Quercetin protects against inflammation, MMP-2 activation and apoptosis induction in rat model of cardiopulmonary resuscitation through modulating Bmi-1 expression

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Abstract. With extensive pharmacological actions, quercetin has anti-oxidant, free radical scavenging, anti-tumor, anti-inflammatory, anti-bacterial and anti-viral activity. Quercetin also reduces blood glucose and reduces high blood pressure, and has immunoregulation and cardiovascular protection functions. Additionally, it has been reported that it can reduce depression. The current study evaluated whether quercetin protects against inflammation, matrix metalloproteinase-2 (MMP-2) activation and apoptosis induction in a rat model of cardiopulmonary resuscitation (CPR), and whether Bmi-1 expression was involved in the effects. In CPR model rats, treatment with quercetin significantly recovered left ventricular ejection fraction, left ventricular fractional shortening, ejection fraction (%), and left ventricle weight/body weight. Treatment with quercetin significantly inhibited ROS generation, inflammation and MMP-2 protein expression in the rat model CPR. Finally, quercetin significantly suppressed caspase-3 activity and activated Bmi-1 protein expression in the rat model of CPR. The results demonstrated that quercetin protects against inflammation, MMP-2 activation and apoptosis induction in a rat model of CPR, and that this may be mediated by modulating Bmi-1 expression.

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Introduction

Cardiopulmonary arrest is common seen in emergency practice. With the standardization of cardiopulmonary resuscitation (CPR), the rate of resuscitation is increased (1). However, ~60% of patients experience cerebral resuscitation failure and 2-3% of patients that survive suffer from severe nerve function deficits (1). The prognosis of resuscitated patients depends on the levels of cerebral ischemic injuries (2). Reperfusion injury following complete cerebral ischemia is the major cause of cerebral injury. Following CPR, patients experience ischemia-reperfusion injury (3). It is established that the mechanisms of ischemia-reperfusion injury are associated with oxygen radicals and calcium overload (4). Following CPR, various inflammatory cells are activated and produce cytokines that participate in the damaging effects of ischemia-reperfusion (5). At the early stage of CPR, the increase of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) indicates that the inflammatory response system is activated following CPR. The release of cytokines can be considered as a stress response to cardiac arrest (6).

The uniform conduction of electrical activity depends on electrical coupling among myocardial cells and is influenced by the extracellular matrix (ECM) (7). Under normal physiological conditions, the synthesis and degradation of ECM is in dynamic equilibrium (8). Its excessive synthesis or abnormal degradation may alter the mechanical properties of myocardium and electrophysiological structures, which may affect its uniform conduction (9). Previous studies revealed that the regulation of matrix metalloproteinases (MMPs) have an important role in the synthesis and degradation of ECM (7,10).

B-cell specific moloney leukemia virus insertion site 1 (Bmi-1) is an important member of the Polycomb-group protein family (11). It has essential actions in repairing DNA damage, cell cycle control, the stability of chromatin, the activation of genetic transcription and apoptosis (12).

Patients with cardiac arrest have obvious central lesions following CPR. Following complete cerebral ischemia-reperfusion injury, hydromechanics, pathomorphology and metabolic

disorders may form encephaledema, and severity influences patient prognosis (13). Clinic trials demonstrated that even when autonomic circulation recovered following CPR, some patients still experienced loss of consciousness (14).

Quercetin is a polyhydroxy flavonoid widely present in flowers, leaves and fruits, with various biological activities and high pharmaceutical value (15). Quercetin is present in >100 types of Chinese herbal medicine, including sophora flower bud, sophora flower, the root bark of the peony tree, chrysanthemum, *Hypericum japonicum*, Parasemia plantaginis, parasitic *Loranthus*, hairyvein agrimony, Perfoliate knotweed herb, *Gynostemma pentaphyllum*, maythorn, *Hypericum perforatum*, semen cuscutae, ginkgo leaf, *Aesculus wilsonii* rehd, *Oldenlandia diffusa*, cockscomb, *Houttuynia cordata*, emblic leafflower fruit, *Saururus chinensis*, *Psidium guajava* leaf, phyllan lhus mat shilllirae, Chinese violet and *Sedum sarmentosum* (16-19). The results of the current study demonstrated that quercetin inflammation, MMP-2 activation and apoptosis induction in a rat model of CPR and investigated the mechanism of the protective effect of quercetin following CPR.

