Emodin Attenuates Acetaminophen-Induced Hepatotoxicity via Cgas-STING Pathway

Pan Shen

Institute of Integarted Traditional Chinese and Western Medicine, Tongji Hospital, Tongji Medical College of Huazhong University of Science and Technology

Zhe Cheng

Institute of Integrated Traditional Chinese and Western Medicine, Tongji Hospital, Tongji Medcial College of Huazhong University of Science and Technology

Qiong Liu (liuqtjh@163.com)

Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology

https://orcid.org/0000-0001-7098-5704

Research Article

Keywords: Emodin, Acetaminophen, Hepatotoxicity, cGAS-STING, Inflammatory response.

Posted Date: May 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-419469/v1

Version of Record: A version of this preprint was published at Inflammation on August 18th, 2021. See the published version at https://doi.org/10.1007/s10753-021-01529-5.

Abstract

Background:

Emodin, a natural bioactive compound from traditional Chinese herbs, exerts anti-inflammatory, anti-oxidants, anticancer, hepatoprotective, and neuroprotective effects. However, its protective effects in acetaminophen (APAP)-induced hepatotoxicity still unclear.

Aim:

The study explored the effects of emodin on APAP-induced hepatotoxicity and investigated the potential molecular mechanisms.

Materials and Methods:

C57BL/6 mice with pre-treatment of emodin (15, 30 mg/kg) for consecutive 5 days, then were given APAP (300 mg/kg) to make APAP-induced liver injury model. Mice were sacrificed to detected the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin (ALB), and the liver tissues levels of glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD). Histological assessment, Western blot and ELISA were conducted.

Results:

Emodin pretreatment significantly reduced the levels of ALT, AST, and ALP, increased the levels of ALB, alleviated hepatocellular damage and apoptosis, attenuated the exhaustion of GSH, SOD, and accumulation of MDA, increased the expression of antioxidative enzymes, including nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase 1 (HO-1), and NAD(P)H quinone dehydrogenase 1(NQO1). Additionally, emodin inhibited the expression of NLRP3 and reduced the levels of pro-inflammatory factors, including interleukin-1 beta (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α). Emodin inhibited Cyclic GMP-AMP synthase (cGAS) and its downstream signaling effector stimulator of interferon genes (STING) expression for liver protection against APAP-induced inflammatory responses and apoptosis.

Conclusion:

The above results suggested that emodin protected hepatocytes from APAP induce liver injury via the up-regulation of Nrf2 mediated anti-oxidative stress pathway, the inhibition of NLRP3 inflammasome, and the down-regulation of the cGAS-STING signaling pathway.

Introduction

Acetaminophen (N-acetyl-p-aminophenol, APAP), a widely used acetanilide analgesic and antipyretic drug all over the world, which is primarily used against cold or influenza-induced headache and fever [1,2]. Although APAP has been considered highly safe at a recommended dose, its intentional or unintentional overdose can cause severe nephrotoxicity and hepatotoxicity, leading to life-threatening acute kidney injury and liver failure [3,4]. Every year, more than 200 million people use APAP, APAP-induced acute hepatic failure results in 200 deaths. However, the treatment for APAP poisoning is mainly limited to N-acetyl-L-cysteine (NAC), which is a nonspecific antidote that restores endogenous glutathione (GSH) [5]. Therefore, it is of great significance to explore the possible molecular mechanism of liver damage caused by APAP for its clinical application, as well as the potential therapeutic drugs against its toxicity.

Inflammatory response and oxidative stress are considered to be the main mechanisms of APAP-induced liver failure [6,7]. When patients take an overdose of APAP, most drugs are metabolized in the liver by UDP-glucuronidase (UGT) and sulfotransferase (SULT) enzymes into non-toxic compounds, which are subsequently excreted in the urine and bile [8]. The remaining APAP is oxidized by CYP450 enzyme into a toxic intermediate metabolite, N-acetyl-p-benzo-quinoneimine (NAPQI), which can lead to the deplete of GSH and the generation of protein adduct in liver [9]. The depletion of GSH and the NAPQI adducts cause mitochondrial dysfunction and massive reactive oxygen species (ROS) secreted from the injury hepatocytes, which in turn leads to hepatocellular apoptosis[10,11]. Intercellular contents released from these damaged cells, called damage-associated molecular patterns (DAMPs), which can stimulate non-parenchymal cells to produce and release inflammatory mediators and chemokines[12]. Under the action of chemokines, a variety of immune and inflammatory cells, such as monocytes and neutrophils, are recruited into the liver and promote inflammatory responses via the activation of innate immune signal transduction pathways, resulting in the necrosis and apoptosis of liver cells[13]. Importantly, blocking oxidative stress and inhibiting inflammation are important targets for protecting hepatocytes from hepatotoxicity of APAP.

Rheum palmatum L. (RP, Dahuang in Chinese) is one of the most popular plant products used in traditional Chinese medicine (TCM). RP-composed formulas have been widely used for haematemesis, constipation, enteritis, liver injury, and menorrhagia for many years in China[14,15]. Emodin (1,3,8-trihydroxy-6-methylanthraquinone) (Fig. 1), a natural anthraquinone derivative, is the main active component and the quality control index of RP[16,17]. Emodin has been shown to have various biological activities, such as hepatoprotective, anticancer, antibacterial, neuroprotective, antidiabetic, anti-inflammation, and anti-oxidants[18,19].

In the present study, we investigated the protective effects of emodin on APAP-induced liver injury, evaluated its activity of anti-inflammatory, anti-oxidative stress, and anti-apoptosis, and explored the role of cGAS-STING pathway in the beneficial effects of emodin.

Materials And Methods

Reagents and chemicals

Emodin (purity > 98%) and APAP (purity > 98%) were obtained from Yuanye Biotech Co., LTD (Shanghai, China). The alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), alkaline phosphatase (ALP), GSH, malondialdehyde (MDA), superoxide Dismutase (SOD), assay kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). The enzyme-linked immunosorbent assay (ELISA) kits for detecting tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-10, and IL-1β were purchased from Bioswamp Biotech Co., LTD (Wuhan, China). transferase-mediated nucleotide nick-end labeling (TUNEL) kit was purchased from Beyotime Biotech Co., LTD (Shanghai, China). Primary antibodies against nuclear factor erythroid 2-related factor 2, (Nrf2, #12721, 1:1000), hemeoxygenase-1 (HO-1, #86806, 1:1000), NLRP3 (#15101, 1:1000), Caspase1 (#24232, 1:1000), IL-1β, Bcl-2 (#3498, 1:1000), Bcl-2-associated X protein (Bax) (#2772, 1:1000), phosphor (P)-TBK1 (#5483, 1:1000), P-IRF3 (#29047, 1:1000), cGAS, STING (#31659, 1:1000), GAPDH (#5174, 1:1000) were bought from Cell Signaling Technology (Danvers, MA, USA). Primary antibodies against CYP2E1 (#19937-1-AP 1: 2000), NAD(P)H quinone dehydrogenase 1 (NQO1, #67240-1-Ig, 1: 2000), was purchased from Protenintech Biotech Co., LTD (Wuhan, China).

Animal experiments

A total of 32 male (6–8 weeks) C57BL/6 mice weighting 17-23g were supplied by Weitonglihua Biotechnology Co., Ltd (Hangzhou, China) and kept in the Experimental Animal Center of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. All animals were fed under the rearing room with a temperature of 22 ± 3℃, a relative humidity of 55 ± 5%, and a day-night cycle at 12 hours. After 7 days adaptive feeding, 32 mice were randomly distributed in four groups of 8 mice each, healthy control group (control), APAP group (APAP), Emodin low-dose group (Emo-L), and Emodin high-dose group (Emo-H).

The mice in Emo-L, and Emo-H group were orally administration with emodin for consecutive 5 days (15 and 30 mg/ kg/day respectively). Emodin was dissolved in 40% polyethylene glycol (PEG). The control and APAP group received the same volume of vehicle. Two hours after the last emodin administration, APAP were used to (intraperitoneally injection at the concentration of 300 mg/kg body weight) induce acute hepatic injury. APAP was dissolving in saline. The control group received the same volume of vehicle. Twenty-four hours later, all the mice were euthanized with overdose of 1% pentobarbital sodium and specimens were collected immediately. All procedures of animal experiments were approved by Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology.

Tissue collection

Serum samples were obtained from peripheral blood by centrifugation at 3000 rpm for 10 min at 4 ℃. Half of left lobe of liver samples were also separated and collected. All above samples were stored at -80 ℃. Remaining left lobe of liver samples were fixed with 4% paraformaldehyde and embedded in paraffin. All samples were processed on ice as soon as possible to prevent protein degradation.

