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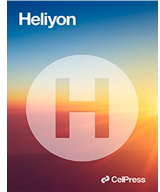

Review article

Advances in the mechanism of emodin-induced hepatotoxicity

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ABSTRACT

Emodin is a naturally occurring anthraquinone derivative and serves as an active component in various traditional Chinese herbal medicines. It is widely known for its broad pharmacological effects, including anti-inflammatory, antioxidant, and anticancer properties. However, high doses and long-term use of emodin can also lead to liver toxicity. Nevertheless, the mechanism of emodin-induced liver toxicity remains unclear at present. This article aims to summarize the toxicological research progress on emodin, with a particular focus on elucidating the mechanisms underlying emodin-induced hepatocyte injury. By providing essential information, the study intends to facilitate further research and safe usage of emodin for researchers and clinical practitioners.

1. Introduction

Emodin ($C_{15}H_{10}O_5$), whose chemical structure is illustrated in Fig. 1, is a naturally occurring anthraquinone derivative. It serves as a vital constituent in various traditional Chinese herbal remedies, including Polygonum multiflorum, Rheum palmatum, and Cassia seeds. Emodin is prevalent in more than 800 types of Chinese medicinal formulations [1](as shown in Table 1). A multitude of prior investigations have ascertained that emodin exhibits a diverse array of pharmacological effects, encompassing its roles as an anticancer agent [2], an anti-inflammatory agent [3], an antiviral agent [4], and an antioxidant [5]. These properties have rendered emodin a valuable ingredient in Chinese medicinal preparations and dietary supplements. However, several experimental and clinical studies have found that high doses and prolonged use of emodin or emodin-containing herbal medicines are associated with hepatocellular damage, and abnormalities in liver function [6–10]. This has triggered the need for evaluation and in-depth studies of the hepatotoxic effects of emodin. Currently, studies on emodin-induced hepatocellular injury have focused on emodin-induced hepatic mitochondrial dysfunction, which leads to oxidative stress and apoptosis, while other aspects, such as emodin-induced endoplasmic reticulum stress, disruption of bile acid metabolism in the hepatocytes, and hepatocellular steatosis, have yet to be investigated in depth. In-depth studies of these aspects may be important for our comprehensive understanding of emodin-induced hepatocyte injury. Table 2 summarizes studies of emodin-induced hepatotoxicity over the past decades.

In this review, we conducted a search using “Emodin, Frangulic Acid, Hepatocyte injury, Liver injury, Hepatotoxicity, and Chemical and Drug-induced Liver Injury” as key words in the Pubmed and Embase databases, resulting in the identification of 31 articles. We comprehensively examined the relationship between emodin and hepatotoxicity and discussed its potential mechanisms. Additionally,

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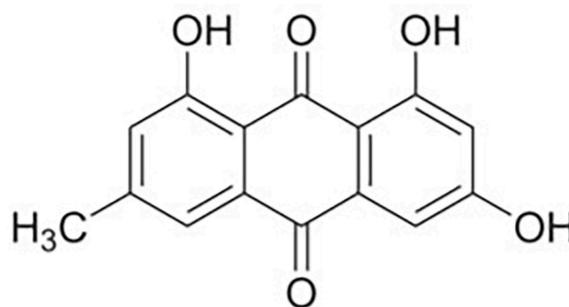

Fig. 1. Chemical structure of emodin.

we examined the influence of factors such as emodin metabolism, drug interactions, dose-response relationships, and individual differences on the hepatotoxic effects of emodin. We aim to provide necessary information to researchers and clinicians by delving into the mechanisms underlying emodin-induced hepatotoxicity, thereby promoting the safe use of emodin and emodin-containing traditional Chinese medicine preparations.

Table 1. Chinese herbal medicines containing emodin and related Chinese medicinal preparations. The Chinese herbal medicines containing emodin and the related Chinese medicinal formulations were searched and compiled from several authoritative databases, such as the Chinese Medicine Pharmacology Database, the Taiwan Chinese Medicine Pharmacology Database, Pharmacopoeia, and China National Knowledge Internet.

