







# Role of oxidative stress and endoplasmic reticulum stress in drug-induced liver injury

Hanghang Wu<sup>1†</sup>, Xiyuan Bao<sup>1,2†</sup>, Alejandro H. Gutierrez<sup>1†</sup>, Yulia A. Nevzorova<sup>1,3,4</sup>, Francisco Javier Cubero<sup>1,3,4\*</sup>

<sup>1</sup>Department of Immunology, Ophthalmology and ENT, Complutense University School of Medicine, 28040 Madrid, Spain

<sup>2</sup>Department of General Surgery, No. 924 Hospital of Joint Logistics Support Force of PLA, Guilin 541002, Guangxi Zhuang Autonomous Region, China

<sup>3</sup>Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), 28029 Madrid, Spain

<sup>4</sup>Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), 28007 Madrid, Spain

<sup>†</sup>These authors share the first authorship.

\***Correspondence:** Francisco Javier Cubero, Department of Immunology, Ophthalmology and ENT, Complutense University School of Medicine, c/Dr. Severo Ochoa, 9, 28040 Madrid, Spain. [fcubero@ucm.es](mailto:fcubero@ucm.es)

**Academic Editor:** Wen-Xing Ding, University of Kansas Medical Center, USA

**Received:** February 11, 2023 **Accepted:** March 22, 2023 **Published:** June 28, 2023

**Cite this article:** Wu H, Bao X, Gutierrez AH, Nevzorova YA, Cubero FJ. Role of oxidative stress and endoplasmic reticulum stress in drug-induced liver injury. *Explor Dig Dis.* 2023;2:83–99. <https://doi.org/10.37349/edd.2023.00020>

## Abstract

The pathogenesis of drug-induced liver injury (DILI) is still in an early stage of research. However, investigators have shown that both oxidative stress and endoplasmic reticulum (ER) stress play a significant role in the pathological mechanism. However, there is little in-depth literature about these two mechanisms. In order to prevent and improve the clinical symptoms of DILI, it is particularly important to study its pathogenesis. In this review article, the role of ER and oxidative stress in DILI is thoroughly discussed.

## Keywords

Reactive nitrogen species, reactive oxygen species, endoplasmic reticulum stress, unfolded protein response, drug-induced liver injury

## Introduction

Drug-induced liver injury (DILI) plays an essential role both in the clinic and in drug development [1]. The incidence of DILI has continued to increase, and, it has become a major public health problem [2]. There are two types of toxic-related effects, intrinsic and idiosyncratic [3, 4]. Intrinsic DILI is caused by drugs or chemicals directly and its related hepatotoxicity is mostly predictable, reproducible, dose-dependent, and also can be different among individuals [5]. Acetaminophen (APAP) is one of the most widely used painkillers in the world. Although it is a safe drug, APAP is a dose-dependent hepatotoxin which is the most common cause of this type of DILI [6–8]. On the other hand, idiosyncratic DILI causes injury in an unpredictable way, not strictly dose-dependent. It is determined by the interaction of environmental and unique host characteristics, usually occurring in less than 1 of every 10,000 exposed individuals, and it is

© The Author(s) 2023. This is an Open Access article licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



associated with a long latency period (from a few days to several months) [9, 10]. Clinical observation has blurred the line to distinguish the different forms of hepatotoxicity. Therefore, unless stated otherwise, the term DILI is used for both intrinsic and idiosyncratic lesions in this review.

The pathogenesis of DILI is a complex process, which can develop from asymptomatic liver laboratory abnormalities to acute liver failure (ALF) and death [8]. Hepatotoxicity is a major cause of ALF in Western countries [11], which results in cell death and inflammation in liver. At present, it is speculated that the occurrence of DILI mainly involves immune mechanism and non-immune mechanism [12]. The immune mechanism involves allergic reactions caused by drug properties, dosage, and individual differences. Exposure of hepatocytes to drugs or their reactive metabolites may bring out a series of specific intracellular stress responses and adaptive mechanisms, which will activate the innate and adaptive immune systems. The innate and adaptive immune systems are involved in the process of cell death induced by idiosyncratic DILI, activating death receptor (DR) signaling [13]. The non-immune mechanism mainly involves the metabolic reaction of drugs: direct and indirect damage to hepatocytes. Toxins directly impact hepatocytes likely inducing oxidative organelle stress [such as endoplasmic reticulum (ER)] leading to cell death. Recent research showed that the crosstalk between drug-responsive metabolite-mediated intracellular stress responses and cytokine-mediated pro-apoptotic signaling are important components of DILI pathophysiology [1, 14, 15]. Tumor necrosis factor (TNF) seriously enhances liver injury caused by various exogenous substances [16–18]. Drug-induced ER stress and the oxidative stress response are sensitive to TNF-mediated hepatotoxicity [1].