Materials and methods

Experimental animals. The protocol of animal experiments was approved by the University Laboratory Animal Research Committee of The First Hospital of Jilin University (Changchun, China). Adult Sprague-Dawley (SD) rats (250±30 g; n=30) were purchased from Qinghai Experimental Animal Center (Qinghai, China) and maintained in a specific pathogen-free environment. The CPR model was established by an asphyxia method and animals anaesthetized with intraperitoneal injection of chloral hydrate (Shanghai Ruiqi Biological Engineering Research Center, Shanghai, China). Tracheal intubation was closed at the end of expiration for 5 min. SD rats were randomly divided into sham operation group (sham; n=6), quercetin group (QCT; n=6), Model group (Model, n=8) and Model +quercetin group (Model + QCT; n=8). In the QCT group and Model + quercetin group, normal or CPR model SD rats were treated with intragastric injection of 50 mg/kg quercetin once a day for 5 days, respectively. The chemical structure of quercetin is presented in Fig. 1 and was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). The rats of Model and QCT group were anaesthetized with 35 mg/kg pentobarbital sodium, tracheal intubation was closed at the end of expiration for 5 min and cardiopulmonary resuscitation was performed. The rats of sham group were anaesthetized with 35 mg/kg pentobarbital sodium without cardiopulmonary resuscitation.

Hemodynamic changes. After treatment with quercetin, surgical procedures were performed on all SD rats to measure hemodynamic parameters. Left ventricular dysfunction systolic (LVDs), left ventricular dysfunction diastolic (LVDd), stroke volume (SV) and cardiac output (CO). Ejection fraction (EF%) and left ventricular shortening fraction (FS%) was calculated using the same area-length method as previously described (19).

ELISA. Serum was separated from venous blood of each rat following intragastric injection treatment with quercetin and used to measure reactive oxygen species (ROS) generation
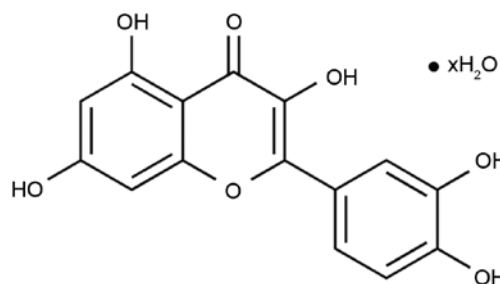

Figure 1. Chemical structure of quercetin.

(S0033), IL-6 (PI328) and TNF-α (PT516) activities using assay kits according to the manufacturer's instructions (Beyotime Institute of Biotechnology, Haimen, China). Caspase-3 activity was measured using a caspase-3 activity detection kit (C1116, Beyotime Institute of Biotechnology, Haimen, China).

Left ventricle weight/body weight. After 4 weeks, body weight was measured and recorded. Subsequently, left ventricle weight was acquired. Left ventricle weight/body weight was calculated.