Hepatic histological analysis

Sections of fixed hepatic samples embedded in paraffin were used for routine hematoxylin and eosin (H&E) staining. Sections were observed and photographed by the optical microscope (Olympus, Japan). Injury grades of hepatic samples were evaluated by Suzuki's score according to H&E staining results [20].

Biochemical assays

Serum levels of ALT, AST, ALP and ALB, and SOD, MDA, as well as the levels of GSH in liver tissues were tested by the biochemical kits.

Enzyme-linked immunosorbent assay (ELISA) analysis

The levels of IL-1β, TNF-α, IL-6, IL-10 in hepatic tissues were detected by ELISA kits followed the protocol offered by manufacture.

Protein extraction and western-blot analysis

Total proteins were extracted from hepatic tissues using RIPA buffer. The concentration of total proteins was quantified by bicinchoninic acid assay. Total proteins were subjected to 10–12% SDS-PAGE electrophoresis, and the proteins were transferred to PVDF membranes (Sigma, MA, USA). PVDF membranes were blocked with 5% non-fat milk for 1.5 hours at room temperature. Then the membranes were incubated with primary antibodies overnight at 4 ℃. PVDFs were incubated with corresponding secondary antibodies. Gray-scale values of straps was analyzed by Image J software.

TUNEL staining analysis

TUNEL staining were used to detect the apoptosis rate of hepatocytes. Firstly, 6-micron sections of hepatic tissue were deparaffinized and rehydrated. The sections were treated with proteinase K for 15 mins, followed by incubation with TdT at 37℃ for 2 hours. Finally, results of TUNEL staining were observed and photographed by the optical microscope. The hepatic TUNEL positive cell number was assessed by Image Picture Pro software.

Statistical analysis

All experimental data in this study were come from at least 3 independent experiments and showed as means ± standard division (SD). One way ANOVA was performed to compare the differences among the four groups. A $p \leq 0.05$ was considered statistically significant.

Results

Emodin alleviated APAP-induced liver injury

Figure 2A shows that widely inflammatory infiltration in hepatic tissue, severe hepatocytes ballooning degeneration, and extensive hepatocytes necrosis in APAP group, which had not presented in control

group. As is shown in Fig. 2B, Suzuki's score in APAP group increased compared to that in the control group ($p < 0.05$, $p < 0.01$). APAP group showed higher levels of serum ALT, AST, ALP, and lower levels of ALB compare to control group ($p < 0.05$, $p < 0.01$) (Fig. 2C-F). The above differences indicated the model of APAP-induced liver injury was successfully established in mice.

Compared to those indicators in APAP group, serum levels of ALB, ALT and ALP decreased, and the levels of ALB was upregulated in Emo-H group ($p < 0.05$). The results of Suzuki's score indicating that the hepatic injury was alleviated significantly under the administration of high-dose Emo ($p < 0.05$) (Fig. 2).

Emodin inhibited APAP-induced oxidative stress of liver tissues

It is considered that hepatic damage is associated with the upregulation of oxidative stress. Our results exhibited that the levels of SOD, GSH in APAP group were downregulated and the levels of MDA was up-regulated compared with control group (Fig. 3A-C) ($p < 0.05$, $p < 0.01$). However, the levels of SOD, MDA, and GSH were improved significantly in Emo-H group, which indicated that emodin could inhibit the oxidative stress caused by APAP-mediated liver injury. Previous studies indicated that transcription factor Nrf2 and its downstream proteins HO-1 and NQO1 exert anti-oxidant properties in cellular. So, we also detected the levels of Nrf2, HO-1, and NQO1 in hepatic tissues. Nrf2, HO-1, and NQO1 were downregulated in APAP group (Fig. 3D and E) ($p < 0.05$, $p < 0.01$), which indicated that they were failed to fulfill their protective roles in APAP-mediated hepatic injury. Nevertheless, the expression levels of Nrf2, HO-1, and NQO1 were partially recovered under the intervention of high-dose Emo ($p < 0.05$). Additionally, we also detected the hepatic levels of CYP2E1 which is responsible for the metabolism of APAP to the toxicant NAPQI. The results shown a significant increase in the levels of CYP2E1 in the APAP group compared to the control group, Emo-H treatment suppressed them to a lower level (Fig. 3D and E) ($p < 0.05$, $p < 0.01$).

Emodin suppressed APAP-induced hepatic inflammation

To determine whether emodin could inhibit inflammation in APAP-induced hepatic damage, levels of pro-inflammatory cytokines, including IL-1β, IL-6, TNF-α and anti-inflammatory cytokine (IL-10) in hepatic tissues were examined. In APAP group, the levels pro-inflammatory factors of IL-1β, IL-6, and TNF-α were upregulated (Fig. 4A-C) ($p < 0.01$), and the level of anti-inflammatory factor IL-10 (Fig. 4D) ($p < 0.01$) was downregulated, which indicated that there was robust inflammation caused by APAP in liver. Excitingly, the pro-inflammatory factors IL-1β, IL-6, TNF-α were lower and the anti-inflammatory factor IL-10 were higher in Emo-H group than those in APAP group ($p < 0.05$, $p < 0.01$). Furthermore, we detected the expression of NLRP3 inflammasome-associated proteins, including NLRP3, Caspase1 and pro-IL-1β. As shown in Fig. 4E and F, the results showed a significant increase in the NLRP3 protein levels in the APAP group compared to the control group (Fig. 4E and F) ($p < 0.05$, $p < 0.01$). In contrast, emodin treatment significantly reduced NLRP3 protein levels (Fig. 4E and F) ($p < 0.05$).

Emodin-mediated APAP-induced hepatocellular apoptosis

Apoptosis is a programmed cell death procedure when cells confront harsh environment or suffered severe destroy. In APAP-induced hepatic injury, hepatocytes underwent strict oxidative stress and inflammation, which might lead to hepatocellular apoptosis ultimately. TUNEL staining is an effective method to label fragmented DNA emerged by cellular apoptosis. Figure 5A and B illustrated that there were more fragmented DNA in APAP group than those in control group and Emo-H group ($p < 0.05$, $p < 0.01$).

The results shown that the levels of Bax in APAP group were up-regulated ($p < 0.01$), and reduced in Emo-H group ($p < 0.05$). The levels of Bcl-2, a kind of antiapoptotic proteins, had opposite variation trends with Bax both in APAP group and in Emo-H group ($p < 0.05$, $p < 0.01$). The above results presented that APAP-induced hepatic injury also had severe hepatocellular apoptosis like other acute hepatic injury, and emodin could alleviate the apoptosis by rectifying the oxidative stress and inflammations.

Emodin attenuated the activity of cGAS-STING signaling pathway

As is shown in Fig. 6A and B, compare to Control group, the expression of cGAS-STING signaling pathway related proteins, including P-TBK1, P-IRF3, cGAS, and, STING significantly decreased in APAP group ($p < 0.05$, $p < 0.01$). This suggested that the cGAS-STING signaling pathway was activated in model mice. The expression of those proteins was significantly reduced ($p < 0.05$, $p < 0.01$) in Emo-H group, indicating that emodin could inhibit the hepatocellular injury caused by APAP via regulating the activity of cGAS-STING signaling pathway.

Discussion

APAP is one of the most widely used analgesic and antipyretic drugs around the world [21]. However, it has been reported that overdosage of APAP could cause liver injury even death [22,23]. Accumulation of NAPQI, one of the intermediate metabolites of APAP in liver, could actuate the liver injury by promoting oxidative stress and inflammation of hepatic tissues, which finally triggers the hepatocellular apoptosis [22,24,25]. In the present study, abnormal pathologic alternations, increased Suzuki's score, upregulated expression of AST, ALT and ALP, as well as downregulated ALB level in APAP group of mice indicated that the modelling of acute hepatic injury had been successfully established.

Emodin is the major component and one of quality control indexes of RP, a traditional Chinese herb [26–28]. Emodin has been reported that it has biologic activities and beneficial effects, such as hepatoprotective, anti-inflammatory responses, antibacterial, antivirus, as well as neuroprotective [19,29–31]. Previous studies demonstrated that emodin could protect against APAP-induced hepatic injury via multiple targets, including cytochrome P450 (CYP), and AMP-activated protein kinase (AMPK)/ Yes-associated protein (YAP) signaling pathway [17]. Our study suggested that emodin attenuated APAP-induced hepatic injury by activating Nrf2 anti-oxidant pathway and inhibiting NLRP3 via downregulating cGAS-STING signaling pathway.

