Emodin Attenuates Acetaminophen-Induced Hepatotoxicity via the cGAS-STING Pathway

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INTRODUCTION

Acetaminophen (N-acetyl-p-aminophenol, APAP) is an acetanilide analgesic and antipyretic drug used worldwide that is primarily used against cold- or...
influenza-induced headache and fever [1, 2]. Although APAP is considered highly safe at recommended doses, its intentional or unintentional overdose causes severe nephrotoxicity and hepatotoxicity, which lead to life-threatening acute kidney injury and liver failure [3, 4]. More than 200 million people use APAP annually, and APAP-induced acute hepatic failure results in 200 deaths. However, treatment for APAP poisoning is primarily limited to N-acetyl-L-cysteine (NAC), which is a non-specific antidote that restores endogenous glutathione (GSH) [5]. Therefore, it is of great significance to examine the possible molecular mechanism of APAP-induced liver damage for clinical applications and the development of potential therapeutic drugs against APAP toxicity.

The inflammatory response and oxidative stress are the main mechanisms of APAP-induced liver failure [6, 7]. UDP-glucuronicidase (UGT) and sulfotransferase (SULT) enzymes in the liver metabolize most ingested APAP into non-toxic compounds that are subsequently excreted in the urine and bile [8]. The CYP450 enzyme metabolizes the remaining APAP into a toxic intermediate metabolite, N-acetyl-p-benzo-quinoneimine (NAPQI), which may lead to the depletion of GSH and the generation of a protein adduct in the liver [9]. The depletion of GSH and NAPQI adducts causes mitochondrial dysfunction and massive reactive oxygen species (ROS) secretion from injured hepatocytes, which lead to hepatocellular apoptosis [10, 11]. Intercellular contents released from these damaged cells, called damage-associated molecular patterns (DAMPs), stimulate non-parenchymal cells to produce and release inflammatory mediators and chemokines [12]. These chemokines recruit a variety of immune and inflammatory cells, such as monocytes and neutrophils, into the liver and promote inflammatory responses via the activation of innate immune signal transduction pathways, which results in the necrosis and apoptosis of liver cells [13]. The recognition of DAMPs is essential for defense and triggers signaling cascades that lead to the production of pro-inflammatory cytokines and type I interferons (IFN-α and IFN-β) [14]. Therefore, the blockade of oxidative stress and inhibition of inflammation are important targets for protecting hepatocytes from APAP hepatotoxicity.

The activation of endogenous substances induces inflammation in APAP-induced liver damage. The cyclic GMP-AMP synthase (cGAS)-stimulator of interferon gene (STING) pathway plays a role in the activation of the innate immune response. cGAS is a cytosolic sensor of DNA and activates STING, which leads to the phosphorylation of transcription factors, including interferon regulatory factor (IRF)3 and nuclear factor (NF)-κB, and the activation of the transcription of innate immunity-related genes, including type I IFN [15, 16]. cGAS-STING signaling was also implicated in several pathogenic processes, such as alcohol intoxication, autoimmune diseases, and kidney injury [17, 18]. The activation of the cGAS-STING signaling pathway promotes cell apoptosis, inflammation, and oxidation [19, 20], and it likely plays a key role in hepatic injury. Little is known about the role of cGAS-STING signaling in liver injury, and it is valuable to examine the mechanisms to develop treatments. The present study used an APAP-induced liver damage model to study the pathogenic role of cGAS-STING-dependent inflammation in liver damage.

_Rheum palmatum_ L. (RP, Dahuang in Chinese) is one of the most popular plant products in traditional Chinese medicine (TCM). RP-composed formulas have been widely used for the treatment of hematemeses, constipation, enteritis, liver injury, and menorrhagia for many years in China [21, 22]. Emodin (1,3,8-trihydroxy-6-methylanthraquinone) (Fig. 1) is a natural anthraquinone derivative, and it is the main active component of RP [23, 24]. Emodin has various biological activities, such as hepatoprotective, anticancer, antibacterial, neuroprotective, antidiabetic, anti-inflammatory, and antioxidant effects [25, 26]. Bhadaura et al. reported that emodin reversed APAP-induced toxicity similarly to silymarin in a rat model [27]. Emodin inhibited the death of HepG2 cells in vitro, attenuated the degeneration of anti-apoptotic proteins, and improved mitochondrial membrane potential [24]. Emodin also promoted the phosphorylation of Yes-associated protein 1 (YAP1), which is the main downstream target of Hippo that mediates oxidative stress. Many studies reported these natural entities and potential benefits to human health. Preliminary results indicated that emodin possessed several biological activities. However, systemic investigations to examine the protective effect on hepatic disorders in vivo are lacking. More studies are needed to determine the precise roles of emodin in hepatic injury and provide more evidence for the clinical application of relevant products.

