldehyde-phosphate dehydrogenase.

2.5 Enzyme-linked immunosorbent assay (ELISA)

Serum samples were collected following different treatments. The levels of inflammatory factors in these samples were measured using ELISA. tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) levels were quantified using the TNF-α ELISA kit (ab236712; Abcam, Cambridge, MA, USA), IL-1β ELISA kit (ab255730; Abcam, Cambridge, MA, USA), and IL-6 ELISA kit (ab234570; Abcam, Cambridge, MA, USA), respectively, according to the manufacturer's instructions.

2.6 Detection of oxidative stress

The myocardial tissues were collected, and the levels of lipid peroxidation (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) were assessed using the MDA assay kit (ab118970; Abcam, Cambridge, MA, USA), SOD activity assay kit (ab65354; Abcam, Cambridge, MA, USA), CAT activity assay kit (ab118184; Abcam, Cambridge, MA, USA), and GSH assay kit (ab65322; Abcam, Cambridge, MA, USA), respectively, following the provided protocols.

2.7 TUNEL staining

The myocardial tissues were collected and sectioned into 10 μm slices, fixed in 4% paraformaldehyde (P6148; Sigma, St. Louis, MO, USA) for 30 minutes, and permeabilized with 0.1% TritonX-100 (9036-19-5, Sigma, St. Louis, MO, USA) for 15 minutes. TUNEL staining was performed using a TUNEL kit (T7167; Sigma, St. Louis, MO, USA). Briefly, the slices were incubated with the TdT mixture (T7167; Sigma, St. Louis, MO, USA) for 2 hours, followed by staining with diamidinyl phenylindole (DAPI) (T7167; Sigma, St. Louis, MO, USA) for 10 minutes. The slices were then examined under a fluorescent microscope (LWD300-38LFT, Nikon, Tokyo, Japan).

2.8 Western blot

The myocardial tissues were lysed using the radioimmunoprecipitation assay (RIPA) lysis buffer (R0278; Sigma, St. Louis, MO, USA), and the protein concentrations were determined using a bicinchoninic acid (BCA) kit (B9643; Sigma, St. Louis, MO, USA). Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a Gel Electrophoresis System (Thermo Fisher Scientific, Rockville, MD, USA) and transferred to polyvinylidene fluoride (PVDF) membranes (Sigma) using a Blotting Transfer System (Thermo Fisher Scientific). After blocking, the membranes were incubated at 4 °C overnight with primary antibodies: anti-Bax (ab32503; 1:1000; Abcam), anti-Bcl-2 (ab194583; 1:1000; Abcam), anti-MAPK1 (ab32081; 1:1000; Abcam) and anti-GAPDH (ab8245; 1:1000; Abcam), following which the membranes were probed with a secondary antibody (ab205718; 1:2500; Abcam) for 1 hour. The protein bands were visualized using an efficient chemiluminescence (ECL) kit (ECL1; Sigma, St. Louis, MO, USA).

2.9 Statistical assay

Statistical analyses were conducted using GraphPad Prism 7 (GraphPad Inc., La Jolla, CA, USA). Data are presented as mean $\pm$ standard deviation, with each experiment performed at least three times. Student's *t*-test or analysis of variance (ANOVA) was used for pairwise or multiple comparisons, respectively. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1 PAE alleviated myocardial damage in I/R rats

The effects of PAE on myocardial injury induced by I/R were investigated using our established I/R rat model. The results showed that serum levels of LDH and CK were significantly increased in the I/R group compared to the sham group, with LDH rising 1.9-fold and CK increasing 2.4-fold. PAE treatment reduced these levels in a dose-dependent manner (Fig. 1A). Additionally, the infarct size was significantly greater in the I/R group (increased 19.5-fold) compared to the sham group, but was reduced in a dose-dependent manner following PAE treatment (Fig. 1B). These findings indicate that PAE effectively mitigates myocardial damage in I/R rats.

3.2 PAE alleviated myocardial inflammation in I/R rats

Next, we investigated the effects of PAE on myocardial inflammation induced by I/R by determining the levels of proinflammatory factors in myocardial tissues using qRT-PCR. Our analysis revealed that TNF-α, IL-1β and IL-6 were significantly upregulated in the I/R group, with increases of 4.2-fold, 3.1-fold and 3.2-fold, respectively, compared to the sham group. PAE treatment reduced the levels of these proinflammatory factors in a dose-dependent manner (Fig. 2A). Additionally, serum levels of TNF-α, IL-1β and IL-6, as assessed by ELISA, were significantly elevated in the I/R group, with increases of 2.5-fold, 2.1-fold and 1.3-fold, respectively. PAE treatment attenuated these elevations in a dose-dependent manner (Fig. 2B). These results demonstrate that PAE effectively mitigates myocardial inflammation in I/R rats.

3.3 PAE alleviated myocardial oxidative stress in I/R rats

We also assessed the impact of PAE on oxidative stress induced by I/R based on the levels of oxidative stress-related factors in myocardial tissues. The results showed that I/R induction significantly increased the abundance of MDA (2.5-fold) and decreased the levels of SOD (47%), CAT (37%) and GSH (32%) compared to the sham group, and that PAE treatment could mitigate these effects in a dose-dependent manner (Fig. 3). These findings suggest that PAE reduces oxidative stress in myocardial tissues affected by I/R.