Oxidative stress is one of landmark events of APAP-induced acute hepatic injury [32]. In experiments of rodent model, routine dose of APAP were mainly involved in glucuronidation and sulfation, and the non-toxic metabolites were then excreted through vile and urine [33,34]. However, when excessive APAP is oxidated to NAPQI by cytochrome P450 (CYP), which binds with GSH to inhibit toxic responses [35,36]. The accumulation of NAPQI result in the depletion of GSH in liver, leading to the decrease of anti-oxidant enzyme activities, and the massive production of ROS [37]. ROS directly cause cytoplasmic vacuolation, hepatocyte apoptosis, and liver failure [38]. SOD, MDA and GSH are the commonly used indexes to measure the levels of intracellular oxidative stress, and SOD and GSH are involved in the anti-oxidant processes, the levels of MDA represent the extents of oxidative injury [39,40]. In our study, the levels of SOD and GSH were significantly increased with the treatment of emodin, the concentration of MDA was reduced. Anti-oxidant enzymes like HO-1 and NQO1, transcription factor Nrf2 is closely related to oxidative stress-associated cellular damage. The loss of Nrf2 in mice caused severe hepatic injury in chlorogenic acid induced acute liver injury [41]. Nrf2 can translocate to nucleus under the stimulation of ROS, and binds to anti-oxidant response element (ARE), leading to the transcription of anti-oxidant enzymes, including NQO1 and HO-1 [42,43]. Our results shown that emodin downregulated CYP2E1 expression and upregulated Nrf2, HO-1, NQO1 expression.

In APAP-induced hepatic injury, oxidative stress causes the activation of inflammatory related-signaling pathways, which further aggravates liver injury [44]. NLRP3 has been considered as an important pro-inflammatory factor activated by oxidative stress [45,46]. It has been reported that NLRP3 is one of potential inflammatory mediators in APAP-induced hepatic damage, partly because of lower levels of NQO1 in the liver [47,48]. Besides, immune cells in hepatic activated by damage-associated molecular patterns (DAMPs), which was conducted with mitochondrial DNA (mtDNA) and fragmented nucleus DNA as well as other proteins released from injured cell, could also be involved in hepatic inflammation [32,49]. Inflammation in APAP-induced damage model is thought to be amplified by IL-1β, IL-6, and TNF-α, produced by Kupffer cells and hepatic dendric cells [12]. IL-10 is known as an anti-inflammatory cytokine and could suppress the acute hepatic injury, moreover, IL-10 deficiency mice showed more severe hepatic damage. In the present study, APAP was found to initiated the activation of NLRP3 inflammasome, which was inhibited by treatment with emodin.

APAP-induced liver damage causes hepatocyte death via necrosis and apoptosis [50]. Bax and Bcl-2 can regulate the of progression apoptosis[51]. The excessive APAP-adducts have been proved to promote hepatocellular apoptosis [52]. We found that emodin alleviated hepatocyte necrosis and apoptosis induced by APAP, as well as inhibited Bax/Bcl-2 ratio. Above results indicated that emodin could inhibit APAP-induced hepatic injury via multiple processes.

Cyclic GMP-AMP synthase (cGAS), a sensor of DNA, which could be activated by virus DNA or aberrant intracellular DNA [53]. cGAS can recognize DNA via electrostatic action and hydrogen-bonding interaction [54], and catalyze the synthesis of Cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) from adenosine triphosphate (ATP) and guanosine triphosphate (GTP) after recognizing DNA [55]. Stimulator of interferon genes (STING) is found on the outer mitochondrial membrane and

endoplasmic reticulum in the form of a dimer in inactivated state [56]. Dimer STING can bind with cGAMP catalyzed by cGAS and subsequently translocated to the vesicles around perinuclear region from endoplasmic reticulum by Golgi body [57]. TANK-binding kinase 1 (TBK1) were enrolled into the vesicles to phosphorylated and activated STING [58]. Furthermore, phosphorylated STING can phosphorylate transcription factor interferon regulatory factor 3 (IRF3) [58], one of the most important downstream transcription factors of cGAS-STING signaling pathway, which is closely related to inflammation and apoptosis [59]. Finally, phosphorylated IRF3 enter the nucleic and promote the transcription of IFN-α [58]. In addition to cGAMP, STING can also be activated by second messenger cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) [60].

It is noteworthy that cGAS-STING signaling pathway also participate in multiple kinds of acute and chronic hepatic injury, including radiation-induced liver injury [61], non-alcoholic fatty liver disease (NAFLD) and high fat diet-associated hepatic injury [62], and hepatitis B virus (HBV) infection-associated liver injury [63]. However, it is interesting that STING mainly expressed in hepatic nonparenchymal cells like intrahepatic macrophages instead of hepatic parenchymal cells, which resulted that the resistance to HBV infection is failed in hepatocellular [63]. It plays vital role in APAP-induced hepatic injury that necrosis hepatocellular released generous mtDNA and fragmented nucleus DNA to intracellular space, and the DNA might amplify the hepatic injury as DAMPs [64]. The cGAS-STING signaling pathway, is associated with innate immune response and DNA recognition. Araujo et al. found that the activation of cGAS-STING signaling pathway plays an important role in APAP-induced hepatic injury [65]. The levels of cGAS and STING were upregulated in hepatic parenchymal cells, and the levels of STING were also consistently increased in hepatic nonparenchymal cells. Simultaneously, massive mtDNA accumulated in extracellular space, which is one of the causes of the activation of cGAS-SITNG signaling pathway in hepatocellular. Activated cGAS-STING signaling pathway in hepatic parenchymal cells promotes inflammation, apoptosis, and necrosis of hepatic tissues [65]. In addition, hepatic nonparenchymal cells with activated cGAS-STING signaling pathway secret IFN-α, which also aggravates the liver damage. Therefore, inhibiting the cGAS-STING signaling pathway is a potential therapeutic method of APAP-induced hepatic injury. Our study shown that emodin could inhibit the expression of cGAS, STING, P-IRF3, and P-TBK1 in liver tissues. These results suggested that the protective effect of emodin on APAP-induced liver damage might be associated with the inhibition of the cGAS-STING signaling pathway.

Conclusion

In the present study, the results shown that administration of emodin attenuated APAP-induced liver injury mainly by alleviating hepatic pathological damage, apoptosis, oxidative stress, and inhibiting the inflammatory response. Additionally, we found that emodin suppressed the cGAS-STING signaling pathway against APAP-induced inflammatory responses and apoptosis. This study supports more evidences for the application of emodin and RP. Building on prior research, it is reasonable to speculate that emodin might be a potential candidate for the prevention and treatment of APAP.

Declarations

COMPETING INTERESTS

None.

DATA AVAILABILITY

The data used to support the findings of this study are included in the paper.

Ethics Approval and Consent to Participate.

All experiment procedures were approved and carried out in accordance with Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology Care Committee guidelines.

FUNDING

This research was supported by The National Natural Science Foundation of China (No.81903965).

DATA AVAILABILITY

The data used to support the findings of this study are included in the paper.

AUTHOR CONTRIBUTIONS

Author Contributions: Conceptualization, Qiong Liu, methodology, Pan Shen, validation, Zhe Cheng, investigation, Pan Shen, data curation, Pan Shen, writing—original draft preparation, Pan Shen, writing—review and editing, Qiong Liu, visualization, Pan Shen, supervision, Qiong Liu.

CONSENT FOR PUBLICATION

Not Applicable.

ACKNOWLEDGEMENTS

Graphic abstract was created by www.BioRender.com.

References

1. Im, Y.J., Jeon, J.Y., Kim, E.Y., Kim, Y., Oh, D.J., Yoo, J.S., Shin, D.H., Chae, S.W., Kim, M.G. An assessment of the pharmacokinetics of a sustained-release formulation of a tramadol/acetaminophen combination in healthy subjects. *Clinical therapeutics* **2015**, *37*, 376-389, doi:10.1016/j.clinthera.2014.12.007.
2. Msolli, M.A., Sekma, A., Toumia, M., Bel Haj Ali, K., Khalil, M.H., Grissa, M.H., Bouida, W., Beltaief, K., Zorgati, A., Methamem, M., et al. Acetaminophen, Nonsteroidal Anti-inflammatory Drugs, or

Combination of Both Analgesics in Acute Posttrauma Pain: A Randomized Controlled Trial. *Academic emergency medicine : official journal of the Society for Academic Emergency Medicine* **2020**, 10.1111/acem.14169, doi:10.1111/acem.14169.

