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Original Article

Corilagin reduces acetaminophen-induced hepatotoxicity through MAPK and NF-κB signaling pathway in a mouse model

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Abstract: Corilagin is a major active polyphenolic tannins extracted from *Phyllanthus urinaria*, an important herb used in traditional medicine. Previous reports demonstrated that corilagin possesses antioxidant and anti-inflammatory properties. Therefore, this study aimed to evaluate its hepatoprotective effects and mechanisms on acetaminophen (APAP)-induced liver injury in mice. Mice included in this study were intraperitoneally injected with a hepatotoxic APAP dose (300 mg/kg). After a 30 min of APAP administration, corilagin was injected intraperitoneally at concentrations of 0, 1, 5, 10, and 20 mg/kg. Then, after 16 h of corilagin treatment, mice were sacrificed for further analysis. APAP overdose significantly elevated the serum ALT level, hepatic myeloperoxidase (MPO) activity, cytokines (TNF-α, IL-1β, and IL-6) production, malondialdehyde (MDA) activity, and ERK/JNK MAPK and NF-κB protein expressions. Corilagin treatment significantly decreased these parameters in a dose-dependent manner (1-20 mg/kg). This study demonstrated that corilagin may be a potential therapeutic target for the prevention of APAP-induced hepatotoxicity by down-regulating the inflammatory response and by inhibiting ERK/JNK MAPK and NF-κB signaling pathways.

Keywords: Corilagin, acetaminophen, liver injury, inflammation, oxidative stress, MAPK, NF-κB

Introduction

Liver is considered as the major site of drug metabolism. However, drug-induced liver injury is also one of the common factors that may cause severe hepatotoxicity and even death. Acetaminophen (N-acetyl-p-aminophenol or APAP) is a frequently used analgesic and antipyretic medication worldwide. Although this drug has been considered highly safe, its intentional or unintentional overdose can cause life-threatening acute liver failure [1, 2]. Despite the number of efforts to reduce the incidence of APAP-induced liver injury, the number of liver failure cases reported in the literature remains a serious public health problem.

At therapeutic dosages, 85-90% of APAP metabolism conjugates with glucuronide or sulfate to form non-toxic metabolites in hepatocytes, and subsequently excreted in the urine and bile juice. Other APAPs are converted into a highly toxic, reactive intermediate metabolite, N-acetyl-p-benzoquinone imine (NAPQI) by the cytochrome P450 system. Then NAPQI produced from normal doses of APAP is depleted by the hepatic glutathione (GSH) antioxidant system [3]. However, under toxic APAP doses, sulfation and glucoronidation pathways become insufficient and hepatic GSH is depleted due to excessive NAPQI. Consequently, it binds to cellular proteins in hepatocytes, resulting in oxidant stress formation and mitochondrial dysfunction, which causes centrilobular hepatocyte death [4, 5]. After the initial damage to parenchymal hepatocytes, innate immunity and oxidative stress participates in the progression of APAP-induced inflammation and contribute to its severity. Activated Kupffer cells triggered by hepatocyte damage may lead to increased release of oxidative stress and pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6.

Then, the number of immune cells (infiltrating macrophages and neutrophils) recruited into liver vasculature increased, which aggravates liver inflammation [6, 7].

APAP-induced hepatotoxicity is related to oxidative stress, inflammatory response, and apoptosis. Recent studies reported that inflammatory mediators, including cytokines and reactive oxygen species (ROS) have been associated with the activation of mitogen-activated protein kinase (MAPK) that regulates intracellular signal transduction pathway in APAP-induced liver injury [8, 9]. Evidence that extracellular signal-regulated kinase (ERK), one of MAPK pathways, participates in the regulation of inflammation and cell apoptosis is increasing [10]. Moreover, oxidative stress has also been shown to activate signal pathways such as nuclear factor kappa B (NF-κB), an important transcription factor in the nucleus. APAP overdose caused NF-κB activation, and then nuclear translocation and binding specific sites in the promoter regions of target genes. It up-regulates gene expressions of numerous pro-inflammatory cytokines and inflammatory mediators including TNF-α, IL-1β, and cyclooxygenase-2 (COX-2) [11, 12], which have been implicated in the inflammatory response to hepatotoxicity.

Corilagin is a major active polyphenolic tannins extract from *Phyllanthus urinaria*, an important herb used in traditional medicine. Previous reports demonstrated that it possesses antioxidant and anti-inflammatory properties [13, 14], and its safety has been clinically proven. Previous reports have shown that corilagin can inhibit TNF-α expression and radiation-induced microglia activation by restraining the NF-κB pathway [15]. It is also a potential component to relieve cholestasis through anti-inflammation and anti-oxidation related pathways in a rat model of acute cholestasis [16]. Furthermore, corilagin has hepatoprotective effects against LPS-induced liver injury by suppressing of oxidative stress and apoptosis [17]. These studies suggested that corilagin may play important roles in the oxidative stress and inflammatory reaction. However, its pharmacological effects in APAP-induced liver injury have not yet been investigated. Therefore, further studies should be conducted on the effects and mechanisms of corilagin following an APAP-induced liver injury in a mouse model.

Materials and methods

Animals

The C57BL/6 (B6) mice were purchased from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan). All procedures used in this proposal have been approved by the Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital. All animal experiments were performed according to the guidelines of the Animal Welfare Act and the Guide for Care and Use of Laboratory Animals from the National Institutes of Health.

Experimental protocols and drug treatment

All animals were housed in an environmentally controlled room and fasted overnight before the procedure. APAP (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in normal saline at a concentration of 20 mg/mL. The mice were intraperitoneally injected with a hepatotoxic dose of APAP (300 mg/kg), whereas the control mice were administered with an equal volume of normal saline. After 30 min of APAP administration, the mice were intraperitoneally injected with corilagin (Sigma) at concentrations of 0, 1, 5, 10, and 20 mg/kg. Then, 16 h after the corilagin treatment, the animals were sacrificed via cervical dislocation under isoflurane anesthesia. Blood was drawn from the vena cava into heparinized syringes and centrifuged. The serum was used to determine liver enzyme activities. Immediately after collecting the blood, the livers were excised and rinsed with saline. A small section from each liver was placed in 10% phosphate-buffered formalin. The remaining liver was frozen in liquid nitrogen and stored at -80°C. Blood and liver samples were obtained for further analysis.

Measurement of serum enzyme

Serum glutamyl pyruvic transaminase (GPT, also known as alanine aminotransferase or ALT) was measured to determine hepatic injury using a Vitros DT60 II Chemistry System (Ortho-Clinical Diagnostics; Johnson & Johnson, New York, NY). All samples processing and procedures are following the manufacture's manual.