2. Mechanism of emodin-induced hepatotoxicity

2.1. Emodin induces hepatocyte apoptosis

2.1.1. Emodin induces mitochondrial damage

Hepatocyte apoptosis refers to an orderly, programmed cell death process, usually achieved through the activation of apoptosis signaling pathways. These pathways include intrinsic and extrinsic pathways. The intrinsic pathway primarily involves a series of proteins and signaling molecules within the cell, such as apoptosis-related proteins associated with mitochondria. The extrinsic pathway typically involves cell death signals triggered by external stimuli, such as cytokines and cell death ligands [30]. Mitochondria are abundant in hepatocytes and are involved in a wide range of cellular physiological activities, including regulation of electrolyte homeostasis, ion transport across membranes, oxygen radical production, and cell signaling. Furthermore, mitochondria provide 80 % of the energy required for cellular life activities, and they are also the main organelles for cellular bio-oxidation and energy conversion [15,22,24,29,31]. Mitochondrial damage may be an obvious trigger for apoptosis.

Research indicates emodin exhibits concentration- and time-dependent cytotoxic effects on normal hepatocytes and hepatocellular carcinoma cells [32,33]. It is noteworthy that the primary mechanism by which emodin exerts hepatocellular cytotoxicity is through inducing apoptosis [11–26,34]. A study in 2016 found that emodin could increase the ratio of Bax/Bcl2 proteins in HepG2 and primary human hepatocytes, leading to disruption of mitochondrial membrane potential (MMP) and consequent mitochondrial damage. Furthermore, emodin can modulate mitochondria-dependent and death receptor-mediated apoptotic signals by inhibiting the PI3K/AKT pathway and activating the MAPK P38 signaling pathway [24]. A study in 2017 discovered that emodin could accumulate in the mitochondria of HepG2 cells and induce mitochondrial dysfunction by upregulating the mitochondrial matrix protein Cyclophilin D (CypD) [31]. In a study by Ni Jian et al., it was found that emodin could induce apoptosis in HepaRG cells by damaging mitochondria, consistent with the results observed by Liu et al. [22,24]. Additionally, emodin can induce mitochondrial damage and cell apoptosis by inhibiting the oxidative phosphorylation pathway of L02 cells, leading to disruption of adenosine triphosphate (ATP)

Table 1
Chinese herbal medicines containing emodin and related Chinese medicine preparations.

Name of Herbs	Number of relevant formulations	Examples
Rheum Officinale	2805	*Daiokanzoto; Rhubarb draining liver soup*
Rhizoma Dioscoreae	269	*Rhizoma Dioscoreae Detoxifying Decoction*
Polygonum Multiflorum	268	*Four substance decoction; Liuwei Dihuang Pill*
Lilium Brownie	209	*Lily Snow fungus Decoction*
Polygonum Cuspidatum	98	*Quyu-Huoxue Decoction*
Semen Cassia	85	*Cassia Seed Xiegan Decoction*
Buckwheat	31	*Buckwheat Fire Reducing Decoction*
Vine of Multiflower Knotweed	24	*Shouwu Teng Decoction*
Rumex Japonicus Houtt	19	*Rumex Japonicus Houtt fire reducing Decoction*
Ramie Root	16	*Ramie Root Qingre Jiedu Decoction*
Artemisia Scoparia	3	*Artemisinin Decoction*

Table 2
Studies on the emodin-induced hepatotoxicity.