## Metabolic mechanisms in pathophysiology

The liver is a highly dynamic metabolic organ that plays a key role in drug and xenobiotic metabolism and detoxification, protecting potential toxic chemicals against organisms [19, 20]. Under pathological conditions, the following three main mechanisms (oxidative stress, ER stress, and mitochondrial dysfunction) can affect drug metabolism of the liver, and drug metabolites further aggravate liver injury. Bioactivation mechanisms of drugs metabolites and the detoxification and excretion processes of xenobiotics are crucial for understanding the mechanism of DILI [8, 21].

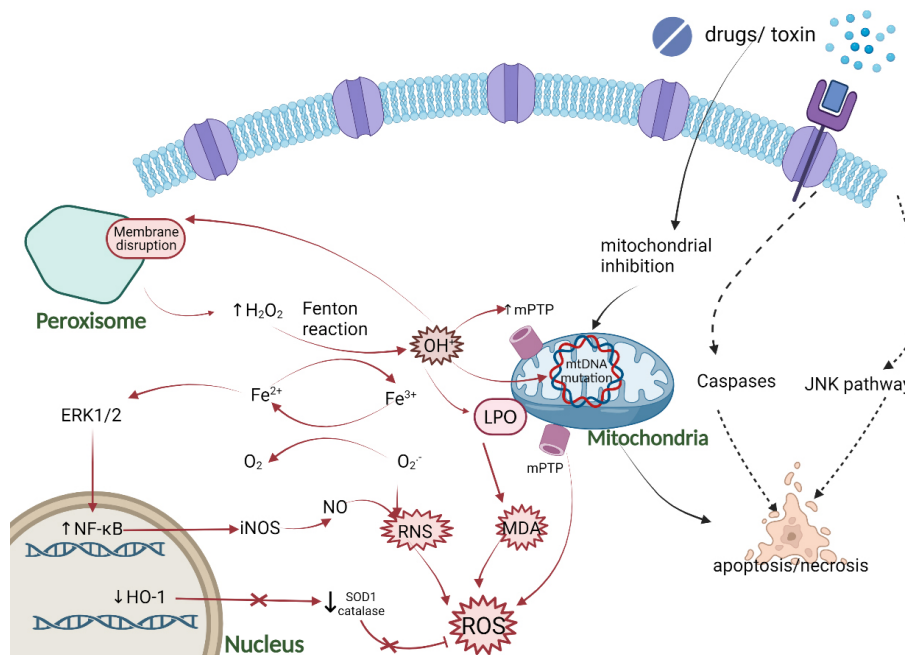
### Oxidative stress

The occurrence of oxidative stress is a sign of DILI, which can activate cell death related pathways [22, 23]. The harmful compounds produced by the oxidation reaction of the body possess strong oxidative properties, damage the tissues and cells of the body, and cause chronic diseases and aging. Oxygen free radicals involved in oxidation reaction mainly exist in organisms and are collectively called reactive oxygen species (ROSs) which are short-lived molecules containing unpaired electrons, highly reactive oxygen-containing molecules that induce DNA damage and activate the DNA damage response (DDR) [24, 25]. ROS is a by-product of normal metabolism and plays an important role in cell signal transduction and homeostasis. Some DILI-causing drugs increase ROS accumulation through a variety of mechanisms, ultimately inducing oxidative stress [26]. It is noteworthy that the increase in ROS generation directly induces ER stress, which activates ER-related apoptosis that is characterized by the release of ER calcium and the activation of caspase-12 [27]. In addition, other small molecules, reactive nitrogen species (RNSs), can also lead to oxidative stress. With the help of the enzymatic activity of the nitric oxide synthase (NOS), RNS mainly come from arginine. NOS produces nitric oxide free radical (NO<sup>\*</sup>) and then reacts with superoxide anion radical (O<sub>2</sub><sup>\*-</sup>) to produce peroxynitrite (ONOO<sup>-</sup>), which is a powerful oxidant and nitrifier [28, 29]. Furthermore, the mechanism of DILI cell death may involve lipid peroxidation (LPO) accumulation and ferroptosis.

