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CircPAN3/miR-221/PTEN axis and apoptosis in myocardial Infarction: Quercetin's regulatory effects

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ABSTRACT

The circular RNA/microRNA/mRNA axis is a new layer of non-coding RNA(ncRNA)-based regulatory gene expression networks upstream of numerous cell signaling pathways. Circular RNA *PAN3* (*circPAN3*) is involved in autophagy, fibrosis and apoptosis which are responsible for the reduction in cardiac functional capacity - following myocardial infarction (MI). However, the molecular mechanism of *circPAN3* association with apoptosis is unknown. In addition, the relationship between quercetin as a cardioprotective factor in MI and circular RNA-dependent regulatory pathways has not yet been elucidated. MI was induced in Wistar rats using the left anterior descending artery (LAD) ligation method. One day after surgery, quercetin (30 mg/kg) was injected intraperitoneal (IP) every other day for two weeks. The expression of *circPAN3* was increased in the MI group ($P < 0.05$). The increase in *circPAN3* was accompanied by a decrease in *miR-221* ($P < 0.0001$), an increase in *PTEN* ($P < 0.0001$), and cleaved *caspase 3* ($P < 0.001$). Quercetin effectively reduced the expression of *circPAN3* ($P < 0.05$), PTEN ($P < 0.0001$), and cleaved *caspase 3* ($P < 0.001$), and increased the expression of *miR-221* ($P < 0.0001$) and the ratio of *p-AKT* to *p-PI3K* ($P < 0.001$). The *circPAN3/miR-221/PTEN* pathway is an ncRNA-dependent apoptotic pathway in MI cardiac tissue. Quercetin effectively modulated this pathway, resulting in a reduction of cardiac tissue death and improvement in cardiac function after MI. This suggests that the *circPAN3/miR-221* axis plays a role in apoptosis in MI, and quercetin can act as a protective candidate by modulating this pathway.

1. Introduction

Myocardial infarction (MI) remains a leading cause of death worldwide. Despite advancements in detecting and controlling symptoms, mortality rates following heart failure post-MI continue to rise (Salari et al., 2023). A key protective strategy against MI is to prevent early-phase cardiomyocyte cell death and necrosis caused by oxygen deprivation stress, which can result in the replacement of functional myocardium with fibrotic tissue, and increase the risk of heart failure in the long term after MI (Raziyeva et al., 2022). Therefore, it is of great importance to identify the factors that minimize the primary damages at the times close to the onset of MI and have a protective role in post-ischemic cardiac remodeling. Phosphatase and tensin homolog (*PTEN*) - also referred to as *MMAC1* (mutated in multiple advanced cancers) dephosphorylating lipids and proteins on serine, threonine, and tyrosine residues to regulate cellular signaling pathways involved in cell survival, proliferation, and metastasis (Liang,et al., 2021). *PTEN* primarily targets phosphatidylinositol (3,4,5)-triphosphate (PIP3), whose dephosphorylation leads to the inactivation of the phosphatidylinositol-3-kinase (*PI3K*)/*AKT* pathway. On the other hand, activation of the *PI3K/AKT*

Abbreviations: MI, Myocardial infarction; PTEN, Phosphatase and tensin homolog; MMAC1, mutated in multiple advanced cancers; ncRNA, non-coding RNA; lncRNA, long noncoding RNA; miRNA, microRNA; circRNA, circular RNA; PI3K/AKT, phosphatidyl inositol 3phosphate/ protein kinase B; LAD, Left ventricular anterior descending coronary artery; LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.

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pathway has been shown to protect cardiomyocytes from apoptosis, reduce cardiac injury, and increase survival rate (Cheng et al., 2019). *AKT* plays a negative regulatory role in pro-apoptotic signaling pathways and a positive regulatory role in anti-apoptotic pathways, which ultimately promotes cell survival. Additionally, animal models of MI have demonstrated that *AKT* can improve cardiac contractility and reduce infarct size (Matsui,et al., 2003). However, in animal models of MI, *PTEN* expression and activity increased, inhibiting the *PI3K/AKT* signaling pathway and exacerbating myocardial damage. Conversely, pharmacological inhibition of *PTEN* has been shown to reduce myocardial damage and improve cardiac function in animal models of MI (Oudit et al., 2004). Until recently, investigating the role of proteins as cellular effectors was considered sufficient for understanding the molecular mechanisms of diseases (Jafarinejad-Farsangi et al., 2015). In recent years, it has become evident that protein expression and function are regulated by a group of non-coding RNAs that do not translate into proteins(Gharbi et al., 2022). Non-coding RNAs encompass a diverse group of RNAs with varying lengths and functions, which create important regulatory networks upstream of proteins in the cell. 80 % of the genome is functional ENCODE Project Consortium, 2012 and transcribed into RNAs and less than 3 % of transcripts are translated to proteins (Li and Liu,2019). The majority of transcripts are non-coding RNAs (ncRNAs) (Hon et al., 2017). Long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and small non-coding RNAs (including miRNAs) are the main RNA-based regulatory systems of gene expression (Ackah et al., 2022; Sun et al. 2020) microRNA (19–25 nt) directly binds to mRNAs and in most cases enhances degradation or inhibits the translation process. One-third of the human genome is regulated by microRNAs (Ardekani et al. 2010; Urbich,et al., 2008). CircRNAs are abundant and conserved among species. They originate from the back splicing process and are stable from degradation because of the lack of the 5′ and 3′ ends. CircRNAs can regulate gene expression by influencing mRNA transcription, turnover, and translation through direct binding to RNA-binding proteins and microRNAs, commonly called “sponging” (Panda, 2018). CircRNAs act as competing endogenous RNAs (ceRNAs) by directly binding to microRNAs and preventing them from interacting with their target mRNA. This can lead to positive regulation of the target mRNA and influence corresponding signaling pathways. The regulatory role of circular RNAs as microRNA sponges is an active area of investigation in many RNA-based physiological and pathological research. *Circ_0040414*, which is upregulated in the blood of heart failure patients, inhibits the *AKT* pathway by sequestering *miR-186-5p*. *miR-186-5p* directly inhibits *PTEN*, an upstream regulator of *AKT*. Ultimately, this reduces proliferation, cell death, and hypertrophy in cardiomyocyte cells (Feng et al., 2021). Several studies have investigated circRNA-miRNA-mRNA network in animal models of MI and found that mRNAs *Arl2*, *BCL2L11*, and *TEAD1* which are involved in survival and suppression of apoptosis in cardiomyocytes are targeted by *miR-15b*, *miR-92a*, and *miR-214-5p* respectively. At a higher level, circular RNAs *Ttc3*, *Rbms1*, and *circSLC8a1* negatively regulate these miRNAs through sponging and subsequently increase the expression of their target proteins (Jin et al., 2022; Lan et al., 2022; Cai et al., 2019). Quercetin, a flavonoid present in fruits and vegetables like onions, peppers, mangoes, and berries, has antioxidant, anti-inflammatory, and anti-apoptotic effects against myocardium damage in acute MI (B. Li et al., 2016). In ischemic/reperfusion (I/R) models of rats, treatment with quercetin resulted in a significant reduction in infarcted area and improvements in hemodynamic parameters. In a randomized clinical trial quercetin supplementation in post-MI patients for 8 weeks elevated total antioxidant capacity and improved the insecurity dimension of quality of life (Dehghani et al., 2021). To the best of our knowledge, there is no report about the relation between quercetin and *PTEN/PI3K/AKT* pathway in myocardial infarction. In addition, it has not yet been investigated if the beneficial effect of quercetin is mediated through a circular RNA-miRNA-mRNA network.