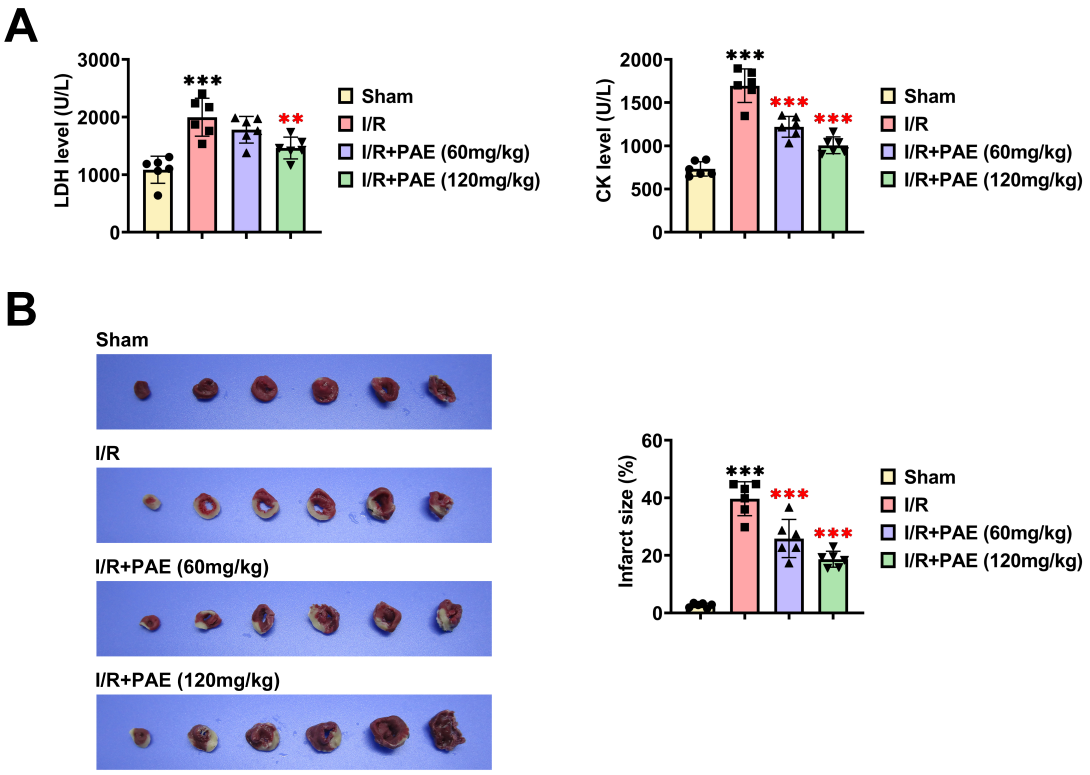

FIGURE 1. PAE alleviates myocardial damage in I/R rats. (A) Serum levels of LDH and CK were quantified using commercial assay kits. (B) Infarct size was measured using TTC staining. $^{**}p < 0.01$, $^{***}p < 0.001$. Black asterisks denote comparisons with the sham group; red asterisks denote comparisons with the I/R group. LDH: levels of lactate dehydrogenase; I/R: ischemia-reperfusion; PAE: perillaldehyde; CK: creatine kinase.

