



Dihydroquercetin ameliorated acetaminophen-induced hepatic cytotoxicity via activating JAK2/STAT3 pathway and autophagy

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Abstract

Acetaminophen (APAP) overdose is currently the leading cause of acute liver disease, but therapeutic treatment strategies are commonly limited. Although dihydroquercetin (DHQ) is an attractive botanical antioxidant, its protective potential for liver disease remains elusive. The present study investigated the protective effects of DHQ against APAP-induced hepatic cytotoxicity. Primary mouse hepatocytes were treated with different concentrations of DHQ followed by APAP administration. Our data showed that DHQ relieved APAP-induced growth inhibition and lactate dehydrogenase (LDH) release in a dose-dependent manner, as well as inhibited APAP-induced necrosis and extracellular signal regulated kinase-c-Jun-N-terminal kinase (ERK-JNK) stress. In addition, reactive oxygen species (ROS) accumulation and mitochondria dysfunction were also reversed by DHQ treatment. Further study revealed that DHQ induced phosphorylation of Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) cascade and thus modulated expression of anti-apoptotic Bcl-2 family proteins. Moreover, DHQ induced autophagy which mediated its protective effects in hepatocytes. The protection was abrogated through pharmacological blockage of autophagy by chloroquine (CQ). These studies demonstrated, for the first time, that DHQ possessed hepatocellular protective effects in the context of APAP-induced cytotoxicity and subsequently revealed that the mechanisms comprised activation of JAK2/STAT3 signaling pathway and autophagy. These altogether highlighted the significant therapeutic potential of this agent during acute liver failure and other types of liver diseases.

Keywords Dihydroquercetin · Acetaminophen · Hepatic cytotoxicity · JAK2/STAT3 · Autophagy

Introduction

Dihydroquercetin (DHQ) is a typical flavanone constituent of *Pseudotsuga taxifolia*, *Taxus chinensis*, *Cedrus deodara*, and so on (Kuang et al. 2017). It is described to possess series of

biological capacities including antioxidant, anti-inflammation, anti-tumor, and cardiovascular protective properties (Sun et al. 2014). Although barely discernable, DHQ is simultaneously found in the approved drug Silymarin, which as a hepatoprotective product used for centuries in the world (Kroll et al. 2007; Theriault et al. 2000). As the efficacy of Silymarin is always attributed to the major flavonolignan constituents, DHQ is ignored as an impurity and its property to protect against liver injury is rarely investigated (Weidmann 2012). In fact, DHQ is described as “particularly noteworthy” in a study screening the potency of pure compounds derived from Silymarin extract against hepatitis C virus infection (Polyak et al. 2010). Previous studies have also revealed the protective actions of DHQ in tetrachloromethane-induced hepatitis and concanavalin A-induced autoimmune hepatitis (Teselkin et al. 2000; Zhao et al. 2015). However, the precise mechanisms remain elusive and whether DHQ can be explicated as an ideal hepatoprotective agent needs further exploration.

Wenjing Zai and Wei Chen contributed equally to this work.

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Acetaminophen (APAP) overdose is the leading cause of acute liver disease. Although the pathogenesis of APAP-induced toxicity has been well studied, there are still lack of efficient methods to cure this acute disease clinically (Bunchorntavakul and Reddy 2013). Mechanistically, the majority of APAP undergoes phase II metabolism to non-toxic compounds and then excreted, but some proportions are bioactivated by cytochrome P-450 (CYP450) enzyme system to reactive *N*-acetyl-*p*-benzoquinone imine (NAPQI). NAPQI in turn depletes glutathione (GSH) and causes oxidative stress, mitochondrial dysfunction, DNA fragmentation, and finally cell necrosis (Antoine et al. 2008; McGill et al. 2012; Jaeschke et al. 2012). Currently, the best therapeutic option for APAP overdose is *N*-acetyl-L-cysteine (NAC), a cysteine prodrug which supports the detoxification of NAPQI via restoring GSH pool (Saito et al. 2010). However, NAC possesses several side effects and it must be administered very early considering its short-time window (Athuraliya and Jones 2009). Further research for alternative and efficacious antidotes against APAP toxicity is warranted.

In the present study, we investigated the protective function of DHQ on APAP-induced hepatic cytotoxicity using primary mouse hepatocytes. Our results demonstrated that DHQ effectively ameliorated APAP-induced hepatocellular necrosis via free radical elimination and mitochondrial protection. Further investigation revealed an involvement of Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) cascade and autophagy in DHQ-mediated hepatocellular protective actions. Thus, DHQ was proved to be a promising therapeutic candidate for liver diseases.

Materials and methods

Reagents and antibodies

Dihydroquercetin and acetaminophen were purchased from MedChem Express (Shanghai, China); NAC was obtained from Beyotime Institute of Biotechnology (Jiangsu, China); and chloroquine (CQ) and rapamycin were obtained from Sigma-Aldrich (St. Louis, MO, USA). The antibodies including anti- β -Actin, anti-phospho-JNK (Thr183/Tyr204), anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204), anti-phospho-JAK2, anti-phospho-STAT3 (Tyr705), anti-Bax, anti-Bcl-2, anti-LC3B, and anti-p62 were all purchased from Cell Signaling Technology (Danvers, MA, USA) (1:1000 dilutions). The anti-CYP2E1 antibody was purchased from Sangon Biotech (Shanghai, China) (1:300 dilutions). Horseradish peroxidase (HRP)-conjugated secondary antibodies were obtained from MR Biotech (Shanghai, China).

Primary mouse hepatocyte isolation and cell culture

Primary mouse hepatocytes were isolated from C57BL/6 mice by retrograde perfusion (a two-step isolation procedure) with 0.05% Collagenase IV (Yeasten Institute of Biotechnology, Shanghai, China); then, the isolated cells were purified with 40% Percoll (GE Healthcare Life Sciences, Shanghai, China). Cells were suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin and then seeded in collagen-coated plates subsequently. Hepatoma-derived HepG2 cell line was obtained from Cell Bank of Chinese Academy of Science, Shanghai branch (Shanghai, China), and cultured in complete DMEM. After plating overnight, the medium was replaced with serum-free DMEM medium and proposed compounds were added for further detection.