Western blotting analysis. Heart tissue samples were homogenized in radioimmunoprecipitation assay buffer (10 μg/ml, Beyotime Institute of Biotechnology) in the presence of protease inhibitors (EMD Millipore, Billerica, MA, USA). The supernatant was collected to measure total proteins using the bicinchoninic acid method (Beyotime Institute of Biotechnology). Total proteins (50 μg) were separated by 12% SDS-PAGE and transferred to nitrocellulose membranes. Following blocking with 5% non-fat milk in TBS-Tween at 37°C for 1 h, membranes were incubated with anti-MMP-2 (sc-10736, 1:5,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), anti-Bmi-1 (sc-10745, 1:2,000; Santa Cruz Biotechnology, Inc.), anti-inducible nitric oxide synthase (iNOS;sc-649, 1:3,000; Santa Cruz Biotechnology, Inc.) and β-actin (sc-7210, 1:2,000; Santa Cruz Biotechnology, Inc.) at 4°C overnight, followed by incubation with goat anti-rabbit IgG-HRP secondary antibodies (sc-2004, 1:5,000; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. The blot images were observed using BeyoECL Moon kit (P0018F; Beyotime Institute of Biotechnology) and analyzed with Image_Lab version 3.0 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Statistical analysis. The values are presented as the mean ± standard error. One-way analysis of variance followed by a Tukey post hoc test was used to analyze the differences between groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Quercetin protects LVDs and LVDd in a rat model of CPR. CPR rat were treated with 50 mg/kg quercetin, and LVDs and LVDd levels were measured to evaluate that quercetin protects against CPR. As presented in Fig. 2A, the LVDs of CPR model rats was higher than that of the sham group. However, quercetin significantly inhibited the LVDs of CPR model rats
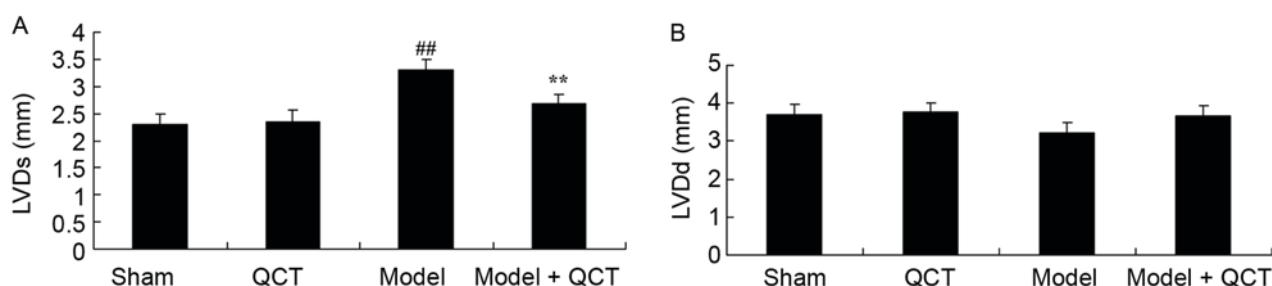
Figure 2. Quercetin protects against LVDs and LVDd levels in rat model of CPR. (A) LVDs and (B) LVDd levels were determined in the rat model of cardiopulmonary resuscitation. ##P<0.01 vs. sham group; **P<0.01 vs. model group. LVDs, left ventricular dysfunction systolic; QCT, quercetin group; Model, CPR model; Model + QCT, CPR model + quercetin group; LVDd, left ventricular dysfunction diastolic; CPR, cardiopulmonary resuscitation.
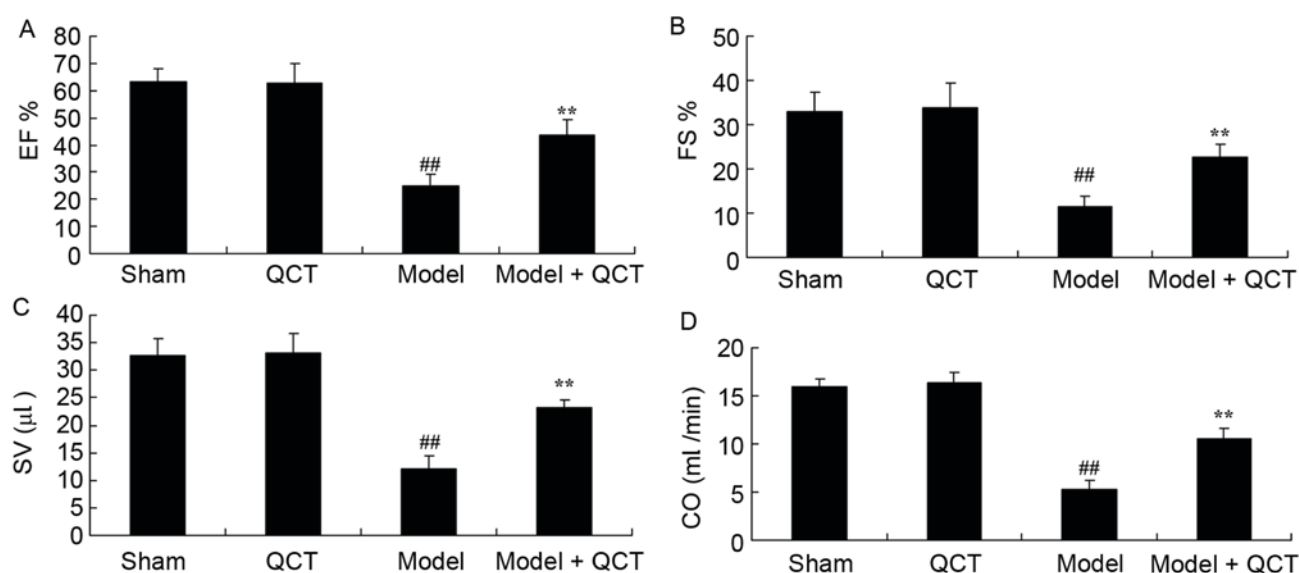