### ROSs

The excessive accumulation of ROS is the main cause of oxidative stress, which can be caused by the DILI-causing drugs through different mechanisms [26]. Mitochondria are the main sites for ROS, which includes peroxides, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl groups, produced in the

cell (Figure 1) [30, 31]. Under physiological conditions, most of the electrons donated to mitochondrial outer membrane (MOM) migrate downward to cytochrome c oxidase, where they react with  $O_2$  and  $H^+$  to yield  $H_2O_2$ . However, some electrons may react directly with  $O_2$  to form  $O_2^{\bullet-}$ , and spontaneously disproportionate to  $O_2$  and  $H_2O_2$  with manganese superoxide dismutase (MnSOD) [32].  $H_2O_2$  is detoxified into  $H_2O$  via mitochondrial glutathione (GSH) [33] peroxidase and peroxiredoxins, or active myeloperoxidase reacts with iron ( $Fe^{2+}$ ) to become the highly reactive hydroxyl radical ( $OH^{\bullet}$ ) [31, 34–36]. ROS is not only a signal transduction molecule under pathological conditions, but also important for maintaining physiological balance, depending on the intensity and duration of oxidative stress. Low-intensity ROS participates in immune regulation and signal transduction, maintains energy level, kills bacteria and parasites, and eliminates toxins, and high-level ROS promotes cell apoptosis or autophagy which cause damage to normal cells and tissues [37, 38].



**Figure 1.** Oxidative stress mechanism caused by DILI. The main pro-oxidation mechanism is characterized by the production of  $OH^{\bullet}$  from  $H_2O_2$  and  $Fe^{2+}$  through the Fenton reaction. The main source of  $H_2O_2$  is the peroxisome of long-branched fatty acids  $\beta$ -oxidation.  $OH^{\bullet}$  can cause LPO of organelle membranes, damage mitochondrial metabolism through the production of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (mPTP) and mitochondrial DNA (mtDNA) mutation, increase the pro-apoptotic activity, and produce malondialdehyde (MDA). In addition, chronic  $Fe^{2+}$  overload may also activate nuclear factor-kappa B (NF- $\kappa$ B) enhancing the production of inducible nitric oxide synthase (iNOS), resulting in the increase of nitric oxide (NO), which leads to the reaction with the superoxide anion RNS. Figure was created with [BioRender.com](https://www.biorender.com). ERK1/2: extracellular signal-regulated kinase 1/2; HO-1: heme oxygenase-1; JNK: c-Jun N-terminal kinase; SOD1: superoxide dismutase 1

The liver is a crucial organ for the production of ROSs, which are either converted from endogenous products during liver metabolism or by intake (e.g., APAP). APAP is a non-steroidal anti-inflammatory drug (NSAID) with definite hepatotoxicity in clinical application [12]. After rapid absorption, APAP is mainly metabolized through phase II reaction (sulfation or glucuronidation), converting into non-toxic compounds and excreting into urine. At present, the major reason of hepatotoxicity caused by APAP is its metabolite *N*-acetyl-*p*-benzoquinoneimine (NAPQI). A small amount of APAP is metabolized by cytochrome P450 isoenzyme 2E1 (Cyp2E1) to produce intermediates, which produce NAPQI. NAPQI is directly excreted into bile by interaction with GSH to play a detoxification role. After overdose, APAP overwhelms phase II reaction pathway. After NAPQI depletes GSH, excessive NAPQI interferes with the complex I/II of mitochondrial electron transfer chain (ETC), resulting in the leakage of electrons from ETC to  $O_2$ , thus forming  $O_2^{\bullet-}$  [39, 40]. The interaction of NAPQI with target DNA and protein in mitochondria and the formation of protein adducts lead to oxidative stress, mitochondrial dysfunction [26, 41]. Furthermore, other drugs produce ROS through different mechanisms. Diethylaminoethoxyhexestrol, perhexiline,

amiodarone, and tamoxifen selectively accumulate and reach high concentrations in the mitochondrial matrix, thereby blocking the transfer of electrons along the MOM, increasing production of ROS [42–44].

As mentioned earlier, cytochrome P450 (Cyp450) plays a major role in the detoxification process of APAP. The latest literature also shows that the liver injury caused by polymedication in coronavirus disease 2019 (COVID-19) patients is also considered to be concentrated in the catalytic cycle of Cyp450 with its isoforms and the production of ROS in the oxidative stress environment [45]. In some cases of idiosyncratic DILI caused by drugs, anti-Cyp450 was detected in the blood of patients, representing an association between these drugs and Cyp450 in line with the proposed involvement of the adaptive immune system [46]. Oxidative stress in idiosyncratic DILI includes mitochondrial stress and microsomal stress, which may be related to ROS produced by Cyp450 in these subcellular domains of hepatocytes, but the specific ROS type involved has not been determined [46].