Cell viability and LDH release assay

Cells were seeded in 96-well plates overnight; different concentrations of DHQ (containing 0.1% DMSO) or NAC were added 1 h before APAP (10 mM) treatment. Cell viability was measured by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. LDH release was detected via CytoTox 96® Non-Radio Cytotoxicity Assay (Promega, WI, USA) according to the manufacture's instruction.

Determination of necrosis

After indicated treatment, cells were doubly stained with Hoechst 33342 and propidium iodide (PI) dye (Beyotime, Jiangsu, China) for 15 min and then observed by a fluorescent microscopy (Zeiss Inc., MN, USA). The proportion of necrosis cells was quantified via ImageJ software (National Institutes of Health, MD, USA).

Analysis of intracellular GSH content

GSH concentration was measured with a GSH assay kit (Nanjing JianCheng Bioengineering Institute, Jiangsu, China). After treatment, cells were lysed and subjected to a cycling reaction; the GSH levels were determined by spectrophotometry (Sigma, MO, USA). The concentration of total protein was measured by bicinchoninic acid (BCA) protein assay kit.

ROS detection

2',7'-Dichlorofluorescein diacetate (DCFH-DA) assay kit (Beyotime, Jiangsu, China) was used for detection of intracellular ROS levels. Primary mouse hepatocytes were seeded in a 96-well black plate and were incubated overnight for

attachment. After treatment with intensive drugs, the medium was replaced with serum-free DMEM containing fluorescent probes for 30 min at 37 °C. The DCF fluorescent intensity was detected at Ex./Em. = 488/525 nm using a fluorescence spectrophotometer (Tecan). Cells were stained with MitoSox™ Red Dye (Beyotime, Jiangsu, China) and Hoechst 33342 for the observation by confocal microscopy (Carl Zeiss LSM710, Carl Zeiss, Germany) following the manufacturer's instructions. Relative fluorescent intensity was quantified by ImageJ software.

MMP measurement

MMP was measured via JC-1 assay kit (Beyotime, Jiangsu, China). After treatment, cells were stained with JC-1 at 37 °C for 30 min, according to the manufacture's protocol, and then photographed using fluorescence microscopy (Zeiss Inc., MN, USA). JC-1 aggregates fluorescent red, representing normal membrane potential, whereas monomers green, indicating the depolarized mitochondria. The fluorescent intensities were calculated using ImageJ software.

Autophagy detection via confocal microscopy

Cells were seeded in glass bottom culture dishes. After indicated treatments, cells were disposed with Cyto-ID® Autophagy Detection Kit (Enzo Life Science, Inc., Farmingdale, NY, USA) following the manufacture's instruction and then observed via confocal microscopy.

Western Blot analysis

Equivalent amounts of protein extracted from primary hepatocytes were loaded and separated by sodium dodecyl sulfate (SDS) polyacrylamide gels. The protein was subsequently transferred to polyvinylidene difluoride (PVDF) membranes and blocked with 5% BSA in Tris-buffered saline (containing 0.1% Tween 20) (TBST). The blots were then probed with primary antibodies for 4 h followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies for another 2 h in room temperature. The membranes were visualized by enhanced chemiluminescent detection kit (Pierce, Rockford, IL, USA). Intensity of protein bands was quantified by ImageJ software.

Statistical analysis

Data was analyzed using one-way analysis of variance (ANOVA) or two-tailed Student's test and presented as mean ± standard deviations (S.D.). Values of $P < 0.05$ were considered statistically significant.

Results

DHQ inhibited APAP-induced cytotoxicity in hepatocytes

Cultured primary mouse hepatocytes were pretreated with different concentrations of DHQ for 1 h and then incubated with APAP (10 mM) for another 24 h. The result depicted a potent suppression of hepatocytes triggered by APAP, whereas DHQ prevented the cytotoxicity in a concentration-dependent manner (Fig. 1a). NAC was used here as a positive control. Meanwhile, APAP treatment increased the percentage of LDH release in hepatocytes, which was significantly ameliorated by co-treatment with DHQ (Fig. 1b). Cytotoxicity dose-response experiment showed that DHQ was well tolerated by primary mouse hepatocytes up to 200 μM (Fig. S1A). Similar protection of DHQ was also detected in HepG2 cell lines (Fig. S1B).

These results suggested that DHQ could prevent APAP-induced cellular injury both in primary mouse hepatocytes and in hepatoma carcinoma cells.

DHQ relieved APAP-induced necrosis and suppressed ERK/JNK stress responses

Exposure of hepatocytes to APAP led to a remarkable increase in propidium iodide (PI)-positive cell numbers, indicating cell

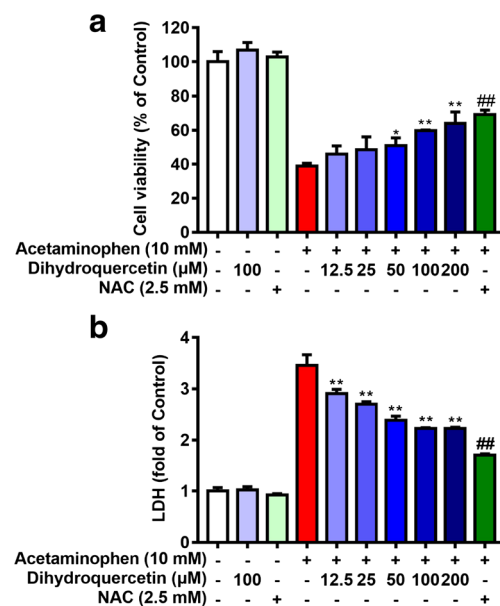


Fig. 1 Dihydroquercetin inhibited APAP-induced cytotoxicity in hepatocytes. **a** Primary mouse hepatocytes were pretreated with different concentrations of DHQ or NAC (2.5 mM) for 1 h and then incubated with APAP (10 mM) for another 24 h. Cell viability was measured via MTT assay. **b** Primary mouse hepatocytes were incubated with DHQ, NAC, and/or APAP (10 mM); the LDH release was quantified using LDH assay. Values were expressed as mean ± S.D. ($n = 3$). ** $P < 0.01$, * $P < 0.05$ compared to APAP; ## $P < 0.01$ compared to APAP

death and DNA damage, while DHQ dramatically relieved the extent of PI staining (Fig. 2a, b). In addition, APAP treatment also caused a significant phosphorylated activation of c-Jun-N-terminal kinase (JNK) but this effect was drastically attenuated by DHQ in a dose-dependent manner (Fig. 2c). The densitometric analysis of p-JNK1/2 protein bands confirmed

the above alternation (Fig. 2d). Similarly, extracellular signal regulated kinase (ERK) was activated as a consequence of APAP toxicity, which was reversed by co-treatment with DHQ (Fig. 2e, f).

