

## Original Research Article

# $\alpha$ -Mangostin protects against myocardial ischemia reperfusion injury by suppressing the activation of HIF-1 $\alpha$

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Sent for review: 29 July 2019

Revised accepted: 20 December 2019

### Abstract

**Purpose:** To investigate the cytoprotective effect of  $\alpha$ -mangostin on myocardial tissues in ischemic rats, and the underlying mechanism.

**Methods:** Histopathological changes in myocardial tissues were determined using inverted microscope. Protein expressions were measured by western blotting, while enzyme-linked immunosorbent assay (ELISA) was used to assay the expression levels of caspase-3, caspase-9 and caspase-8.

**Results:** Treatment with  $\alpha$ -mangostin (20 mg/kg) suppressed production of reactive oxygen species (ROS) and lipid peroxides in myocardial tissues of MI/R rats, and significantly alleviated MI/R injury-mediated reduction in ATP levels in cardiac tissues ( $p < 0.05$ ).  $\alpha$ -Mangostin treatment of MI/R injury rats suppressed HIF-1 $\alpha$  activation, and markedly elevated BNIP3 levels, relative to model group. Moreover, MI/R-induced cardiomyocyte apoptosis was significantly alleviated by  $\alpha$ -mangostin treatment ( $p < 0.05$ ). Treatment with  $\alpha$ -mangostin also suppressed I/R-induced increases in caspase-8 and caspase-3 activation in myocardial tissues, improved Nrf-2 activation, and promoted HO-1 and GST levels in MI/R injury rats ( $p < 0.05$ ).

**Conclusion:** These results suggest that  $\alpha$ -mangostin protects rat cardiac tissues from MI/R-induced oxidative damage via reduction of HIF-1 $\alpha$  expression, inhibition of ROS generation and suppression of apoptosis. Therefore,  $\alpha$ -mangostin may be of therapeutic importance for the management of myocardial ischemia in humans.

**Keywords:**  $\alpha$ -Mangostin, Hypoxia, Inflammation, Nrf-2, Oxidative stress, Reperfusion

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## INTRODUCTION

Myocardial ischemia accounts for the highest mortality among various diseases of cardiovascular system worldwide [1]. Myocardial ischemia followed by reperfusion injury (MI/R

injury) causes severe damage to cardiac tissues, leading to oxidative-nitrate stress and activation of inflammatory responses [2,3]. Excess accumulation of ROS, Ca<sup>2+</sup> overload and ATP depletion initiate mitochondrial dysfunction. After reperfusion injury, cardiomyocytes are prone to

increased mitochondrial stress which triggers apoptosis [2,4].

Hypoxia-inducing factor (HIF-1) plays a major role in MI/R injury [5]. Under normoxic conditions, HIF-1 is hydroxylated and acts as target for ubiquitinated proteasomal degradation. However, HIF-1 is stabilized and upregulated under low oxygen environments. Hypoxia inducing factor-1 $\alpha$  regulates hypoxia-induced transcription of number of genes and regulates cardiac apoptosis [6]. Thus, hypoxia-induced gene alterations and redox imbalance act as important contributors in myocardial infarction. An effective approach to protecting the cardiomyocytes from I/R injury could be achieved through pharmacological intervention. Some studies have shown the importance of antioxidants as protective agents against myocardial infarction [7-9]. Studies have revealed the significance of  $\alpha$ -mangostin as potent antioxidant with free radical scavenging property [10,11]. It has been reported that alpha-mangostin exerts protective effect against cisplatin-induced nephrotoxicity by reducing oxidative stress and inflammatory responses [12]. In addition, studies have demonstrated that  $\alpha$ -mangostin protects renal tubular cells against CDDP-induced apoptosis [13]. Alpha-mangostin mediates cardio-protection against  $\beta$ -adrenergic catecholamine-induced myocardial toxicity, and prevents oxidative stress and post-ischemic injury [14,15].