Reference	Cell lines/Animal model	Dose of administration	duration of administration	Pathological characteristic
Yu et al. [11]	L02	0–300 μM	48h	AST, ALT and ALP abnormal; Decreased cell survival rate;
Cui et al. [12]	L02	0–120 μM	24, 48, 72h	Decreased cell survival rate; Apoptosis;
Jiang et al. [13]	PHH/Rat	0–120 μM/200 mg/kg	48h/4d	Decreased cell survival rate; AST abnormal; Inflammatory infiltration; Steatosis;
Wu et al. [14]	HepG2/Rat	0–100 μM/150 mg/kg	48h/28d	AST, ALT abnormal; Decreased cell survival rate; Inflammatory infiltration; Apoptosis;
Lin et al. [15]	L02	50 μM	24, 48h	Increased ROS; Mitochondrial damage; Apoptosis;
Wang et al. [16]	PMH/Rat/Mice	50μM/150 mg/kg/30, 90, 300 mg/kg	6h/7d/14d	Bile acid increased significantly
Hu et al. [17]	L02/HepaRG	0–60 μM	48h	Apoptosis; Increased ROS; Calcium ion overload;
Zhang et al. [18]	HepG2	0–70 μM	24h	AST, ALT abnormal; Increased ROS;
Chen et al. [19]	HepG2	0–200 μM	48h	Decreased cell survival rate; BUN increased;
Li et al. [20]	PRH	10 μM	24h	Apoptosis; Up-regulation on bile acids synthesis
Wang et al. [21]	L02/Rat/Mouse,	50μM/20,60,180 mg/kg /40,120,360 mg/kg	30d/30d	Apoptosis; Increased ROS; Calcium ion overload;
Dong et al. [22]	HepaRG	0–20 μM	8h	Increased ROS; Apoptosis; Cell cycle arrest
Lin et al. [23]	L02/Mouse	0–200μM/25,50 mg/kg	12,24,48h/31d	Decreased cell survival rate; Apoptosis; MAPK, signal pathway activated
Cui et al. [24]	HepG2/L02	0–80 μM	24h	Apoptosis; PI3K/AKT signal pathway inhibited
Ni et al. [25]	L02/Zebrafish	50/1.8 μM	48/72h	Increased ROS;
Wang et al. [26]	HepaRG	100–800 μM	0-7d	Block bile acid metabolism pathway;
Chen et al. [27]	Rat	1500 mg/kg	28d	Steatosis; Cholestasis;
Yang et al. [28]	Rat	150,500,1500 mg/kg	28d	Inflammatory infiltration; Steatosis;
Zhang et al. [29]	Rat	150 mg/kg	28d	Fatty acid β oxidation disorder; Oxidative stress

PHH: Primary human hepatocytes; ROS: Reactive oxygen species; PMH: Primary human hepatocytes; BUN: Blood urea nitrogen; PRH: Primary rat hepatocytes.

synthesis [15]. The mechanisms by which emodin induces apoptosis in hepatocytes by damaging mitochondria are illustrated in Fig. 2.

2.1.2. Emodin induces DNA damage

DNA damage refers to situations where the structure of DNA molecules is compromised or altered. DNA damage can potentially lead to cellular dysfunction and even cell death. Currently, there are numerous reports about emodin causing DNA damage to hepatocytes, but the underlying mechanisms remain unclear [35]. Research has found that emodin exhibits DNA-damaging properties by stabilizing the TopoII-DNA cleavage complex and inhibiting ATP hydrolysis [36]. Additionally, emodin can significantly upregulate various proteins related to DNA damage detection, DNA damage signal transduction, and DNA damage repair in L02 cells. Excessive reactive oxygen species (ROS) can also cause oxidative DNA damage, leading to cell cycle arrest or delay [37,38]. Similarly, emodin may induce DNA damage in hepatocytes by generating ROS, although this requires further experimental validation. Furthermore, although research has indicated that emodin induces DNA double-strand breaks in mammalian cells in a concentration-dependent manner, whether emodin-induced DNA damage in hepatocytes is correlated with its concentration and duration of exposure remains to be further investigated.