These data further confirmed the hepato-protective action of DHQ in the context of APAP toxicity.

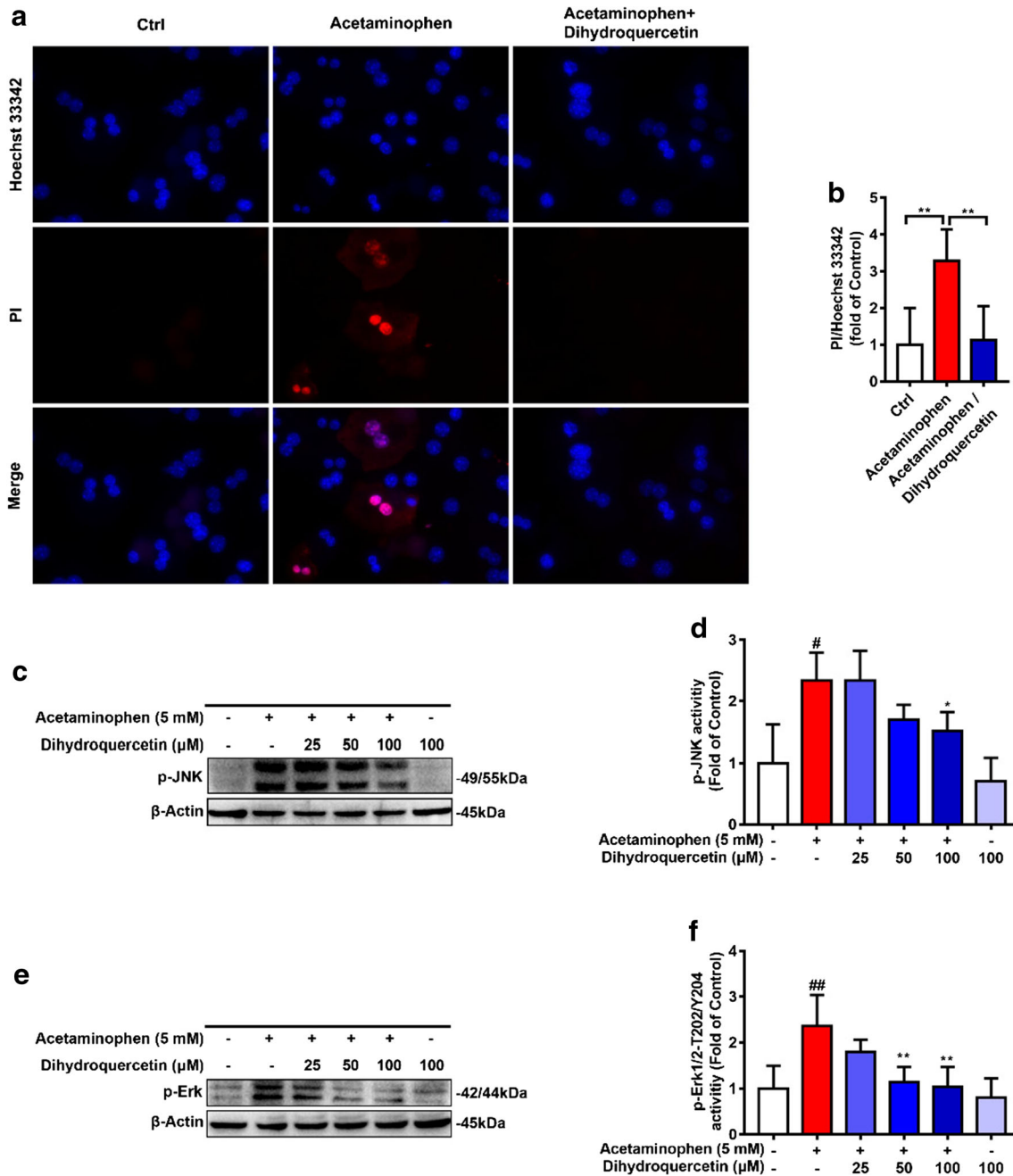


Fig. 2 Dihydroquercetin relieved APAP-induced necrosis and suppressed ERK/JNK stress responses. **a** Primary mouse hepatocytes were pretreated with DHQ (100 μ M) for 1 h and then incubated with APAP (5 mM) for another 12 h. After treatment, cells were subjected to Hoechst 33342 and PI staining to detect cell necrosis. **b** The statistics of PI-positive cells. Primary mouse hepatocytes were pretreated with DHQ (0–100 μ M) for 1 h, followed by treatment with APAP (5 mM) for 24 h. **c** Western Blot

analysis for phosphor-JNK1/2, β -Actin as a loading control. **d** Densitometric values of p-JNK1/2 were qualified by ImageJ software. **e** Western Blot analysis for phosphor-Erk1/2, β -Actin as a loading control. **f** Densitometric values of p-Erk1/2. Values were expressed as mean \pm S.D. ($n = 3$). $**P < 0.01$, $*P < 0.05$ compared to APAP, $###P < 0.01$ compared to normal