Figure 3. Quercetin protects EF, FS, SV and CO in rat model of CPR. (A) EF, (B) FS, (C) SV and (D) CO in rat model of cardiopulmonary resuscitation. ##P<0.01 vs. sham group; **P<0.01 vs. model group. EF, ejection fraction; QCT, quercetin group; Model, CPR model; Model + QCT, CPR model + quercetin group; FS, left ventricular shortening fraction; SV, stroke volume; CO, cardiac output; CPR, cardiopulmonary resuscitation.
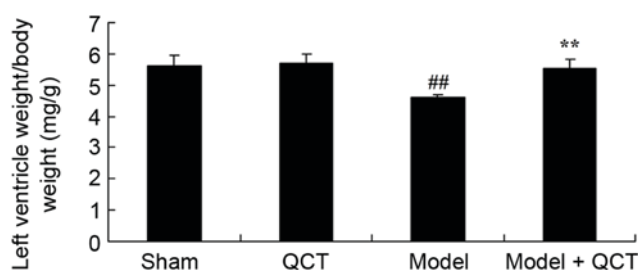

Figure 4. Quercetin protects left ventricle weight/body weight in a rat model of CPR. ##P<0.01 vs. sham group; **P<0.01 vs. model group. QCT, quercetin group; Model, CPR model; Model + QCT, CPR model + quercetin group; CPR, cardiopulmonary resuscitation.

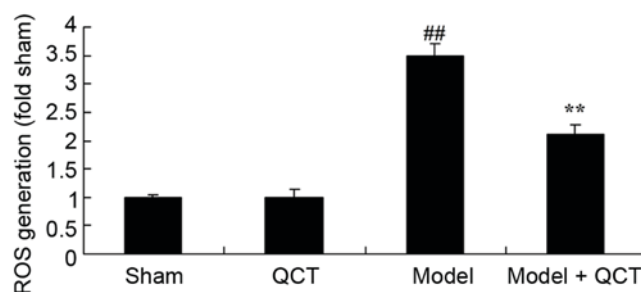Figure 5. Quercetin protects against ROS generation in rat model of CPR. ##P<0.01 vs. sham group; **P<0.01 vs. model group. ROS, reactive oxygen species; QCT, quercetin group; Model, CPR model; Model + QCT, CPR model + quercetin group; CPR, cardiopulmonary resuscitation.

(Fig. 2A). There was no significant difference in the LVDd between the sham, QCT, CPR model group and model + QCT groups (P>0.05; Fig. 2B).