Measurement of tissue myeloperoxidase (MPO) activity

Myeloperoxidase is released from the neutrophils into the phagosome and acts a biomarker

of oxidative stress and inflammation. Briefly, liver tissues of mice were homogenized with a Tekmar tissue grinder and centrifuged at 15000 *g* for 15 min at 4°C. The pellet was resuspended in 10 volumes of room temperature 50 mM KPO_4 buffer, and then incubated for 2 h at 60°C. The homogenate was sonicated for 10 s using the sonicator (QSONICA Q700) and underwent 3 cycles of freeze/thaw. The suspension was centrifuged at 15000 *g* for 15 min at 4°C. Subsequently, the supernatant was transferred to phosphate buffer containing *o*-dianisidine hydrochloride (10 mg/mL), 0.3% hydrogen peroxide, and 50 mM KPO_4, pH 6.0. The change in light absorbance was measured at 460 nm for a period of 5 min and expressed in units per gram tissue.

Histology examination

The livers were harvested, fixed in 4% paraformaldehyde in PBS pH 7.4, and embedded in paraffin. Sections of thickness 4 μm were subjected to standard hematoxylin and eosin (H&E) staining for histology examination.

Immunohistochemistry on liver tissue

Liver sections were blocked with blocking buffer for 30 min and incubated with a specific primary antibody. For immunostaining of neutrophils, tissue sections were incubated with rat anti-mouse primary antibody (Ly6G for neutrophils). After washing for 5 min twice, samples were incubated with biotinylated goat anti-rat secondary antibody for 1 h. Then the peroxidase reaction was performed following the manufacturer's protocol (Millipore IHC select kit) and the reaction times for all sections were identical.

Measurement of tissue cytokine by ELISA

Liver tissue homogenates were used to determine TNF-α, IL1β, and IL-6 expression. The tissues were homogenized on ice, centrifuged for 10 min (12000 *g*, 4°C), and the supernatants were assayed for cytokines expressions using the eBiosciences ELISA Kit (San Diego, CA, USA). 96 well plates were precoated with 2 μg/mL primary antibodies overnight and were blocked with commercial blocking buffer for 1 h. Then, samples were added into each well and incubated at room temperature for 2 h. After washing for several times, biotinylated detection antibody was added for 1 h. Then, after incubation with HRP substrate for 30 min, the reaction was stopped by adding 2N H_2SO_4 and the absorbance was read at 450 nm using TECAN infinite 200.

Measurement of tissue malondialdehyde (MDA) levels

The tissue samples were homogenized in ice-cold condition and centrifuged at 1000 *g* for 15 min at 4°C. The supernatant was removed and re-centrifuged at 35000 *g* at 4°C for 8 min. We measured MDA generation as the indicator of lipid peroxidation using a Bioxytech MDA-586 Kit (OxisResearch, Portland, OR, USA). Lipid peroxide levels were expressed in terms of MDA equivalents as nmol MDA/g tissue.

Western blotting

The tissue were lysed in buffer containing 20 mM Tris-HCl (pH 7.5), 1% Triton X-100, 137 mM sodium chloride, 10% glycerol, 0.02% NaN_3, 2 mM EDTA, 1 mM sodium orthovanadate, 25 Mm b-glycerophosphate, 2 mM sodium pyrophosphate, 1 mM phenylmethylsulfonylfluoride, 1 ug/ml aprotinin and 0.1 ug/ml leupeptin. The cell lysates were centrifuged at 12000 *g* for 10 min. Equal quantity of protein from each group was separated on 10% sodium dodecylsulfate polyacrylamide gel (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membrane (Schleicher & Schuell, Middlesex, UK). The membrane was blocked with 5% fat-free milk solution and rinsed 3 times using Tris-buffer with 1% Tween 20. The membrane was incubated with antibodies to ERK, JNK, p38, NF-κB, phospho-ERK, phospho-JNK, phospho-p38, and phospho-NF-κB (Cell Signaling Technology, MA, USA) primary antibodies overnight at 4°C. After washing with horseradish peroxidase-conjugated secondary antibody, proteins were detected using an enhanced chemiluminescence system (Amersham, Piscataway, NJ, USA).

Statistical analysis

All data were expressed as mean ± SEM. Calculations were performed with GraphPad Prism 6.0 Software (GraphPad Software Inc., San Diego, USA). The differences of measurement data among groups were analyzed using one-way analysis of variance (ANOVA) followed