DHQ prevented APAP-triggered ROS accumulation and mitochondria dysfunction

GSH depletion by NAPQI was the early step of redox balance disruption and could result in further mitochondrial dysfunction (Lee et al. 2015). APAP (5 mM) treatment for 6 h caused a consumption of GSH in hepatocytes, not DHQ (100 μ M) but NAC (2.5 mM) restored GSH levels (Fig. S2A). Similarly, cytochrome P450 (CYP)2E1, the major CYP form that mediated the metabolism of APAP, remained unchanged after the addition of DHQ (Fig. S2B, C). These results indicated that the protection of DHQ was not simply due to the modulation of APAP metabolism. We next examined the function of DHQ on APAP-triggered oxidative stress. The intracellular ROS levels were measured via quantification of fluorescent DCFH by a spectrometer. Hepatocytes showed a marked ROS accumulation after 24 h treatment of 5 mM APAP, while DHQ ameliorated cellular ROS levels in a dose-dependent manner (Fig. 3a). To confirm the anti-oxidative function of DHQ, the superoxide production was measured with MitoSOX Red dye and analyzed by confocal microscopy. Similarly, MitoSOX Red-derived fluorescence in APAP-treated cells distinctively increased, which was significantly attenuated by DHQ through eliminating ROS (Fig. 3b, c). Mitochondria dysfunction was a major contributor of ROS, and early oxidative stress could further amplify mitochondria damage (Lin et al. 2014). The result exhibited that JC-1 dye formed red fluorescent aggregates in normal cells, whereas APAP treatment caused an increase of JC-1 green monomer and a decrease of JC-1 aggregates, indicating the loss of mitochondria membrane potential (MMP). As expected, DHQ improved the proportion of red fluorescence via reversing the depolarization of MMP (Fig. 3d, e).

Altogether, these data showed that DHQ could prevent against APAP-triggered ROS accumulation and mitochondrial dysfunction.

DHQ activated JAK2/STAT3 pathway in APAP-treated hepatocytes

Multiple evidence showed that JAK2/STAT3 cascade was closely related to the regulation of oxidative stress responses and mitochondrial function (Cai et al. 2016; Yu et al. 2011). The action of DHQ on JAK2/STAT3 signaling against APAP toxicity was further studied by Western Blot analysis. The expression of p-JAK2 and p-STAT3 was slightly increased after APAP treatment, while DHQ pretreatment further increased JAK2 and STAT3 phosphorylation levels (Fig. 4a–d). As STAT3 was an anti-apoptotic factor regulating the expression of Bcl-2 family protein (Tian et al. 2011), the pro-apoptotic protein Bax and the anti-apoptotic protein Bcl-2 were next detected. Our results showed that the expression of Bax was increased by APAP treatment, whereas the

expression of Bcl-2 was decreased. But these changes were dramatically attenuated in the presence of DHQ (Fig. 4e–h).

Collectively, these data suggested that the protective effects of DHQ were partly the result of activating JAK2/STAT3 cascade.

DHQ attenuated APAP toxicity via activation of autophagy

Previous studies revealed that autophagy served as an adaptive response to counteract APAP-induced liver injury, mainly via autophagic removal of damaged mitochondria as well as toxic protein aggregates (Ni et al. 2012, 2016). We next investigated whether autophagy was participated in the protective effect of DHQ against APAP toxicity. As evidenced by Fig. 5a, a punctuated pattern of Cyto-ID labeled autophagic vacuoles was detected via confocal microscopy after DHQ treatment. Further autophagy induction was ensured by Western Blot analysis of light chain 3 (LC3) expression (Fig. 5b). In consistent with previous studies, APAP treatment induced the elevation of LC3-II levels and Cyto-ID-positive vacuoles. Our results showed that DHQ further increased LC3-II levels and induced the formation of autophagosomes (Fig. 5c–e). To determine the role of autophagy in DHQ-mediated hepatoprotection, CQ was applied to pharmacologically suppress autophagy. As expected, the protective function of DHQ was significantly compromised when autophagy was inhibited (Fig. 6).

Consequently, autophagy induced by DHQ was likely participated in the protection against APAP-induced hepatotoxicity.

Discussion

Dihydroquercetin (DHQ), also known as taxifolin, is a widely distributed flavonoid and is attractive for its potent antioxidant property (Xie et al. 2017). Although this compound exhibits multiple healthy benefits such as anti-inflammatory, anti-allergic, antibacterial, anticancer activity, and so on (Kuang et al. 2017), its protective potential for liver injury remains neglected. To our best knowledge, this study is unique in determining the hepatocellular protective function of DHQ in the context of APAP-induced cytotoxicity. This botanical extract DHQ displayed potent activity against APAP-mediated lethal cellular injury as evidenced by cell viability and LDH release assay. In consistent with these results, further PI staining showed that DHQ significantly decreased the degree of APAP-triggered hepatocyte necrosis. Besides, we also found that DHQ could provide protection against palmitic acid and poly-amidoamine (PAMAM) dendrimer-induced hepatocyte injury (Fig. S3). Given its safety and easy availability, DHQ may be a potential therapeutic candidate for liver diseases.