Quercetin protects the EF, FS, SV and CO in a rat model of CPR. It was evaluated whether quercetin protects EF, FS, SV and CO in the rat model of CPR. There was no significant difference between the sham group and QCT group (Fig. 3). However, the levels of EF, FS, SV and CO in the CPR model rats were significantly lower than those of the sham group (Fig. 3). Treatment with quercetin significantly increased the EF, FS, SV and CO levels in CPR model rats (Fig. 3).
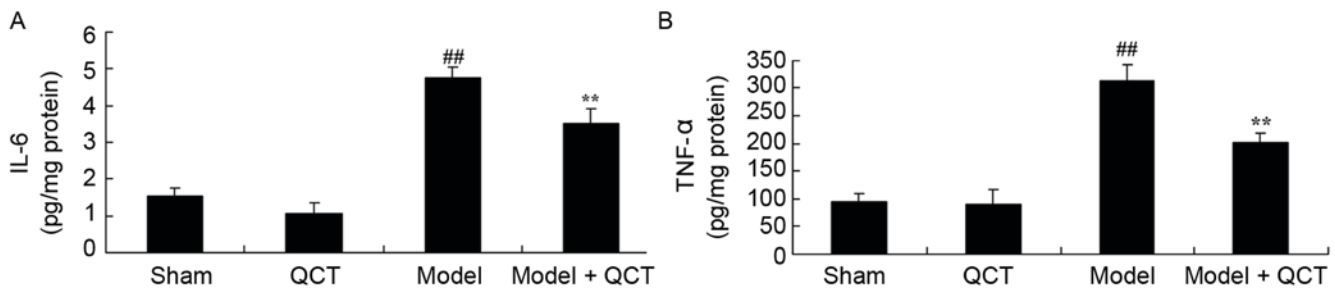
Figure 6. Quercetin protects against inflammation in rat model of CPR. (A) IL-6 and (B) TNF-α were measured in a rat model of CPR. ##P<0.01 vs. sham group; **P<0.01 vs. model group. IL-6, interleukin; QCT, quercetin group; Model, CPR model; Model + QCT, CPR model + quercetin group; TNF-α, tumor necrosis factor-α; CPR, cardiopulmonary resuscitation.

Quercetin protects the left ventricle weight/body weight in a rat model of CPR. Subsequently, it was verified whether quercetin protects the left ventricle weight/body weight in a rat model of CPR. As presented in Fig. 4, there was no significant difference in left ventricle weight/body weight between sham group and QCT group. The left ventricle weight/body weight in the CPR model group was significantly lower than that of the sham group (Fig. 4). Treatment with quercetin significantly enhanced left ventricle weight/body weight in CPR model rats (Fig. 4).

Quercetin protects against ROS generation in rat model of cardiopulmonary resuscitation. The results demonstrated that there was no significant change in ROS generation between the sham group and QCT group (P>0.05; Fig. 5). The CPR model significantly induced ROS generation in rats compared with the sham group (Fig. 5). Treatment with quercetin significantly suppressed ROS generation in CPR model rats (Fig. 5).

Quercetin protects against inflammation in rat model of cardiopulmonary resuscitation. To determine the anti-inflammatory effect of quercetin in CPR model rats, IL-6 and TNF-α levels were measured. IL-6 and TNF-α were similar in the sham group and QCT group (P>0.05; Fig. 6). IL-6 and TNF-α levels were significantly induced in the CPR rat model group compared with the sham group (Fig. 6). Treatment with quercetin significantly inhibited the activation of IL-6 and TNF-αin CPR model rats (Fig. 6).

Quercetin reduces MMP-2 and iNOS protein expression and induces Bmi-1 protein expression in a rat model of CPR. It was evaluated whether quercetin protects against increased MMP-2, iNOS and Bmi-1 protein expression in a rat model of CPR using western blotting analysis. There was no significant difference in MMP-2, iNOS and Bmi-1 protein expression between the sham group and QCT group (Fig. 7). However, CPR significantly induced MMP-2 and iNOS protein expression and suppressed Bmi-1 protein expression in compared with the sham group (Fig. 7). Quercetin treatment significantly inhibited MMP-2 and iNOS protein expression, and induced Bmi-1 protein expression in cardiopulmonary resuscitation rat (Fig. 7).

Quercetin protects against caspase-3 activity in rat model of CPR. The current results revealed that there was no

significant difference in caspase-3 activity between the sham group and QCT group (P>0.05; Fig. 8). Caspase-3 activity was significantly increased in the CPR model group compared with the sham group (Fig. 8). Quercetin treatment significantly inhibited caspase-3 activity in CPR model rats (Fig. 8).