## RNS

Posttranslational modifications involving various active nitrogen species have a common precursor, NO that is the most well-known free radical acting as a signaling molecule. RNS includes nitrogen free radicals and non-free radicals, which are derived from NO and  $O_2^{\bullet-}$  by *iNOS* and nicotinamide adenine dinucleotide phosphate (NADPH), respectively (Figure 1) [47, 48]. Under steady-state, *NOS* expression is regulated by signal pathways including mitogen-activated protein kinase (MAPK) and JNK/signal transducer of activators of transcription (STAT), which indicates that the inducible production of NO is strictly controlled [49]. In the pathological state,  $O_2^{\bullet-}$  reacts with NO to increase ONOO<sup>-</sup> via an up-regulation of *NOS* and endothelial *NOS* [50]. Upon APAP overdose, excessive NAPQI can also induce the formation of OH<sup>•</sup> free radicals and react with NO to form ONOO<sup>-</sup> [51]. Since  $O_2^{\bullet-}$  hardly passes through the hepatocyte membrane, the production of ONOO<sup>-</sup> only occurs in mitochondria. The ONOO<sup>-</sup> can react with GSH to detoxify [52]. Consequently, GSH is exhausted due to these excessive reactions, resulting in the accumulation of ONOO<sup>-</sup> [53]. High reactivity and powerful oxidant ONOO<sup>-</sup> can cause nitration of protein tyrosine residues, resulting in mtDNA damage and membrane pore opening [53]. In addition ONOO<sup>-</sup> reacts directly with carbon dioxide producing nitrosoperoxy carbonate (ONOOOCO<sub>2</sub><sup>-</sup>), which decomposes into nitrogen dioxide (NO<sub>2</sub>) and carbonate (<sup>•</sup>CO<sub>3</sub><sup>-</sup>) with a reactivity similar to that of OH<sup>•</sup> radicals [54]. The production of <sup>•</sup>CO<sub>3</sub><sup>-</sup> and OH<sup>•</sup> radicals and the capacity of ONOO<sup>-</sup> to react with metal centers leads to hepatocyte toxicity [49].

## The JNK signaling pathway

The JNK is a subclass of the MAPK family, which was confirmed to play a vital role in APAP hepatotoxicity by mediating APAP-induced mitochondrial targeting and oxidative stress (Figure 1) [55, 56]. JNK has 10 subtypes from three genes, only *JNK1* and *JNK2* genes are widely expressed in the liver [57]. During JNK activation the first step occurs when the  $O_2^{\bullet-}$  production from mitochondrial respiratory complex III spills into the cytosol and triggers a signaling cascade [58]. Then, NAPQI and subsequent mitochondrial-derived ROS activates upstream kinases, including protein kinase C (PKC), MAPK, such as mixed lineage kinase 3 (MLK3) and apoptosis signal-regulated kinase 1 (ASK1), eventually leading to JNK activation. MLK3 is activated by oxidative stress and regulates the early stage of JNK activation; however, ASK1 controls the late stage of JNK activation induced by APAP [13, 59, 60]. The activated JNK [phospho-c-Jun N-terminal kinase (p-JNK)] combines with its target SH3 domain-binding protein (Sab) on the mitochondrial membrane, leading to a self-sustaining activation circuit of ROS production and JNK activation, which eventually leads to the opening of mitochondrial membrane permeability transition pore (MPT) [61]. After the toxic stress of APAP, JNK phosphorylates Sab, which causes Sab to release type 6 protein tyrosine phosphatase (PTPN6) [62, 63]. The released PTPN6 is activated and transferred to the intima, where it dephosphorylates and inactivates Src in the membrane gap. With the deactivation of Src, the ETC chain is blocked and the generation of ROS is enhanced [13]. Switching on MPT induces the collapse of mitochondrial membrane potential, thus ceasing ATP synthesis and releasing the membrane proteins which initiate necrosis [64].