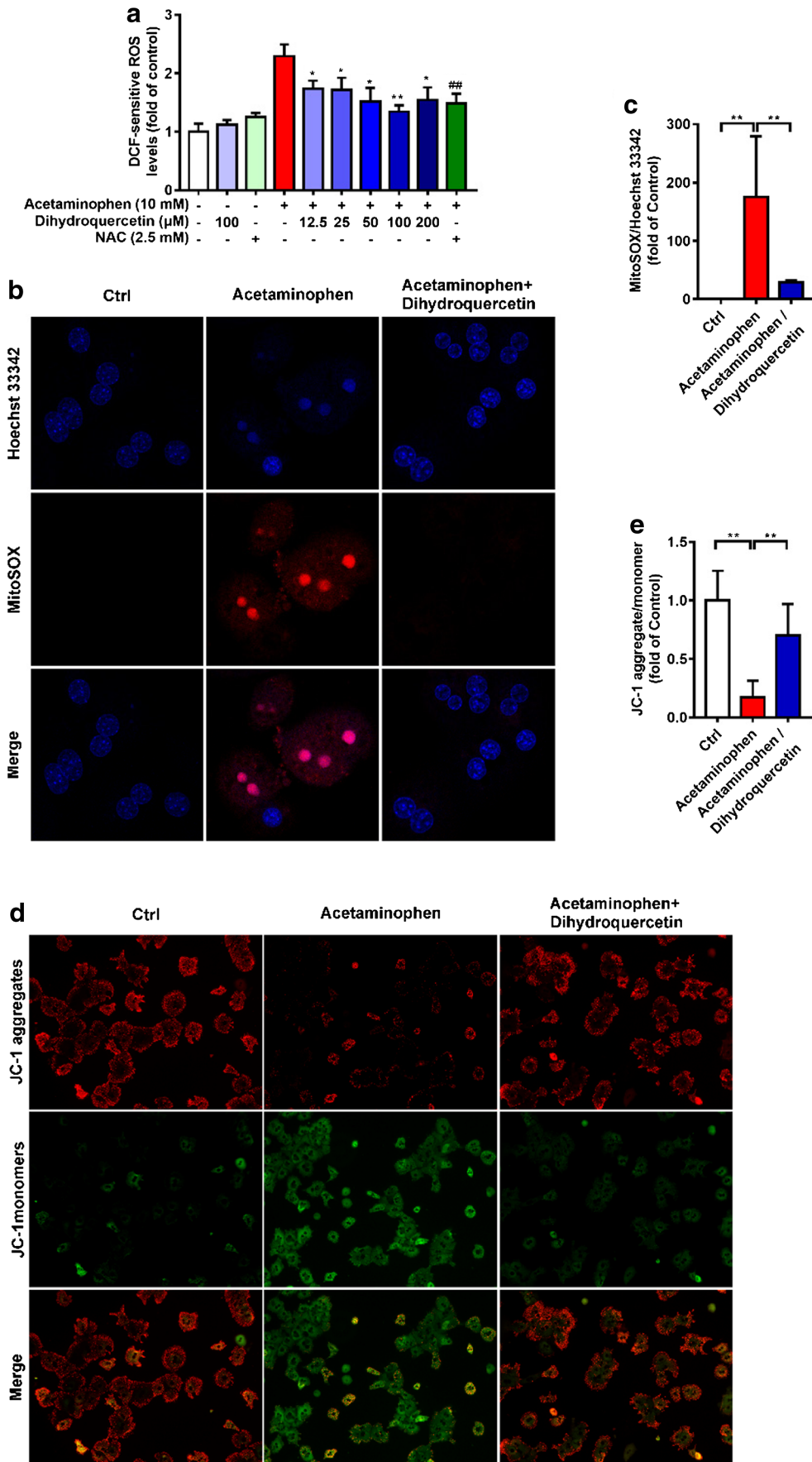


Fig. 3 Dihydroquercetin prevented APAP-triggered ROS accumulation and mitochondria dysfunction. **a** Primary mouse hepatocytes were incubated with DHQ (0–200 μ M), NAC (2.5 mM), and/or APAP (10 mM) for 24 h; the intracellular ROS levels were measured by DCFH-DA assay. **b** Hepatocytes were incubated with DHQ (100 μ M) for 1 h, followed by APAP (5 mM) treatment for 12 h; the cells were then stained by MitoSOX and Hoechst 33342 to visualize intracellular ROS. **c** The statistics of MitoSOX-positive staining compared to Hoechst 33342. **d** Hepatocytes were treated with DHQ (100 μ M) and/or APAP (5 mM) for 12 h and then stained by JC-1 to detect the cellular MMP. **e** The statistics of JC-1 aggregates compared to JC-1 monomers. Values were expressed as mean \pm S.D. ($n = 3$). ** $P < 0.01$, * $P < 0.05$; ### $P < 0.01$ compared to APAP

APAP overdose-induced hepatotoxicity is the most common acute liver injury in the world, but this severe fatal disease lacks efficient antidotes. Mitogen-activated protein kinases (MAPK) such as JNK and ERK are reported to

participate in the development of hepatic damage and amplify APAP toxicity (Latchoumycandane et al. 2007; Xie et al. 2016; McGarry et al. 2015). Activation of JNK is an important mediator of APAP toxicity, by which ER stress could be conveyed to mitochondria triggering further necrosis (Zhang et al. 2016; Saberi et al. 2014). In this study, exposure to APAP induced activation of ERK-JNK cascade in cultured hepatocytes, which was attenuated by DHQ co-treatment. These results confirmed the hepatocellular protective function of this plant-derived antioxidant DHQ in vitro.

Previous studies have demonstrated that the metabolic product NAPQI contributes to the major pathogenesis of APAP overdose-induced liver injury. GSH depletion by NAPQI renders uncontrolled generation of reactive oxygen species and mitochondria dysfunction (Pang et al. 2016). Oxidative stress is proven to be the key factor of APAP-

