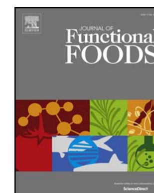
Journal of Functional Foods 40 (2018) 544–553

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff



γ-Mangostin, a xanthone from mangosteen, attenuates oxidative injury in liver via NRF2 and SIRT1 induction

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ARTICLE INFO

Keywords:
γ-mangostin
Mangosteen
Liver oxidative injury
NRF2
SIRT1
PGC-1α

ABSTRACT

γ-Mangostin (γ-man), an active compound from *Garcinia mangostana* L., has been discovered as a hepatoprotective agent against oxidative injury. However, the underlying mechanisms remained unclear. The current study showed that γ-man stimulated the nuclear translocation of nuclear factor erythroid 2-related factor 2 (NRF2) to enhance antioxidant capacity under oxidative stress, which was partially reversed by treatment of the NRF2 inhibitor, all-trans-retinoic acid. Moreover, γ-man increased the expression and activity of SIRT1 (silent mating type information regulation 2 homolog 1), which facilitated the deacetylation of peroxisome proliferator-activated receptor γ coactivator 1α to improve the mitochondrial function in L02 cells. The protective effect of γ-man was partially blocked by treatment of the SIRT1 inhibitor tenovin-1 or SIRT1 knockdown. *In vivo* studies showed γ-man protected mice from carbon tetrachloride-induced acute liver injury, through up-regulation of NRF2 and SIRT1. Thus, γ-man might be a candidate to protect liver from acute oxidative injury.

1. Introduction

Many factors, such as hepatic virus infection (Choi & Ou, 2006), excessive alcohol consumption (Wu & Cederbaum, 2003) and drug intake (Bissell, Gores, Laskin, & Hoofnagle, 2001), can cause acute liver injury. The rise of oxidative stress in liver is considered as one of the main causes of liver damage. More and more research focuses have been attracted on alleviation and/or prevention of excess accumulation of reactive oxygen species (ROS) in liver.

Nuclear factor erythroid 2-related factor 2 (NRF2) is the master regulator of the antioxidant responsive element (ARE)-mediated induction of phase II detoxification and anti-oxidative enzyme genes expression (Gorrini, Harris, & Mak, 2013). Under basal condition, the NRF2 inhibitor KEAP1 (Kelch-like ECH-associated protein 1) binds and retains NRF2 in cytoplasm where it can be targeted for ubiquitin-mediated degradation (Cullinan, Gordan, Jin, Harper, & Diehl, 2004; Itoh et al., 1999). Under oxidative stress, NRF2 is released and translocated to nucleus and binds to ARE-containing genes, which activates the transcriptions of heme oxygenase-1 (HO-1) and superoxide dismutase 2 (SOD2) to against oxidative stress (Liu et al., 2017; Nguyen, Sherratt, Nioi, Yang, & Pickett, 2005). The induction of HO-1 and SOD2 have been implicated in numerous disease states, such as Alzheimer disease, and liver diseases (Cullinan et al., 2004; Ke et al., 2012; Schipper, Gupta, & Szarek, 2009).

The silent mating type information regulation 2 homologs (Sirtuins) are nicotinamide adenine dinucleotide (NAD^+)-dependent protein deacetylases. Sirtuin 1 (SIRT1) is implicated in the regulation of many cellular processes, including apoptosis, cellular senescence, glucose homeostasis, aging and longevity, and its targets include p53 (Vaziri et al., 2001), forkhead transcription factors (Brunet et al., 2004), peroxisome proliferator-activated receptor gamma (PPARγ) (Picard et al., 2004), and the PPARγ coactivator-1α (PGC-1α) (Rodgers et al., 2005). SIRT1 also plays a key role in improving oxidative stress (Li, 2014). SIRT1 stimulates mitochondrial biogenesis through PGC-1α, reduces ROS generation from the respiratory chain, and enhances SOD2 expression in mitochondria, resulting in increased mitochondrial

Abbreviations: ALT, alanine aminotransferase; ARE, antioxidant responsive element; AST, aspartate transaminase; ATRA, all–retinoid acid; CCl_4, carbon tetrachloride; DCFH-DA, 2′,7′-dichlorofluorescin diacetate; γ-man, γ-mangostin; GSH, glutathione; HO-1, heme oxygenase-1; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide; KEAP1, Kelch-like ECH-associated protein 1; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazolium bromide; NRF2, nuclear factor erythroid 2-related factor 2; PEG 400, polyethylene glycol 400; PFA, paraformaldehyde; PGC-1α, peroxisome proliferators-activated receptor-γ coactivator-1α; PPARγ, peroxisome proliferator-activated receptor γ; ROS, reactive oxygen species; SIRT1, silent mating type information regulation 2 homolog 1; SOD, superoxide dismutase; *t*-BHP, *tert*-butyl hydroperoxide

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https://doi.org/10.1016/j.jff.2017.11.047
Received 7 April 2017; Received in revised form 31 October 2017; Accepted 28 November 2017
Available online 01 December 2017
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superoxide dismutation (Hsu et al., 2010; Nemoto, Fergusson, & Finkel, 2005; St-Pierre et al., 2006; Tanno et al., 2010).

Garcinia mangostana L. (Clusiaceae) is an evergreen tree in Southeast Asia, and its fruits, mangosteen, are called as "Queen of Fruit" due to its sweet taste, pleasant aroma and potential role in promoting health (Liu, Wang, & Lin, 2015). Many healthy botanical dietary supplements have been developed from mangosteen, such as Xango, Verve and TraXan, which ranked 12th amongst the top-selling dietary supplements in the US according to NBJ's Supplement Business Report, U.S, 2013. A series of xanthones have been identified from different parts of *G. mangostana*, with a wide range of bioactivities including anti-oxidation, anti-inflammation and anti-cancer (Akao, Nakagawa, & Nozawa, 2008; Jung, Su, Keller, Mehta, & Kinghorn, 2006; Liu et al., 2016). In our previous study, γ-mangostin (γ-man) was identified as the chemical principle of *G. mangostana* in protecting hepatocytes from being damaged by *tert*-butyl hydroperoxide (*t*-BHP) with low cytotoxicity (Wang, Liu, Ye, Wang, & Lin, 2015). However, the underlying mechanisms remain unclear. Herein, we attempt to disclose the underlying mechanisms for the hepatoprotective effect of γ-man *in vitro* and *in vivo*, and provide basis for further develop of this compound as functional food for treatment or prevention of liver diseases.

2. Materials and methods

2.1. Chemicals and reagents

Phosphate-buffered saline (PBS) powder, RPMI 1640 medium, penicillin-streptomycin (P/S), 0.25% (w/v) trypsin/1 mM EDTA and fetal bovine serum (FBS) were purchased from Gibco (Waltham, MA, USA). *t*-BHP, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide (JC-1), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazolium bromide (MTT), Hoechst 33342, 2′,7′-dichlorofluorescin diacetate (DCFH-DA), polyethylene glycol 400 (PEG 400), paraformaldehyde (PFA), all-trans-retinoic acid (ATRA) and carbon tetrachloride (CCl_4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). RIPA lysis buffer was purchased from Beyotime Biotechnology Company (Shanghai, China). Protein A/G agarose, SIRT1 shRNA and SIRT1 primary antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Goat anti-Rabbit IgG secondary antibody and Lipofectamine 2000 were purchased from Thermo Scientific (Waltham, MA, USA). All other primary and secondary antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Tenovin-1 was purchased from Selleck Chemicals (Houston, TX, USA). γ-Mangostin (γ-man, ≥98%) was separated from the pericarps of *G. mangostana* as previously reported (Liu et al., 2016). γ-Man was well dissolved in DMSO at a concentration of 10 mM as stock solution. A small volume of γ-man stock solution was added in each well based on the concentrations indicated in figures, and the same volume of DMSO was also added as the vehicle control. The concentration of DMSO is less than 0.1% in all experiments.

2.2. Cell culture

Human immortalized hepatocyte cell line L02 was obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). L02 cells were routinely cultured in RPMI 1640 medium supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified incubator under 95% air and 5% CO_2 at 37 °C.

2.3. Cell viability assay

The cell viability was evaluated by MTT assay. L02 cells were seeded in 96-well plates at a density of 5×10^3 cells/well. When reaching approximately 70–80% confluence, cells were treated with or without different concentrations of indicated compounds for 12 h. Subsequently, the culture medium was discarded and cells were cultured with or without 200 μM *t*-BHP for additional 6 h. Then cell viability was determined by being incubated with medium containing MTT (1 mg/ml) for 4 h, followed by dissolving the formazan crystals with DMSO. The absorbance at 570 nm was determined by a microplate reader (SpectraMax M5, Molecular Devices, USA) and presented as relative cell viability. The results were analyzed based on at least three independent experiments.