Discussion

CPR causes hypoxia-ischemia. Effective and timely CPR may restore the heart beat for the majority of patients (20). However, cerebral injury following resuscitation remains difficult issue for complete rehabilitation (21). Following successful CPR, the autonomic circulation recovery of the cerebral blood flow causes cerebral-reperfusion injury and may cause further aggravation of the prognosis of cerebral functions and mortality (22). In present study, quercetin significantly reduced the LVDs, increased the EF, FS, SV and CO, and enhanced left ventricle weight/body weight in CPR model rats.

Following cardiac arrest, organisms are in severe and general hypoxic-ischemic states, which lead to reperfusion injury and secondary lesions of visceral organs (23). During CPR, organisms produce stress reactions. Under strong pathological stimuli, cytokines are produced and cascade reactions are triggered. Reperfusion injury following cardiac arrest is closely associated with pro-inflammatory cytokines, including TNF-α, IL-1 and IL-6 (21). The current study demonstrated that treatment with quercetin significantly inhibited the activation of IL-6 and TNF-α release and suppressed ROS generation in CPR model rats. Liu *et al* (24) reported that quercetin suppressed insulin-mediated glucose disposal during inflammatory conditions in skeletal muscle tissue/cells.

In cardiac muscle tissues, MMPs and TIMPs are tightly balanced. If the level of MMPs increases, the balance is disrupted, which may cause the remodeling of ECM (25). Previous studies reported that MMPs have an important role in left atrioventricular remodeling following acute myocardial infarction, chronic heart failure, hypertension, diastolic cardiomyopathy and atrial fibrillation (25,26). MMPs also participate in pathophysiological processes, including platelet aggregation, angiotasis regulation, inflammation and ischemia-reperfusion injury (27). However, studies on the
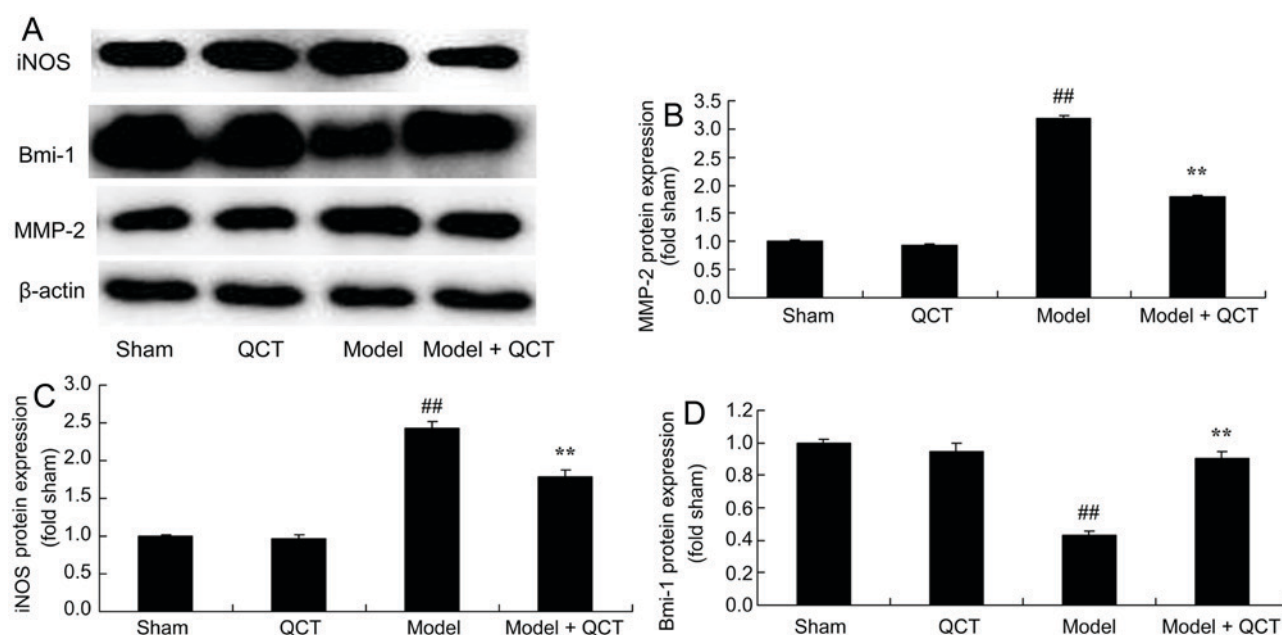
Figure 7. Quercetin reduces MMP-2 and iNOS protein expression and induced Bmi-1 protein expression in a rat model of CPR. (A) Western blotting and (B-D) densitometry analysis of MMP-2, iNOS and Bmi-1 protein expression in a rat model of CPR. ##P<0.01 vs. sham group; **P<0.01 vs. model group. MMP-2, matrix metalloproteinase-2; QCT, quercetin group; Model, CPR model; Model + QCT, CPR model + quercetin group; CPR, cardiopulmonary resuscitation.