The present study investigated the protective effects of emodin on APAP-induced liver injury: evaluated its anti-inflammatory, antioxidative stress, and anti-apoptosis effects; and examined the role of the 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). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), alkaline phosphatase (ALP), GSH, malondialdehyde (MDA), and superoxide dismutase (SOD) assay kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). Enzyme-linked immunosorbent assay (ELISA) kits for detecting tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-10, IFN-α, and IL-1β were purchased from Bioswamp Biotech Co., Ltd. (Wuhan, China). A 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, no. 12721, 1:1000), heme oxygenase-1 (HO-1, no. 86806, 1:1000), NLRP3 (no. 15101, 1:1000), caspase 1 (no. 24232, 1:1000), IL-1β, Bcl-2 (no. 3498, 1:1000), Bcl-2-associated X protein (Bax) (no. 2772, 1:1000), phosphor (P)-TBK1 (no. 5483, 1:1000), P-IRF3 (no. 29047, 1:1000), cGAS, STING (no. 31659, 1:1000), and GAPDH (no. 5174, 1:1000) were purchased from Cell Signaling Technology (Danvers, MA, USA). Primary antibodies against CYP2E1 (no. 19937–1-AP 1: 2000) and NAD(P)H quinone dehydrogenase 1 (NQO1, no. 67240–1-lg, 1: 2000) were purchased from Proteintech Biotech Co., Ltd. (Wuhan, China).

Animal Experiments

Thirty-two male (6–8 weeks) C57BL/6 mice weighing 17–23 g 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 in the rearing room with a temperature of 22 ± 3 °C, a relative humidity of 55 ± 5%, and a day-night cycle of 12 h. After 7 days of adaptive feeding, the 32 mice were randomly distributed into 4 groups of 8 mice each: a healthy control group (control), APAP group (APAP), emodin low-dose group (Emo-L), and emodin high-dose group (Emo-H). Mice in the Emo-L and Emo-H groups were orally administered emodin for 5 consecutive days (15 and 30 mg/kg/day, respectively). Emodin was dissolved in 40% polyethylene glycol (PEG). The control and APAP groups received the same volume of vehicle. Two hours after the last emodin administration, APAP was intraperitoneally injected at a concentration of 300 mg/kg body weight to induce acute hepatic injury. APAP was dissolved in saline. The control group received the same volume of vehicle. Twenty-four hours later, all of the mice were euthanized with an overdose of 1% sodium pentobarbital, and specimens were collected immediately. The Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology approved all animal procedures and experiments.

Fig. 1 Chemical structure of emodin.
Tissue Collection

Serum samples were obtained from peripheral blood via centrifugation at 3000 rpm for 10 min at 4 °C. Half of the liver samples from the left lobe were separated and collected. All of these samples were stored at −80 °C. The remaining liver samples from the left lobe were fixed in 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 using optical microscopy (Olympus, Japan). Injury grades of hepatic samples were evaluated using Suzuki’s score based on the H&E staining results [28].

Biochemical Assays

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

ELISA Analysis

The levels of IL-1β, TNF-α, IL-6, and IL-10 in hepatic tissues and the serum levels of IFN-α were detected using ELISA kits following the protocol provided by the manufacturer.

Protein Extraction and Western Blot Analysis

Total proteins were extracted from hepatic tissues using RIPA buffer. The concentration of total proteins was quantified using the 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 h at room temperature. The membranes were incubated with primary antibodies overnight at 4 °C. PVDFs were incubated with the corresponding secondary antibodies. Grayscale values of bands were analyzed using ImageJ software.

