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Original Research Article

γ-Mangostin alleviates myocardial ischemia-reperfusion injury by up-regulating SIRT3

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Abstract

Purpose: *To determine the effect of γ-mangostin on myocardial ischemia (MI) -induced injury of myocardial cells, and the possible involvement of SIRT3 in myocardial cell apoptosis in Sprague-Dawley rats after ischemia-reperfusion (I/R).*
Methods: *Ischemic reperfusion (I/R) model of rat was established, followed by TTC staining. The serum levels of CK-MB and LDH were also assessed. In addition, inflammatory response and oxidative stress were evaluated by quantitative PCR and enzyme linked immunosorbent assay (ELISA), while cell apoptosis was assessed using TdT-mediated dUTP nick end labeling (TUNEL) assay and western blot. The mechanism of action of γ-mangostin by which it mediated improvement in cardiac injury was investigated by ELISA and western blot.*
Results: *γ-Mangostin attenuated myocardial injury and reduced myocardial inflammation in I/R rats ($p < 0.05$). In addition, it alleviated oxidative stress in I/R rat myocardial tissues and suppressed apoptosis. Furthermore, γ-mangostin improved myocardial injury probably by targeting SIRT3 ($p < 0.05$).*
Conclusion: *γ-Mangostin has potentials for use as a therapeutic agent for the treatment of myocardial I/R injury. However, there is a need for clinical trials on the compound.*

Keywords: *Myocardial ischemia (MI), γ-Mangostin, Oxidative stress, Apoptosis, SIRT3, Inflammatory response*

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INTRODUCTION

Myocardial ischemia (MI) causes fatality worldwide, and ischemic reperfusion (I/R) is the current standard strategy for its treatment. However, approximately 30 % of patients experience myocardial reperfusion (I/R)-induced injury [1]. In most cases, IR damage is due to immunity-induced accumulation of inflammatory cells in the infarction area under reperfusion. Several factors such as oxidative stress play a role in myocardial I/R injury. Moreover, cardiac apoptosis and inflammation are considered as markers of myocardial I/R injury [2,3]. To combat this disease, more effective drugs are critically needed.

Mangosteen (MS) has long been used as food and medicine, and the dried skin of the fruit is especially used to treat skin-related diseases. The yellow resin that exudes from MS is a rich source of xanthanone and many bioactive

substances, such as α-, β- and γ -mangosteins (γ-MS) [4]. γ-Mangostin is a key bioactive compound found in mangosteen, which inhibits inflammation and improves osteoarthritis [5]. γ -Mangostin inhibited lipid peroxidation and DPPH free radical formation, and enhanced SIRT3 expression in liver fibrosis induced by oxidative stress [6]. However, its possible effects on myocardial I/R injury are still unclear.

Sirtuin-3 (SIRT3) is a highly conserved nicotinamide adenine dinucleotide (NAD+) dependent deacetylase [7]. SIRT3, SIRT4 and SIRT5 are three members of the sirtuin family, among which SIRT3 was the first to be discovered [8]. The activation of SIRT3 signaling pathway reduces oxidative stress and apoptosis and so ameliorates MI/R injury [9]. SIRT3 mitigates MI/R damage by stimulating specific downstream targets such as FoxO3a [10]. However, the role and mechanism of γ-mangostin in myocardial ischemia-reperfusion remain unclear.

In this study, the possible effects of γ-mangostin was investigated on myocardial I/R injury.

EXPERIMENTAL

Animals and surgical procedures

This study was approved by the Ethics Committee of The First People's Hospital of Fuyang Hangzhou, China for the use of animals (approval no. 2021011) and performed in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines [11]. For establishment of the I/R rat model, male Sprague–Dawley rats (250 - 300 g) were anesthetized with pentobarbital (80 mg/kg, ip). The animals were randomly divided into sham, I/R, I/R+γ-mangostin (50 mg/kg), I/R+γ-mangostin (100 mg/kg), and I/R+γ-mangostin (200 mg/kg) groups. An intravenous 24-gauge catheter was then installed in the left jugular vein for γ-mangostin administration (30 min before I/R injury).