Based on the importance of RNA-based gene expression regulatory networks, bioinformatics studies have predicted the relevance of extensive network analyses in cardiovascular diseases, including MI (Wu et al., 2022; Chen et al., 2022; Z. Zhang et al. 2022a). Experimental investigation of these regulatory networks and identifying drugs and factors that regulate these pathways can shed light on the molecular mechanisms of MI in its early stages and in the long-term phases that can lead to heart failure. We aimed to investigate the relation between the beneficial effect of quercetin on the *circPAN3/miR-221/PTEN/PI3K/AKT* in the hearts of rats with myocardial infarction.

2. Methods

This study was conducted on 24 Wistar male rats (n = 8 in each group) with a weight range of 220–250 g (6–8 weeks old) purchased from Kerman University of Medical Science. The rats were placed in conditions of free access to food and water, 12 h of darkness and 12 h of light. The Ethics Committee of Kerman University of Medical Sciences approved the experimental protocol with the code IR.KMU.AEC.1402.012. Rats were divided into 3 groups of 8: Sham, Myocardial Infarction + vehicle (MI + Veh), and Myocardial Infarction with Quercetin treatment (MI + Q). The administration of quercetin (Q4951-Sigma-Aldrich) was performed one day after MI induction via intraperitoneal injection at an every-other-day dose of 30 mg/kg in a saline solution containing 5 % ethanol for two weeks, as previously described (Rajabi et al., 2021) (Fig. 1). The vehicle group received injections of saline solution containing 5 % ethanol using the same injection schedule.

2.1. Myocardial infarction induction

Left ventricular anterior descending coronary artery (LAD) was occluded permanently to induce myocardial infarction (Farsangi et al. 2021) in rats that were first anesthetized with ketamine (80 mg/kg, BREMER PHARMA GMBH, Germany) and xylazine (10 mg/kg, Alfasan, Holland). The hearts were then exposed through an incision in the fourth intercostal space, and the LAD was ligated 2 mm below the origin. Infarction was confirmed by the pale appearance of the ischemic area and the elevation of the ST segment. The sham group underwent the same procedure without LAD ligation. Rats were mechanically ventilated during the experiment using an animal ventilator. Thirty minutes before the surgery, buprenorphine was injected at a dose of 1.1 mg/kg. Additionally, it was administered every 12 h for 24 h for pain control.

2.2. Measurement of hemodynamic and heart function indicators

Animals were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg). A polyethylene catheter filled with heparin (7 U/mL) was inserted through the right carotid artery and advanced to the left ventricle to measure left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), maximum rate of increase in left ventricular pressure (dp/dt max) as an indicator of contraction, and maximum speed of left ventricular pressure reduction (-dp/dt max) as an indicator of left ventricular relaxation (Rostamzadeh et al., 2021). Another catheter was placed in the femoral artery to measure systolic blood pressure (SBP) and diastolic blood pressure (DBP). The Powerlab physiograph system was used to record these measurements.

2.3. Measurement of histopathological scores

After the treatment period, which was one day after the last injection, the animal’s heart was removed and weighed under deep anesthesia with thiopental sodium (50 mg/kg) Farzaneh et al., 2017. The ratio of heart weight (mg) and left ventricular weight (mg) to body weight (gr) was used to calculate the index of heart and left ventricular hypertrophy. The left ventricle was fixed in 10 % buffered formalin (pH 7.4) and embedded in paraffin. The molded tissue was cut into 5 μm