TUNEL Staining Analysis

TUNEL staining was used to detect the apoptosis rate of hepatocytes. Six-micron sections of hepatic tissue were deparaffinized and rehydrated. The sections were treated with proteinase K for 15 min, followed by incubation with TdT at 37 °C for 2 h. The results of TUNEL staining were observed and photographed using optical microscopy. Hepatic TUNEL-positive cell numbers were assessed using Image Picture Pro software.

Statistical Analysis

All experimental data in this study were obtained from at least 3 independent experiments and are shown as the means ± standard deviations (SDs). One-way ANOVA was performed to compare between-group differences. A p ≤ 0.05 was considered statistically significant.

RESULTS

Emodin Alleviated APAP-Induced Liver Injury

Figure 2A shows widespread inflammatory infiltration in hepatic tissue, severe hepatocyte ballooning degeneration, and extensive hepatocyte necrosis in the APAP group, which were not present in the control group. As shown in Fig. 2B, Suzuki’s score in the APAP group was increased compared to the control group (p < 0.01). The APAP group showed higher levels of serum ALT, AST, and ALP and lower levels of ALB than the control group (p < 0.01) (Fig. 2C–F). These differences indicated that the APAP-induced liver injury model was successfully established in mice.

Compared to the APAP group, serum levels of ALB, ALT, and ALP were decreased, and the levels of ALB were upregulated in the Emo-H group (p < 0.05) (Fig. 2C–F). Suzuki’s scores indicated that the administration of high-dose Emo significantly alleviated the hepatic injury (p < 0.05) (Fig. 2B).
Emodin Inhibited APAP-Induced Oxidative Stress in Liver Tissues

Hepatic damage is associated with the upregulation of oxidative stress. Our results showed that the levels of SOD and GSH in the APAP group were downregulated, and the levels of MDA were upregulated compared to those in the control group (Fig. 3A–C) \((p < 0.01)\). However, the levels of SOD and GSH were increased, and MDA levels were decreased in the Emo-H group, which indicated that emodin inhibited oxidative stress in APAP-mediated liver injury. Previous studies indicated that the transcription factor Nrf2 and its downstream proteins HO-1 and NQO1 exerted antioxidant properties in cells. Therefore, we detected the levels of Nrf2, HO-1, and NQO1 in hepatic tissues. Nrf2, HO-1, and NQO1 were downregulated in the APAP group (Fig. 3D and E) \((p < 0.01)\), which indicated that these proteins failed to fulfill their protective roles in APAP-mediated hepatic injury. However, the expression levels of Nrf2, HO-1, and NQO1 were partially recovered after high-dose Emo intervention \((p < 0.05)\). We also detected the hepatic levels of CYP2E1, which is responsible for the metabolism of APAP to the toxicant NAPQI. The results showed a significant increase in the levels of CYP2E1 in the APAP group compared to the control group, and Emo-H treatment suppressed this increase (Fig. 3D and E) \((p < 0.05)\).

Fig. 3 The protective effects of emodin on APAP-induced oxidative stress in mice. Hepatic contents of SOD A, MDA B, and GSH C \((n = 8)\). D, E Hepatic protein expression of CYP2E1, Nrf2, HO-1, and NQO1 was measured using Western blotting \((n = 3)\). All values are presented as the mean \pm SD. \#\# \(p < 0.01\) vs. the control group; * \(p < 0.05\) vs. the APAP group.
Emodin Suppressed APAP-Induced Hepatic Inflammation

To determine whether emodin inhibited inflammation in APAP-induced hepatic damage, the levels of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α, and an anti-inflammatory cytokine (IL-10) in hepatic tissues were examined. Levels of the pro-inflammatory factors IL-1β, TNF-α, and IL-6 were upregulated in the APAP group (Fig. 4A–C) \( p < 0.01 \), and the level of the anti-inflammatory factor IL-10 (Fig. 4D) \( p < 0.01 \) was downregulated, which indicated that APAP induced robust inflammation in the liver. Notably, the pro-inflammatory factors IL-1β, IL-6, and TNF-α were lower and the anti-inflammatory factor IL-10 was higher in the Emo-H group than in the APAP group \( p < 0.05 \). We also detected the expression of NLRP3 inflammasome-associated proteins, including NLRP3, caspase1, and pro-IL-1β. Figure 4E and F show a significant increase in NLRP3 protein levels in the APAP group compared to the control group \( p < 0.01 \).