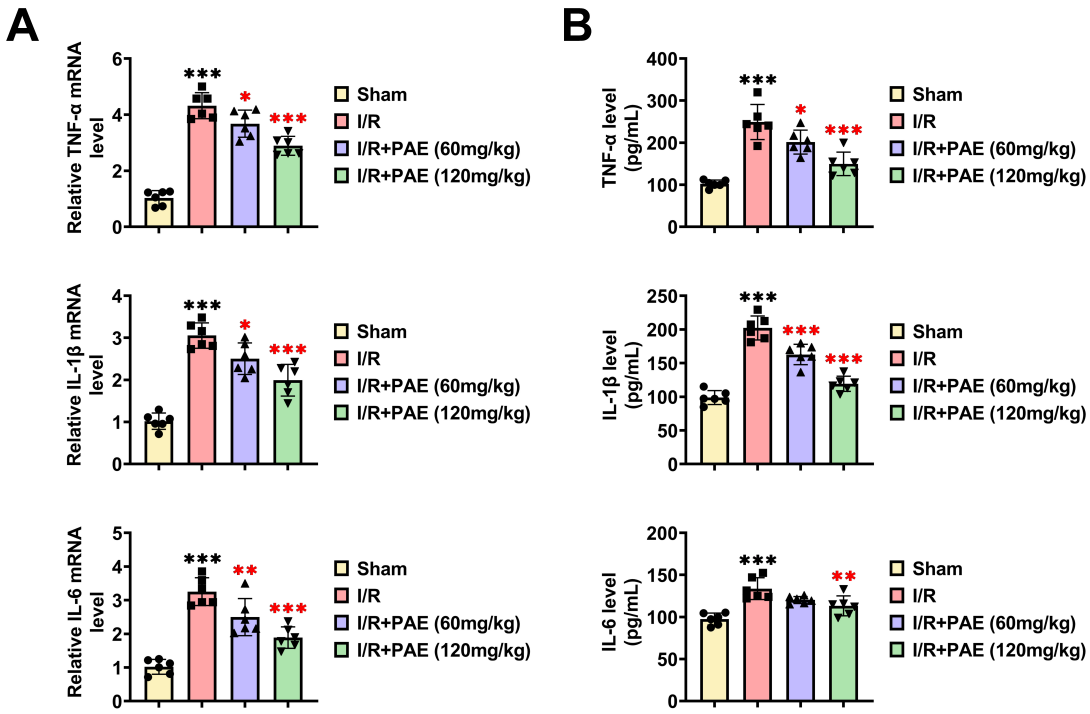

FIGURE 2. PAE reduces myocardial inflammatory response in I/R rats. (A) The mRNA expression levels of proinflammatory factors in myocardial tissues were measured by qRT-PCR. (B) Serum levels of proinflammatory factors were determined using ELISA. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. The black asterisks denote comparisons with the sham group, and the red asterisks denote comparisons with the I/R group. I/R: ischemia-reperfusion; PAE: perillaldehyde; CK: creatine kinase; TNF-α: tumor necrosis factor-alpha; IL-1β: interleukin-1 beta; IL-6: interleukin-6.

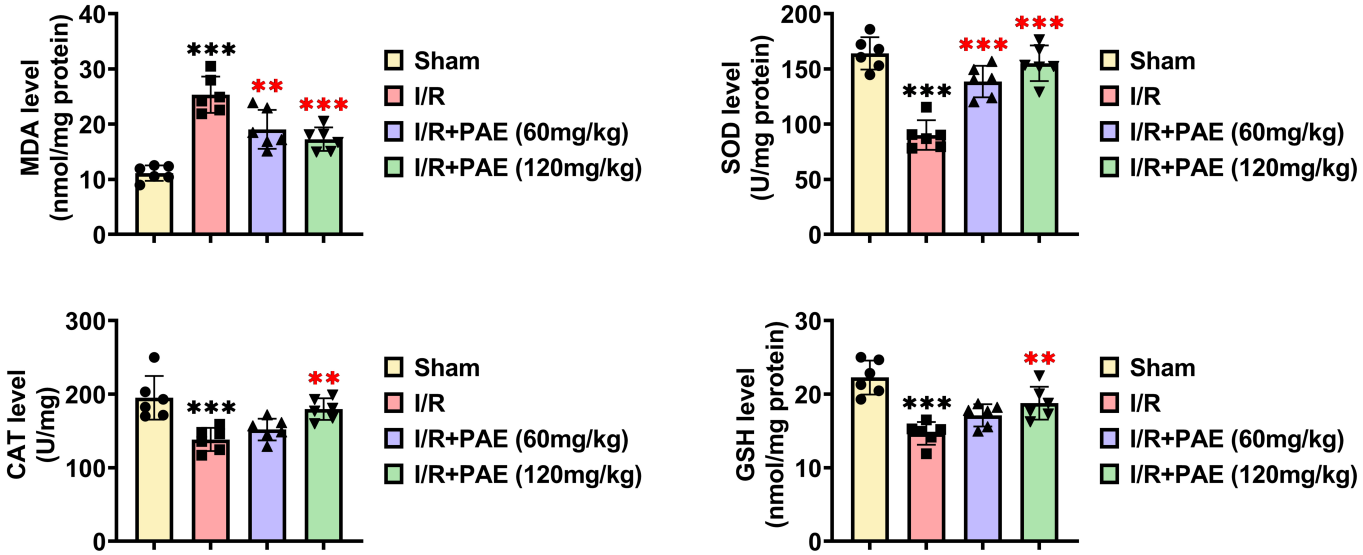

FIGURE 3. PAE mitigates myocardial oxidative stress in I/R rats. The levels of oxidative stress-related factors (MDA, SOD, CAT and GSH) in myocardial tissues were assessed using commercial kits. $^{**}p < 0.01$, $^{***}p < 0.001$. The black asterisks denote comparisons with the sham group, and the red asterisks denote comparisons with the I/R group. MDA: levels of lipid peroxidation; SOD: superoxide dismutase; CAT: catalase; GSH: glutathione; I/R: ischemia-reperfusion; PAE: perillaldehyde.

3.4 PAE inhibited myocardial cell apoptosis in I/R rats

Moreover, we determined the influence of PAE on myocardial cell apoptosis induced by I/R and observed that the number of TUNEL-positive myocardial cells was significantly increased in the I/R group compared to the sham group (Fig. 4A) and that PAE treatment reduced the number of TUNEL-positive cells in a dose-dependent manner (Fig. 4A). Additionally, I/R induction led to a significant increase in Bax levels (8.3-fold) and a decrease in Bcl-2 levels (81%) in myocardial tissues, while PAE treatment counteracted these changes in a dose-dependent manner (Fig. 4B). These results indicate that PAE may effectively reduce myocardial cell apoptosis in I/R rats.