Measurement of myocardial infarct size

After reperfusion, the hearts were excised, snap-frozen immediately, and then cut into 2 – 3 mm thick slices along the sagittal plane of the heart. They were stained in TTC solution for 30 min, and then fixed in 10 % formaldehyde solution for 8 h. The cerebral peduncle was delineated by Image J and the area of cerebral peduncle was calculated

Assessment of serum levels of CK-MB and LDH

Following reperfusion, blood samples were collected from the carotid artery and left for 0.5 h; serum was then obtained by centrifugation at 3,000 × *g* at 4 °C for 15 min and stored at -70 °C until used. For CK-MB determination, 100 µl of each standard or samples were added to each well and incubated for 1.5 h at 37 ºC. The cover was removed and the contents of the plate was discarded.

An aliquot (0.1 mL) of Biotin-detection antibody work solution was added to the wells, the plate was sealed and incubated at 37 ºC for 60 min, followed by washing with wash buffer. An aliquot (0.1 mL) of SABC working solution was added to each well, and incubated at 37 ºC for 30 min. Next, TMB substrate was added to each well, and incubated at 37 ºC in the dark for 30 min. Then, 50 µl of Stop Solution was added to each well. The absorbance was raed at 450 nm within 20 min.

For LDH assessment, a master mix of the reaction mix was prepared, and 50 µL of the reaction mix was added to each standard, sample and positive control sample wells. Absorbance was measured at 450 nm (T1) on a microplate reader in a kinetic mode, every 3 min for at least 30 min at 37 °C, protected from light.

Enzyme linked immunosorbent assay (ELISA)

The concentrations of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) in the serum were measured with ELISA kit following the protocols. All samples or standards, were added to the respective ELISA plates and incubated simultaneously. After washing, the enzyme streptavidin-peroxidase that binds to the aforementioned biotinylated antibody was added to each well. To induce a colored reaction product, a TMB (3, 3', 5, 5'-Tetramethylbenzidine) substrate solution was added. A linear standard curve was used for the determination of TNF-α, IL-1β, and IL-6 concentration.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Heart total RNA was extracted with TRIzol reagents (Thermo, Rockford, USA), and total RNA was reverse-transcribed into cDNA using M-MLV reverse transcriptase (Promega, USA). The cDNA was amplified using the primers shown in Table 1.

Table 1: PCR primer sequences

Gene	Sequences
TNF-α-fp	GGTGCCTATGTCTCAGCCTCTT
TNF-α-rp	GCCATAGAACTGATGAGAGGGAG
IL-1β-fp	ACAAGGAGAAGAAAGTAATGAC
IL-1β-rp	GCTGTAGAGTGGGCTTAT
IL-6-fp	AGACAGCCACTCACC
IL-6-rp	TTCTGCCAGTGCCTCTT
caspase-1-fp	ATGGCCGACAAGGTCCTGA
caspase-1-rp	TTTAATG TCCTGGGAAGAGGTAGA
GAPDH-fp	AGAAGGCTGGGGCTCATTTG
GAPDH-rp	AGGGGCCATCCACAGTCTTC

Evaluation of superoxide dismutase (SOD), malondialdehyde (MDA), GSH-px and reactive oxygen species (ROS)

Heart tissues were collected for the determination of MDA, SOD, GSH-px, and ROS using the appropriate commercial kits (Jiancheng Bioengineering Institute of Nanjing, China).

TUNEL assay

Frozen heart slices (8 μm thick) were fixed with 4 % paraformaldehyde for 1 h at 25 °C, immersed in 0.1 % Triton X-100 for 2 min on ice, and then incubated with 50 μl TUNEL reaction mixture for 1 h at 37 °C in the dark. The numbers of TUNEL-positive cells were counted.

Western blot assay

Total proteins from brains were extracted with RIPA buffer (Beyotime, Shanghai, China). Then the samples were collected and electrophoresed by 10 % SDS-PAGE, transferred onto PVDF membranes, and then blocked with 5 % fat-free milk. Subsequently, the membranes were incubated with primary antibodies targeting SIRT3 (Mouse, 1:1000, Abcam), Bax (Mouse, 1:1000), Bcl-2 (Mouse, 1:1000), cleaved caspase-3 (Mouse, 1:1000), p-p65 (Mouse, 1:1000), p65 (Mouse, 1:1000), and β-actin (Mouse, 1:10000) for 1 h. The membranes were conjugated with the anti-mouse IgG and anti-rabbit IgG (Abcam) for 1 h. Specific proteins were visualized with enhanced chemiluminescence detection kit (ECL, USA).