2.4. ROS determination

A fluorescent probe, DCFH-DA, was used for determination of intracellular ROS levels. L02 cells were seeded in 96-well black plates at a density of 5×10^3 cells/well for 24 h. Cells were pretreated with or without different concentrations of γ-man or inhibitors for 12 h and then incubated with *t*-BHP for additional 4 h. Then cells were washed with PBS for three times and incubated with DCFH-DA (10 μM) at 37 °C for 15 min. Subsequently, cells were washed with PBS for three time and the intracellular fluorescent intensity was determined by a microplate reader (SpectraMax M5) with the excitation and emission wave lengths at 485 and 535 nm, respectively. Meanwhile, the Incell Analyzer 2000 was used to observe the intracellular fluorescence. Results were presented as relative fluorescent intensity based on at least three independent experiments.

2.5. Mitochondrial membrane potential (MMP) assay

The cationic dye, JC-1, was used to determine the MMP of L02 cells. L02 cells were seeded in 24-well plates for 24 h, followed by treatment with or without different concentrations of γ-man for 12 h. Then cells were incubated with or without 200 μM *t*-BHP for additional 4 h, followed by JC-1 (10 μg/ml) staining for 10 min and gently washed with PBS for three times. Then cells were suspended in PBS for flow cytometry analysis (Becton Dickinson, USA). Meanwhile, the Incell Analyzer 2000 was used to observe the intracellular fluorescence. The MMP of cells was expressed as the ratio of red to green fluorescent intensity for each sample.

2.6. Nuclear and cytoplasmic protein Extraction

The nuclear and cytoplasmic protein was collected with the Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime Biotechnology company, Shanghai, China), according to the manufacture's instruction.

2.7. Immunoprecipitation (IP)

To examine the acetylation level of PGC-1α, the IP/Western blot analyses were performed. The detailed procedure was described as followings: the cellular protein was extracted and precleared by incubation with protein A/G agarose beads at 4 °C for 1 h. Next, the sample was incubated with the beads coupled to the PGC-1α antibody at 4 °C overnight. The immune complex was washed by RIPA lysis buffer for three times and boiled in protein loading buffer for 5 min at 95 °C. Finally, the immunoprecipitate was separated with SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes.

2.8. Immunofluorescent assay

L02 cells were cultured in dish for 24 h and then treated with or without different concentrations of indicated compounds for 12 h. Subsequently, the culture medium was discarded and cells were cultured with or without 200 μM *t*-BHP for additional 6 h. And then cells were fixed with 4% (v/v) PFA for 1 h at room temperature and permeabilized with 0.1% Triton X-100 for 1 h at room temperature. The cells were blocked with PBS supplemented with 0.5% (w/v) BSA, thereafter, incubated with anti-NRF2 antibody overnight at 4 °C. After washing with PBS, the cells were incubated for 1 h at room temperature

with a Texas red-conjugated secondary antibody (1:1000 dilution; Molecular Probes, Eugene, OR, USA). Finally, the cells were washed twice with PBS and the nuclei were counterstained with Hoechst 33342 for 15 min at room temperature. Observation of the intracellular fluorescence was performed on a Leica TCS SP8 Confocal Laser Scanning Microscope System.

2.9. Generation of SIRT1 knockdown cell line

L02 cells were cultured in 6-well plate and incubated with antibiotics free medium for 24 h. shRNA targeting *SIRT1* and empty vector were transfected to cells using Lipofectamine 2000, following the manufacturer's instruction. Six hours after transfection, medium was changed to fresh culture medium. Forty-eight hours after transfection, 10 μg/ml puromycin was added to the medium to select positive cells for 14 days. Medium was changed every other day. The positive cells were pooled together.

2.10. Western blot analysis

Cell lysates were prepared in RIPA buffer on ice. The protein concentrations were determined by BCA Protein Assay Kit (Pierce Biotechnology). Equal amounts of protein were separated by SDS-PAGE, and transferred to PVDF membranes. The membranes were blocked with 5% nonfat milk for 3 h at room temperature and immunoblotted with polyclonal primary antibodies. After washed with TBST for three times, the immunoblots were incubated with peroxidase-conjugated secondary antibodies (diluted 1:1000) at room temperature for 3 h. The proteins were detected with SuperSignal™ West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, Waltham, MA, USA).

2.11. CCl_4-induced acute liver injured mice model

Fifty C57BL/6 mice were purchased from the animal facility of Faculty of Health Science, University of Macau. The mice were housed at 22 ± 1 °C with 12 h light–dark cycles. Food and water were available *ad libitum*. The mice were randomly divided into five groups (n = 10). Groups I (blank control) and III (CCl_4) were administrated PEG 400-water (6:4, v/v) (10 ml/kg body weight). Groups II (γ-man control), IV (low dosage) and V (high dosage) were received 10, 5 and 10 mg/kg γ-man (dissolved in PEG 400-water (6:4, v/v), 10 ml/kg body weight), respectively. The mice were intraperitoneally injected once a day for 7 days. Two hours after the final administration, the mice from Groups III–V were injected intraperitoneally with 5% (v/v) CCl_4 (10 ml/kg, dissolved in olive oil), and those from Groups I and II were given the same volume of olive oil (i.p.). After twenty hours, the blood samples were collected and the livers were dissected. A part of liver was fixed with 4% PFA for histological analysis, and the remained part was refrozen in −80 °C for biochemical parameters measurement and proteins determination. All the procedures were approved by the Animal Ethics Committee of University of Macau, and performed in strictly accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Macau.

2.12. H&E staining

H&E staining was performed using standard procedures. Briefly, liver pieces were rapidly removed from 4% PFA solution and embedded in paraffin wax. The sections were cut to appropriate thickness followed by H&E staining.

2.13. Measurement of biochemical parameters

The biochemical parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT) in serum, superoxide dismutase (SOD) and glutathione (GSH) in liver tissues, were measured with enzymatic assays, according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The results of GSH and SOD were expressed as μg/mg protein and U/mg protein, respectively. And the results of AST and ALT were expressed as unit per liter (U/l). The protein contents were determined with a BCA Kit.

2.14. Statistical analysis

Data were expressed as mean ± SEM based on at least three independent experiments and analyzed on Graphpad Prism 6 (GraphPad Software, San Diego, CA, USA). The significance of differences between groups was assessed by one-way analyses of variance (ANOVA) using SPSS software 16.0 (Chicago, IL, USA). $^{*}p < .05$ and $^{**}p < .01$ were considered as significant difference.

3. Results

3.1. γ-Man induced expression and nuclear localization of NRF2

Our previous study evaluated the hepatoprotective effect of a series of xanthones from *G. mangostana*, and found γ-man showed obvious protective effect in L02 cells against *t*-BHP through decreasing intracellular ROS accumulation (Wang et al., 2015). As the master regulator of antioxidant responses, NRF2 mediates the anti-oxidative activity of many natural compounds. Hence, the expression of NRF2 in L02 cells was detected. Treatment of γ-man upregulated the NRF2 protein level in L02 cells, which induced the expressions of the antioxidant proteins HO-1 and SOD2 (Fig. 1A). Challenging with oxidative stress, cytoplasmic NRF2 translocates to nuclei and binds to the ARE to transcript the anti-oxidation elements. As shown in Fig. 1B, the expression of NRF2 (Texas–red fluorescence indicated) was suppressed in L02 cells treated with *t*-BHP compared with that of control cells. When treated with γ-man (10 μM), the expression of NRF2 was obviously increased and more NRF2 was translocated to nuclei, compared with that of *t*-BHP treated cells. To further confirm the distribution of NRF2 protein, nuclei and cytoplasm proteins were separated and NRF2 was determined by Western blot. Interestingly, the cytoplasmic NRF2 was unchanged in either *t*-BHP or γ-man treated cells, while the nuclear NRF2 level was decreased in *t*-BHP treated cells compared with that of control cells, and pre-treatment with γ-man increased the nuclear NRF2 level in a concentration-dependent manner (Fig. 1C).

To confirm whether the hepatoprotective effect of γ-man was mediated by NRF2, all-trans-retinoid acid (ATRA), an NRF2 inhibitor, was recruited. The viability was significantly decreased in *t*-BHP and ATRA co-treated cells, compared with that of *t*-BHP treated cells, indicating that ATRA treatment significantly decreased the ability of counteracting oxidative stress in L02 cells (Fig. 2A). As expected, the protective effect of γ-man was partially impaired when co-treated with ATRA (Fig. 2A). Next, treatment with *t*-BHP or co-treatment with *t*-BHP and ATRA significantly increased the intracellular ROS accumulation in L02 cells, which was eliminated by γ-man treatment (Fig. 2B). The ROS level was significantly increased in the cells co-treated with γ-man and ATRA compared with that in γ-man treated cells. Compared with the untreated cells, the expression of HO-1 was decreased in *t*-BHP treated, as well as *t*-BHP and ATRA co-treated cells. And γ-man treatment increased the levels of HO-1 and SOD2, which were slightly decreased by the treatment of ATRA (Fig. 2C). Taken together, γ-man increased expression of NRF2 and promoted its nuclear localization to enhance antioxidant capacity in L02 cells.