3. Agrawal, S., Khazaeni, B. Acetaminophen Toxicity. In *StatPearls*, StatPearls Publishing Copyright © 2020, StatPearls Publishing LLC.: Treasure Island (FL), 2020.
4. Hua, H., Ge, X., Wu, M., Zhu, C., Chen, L., Yang, G., Zhang, Y., Huang, S., Zhang, A., Jia, Z. Rotenone Protects Against Acetaminophen-Induced Kidney Injury by Attenuating Oxidative Stress and Inflammation. *Kidney & blood pressure research* **2018**, *43*, 1297-1309, doi:10.1159/000492589.
5. Athuraliya, T.N., Jones, A.L. Prolonged N-acetylcysteine therapy in late acetaminophen poisoning associated with acute liver failure--a need to be more cautious? *Critical care (London, England)* **2009**, *13*, 144, doi:10.1186/cc7800.
6. Woolbright, B.L., Jaeschke, H. Role of the inflammasome in acetaminophen-induced liver injury and acute liver failure. *Journal of hepatology* **2017**, *66*, 836-848, doi:10.1016/j.jhep.2016.11.017.
7. Jaeschke, H., McGill, M.R. Cytochrome P450-derived versus mitochondrial oxidant stress in acetaminophen hepatotoxicity. *Toxicology letters* **2015**, *235*, 216-217, doi:10.1016/j.toxlet.2015.04.002.
8. Jiang, Z., Guo, X., Zhang, K., Sekaran, G., Cao, B., Zhao, Q., Zhang, S., Kirby, G.M., Zhang, X. The Essential Oils and Eucalyptol From Artemisia vulgaris L. Prevent Acetaminophen-Induced Liver Injury by Activating Nrf2-Keap1 and Enhancing APAP Clearance Through Non-Toxic Metabolic Pathway. *Frontiers in pharmacology* **2019**, *10*, 782, doi:10.3389/fphar.2019.00782.
9. Connolly, M.K., Ayo, D., Malhotra, A., Hackman, M., Bedrosian, A.S., Ibrahim, J., Cieza-Rubio, N.E., Nguyen, A.H., Henning, J.R., Dorvil-Castro, M., et al. Dendritic cell depletion exacerbates acetaminophen hepatotoxicity. *Hepatology (Baltimore, Md.)* **2011**, *54*, 959-968, doi:10.1002/hep.24429.
10. Du, K., Ramachandran, A., Jaeschke, H. Oxidative stress during acetaminophen hepatotoxicity: Sources, pathophysiological role and therapeutic potential. *Redox biology* **2016**, *10*, 148-156, doi:10.1016/j.redox.2016.10.001.
11. Yan, M., Huo, Y., Yin, S., Hu, H. Mechanisms of acetaminophen-induced liver injury and its implications for therapeutic interventions. *Redox biology* **2018**, *17*, 274-283, doi:10.1016/j.redox.2018.04.019.
12. Krenkel, O., Mossanen, J.C., Tacke, F. Immune mechanisms in acetaminophen-induced acute liver failure. *Hepatobiliary surgery and nutrition* **2014**, *3*, 331-343, doi:10.3978/j.issn.2304-3881.2014.11.01.
13. Samra, Y.A., Hamed, M.F., El-Sheakh, A.R. Hepatoprotective effect of allicin against acetaminophen-induced liver injury: Role of inflammasome pathway, apoptosis, and liver regeneration. *Journal of biochemical and molecular toxicology* **2020**, *34*, e22470, doi:10.1002/jbt.22470.
14. Tan, Z.B., Fan, H.J., Wu, Y.T., Xie, L.P., Bi, Y.M., Xu, H.L., Chen, H.M., Li, J., Liu, B., Zhou, Y.C. Rheum palmatum extract exerts anti-hepatocellular carcinoma effects by inhibiting signal transducer and

activator of transcription 3 signaling. *Journal of ethnopharmacology* **2019**, *232*, 62-72, doi:10.1016/j.jep.2018.12.019.

15. Ma, L., Shen, Z., Hu, H., Zhou, H., Yu, L., Jiang, H., Zeng, S. Effects of rhein and Rheum palmatum L. extract on the pharmacokinetics and tissue distribution of aristolochic acid I and its demethylated metabolite in rats. *Journal of ethnopharmacology* **2021**, *267*, 113537, doi:10.1016/j.jep.2020.113537.
16. Wang, J.B., Zhao, H.P., Zhao, Y.L., Jin, C., Liu, D.J., Kong, W.J., Fang, F., Zhang, L., Wang, H.J., Xiao, X.H. Hepatotoxicity or hepatoprotection? Pattern recognition for the paradoxical effect of the Chinese herb Rheum palmatum L. in treating rat liver injury. *PloS one* **2011**, *6*, e24498, doi:10.1371/journal.pone.0024498.
17. Lee, E.H., Baek, S.Y., Park, J.Y., Kim, Y.W. Emodin in Rheum undulatum inhibits oxidative stress in the liver via AMPK with Hippo/Yap signalling pathway. *Pharmaceutical biology* **2020**, *58*, 333-341, doi:10.1080/13880209.2020.1750658.
18. Dong, X., Fu, J., Yin, X., Cao, S., Li, X., Lin, L., Ni, J. Emodin: A Review of its Pharmacology, Toxicity and Pharmacokinetics. *Phytotherapy research : PTR* **2016**, *30*, 1207-1218, doi:10.1002/ptr.5631.
19. Hu, N., Liu, J., Xue, X., Li, Y. The effect of emodin on liver disease -- comprehensive advances in molecular mechanisms. *European journal of pharmacology* **2020**, *882*, 173269, doi:10.1016/j.ejphar.2020.173269.
20. Suzuki, S., Toledo-Pereyra, L.H., Rodriguez, F.J., Cejalvo, D. Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Modulating effects of FK506 and cyclosporine. *Transplantation* **1993**, *55*, 1265-1272, doi:10.1097/00007890-199306000-00011.
21. Esh, C.J., Mauger, A.R., Palfreeman, R.A., Al-Janubi, H., Taylor, L. Acetaminophen (Paracetamol): Use beyond Pain Management and Dose Variability. *Frontiers in physiology* **2017**, *8*, 1092, doi:10.3389/fphys.2017.01092.
22. Yoon, E., Babar, A., Choudhary, M., Kutner, M., Pyrsopoulos, N. Acetaminophen-Induced Hepatotoxicity: a Comprehensive Update. *Journal of clinical and translational hepatology* **2016**, *4*, 131-142, doi:10.14218/jcth.2015.00052.
23. Nadler, A., Fein, D.M. Acetaminophen Poisoning. *Pediatrics in review* **2018**, *39*, 316-318, doi:10.1542/pir.2017-0093.
24. Chun, L.J., Tong, M.J., Busuttil, R.W., Hiatt, J.R. Acetaminophen hepatotoxicity and acute liver failure. *Journal of clinical gastroenterology* **2009**, *43*, 342-349, doi:10.1097/MCG.0b013e31818a3854.
25. Kon, K., Ikejima, K., Okumura, K., Aoyama, T., Arai, K., Takei, Y., Lemasters, J.J., Sato, N. Role of apoptosis in acetaminophen hepatotoxicity. *Journal of gastroenterology and hepatology* **2007**, *22 Suppl 1*, S49-52, doi:10.1111/j.1440-1746.2007.04962.x.
26. Jin, J., Xue, H., Sun, X., Zan, B., Li, Y., Wang, T., Shi, R., Ma, Y. Simultaneous determination of multiple compounds of Da-Huang-Xiao-Shi decoction in rat plasma by LC-MS/MS and its application in a pharmacokinetic study. *Journal of pharmaceutical and biomedical analysis* **2019**, *174*, 8-18, doi:10.1016/j.jpba.2019.05.050.