3.5 PAE regulated miR-133a-3p/MAPK1 in I/R rats

Lastly, to explore the molecular mechanisms underlying the effects of PAE, we examined the levels of miR-133a-3p and MAPK1. qRT-PCR and Western blot analyses revealed that I/R induction significantly decreased the abundance of miR-133a-3p (75%) and increased MAPK1 levels (8.5-fold) in myocardial tissues. PAE treatment attenuated these changes in a dose-dependent manner (Fig. 5). These findings suggest that MAPK1 is a downstream target of miR-133a-3p, and PAE may exert its protective effects by upregulating miR-133a-3p and subsequently decreasing MAPK1 levels.

4. Discussion

In this study, we demonstrated that PAE could mitigate myocardial injury in I/R rats by reducing the levels of LDH and CK and decreasing infarct size. Additionally, PAE was found to alleviate myocardial inflammation in I/R rats by decreasing the levels of proinflammatory cytokines TNF-α, IL-1β and IL-6. PAE also alleviated oxidative stress in myocardial tissue and inhibited apoptosis of myocardial cells. Furthermore, our findings suggest that PAE may reduce the abundance of MAPK1 through the upregulation of miR-133a-3p. Collectively, PAE demonstrated promising ability to attenuate MI/RI, myocardial inflammation, oxidative stress, and apoptosis in I/R rats by inhibiting MAPK1.

AMI is often a result of coronary artery disease, characterized by the narrowing and formation of plaques within the coronary arteries [20]. These obstructions can lead to reduced or interrupted blood flow to the myocardium, resulting in myocardial ischemia and necrosis [20, 21]. Common symptoms of AMI include severe, prolonged chest pain, shortness of breath, nausea, vomiting, cold sweats and anxiety and may also be accompanied by irregular heartbeats or palpitations [22]. It can be diagnosed by electrocardiography (ECG), which can detect changes in cardiac electrical activity, as well as blood tests (measure cardiac biomarkers such as troponin) and echocardiography (assess heart structure and function) [23]. Immediate medical intervention is critical for AMI and may involve the use of medications such as analgesics, anticoagulants and antiplatelet drugs to relieve pain and prevent thrombus formation. Procedures such as percutaneous coronary intervention can be performed to restore coronary artery patency and reperfuse the myocardium [24, 25]. In addition, continuous monitoring and a comprehensive rehabilitation plan are essential to prevent future cardiac events. Moreover, preventive measures such as maintaining a healthy lifestyle, managing cardiac risk factors and undergoing regular cardiac health check-ups are essential for improving patient outcomes and survival [26].

MI/RI is a form of cardiac impairment that occurs during the reperfusion of myocardial tissue following a period of ischemia [27]. This condition commonly arises in the context of treatments for cardiac patients, such as coronary artery