Statistical analysis

GraphPad 6.0 was used for the statistical analysis. Three replicates were performed for each experiment, and one-way ANOVA and Student's *t*-test were used for statistical comparisons. $P < 0.05$ was taken as significant.

RESULTS

γ-Mangostin ameliorates cardiomyocyte damage induced by I/R injury

There was a significant increase of serum CK-MB and LDH in I/R rats (Figure 1 A). However, γ-mangostin treatment significantly reduced the levels in CK-MB and LDH levels (Figure 1 A). Infarction size is displayed in Figure 1 B. I/R injury significantly induced heart infarction, but γ-mangostin preconditioning significantly reduced the infarction size compared to the I/R groups (Figure 1 B).

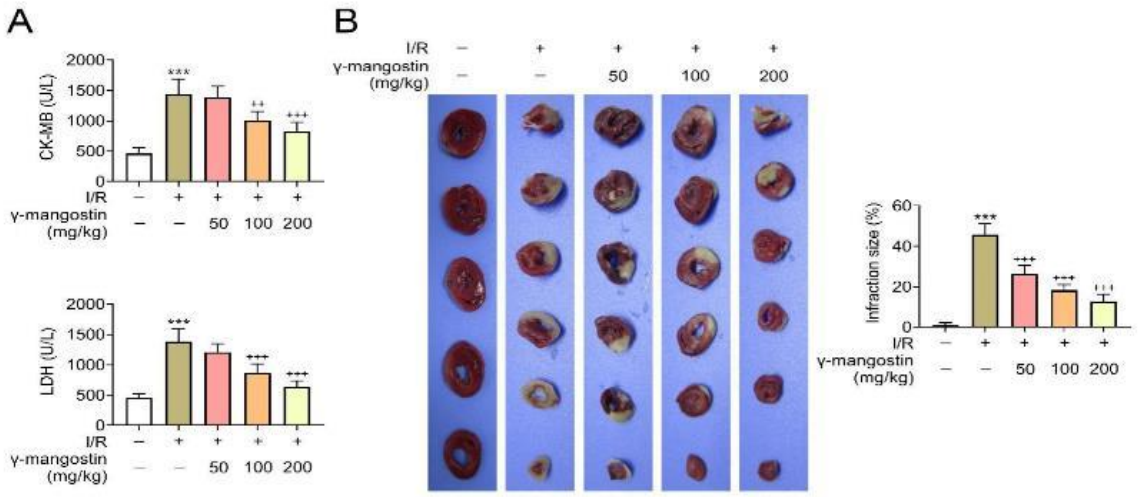

Figure 1: γ-Mangostin mitigated the cardiomyocyte damage induced by I/R injury. (A) CK-MB and LDH in sham, I/R, I/R+γ-mangostin (50 mg/kg), I/R+γ-mangostin (100 mg/kg), I/R+γ-mangostin (200 mg/kg) group. (B): TTC staining shows that γ-mangostin preconditioning reduced infarct size compared to I/R groups. Data are displayed as mean ± SD (n = 5). ***$P < 0.01$ versus control group; ++$p < 0.01$, +++$p < 0.001$; I/R+γ-mangostin versus I/R group

γ-Mangostin inhibited inflammation induced by I/R injury

The I/R injury was accompanied by increased inflammation response. Therefore, the inflammation status in the I/R models were analyzed. The level of IL-6, IL-1β and TNF-α were enhanced in I/R rats. However, γ-mangostin significantly lowered the level of IL-6, IL-1β and TNF-α in I/R rats, both in both serum and cardiomyocytes in a dose-dependent manner (Figure 2 A and B).

γ-Mangostin suppressed oxidative stress induced by I/R injury

Since oxidative stress plays a vital role in I/R injury, the level of ROS, MDA, GSH-px and SOD levels were analyzed in different groups. The induction of MDA and ROS, and reduction of SOD and GSH-px were observed in the I/R group. Treatment with γ-mangostin reversed the changes in the level of SOD, MDA, GSH-px and ROS in a dose-dependent manner (Figure 3). Therefore, γ-mangostin is associated with reduced oxidative stress in I/R rats.