27. Bai, J., Wu, J., Tang, R., Sun, C., Ji, J., Yin, Z., Ma, G., Yang, W. Emodin, a natural anthraquinone, suppresses liver cancer in vitro and in vivo by regulating VEGFR(2) and miR-34a. *Investigational new drugs* **2020**, *38*, 229-245, doi:10.1007/s10637-019-00777-5.
28. Du, C., Shi, L., Wang, M., Mao, P., Wang, J., Wei, Y., Hou, J., Wang, M. Emodin attenuates Alzheimer's disease by activating the protein kinase C signaling pathway. *Cellular and molecular biology (Noisy-le-Grand, France)* **2019**, *65*, 32-37.
29. Monisha, B.A., Kumar, N., Tiku, A.B. Emodin and Its Role in Chronic Diseases. *Advances in experimental medicine and biology* **2016**, *928*, 47-73, doi:10.1007/978-3-319-41334-1_3.
30. Dang, S.S., Jia, X.L., Song, P., Cheng, Y.A., Zhang, X., Sun, M.Z., Liu, E.Q. Inhibitory effect of emodin and Astragalus polysaccharide on the replication of HBV. *World journal of gastroenterology* **2009**, *15*, 5669-5673, doi:10.3748/wjg.15.5669.
31. Jessick, V.J., Xie, M., Pearson, A.N., Torrey, D.J., Ashley, M.D., Thompson, S., Meller, R. Investigating the role of the actin regulating complex ARP2/3 in rapid ischemic tolerance induced neuro-protection. *International journal of physiology, pathophysiology and pharmacology* **2013**, *5*, 216-227.
32. McGill, M.R., Sharpe, M.R., Williams, C.D., Taha, M., Curry, S.C., Jaeschke, H. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. *The Journal of clinical investigation* **2012**, *122*, 1574-1583, doi:10.1172/jci59755.
33. Nelson, S.D. Molecular mechanisms of the hepatotoxicity caused by acetaminophen. *Seminars in liver disease* **1990**, *10*, 267-278, doi:10.1055/s-2008-1040482.
34. Yoshioka, H., Ichimaru, Y., Fukaya, S., Nagatsu, A., Nonogaki, T. Potentiating effect of acetaminophen and carbon tetrachloride-induced hepatotoxicity is mediated by activation of receptor interaction protein in mice. *Toxicology mechanisms and methods* **2018**, *28*, 615-621, doi:10.1080/15376516.2018.1485804.
35. Jaeschke, H., Ramachandran, A., Chao, X., Ding, W.X. Emerging and established modes of cell death during acetaminophen-induced liver injury. *Archives of toxicology* **2019**, *93*, 3491-3502, doi:10.1007/s00204-019-02597-1.
36. Guo, H., Sun, J., Li, D., Hu, Y., Yu, X., Hua, H., Jing, X., Chen, F., Jia, Z., Xu, J. Shikonin attenuates acetaminophen-induced acute liver injury via inhibition of oxidative stress and inflammation. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* **2019**, *112*, 108704, doi:10.1016/j.biopha.2019.108704.
37. Lee, K.K., Imaizumi, N., Chamberland, S.R., Alder, N.N., Boelsterli, U.A. Targeting mitochondria with methylene blue protects mice against acetaminophen-induced liver injury. *Hepatology (Baltimore, Md.)* **2015**, *61*, 326-336, doi:10.1002/hep.27385.
38. Clemens, M.M., Kennon-McGill, S., Apte, U., James, L.P., Finck, B.N., McGill, M.R. The inhibitor of glycerol 3-phosphate acyltransferase FSG67 blunts liver regeneration after acetaminophen overdose by altering GSK3β and Wnt/β-catenin signaling. *Food and chemical toxicology : an international*

journal published for the British Industrial Biological Research Association **2019**, *125*, 279-288, doi:10.1016/j.fct.2019.01.014.

39. Marrocco, I., Altieri, F., Peluso, I. Measurement and Clinical Significance of Biomarkers of Oxidative Stress in Humans. *Oxidative medicine and cellular longevity* **2017**, *2017*, 6501046, doi:10.1155/2017/6501046.
40. Porto, H.K.P., Grando, M.D., Ramalho, L.N.Z., Valadares, M.C., Bendhack, L.M., Batista, A.C., Rocha, M.L. Exposure to acetaminophen impairs vasodilation, increases oxidative stress and changes arterial morphology of rats. *Archives of toxicology* **2019**, *93*, 1955-1964, doi:10.1007/s00204-019-02463-0.
41. Shi, A., Shi, H., Wang, Y., Liu, X., Cheng, Y., Li, H., Zhao, H., Wang, S., Dong, L. Activation of Nrf2 pathway and inhibition of NLRP3 inflammasome activation contribute to the protective effect of chlorogenic acid on acute liver injury. *International immunopharmacology* **2018**, *54*, 125-130, doi:10.1016/j.intimp.2017.11.007.
42. He, F., Ru, X., Wen, T. NRF2, a Transcription Factor for Stress Response and Beyond. *International journal of molecular sciences* **2020**, *21*, doi:10.3390/ijms21134777.
43. Lee, S., Hu, L. Nrf2 activation through the inhibition of Keap1-Nrf2 protein-protein interaction. *Medicinal chemistry research : an international journal for rapid communications on design and mechanisms of action of biologically active agents* **2020**, *29*, 846-867, doi:10.1007/s00044-020-02539-y.
44. Yang, S., Kuang, G., Jiang, R., Wu, S., Zeng, T., Wang, Y., Xu, F., Xiong, L., Gong, X., Wan, J. Geniposide protected hepatocytes from acetaminophen hepatotoxicity by down-regulating CYP 2E1 expression and inhibiting TLR 4/NF-κB signaling pathway. *International immunopharmacology* **2019**, *74*, 105625, doi:10.1016/j.intimp.2019.05.010.
45. Abais, J.M., Xia, M., Zhang, Y., Boini, K.M., Li, P.L. Redox regulation of NLRP3 inflammasomes: ROS as trigger or effector? *Antioxidants & redox signaling* **2015**, *22*, 1111-1129, doi:10.1089/ars.2014.5994.
46. Li, S., Lin, Q., Shao, X., Mou, S., Gu, L., Wang, L., Zhang, Z., Shen, J., Zhou, Y., Qi, C., et al. NLRP3 inflammasome inhibition attenuates cisplatin-induced renal fibrosis by decreasing oxidative stress and inflammation. *Experimental cell research* **2019**, *383*, 111488, doi:10.1016/j.yexcr.2019.07.001.
47. Shan, S., Shen, Z., Zhang, C., Kou, R., Xie, K., Song, F. Mitophagy protects against acetaminophen-induced acute liver injury in mice through inhibiting NLRP3 inflammasome activation. *Biochemical pharmacology* **2019**, *169*, 113643, doi:10.1016/j.bcp.2019.113643.
48. Liu, X., Zhang, X., Ding, Y., Zhou, W., Tao, L., Lu, P., Wang, Y., Hu, R. Nuclear Factor E2-Related Factor-2 Negatively Regulates NLRP3 Inflammasome Activity by Inhibiting Reactive Oxygen Species-Induced NLRP3 Priming. *Antioxidants & redox signaling* **2017**, *26*, 28-43, doi:10.1089/ars.2015.6615.
49. Roh, J.S., Sohn, D.H. Damage-Associated Molecular Patterns in Inflammatory Diseases. *Immune network* **2018**, *18*, e27, doi:10.4110/in.2018.18.e27.