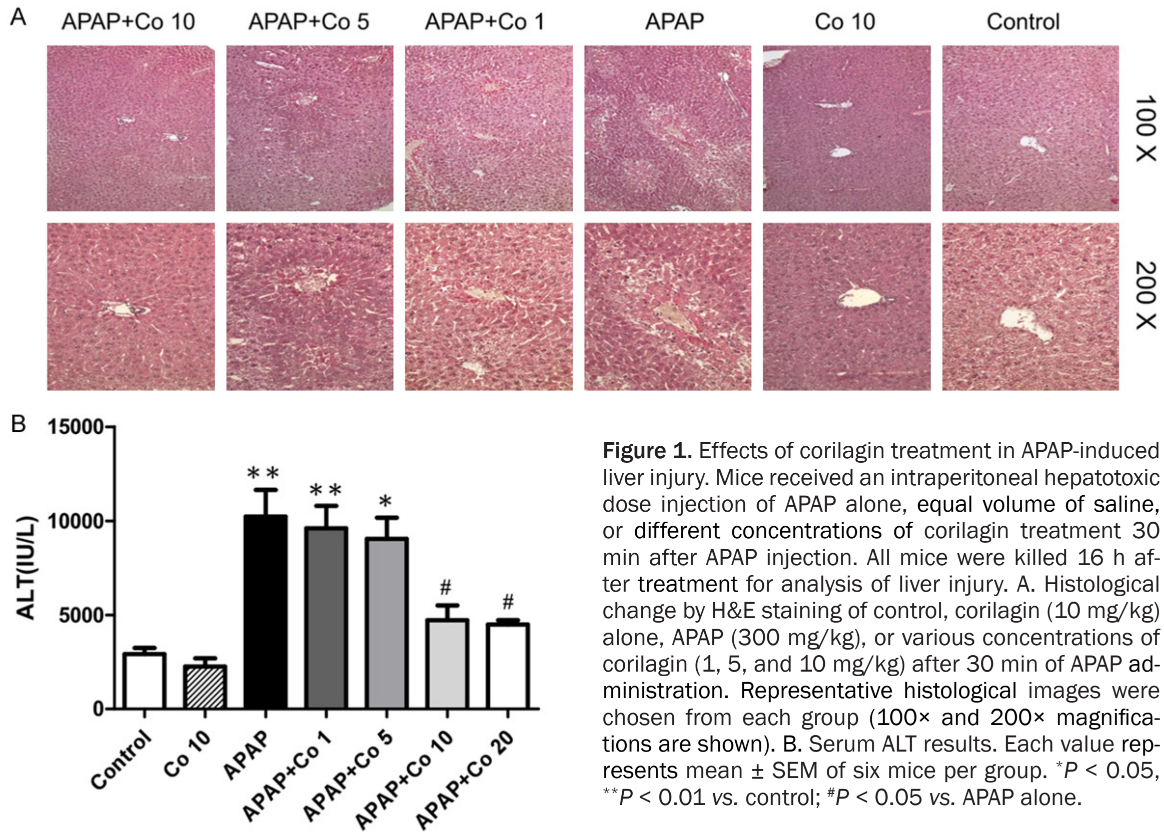

Figure 1. Effects of corilagin treatment in APAP-induced liver injury. Mice received an intraperitoneal hepatotoxic dose injection of APAP alone, equal volume of saline, or different concentrations of corilagin treatment 30 min after APAP injection. All mice were killed 16 h after treatment for analysis of liver injury. A. Histological change by H&E staining of control, corilagin (10 mg/kg) alone, APAP (300 mg/kg), or various concentrations of corilagin (1, 5, and 10 mg/kg) after 30 min of APAP administration. Representative histological images were chosen from each group (100× and 200× magnifications are shown). B. Serum ALT results. Each value represents mean ± SEM of six mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* control; $^{\#}P < 0.05$ *vs.* APAP alone.

by Tukey-Kramer multiple comparison tests. The significance level was set at $P < 0.05$ for all tests.

Results

Protective effects of corilagin against APAP-induced hepatotoxicity

A single toxic dose of APAP (300 mg/kg) markedly increased the serum ALT levels compared with the control animals (**Figure 1B**). After the 30-min APAP administration, corilagin treatment significantly decreased the serum ALT enzyme activity. Serum ALT levels were markedly lower in the corilagin treatment groups (10 and 20 mg/kg) than that in the APAP-only group. Effects of corilagin were similar whether administered at a dose of 10 or 20 mg/kg. Histopathological analysis of the liver parenchyma in the APAP group showed centrilobular necrosis and fatty infiltration as evidenced in the H&E staining (**Figure 1A**). Corilagin treatment (10 mg/kg) significantly decreased these pathological findings and showed well preserved hepatocytes with less area of necrosis.

Effects of corilagin on neutrophil infiltrations in APAP-induced hepatotoxicity

Immunohistochemistry staining of the liver tissue with a granulocyte-specific marker, Ly6G antibody, was used to investigate neutrophil infiltration in APAP-induced liver injury. APAP-only treated animals demonstrated obvious infiltrated neutrophils around the necrotic area in the liver parenchyma as compared with the control animals (**Figure 2A**). Corilagin-treated (1, 5, and 10 mg/kg) animals after APAP injection had significantly lower liver neutrophil accumulation than APAP-only treated animals.

Protective effects of corilagin on MPO activity in liver tissues

Hepatic expression of MPO was obviously elevated in the APAP (300 mg/kg) group than that in the control group ($P < 0.005$, **Figure 2B**). After the 30-min APAP administration, corilagin treatment (1, 5, and 10 mg/kg) significantly decreased hepatic MPO levels compared with the APAP-only group ($P < 0.01$ or 0.005). This result demonstrates that corilagin treatment

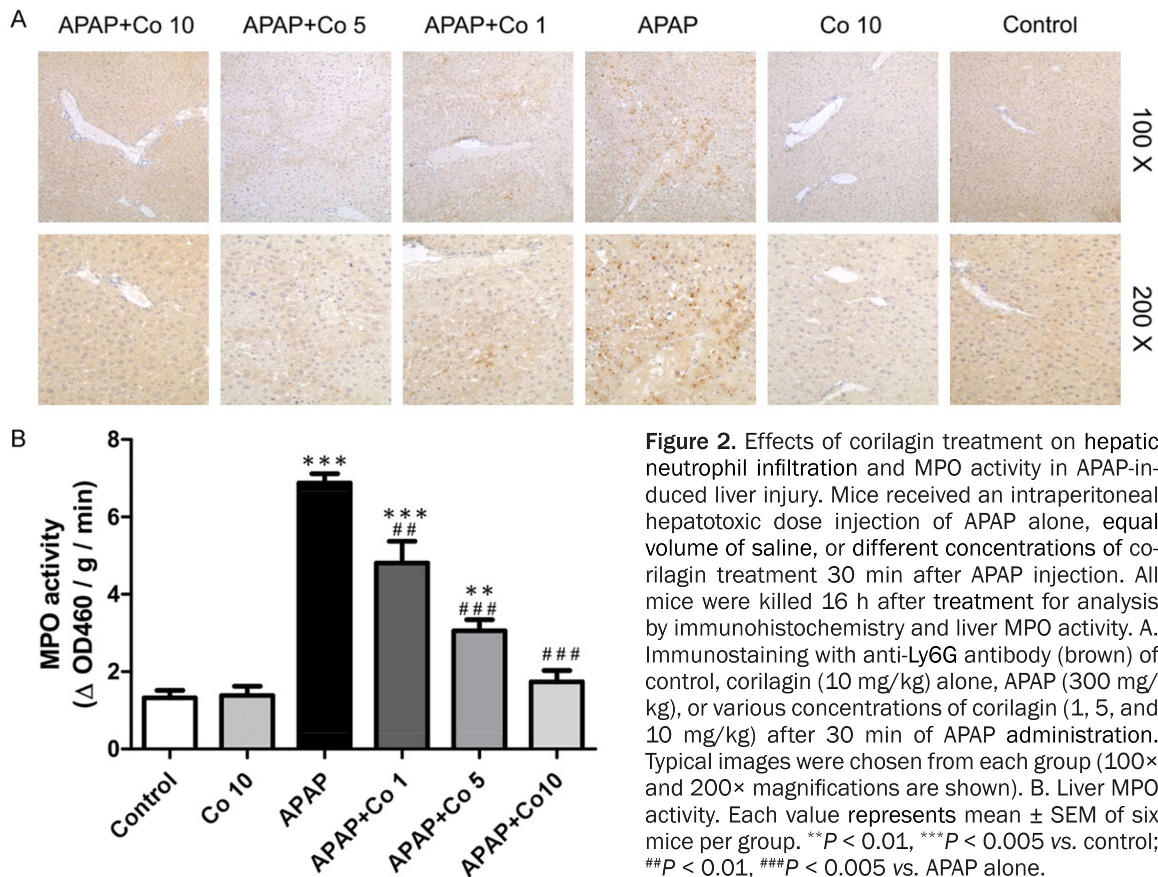

Figure 2. Effects of corilagin treatment on hepatic neutrophil infiltration and MPO activity in APAP-induced liver injury. Mice received an intraperitoneal hepatotoxic dose injection of APAP alone, equal volume of saline, or different concentrations of corilagin treatment 30 min after APAP injection. All mice were killed 16 h after treatment for analysis by immunohistochemistry and liver MPO activity. A. Immunostaining with anti-Ly6G antibody (brown) of control, corilagin (10 mg/kg) alone, APAP (300 mg/kg), or various concentrations of corilagin (1, 5, and 10 mg/kg) after 30 min of APAP administration. Typical images were chosen from each group (100× and 200× magnifications are shown). B. Liver MPO activity. Each value represents mean ± SEM of six mice per group. $^{**}P < 0.01$, $^{***}P < 0.005$ *vs.* control; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.005$ *vs.* APAP alone.

dose-dependently reduces neutrophil accumulation and inflammation in the liver.