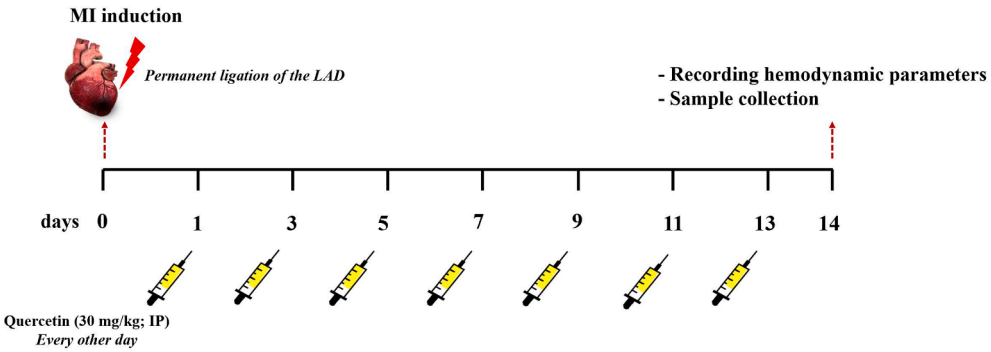

Fig. 1. Timeline of experimental procedures. Myocardial infarction, MI; left anterior descending artery. LAD; intrapritoneal injection, IP.

thick sections and stained with hematoxylin and eosin (H&E) and also Masson 's trichrome. The total histopathological score was evaluated and calculated microscopically (Olympus, CX33) by a pathologist based on four indices: congestion and hemorrhage, hypereosinophilic bundles, leukocyte infiltration, and cardiomyocyte necrosis, which were categorized as severe (+++), moderate (++), mild (+), and normal (0). Each animal was assigned one score, with a total of 12 histological scores (Najafipour et al., 2010). ImageJ software measured the percent of fibrosis (version 1.53 t).

2.4. Quantitative Real-Time PCR analysis

Total RNA, including circular RNAs and miRNAs, was extracted from rat heart tissue using a total RNA extraction kit (QIAGEN, USA) following the manufacturer's instructions. Briefly, the heart tissues were cut into small pieces on ice using a razor blade. Subsequently, 750 µl of extraction buffer (RL) was added to a 1.5 ml tube containing the tissue. The tissue was then homogenized on ice using an ultrasonic homogenizer with 0.5-second cycles and 50 % amplitude for 10 s (UP200H, 50/60 Hz, Hielscher, Germany). After incubating for 5 min at room temperature, 200 µl of chloroform was added, and the mixture was centrifuged at 13,000 rpm for 12 min at 4 °C to separate the aqueous phase containing RNA. Next, 70 % ethanol was added in the same volume as the aqueous phase, and the solution was transferred to a spin column. The column was washed twice with washing buffer (RW buffer), and the column-bound RNAs were then released by adding 50 µl of DEPC water and centrifuging the column.

The complementary DNA synthesis (cDNA) was performed using a cDNA synthesis kit (Parstous Biotechnology, Iran). The expression of circRNAs was measured with a StepOnePlus instrument (Applied Biosystems, USA) using RealQ Plus 2 × Master Mix Green (Amplicon, Denmark). *GAPDH* (Mohseni-Moghaddam et al. 2022) was used as an internal control for *circPAN3* (F. Li et al., 2020), *18S rRNA* for *PTEN* (Rajabi et al., 2022; Zhuang et al., 2018), and *RNU6* for *miR-221* (Rajabi et al., 2020; Yao et al., 2014). Divergent primers are used to measure the expression of circRNAs in RT-qPCR (F. Li et al., 2020). The sequence of the primers is given in Table 1.

2.5. Western blot

Western blot analysis was conducted as previously described with some modifications (Babaei et al., 2018). The heart tissues were lysed using Ripa buffer and centrifuged at 14,000 rpm for 20 min at 4 °C. According to the manufacturer's instructions, the supernatant protein concentration was determined using the Bradford Protein Quantification kit (DB0017, DNAbioTech, Iran). Protein samples were mixed with an equal volume of 2X Laemmli sample buffer and subjected to SDS-PAGE after a 5-minute boiling step. The separated proteins were then transferred to a 0.2 µm immune-Blot™ polyvinylidene difluoride (PVDF) membrane (Cat No: 162–017777; Bio-Rad Laboratories, CA, USA). The membranes were blocked in 5 % BSA buffer (Cat No: A-7888; Sigma Aldrich, MO, USA) containing 0.1 % Tween 20 for 1 h. They were then incubated with primary antibodies against *PTEN* (Cat No: ab267787, Abcam), *AKT1* (Cat No: ab238477, Abcam), *PI3 Kinase p85 alpha* (phospho Y607) (Cat No: ab182651, Abcam), Cleaved *Caspase-3* (Cat No: 9661 T, cell signaling), *AKT1* (phospho S473) (Cat No: ab81283, Abcam), *PI3 Kinase* (Cat No: ab191606, Abcam), and *β-actin* loading control antibodies (Cat No: ab8227, Abcam) for 1 h at room temperature. After incubation, the membranes were washed three times with TBST and incubated with a secondary antibody, goat anti-rabbit IgG H&L horseradish peroxidase (HRP) (Cat No: ab6721; Abcam). Protein bands were detected by enhanced chemiluminescence (ECL), and the expression of proteins was normalized to the expression of *β-actin*. The densitometry of protein bands was performed using GelAnalyzer Version 2010a software (NIH, USA), and the relative expression of proteins was evaluated according to Bashiri et al. (Bashiri et al., 2023).

Table 1
primer list.

Genes	Primer sequence (5 – 3)	References
Circ*PAN3*	F: 5′-TGACTTCGGTGCCCTCAA-3′ R: 5′-CTGGTGCTGAAATAGGACTCTG-3′	(Li et al.,2020)
GAPDH	F: 5′-ATGCCAGTGAGCTTCCCGTTCAGC-3′ R: 5′-GTCTTCACCACCATGGAGAAGGC-3′	(Mohseni-Moghaddam et al.,2022)
miR-221	F: 5′-CGCAGCTACATTGTCTGCTGG-3′ R: 5′-GTGCAGGGTCCGAGGT-3′ RT: 5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGAAACC-3′	(Yao et al.,2014)
RNU6	F: 5′-CTCGCTTCGGCAGCACA-3′ R: 5′-AACGCTTCACGAATTTGCGT-3′	(Rajabi et al.,2020)
18S rRNA	F: 5′-AGTCCCTGCCCTTTGTACACA-3′ R: 5′-GATCCGAGGGCCTCACTAAAC-3′	(Rajabi et al.,2022)
PTEN	F: 5′-GGAAAGGACGGACTGGTGTA-3′ R: 5′-TGCCACTGGTCTGTAATCCA-3′	(Zhuang et al.,2018)