The anti-cancer effects of  $\alpha$ -mangostin have been reported in a wide range of cancers including human leukemia cell line HL60 [16], human colon cancer [17,18], prostate carcinoma [19] and head and neck squamous cell carcinoma [20]. The present study evaluated the antioxidant capacity of  $\alpha$ -mangostin and its cytoprotective effect against myocardial I/R injury in rats.

## EXPERIMENTAL

### Establishment of myocardial ischemia/reperfusion injury model

Male Sprague-Dawley rats were housed in standard laboratory conditions (temperature and humidity of  $25 \pm 2^\circ\text{C}$  and 70 %, respectively, with alternating 12 h light/12 h and dark cycles) and *ad libitum* access to standard feed and clean drinking water. All animal experimental protocols were approved by the ethics committee of Qingdao Hiser Hospital, China. The study followed the guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) for experimentation and animal use.

Rats weighing 200 - 250 g were randomly divided into 4 groups: sham,  $\alpha$ -mangostin, myocardial I/R injury, and  $\alpha$ -mangostin + myocardial I/R injury groups. After the period of acclimatization, the rats were anesthetized with sodium pentobarbital intraperitoneally (*i.p.*) at a dose of 40 mg/kg body weight. Myocardial I/R injury was established via left thoracic incision, followed by slipknot around left anterior descending coronary artery (LAD). The slipknot was removed after 30 min, after which a 2-h reperfusion was carried out. Similar surgical procedures were done for control animals, but without LAD occlusion. Alpha-mangostin ( $\geq 98$  %, Sigma Aldrich) was dissolved in mixture of DMSO and ethanol, and administered intravenously to the rats at a dose of 20 mg/kg 15 min before reperfusion. Based on preliminary studies using different doses (5, 10, 20 and 30 mg/kg), the dose of 20 mg/kg was selected. Doses above 20 mg/kg showed similar cytoprotective effects. Thus, the lower dose with significant cytoprotection was chosen for further detailed studies.

### Histopathological studies

Heart tissues were fixed in 4 % paraformaldehyde and embedded in paraffin. The tissues were cut into thin sections (2- $\mu\text{m}$ ) and subsequently de-paraffinized in boiling xylene prior to hematoxylin and eosin (H&E) staining. The stained tissues were analyzed for histopathological changes with the aid of light microscopy.

### Determination of ROS levels

Hearts were excised from the rats, washed with PBS, and then embedded in O.C.T (SAKURA). The tissues were cut into thin sections 2- $\mu\text{m}$  thick, and subsequently subjected to determination of ROS levels.

### Measurement of lipid peroxidation

Homogenized cardiac tissues were analyzed for lipid peroxide content using commercially available Lipid Peroxidation Assay Kit (Sigma Aldrich, St. Louis, MO) according to instructions from the manufacturer. The lipid peroxide levels were expressed as TBARS (ng/mg protein).

### Determination of adenosine triphosphate (ATP) contents

Levels of ATP in the homogenized cardiac tissue samples were measured using Cayman's ATP Detection Assay Kit, as per manufacturer's instructions.

## Evaluation of antioxidant enzyme activities

The activities of antioxidant enzymes and GSH levels in homogenized heart tissues were assayed using Sigma Aldrich assay kits for SOD (19160), GST (CS0410) and GPx (CGP1) and GSH (CS0260). The assays were performed as per the technical instructions given by the manufacturer (Sigma Aldrich, St. Louis, MO). The results are expressed as Units/mg of protein.

## Assay of caspase 8, 9 and 3 activities

Heart homogenates were analyzed for caspase expressions using ELISA assay kits for caspase 8 (ab119507, Abcam), caspase 9 (ab119508, Abcam) and caspase 3 (Human Active Caspase-3 ELISA Kit, Thermo Scientific), as per manufacturer's instructions.