Effects of corilagin on the inflammatory cytokine expressions in liver tissues

Essential pro-inflammatory cytokine expressions including TNF-α, IL-1β, and IL-6 in APAP-induced liver injury were measured. As shown in **Figure 3A-C**, APAP (300 mg/kg) administration for 16 h significantly increased these cytokines compared with the control group. After 30 min of APAP overdose, corilagin treatment (1 mg/kg) significantly decreased the hepatic TNF-α and IL-6 levels ($P < 0.05$ and $P < 0.005$ respectively). In addition, treatment with a higher dose of corilagin (5 mg/kg) markedly lowered TNF-α, IL-1β, and IL-6 levels ($P < 0.01$, $P < 0.01$, and $P < 0.005$ respectively) compared with the APAP-only treated group. These results indicated that corilagin treatment might attenuate the production and release of these pro-inflammatory cytokines in APAP-induced liver injury.

Effects of corilagin on oxidative stress in APAP-induced hepatotoxicity

APAP overdose results in the generation of oxidative stress. The production of MDA was considered as the marker of lipid peroxidation. After APAP (300 mg/kg) administration for 16 h, MDA concentrations were significantly higher in the liver tissues compared with the control group (**Figure 3D**). In mice receiving corilagin of 1 mg/kg plus APAP, MDA levels were not significantly lower than that in with the APAP-treated mice. However, treatment with higher dose of corilagin (5 and 10 mg/kg) significantly alleviated APAP-induced MDA production ($P < 0.005$).

Effects of corilagin on immunohistochemistry evidence of NF-κB expression in APAP-induced hepatotoxicity

To investigate the possible anti-inflammatory mechanism of corilagin in APAP-induced hepatotoxicity, liver tissues were immunohistochemistry stained with NF-κB antibody. The

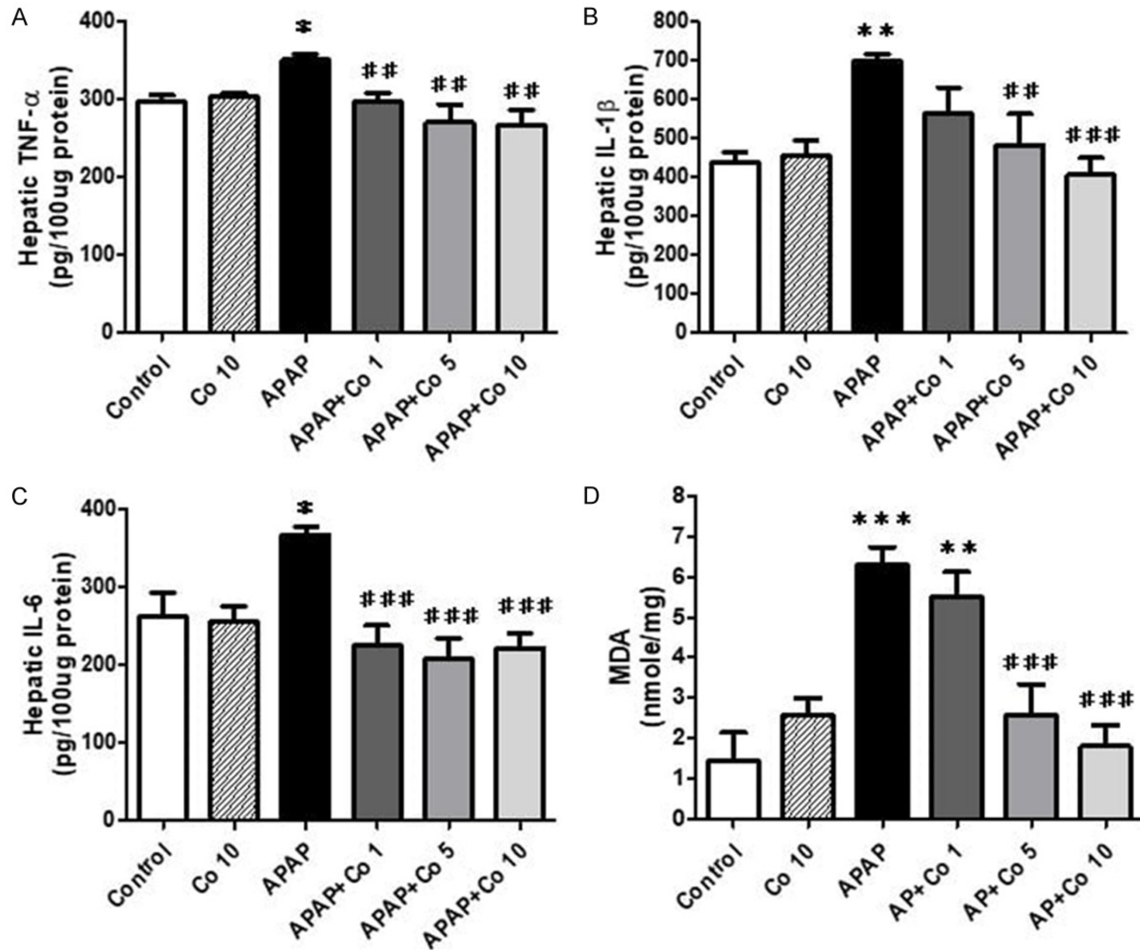

Figure 3. Effects of corilagin treatment on hepatic TNF-α (A), IL-1β (B), IL-6 (C), and MDA (D) expressions in APAP-induced liver injury. Mice received an intraperitoneal hepatotoxic dose injection of APAP (300 mg/kg) alone, equal volume of saline (control), or various concentrations of corilagin treatment (1, 5, and 10 mg/kg) 30 min after APAP injection. All mice were killed 16 h after treatment for analysis of these biochemical markers. Each value represents mean ± SEM of six mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.005$ *vs.* control; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.005$ *vs.* APAP alone.

APAP-only treated group showed significantly higher NF-κB expressions in liver tissues compared with the control animals (**Figure 4C**). Corilagin (5 and 10 mg/kg)-treated group after APAP injection had a significantly lower NF-κB expression around the inflammatory area of hepatotoxicity in the liver parenchyma (**Figure 4E**, **4F**).