3.2. γ-Man exerted protective effect by enhancing the activity of SIRT1 and maintaining the mitochondrial membrane potential (MMP) in L02 cells

Due to the involvement of SIRT1 in counteracting oxidative stress, we speculated that the hepatoprotective effect of γ-man might be

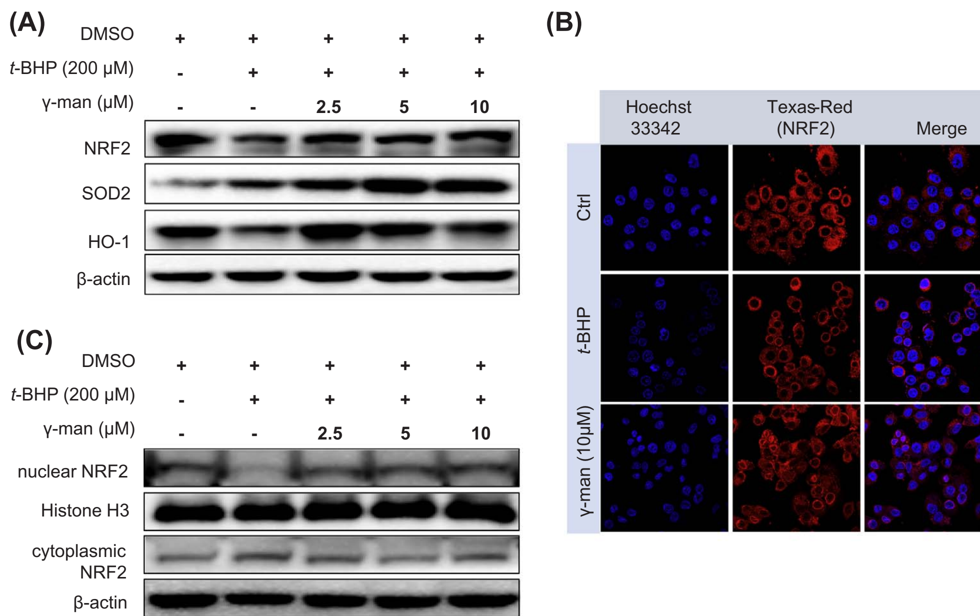

Fig. 1. γ-Man induced the expression and nuclear translocation of NRF2 in L02 cells. (A) γ-Man increased the expressions of NRF2 and antioxidant enzymes in *t*-BHP treated L02 cells. (B) The immunofluorescence of NRF2 in L02 cells. (C) The nuclear and cytoplasmic NRF2 expressions in L02 cells treated with or without γ-man.

mediated through activating SIRT1. As expected, γ-man reversed *t*-BHP induced suppression of SIRT1 expression in a concentration-dependent manner (Fig. 3A). PGC-1α, one of the deacetylation substrates of SIRT1, was also involved in alleviation of excessive ROS accumulation in cells, and the deacetylated PGC-1α was the biologically activated form (St-Pierre et al., 2006). Therefore, the total and acetylated PGC-1α in L02 cells were investigated. As shown in Fig. 3B, treatment of γ-man significantly increased total PGC-1α level, and decreased the acetylated PGC-1α level in a concentration-dependent manner. These results indicated that γ-man stimulated SIRT1 expression and enhanced its deacetylation activity on PGC-1α, resulting in protective effect against oxidative stress in L02 cells.

Excess oxidative stress can change the MMP of cells, and further induce cell apoptosis (Wei et al., 2001). SIRT1 and PGC-1α are reported to regulate the mitochondrial function (Gerhart-Hines et al., 2007; Lagouge et al., 2006). Thus, the protective role of γ-man might be reflected by the MMP in L02 cells. Using JC-1 staining, the polarized mitochondria (normal status) were markedly displayed punctate orange-red fluorescence, which was replaced by diffuse green monomer fluorescence on depolarization (abnormal status) (Smiley et al., 1991).

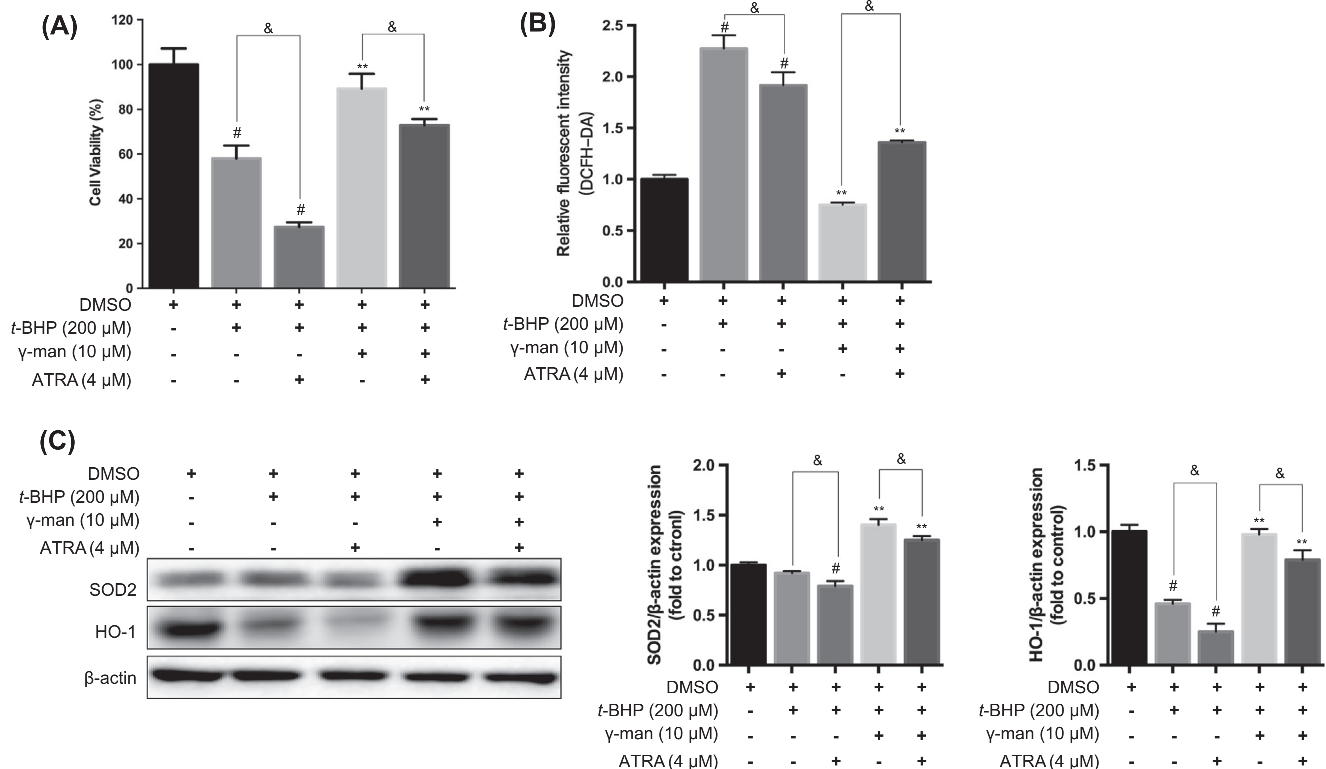

Fig. 2. The hepatoprotective effect of γ-man is partially mediated through NRF2. The effect of NRF2 inhibitor (ATRA) on cell viability (A), intracellular ROS accumulation (B) and expressions of HO-1 and SOD2 (C) in L02 cells treated with or without γ-man. $^{\#}p < .01$ *vs.* normal control group; $^{**}p < .01$ *vs.* *t*-BHP treated group; $^{\&}p < .01$ *vs.* without ATRA treatment.