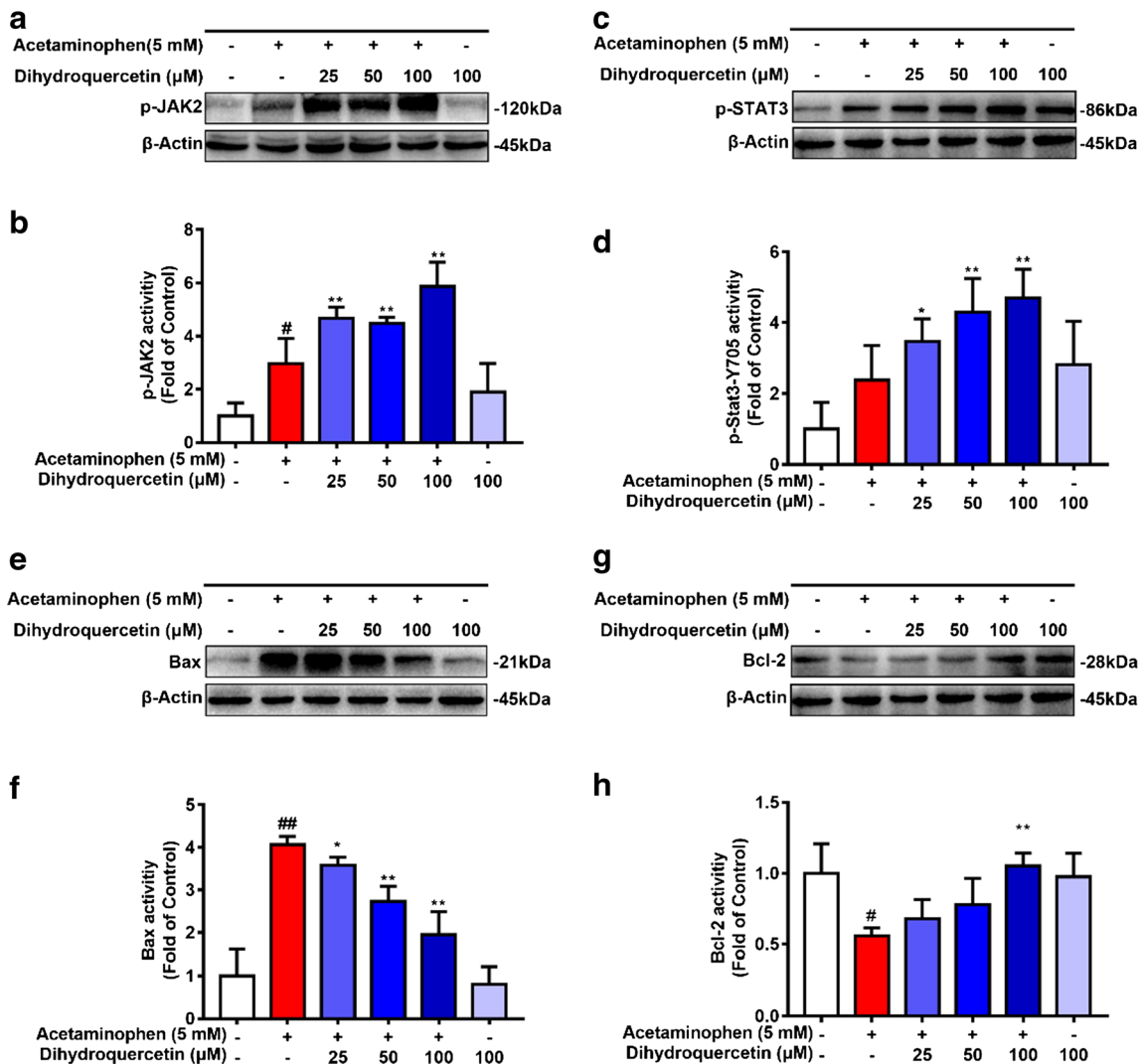


Fig. 4 Dihydroquercetin activated JAK2/STAT3 pathway in APAP-treated hepatocytes. Primary mouse hepatocytes were incubated with DHQ (0–100 μ M) and/or APAP (5 mM) for 24 h; Western Blot analysis for **a** phosphor-JAK2, **c** phosphor-STAT3, **e** Bax, and **g** Bcl-2; and β -

Actin as a loading control. Densitometric values of **b** p-JAK2, **d** p-STAT3, **f** Bax, and **h** Bcl-2. Values were expressed as mean \pm S.D. ($n = 3$). ** $P < 0.01$, * $P < 0.05$ compared to APAP, ### $P < 0.01$ compared to normal