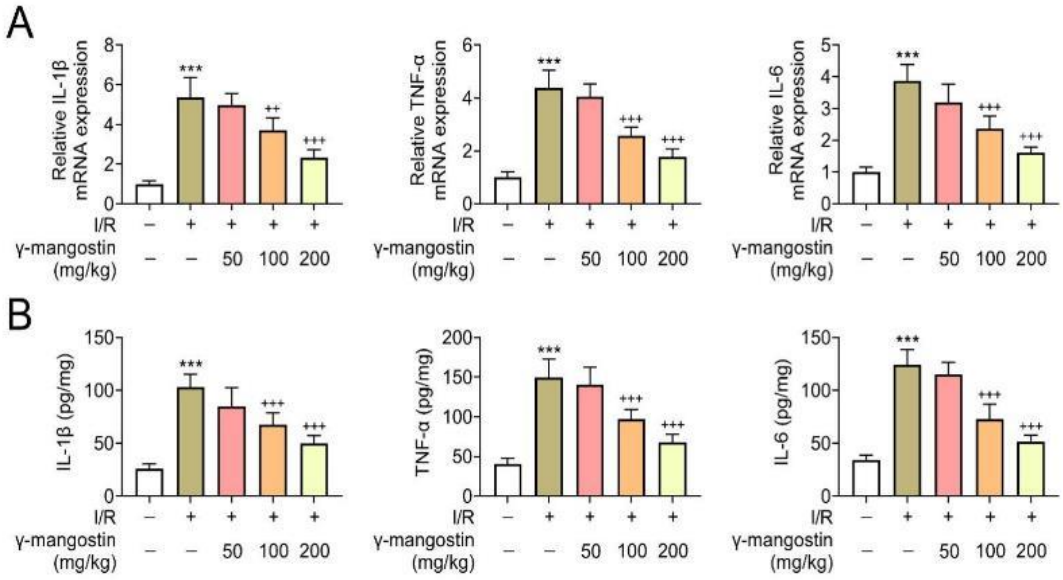

Figure 2: γ-Mangostin inhibited inflammation induced by I/R injury (A) Relative mRNA expression levels of IL-1b, IL-6 and TNF-a in each group (B) Serum level of IL-1b, IL-6 and TNF-a. Data are displayed as mean ± SD; ***p < 0.01 versus control group; +p < 0.05, ++p < 0.01, +++p < 0.001, I/R+γ-mangostin versus I/R group, respectively

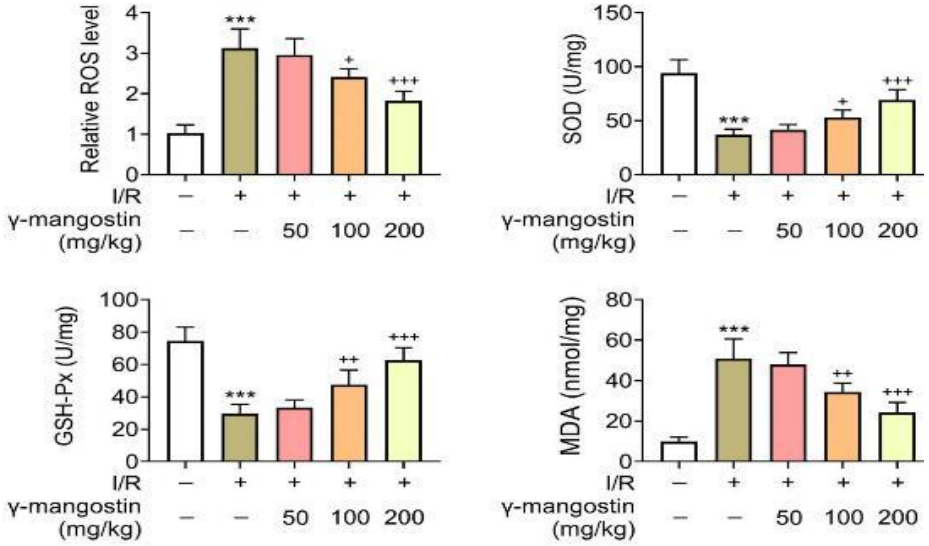

Figure 3: γ-Mangostin suppressed oxidative stress induced by I/R injury. Data are displayed as mean ± SD. ***P < 0.01 versus control group, ++p < 0.01, +++p < 0.001, I/R+γ-mangostin versus I/R group

γ-Mangostin reduces cell apoptosis in rats exposed to I/R

To reveal the role of γ-mangostin in cell apoptosis in cardiomyocytes exposed to I/R injure, TUNEL assay was performed. Compared to the I/R group, γ-mangostin significantly suppressed cell apoptosis revealed by TUNEL assay (Figure 4 A). Moreover, γ-mangostin significantly reduced levels of bax and cleaved caspase3 level and enhanced Bcl-2 exposed to I/R injury (Figure 4 B). These results suggest that γ-mangostin mediated cell apoptosis in I/R model.