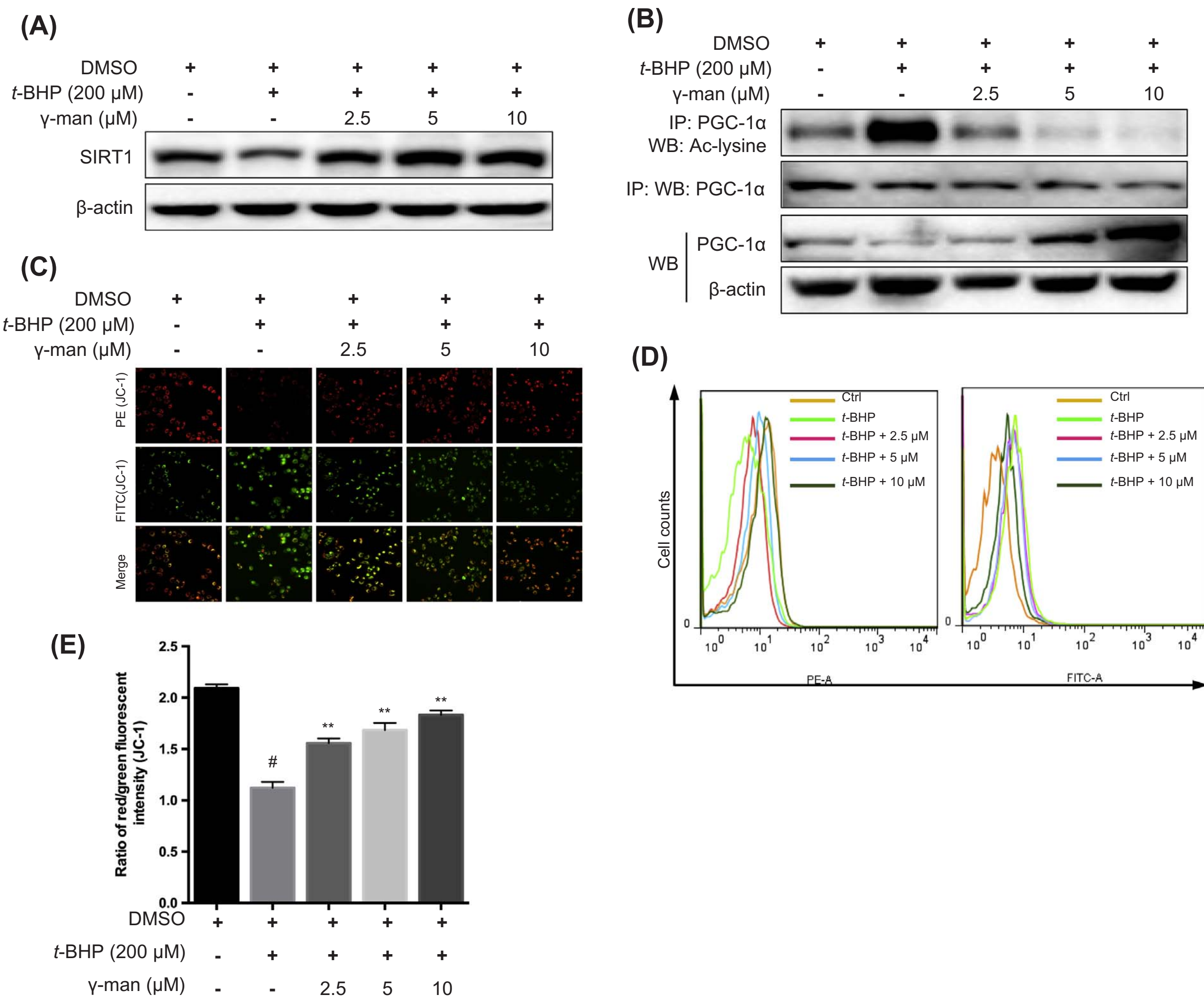

Fig. 3. γ-Man increased SIRT1 expression and activity, and enhanced mitochondrial function in L02 cells. (A) γ-Man increased expression of SIRT1 in L02 cells. (B) γ-Man increased PGC-1α expression and decreased its acetylation level in *t*-BHP treated L02 cells. (C) γ-Man protected L02 cells from *t*-BHP-induced depolarization of mitochondrial membrane. The polarized (PE, red fluorescence) and depolarized (FITC, green fluorescence) mitochondrial membrane was stained by JC-1. (D) The polarized (PE, red fluorescent) and depolarized (FITC, green fluorescent) mitochondrial membrane of L02 cells was determined by flow cytometer. (E) Quantitative determination of the ratio of red to green fluorescent intensity. $^{\#}p < .01$ *vs.* normal control group; $^{**}p < .01$ *vs.* *t*-BHP treated group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

As shown in Fig. 3C, the red fluorescence was significantly decreased accompanied with rise of green fluorescence in L02 cells after treatment with *t*-BHP, compared with those of control cells, which indicated that a great proportion of mitochondria were at depolarization status. Pretreatment of γ-man obviously reversed the change of fluorescence, which suggested the polarized status of mitochondria was maintained. The relative intensity of red to green fluorescence was analyzed quantitatively on a flow cytometer. The great shift of red to green fluorescence was observed in *t*-BHP treated cells, which was gradually reversed in γ-man pretreated cells (Fig. 3D). The ratio of red to green fluorescent intensity in *t*-BHP treated cells was around half of that of control cells, which was significantly increased by γ-man treatment in a dose dependent manner (Fig. 3E). Therefore, it was deduced that the hepatoprotective effect of γ-man might be mediated by maintaining the polarized status of mitochondria.

3.3. SIRT1 inhibitor or SIRT1 knockdown partially blocked the hepatoprotective effect of γ-man in L02 cells

To further confirm the role of SIRT1 in hepatoprotective effect of γ-man, tenovin-1, a SIRT1 inhibitor, was recruited. The cell viability was significantly decreased by treatment of *t*-BHP or co-treatment of *t*-BHP and tenovin-1, compared with that of control cells (Fig. 4A). γ-Man dramatically rescued L02 cells from *t*-BHP induced oxidative injury, while tenovin-1 partially blocked the protective effect of γ-man, which indicated that the hepatoprotective effect was partially through SIRT1 (Fig. 4A). Western blot analysis showed decreased total PGC-1α level and increased acetylated PGC-1α level in L02 cells treated with *t*-BHP or co-treated with *t*-BHP and tenovin-1, compared with those of control cells (Fig. 4B). Interestingly, tenovin-1 only partially abolished the effect of γ-man on acetylation level of PGC-1α, but not total PGC-1α (Fig. 4B).

Quantitative analysis results showed that treatment with γ-man significantly decreased *t*-BHP induced ROS accumulation in L02 cells (Fig. 4C). And the ROS level was significantly increased by the treatment of tenovin-1 compared with that of γ-man treated cells (Fig. 4C). Furthermore, γ-man treatment increased the expressions of HO-1 and SOD2 in *t*-BHP treated L02 cells, which was partially abolished by the SIRT1 inhibitor (Fig. 4D).

Next, *SIRT1* shRNA interfering experiment was performed in L02 cells. As shown in Fig. 5A, the cell viability of L02 cells were significantly decreased by treatment with *t*-BHP both in vector control (VC) and *SIRT1* knockdown group compared with that of control group. And 20% more cells were damaged in *SIRT1* knockdown cells compared with that of VC cells. The protective effect of γ-man was impaired by *SIRT1* knockdown, in which the cell viability was decreased about 20%