Effects of corilagin on hepatic ERK, JNK, and NF-κB expressions and activity

The hepatic MAPK family protein, including ERK, JNK, and p38 kinase, and NF-κB expressions in APAP-induced hepatotoxicity were investigated. The ERK and JNK activity, as determined by their phosphorylation, was significantly higher in the APAP group than that in the control group (**Figure 5A**, **5B**). However, no significant differences in hepatic p38 protein expressions were observed (data not shown). Corilagin treatment (10 mg/kg) after 30 min of APAP challenge significantly decreased hepatic phosphorylated ERK and JNK expressions compared with the APAP-only group. We next looked at another intracellular signal protein NF-κB. Our result (**Figure 5C**) revealed that phosphorylated NF-κB significantly increased after the APAP administration ($P < 0.005$). Corilagin treatment (10 mg/kg) effectively suppressed the phosphorylation of NF-κB proteins after the APAP challenge ($P < 0.005$).

Discussion

APAP is a widespread and very effective drug used as an analgesic and antipyretic. However,

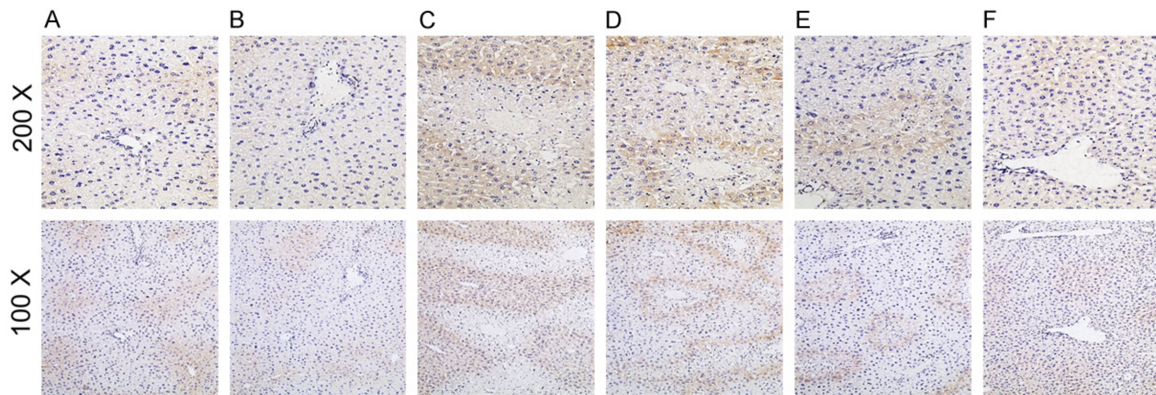

Figure 4. Effects of corilagin treatment on hepatic NF-κB expression in APAP-induced liver injury. Mice were treated with (A) normal saline (control), (B) corilagin (10 mg/kg) alone, (C) APAP (300 mg/kg) alone or various concentrations of corilagin (D: 1 mg/kg; E: 5 mg/kg; F: 10 mg/kg) after 30 min of APAP administration, and were sacrificed 16 h after treatment for analysis by immunohistochemistry. Liver tissues were immunostained with anti-NF-κB antibody (brown). Typical images were chosen from each group (100× and 200× magnifications are shown).

its acute overdose is considered as the major cause of severe liver damage and even liver failure. In our study, the protective effects of corilagin, an important component purified from Phyllanthus urinaria extract, were investigated in APAP overdose-induced hepatotoxicity in mice. Serum ALT concentration, hepatic MPO expressions, pro-inflammatory cytokines, and oxidative stress and intracellular signal transduction parameters were increased after the APAP challenge for 16 h. Corilagin treatment significantly attenuated the elevation of these hepatic parameters. In addition, the protective effects of corilagin treatment in a dose-dependent manner in APAP-induced liver injury were also observed.

APAP-induced hepatotoxicity is characterized by mechanisms of innate immune response. Excessive electrophilic metabolite NAPQI may cause mitochondrial dysfunction and hepatocyte necrosis. Hepatocyte death results in the release of damage associated molecular pattern (DAMP) molecules that are recognized by the resident and infiltrating hepatic macrophages through toll-like receptors [18, 19]. Activated macrophages would release pro-inflammatory cytokines and C-X-C motif (CXC) chemokines to recruit monocytes and neutrophils into the necrotic areas of the liver [20]. Recent studies reported that TNF-α and IL-6 mediated acute inflammatory response and increased neutrophil accumulation in hepatic ischemia-reperfusion injury models [21]. Neutrophil infiltration and transmigration into the liver parenchyma were associated with increased cytokine and chemokine release in a model of drug-induced liver injury [22]. Therefore, macrophage activation together with neutrophil infiltration into the hepatic vasculature is a vital component of APAP-induced liver injury. Previous evidence showed that major extracts of *Phyllanthus urinaria* exhibited strong immunomodulatory effects on neutrophil and macrophage cells. Another report also suggested that they have a strong inhibitory activity against ROS formation and neutrophil chemotaxis [23, 24]. In our results, early corilagin treatment after an APAP overdose attenuated macrophage and neutrophil accumulation, hepatic pro-inflammatory cytokine expressions (i.e., TNF-α, IL-1β, and IL-6), and MPO activity. It suggested that the protective effects of corilagin in APAP-induced liver injury may be mediated by reduced activation of macrophage and downstream effectors of inflammatory cytokine production and neutrophil infiltration.

Evidence that oxidative stress plays a key role in APAP-induced hepatotoxicity is increasing. Excessive NAPQI depletes GSH in the cellular storage and inhibits the antioxidant enzyme activity, which results in increased oxidative stress and ROS formation in APAP toxicity [4, 25]. Previous studies demonstrated that corilagin might exert hepatoprotective effects for hepatitis C virus-infected liver in oxidative stress modulation [26]. Another study also showed that corilagin could relieve acute cholestasis through its anti-oxidative effects [16]. Our results showed that APAP significantly increase the MDA level, indicating ROS production and accumulation of lipid peroxidation. However, after the corilagin treatment, this