A great quantity of experiments demonstrated that hepatocytes can be protected from cell death by knocking down, knocking out, or inhibiting JNK/Sab to interfere with this pathway at any point [59–61, 65]. The key point is that docking site of Sab or inhibiting the binding between JNK and Sab can protect against APAP toxicity, indicating that the interaction of these two proteins is the key step of cell death [13, 66]. In Sab knockout mice, the mitochondrial translocation of p-JNK was totally inhibited regardless of various isoforms of JNK [66, 67]. Therefore, in animal studies, fomepizole can attenuate APAP-induced liver injury by inhibiting JNK during post-metabolic treatment, and its interference with Cyp2E1 metabolism [68, 69]. The latest research found that platanoides may reduce oxidative stress and nitrification stress by preventing continuous JNK activation, which can effectively prevent APAP-related hepatotoxicity [23]. Collectively, this evidence demonstrated that JNK plays an important role in activating oxidative stress and triggering DILI, which also provides targeted targets for clinical treatment.

## LPO

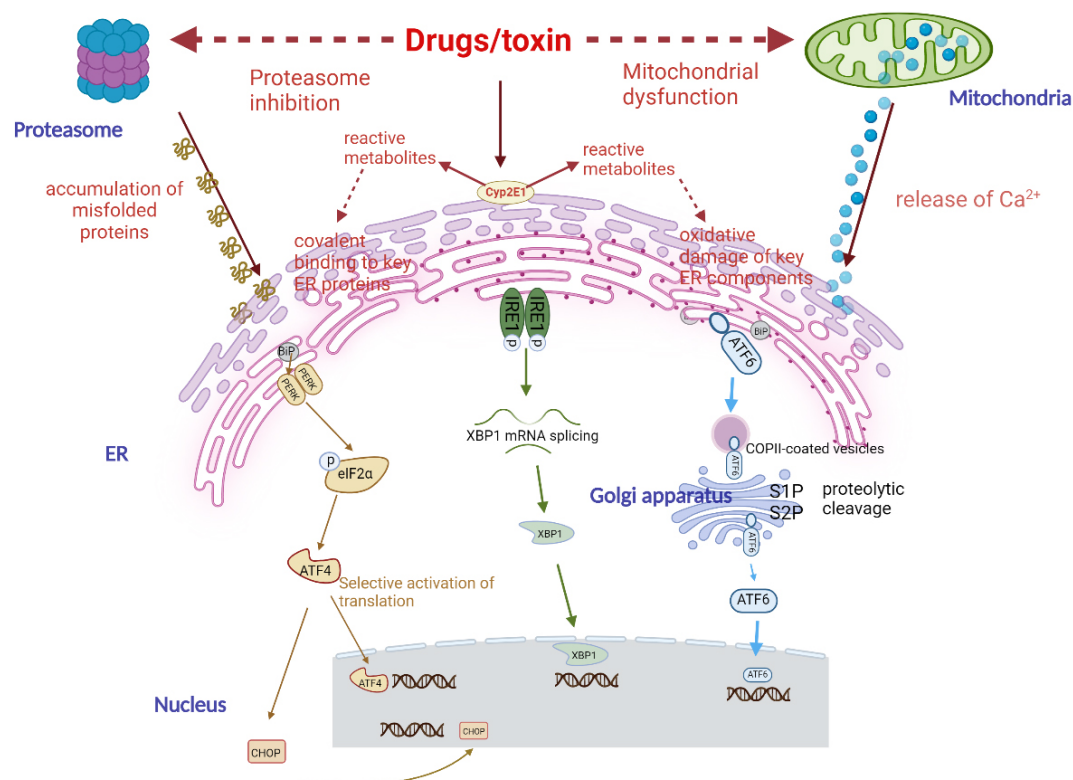
Lipid peroxide acts as a vital role in the balance of oxidative stress between pro-oxidation and antioxidant activities, LPO may be involved in the mechanism of cell death during DILI [8]. Lipids present hydrophobicity in cells and serve as metabolic fuel, supporting membrane structure and selective cell membrane transport. When the oxidative metabolism in mitochondria is abnormal, the electronic leakage increases the uncoupling of oxidative phosphorylation, resulting in the production of oxygen free radicals which may induce LPO reactions (Figure 1) [70]. LPO is a free radical reaction process, which is triggered by the formation of OH<sup>•</sup> radicals (Fe<sup>2+</sup>-dependent Fenton reaction) from H<sub>2</sub>O<sub>2</sub> combined with the production of lipid radicals, leading to the destruction of polyunsaturated fatty acids (PUFAs) [71]. LPO triggered subsequently rapid destruction of membrane potential and ionic gradient, and promoted cell necrosis. This is also one of the mechanisms of DILI caused by anti-epileptic drugs [12]. Mitochondrial oxidative metabolism is blocked by drugs, resulting in abnormal metabolism of free fatty acids (FFAs), increased anaerobic fermentation, lactic acid accumulation, and generation of ROS. ROS oxidizes liposomes and causes LPO, induces mtDNA damage by changing mitochondrial membrane permeability, further promoting liver injury [72]. Furthermore, experimental models of APAP-induced hepatotoxicity showed that vitamin E pre-treatment can prevent massive LPO induce to liver damage [73]. However, this is a matter of debate since there are conflicting results and it is still uncertain whether vitamin E protects against liver injury caused by APAP [74–76]. Research showed that animals fed a regular diet with sufficient fat-soluble antioxidants to prevent a robust increase of LPO, therefore LPO was not the relevant injury mechanism of APAP-induced liver injury in quantity, in addition, LPO was the cause of the injury rather than the outcome [73, 77–80].