2.6. Statistical analysis

The results are expressed as the mean ± standard error of the mean (SEM) in both the tables and figures. The normality of the data was assessed using the Kolmogorov-Smirnov test, and group comparisons were performed using the One-way ANOVA test, followed by Tukey's post hoc test. Nonparametric tests were utilized for analyzing categorical data, such as histopathological scores. A p-value of less than 0.05 was considered statistically significant. Fold changes in gene expression were determined using the $2^{-\Delta\Delta Ct}$ method. The infarct size was measured by ImageJ software.

3. Results

3.1. Measurement of hemodynamic and cardiac performance indices

The hemodynamic findings and cardiac performance indices indicated an improvement in cardiac function following treatment with quercetin. In the MI group, SBP (systolic blood pressure) and DBP (diastolic blood pressure) pressures were significantly lower compared to the sham group ($P > 0.05$; $P > 0.001$ respectively) (Fig. 2 A, B). Quercetin significantly increased both SBP ($P > 0.01$) and DBP ($P > 0.05$) in the MI group, bringing them closer to the sham group. Heart rate (Fig. 2C) did not show significant differences among the study groups. Following MI, LVEDP (left ventricular end-diastolic pressure) and LVSP (left ventricular systolic pressure) (Fig. 2D and E) values which are the cardiac performance indices showed a significant increase ($P > 0.01$) and decrease ($P > 0.01$), respectively, compared to the sham group. Treatment with quercetin partially improved these indices (both $P < 0.05$). Another index examined in this study was myocardial contractility and relaxation. + dp/dt max (maximum rate of rising in LV pressure) and -dp/dt max (maximum rate of decline in LV pressure) were significantly ($P > 0.01$ and $P > 0.005$ respectively) reduced in the MI group compared to the control group. Quercetin treatment increased the reduction of + dp/dt max and -dp/dt max that happened on MI ($P < 0.01$, $P < 0.5$ respectively).

3.2. Effect of quercetin treatment on infarct size, cardiac fibrosis, and histopathologic scores

The results of trichrome staining (Fig. 2C) showed an average of 22 % fibrosis in the heart of rats with MI. The administration of quercetin resulted in a decrease in the percentage of fibrosis, in addition, the total histological score (Fig. 3D), was significantly higher ($P > 0.001$) in the MI group than in the sham group. Treatment with quercetin led to a significant reduction ($P > 0.01$) in the total histological score. The infarct size in our permanent LAD model of the MI group was 45 % and quercetin significantly ($P > 0.001$) reduced it to 21 % (Fig. 3E). Body weight, heart weight, and LV + septum weight/body weight ratio were not different among the studied groups (Fig. 3F).

3.3. Effect of quercetin in the expression of circPAN3, miR-221, and PTEN

Based on the results of Real-time PCR (Fig. 4), the expression of *circPAN3* was found to be significantly increased ($P < 0.05$) two weeks post-MI, while it decreased significantly ($P < 0.05$) following quercetin treatment compared to the MI group. The reduction in *circPAN3* expression due to quercetin treatment resulted in no significant difference between the sham and quercetin-treated groups. Given that *miR-221* directly binds to *circPAN3*, it is expected that the expression of *miR-221* would decrease in the presence of *circPAN3*. Real-time PCR results demonstrated that the expression of *miR-221* was significantly decreased ($P < 0.0001$) in the MI group compared to the sham group. However, quercetin treatment significantly increased the expression of *miR-221* ($P < 0.0001$) in the MI animals. Additionally, the expression of *PTEN*,