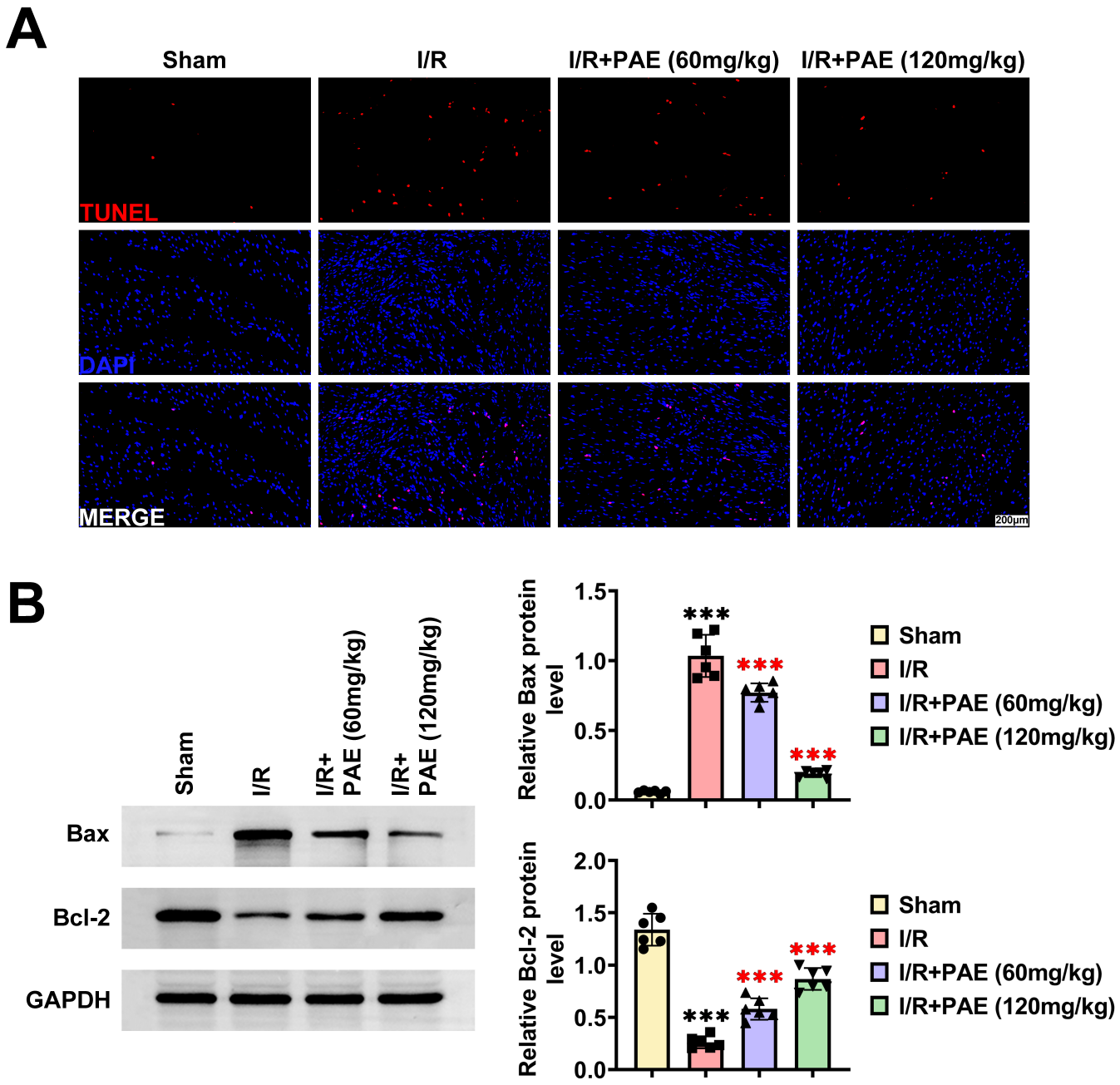

FIGURE 4. PAE inhibits myocardial cell apoptosis in I/R rats. (A) Apoptosis of myocardial cells was evaluated by TUNEL staining. (B) The protein levels of Bax and Bcl-2 in myocardial tissues were analyzed by western blot. Statistical significance is indicated as follows: ***$p < 0.001$. The black asterisks denote comparisons with the sham group, and the red asterisks denote comparisons with the I/R group. I/R: ischemia-reperfusion; PAE: perillaldehyde; TUNEL: terminal-deoxynucleoitidyl transferase mediated nick end labeling; DAPI: diamidinyl phenylindole; MERGE: merger; Bax: BCL2 associated X; Bcl-2: BCL2 apoptosis regulator; GAPDH: reduced glyceraldehyde-phosphate dehydrogenase.

bypass surgery or thrombolytic therapy for coronary artery disease [28]. MI/RI represents a complex pathological process that can lead to substantial damage to cardiac tissues [29]. The mechanisms underlying MI/RI are multifaceted, involving inflammation, the generation of reactive oxygen species, apoptosis and necrosis. These processes contribute to the damage and death of cardiac muscle cells [30]. Reperfusion triggers the activation of immune cells and the release of inflammatory mediators, which further exacerbate myocardial damage [4]. Additionally, reperfusion is associated with an increased production of reactive oxygen species that can inflict damage on cell membranes, proteins, and DNA, thereby intensifying cellular injury [31]. Moreover, MI/RI can lead to myocardial infarction and, in severe cases, may result in sudden cardiac death [32]. Taken together, MI/RI is a critical cardiac condition with significant health implications. Therefore, a thorough understanding of its underlying mechanisms and the implementation of preventive strategies are essential for mitigating injury and improving the prognosis for cardiac patients.