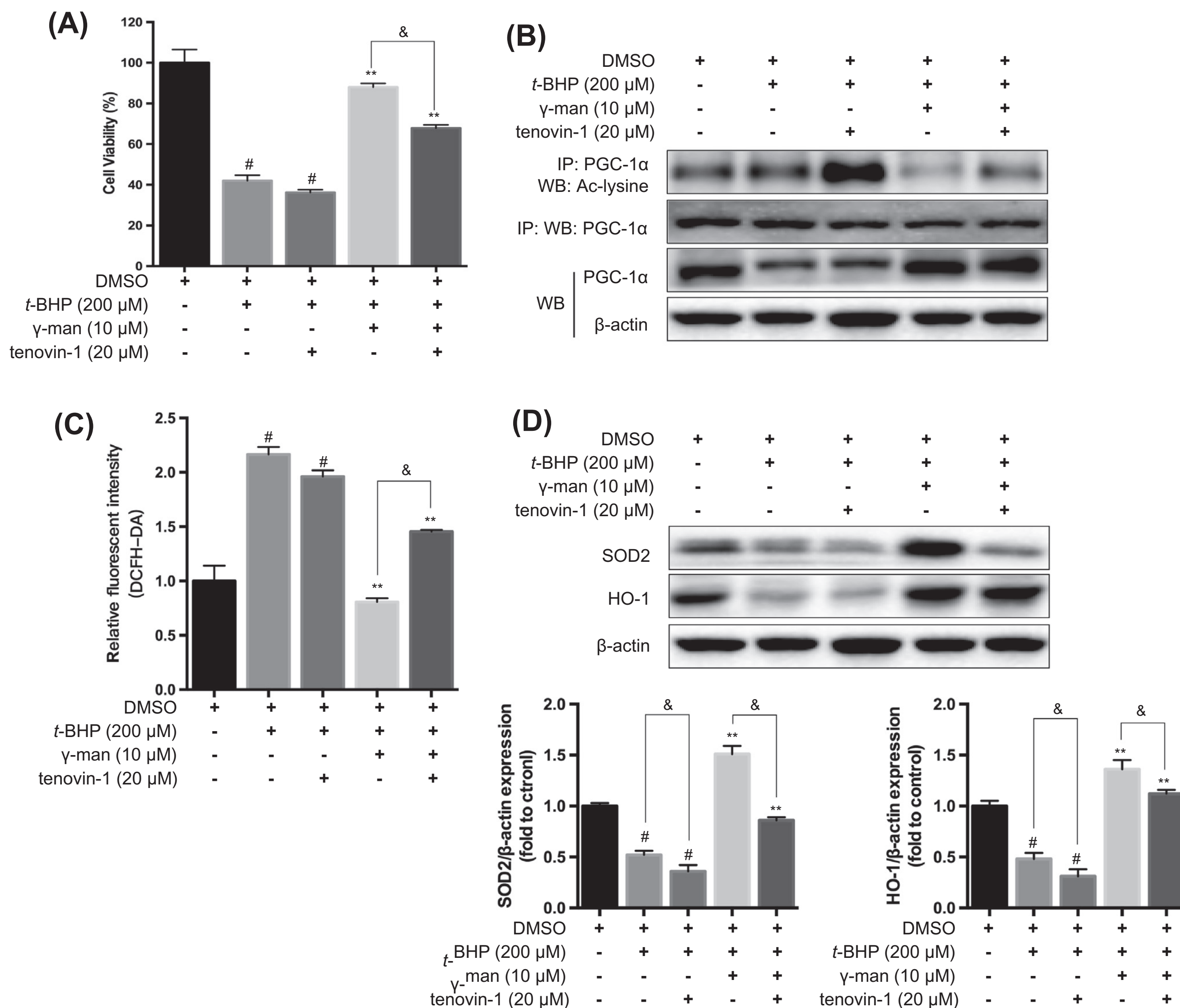

Fig. 4. The SIRT1 inhibitor (tenovin-1) partially blocked the protective effect of γ-man in L02 cells. The effect of tenovin-1 on cell viability (A), expression and acetylation level of PGC-1α (B), intracellular ROS accumulation (C) and expressions of HO-1 and SOD2 (D) in L02 cells treated with or without γ-man. $^{\#}p < .01$ *vs.* normal control group; $^{**}p < .01$ *vs.* *t*-BHP treated group, $^{\&}p < .01$ *vs.* without tenovin-1 treatment.

compared with that of VC cells. These results further indicated that the protective effect of γ-man in L02 cells was involved in SIRT1. Western blot results showed that *SIRT1* knockdown significantly decreased the expression of SIRT1 (Fig. 5C), which induced the increased acetylation level of PGC-1α in control and *t*-BHP cells (Fig. 5B). γ-Man treatment significantly increased the expression and activity of SIRT1, and the effect of this compound was blunted by *SIRT1* knockdown. It was also found that the induction of HO-1 and SOD2 was influenced by *SIRT1* ablation. As shown in Fig. 5C, *SIRT1* knockdown did not influence the expressions of HO-1 and SOD-2 in normal condition. When challenging with oxidative stress, it was found the induction of these two enzymes by γ-man was significantly decreased in *SIRT1* knockdown cells. Taken together, it was deduced that γ-man increased the expression and activity of SIRT1, which further facilitated the deacetylation of PGC-1α to resist oxidative damage.

3.4. γ-Man attenuated CCl_4 induced acute liver injury in mice

CCl_4 is known to cause acute liver injury characterized with centrilobular necrosis (Morio et al., 2001). The main underlying mechanisms includes cytochrome P450-mediated metabolism of CCl_4 to a highly reactive trichloromethyl radical, which initiates lipid peroxidation and leads to hepatocellular membrane damage (Koch, Glende, & Recknagel, 1974). The hepatoprotective effect of γ-man was further validated in CCl_4-treated C57BL/6 mice. The experimental procedure was briefly described in Fig. 6A. The H&E staining was performed to observe the change of liver architecture. More necrosis and inflammation hepatocytes were found in the liver specimens from CCl_4 treated mice, compared with those of control mice (Fig. 6B). However, pretreatment of γ-man for one week obviously protected mice from CCl_4-induced liver toxicity. As shown in Fig. 6B, the liver tissues from low and high dosage of γ-man group of mice (LD and HD) contained less necrosis and inflammation hepatocytes. Moreover, the serum AST and ALT levels were significantly increased by treatment with CCl_4 (Fig. 7A). When pre-treated with γ-man, the AST and ALT levels were significantly decreased compared with that of the CCl_4 group, no matter in LD or HD groups (Fig. 7A). Meanwhile, the SOD2 activity and GSH content in liver tissues were significantly increased in γ-man treated group compared with those of CCl_4 group (Fig. 7A). The biochemical parameters were not changed when the mice were challenged with γ-man only, compared with those of control group, which demonstrated that γ-man did not affect the liver physiological function in mice

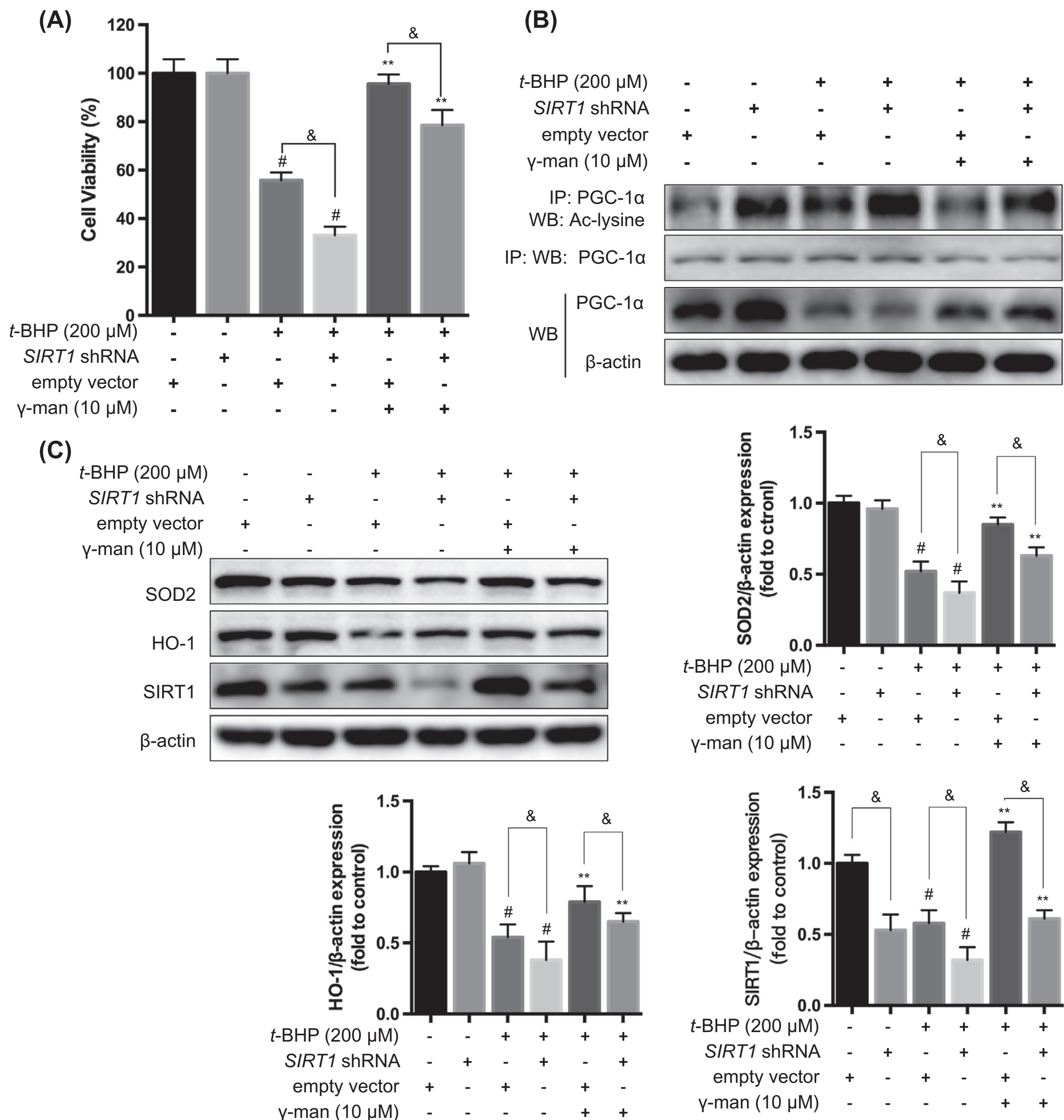

Fig. 5. *SIRT1* knockdown partially blocked the protective effect of γ-man in L02 cells. (A) *SIRT1* knockdown decreased the cell viability in L02 cells treated with or without γ-man. (B) *SIRT1* knockdown decreased the acetylation level of PGC-1α, but didn't change the total PGC-1α level in L02 cells treated with γ-man. (C) *SIRT1* knockdown decreased the expressions of HO-1 and SOD2 in L02 cells treated with γ-man. $^{\#}p < .01$ *vs.* normal control group; $^{**}p < .01$ *vs. t*-BHP treated group, $^{\&}p < .01$ *vs.* without *SIRT1* knockdown.