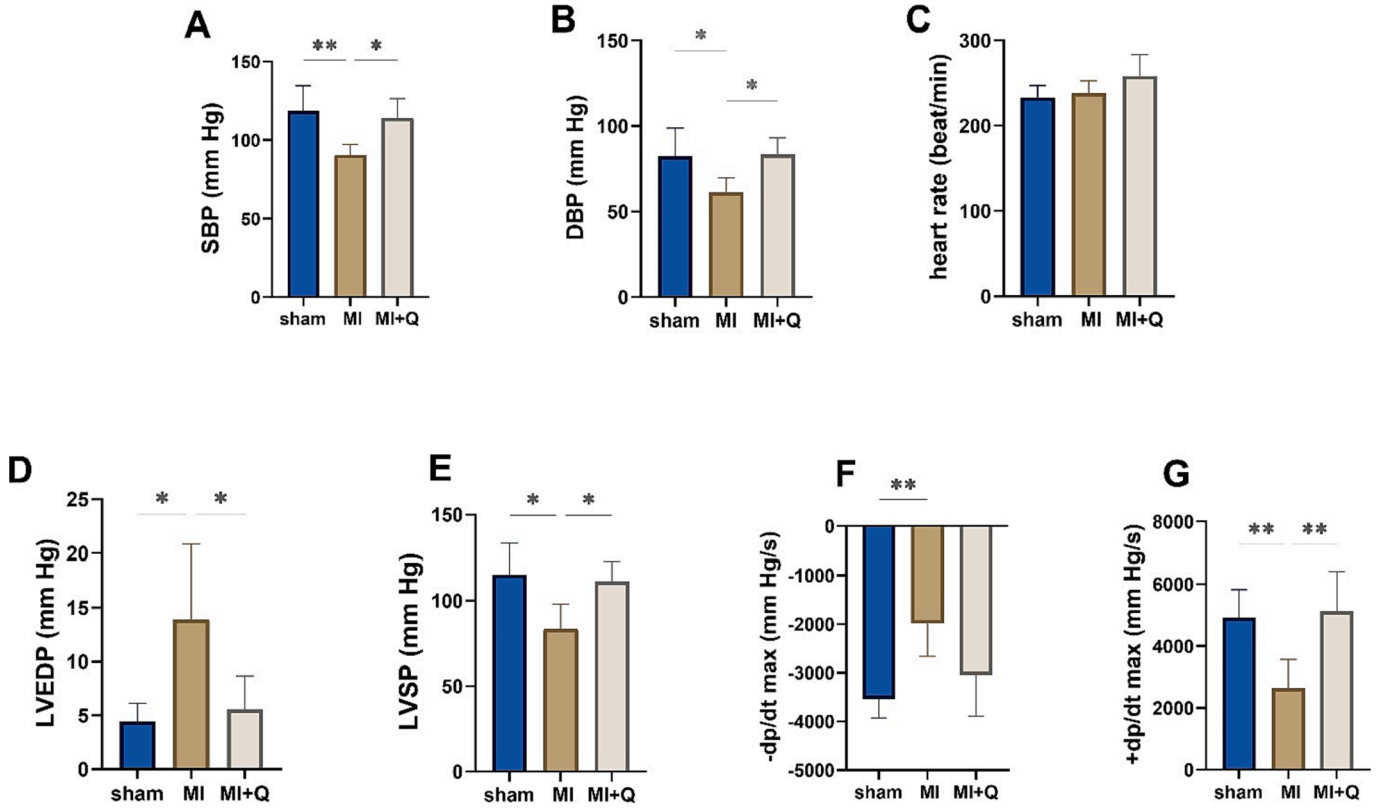

Fig. 2. Hemodynamic parameters and left ventricular contraction indices. HR: heart rate, LVSP: left ventricular systolic pressure, LVEDP: left ventricular end-diastolic pressure, MI: myocardial infarction, SBP: systolic blood pressure, DBP: diastolic blood pressure, Q: Quercetin. * $P < 0.05$, ** $P < 0.01$.

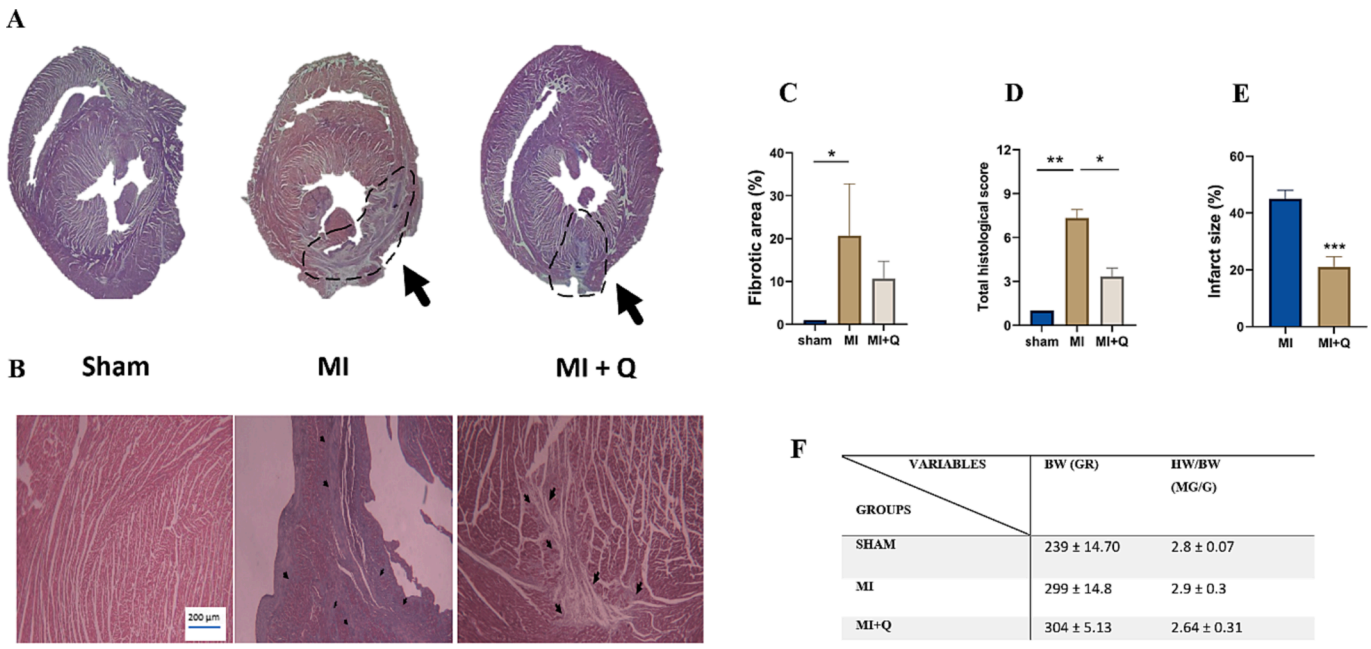

Fig. 3. Masson's trichrome staining showed that two weeks of quercetin treatment reduced the percent of fibrosis and histopathological indices in the heart with MI. (**A**) Representative stereomicroscopic images show infarct areas indicated by arrows in one animal in each group, and (**B**) microscopic images of Masson 's trichrome staining showing reduced fibrosis (bluish area) in the quercetin treatment group (×100 magnification, scale bar = 200 µm). (**C**) Quantification of the percent of fibrotic and (**D**) quantification of the total histopathological score indicates quercetin improved histopathological changes. (**E**) Infarct size in permanent LAD model after two weeks and (**F**) body weight and heart and lung weight to body weight ratios in the studied groups. MI: myocardial infarction, Q: Quercetin. * $P < 0.05$, ** $P \leq 0.01$, *** $P < 0.001$.