## Western blot studies

The cardiac tissues were minced, washed with cold D-PBS and subsequently suspended in 1 % collagenase. Following 50-min collagenase treatment at 37 °C, the tissues were suspended in 0.25 % trypsin for 10 min. The lysate was centrifuged for 30 min at 4 °C at 12000 g, and the protein content of the supernatant was measured using bicinchoninic acid (BCA) assay. Then, protein samples (60 µg) were subjected to 12% SDS-polyacrylamide gel electrophoresis. The separated proteins were transferred to PVDF membrane and blocked in 5 % skimmed milk for 1 h. The membrane was washed 5 times with TBST, and then incubated with primary antibodies overnight at 4°C. The antibodies used were: anti-NF-kb-p65 (ab16502), anti-Nrf-2 (sc-722), anti-HO-1 (sc-10789), anti-GST (ab9085), anti-COX-2 (ab15191), anti-Bnip3 (ab10433) and anti-HIF-1α. After incubation, the membrane was washed with TBST, and then incubated with peroxidase-conjugated goat anti-mouse or rabbit IgG secondary antibody for 2 h at room temperature. The membrane was visualized with enhanced chemiluminescent system, and images were scanned for densitometric analysis using Image J software (Supersignal; Pierce, IL).

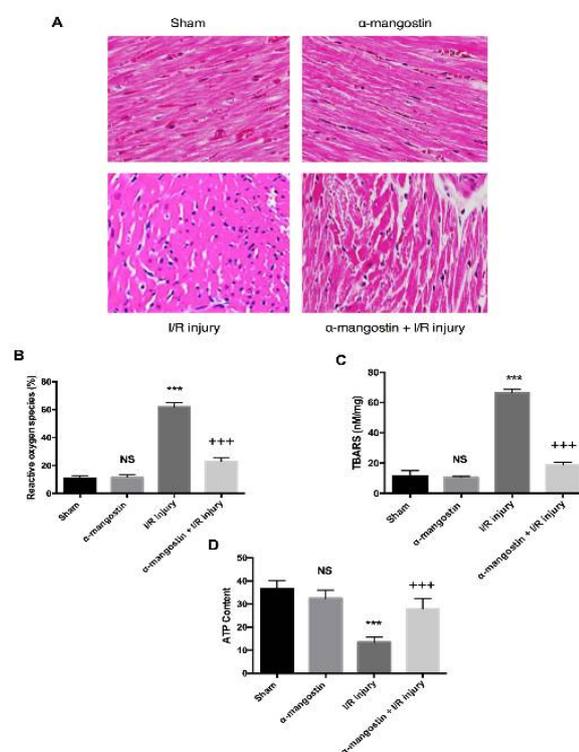
## Statistical analysis

All the experiments were performed in triplicate. The data were analyzed using one-way analysis of variance (ANOVA) and Student's *t*-test. Differences were considered statistically significant at  $p < 0.05$

## RESULTS

### α-Mangostin protected cardiac tissues against myocardial I/R

Myocardial I/R injury significantly induced degeneration of cardiac tissues in rats, when compared to sham control (Figure 1 A). However, histopathological changes were absent in the cardiac tissues of control and α-mangostin treatment groups. The production of reactive oxygen species and lipid peroxides were markedly enhanced in rat cardiac tissues after myocardial I/R (Figure 1 B). However, treatment of myocardial I/R rats with α-mangostin significantly inhibited production of ROS and lipid peroxides.