2.1.3. Emodin induces cell cycle arrest

Cell cycle arrest refers to the phenomenon where a cell halts progression to the next phase in its normal lifecycle. Numerous factors contribute to cell cycle arrest, including physiological factors and pathological factors (e.g., DNA damage and abnormal expression of cell cycle regulatory proteins). Fig. 3 depicts the classical regulatory model of the cell cycle: Mitogens initially stimulate the MAPK pathway, leading to the expression of Cyclin D (CycD) and the activation of Cyclin-dependent kinase 4/6 (CDK4/6). Subsequently, the

Fig. 2. Mechanistic model of emodin-induced apoptosis in hepatocytes. MMP: mitochondrial membrane potential; Cytc: cytochrome C; MPTP: mitochondrial permeability transition pore; PARP: poly (ADP-ribose) polymerase.

CDK4/6-CycD complex phosphorylates the retinoblastoma protein (Rb1), releasing E2F to promote the transcription of Cyclin E and Cyclin A (CycE, CycA), and activating CDK2. Then, activated CDK2 hyper-phosphorylates Rb1, establishing an efficient and sustained positive feedback loop to ensure the continuous expression of CycE, CycA, and other proteins crucial for the S phase. Upon completion of the S phase, Cyclin A and Cyclin B form complexes with CDK1, driving the progression into mitosis.

A recent study by Sabrina L. has shown that inhibition of CycD -Dependent Kinases 2 (CDK2) alone does not cause cell cycle arrest, as an enhanced CycD -Dependent Kinases 4/6 (CKD4/6) activity allows cells to rapidly overcome the detrimental effects of CDK2 inhibition and complete the cell cycle through a CDK2/4/6-Rb-E2F-dependent mechanism, only the simultaneous inhibition of CDK2

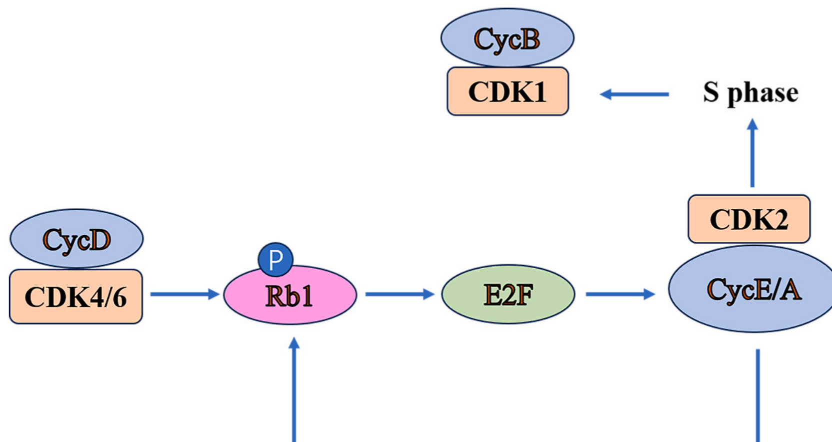

Fig. 3. Classic model of cell cycle regulation.

and CDK4/CDK6 can cause cell cycle arrest [39]. Emodin can induce cell cycle arrest at the S and G2/M phases in hepatocytes by damaging DNA and regulating the expression of cell cycle proteins [40]. The previous section has discussed emodin's DNA-damaging effects, so I won't delve into that further here. Studies have shown that emodin induces apoptosis in HepaRG cells while significantly upregulating the expression of Cyclin E and downregulating the expression of Cyclin A and CDK2 [22]. Similarly, emodin induces apoptosis in L02 cells while significantly downregulating the expression of CDK6 protein [15]. Additionally, Manoharan's research group found that emodin can inhibit the expression of CDK4 protein in cells. It is noteworthy that aloe-emodin, which shares a similar chemical structure with emodin, significantly inhibits the expression of CDK4/CDK6 in HepG2 cells [15,22]. Ni Jian et al. discovered that emodin can induce abnormal expression of various proteins in L02 cells that play crucial roles in cell cycle regulation, cell cycle arrest, cell cycle checkpoints, mitotic control, and cell cycle signal transduction [15]. Unfortunately, there is currently no research confirming the concentration-dependent induction of hepatocyte cycle arrest by emodin. However, it has been observed in chronic myelogenous leukemia cells (K562) that emodin increases the proportion of cells in the G0/G1 phase while reducing the proportion of cells in the S and G2/M phases, and this effect is dose-dependent [41].