When myocardial cells are damaged or undergo cell death,

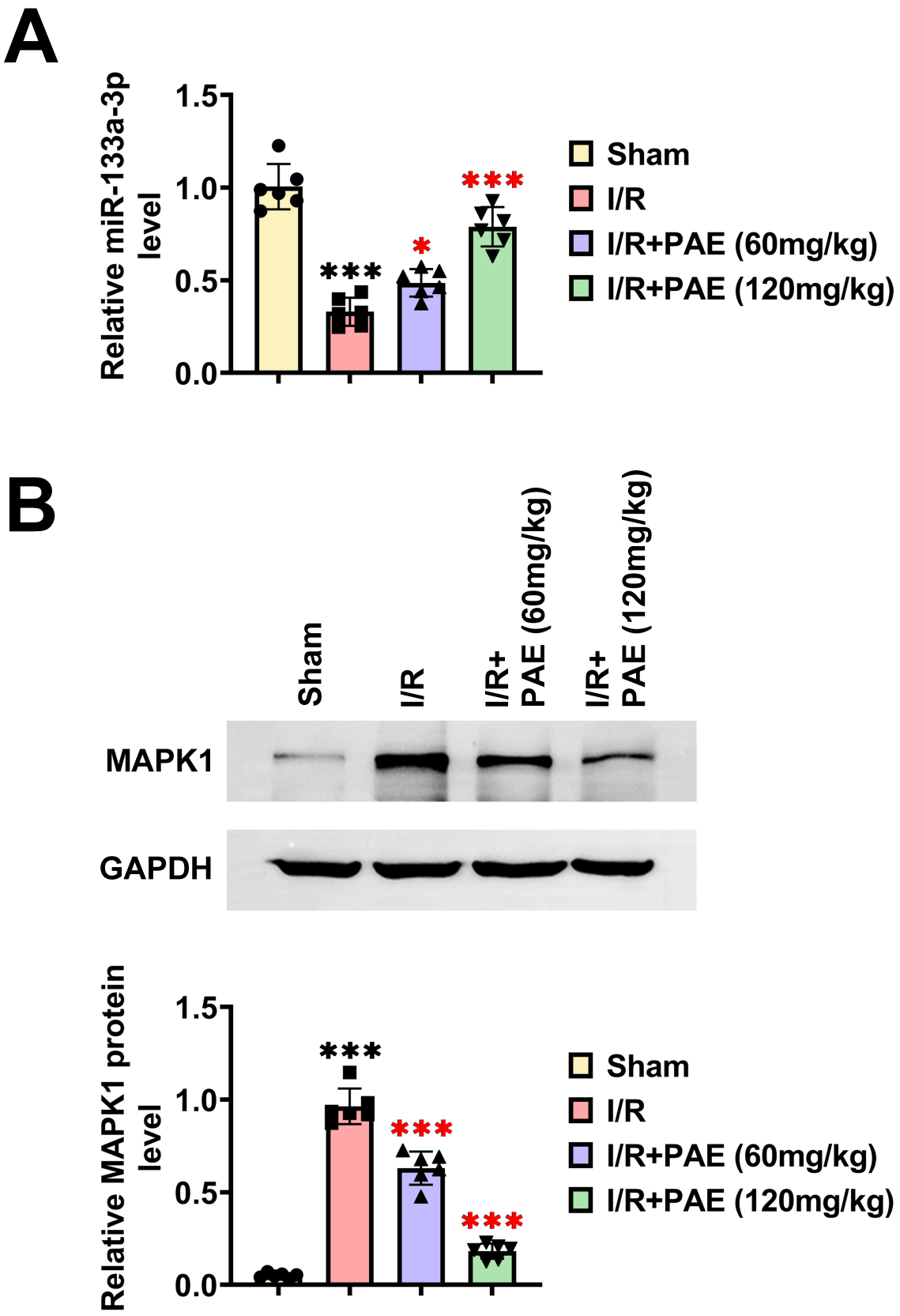

FIGURE 5. PAE regulates the miR-133a-3p/MAPK1 axis in I/R rats. (A) The abundance of miR-133a-3p in myocardial tissues was measured by qRT-PCR. (B) The protein content of MAPK1 was analyzed by western blot. $*p < 0.05$, $***p < 0.001$. The black asterisks denote comparisons with the sham group, and the red asterisks denote comparisons with the I/R group. I/R: ischemia-reperfusion; PAE: perillaldehyde; MAPK1: mitogen-activated protein kinase 1; GAPDH: reduced glyceraldehyde-phosphate dehydrogenase.

LDH and CK are released from the affected cells into the bloodstream, leading to elevated plasma levels of these enzymes. Thus, plasma levels of LDH and CK can be utilized to diagnose myocardial injury, including myocardial infarction. Elevated levels of LDH are indicative of cardiac muscle cell damage or death [33]. In this study, we demonstrated that PAE alleviated myocardial injury in I/R rats by reducing LDH and CK levels and decreasing infarct size, which confirms that PAE mitigates myocardial damage induced by I/R, consistent with the findings of previous studies. For instance, Zheng *et al.* [34] reported that PAE could alleviate spinal cord I/R damage by mitigating inflammatory responses and oxidative stress. Similarly, Xu *et al.* [35] showed that PAE attenuated cerebral I/R damage by reducing levels of IL-1β, IL-6 and TNF-α, and by inhibiting apoptotic cell death. Consistent with these studies, our results indicate that PAE alleviates myocardial inflammatory response in I/R rats by reducing TNF-α, IL-1β and IL-6 levels. Furthermore, PAE also reduced myocardial oxidative stress and cell apoptosis in I/R rats, which also align with the findings of Zheng *et al.* [34] and Xu *et al.* [35].

Numerous studies have suggested that miRNAs hold potential as clinical biomarkers for the detection and screening of human diseases [13, 36, 37]. Specifically, the upregulation of miR-133a has been shown to prevent apoptosis in cardiomyocytes subjected to I/R by modulating death associated protein kinase 2 (DAPK2) [38]. Our previous investigation identified MAPK1 as a target of miR-133a-3p [18]. Additionally, Yu *et al.* [19] demonstrated that PAE regulates glycogen synthase kinase 3 beta (GSK-3β) through the upregulation of miR-133a-3p, thereby improving outcomes in diabetic cardiomyopathy. In this study, we have further elucidated the role of PAE in myocardial protection by showing that PAE reduces MAPK1 levels through the upregulation of miR-133a-3p. These findings are consistent with our prior investigation [18] and align with the results reported by Yu *et al.* [19]. In addition, our present study provides novel evidence that PAE can alleviate myocardial damage induced by I/R through modulation of the miR-133a-3p/MAPK1 axis and expand the current understanding of PAE's regulatory mechanisms by providing a theoretical basis for the potential development of future therapeutic interventions. However, this study had some limitations that should be clarified. The effects of PAE were evaluated only in animal models and validation of our presented findings in clinical settings is still required to fully assess the translational potential of PAE for human therapeutic applications.

5. Conclusions

In conclusion, our findings indicate that PAE attenuates MI/RI by mitigating myocardial inflammatory response, oxidative stress, and apoptosis in I/R rats, primarily through the inhibition of MAPK1. In addition, PAE effectively reduces MI/RI *in vivo*, demonstrating promising potential as a therapeutic agent for the management of MI/RI and for future drug development.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article. The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

WC—conceptualization, methodology and writing-original draft. JH—formal analysis, resources and investigation. QKL—formal analysis, visualization and data curation. QW—project administration, supervision and validation. CWZ, RY—validation, supervision and writing-review & editing. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from the Ethics Committee of the Second Affiliated Hospital of Chengdu Medical College (Approval No. 20230137).