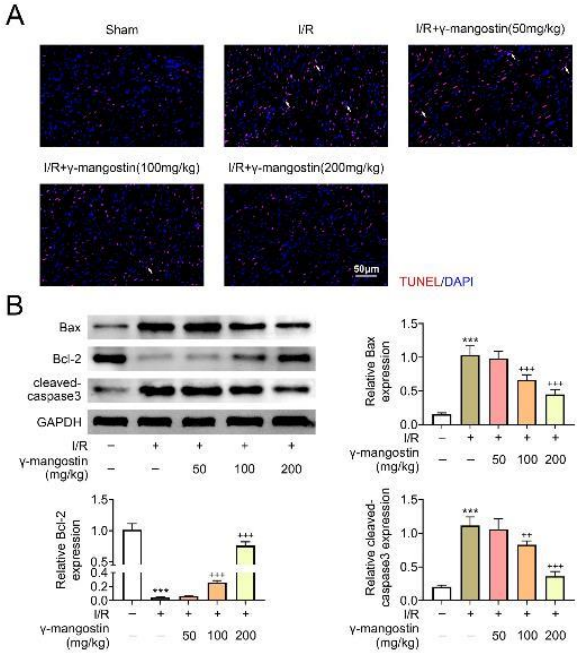

Figure 4: γ-Mangostin reduces cell apoptosis in rats exposed to I/R (A) TUNEL staining displayed reduced cell apoptosis exposed to γ-mangostin in I/R injured rats (B) Data are displayed as mean ± SD. ***P < 0.01 versus control group; +p < 0.05, ++p < 0.01, +++p < 0.001, I/R+γ-mangostin versus I/R group, respectively

γ-Mangostin inhibited myocardial injury, inflammation and oxidative stress

To further understand the regulation of γ-mangostin-induced cardioprotection, the SIRT3 level was evaluated. SIRT1 level was inhibited upon I/R stimulation, and was reversed by γ-mangostin treatment (Figure 5 A). Improvement in serum CK-MB and LDH levels by γ-mangostin was abolished by 3-TYP (Figure 5 B). γ-Mangostin pretreatment significantly decreased the secretion of inflammatory cytokines in cells exposed to I/R. However, these protective effects were weakened by SIRT3 inhibitor (Figure 5 D). Similarly, SOD and MDA levels improved in γ-mangostin pretreated I/R injured cells, but SOD and MDA levels were significantly countered by SIRT3 inhibitor (Figure 5 C). Furthermore, while γ-mangostin pretreatment significantly decreased the level of Bax and cleaved caspase 3 in cells exposed to I/R, these effects were reversed by the treatment of SIRT3 inhibitor (Figure 5 E).