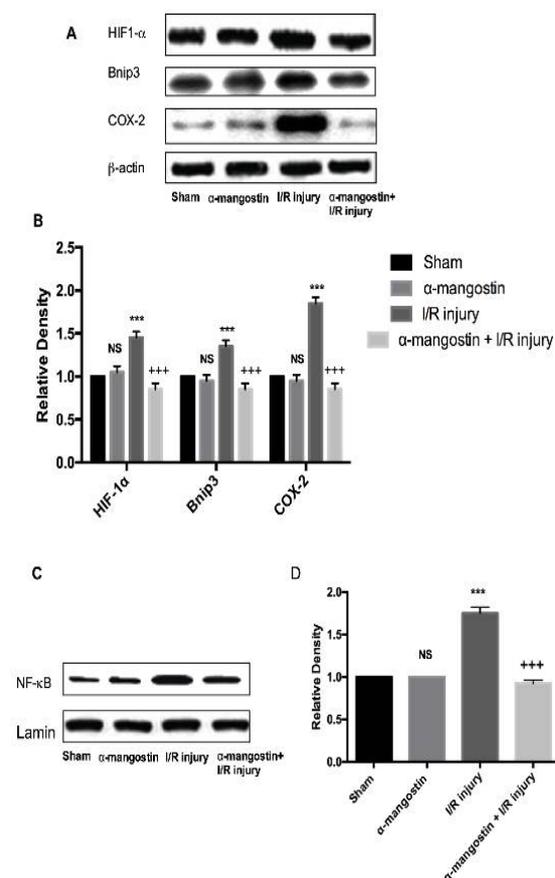


**Figure 1:** Effect of α-mangostin on oxidative stress and myocardial damage in I/R injury rats. (A) Histopathological changes in I/R injury rats. (B) Production of ROS. (C) Lipid peroxide levels. (D) Relative ATP levels. \*\*\*  $p < 0.001$ , when compared to sham group; +++ $p < 0.001$  when compared to I/R injury group

### α-Mangostin suppressed myocardial I/R injury -induced activation of HIF-1α and inflammation

Myocardial I/R injury significantly increased hypoxia inducible factor-1α expression, when compared to control rats. The expression of Bnip3, a pro-apoptotic protein involved in regulation of apoptosis, was significantly

increased in I/R injury rats, relative to sham group. Moreover, I/R injury induced inflammatory activation by up-regulating NF-κB and COX-2 expressions, when compared to sham rats. However, pre-treatment with α-mangostin prevented HIF-1α activation and subsequent downregulation of inflammatory markers, when compared to myocardial I/R injury rats (Figures 2 A - D).

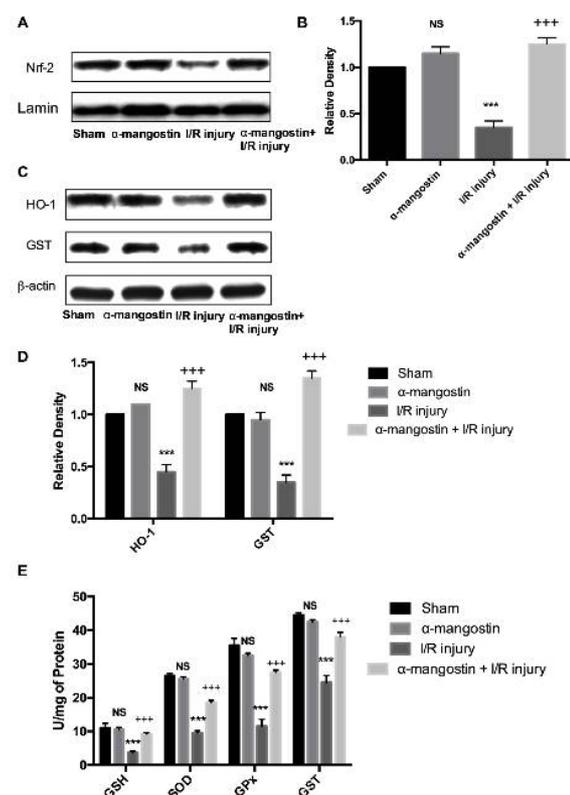


**Figure 2:** Effect of α-mangostin on HIF-1α and inflammation activation. (A) HIF-1α, Bnip3 and COX-2 expressions in rats treated with α-mangostin. (B) Relative protein expressions normalized to β-actin. (C) Suppression of NF-κB expression. (D) Relative NF-κB expression; \*\*\*  $p < 0.001$ , when compared to sham rats; +++  $p < 0.001$ , when compared to I/R injury group

**α-Mangostin prevented myocardial I/R injury-mediated suppression of Nrf-2 target gene expressions**

Nrf-2 and its downstream genes are important regulators of redox balance. In the present study, myocardial I/R injury significantly down-regulated expressions of Nrf-2, HO-1, GST and antioxidant enzyme activities, when compared to sham group. However, pre-treatment with α-mangostin significantly improved the overall redox balance by up-regulating Nrf-2 target genes and

antioxidant levels. These results are depicted in Figures 3 A - E).