ACKNOWLEDGMENT

Not applicable.

FUNDING

This work was supported by research grants from the Sichuan Provincial Administration of Traditional Chinese Medicine Science and Technology Research Special Project (No. 2023MS177), grants from the Chengdu Medical Research Project (No. 2023189) and the Sichuan Provincial Medical Research Project (No. S23030).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Damluji AA, van Diepen S, Katz JN, Menon V, Tamis-Holland JE, Bakitas M, *et al.* Mechanical complications of acute myocardial infarction: a scientific statement from the American heart association. Circulation. 2021; 144: e16–e35.

[2] Kapur NK, Thayer KL, Zweck E. Cardiogenic shock in the setting of acute myocardial infarction. Methodist DeBakey Cardiovascular Journal. 2020; 16: 16–21.

[3] Dauerman HL, Ibanez B. The edge of time in acute myocardial infarction. Journal of the American College of Cardiology. 2021; 77: 1871–1874.

[4] Algoet M, Janssens S, Himmelreich U, Gsell W, Pusovnik M, Van den Eynde J, *et al.* Myocardial ischemia-reperfusion injury and the influence of inflammation. Trends in Cardiovascular Medicine. 2023; 33: 357–366.

[5] Li H, Zheng F, Zhang Y, Sun J, Gao F, Shi G. Resveratrol, novel application by preconditioning to attenuate myocardial ischemia/reperfusion injury in mice through regulate AMPK pathway and autophagy level. Journal of Cellular and Molecular Medicine. 2022; 26: 4216–4229.

[6] Ahmed HM. Ethnomedicinal, phytochemical and pharmacological investigations of *Perilla frutescens* (L.) britt. Molecules. 2018; 24: 102.

[7] Erhunmwunsee F, Pan C, Yang K, Li Y, Liu M, Tian J. Recent

development in biological activities and safety concerns of perillaldehyde from perilla plants: a review. Critical Reviews in Food Science and Nutrition. 2022; 62: 6328–6340.

[8] Zhang Y, Long Y, Yu S, Li D, Yang M, Guan Y, *et al*. Natural volatile oils derived from herbal medicines: a promising therapy way for treating depressive disorder. Pharmacological Research. 2021; 164: 105376.

[9] Zielinska-Blajet M, Pietrusiak P, Feder-Kubis J. Selected monocyclic monoterpenes and their derivatives as effective anticancer therapeutic agents. International Journal of Molecular Sciences. 2021; 22: 4763.

[10] Zhou F, Dai O, Peng C, Xiong L, Ao H, Liu F, *et al.* Pro-angiogenic effects of essential oil from *Perilla frutescens* and its main component (perillaldehyde) on zebrafish embryos and human umbilical vein endothelial cells. Drug Design, Development and Therapy. 2021; 15: 4985–4999.

[11] Yu L, Liu H. Perillaldehyde prevents the formations of atherosclerotic plaques through recoupling endothelial nitric oxide synthase. Journal of Cellular Biochemistry. 2018; 119: 10204–10215.

[12] Yin Y, Niu Q, Hou H, Que H, Mi S, Yang J, *et al*. PAE ameliorates doxorubicin-induced cardiotoxicity via suppressing NHE1 phosphorylation and stimulating PI3K/AKT phosphorylation. International Immunopharmacology. 2022; 113: 109274.

[13] Zhang L, Ding H, Zhang Y, Wang Y, Zhu W, Li P. Circulating microRNAs: biogenesis and clinical significance in acute myocardial infarction. Frontiers in Physiology. 2020; 11: 1088.

[14] Hepworth EMW, Hinton SD. Pseudophosphatases as regulators of MAPK signaling. International Journal of Molecular Sciences. 2021; 22: 12595.

[15] Gao T, Li J, Shi L, Hu B. Rosavin inhibits neutrophil extracellular traps formation to ameliorate sepsis-induced lung injury by regulating the MAPK pathway. Allergologia et Immunopathologia. 2023; 51: 46–54.

[16] Yuan W, Shi Y, Dai S, Deng M, Zhu K, Xu YM, *et al*. The role of MAPK pathway in gastric cancer: unveiling molecular crosstalk and therapeutic prospects. Journal of Translational Medicine. 2024; 22: 1142.

[17] Guo YJ, Pan WW, Liu SB, Shen ZF, Xu Y, Hu LL. ERK/MAPK signalling pathway and tumorigenesis. Experimental and Therapeutic Medicine. 2020; 19: 1997–2007.

[18] Wang Z, Luo W, Zhong P, Feng Y, Wang H. lncRNA HAGLR modulates myocardial ischemia-reperfusion injury in mice through regulating miR-133a-3p/MAPK1 axis. Open Medicine. 2022; 17: 1299–1307.