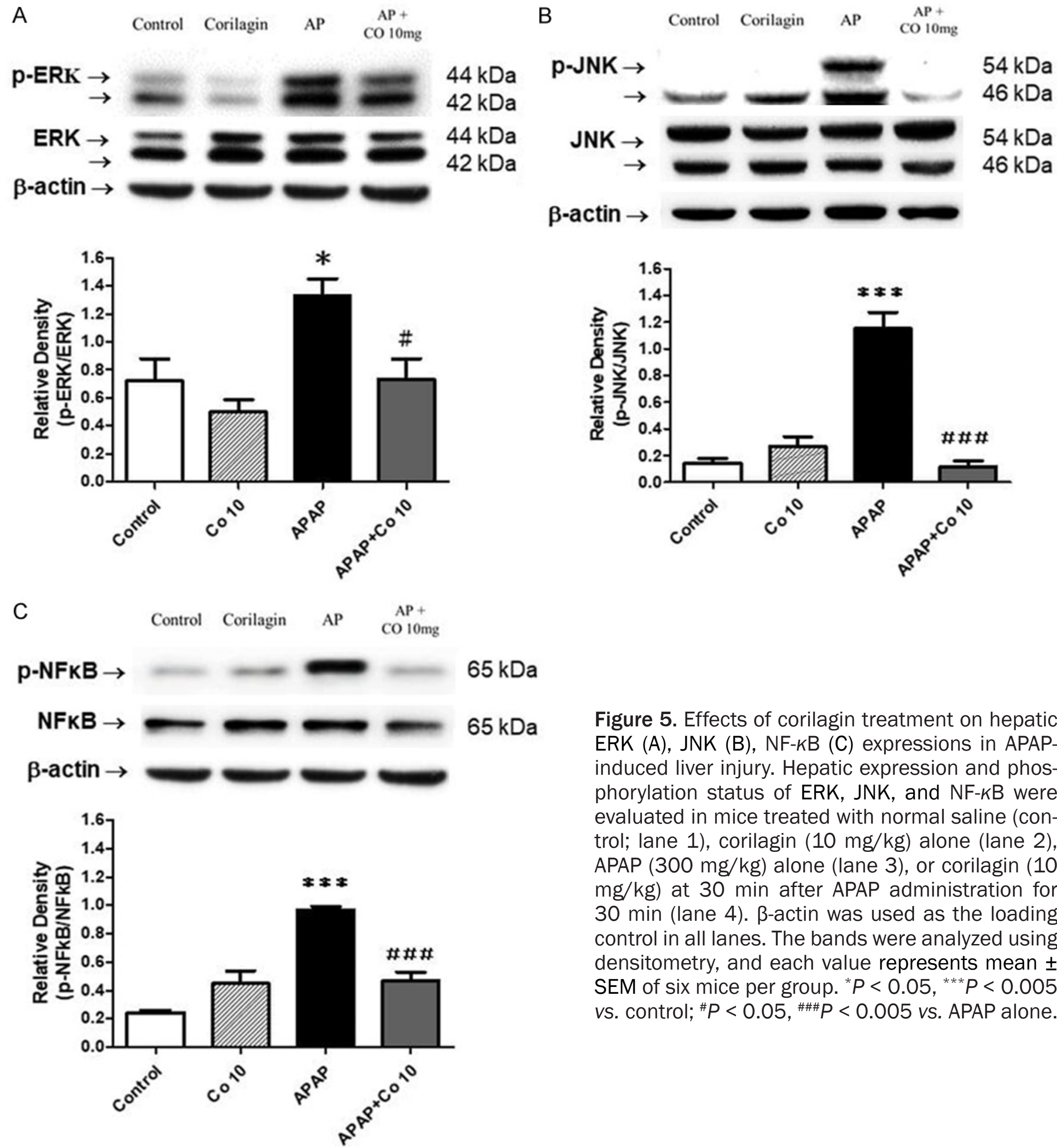

Figure 5. Effects of corilagin treatment on hepatic ERK (A), JNK (B), NF-κB (C) expressions in APAP-induced liver injury. Hepatic expression and phosphorylation status of ERK, JNK, and NF-κB were evaluated in mice treated with normal saline (control; lane 1), corilagin (10 mg/kg) alone (lane 2), APAP (300 mg/kg) alone (lane 3), or corilagin (10 mg/kg) at 30 min after APAP administration for 30 min (lane 4). β-actin was used as the loading control in all lanes. The bands were analyzed using densitometry, and each value represents mean ± SEM of six mice per group. $^{*}P < 0.05$, $^{***}P < 0.005$ *vs.* control; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.005$ *vs.* APAP alone.

APAP-induced change in the MDA level was reduced, suggesting that corilagin can be used to protect against APAP-induced hepatotoxicity by decreasing oxidative stress.

ROS can further elicit the MAPK activation, a family of serine-threonine protein kinases, that plays an essential role in intracellular signaling pathways in APAP-induced hepatotoxicity [8, 9]. The MAPK family is associated with cell death, especially ERK and JNK. They are also responsible for the ROS and pro-inflammatory cytokine production [27]. Previous studies showed that ERK is one of the essential members of the MAPK family and associated with oxidative stress and apoptotic event [10, 28]. The protection against APAP-induced hepatotoxicity was mediated by regulating pro-inflammatory cytokine and suppressing the activation of ERK signaling pathways [29]. A recent study also demonstrated that ERK pathway inhibition protects against APAP-induced liver injury and this is accompanied by reduced ROS production [30]. In addition, JNK activation promotes mitochondrial dysfunction and further contributes to the mitochondrial oxidant stress and ROS formation, which led to hepatocyte apoptosis in APAP overdose [31, 32]. Blockade of JNK phosphory-

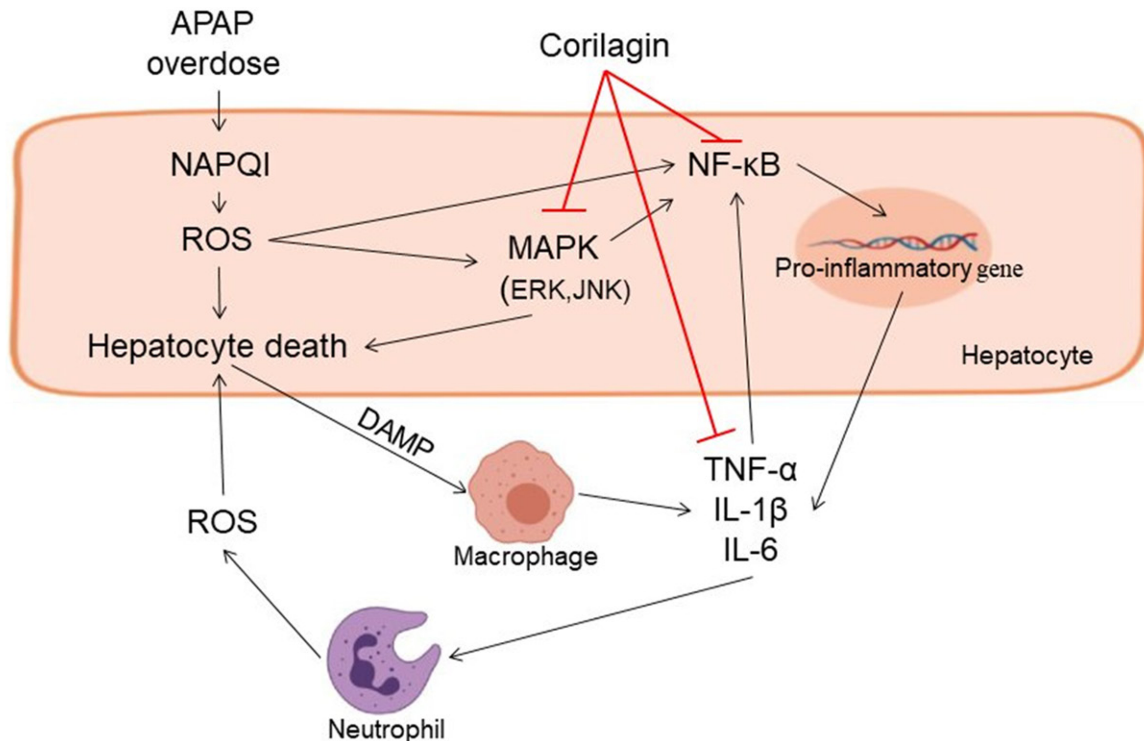

Figure 6. Schematic summary of possible mechanism underlying the protective effects of corilagin treatment in APAP-induced liver injury. Corilagin treatment effectively protects against APAP-induced liver injury in mice through anti-inflammatory and anti-oxidation mechanisms, and by inhibition of ERK/JNK MAPK and NF-κB pathways.