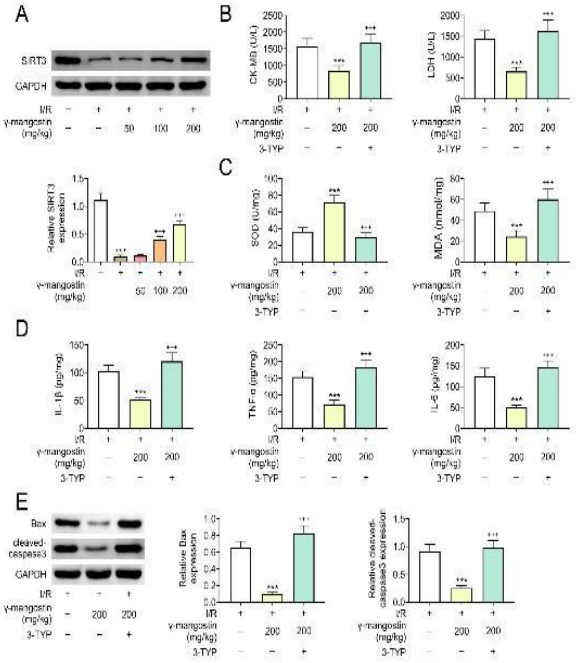

Figure 5: γ-Mangostin inhibited myocardial injury, inflammation and oxidative stress by enhancing SIRT3 expression in myocardial I/R rats. (A): SIRT3 protein expression was determined in each group through Immunoblot assays. (B): CK-MB and LDH levels were determined in the indicated groups. (C): SOD and MDA levels were determined in each group. (D): IL-1b, TNF-a and IL-6 level was measured in rats exposed to I/R, γ-mangostin and 3-TYP the indicated groups. (E): Bax and cleaved caspase-3 protein expression was determined the indicated groups. Data are displayed as mean ± SD. ***P < 0.01 versus control group; +++p < 0.001, I/R+γ-mangostin versus I/R group

DISCUSSION

Myocardial post-ischemia-reperfusion injury refers to the pathological process of progressive aggravation injury [12]. Myocardial post-ischemia-reperfusion injury could cause myocardial ultrastructure, energy metabolism, cardiac function, electrophysiology and a series of traumatic changes [13]. Several factors such as oxidative stress, also play important roles in myocardial I/R injury [14]. In addition, cardiac apoptosis and inflammation have been considered as markers of myocardial I/R injury [15]. Several drugs treat this disease by suppressing oxidative stress/inflammatory response and apoptosis.

In this study, γ-mangostin inhibited myocardial injury, inflammation and oxidative stress in myocardial ischemia-reperfusion rats. Therefore, γ-mangostin may serve as a drug for myocardial I/R injury. The left anterior descending branch (LAD) coronary artery using 6-0 silk suture at 2 - 3 mm below the left auricle was set. ST segment elevation is considered to be a successful ischemia model. Moreover, 30 min after LAD occlusion, artery reperfusion was affected for 180 min, thus indicating successful establishment of the rat model of ischemia-reperfusion injury.

Furthermore, based on TTC staining, γ - mangostin attenuated myocardial injury in I/R rats, mitigated the inflammation of myocardial injury in I/R rats. In addition, treatment of γ-mangostin suppressed the apoptosis of myocardial injury in I/R rats. The biological activities of γ-mangostin, such as anti-inflammation and anti-apoptosis have been widely reported [16]. The protective effects of γ-mangostin against glutamate-stimulated cytotoxicity were seen in human HT22 neuronal cells. In addition, γ-mangostin attenuated Aβ42-stimulated neuroinflammation and oxidative stress in microglia-like BV2 cells via MAPK pathway [17]. Whether γ-mangostin attenuated inflammation as well as oxidative stress in myocardial I/R injury rats via this pathway will require further study. However, γ-mangostin inhibited lipid peroxidation and DPPH free radical formation. Thus, it seems that treatment of γ - mangostin exerts therapeutic activity in several diseases, by mediating inflammation and oxidative stress.

γ-Mangostin enhanced SIRT3 expression in liver fibrosis induced by oxidative stress, and may also inhibit myocardial injury, inflammation as well as oxidative stress in myocardial ischemia-reperfusion rats via the mediation of SIRT3. MI/R induced the significantly decreased SIRT3 expression and activity in myocardium, and the activation of the SIRT3 signaling pathway reduced oxidative stress and apoptosis, and thus ameliorated MI/R injury [18]. This evidence suggest that SIRT3 intervention can potentially treat ischemia-reperfusion injury more effectively. The results in the present study further support this view. However, its detailed regulatory mechanism of action requires further study.

CONCLUSION

The findings of this study show that γ-mangostin inhibits myocardial injury, inflammation and oxidative stress by enhancing SIRT3 expression in myocardial ischemia-reperfusion rats. Thus, γ-mangostin is a potential drug for the treatment of myocardial ischemia-reperfusion injury. However, clinical trials in humans are required to validate the results.

DECLARATIONS

Acknowledgements

None provided.

Funding

None provided.

Ethical approval

The study was approved by the Ethics Committee of The First People's Hospital of Fuyang Hangzhou, China for the use of animals (approval no. 2021011).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors contributed to the study conception and design, material preparation, the experiments, data collection and analysis. All authors wrote the first draft of the manuscript and commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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