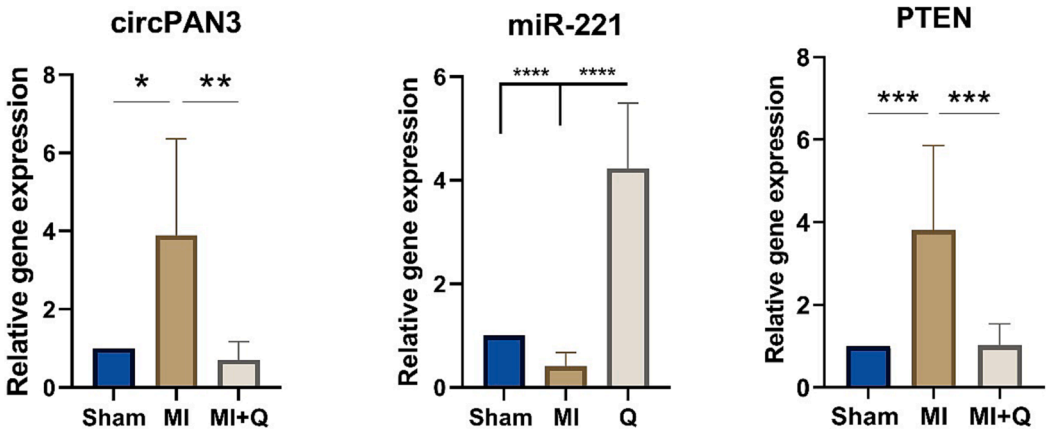

Fig. 4. Real-time PCR results for *circPAN3*, *miR-221*, and *PTEN* expression pattern in MI. MI: myocardial infarction, Q: Quercetin. *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

which is a direct target of *miR-221*, showed a significant increase ($P < 0.0001$) in the MI group compared to the control group. However, quercetin treatment significantly decreased the expression of *PTEN* ($P < 0.0001$) compared to the MI group, to the point where there was no significant difference from the control group. Considering that *circPAN3* targets *miR-221* and *miR-221* targets *PTEN*, the changes in *PTEN* expression are expected to be similar to *circPAN3* and opposite to *miR-221*. Our results demonstrated such changes in expression.

3.4. Effect of quercetin on PTEN/AKT/PI3K pathway

The protein levels of *PTEN*, *AKT*, *p-AKT*, *PI3K*, *p-PI3K*, and cleaved *caspase 3* were investigated in response to quercetin treatment to examine the *PTEN/AKT/PI3K* pathway (Fig. 5). Based on the results, the level of *PTEN* increased in the MI group ($P < 0.001$) compared to the sham group, while the levels of *p-AKT* ($P < 0.001$) and p-PI3K ($P < 0.001$) decreased about the total amount. Quercetin treatment reduced *PTEN* levels and increased the ratio of *p-AKT/AKT* ($P < 0.001$) and *p-PI3K/PI3K* ($P < 0.001$). Additionally, the expression of cleaved *caspase 3*, as a marker of apoptosis, which was significantly increased ($P < 0.001$) in the MI group, decreased ($P < 0.001$) following quercetin treatment.

4. Discussion

Preventing apoptosis of cardiac tissue early after MI plays a crucial role in protecting against the development and stabilization of MI pathogenesis. The circRNA-miRNA-mRNA regulatory network, based on RNA, holds significant importance in understanding the mechanism of gene expression regulation in various diseases, including MI. Drugs and