(Fig. 7A). These biochemical parameters measurements were in accordance with the pathological observations, and the results showed that γ-man attenuated the CCl_4-induced acute liver injury in mice.

To further confirm the potential molecular mechanisms of γ-man in counteracting CCl_4-induced liver toxicity in mice, NRF2 and SIRT1 expressions were detected in livers. The expressions of NRF2 and SIRT1 were significantly increased in LD and HD groups, resulting in increased of antioxidant enzymes HO-1 and SOD2 (Fig. 7B). Based on these results, it was deduced that the increased expressions of NRF2 and SIRT1 might mediate the hepatoprotective effects of γ-man in mice.

4. Discussion

In the current study, γ-man was found to upregulate the expressions of NRF2 and SIRT1 *in vitro* and *in vivo* when challenging with oxidative stress. Recent studies reported NRF2 can be deacetylated by SIRT1 to further induce less nuclei localization in HepG2 cells (Kawai, Garduño, Theodore, Yang, & Arinze, 2011) and glomerular mesangial cells (Huang et al., 2013). However, the current data showed the nuclear localization of NRF2 and induction of antioxidant elements were significantly increased by γ-man treatment, which was accompanied with the increased expression and activity of SIRT1. The contrary observation might be due to the cells and animals in current study were challenged by oxidative stress (*t*-BHP or CCl_4). Actually, several other

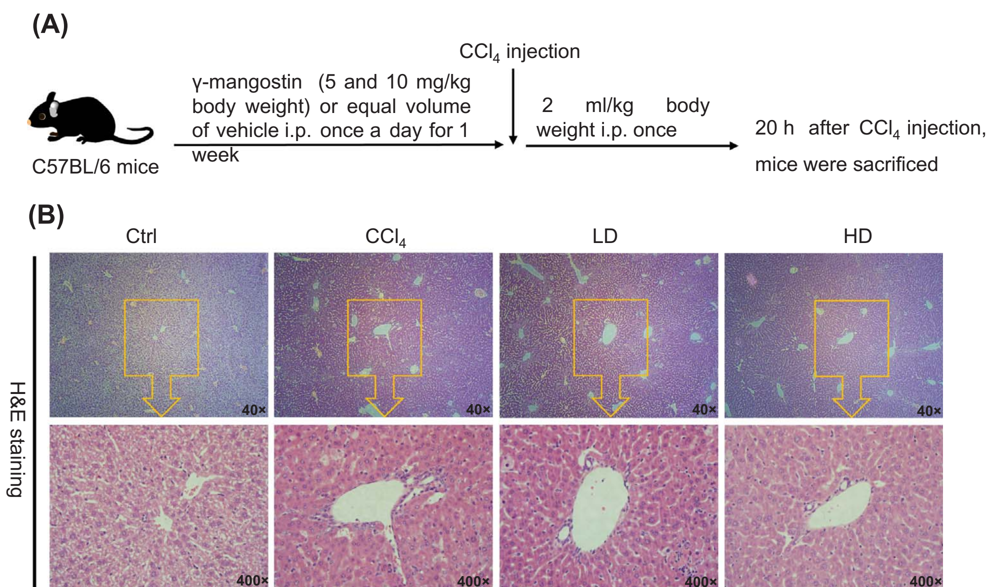

Fig. 6. γ-Man protected mice from CCl_4 induced acute liver injury. (A) The animal experimental procedures. (B) H&E staining of liver tissues (ctrl, normal control group; LD, low dosage group, 5 mg/kg BW i.p; HD, high dosage group, 10 mg/kg BW i.p).

studies also demonstrate that SIRT1 and NRF2 simultaneously enhanced antioxidant capacity in several animal and cell models, including LPS-induced oxidative stress in rat brain, rat glomerular mesangial cells and coronary arterial endothelial cells (Huang et al., 2015; Shah et al., 2017; Ungvari et al., 2010). Till now the direct interaction relationship between SIRT1 and NRF2 is merely investigated.

The current data showed either the NRF2 inhibitor (ATRA) or the SIRT1 inhibitor (tenovin-1) could only partially block the protective effect of γ-man in L02 cells. Hence it was deduced that NRF2 and SIRT1 might regulate the hepatoprotective effect of γ-man parallelly. To validate this hypothesis, the protective effect of γ-man in L02 cells was evaluated with co-treatment of ATRA and tenovin-1. As shown in Fig. S1, more L02 cells were damaged by treatment with ATRA (4 μM) or tenovin-1 (20 μM) compared with that of *t*-BHP treated cells. Co-treatment of ATRA and tenovin-1 in L02 cells induced more severe cell death, which demonstrated that NRF2 and SIRT1 signaling pathways functioned parellelly. Surprisingly, co-treatment with ATRA and tenovin-1, together with γ-man, only partially decreased the cell viability compared with the γ-man treated cells. It indicated that γ-man might competitively bind to the binding domains of NRF2 and SIRT1, resulting in impaired the inhibitory effects of these two inhibitors. On the other hand, other signaling pathways responsible for the cell survive and mitochondrial ROS production might also mediate the hepatoprotective effect of γ-man, such as B-cell lymphoma 2 (Bcl-2) (Hockenbery, Oltvai, Yin, Milliman, & Korsmeyer, 1993), Bcl-xL (Gross, McDonnell, & Korsmeyer, 1999), p53 (Finkel & Holbrook, 2000), Forkhead box O3a (FoxO3a) (Li et al., 2008) and hypoxia-inducible factor 1α (HIF-1α) (Sack & Finkel, 2012). Thus, the treatment of SIRT1 and NRF2 inhibitors could not totally block the protective effect of γ-man in *t*-BHP challenged L02 cells. Recently, mangiferin, a xanthone isolated from

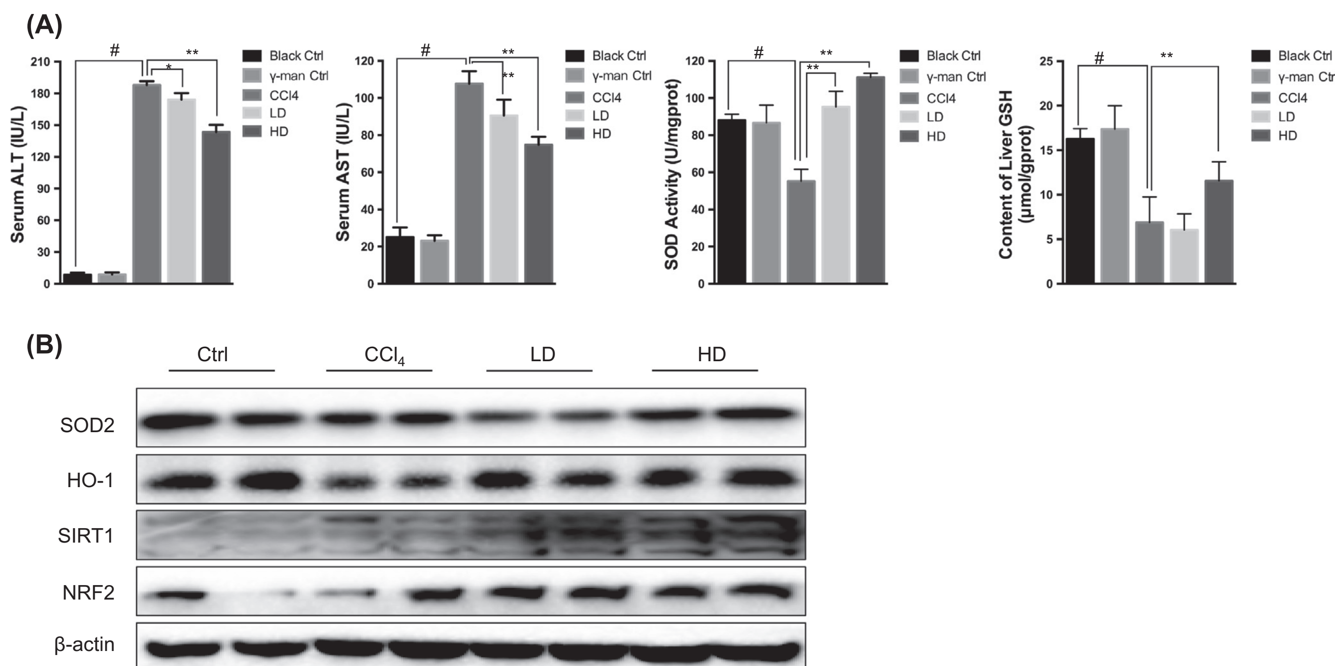

Fig. 7. γ-Man attenuated liver oxidative stress through SIRT1 and NRF2 *in vivo*. (A) The serum AST and ALT levels, and the liver SOD activity and GSH content. (B) γ-Man increased the expressions of anti-oxidative enzymes, SIRT1 and NRF2 in acute liver injured mice. $^{\#}p < .01$ *vs.* normal control group; $^{*}p < .05$ and $^{**}p < .01$ *vs.* CCl_4 treated group.