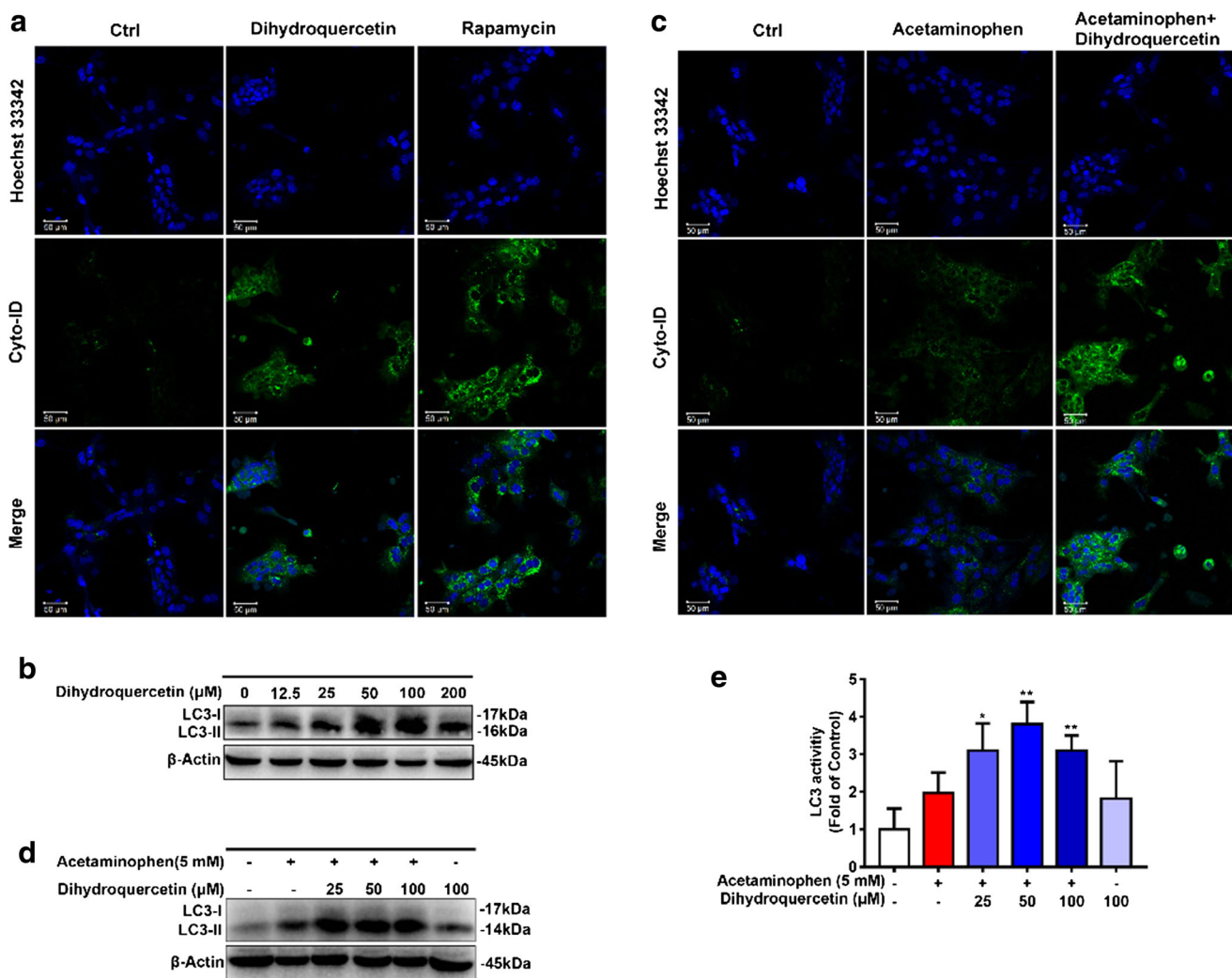


Fig. 5 Dihydroquercetin attenuated APAP toxicity via activation of autophagy. **a** Primary mouse hepatocytes were incubated with DHQ (100 μM) or rapamycin (50 nM) for 24 h; then, cells were stained with Cyto-ID® Green autophagy dye and Hoechst 33342. **b** Primary mouse hepatocytes were incubated with various concentrations of DHQ (0–200 μM) for 24 h, Western Blot analysis for LC3, and β-Actin as a loading control. **c** Hepatocytes were treated with DHQ (100 μM) and/or

APAP (5 mM) for 24 h and then detected for autophagy by confocal microscopy. **d** Primary mouse hepatocytes were pretreated with DHQ (0–100 μM) for 1 h and then treated with APAP (5 mM) for another 24 h, Western Blot analysis for LC3, and β-Actin as a loading control. **e** Densitometric values of LC3. Values were expressed as mean ± S.D. ($n = 3$). ** $P < 0.01$, * $P < 0.05$ compared to APAP

induced hepatotoxicity. Consistently, mitochondrial membrane potential collapse further amplifies oxidative stress signaling pathways and leads to the necrotic cell death (Wang et al. 2016). In the current study, pretreatment with DHQ did not inhibit the consumption of GSH or inhibit the CYP2E1 expression, indicating that its protection was not mediated via modulation of APAP metabolism. Instead, DHQ abrogated APAP-induced ROS accumulation in hepatocytes, suggesting that the hepatoprotective properties of DHQ were partly ascribed to its antioxidant ability. Moreover, DHQ restored the depolarization of mitochondria membrane potential, which further blocked mitochondrial oxidative stress cascade.

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway is an evolutionarily

conserved pathway and is well studied in tissue homeostasis modulation (Chen et al. 2017). It participates in different cellular processes, such as growth, survival, development, and differentiation (Mahmoud and Abd El-Twab 2017). Previous studies have demonstrated that JAK2/STAT3 pathway plays a vital role both in myocardial and hepatic ischemia/reperfusion injury (Yu et al. 2011). Activated STAT3 not only exerts antioxidant property, but also stabilizes mitochondrial membrane (Wu et al. 2016). Furthermore, STAT3 is also recognized as an anti-apoptotic factor as it regulates several apoptosis-related genes, such as Bcl-2 and Bcl-xL (Tian et al. 2011). During cellular stress, JAK2/STAT3 could be phosphorylated thus induce protective and survival signals and restrict the extent of damage (Boengler et al. 2008). The present study showed that APAP

Fig. 6 Suppression of autophagy blocked the effect of dihydroquercetin against APAP toxicity. Primary mouse hepatocytes were pretreated with DHQ (100 μ M) and/or autophagy inhibitor CQ (10 μ M) for 1 h and then treated with APAP (5 mM) for another 24 h. **a** Cell viability was measured via MTT assay. **b** LDH release by LDH assay. **c** Western Blot analysis for Bax and p62 and β -Actin as a loading control. Values were expressed as mean \pm S.D. (n = 3). **P* < 0.05 compared to APAP; ###*P* < 0.01, ##*P* < 0.01 compared to normal