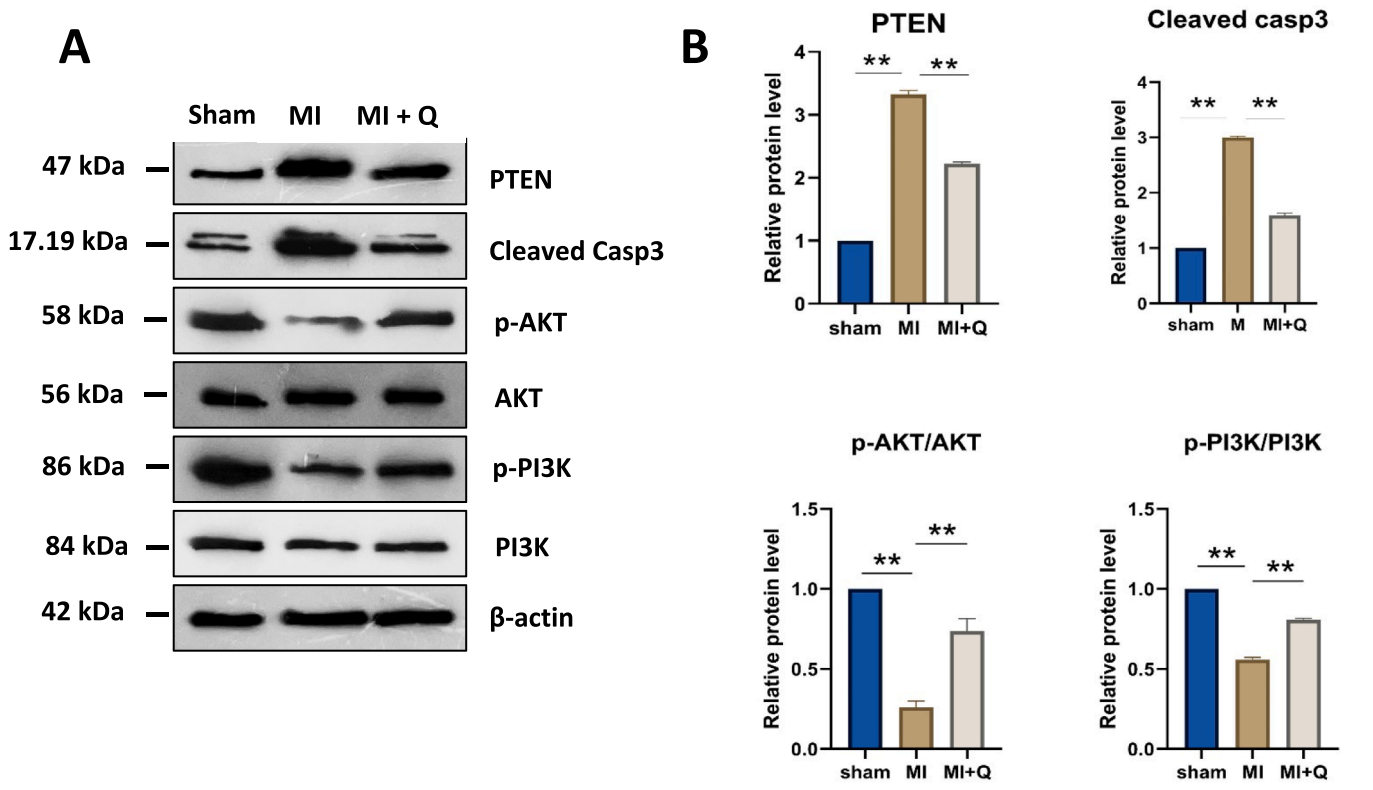

Fig. 5. Modulation of the level of proteins involved in the process of apoptosis. (**A**) The western blot results of *PTEN*, *caspase 3*, *p-AKT*, *AKT*, *p-PI3K*, *PI3K*, and *β-actin*. (**c**) Analysis of the level of *PTEN*, *caspase 3*, *p-AKT*, and *p-PI3K*. MI: myocardial infarction, Q: Quercetin. $^{**}P < 0.001$.

agents that can impact these pathways can be considered protective and therapeutic options. In this study, we demonstrated that quercetin exhibited a protective role against the development of infarct size and improved cardiac function in rat hearts with MI. Furthermore, we showed that its protective role may be attributed to its modulatory effect on the *circPAN3/miR-221/PTEN* pathway. *PTEN* serves as an important apoptotic protein in the heart tissue of rats with myocardial infarction, regulated by *miR-221* and circular *PAN3*. The cardioprotective effects of quercetin on cardiac function were associated with the modulation of the *circPAN3/miR-221/PTEN* pathway. Our results have demonstrated that treatment with quercetin improved cardiac indices and reduced the fibrotic area in the infarcted region two weeks after MI. Another study conducted by Albadrani et al. has also shown that quercetin prevents LV remodeling and fibrosis in remote myocardium (Albadrani et al., 2021). The reduction in fibrotic area and improvement in cardiac function is largely associated with the decrease in cardiomyocyte death and elimination from the myocardium, with the *PI3K/Akt* pathway being involved in this process (Walkowski et al., 2022; Huang et al., 2020). Enhancing this pathway, which involves phosphorylated and activated forms of *Akt* and *PI3K*, targets proteins involved in apoptosis such as caspases, leading to cell survival (Khezri,Ghasemnejad-Berenji,and Moloodsouri, 2023; Liu et al., 2020). Our results have shown that in the MI group, the phosphorylated and active forms of *PI3K/AKT* were decreased, while the level of cleaved caspase-3, indicative of apoptosis occurrence, was increased. *PTEN* is the most important inhibitor of the *PI3K/AKT* pathway and has a direct relationship with the occurrence of apoptosis in cardiomyocytes (Fujio et al., 2000). The increased expression of this protein in the MI group confirms that dephosphorylation of *PI3K/AKT* leads to an increase in cleaved *caspase-3* and apoptosis. However, there is no report available on the role of quercetin in regulating *PTEN/PI3K/AKT* in human diseases or animal models of human diseases. Our results have shown that quercetin treatment reduces *PTEN* expression in the heart tissue of rats with myocardial infarction. This decrease in expression is accompanied by an increase in the *P-Akt/P-PI3K* ratio and a decrease in cleaved caspase-3. In addition to demonstrating the relationship between *PTEN/PI3K/AKT* following MI, these results also confirm the modulation of this pathway by quercetin. Our results have also shown that this pathway is regulated by the ncRNA *circPAN3/miR-221*. *CircPAN3* is produced from the *PAN3* gene via back splicing (L. Zhang et al. 2022b). *CircPAN3* modulates autophagy and apoptosis, and whether apoptosis or autophagy ultimately occurs depends on the type of disease, the type of cell being studied, and the drug being used. In drug-resistant AML cell lines, the level of *circPAN3* is increased and is directly proportional to increased autophagy and drug resistance (Shang et al., 2019). On the other hand, Ji et al. have shown that treatment of cardiomyocyte H9c2 cell lines with doxorubicin, an important chemotherapeutic drug that can cause cardiotoxicity, reduces *circPAN3* and doxorubicin-induced apoptosis. They have shown that doxorubicin increases the expression of *miR-31-5p*, which directly inhibits the synthesis of *quaking* (*QKI*) and prevents the formation of the circular form of *PAN3*. In other words, *miR-31-5p* inhibits the formation of the circular form of *circPAN3* and acts upstream of *circPAN3* (Ji et al., 2020). Under these conditions, it has been shown that increasing *circPAN3* prevents cell death due to the cytotoxic effects of doxorubicin. Li et al. conducted a comprehensive study on rats with LAD ligation MI and identified *circPAN3* as a fibrotic factor (F. Li et al., 2020). *CircPAN3* had a direct relationship with fibrosis, autophagy, and apoptosis in the infarcted and border zone areas of the heart with MI. They created a cellular model of cardiac fibrosis by treating cardiac fibroblasts with *TGF-β* and observed that *TGF-β* increases the expression of *circPAN3* and inhibiting *circPAN3* can inhibit the fibrotic effects of *TGF-β*. By identifying the molecular pathway involved, they found that the autophagy and fibrotic pathways were interrelated, and *circPAN3* inhibits fibrosis by inhibiting autophagy. *CircPAN3* has a binding site for *miR-221* for sponging and inhibits its expression (F. Li et al., 2020). *miR-221* targets the *FoxO3* gene, and *FoxO3*, as a transcription factor, binds to the *ATG7* promoter and reduces its expression which is an important autophagy-related protein. Regarding the relationship between *circPAN3* and