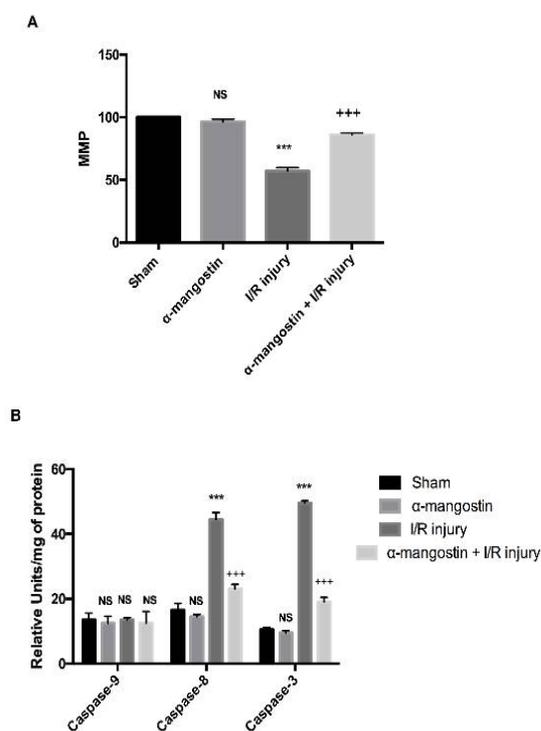
**Figure 3:** Effect of α-mangostin on Nrf-2 expression and antioxidant pool. (A) The α-mangostin increased Nrf-2 expressions. (B) Relative Nrf-2 expression compared to Lamin. (C) α-mangostin up regulated GST and HO-1 expressions. (D) Densitometry of GST and HO-1 expressions. (E) α-mangostin improved antioxidant pool in I/R injury mice; \*\*\*  $p < 0.001$ , when compared to sham rats; +++  $p < 0.001$ , when compared to I/R injury group

**Myocardial I/R injury induced apoptosis was prevented by α-mangostin**

Myocardial I/R injury significantly increased the expressions of caspase-8 and caspase-3, relative to sham group, suggesting activation of the intrinsic apoptotic pathway. However, pre-treatment with α-mangostin suppressed apoptosis, maintained membrane potential, and down-regulated caspase expressions (Figures 4 A and B).

**DISCUSSION**

The present study demonstrates that α-mangostin pre-treatment prevents degeneration of myocardial tissues in myocardial I/R injury in rats via inhibition of oxidative stress. The myocardial I/R injury-induced HIF-1α and



**Figure 4:** Effect of  $\alpha$ -mangostin on apoptosis in I/R injury mice. (A) Effect of  $\alpha$ -mangostin and I/R injury on MMP levels. (B) Down-regulation of caspase expressions in I/R injury rats by  $\alpha$ -mangostin; \*\*\* $p$ <0.001, when compared to sham mice; +++ $p$ <0.001, when compared to I/R injury group

inflammation activation were suppressed by  $\alpha$ -mangostin treatment.  $\alpha$ -Mangostin also prevented myocardial I/R injury-induced apoptosis. Myocardial ischemic injury/reperfusion leads to exacerbated myocardial damage. Oxidative stress is one of the important contributors to such events. Thus, antioxidant treatment can offer protection against myocardial injury [21].

In the present study, oxidative stress was significantly increased under I/R injury, with increased levels of ROS and lipid peroxides, and decline in ATP content. These results are in line with previous reports implicating oxidative stress as a major regulator of myocardial I/R injury [22,23]. Treatment with  $\alpha$ -mangostin significantly reduced the oxidative stress, increased myocardial ATP levels, and decreased cell degeneration in myocardial tissue. It has been reported that  $\alpha$ -mangostin exhibits cytoprotective effect through reduction of oxidation of low-density lipoproteins, as well as free radical scavenging and inhibition of apoptosis [24,25].