mango, was found to protect murine liver from being damaged by oxidative stress, and suppress cell apoptosis induced by plumbum through activation of mitogen-activated protein kinases (MAPK) and nuclear factor-κB (NF-κB) pathways (Pal, Sinha, & Sil, 2013). In our study, the oxidative stress induced by oxidants (*t*-BHP) or toxicants (CCl_4) is considered as the major reason for loss of cell viability in L02 cells or liver function in mice. And our results demonstrated γ-man as a potent candidate in counteracting with oxidative stress both *in vitro* and *vivo*. Thus, xanthones might be a new class of candidates to develop as hepatoprotective agents.

PGC-1α, a deacetylation substrate of SIRT1, regulates mitochondrial biogenesis and antioxidant effect with the involvement of nuclear respiratory factors (NRFs) and peroxisome proliferators-activated receptor-β (PPARβ) (St-Pierre et al., 2003; Ventura-Clapier, Garnier, & Veksler, 2008). It has been demonstrated that deacetylated PGC-1α translocates to mitochondria and induces the expression and activation of SIRT3, which further facilities the activation of SOD2 (deacetylation) to counteract with ROS (Qiu, Brown, Hirschey, Verdin, & Chen, 2010; Shi, Wang, Stieren, & Tong, 2005; St-Pierre et al., 2006). In our study, it was found that the expression of SOD2 was not always consistent with the ROS level in cells. It was deduced that apart from the expression of SOD2, the activity of this enzyme might also affect its antioxidant ability. Meanwhile, other signaling pathways, such as p53, FoxO3a and HIF-1α, might be also responsible for ROS scavenging, which need further investigation and exploration.

Besides γ-man, the hepatoprotective activity of other xanthones from *G. mangostana* have also been investigated, especially α-mangostin. Previous study reported α-mangostin attenuated hepatic steatosis in high fat diet induced obese mice through SIRT1 and PPARγ (Choi et al., 2015). In the current study, we found that γ-man, one of analogues of α-mangostin, could also upregulate the expression of SIRT1 in liver to counteract with CCl_4 induced acute liver injury. Using the “Fluor-de-Lys” substrate, the direct activating effect of γ-man on SIRT1 deacetylation activity was evaluated. The result showed that γ-man did not change SIRT1 deacetylation activity obviously (data no shown). However, other studies challenged the overall utility of the above assay to screen SIRT1 activator, since the authors considered that the Fluor de Lys-p53 peptide is an artificial SIRT1 substrate (Beher et al., 2009). Resveratrol, one of the activators of SIRT1, is widely investigated in *in vitro* and *in vivo* studies (Howitz et al., 2003). Report also indicated the activation effects of resveratrol on SIRT1 deacetylation depend on substrate sequence, and suggested substrates relevant for *in vivo* effects (Lakshminarasimhan, Rauh, Schutkowski, & Steegborn, 2013). In our study, γ-man activated SIRT1 to facilitate deacetylation of PGC-1α challenging with *t*-BHP in a concentration-dependent manner. Till now, there is no report to clearly elucidate the activation mechanism of SIRT1 by neither α-mangostin nor γ-man. Based on current results, several questions about the activation mechanism of γ-man, including whether γ-man directly binds and activates SIRT1, which binding site is effective for γ-man and the structure-activity relationship between xanthones, still need to be resolved and investigated.

5. Conclusion

In summary, γ-man attenuated oxidative injury in *t*-BHP-treated hepatocytes and CCl_4-challenged mice. Mechanisms study demonstrated that γ-man increased the nuclear translocation of NRF2 and expressions of SOD2 and HO-1 to enhance antioxidant capacity. Meanwhile, γ-man stimulated the expression of SIRT1 and enhanced its deacetylation activity, which further facilitated the decrease of acetylated PGC-1α to regulate the mitochondrial function. Taken together, γ-man might be a potential candidate for clinical prevention and treatment of acute liver oxidative injury.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgement

Financial support by Science and Technology Development Fund, Macao S.A.R (FDCT 120/2013/A3) and the Research Fund of University of Macau (MYRG2015-00153-ICMS-QRCM and MYRG2017-00109-ICMS) are gratefully acknowledged.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jff.2017.11.047.

References

Akao, Y., Nakagawa, Y., & Nozawa, Y. (2008). Anti-cancer effects of xanthones from pericarps of mangosteen. *International Journal of Molecular Sciences, 9*, 355–370.
Beher, D., Wu, J., Cumine, S., Kim, K. W., Lu, S. C., Atangan, L., & Wang, M. (2009). Resveratrol is not a direct activator of SIRT1 enzyme activity. *Chemical Biology & Drug Design, 74*, 619–624.
Bissell, D. M., Gores, G. J., Laskin, D. L., & Hoofnagle, J. H. (2001). Drug-induced liver injury: Mechanisms and test systems. *Hepatology, 33*, 1009–1013.
Brunet, A., Sweeney, L. B., Sturgill, J. F., Chua, K. F., Greer, P. L., Lin, Y., ... Cohen, H. Y. (2004). Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science, 303*, 2011–2015.
Choi, Y. H., Bae, J. K., Chae, H.-S., Kim, Y.-M., Sreymom, Y., Han, L., ... Chin, Y.-W. (2015). α-Mangostin regulates hepatic steatosis and obesity through SirT1-AMPK and PPARγ pathways in high-fat diet-induced obese mice. *Journal of Agricultural and Food Chemistry, 63*, 8399–8406.
Choi, J., & Ou, J.-H. J. (2006). Mechanisms of liver injury. III. Oxidative stress in the pathogenesis of hepatitis C virus. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 290*, G847–G851.
Cullinan, S. B., Gordan, J. D., Jin, J., Harper, J. W., & Diehl, J. A. (2004). The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: Oxidative stress sensing by a Cul3-Keap1 ligase. *Molecular and Cellular Biology, 24*, 8477–8486.
Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature, 408*, 239–247.
Gerhart-Hines, Z., Rodgers, J. T., Bare, O., Lerin, C., Kim, S. H., Mostoslavsky, R., ... Puigserver, P. (2007). Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1α. *The EMBO Journal, 26*, 1913–1923.
Gorrini, C., Harris, I. S., & Mak, T. W. (2013). Modulation of oxidative stress as an anticancer strategy. *Nature Reviews Drug Discovery, 12*, 931–947.
Gross, A., McDonnell, J. M., & Korsmeyer, S. J. (1999). BCL-2 family members and the mitochondria in apoptosis. *Genes & Development, 13*, 1899–1911.
Hockenbery, D. M., Oltvai, Z. N., Yin, X. M., Milliman, C. L., & Korsmeyer, S. J. (1993). Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell, 75*, 241–251.
Howitz, K. T., Bitterman, K. J., Cohen, H. Y., Lamming, D. W., Lavu, S., Wood, J. G., ... Sinclair, D. A. (2003). Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature, 425*, 191–196.
Hsu, C. P., Zhai, P., Yamamoto, T., Maejima, Y., Matsushima, S., Hariharan, N., ... Sadoshima, J. (2010). Silent Information Regulator 1 protects the heart from ischemia/reperfusion clinical perspective. *Circulation, 122*, 2170–2182.
Huang, K. P., Chen, C., Hao, J., Huang, J. Y., Wang, S. G., Liu, P. Q., & Huang, H. Q. (2015). Polydatin promotes Nrf2-ARE anti-oxidative pathway through activating Sirt1 to resist AGEs-induced upregulation of fibronetin and transforming growth factor-β1 in rat glomerular messangial cells. *Molecular and Cellular Endocrinology, 399*, 178–189.
Huang, K. P., Huang, J., Xie, X., Wang, S. G., Chen, C., Shen, X. Y., ... Huang, H. Q. (2013). Sirt1 resists advanced glycation end products-induced expressions of fibronectin and TGF-β1 by activating the Nrf2/ARE pathway in glomerular mesangial cells. *Free Radical Biology and Medicine, 65*, 528–540.
Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., Igarashi, K., Engel, J. D., & Yamamoto, M. (1999). Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes & Development, 13*, 76–86.
Jung, H. A., Su, B. N., Keller, W. J., Mehta, R. G., & Kinghorn, A. D. (2006). Antioxidant xanthones from the pericarp of *Garcinia mangostana* (Mangosteen). *Journal of Agricultural and Food Chemistry, 54*, 2077–2082.
Kawai, Y., Garduño, L., Theodore, M., Yang, J., & Arinze, I. J. (2011). Acetylation-deacetylation of the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) regulates its transcriptional activity and nucleocytoplasmic localization. *Journal of Biological Chemistry, 286*, 7629–7640.
Ke, B. B., Shen, X. D., Ji, H. F., Kamo, N., Gao, F., Freitas, M. C. S., ... Kupiec-Weglinski, J. W. (2012). HO-1–STAT3 axis in mouse liver ischemia/reperfusion injury: Regulation of TLR4 innate responses through PI3K/PTEN signaling. *Journal of Hepatology, 56*, 359–366.
Koch, R. R., Glende, E. A., & Recknagel, R. O. (1974). Hepatotoxicity of