50. Ramachandran, A., Jaeschke, H. Acetaminophen Hepatotoxicity. *Seminars in liver disease* **2019**, *39*, 221-234, doi:10.1055/s-0039-1679919.
51. Alhusaini, A.M., Faddah, L.M., Hasan, I.H., Jarallah, S.J., Alghamdi, S.H., Alhadab, N.M., Badr, A., Elorabi, N., Zakaria, E., Al-Anazi, A. Vitamin C and Turmeric Attenuate Bax and Bcl-2 Proteins' Expressions and DNA Damage in Lead Acetate-Induced Liver Injury. *Dose-response : a publication of International Hormesis Society* **2019**, *17*, 1559325819885782, doi:10.1177/1559325819885782.
52. Volpe, C.M.O., Villar-Delfino, P.H., Dos Anjos, P.M.F., Nogueira-Machado, J.A. Cellular death, reactive oxygen species (ROS) and diabetic complications. *Cell death & disease* **2018**, *9*, 119, doi:10.1038/s41419-017-0135-z.
53. Gao, D., Wu, J., Wu, Y.T., Du, F., Aroh, C., Yan, N., Sun, L., Chen, Z.J. Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. *Science (New York, N.Y.)* **2013**, *341*, 903-906, doi:10.1126/science.1240933.
54. Civril, F., Deimling, T., de Oliveira Mann, C.C., Ablasser, A., Moldt, M., Witte, G., Hornung, V., Hopfner, K.P. Structural mechanism of cytosolic DNA sensing by cGAS. *Nature* **2013**, *498*, 332-337, doi:10.1038/nature12305.
55. Cai, X., Chiu, Y.H., Chen, Z.J. The cGAS-cGAMP-STING pathway of cytosolic DNA sensing and signaling. *Molecular cell* **2014**, *54*, 289-296, doi:10.1016/j.molcel.2014.03.040.
56. Kwon, J., Bakhoum, S.F. The Cytosolic DNA-Sensing cGAS-STING Pathway in Cancer. *Cancer discovery* **2020**, *10*, 26-39, doi:10.1158/2159-8290.Cd-19-0761.
57. Ishikawa, H., Ma, Z., Barber, G.N. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature* **2009**, *461*, 788-792, doi:10.1038/nature08476.
58. Tanaka, Y., Chen, Z.J. STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway. *Science signaling* **2012**, *5*, ra20, doi:10.1126/scisignal.2002521.
59. Qiao, J.T., Cui, C., Qing, L., Wang, L.S., He, T.Y., Yan, F., Liu, F.Q., Shen, Y.H., Hou, X.G., Chen, L. Activation of the STING-IRF3 pathway promotes hepatocyte inflammation, apoptosis and induces metabolic disorders in nonalcoholic fatty liver disease. *Metabolism: clinical and experimental* **2018**, *81*, 13-24, doi:10.1016/j.metabol.2017.09.010.
60. Burdette, D.L., Monroe, K.M., Sotelo-Troha, K., Iwig, J.S., Eckert, B., Hyodo, M., Hayakawa, Y., Vance, R.E. STING is a direct innate immune sensor of cyclic di-GMP. *Nature* **2011**, *478*, 515-518, doi:10.1038/nature10429.
61. Du, S., Chen, G., Yuan, B., Hu, Y., Yang, P., Chen, Y., Zhao, Q., Zhou, J., Fan, J., Zeng, Z. DNA sensing and associated type 1 interferon signaling contributes to progression of radiation-induced liver injury. *Cellular & molecular immunology* **2020**, 10.1038/s41423-020-0395-x, doi:10.1038/s41423-020-0395-x.
62. Luo, X., Li, H., Ma, L., Zhou, J., Guo, X., Woo, S.L., Pei, Y., Knight, L.R., Deveau, M., Chen, Y., et al. Expression of STING Is Increased in Liver Tissues From Patients With NAFLD and Promotes Macrophage-Mediated Hepatic Inflammation and Fibrosis in Mice. *Gastroenterology* **2018**, *155*, 1971-1984.e1974, doi:10.1053/j.gastro.2018.09.010.

63. Thomsen, M.K., Nandakumar, R., Stadler, D., Malo, A., Valls, R.M., Wang, F., Reinert, L.S., Dagnaes-Hansen, F., Hollensen, A.K., Mikkelsen, J.G., et al. Lack of immunological DNA sensing in hepatocytes facilitates hepatitis B virus infection. *Hepatology (Baltimore, Md.)* **2016**, *64*, 746-759, doi:10.1002/hep.28685.
64. He, Y., Feng, D., Li, M., Gao, Y., Ramirez, T., Cao, H., Kim, S.J., Yang, Y., Cai, Y., Ju, C., et al. Hepatic mitochondrial DNA/Toll-like receptor 9/MicroRNA-223 forms a negative feedback loop to limit neutrophil overactivation and acetaminophen hepatotoxicity in mice. *Hepatology (Baltimore, Md.)* **2017**, *66*, 220-234, doi:10.1002/hep.29153.
65. Araujo, A.M., Antunes, M.M., Mattos, M.S., Diniz, A.B., Alvarenga, D.M., Nakagaki, B.N., Carvalho, É., Lacerda, V.A.S., Carvalho-Gontijo, R., Goulart, J., et al. Liver Immune Cells Release Type 1 Interferon Due to DNA Sensing and Amplify Liver Injury from Acetaminophen Overdose. *Cells* **2018**, *7*, doi:10.3390/cells7080088.

Figures

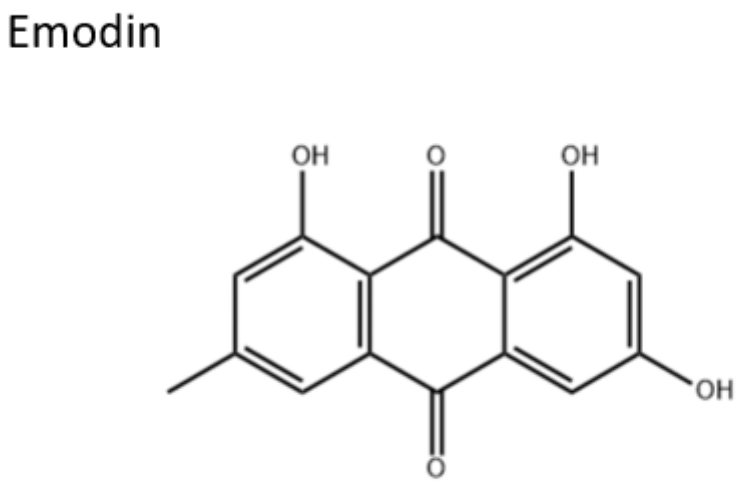

Figure 1

Chemical structure of emodin

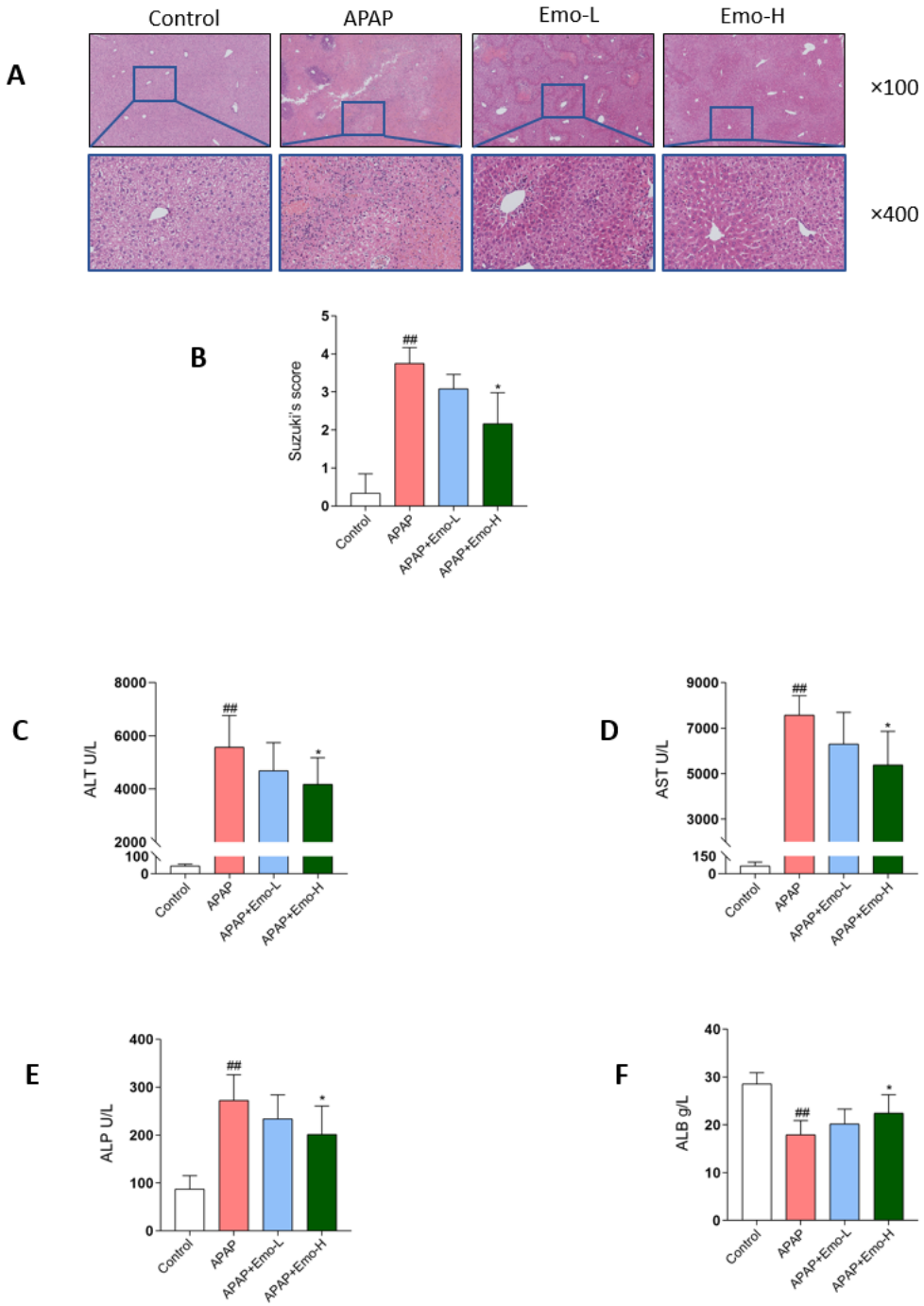

Figure 2

Therapeutic effects of emodin on APAP-induced hepatic injury of mice. (A) Representative images of hepatic H&E staining. (n=6) (B) Histological changes were graded by Suzuki's score. (C) Serum levels of ALT, AST, ALP, ALB were detected by corresponding kits. Data and mean ± standard deviation (SD) values. (n=8). #P < 0.05, ##P < 0.01 vs the control group, *P < 0.05, **P < 0.01vs the APAP group.