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 process that occurs when cells confront harsh environments or suffer severe destruction. Hepatocytes undergo strict oxidative stress and inflammation during APAP-induced hepatic injury, which may lead to hepatocellular apoptosis. TUNEL staining is an effective method to label fragmented DNA that emerges from cellular apoptosis. Figure 5A and B show that there was more fragmented DNA in the APAP group than in the control group and Emo-H group \( p < 0.01 \).

The levels of Bax in the APAP group were upregulated \( p < 0.01 \) and reduced in the Emo-H group \( p < 0.05 \). The levels of the anti-apoptotic protein Bcl-2 exhibited opposite trends with Bax in the APAP group and Emo-H group \( p < 0.05, p < 0.01 \). These results

Fig. 4 Emodin attenuated APAP-induced hepatic inflammation in mice. Hepatic levels of IL-1β A, TNF-α B, IL-6 C, and IL-10 D were detected using ELISA kits \( n = 8 \). E, F Hepatic protein expression of NLRP3, caspase1, and pro-IL-1β was measured using Western blotting \( n = 3 \). All values are presented as the mean ± SD. **\( p < 0.01 \) vs. the control group; *\( p < 0.05 \) vs. the APAP group.
showed that APAP-induced hepatic injury also caused severe hepatocellular apoptosis, similar to other acute hepatic injuries, and emodin alleviated apoptosis by rectifying the oxidative stress and inflammation.

**Emodin Attenuated the Activity of the cGAS-STING Signaling Pathway**

Compared to the control group, the expression of cGAS-STING signaling pathway-related proteins, including P-TBK1, P-IRF3, cGAS, and STING, was significantly increased in the APAP group (p < 0.01) (Fig. 6A and B). The expression of IFN-α in serum was also significantly increased in the APAP group (p < 0.01) (Fig. 6C). These results suggested that the cGAS-STING signaling pathway was activated in the model mice. The expression of these proteins was significantly reduced (p < 0.05) in the Emo-H group, which indicated that emodin inhibited the APAP-induced hepatocellular injury via the regulation of cGAS-STING signaling pathway activity.

**Fig. 5** Emodin alleviated APAP-induced hepatocellular apoptosis. A TUNEL staining of liver tissues (×200). B Quantification of hepatic TUNEL-positive cell numbers. (n=3). C, D Hepatic protein expression of Bax and Bcl2 was measured using Western blotting (n=3). All values are presented as the mean ± SD. **p < 0.01** vs. the control group; *p < 0.05 vs. the APAP group.
DISCUSSION

APAP is one of the most widely used analgesic and antipyretic drugs worldwide [29]. However, overdoses of APAP may cause liver injury and death [30, 31]. The accumulation of NAPQI, one of the intermediate metabolites of APAP in the liver, induces liver injury by promoting oxidative stress and inflammation in hepatic tissues, which ultimately trigger hepatocellular apoptosis [30, 32, 33]. The present study showed abnormal pathological alterations; an increased Suzuki’s score; upregulated expression of AST, ALT, and ALP; and downregulated ALB levels in the APAP group of mice, which indicated successful establishment of the acute hepatic injury model.

Emodin is the major component and one of the quality control indexes of the traditional Chinese herb RP [34–36]. Emodin has biological activities and beneficial effects, such as hepatoprotective, anti-inflammatory, antibacterial, antiviral, and neuroprotective effects [26, 37].

Fig. 6 Emodin inhibited the cGAS-STING signal pathway. A, B Hepatic protein expression of P-TBK1, P-IRF3, cGAS, and STING was measured using Western blotting (n=3). All values are presented as the mean ± SD. ## p < 0.01 vs. the control group; *p < 0.05 vs. the APAP group.
which are associated with mitochondrial DNA (mtDNA) 
[55, 56]. Immune cells in the liver are activated by DAMPs, 
partially because of the lower levels of NQO1 in the liver 
inflammatory mediator in APAP-induced hepatic damage, 
activated by oxidative stress [53, 54]. NLRP3 is a potential 
NLRP3 is an important pro-inflammatory factor that is 
causes the activation of the Nrf2 antioxidant pathway and the 
inhibition of NLRP3 by downregulating the cGAS-STING 
signaling pathway.