bromotrichloromethane—bond dissociation energy and lipoperoxidation. *Biochemical Pharmacology, 23*, 2907–2915.
Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., ... Auwerx, J. (2006). Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. *Cell, 127*, 1109–1122.
Lakshminarasimhan, M., Rauh, D., Schutkowski, M., & Steegborn, C. (2013). Sirt1 activation by resveratrol is substrate sequence-selective. *Aging, 5*, 151–154.
Li, H. (2014). Sirtuin 1 (SIRT1) and oxidative stress. *Systems Biology of Free Radicals and Antioxidants,* 417–435 Springer.
Li, G. H., Lee, Y. F., Liu, S., Cai, Y., Xie, S. Z., Liu, N. C., ... Chang, C. S. (2008). Oxidative stress stimulates testicular orphan receptor 4 through forkhead transcription factor forkhead box O3a. *Endocrinology, 149*, 3490–3499.
Liu, Q. Y., Li, D., Wang, A. Q., Dong, Z., Yin, S., Zhang, Q. W., ... Lin, L. G. (2016). Nitric oxide inhibitory xanthones from the pericarps of *Garcinia mangostana*. *Phytochemistry, 131*, 115–123.
Liu, J. X., Li, D., Zhang, T., Tong, Q., Ye, R. D., & Lin, L. G. (2017). SIRT3 protects hepatocytes from oxidative injury by enhancing ROS scavenging and mitochondrial integrity. *Cell Death and Disease, 8*, e3158.
Liu, Q. Y., Wang, Y. T., & Lin, L. G. (2015). New insights into the anti-obesity activity of xanthones from *Garcinia mangostana*. *Food & Function, 6*, 383–393.
Morio, L. A., Chiu, H., Sprowles, K. A., Zhou, P., Heck, D. E., Gordon, M. K., & Laskin, D. L. (2001). Distinct roles of tumor necrosis factor-α and nitric oxide in acute liver injury induced by carbon tetrachloride in mice. *Toxicology and Applied Pharmacology, 172*, 44–51.
Nemoto, S., Fergusson, M. M., & Finkel, T. (2005). SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1α. *Journal of Biological Chemistry, 280*, 16456–16460.
Nguyen, T., Sherratt, P. J., Nioi, P., Yang, C. S., & Pickett, C. B. (2005). Nrf2 controls constitutive and inducible expression of ARE-driven genes through a dynamic pathway involving nucleocytoplasmic shuttling by Keap1. *Journal of Biological Chemistry, 280*, 32485–32492.
Pal, P. B., Sinha, K., & Sil, P. C. (2013). Mangiferin, a natural xanthone, protects murine liver in Pb (II) induced hepatic damage and cell death via MAP kinase, NF-κB and mitochondria dependent pathways. *PLoS ONE, 8*, e56894.
Picard, F., Kurtev, M., Chung, N. J., Topark-Ngarm, A., Senawong, T., De Oliveira, R. M., ... Guarente, L. (2004). Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-γ. *Nature, 429*, 771–776.
Qiu, X., Brown, K., Hirschey, M. D., Verdin, E., & Chen, D. (2010). Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. *Cell Metabolism, 12*, 662–667.
Rodgers, J. T., Lerin, C., Haas, W., Gygi, S. P., Spiegelman, B. M., & Puigserver, P. (2005). Nutrient control of glucose homeostasis through a complex of PGC-1α and SIRT1. *Nature, 434*, 113–118.
Sack, M. N., & Finkel, T. (2012). Mitochondrial metabolism, sirtuins, and aging. *Cold Spring Harbor Perspectives in Biology, 4*, a013102.
Schipper, H. M., Gupta, A., & Szarek, W. A. (2009). Suppression of glial HO-1 activity as a potential neurotherapeutic intervention in AD. *Current Alzheimer Research, 6*, 424–430.
Shah, S. A., Khan, M., Jo, M. H., Jo, M. G., Amin, F. U., & Kim, M. O. (2017). Melatonin stimulates the SIRT1/Nrf2 signaling pathway counteracting lipopolysaccharide (LPS)-induced oxidative stress to rescue postnatal rat brain. *CNS Neuroscience & Therapeutics, 23*, 33–44.
Shi, T., Wang, F., Stieren, E., & Tong, Q. (2005). SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. *Journal of Biological Chemistry, 280*, 13560–13567.
Smiley, S. T., Reers, M., Mottola-Hartshorn, C., Lin, M., Chen, A., Smith, T. W., ... Chen, L. B. (1991). Intracellular heterogeneity in mitochondrial membrane potentials revealed by a J-aggregate-forming lipophilic cation JC-1. *Proceedings of the National Academy of Sciences, 88*, 3671–3675.
St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J. M., Rhee, J., Jäger, S., ... Spiegelman, B. M. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell, 127*, 397–408.
St-Pierre, J., Lin, J., Krauss, S., Tarr, P. T., Yang, R., Newgard, C. B., & Spiegelman, B. M. (2003). Bioenergetic analysis of peroxisome proliferator-activated receptor γ coactivators 1α and 1β (PGC-1α and PGC-1β) in muscle cells. *Journal of Biological Chemistry, 278*, 26597–26603.
Tanno, M., Kuno, A., Yano, T., Miura, T., Hisahara, S., Ishikawa, S., ... Horio, Y. (2010). Induction of manganese superoxide dismutase by nuclear translocation and activation of SIRT1 promotes cell survival in chronic heart failure. *Journal of Biological Chemistry, 285*, 8375–8382.
Ungvari, Z., Bagi, Z., Feher, A., Recchia, F. A., Sonntag, W. E., Pearson, K., ... Csiszar, A. (2010). Resveratrol confers endothelial protection via activation of the antioxidant transcription factor Nrf2. *American Journal of Physiology-Heart and Circulatory Physiology, 299*, H18–H24.
Vaziri, H., Dessain, S. K., Eaton, E. N., Imai, S.-I., Frye, R. A., Pandita, T. K., ... Weinberg, R. A. (2001). HSIR2SIRT1 functions as an NAD-dependent p53 deacetylase. *Cell, 107*, 149–159.
Ventura-Clapier, R., Garnier, A., & Veksler, V. (2008). Transcriptional control of mitochondrial biogenesis: The central role of PGC-1α. *Cardiovascular Research, 79*, 208–217.
Wang, A. Q., Liu, Q. Y., Ye, Y., Wang, Y. T., & Lin, L. G. (2015). Identification of hepatoprotective xanthones from the pericarps of *Garcinia mangostana*, guided with *tert*-butyl hydroperoxide induced oxidative injury in HL-7702 cells. *Food & Function, 6*, 3013–3021.
Wei, M. C., Zong, W. X., Cheng, E. H. Y., Lindsten, T., Panoutsakopoulou, V., Ross, A. J., ... Korsmeyer, S. J. (2001). Proapoptotic BAX and BAK: A requisite gateway to mitochondrial dysfunction and death. *Science, 292*, 727–730.
Wu, D., & Cederbaum, A. I. (2003). Alcohol, oxidative stress, and free radical damage. *Alcohol Research and Health, 27*, 277–284.