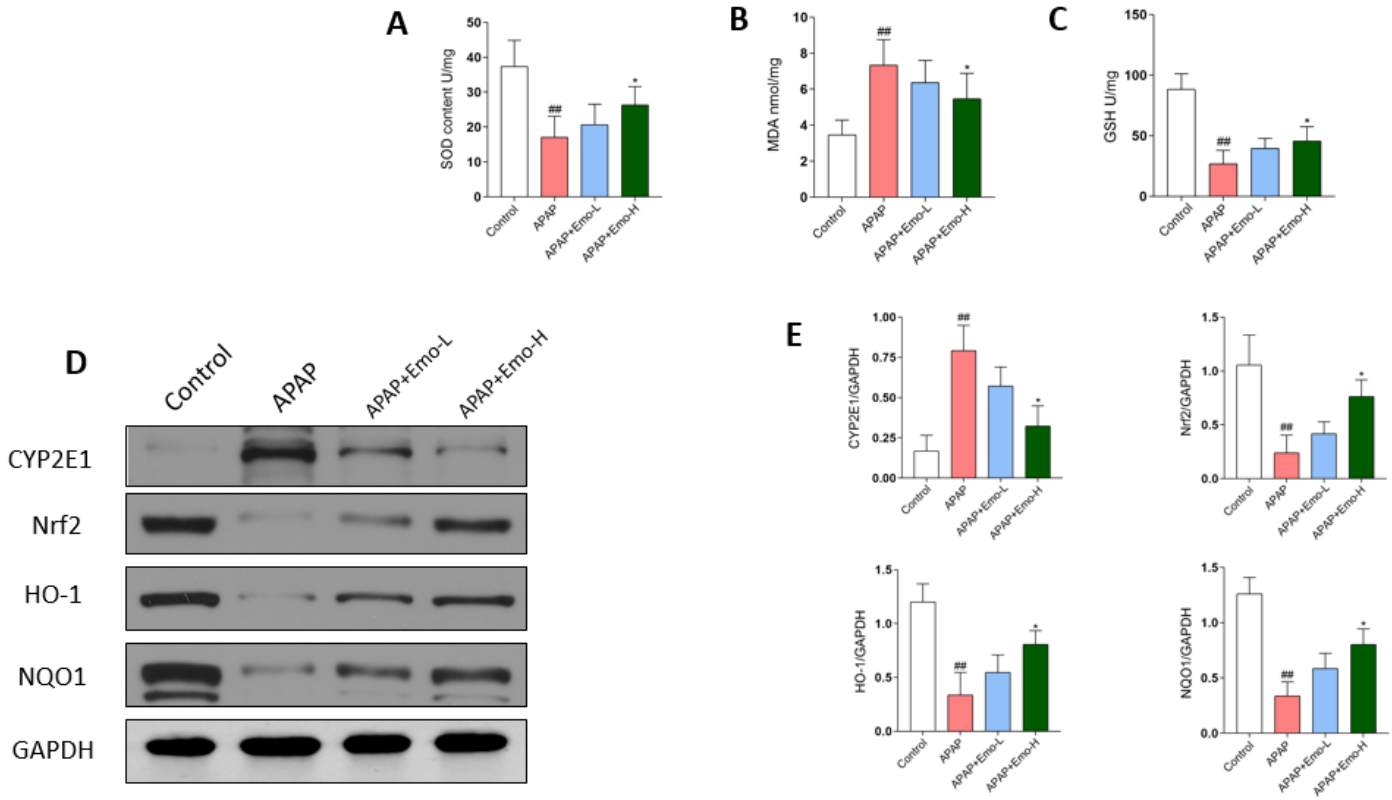

Figure 3

The protective effects of emodin on APAP-induced oxidative stress of mice. Hepatic content of SOD (A), MDA(B), and GSH (C). (n=8). (D) and (E) Hepatic protein expression of CYP2E1, Nrf2, HO-1, and NQO1 was measured by western blot. Data and mean ± standard deviation (SD) values. (n=3). #P < 0.05, ##P < 0.01 vs the control group, *P < 0.05, **P < 0.01vs the APAP group.

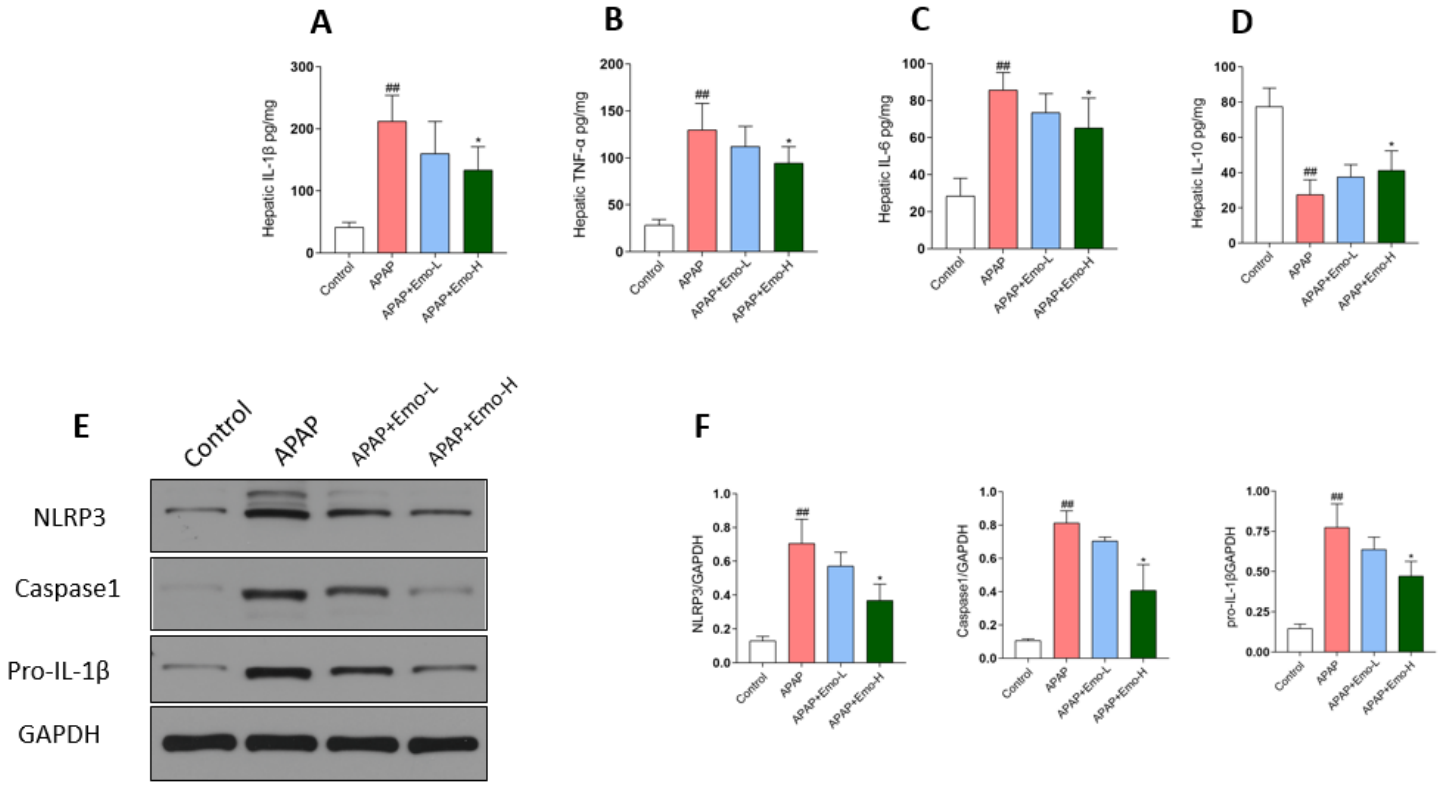

Figure 4

Emodin attenuated APAP-induced hepatic inflammation in mice. Hepatic levels of IL-1β (A), TNF-α (B), IL-10 (C), and IL-6 (D) were detected by ELISA kits (n=8). (E) and (F) Hepatic protein expression of NLRP3, Caspase1, pro-IL-1β was measured by western blot (n=3). Data and mean ± standard deviation (SD) values. #P < 0.05, ##P < 0.01 vs the control group, *P < 0.05, **P < 0.01vs the APAP group.