Based on the evidence presented above, we have reason to believe that the simultaneous inhibition of CDK2, CDK4, and CDK6, along with the modulation of aberrant expression of related cell cycle proteins, may be one of the mechanisms by which emodin induces hepatocyte damage.

2.2. Emodin induces hepatocyte stress injury

2.2.1. Emodin induces oxidative stress

Oxidative stress refers to the phenomenon where the oxidation-reduction balance within cells or tissues is disrupted. The disturbance of the redox state can lead to a series of metabolic disorders, including oxidative stress and disruption of fatty acid oxidation. In recent years, the role of oxidative stress in emodin-induced hepatocyte damage has been confirmed by multiple research groups. For example, the increase in reactive oxygen species (ROS) upregulates the ratio of Bax to Bcl2 in cells, leading to cytochrome C release, which subsequently induces cell death through the mitochondrial apoptotic pathway [42–44]. Additionally, ROS can oxidize two redox-sensitive sites on the mitochondrial permeability transition pore (MPTP), leading to MPTP opening. Subsequently, changes in mitochondrial membrane permeability cause the release of cytochrome C from the mitochondrial matrix, activating related apoptotic factors, specifically inducing cell toxicity and apoptosis [15]. The antioxidant N-acetyl-L-cysteine inhibits cell apoptosis, further demonstrating the damaging effect of ROS on hepatocytes [31]. The nuclear factor erythroid-2 related factor (Nrf2) signaling pathway plays an important regulatory role in cell protection and the expression of antioxidant genes, and is considered a major regulatory mechanism for reducing oxidative stress. Under normal physiological conditions, Nrf2 binds to Kelch-like-ECH-related protein 1 (Keap1), which is a sensor protein against ROS. However, under oxidative stress, Nrf2 dissociates from Keap1 and translocate to the nucleus upon activation. In the nucleus, activated Nrf2 interacts with the muscle aponeurotic fibrosarcoma protein (Maf) and other co-activators, thereby activating genes related to the antioxidant response element (ARE), such as superoxide dismutase (SOD), glutathione S-transferase (GST), heme oxygenase-1 (HO-1), and NAD(P)H quinone dehydrogenase-1 (NQO-1). Studies have shown that high concentrations of emodin can inhibit the nuclear translocation of Nrf2, while low concentrations of emodin have the opposite effect [45–47]. Additionally, similar to acetaminophen, emodin can form complexes with glutathione, leading to glutathione depletion, which may be a key factor in emodin-induced hepatocyte damage [13]. In the latest study, Li et al. reported 1399 genes related to oxidative stress [48]. Building upon their research, we found that 50 μM emodin treatment of L02 cells for 48h resulted in differential expression of 73 proteins related to oxidative stress, further confirming that emodin can induce hepatocyte damage by disrupting the redox balance within hepatocytes.

2.2.2. Emodin induces endoplasmic reticulum stress

Endoplasmic reticulum stress in hepatocytes refers to a state where the endoplasmic reticulum (ER) functions abnormally or becomes overloaded, leading to disruption in protein folding and repair processes within the ER, thereby triggering a cellular stress response. This endoplasmic reticulum dysfunction triggers the activation of a series of cellular stress signaling pathways, including three major sensor proteins: endoplasmic reticulum membrane-associated tyrosine kinase/endoplasmic reticulum membrane-associated ribonucleic acid endonuclease 1 (IRE1), protein kinase RNA-like endonuclease activator (PKR-like endoplasmic reticulum kinase, PERK) and activating transcription factor (ATF). Activation of these sensor proteins triggers a series of responses, including activation of the anti-stress protein CHOP, oxidative stress, inflammation, and regulation of autophagic processes in an attempt to correct endoplasmic reticulum dysfunction and restore cellular homeostasis. However, if endoplasmic reticulum stress persists or is excessively severe, the cell may not be able to respond effectively, leading to cell death or the onset of disease.