association between MMPs and ventricular fibrillation are rare. In the current study, quercetin treatment significantly inhibited MMP-2 protein expression in CPR model rats. Barteková et al (16) demonstrated that quercetin improves post-ischemic recovery of heart function through suppression of MMP-2 and anti-apoptosis.

Reperfusion following CPR can cause server cerebral anoxia and ultimately result in dysneuria. Randomized clinical trial confirmed that low temperature therapy is effective for coma patients following CPR, with ventricular fibrillation to improve dysneuria. It has been reported that fast cooling of the head when conducting CPR can improve survival rates and nervous system functions, while studies on the endogenous protective mechanisms involved are rare (29). Biological behaviors and external information transmission are produced by a series of signal transduction and regulation mechanisms. Signal transduction systems have an essential role in cell differentiation, growth, apoptosis and gene expression (30). In the current study, quercetin treatment significantly inhibited caspase-3 activity in CPR model rats.

It is generally established that Bmi-1 is highly expressed in hematopoietic stem cells and neural stem cells (31). Bmi-1 is involved in maintaining the self-renewal capacities of stem cells and has an important role in stem cell growth (32). In the current study, treatment with quercetin significantly increased the protein expression of Bmi-1 and suppressed the protein expression of iNOS in CPR model rats. Dong et al (32) demonstrated that quercetin attenuates doxorubicin cardiotoxicity through activation of Bmi-1 expression. Zhang et al (15) suggested that quercetin protected endothelial NOS expression in cavernous endothelial cells.

In summary, quercetin significantly inhibited the LVDs, increased EF, FS, SV and CO, and enhanced left ventricle
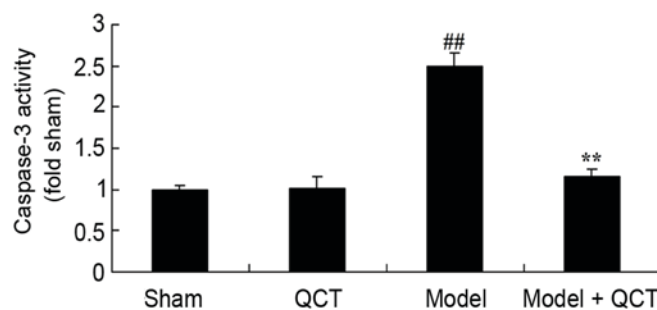

Figure 8. Quercetin reduces caspase-3 activity in rat model of CPR. ##P<0.01 vs. sham group; **P<0.01 vs. model group. QCT, quercetin group; Model, CPR model; Model + QCT, CPR model + quercetin group; CPR, cardiopulmonary resuscitation.

weight/body weight in a rat CPR model. Additionally, quercetin protected against inflammation, MMP-2 activation, iNOS expression and apoptosis, and modulated Bmi-1 expression in rat model of CPR.

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Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

X-LL and NZ conceived and designed the experiment; DW, XL, X-MJ and CY performed the experiments; X-LL and NZ wrote the paper. DW, X-LL and NZ analyzed the data.

Ethics approval and consent to participate

The protocol of animal experiments was approved by the University Laboratory Animal Research Committee of The First Hospital of Jilin University (Changchun, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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