Oxidative stress is a landmark event of APAP- 
duced acute hepatic injury [40]. Routine doses of APAP 
in experimental rodent models primarily induced glucuro-
nidation and sulfation, and the non-toxic metabolites were 
excreted in bile and urine [41, 42]. However, cytochrome 
P450 (CYP) oxidizes excessive APAP to NAPQI, which 
binds with GSH to inhibit toxic responses [43, 44]. The 
accumulation of NAPQI results in the depletion of GSH 
in the liver, which leads to a decrease in antioxidant 
enzyme activities and the massive production of ROS 
[45]. ROS directly causes cytoplasmic vacuolation, hepat-
ocyte apoptosis, and liver failure [46]. SOD, MDA, and 
GSH are commonly used indexes to measure the levels of 
intracellular oxidative stress. SOD and GSH are involved 
in antioxidant processes, and the levels of MDA represent 
the extent of oxidative injury [47, 48]. The levels of SOD 
and GSH were significantly increased with emodin treat-
ment in our study, and the concentration of MDA was 
reduced. Antioxidant enzymes, such as HO-1 and NQO1, 
and the transcription factor Nrf2 are closely related to 
oxidative-stress-associated cellular damage. The loss of 
Nrf2 in mice caused severe hepatic injury in a chloro-
genic acid–induced acute liver injury model [49]. Nrf2 
translocates to the nucleus under ROS stimulation and 
binds to antioxidant response elements (AREs), which 
leads to the transcription of antioxidant enzymes, including 
NQO1 and HO-1 [50, 51]. Our results showed that 
emodin downregulated CYP2E1 expression and upregu-
lated Nrf2, HO-1, and NQO1 expression. These results 
suggest that emodin alleviates Nrf2-dependent oxidative 
stress.

Oxidative stress in APAP-induced hepatic injury 
causes the activation of inflammatory-related signaling 
pathways, which further aggravates liver injury [52]. 
NLRP3 is an important pro-inflammatory factor that is 
activated by oxidative stress [53, 54]. NLRP3 is a potential 
inflammatory mediator in APAP-induced hepatic damage, 
partially because of the lower levels of NQO1 in the liver 
[55, 56]. Immune cells in the liver are activated by DAMPs, 
which are associated with mitochondrial DNA (mtDNA), 
fragmented nuclear DNA, and other proteins that are 
released from injured cells and may also be involved in 
hepatic inflammation [40, 57]. Inflammation in an APAP- 
duced damage model is amplified by IL-1β, IL-6, and 
TNF-α, which are produced by Kupffer cells and hepatic 
dendritic cells [12]. IL-10 is an anti-inflammatory cytokine 
that suppresses acute hepatic injury [58]. IL-10-deficient 
mice show more severe hepatic damage [59]. APAP ini-
tiated the activation of the NLRP3 inflammasome in the 
present study, and treatment with emodin inhibited this 
activation. These results indicated that emodin inhibited 
inflammatory responses via the suppression of the NLRP3 
 inflammasome.

APAP-induced liver damage causes hepatocyte death 
via necrosis and apoptosis [60]. Bax and Bcl-2 regulate 
the progression of apoptosis [61]. Excessive APAP adducts 
promote hepatocellular apoptosis [62]. We found that 
emodin alleviated APAP-induced hepatocyte necrosis and 
apoptosis and decreased the Bax/Bcl-2 ratio. These results 
indicated that emodin inhibited APAP-induced hepatic 
 injury via the regulation of apoptosis.