lation or gene expression decreased hepatic damage in APAP toxicity [33]. A previous study reported that corilagin targeted the MAPK signaling pathways to exert its antioxidant and anti-inflammatory effects in LPS-induced liver injury [34]. In this experiment, our western blot data showed that APAP activated the p-ERK and p-JNK expressions, which result in hepatocyte apoptosis. After the APAP toxicity, corilagin effectively protected liver against injury by suppressing ERK/JNK MAPK pathways.

APAP overdose can trigger ROS-mediated hepatocyte death by activating intrinsic signaling pathways, including MAPK and NF-κB. The transcription factor NF-κB plays a key role involved in cellular inflammatory responses. In the inactivated form, NF-κB is normally sequestered in the cytoplasm. After the stimulation by extracellular stimuli, NF-κB is translocated into the nucleus and leads to the transcription of pro-inflammatory genes [11, 12]. Previous studies showed that APAP-induced liver damage occurred through the up-regulation of myeloid differentiation factor 88 (MyD88) and NF-κB [35, 36]. Macrophages are activated by this transcription signaling pathway and induce the pro-inflammatory gene expression, including those encoding TNF-α, COX-2, IL-1β, and IL-6 [37]. Recent studies also demonstrated that MAPK-dependent activation and up-regulation of NF-κB can cause production of pro-inflammatory mediators and cytokines in LPS-stimulated macrophage and drug-intoxicated liver injury models [38-40]. In apoptosis and anti-tumor pathway, corilagin potentially reduced the nuclear expression of NF-κB/p65 protein, which resulted in the attenuation of downstream transcription of inflammatory mediators in the neural cancer [41]. In our results, corilagin can decrease the NF-κB expression and downstream the inflammatory response. It suggested that the inhibition of ERK/JNK MAPK protein by corilagin may contribute to the suppression of NF-κB-mediated inflammatory pathways.

Conclusions

This study demonstrated that corilagin exerted protective effects against APAP-induced hepatotoxicity. Its mechanism is attributed to anti-inflammatory and anti-oxidation activities by suppressing ERK/JNK MAPK and NF-κB signaling pathways (**Figure 6**). These results suggest that corilagin may be used as a potential target drug for APAP-induced liver injury in the future.

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Disclosure of conflict of interest

None.

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References

[1] Kaplowitz N. Idiosyncratic drug hepatotoxicity. Nat Rev Drug Discov 2005; 4: 489-499.

[2] Larsen FS and Wendon J. Understanding paracetamol-induced liver failure. Intensive Care Med 2014; 40: 888-890.

[3] Bunchorntavakul C and Reddy KR. Acetaminophen-related hepatotoxicity. Clin Liver Dis 2013; 17: 587-607.

[4] Hinson JA, Roberts DW and James LP. Mechanisms of acetaminophen-induced liver necrosis. Handb Exp Pharmacol 2010; 369-405.

[5] McGill MR, Sharpe MR, Williams CD, Taha M, Curry SC and Jaeschke H. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. J Clin Invest 2012; 122: 1574-1583.

[6] Liu ZX, Govindarajan S and Kaplowitz N. Innate immune system plays a critical role in determining the progression and severity of acetaminophen hepatotoxicity. Gastroenterology 2004; 127: 1760-1774.

[7] Krenkel O, Mossanen JC and Tacke F. Immune mechanisms in acetaminophen-induced acute liver failure. Hepatobiliary Surg Nutr 2014; 3: 331-343.

[8] Wang AY, Lian LH, Jiang YZ, Wu YL and Nan JX. Gentiana manshurica Kitagawa prevents acetaminophen-induced acute hepatic injury in mice via inhibiting JNK/ERK MAPK pathway. World J Gastroenterol 2010; 16: 384-391.

[9] Wancket LM, Meng X, Rogers LK and Liu Y. Mitogen-activated protein kinase phosphatase (Mkp)-1 protects mice against acetaminophen-induced hepatic injury. Toxicol Pathol 2012; 40: 1095-1105.

[10] Yu SM and Kim SJ. The thymoquinone-induced production of reactive oxygen species promotes dedifferentiation through the ERK pathway and inflammation through the p38 and PI3K pathways in rabbit articular chondrocytes. Int J Mol Med 2015; 35: 325-332.

[11] Napetschnig J and Wu H. Molecular basis of NF-kappaB signaling. Annu Rev Biophys 2013; 42: 443-468.

[12] Vallabhapurapu S and Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. Annu Rev Immunol 2009; 27: 693-733.

[13] Ding Y, Ren D, Xu H, Liu W, Liu T, Li L, Li J, Li Y and Wen A. Antioxidant and pro-angiogenic effects of corilagin in rat cerebral ischemia via Nrf2 activation. Oncotarget 2017; 8: 114816-114828.

[14] Guo S, Fu Y, Xiong S and Lv J. Corilagin protects the acute lung injury by ameliorating the apoptosis pathway. Biomed Pharmacother 2017; 95: 1743-1748.

[15] Gambari R, Borgatti M, Lampronti I, Fabbri E, Brognara E, Bianchi N, Piccagli L, Yuen MC, Kan CW, Hau DK, Fong WF, Wong WY, Wong RS and Chui CH. Corilagin is a potent inhibitor of NF-kappaB activity and downregulates TNF-alpha induced expression of IL-8 gene in cystic fibrosis IB3-1 cells. Int Immunopharmacol 2012; 13: 308-315.

[16] Jin F, Cheng D, Tao JY, Zhang SL, Pang R, Guo YJ, Ye P, Dong JH and Zhao L. Anti-inflammatory and anti-oxidative effects of corilagin in a rat model of acute cholestasis. BMC Gastroenterol 2013; 13: 79.

[17] Kinoshita S, Inoue Y, Nakama S, Ichiba T and Aniya Y. Antioxidant and hepatoprotective actions of medicinal herb, Terminalia catappa L. from Okinawa Island and its tannin corilagin. Phytomedicine 2007; 14: 755-762.

[18] Martin-Murphy BV, Holt MP and Ju C. The role of damage associated molecular pattern molecules in acetaminophen-induced liver injury in mice. Toxicol Lett 2010; 192: 387-394.