Hypoxia-inducing factor-1 $\alpha$  regulates cellular oxygen homeostasis and imparts adaptation to cellular survival or death. Hypoxia-inducing factor-1 $\alpha$  is degraded under normoxia through

prolyl hydroxylase-mediated ubiquitin-proteasome pathway. During hypoxia, stabilization of HIF-1 $\alpha$  via inhibition of prolyl hydroxylases is followed by its heterodimerization with HIF-1 $\beta$ , and subsequent translocation to the nucleus where it regulates transcription of target genes [26]. Hypoxia-inducing factor-1 $\alpha$  mediates apoptosis through activation of pro-apoptotic proteins [27].

Direct regulatory role of HIF-1 $\alpha$  on the expression of mitochondrial pro-apoptotic protein BNIP3 (Bcl-2/E1B 19 kDa interacting protein 3) is involved in apoptotic induction [28]. Thus, the significant up-regulation in HIF-1 $\alpha$  after myocardial I/R injury is consistent with previous reports [4]. Moreover, there were increases in the expressions of inflammatory markers such as NF- $\kappa$ B and COX-2 following I/R injury. These observations show evidence that myocardial I/R injury evokes hypoxia-induced inflammatory responses. Previous studies reported the involvement of hypoxia and HIF-1 $\alpha$  in inflammatory activation and apoptosis during I/R injury [29,30].

In the present study, myocardial I/R injury resulted in apoptotic induction through significant up-regulation of the expressions of BNIP3, caspase9 and caspase-3. Pre-treatment with  $\alpha$ -mangostin resulted in anti-inflammatory and anti-apoptotic effects against myocardial I/R injury. The inhibitory effect of  $\alpha$ -mangostin against secretion of the pro-inflammatory mediators IL-8 and TNF- $\alpha$  has been previously demonstrated [31]. The anti-cancer effect of  $\alpha$ -mangostin in HT-29 cells is mediated through upregulation of Wnt and Bcl-2 expressions [32]. It has been reported that  $\alpha$ -mangostin suppresses MMP expressions and prevents prostate cancer metastasis through the JNK pathway [33].

Cellular protection against oxidative stress and cell death is attributed to redox homeostasis. In the present study, myocardial I/R injury significantly suppressed antioxidant production in rat myocardial tissues. Nuclear factor erythroid (Nrf-2) is a key transcription factor which binds to antioxidant response elements (ARE) and induces target antioxidant gene expressions [34]. Activated Nrf-2 is highly stable and its aggregation in the nucleus leads to cytoprotection [35].

The present study demonstrated that  $\alpha$ -mangostin significantly improves cardiac function in MI/R injury rats by up-regulation of Nrf-2 and its downstream targets HO-1 and GST. These findings are consistent with reports published earlier on  $\alpha$ -mangostin-mediated protective effect

against age-related macular degeneration through Nrf-2 and MAPK-mediated signaling [25,36].

## CONCLUSION

These results suggest that  $\alpha$ -mangostin exhibits protective effect against myocardial ischemic injury in rats via the regulation of HIF-1 $\alpha$  and Nrf-2 signaling. Therefore,  $\alpha$ -mangostin can potentially be used for the treatment of myocardial ischemic injury.

## DECLARATIONS

### Conflict of interest

No conflict of interest is associated with this work.

### Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Hong Jiang, Yan Chen- conceived and designed the study; Wei Zhang, Jing Yu, Manli Feng, Xiaoyu Wang, Xiaopeng Wang, Yanan Jiao, Chengcheng Wang- collected and analyzed the data; Hong Jiang, Wen li Guo, Dongdong Zhu -wrote the manuscript. Yan Chen-Approved final version of the manuscript. All authors read and approved the manuscript for publication.

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