Research suggests that the hepatotoxicity of emodin may be associated with the aryl hydrocarbon receptor (AhR) - cytochrome P450 1A1 (CYP1A1) - endoplasmic reticulum (ER) stress pathway. Further investigations have revealed that a toxic metabolite of emodin, 5-hydroxyemodin, can induce hepatocyte damage by activating the PERK-ATF4-CHOP signaling pathway [21]. In addition, Emodin can led to the upregulation of various proteins related to ER stress, such as transmembrane protein 214 (TMEM214) and BCL2 interacting protein 1 (BNIP1) [15]. Additionally, Dong et al. confirmed that a structurally similar compound to emodin, aloe emodin, can induce the upregulation of ER stress-related proteins (Caspase 12 and GRP78), subsequently inducing cell apoptosis [49]. Currently, there is limited research on the induction of ER stress in hepatocytes by emodin, and the mechanisms behind emodin-induced ER stress in hepatocytes require further in-depth investigation.

2.2.3. Emodin induces inflammatory stress

Hepatocellular inflammation stress refers to the cellular stress response triggered by stimuli on hepatocytes. As one of the vital organs of the immune system, the liver exhibits specific responsiveness to inflammation. When the liver is exposed to toxic substances, inflammatory reactions can lead to the production of various inflammatory mediators by hepatocytes. The release of these inflammatory mediators activates a series of cellular signaling pathways. The activation of these pathways induces a cascade of inflammatory responses, including the synthesis of inflammatory mediators, infiltration of inflammatory cells, as well as inflammation-related cellular apoptosis and necrosis.

As is well known, low-dose emodin possesses anti-inflammatory effects [50]. However, there are relatively few reports regarding the effects of high-dose emodin on inflammation. It has been shown that 250 μM emodin can induce activation of the NFκB signaling pathway and upregulation of the IL-6 in L02 cells [51]. In addition, aloe emodin can induce liver toxicity by activating the NFκB inflammatory pathway in zebrafish [52]. Further investigation is needed to understand the dose-response relationship between emodin and inflammatory stress in hepatocytes.

2.3. Emodin induces hepatocyte metabolic disorders

2.3.1. Emodin induces cholestasis

Hepatocellular cholestasis refers to the phenomenon where the normal flow of bile within hepatocytes is obstructed, resulting in the accumulation of bile within the liver. Bile secretion relies on the functionality of the transport systems in hepatocytes and bile duct cells' membranes, as well as the integrity of bile duct tree structure and function [53]. Bile accumulation may cause some degree of damage to hepatocytes, but the exact mechanism remains incompletely understood. Studies have shown that high concentrations of bile acids can activate death receptors, thereby inducing apoptosis in hepatocytes [54,55]. Subsequent research reports have indicated that bile acids under different concentration conditions can induce apoptosis or necrosis in cells [56]. Additionally, recent studies indicate that bile acids can induce inflammatory responses and oxidative stress by activating hepatocytes, thereby leading to liver damage [57,58].