Moreover, there is a controversy whether Fe<sup>2+</sup> as a promoter of LPO. Studies shown that protein nitration and LPO can lead to APAP hepatotoxicity after Fe<sup>2+</sup> overload [81]. PUFAs react with ROS to generate lipid hydroperoxides (LOOHs), which react with Fe<sup>2+</sup> via Fenton reaction to produce highly reactive lipid free radicals [80]. Lipid free radicals further react with other PUFAs propagating a chain reaction. If is an uncontrolled episode, it leads to the accumulation of lipid peroxides and induces Fe<sup>2+</sup>-mediated cell death [82]. In addition, Fe<sup>2+</sup> has also been proved to catalyze the nitrification process of ONOO<sup>-</sup> to proteins such as MnSOD, which is beneficial to oxidative stress and mitochondrial dysfunction by APAP-mediated cell death [83, 84]. Deferoxamine can inhibit ONOO<sup>-</sup>-mediated protein nitration, which demonstrates that Fe<sup>2+</sup>chelators have partial protective effect on APAP-induced liver injury [85]. As mentioned earlier, LPO was not sufficient in quantity to cause APAP-induced cell death. Therefore, under normal conditions, ferroptosis is not a cell death mode related to APAP hepatotoxicity [86], but may affect DILI through classical Fenton reaction and other mechanisms.

## ER stress

The ER is an essential component of endomembrane system of cells which provide a great many of functionality, such as regulating cellular calcium (Ca<sup>2+</sup>) concentration, involved in lipid synthesis and transportation, and thus accelerating protein folding (Figure 2) [87]. The tight coupling between the

subunits of new proteins in the ER lumen and the ER folding ability is necessary for effective protein folding in ER [88]. However, protein folding is prone to failure when various cellular stresses occur, leading to the induction of the unfolded protein response (UPR), which is initiated to restore cellular homeostasis after acute stress exposure [89]. In mammalian cells, UPR transmits survival signals through three primary pathways and those major proteins involved in this stress response; those pathways mentioned above include inositol-requiring enzyme 1 (IRE1), the protein kinase RNA (PKR)-like ER kinase (PERK), and the activating transcription factor 6 (ATF6) which remain inactive by attaching to binding immunoglobulin protein (BiP), also known as 78-kDa glucose-regulated protein (GRP78) conditions [90–93]. The activation modes of the three proteins are not identical. IRE1 and PERK are activated by autophosphorylation, while ATF6 is activated by intra-membrane cleavage, which releases its N-terminal fragment for transcription transactivation [94].



**Figure 2.** The potential mechanism of drug-induced ER stress. Drugs can induce ER stress through different mechanisms, including proteasome inhibition, mitochondrial dysfunction, and changes in key ER components. The latter mechanism is suspected to be related to drugs, which can be converted into one or several reactive metabolites and can covalently bind with ER protein and/or induce oxidative damage of ER components secondary to oxidative stress. Cyp2E1 is usually involved in the production of reactive metabolites. When ER stress occurs, cells will initiate an adaptive response called UPR. It first activates three effectors, namely PERK, IRE1, and ATF6, and then removes the companion BiP (GRP78) that keeps them in an inactive state. PERK is a phosphorylation and inactivation elongation initiation factor  $\alpha$ -subunit of eukaryotic initiation factor 2 (eIF2 $\alpha$ ) kinases, resulting in a general reduction in protein translation. However, eIF2 $\alpha$ , it selectively stimulates the translation of ATF4, a transcription factor with a specific structure (uORF) on its messenger RNA (mRNA). Then ATF4 activates chaperone proteins and protein synthesis involved in autophagy, protein secretion, and amino acid metabolism. IRE1 has kinase activity that leads to its own phosphorylation and activation of ribonuclease (RNase) activity. This leads to the splicing of X-box binding protein 1 (XBP1) mRNA, which is then translated into an active transcription factor. The transcription factor ATF6, which is a non-active precursor and binds to ER membrane, is transferred to Golgi apparatus through the coat protein complex II (COPII) vesicles, where it is cut into active forms by site-1 protease (S1P) and S2P proteases. Then XBP1 and ATF6 will activate the transcription of a group of factors in the nucleus to restore ER homeostasis, including chaperones, folding enzymes, and proteins involved in the degradation of unfolded peptides [ER-associated degradation (ERAD)]. If these mechanisms cannot effectively restore ER and cell homeostasis, UPR will eventually activate the mechanism leading to apoptosis, especially through transcription factor CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP)