[19] Yu YN, Ren YY, Shao ZL, Chen BL, Cui BY, Chao CY, *et al.* Perillaldehyde improves diabetic cardiomyopathy by upregulating miR-133a-3p to regulate GSK-3β. European Journal of Pharmacology. 2023; 953: 175836.

[20] Młynarska E, Czarnik W, Fularski P, Hajdys J, Majchrowicz G, Stabrawa M, *et al*. From atherosclerotic plaque to myocardial infarction-the leading cause of coronary artery occlusion. International Journal of Molecular Sciences. 2024; 25: 7295.

[21] Buja LM. Pathobiology of myocardial ischemia and reperfusion injury: models, modes, molecular mechanisms, modulation, and clinical applications. Cardiology in Review. 2023; 31: 252–264.

[22] Moore A, Goerne H, Rajiah P, Tanabe Y, Saboo S, Abbara S. Acute myocardial infarct. Radiologic Clinics of North America. 2019; 57: 45–55.

[23] Zeymer U. Diagnosis and initial management of acute myocardial infarction. MMW—Fortschritte der Medizin. 2019; 161: 34–36.

[24] Saito Y, Oyama K, Tsujita K, Yasuda S, Kobayashi Y. Treatment strategies of acute myocardial infarction: updates on revascularization, pharmacological therapy, and beyond. Journal of the American College of Cardiology. 2023; 81: 168–178.

[25] Bae HE, Yoon YH, Kim JY, Cho YD, Choi SH, Park SJ. Impact of COVID-19 outbreak on patients with ST-segment elevated myocardial infarction undergoing primary percutaneous coronary intervention in a regional emergency center in Seoul, Korea. Signa Vitae. 2023; 19: 165–172.

[26] Gulati R, Behfar A, Narula J, Kanwar A, Lerman A, Cooper L, *et al.* Acute myocardial infarction in young individuals. Mayo Clinic Proceedings. 2020; 95: 136–156.

[27] Tian H, Zhao X, Zhang Y, Xia Z. Abnormalities of glucose and lipid metabolism in myocardial ischemia-reperfusion injury. Biomedicine & Pharmacotherapy. 2023; 163: 114827.

[28] Wu D, Gu Y, Zhu D. Cardioprotective effects of hydrogen sulfide in attenuating myocardial ischemia-reperfusion injury (Review). Molecular Medicine Reports. 2021; 24: 875.

[29] Valikeserlis I, Athanasiou AA, Stakos D. Cellular mechanisms and pathways in myocardial reperfusion injury. Coronary Artery Disease. 2021; 32: 567–577.

[30] Korshunova AY, Blagonravov ML, Neborak EV, Syatkin SP, Sklifasovskaya AP, Semyatov SM, *et al.* BCL2-regulated apoptotic process in myocardial ischemia-reperfusion injury (Review). International Journal of Molecular Medicine. 2021; 47: 23–36.

[31] Wang J, Wang H, Mou X, Luan M, Zhang X, He X, *et al.* The advances on the protective effects of ginsenosides on myocardial ischemia and ischemia-reperfusion injury. Mini-Reviews in Medicinal Chemistry. 2020; 20: 1610–1618.

[32] He J, Liu D, Zhao L, Zhou D, Rong J, Zhang L, *et al.* Myocardial ischemia/reperfusion injury: mechanisms of injury and implications for management (Review). Experimental and Therapeutic Medicine. 2022; 23: 430.

[33] Yuan Y, Huang H, Hu T, Zou CC, Qiao YM, Fang M, *et al*. Curcumin pretreatment attenuates myocardial ischemia/reperfusion injury by inhibiting ferroptosis, autophagy and apoptosis via HES1. International Journal of Molecular Medicine. 2024; 54: 110.

[34] Zheng W, Liu B, Shi E. Perillaldehyde alleviates spinal cord ischemia-reperfusion injury via activating the Nrf2 pathway. Journal of Surgical Research. 2021; 268: 308–317.

[35] Xu L, Li Y, Fu Q, Ma S. Perillaldehyde attenuates cerebral ischemia-reperfusion injury-triggered overexpression of inflammatory cytokines via modulating Akt/JNK pathway in the rat brain cortex. Biochemical and Biophysical Research Communications. 2014; 454: 65–70.

[36] Lalem T, Devaux Y. Circulating microRNAs to predict heart failure after acute myocardial infarction in women. Clinical Biochemistry. 2019; 70: 1–7.

[37] Scarlatescu AI, Micheu MM, Popa-Fotea NM, Dorobantu M. MicroRNAs in acute ST elevation myocardial infarction—a new tool for diagnosis and prognosis: therapeutic implications. International Journal of Molecular Sciences. 2021; 22: 4799.

[38] Li S, Xiao FY, Shan PR, Su L, Chen DL, Ding JY, *et al.* Overexpression of microRNA-133a inhibits ischemia-reperfusion-induced cardiomyocyte apoptosis by targeting DAPK2. Journal of Human Genetics. 2015; 60: 709–716.

How to cite this article: Wei Chen, Juan Huang, Qinke Li, Qi Wu, Chengwei Zhang, Rui Yin. Perillaldehyde reduces myocardial ischemia-reperfusion injury in rats by inhibiting MAPK1. Signa Vitae. 2024; 20(10): 97-105. doi: 10.22514/sv.2024.130.