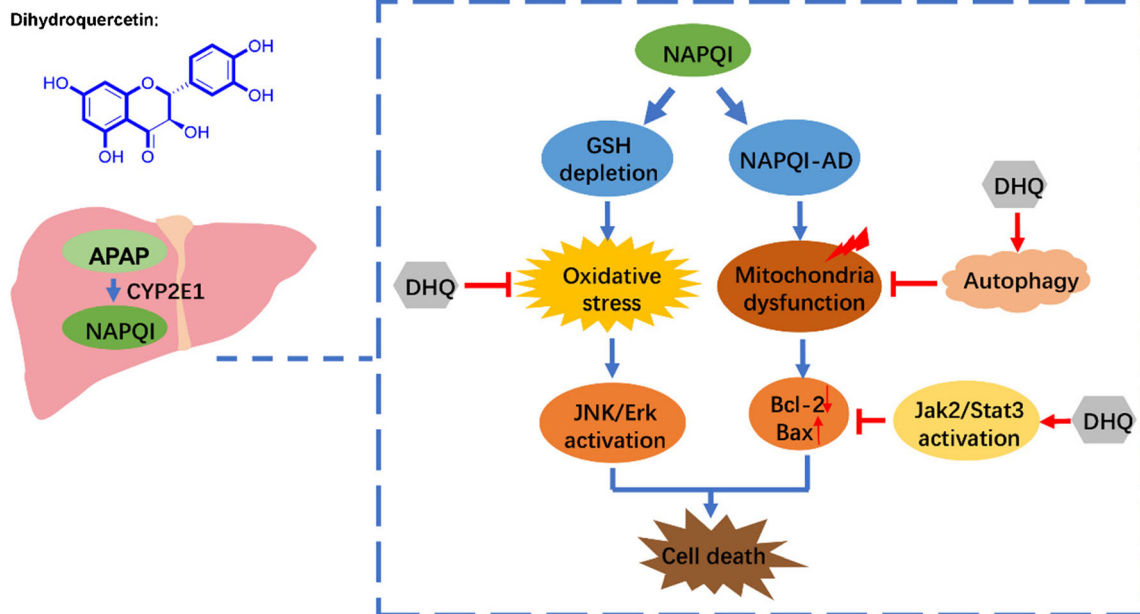
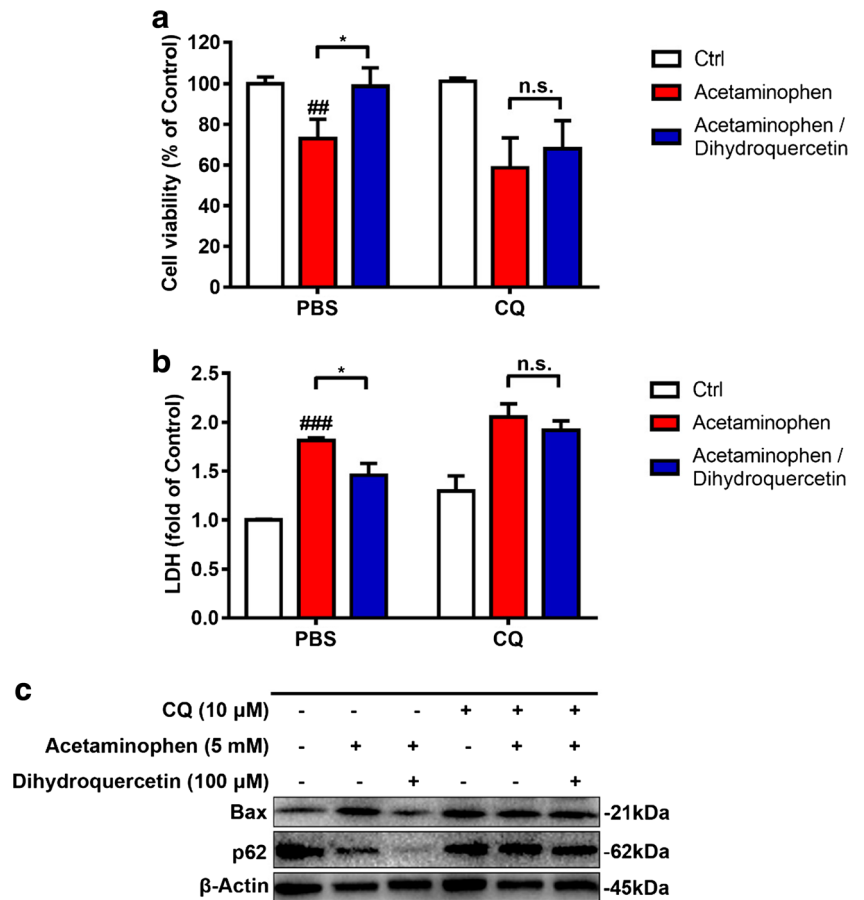


Fig. 7 Potential mechanisms underlying the protective effects of dihydroquercetin in APAP-induced hepatotoxicity. Metabolic NAPQI depletes liver GSH, leading to the accumulation of oxidative stress and mitochondrial dysfunction. DHQ, as a scavenger of ROS, relieves the

ERK/JNK activation caused by oxidative stress. JAK2/STAT3 signaling pathways are involved in the protection of DHQ. Moreover, autophagy is activated by DHQ and subsequently ameliorates APAP-induced hepatotoxicity

treatment caused a minor increase of p-JAK2/p-STAT3 expression and probably triggered by sensing cellular oxidative stress, while pretreatment of DHQ significantly increased the phosphorylation of JAK2/STAT3 cascade. This finding could be supported by a previous study detecting the potential molecular target of DHQ via silico screening and the docking score of DHQ against JAK2 is 0.82 (Oi et al. 2012). Further study demonstrated that expression of anti-apoptotic protein Bcl-2 was elevated whereas the pro-apoptotic protein Bax decreased. These results suggested that the protective effects of DHQ might be related to the activation of the JAK2/STAT3 pathway.

Autophagy is a highly controlled metabolic process which is induced by different stress conditions and is responsible for bulk proteolytic degradation (Zhang et al. 2017). Accumulating evidence supports that autophagy plays an important role in liver physiology and pathology. Selective autophagic degradation of damaged cellular component contributes to quality control of proteins and organisms in hepatocytes, whereas malfunction of autophagy leads to pathological problems (Ueno and Komatsu 2017; Li et al. 2015). Recent studies show that autophagy is activated in response of APAP treatment and serves as an adaptive mechanism to protect against APAP-induced liver injury (Ni et al. 2012). This protection is mediated mainly via removal of damaged mitochondria and toxic APAP-adducted proteins (Williams et al. 2015; Baulies et al. 2015). Autophagy can also relieve oxidative stress and ER stress (Zhang et al. 2016). We next investigated whether autophagy was involved in the protection of DHQ against APAP toxicity. Our results demonstrated that APAP treatment induced elevation of LC3-II protein levels as well as Cyto-ID-positive autophagy vacuoles, while DHQ pretreatment further enhanced the formation of autophagosomes. Suppression of autophagy by CQ remarkably abrogated DHQ-mediated hepatocyte protection, as shown by cell viability and LDH release experiment. These results implied the participation of autophagy in the protective effects of DHQ on APAP-induced cytotoxicity. But the exact mechanisms need further study.

In summary, this is the first report demonstrating that DHQ could be a novel hepatocellular protective candidate against APAP-induced lethal hepatotoxicity. The protective action of DHQ is concerned with its capacity of scavenging oxidative stress, restoring mitochondrial function, activating JAK2/STAT3 cascade, and enhancing autophagy (Fig. 7). Further exploration of DHQ as a therapeutic agent for liver diseases is well worthy. However, the formulation or structural modification of this botanical compound must be solved, because of its poor solubility and bioactivity (Zu et al. 2012). This could also explain why there is a dearth of in vivo assessment of DHQ among existing studies (Weidmann 2012).

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Compliance with ethical standards All animal care and experiment procedures complied with the Animal Management Rules of China and were approved by the Animal Care Committee of Fudan University.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

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