### IRE1 signaling

As an essential ER stress sensor for UPR in animals and plants, IRE1 detects ER homeostasis and triggers UPR through cytoplasmic kinase domain and RNase domain under the stimulation [95–98]. Under ER stress, IRE1 RNase is activated by conformational changes, self-phosphorylation, and higher

oligomerization (Figure 2) [96, 99, 100]. The primary function of IRE1-dependent spliced X-box binding protein 1 (sXBP1) signal transduction is to protect the liver from the stress caused by stimulation [101]. Upon activation of IRE1, multiple downstream signaling of UPR is initiated through unconventional splicing of the transcription factor XBP1 or post-transcriptional modification through controlled inositol-requiring enzyme 1 dependent decay (RIDD) of multiple substrates [97–99]. Splicing occurs through XBP1 mRNA to the translation of sXBP1 protein, which is a transcription factor that induces genes involved in chaperoning proteins and degrades misfolded proteins in ER, thus triggering inflammatory reaction and promoting cell apoptosis mediated by apoptosis ASK1 and JNK [99, 102].

With the main cause of DILI [7, 99, 103]—APAP-mediated hepatotoxicity—a great deal of data indicated a role for ER stress. The mechanism of APAP hepatotoxicity is similar in humans and mice [104]. The sXBP1 or unspliced X-box binding protein 1 (uXBP1) in human HepaRG cells was consistent with the findings in mice [105]. Under ER stress, the IRE1 $\alpha$ -XBP1 arm of the UPR response plays a critical role in APAP-induced liver injury via upregulating the activity of Cyp450 [106]. In XBP1-deleted mice, overexpression of IRE1 in liver led to reduced JNK activation, and protected against APAP via the inhibition of Cyp450 activity [105–107]. This was linked to the recovery of GSH, which prevented severe oxidative stress [105]. In recent years, increasing studies demonstrated that autophagy is activated to respond to APAP-induced liver injury [108].

Furthermore, IRE1 $\alpha$  can also function via the RIDD pathway. IRE1 $\alpha$  accelerates the degradation of mRNAs encoding mostly ER-targeted proteins, resulting in a decrease of protein entering the ER membrane during ER stress [109, 110]. Meanwhile, XBP1-deficient mice showed decreased damage caused by APAP due to enhancement of autophagy and activating RIDD [105, 106].

Once cell stress reaches its critical threshold, over-activation of the JNK signaling pathway and mitochondrial dysfunction are considered as major cellular events in APAP-induced liver injury [111, 112]. p-JNK translocates to the MOM and finally induces mitochondrial dysfunction which contributes to hepatocyte necrosis [80]. Mitochondrial injury is likely to positively regulate the sustained activation of JNK cell death pathways and the onset of ER stress, which may be an intertwined mechanism [113]. After APAP treatment, the intense JNK phosphorylation and upregulated JNK1 expression in JNK-deleted liver correlated with a sharp increase in serum transaminases [22]. Gunawan's study [114] showed that disruption of JNK2 instead of JNK1 can suppress liver injury, indicating the importance of JNK2 in hepatotoxicity caused by APAP. Another paper indicated that extensive necrosis occurred in APAP-treated JNK hepatocyte-specific knockout mice, and further demonstrating that combined activity of JNK1 and JNK2 can prevent APAP-induced necrosis by controlling the generation of oxidative stress [114].