cGAS is a sensor of DNA that is activated by viral 
DNA and aberrant intracellular DNA [63]. cGAS recog-
nizes DNA via electrostatic action and hydrogen-bonding 
interactions [64] and catalyzes the synthesis of cyclic 
guanosine monophosphate–adenosine monophosphate 
(cGAMP) from adenosine triphosphate (ATP) and 
guanosine triphosphate (GTP) after DNA recognition 
[65]. STING is found on the outer mitochondrial mem-
brane and endoplasmic reticulum in the form of a dimer 
in an inactivated state [66]. The STING dimer binds with 
the cGAMP catalyzed by cGAS and subsequently trans-
lates to vesicles around the perinuclear region from 
the endoplasmic reticulum by the Golgi body [67]. TANK-
binding kinase 1 (TBK1) in vesicles phosphorylates and 
activates STING [16]. Phosphorylated STING phospho-
rylates the transcription factor IRF3 [16], which is one of 
the most important downstream transcription factors 
of the cGAS-STING signaling pathway, and it is closely 
related to inflammation and apoptosis [68]. Phosphorylated 
IRF3 enters the nucleus and promotes the transcription 
of IFN-α [16]. STING is also activated by the second 
messenger cyclic guanosine monophosphate (cGMP) and 
cyclic adenosine monophosphate (cAMP) [69].

Notably, the cGAS-STING signaling pathway also 
participates in multiple types of acute and chronic hepatic 
 injury, including radiation-induced liver injury [70], non-
alcoholic fatty liver disease, high-fat diet-associated hepatic 
 injury [71], and hepatitis B virus (HBV) infection-associated
liver injury [72]. However, STING is primarily expressed in hepatic non-parenchymal cells, such as intrahepatic macrophages, instead of hepatic parenchymal cells, which results in hepatocellular resistance to HBV infection [72]. STING plays a vital role in APAP-induced hepatic injury in which necrotic hepatocellular cells release generous amounts of mtDNA and fragmented nuclear DNA into the intracellular space, and this DNA may amplify the hepatic injury via DAMPs [73]. The cGAS-STING signaling pathway is associated with the innate immune response and DNA recognition. Araujo et al. found that activation of the cGAS-STING signaling pathway played an important role in APAP-induced hepatic injury [74]. The levels of cGAS and STING were upregulated in hepatic parenchymal cells, and the levels of STING were consistently increased in hepatic non-parenchymal cells. Simultaneously, massive mtDNA accumulated in the extracellular space, which was one of the causes of cGAS-STING signaling pathway activation in hepatocytes. The activated cGAS-STING signaling pathway in hepatic parenchymal cells promotes the inflammation, apoptosis, and necrosis of hepatic tissues [74]. Hepatic non-parenchymal cells with an activated cGAS-STING signaling pathway secrete IFN-α, which also aggravates liver damage. Therefore, the inhibition of the cGAS-STING signaling pathway is a potential therapeutic method for APAP-induced hepatic injury. Our study showed that emodin inhibited the expression of cGAS, STING, P-IRF3, and P-TBK1 in liver tissues. These results suggest that the protective effect of emodin on APAP-induced liver damage is associated with the inhibition of the cGAS-STING signaling pathway. Compared to existing studies, our study provides new evidence of the protective effects of emodin in APAP-induced hepatic injury and a basis for the development of new drugs from natural products. The cGAS-STING signaling pathway provides new perspectives and directions for the study of hepatic injury mechanisms and may play a key role in liver injury. Our results further clarify the mechanisms of APAP-induced injury to promote the development of new targeted drugs and prevent serious complications. There are some shortcomings in this study, including the lack of a comparison of different dosing durations and evaluations of possible side effects of emodin. Our experiments also lack further and deeper mechanistic investigation, including the silencing and overexpression of cGAS-STING signaling using in vivo and in vitro studies. We will perform more studies in this field to obtain a higher level of evidence for clinical application.

CONCLUSION

The results of the present study showed that the administration of emodin attenuated APAP-induced liver injury primarily by alleviating hepatic pathological damage, apoptosis, and oxidative stress and inhibiting the inflammatory response. We also found that emodin suppressed the cGAS-STING signaling pathway in APAP-induced inflammatory responses and apoptosis. This study provides further evidence for the application of emodin and RP. Building on prior research, it is reasonable to suggest emodin as a potential candidate for the prevention and treatment of APAP.

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AUTHOR CONTRIBUTION

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, Liang Han, Guang Chen; visualization, Pan Shen; supervision, Qiong Liu.

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DATA AVAILABILITY

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

Declarations

Ethics Approval and Consent to Participate All experimental procedures were approved and carried out in accordance with Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology Care Committee guidelines.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.
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