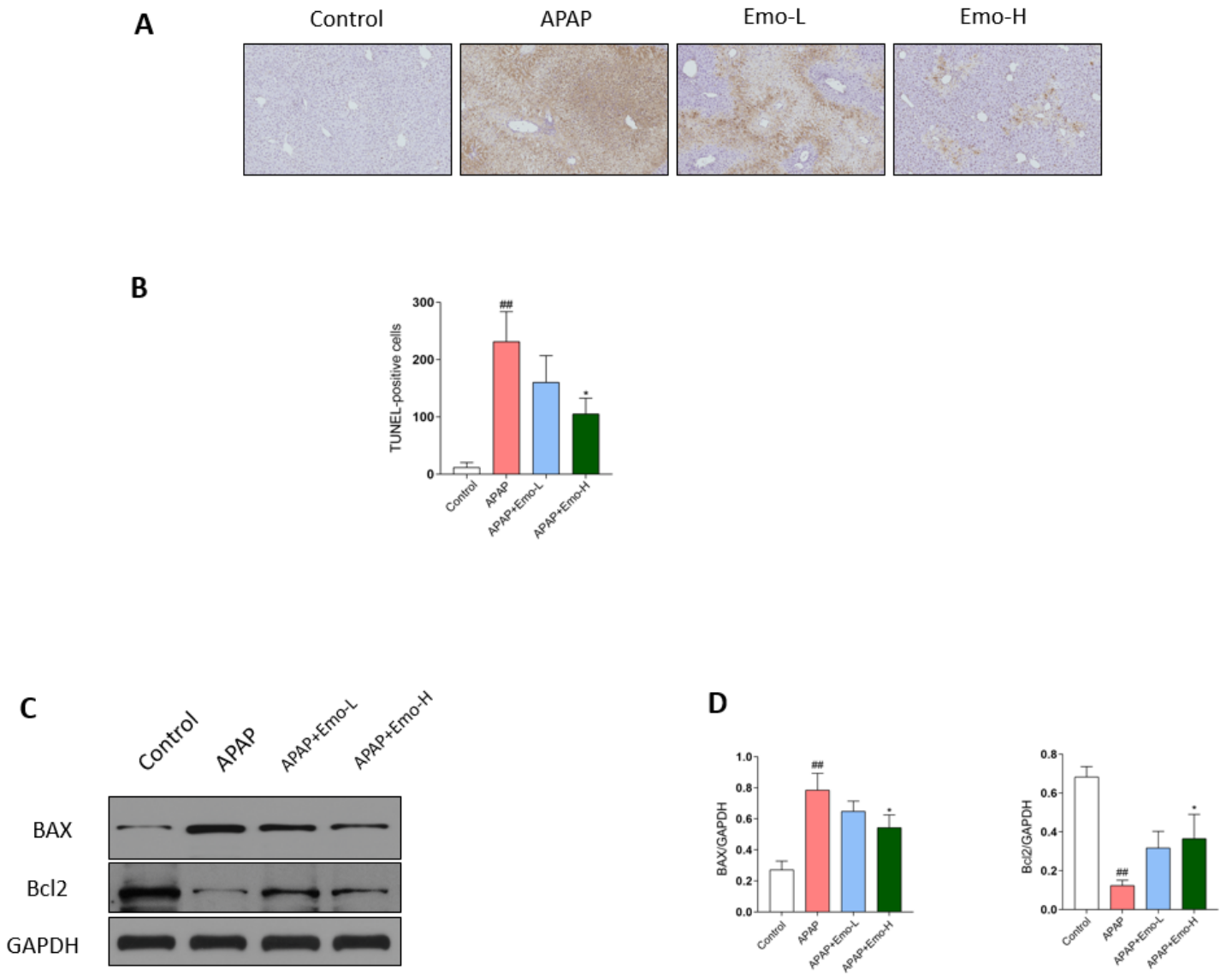

Figure 5

Emodin alleviated APAP-induced hepatocellular apoptosis. (A) TUNEL staining of liver tissues (×200). (B) Quantification of hepatic TUNEL positive cell number. (n=3). (C) and (D) Hepatic protein expression of Bax and Bcl2 was measured by western blot (n=3). Data and mean ± standard deviation (SD) values. #P < 0.05, ##P < 0.01 vs the control group, *P < 0.05, **P < 0.01vs the APAP group.

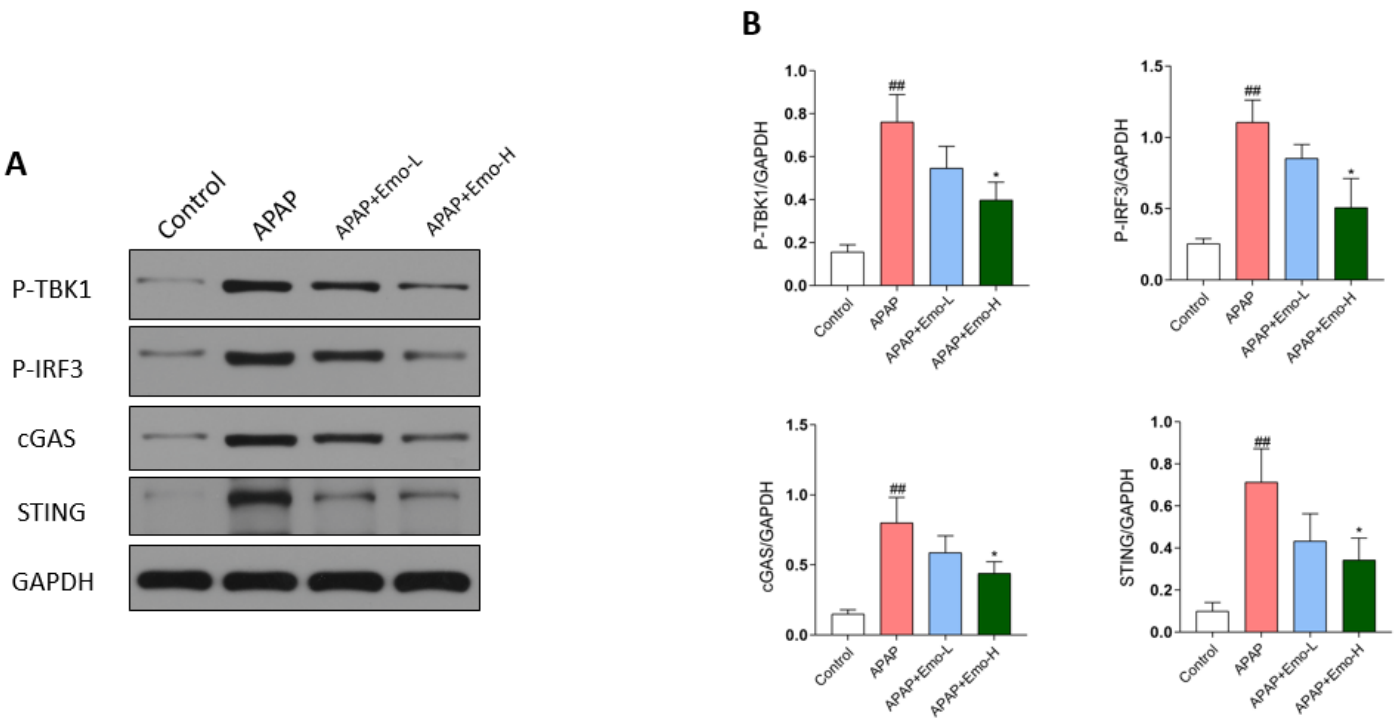

Figure 6

Emodin inhibited the cGAS-STING signal pathway. (A) and (B) Hepatic protein expression of P-TBK1, P-IRF3, cGAS, and STING was measured by western blot (n=3). Data and mean ± standard deviation (SD) values. #P < 0.05, ##P < 0.01 vs the control group, *P < 0.05, **P < 0.01vs the APAP group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Graphicabstract.pptx