apoptosis in MI, it was only observed that high expression of *circPAN3* is associated with apoptosis in cardiomyocytes in the infarcted and border zone areas, and the molecular mechanism of this relationship was not investigated. In this study, we have shown that through the *CircPAN3/miR-221/PTEN/PI3K/AKT* axis, apoptosis is increased in the MI heart tissue (Fig. 6). In other words, the ncRNA *CircPAN3/miR-221* axis can be a common point for apoptosis and autophagy in MI. In addition to targeting *foxo3*, *miR-221* also directly binds to *PTEN* and inhibits its expression (Wang et al., 2018; Kong et al., 2019). which is one of the important apoptotic proteins in cardiomyocytes. In the MI group, the increase in *circPAN3* expression led to the inhibition of *miR-221* and the increase in *PTEN* expression, which ultimately led to an increase in apoptosis, as evidenced by an increase in cleaved *caspase 3*. It appears that the ncRNA *CircPAN3/miR-221* axis may serve as a common point for the events of apoptosis and autophagy in MI.

One limitation of our study is that the effect of quercetin was only investigated for two weeks. Extending the duration of quercetin administration to one month or longer could provide further insights into its protective effects on fibrosis stabilization and hypertrophy. Additionally, it would be beneficial to investigate the effects of quercetin on cardiac regeneration and the proliferation of cardiomyocytes. These aspects could contribute to a more comprehensive understanding of quercetin's potential therapeutic benefits in cardiac health.

5. Conclusion

CircPAN3/miR-221 axis regulates apoptosis in MI through the *circPAN3/miR-221/PTEN/PI3K/AKT* pathway. This highlights the importance of ncRNA regulatory networks, which can have diverse effects on biological processes in MI. Quercetin protectively modulated this RNA-based regulatory pathway by reducing *circPAN3* which subsequently increased *miR-221*, decreased *PTEN*, activated the *PI3K/AKT* pathway, and ultimately decreased apoptosis, improving cardiac function, and reduced the infarct size in MI.

Author contributions

SJF and FR conceptualized the study. SJF, FR, and SG designed and interpreted the experiments. FR established the animal model. MMF and MM performed molecular experiments. EJ performed a pathological section and analyzed data. FR and SJF performed hemodynamic and molecular analysis. SJF, MMF wrote the article. All authors provided comments on this manuscript.

Ethics statement.

All procedures performed in this study were by the ethical standards of the Kerman University of Medical Sciences (KMU) Ethics Committee (IR.KMU.AEC.1402.012) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

CRediT authorship contribution statement

Mohammad Mojtaba Farazi: Writing – original draft, Methodology. **Farzaneh Rostamzadeh:** Validation, Methodology. **Saeideh Jafarinejad-Farsangi:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Maryam Moazam Jazi:** Software, Methodology, Data curation. **Elham Jafari:** Writing – original draft, Methodology. **Sedigheh Gharbi:** Writing – review & editing, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

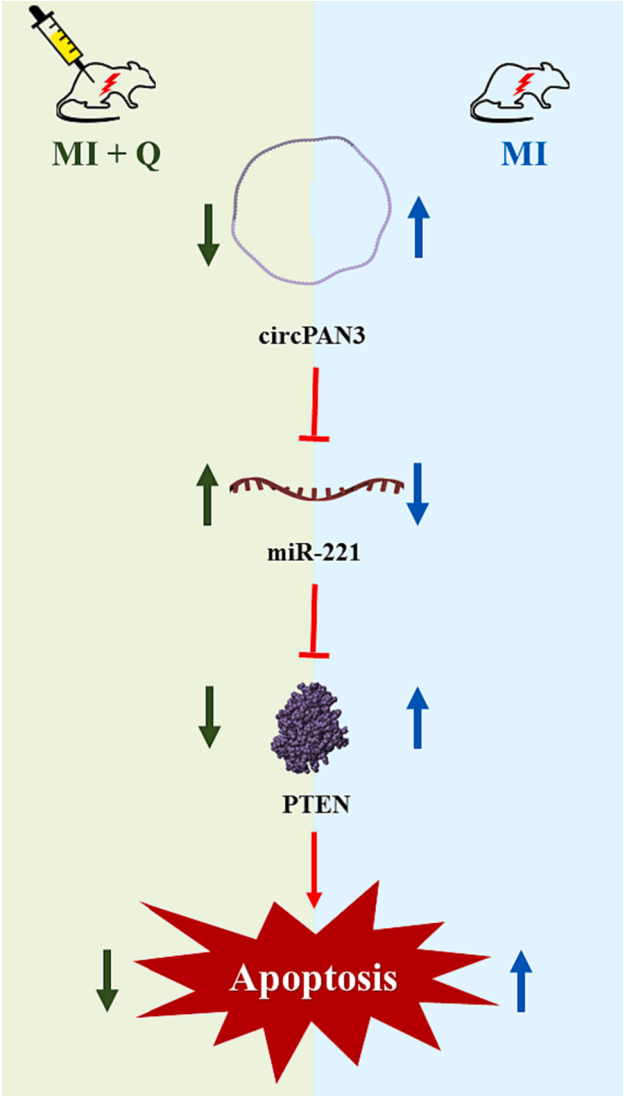

Fig. 6. Schematic illustration of the relationship between *circPAN3* and *miR-221* and *PTEN* in MI and under two weeks of quercetin treatment. According to the obtained results, two weeks of quercetin treatment can significantly reduce the expression of *PTEN* and thus inhibit apoptosis in MI. MI: myocardial infarction, Q: Quercetin.

Data availability

Data will be made available on request.

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