The research conducted by Zhang et al. revealed that emodin inhibits the bile salt export pump (BSEP) potentially by disrupting the crosstalk between AMP-activated protein kinase (AMPK) and Farnesoid X receptor (FXR) in mouse liver, thus indicating a potential pro-cholestasis effect [16]. Multidrug-resistant protein 2 (MRP2), also known as ATP binding cassette subfamily C member 2 (ABCC2), is an ATP-dependent transporter protein that plays a significant role in the transport of various drugs, toxins, and endogenous substances across cell membranes [59]. Studies suggest that MRP2 is crucial for hepatobiliary excretion [60]. Similarly, multidrug-resistant protein 3 (MRP3), or ATP binding cassette subfamily C member 3 (ABCC3), is another ATP-dependent transporter protein that transports various bile salts and provides an alternative pathway for the efflux of bile acids in cholestatic hepatocytes [61]. In in vitro cell experiments, it was found that 50 μM emodin significantly increases the protein expression of MRP2 and MRP3 in HepG2 and L02 cells [14,15]. In in vivo experiments, Tang et al. discovered that continuous oral administration of 150 mg/kg emodin for 28 days induces an increase in both gene expression and protein levels of MRP2 in male rats' livers. On the other hand, Zhang's team found that continuous oral administration of 150 mg/kg emodin for 5 days leads to a decrease in both gene expression and protein levels of MRP2 in the livers of male mice [14,16]. The conflicting results may be attributed to differences in emodin administration duration and animal species.

2.3.2. Emodin induces fatty acid metabolism disorder

Fat acid metabolic disorder refers to abnormalities in processes such as synthesis, oxidation, and transportation of fatty acids, leading to the accumulation of fatty acids in cells. Lipid accumulation resulting from hepatocyte fat acid metabolic disorder can induce lipid peroxidation and ROS generation. Furthermore, lipid accumulation can also trigger endoplasmic reticulum stress and inflammatory responses, causing hepatocyte damage, and even apoptosis. Metabolites produced by abnormal lipid metabolism, such as excess free fatty acids and fatty acid metabolites generated in fatty acid metabolism, exert direct toxic effects on hepatocytes, potentially resulting in cell damage and death.

Currently, research on the induction of hepatocyte fatty acid metabolism disruption by emodin is not common. A few studies suggest that emodin, in combination with dexamethasone (a CYP3A inducer) or BSO (a glutathione depletion agent), can induce hepatic steatosis in rats [13]. Apart from co-administration, emodin alone can also induce hepatic steatosis in rats [14,62]. Additionally, Ni Jian et al.'s research indicates that treatment with 50 μM emodin for 48 h affects the expression of various proteins related to lipid synthesis, lipid transport, lipid storage, and lipid metabolism in L02 cells. This further demonstrates the role of emodin in hepatocyte fat acid metabolic disorder. However, the underlying mechanisms by which emodin induces hepatic steatosis require further investigation.

2.3.3. Emodin induces abnormal expression of hepatocyte metabolic enzymes

The liver is the primary organ for drug metabolism, and metabolic enzymes in hepatocytes play a crucial role in the process. Drug metabolism in the body typically involves two main phases: Phase I metabolism and Phase II metabolism. Phase I metabolism reactions are typically catalyzed by the cytochrome P450 enzyme system in the liver. Phase II reactions typically involve transferases in the liver, such as glucuronyl transferase, sulfotransferase, and methyltransferase. Therefore, abnormalities in the level or activity of hepatic metabolic enzymes may affect cellular metabolic function and trigger cellular damage.

Recent research indicates that the absolute bioavailability of emodin is only 3.2 %. Approximately 56 % of emodin is not absorbed and is primarily excreted in its original form in feces. The absorbed components are mainly metabolized into hydroxylated and