[19] Fisher JE, McKenzie TJ, Lillegard JB, Yu Y, Juskewitch JE, Nedredal GI, Brunn GJ, Yi ES, Malhi H, Smyrk TC and Nyberg SL. Role of Kupffer cells and toll-like receptor 4 in acetaminophen-induced acute liver failure. J Surg Res 2013; 180: 147-155.

[20] Marques PE, Amaral SS, Pires DA, Nogueira LL, Soriani FM, Lima BH, Lopes GA, Russo RC, Avila TV, Melgaco JG, Oliveira AG, Pinto MA, Lima CX, De Paula AM, Cara DC, Leite MF, Teixeira MM and Menezes GB. Chemokines and mitochondrial products activate neutrophils to amplify organ injury during mouse acute liver failure. Hepatology 2012; 56: 1971-1982.

[21] Mendes-Braz M, Elias-Miro M, Jimenez-Castro MB, Casillas-Ramirez A, Ramalho FS and Peralta C. The current state of knowledge of hepatic ischemia-reperfusion injury based on its study in experimental models. J Biomed Biotechnol 2012; 2012: 298657.

[22] Williams CD, Bajt ML, Farhood A and Jaeschke H. Acetaminophen-induced hepatic neutrophil accumulation and inflammatory liver injury in CD18-deficient mice. Liver Int 2010; 30: 1280-1292.

[23] Fang SH, Rao YK and Tzeng YM. Anti-oxidant and inflammatory mediator's growth inhibitory effects of compounds isolated from Phyllanthus urinaria. J Ethnopharmacol 2008; 116: 333-340.

[24] Yuandani, Ilangkovan M, Jantan I, Mohamad HF, Husain K and Abdul Razak AF. Inhibitory effects of standardized extracts of phyllanthus amarus and phyllanthus urinaria and their marker compounds on phagocytic activity of human neutrophils. Evid Based Complement Alternat Med 2013; 2013: 603634.

[25] McConnachie LA, Mohar I, Hudson FN, Ware CB, Ladiges WC, Fernandez C, Chatterton-Kirchmeier S, White CC, Pierce RH and Kavanagh TJ. Glutamate cysteine ligase modifier subunit deficiency and gender as determinants of acetaminophen-induced hepatotoxicity in mice. Toxicol Sci 2007; 99: 628-636.

[26] Reddy BU, Mullick R, Kumar A, Sharma G, Bag P, Roy CL, Sudha G, Tandon H, Dave P, Shukla A, Srinivasan P, Nandhitha M, Srinivasan N and Das S. A natural small molecule inhibitor corilagin blocks HCV replication and modulates oxidative stress to reduce liver damage. Antiviral Res 2018; 150: 47-59.

[27] Chatterjee A and Chatterji U. All-trans retinoic acid ameliorates arsenic-induced oxidative stress and apoptosis in the rat uterus by modulating MAPK signaling proteins. J Cell Biochem 2017; 118: 3796-3809.

[28] Nowak G. Protein kinase C-alpha and ERK1/2 mediate mitochondrial dysfunction, decreases in active Na+ transport, and cisplatin-induced apoptosis in renal cells. J Biol Chem 2002; 277: 43377-43388.

[29] Nguyen NU and Stamper BD. Polyphenols reported to shift APAP-induced changes in MAPK signaling and toxicity outcomes. Chem Biol Interact 2017; 277: 129-136.

[30] Noh JR, Kim YH, Hwang JH, Gang GT, Kim KS, Lee IK, Yun BS and Lee CH. Davallialactone protects against acetaminophen overdose-induced liver injuries in mice. Food Chem Toxicol 2013; 58: 14-21.

[31] Cha H, Lee S, Lee JH and Park JW. Protective effects of p-coumaric acid against acetaminophen-induced hepatotoxicity in mice. Food Chem Toxicol 2018; 121: 131-139.

[32] Saito C, Lemasters JJ and Jaeschke H. c-Jun N-terminal kinase modulates oxidant stress and peroxynitrite formation independent of inducible nitric oxide synthase in acetaminophen hepatotoxicity. Toxicol Appl Pharmacol 2010; 246: 8-17.

[33] Gunawan BK, Liu ZX, Han D, Hanawa N, Gaarde WA and Kaplowitz N. c-Jun N-terminal kinase plays a major role in murine acetaminophen hepatotoxicity. Gastroenterology 2006; 131: 165-178.

[34] Wu H, Pang H, Chen Y, Huang L, Liu H, Zheng Y, Sun C, Zhang G and Wang G. Anti-inflammatory effect of a polyphenol-enriched fraction from acalypha wilkesiana on lipopolysaccharide-stimulated RAW 264.7 macrophages and acetaminophen-induced liver injury in mice. Oxid Med Cell Longev 2018; 2018: 7858094.

[35] Horng CT, Liu ZH, Huang YT, Lee HJ and Wang CJ. Extract from Mulberry (Morus australis) leaf decelerate acetaminophen induced hepatic inflammation involving downregulation of myeloid differentiation factor 88 (MyD88) signals. J Food Drug Anal 2017; 25: 862-871.

[36] Leng J, Wang Z, Fu CL, Zhang J, Ren S, Hu JN, Jiang S, Wang YP, Chen C and Li W. NF-kappaB and AMPK/PI3K/Akt signaling pathways are involved in the protective effects of Platycodon grandiflorum saponins against acetaminophen-induced acute hepatotoxicity in mice. Phytother Res 2018; 32: 2235-2246.

[37] Wang N, Liang H and Zen K. Molecular mechanisms that influence the macrophage m1-m2 polarization balance. Front Immunol 2014; 5: 614.

[38] Song E, Fu J, Xia X, Su C and Song Y. Bazhen decoction protects against acetaminophen induced acute liver injury by inhibiting oxidative stress, inflammation and apoptosis in mice. PLoS One 2014; 9: e107405.

[39] Ganai AA, Khan AA, Malik ZA and Farooqi H. Genistein modulates the expression of NF-kappaB and MAPK (p-38 and ERK1/2), thereby attenuating d-Galactosamine induced fulminant hepatic failure in Wistar rats. Toxicol Appl Pharmacol 2015; 283: 139-146.

[40] Harikrishnan H, Jantan I, Haque MA and Kumolosasi E. Anti-inflammatory effects of Phyllanthus amarus Schum. & Thonn. through inhibition of NF-kappaB, MAPK, and PI3K-Akt signaling pathways in LPS-induced human macrophages. BMC Complement Altern Med 2018; 18: 224.

[41] Yang WT, Li GH, Li ZY, Feng S, Liu XQ, Han GK, Zhang H, Qin XY, Zhang R, Nie QM and Jin F. Effect of corilagin on the proliferation and NF-kappaB in U251 glioblastoma cells and U251 glioblastoma stem-like cells. Evid Based Complement Alternat Med 2016; 2016: 1418309.