glucuronidated metabolites. Enzymes CYP1A2, CYP2E1, UGT1A1, UGT1A9, and UGT2B7 play crucial roles in emodin metabolism [62, 63]. Emodin-3-*O*-β-D-glucuronide is a major glucuronidated metabolite of emodin both in vivo and in vitro and is also a substrate of MRP2. Its toxicity is much lower than that of emodin [14,63]. The study found that emodin can downregulate the gene expression of UGT2B7 in cells, leading to increased exposure of emodin in the body [14]. 5-hydroxyemodin, a hydroxylated metabolite of emodin, has higher toxicity than emodin, and its toxicity mechanism may be related to the activation of the Aryl hydrocarbon receptor (AhR) [21]. The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor of the Per-Arnt-Sim (PAS) superfamily of environmental sensors, playing a significant role in cell proliferation, differentiation, and organ homeostasis. Direct activation of AhR can induce the production of CYP1A1 and CYP1A2, thereby increasing the concentration of 5-hydroxy emodin in the body, leading to hepatocyte damage. This suggests that emodin may act as an activator of CYP1A1 and CYP1A2. However, Tang Yun's research group found that emodin can inhibit CYP1A2 [53]. This contradictory conclusion might be attributed to emodin dosage or other factors, requiring further investigation. Furthermore, drugs that interact with emodin and exacerbate liver damage often affect the expression of hepatic metabolic enzymes, leading to abnormalities. For instance, Li et al. found that when hydroxychloroquine is used in combination with emodin, it further exacerbates emodin-induced liver damage by inhibiting UGTs and MRP2 [64,65]. Resveratrol, on the other hand, can increase the accumulation of emodin in hepatocytes by inhibiting CYP2C19 and CYP3A4, thus enhancing its hepatotoxicity. These studies suggest that emodin may induce hepatocyte damage by affecting the level or activity of hepatic metabolic enzymes, thereby influencing the metabolic function of hepatocytes.

2.4. Emodin induces autophagy in hepatocyte

Autophagy is a cellular process of self-degradation and recycling, aimed at degrading and clearing damaged or aging organelles, proteins, and other components within the cell, to maintain cellular homeostasis and biological balance. The regulation of hepatocyte autophagy involves multiple signaling pathways and key proteins, with the most important being the mammalian target of the rapamycin (mTOR) pathway and adenosine monophosphate-activated protein kinase (AMPK). The mTOR pathway acts as a negative regulator, being suppressed under low-energy conditions to promote autophagy initiation, while AMPK activation stimulates the autophagic process. Dysregulation of autophagy regulation can lead to either excessive activation or inhibition of autophagy, thereby significantly impacting the structure and function of the liver.

Previous studies have indicated that emodin induces autophagy in L02 cells by inhibiting the PI3K/AKT/mTOR signaling pathway [66]. Zhang et al. also confirmed in their study that emodin significantly activates AMPK protein [16]. Additionally, Ni et al. found that emodin markedly upregulates autophagy-related protein 7 (ATG7) [15]. Research on emodin's induction of cellular autophagy is limited, and more in-depth investigations are anticipated in the future.

3. Conclusion

As a monomeric component widely found in traditional Chinese medicinal herbs and classic herbal formulations, the potential hepatotoxic risk of emodin cannot be overlooked. This article comprehensively reviews the mechanisms through which emodin may induce hepatocyte damage, including apoptosis, cell cycle, stress, DNA damage, bile stasis, disruption of fatty acid metabolism, abnormal expression of metabolic enzymes, and autophagy. Current research mainly focuses on apoptosis, the cell cycle, and oxidative stress. In comparison, studies on DNA damage, bile stasis, disruption of fatty acid metabolism, ion channel imbalance, abnormal expression of metabolic enzymes, and autophagy are scarce. Additionally, the existing research primarily centers on cellular and organ tissue levels, definitive toxic targets and damage mechanisms have not yet been identified. Future research should delve deeper into the molecular mechanisms by which emodin triggers liver toxicity, providing concrete evidence for understanding toxicity-induced damage and guiding safe clinical drug usage.

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Data availability

Data will be made available on request.

CRediT authorship contribution statement

Yupeng Wang: Writing – original draft, Visualization, Supervision. **Mengchao Zhao:** Visualization, Supervision, Investigation. **Bo Li:** Writing – review & editing. **Xingchao Geng:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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