### PERK signaling

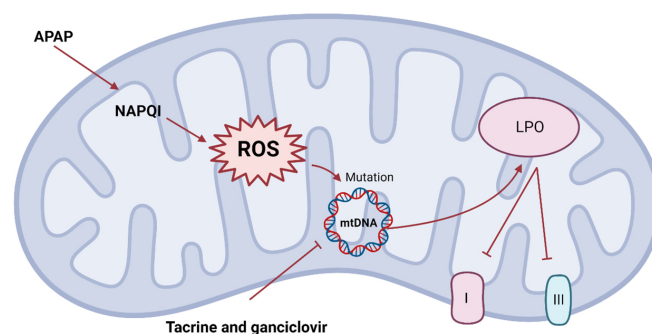
PERK is a member of the eIF2 $\alpha$  kinase family, which protects cells involved in various stimuli by biasing general protein synthesis, but initiating stress-related protein production [115]. In ER stress, after BiP/GRP78 dissociation, increasing expression of phosphorylated eIF2 $\alpha$ /PERK signaling is activated by APAP hepatotoxicity (Figure 2) [94]. PERK temporarily inhibits the translation of general protein via eIF2 $\alpha$  phosphorylation. Notably, phosphorylated eIF2 $\alpha$  selectively enhanced mRNA translation including ATF4 transcription factor to mediate UPR [116–119]. During the ER stress regulation, ATF4 activates *UPR* target gene, which encodes proteins necessary for antioxidant reaction and amino acid synthesis and transport [120]. ATF4 is also a transcriptional activation CHOP, which acts as a transcriptional inhibitor downstream of PERK and IRE1. The main function of CHOP is to induce apoptosis by various kinds of mechanisms in ER stress [117], thereby mediating cell apoptosis via mitochondrial pathway or DR pathway that further exacerbate hepatotoxicity [1, 121]. Conversely, decreased expression of CHOP, BiP/GRP78, and JNK are related to protect against APAP-induced hepatotoxicity which are possibly mediated by increased proliferation [94, 105].

## ATF signaling

Similar to IRE1 and PERK, ATF6 contains an ER luminal stress-sensing domain and a cytoplasmic enzymatic domain, which is one of ER transmembrane proteins. In response to a sublethal dose of APAP-mediated ER stress, the transcription factor ATF6 is activated (Figure 2) [122]. ATF6 dissociates from BiP/GRP78 to Golgi apparatus, where it is hydrolyzed into the nuclear form of ATF6 [123]. The cleavage transcription factor domain of ATF6 regulates UPR in the nucleus, including CHOP, XBP1, and protein chaperones such as BiP/GRP78 [120, 124, 125]. Additionally, ATF6 forms a heterodimer with IRE1-XBP1 pathway to activate the genes involved in ERAD [126].

## Mitochondrial dysfunction

Mitochondria can provide energy for cells and are also the place where many important metabolic processes take place [127, 128]. In addition, mitochondria are the main source of intracellular ROS and the regulatory center of cell apoptosis (Figure 3) [129]. When mitochondria are seriously stimulated, it can induce mitochondrial structure and function abnormalities, which display in the following aspects: morphological and structural changes, abnormal energy metabolism, ROS elevates, mtDNA damage, and abnormal mitophagy [32, 43, 44, 130–134]. Recent studies demonstrated the mechanism of DILI is related to the mitochondria damage of hepatocyte, including antipyretics, NSAIDs, antibiotics, immunosuppressive drugs, and antivirals, and others over 300 drugs [26, 135–138]. The metabolic process of APAP *in vivo* includes the conversion of APAP into the active metabolite NAPQI under the action of Cyp450 [80]. Normally, NAPQI complex can be cleared by GSH, but excessive APAP can lead to excessive production of NAPQI complex, thus depleting GSH and damaging mitochondria [139]. In addition, overdose APAP induces mitochondrial oxidative stress, which results in mitochondrial dysfunction and cell death (Figure 3) [140]. Due to mitochondrial dysfunction induced by APAP hepatotoxicity, the removal of damaged mitochondria through mitochondrial autophagy is a vital mechanism of APAP-induced ALF [141].



**Figure 3.** Mitochondrial dysfunction mechanism. Mitochondria stimulation can trigger changes in mitochondrial structure and function abnormalities. Drugs such as APAP or tacrine and ganciclovir can cause mitochondrial cell death, via the activation of ROS which may cause a damage to mtDNA, resulting in LPO and inhibition of ETC complexes I and III

In addition, other drugs have different mechanisms for mitochondrial dysfunction. Tacrine and ganciclovir alter mtDNA homeostasis from different mechanisms, including inhibition of mtDNA replication and translation, to induce mitochondrial damage [132, 142]. Mitochondrial dysfunction caused by the significant increase of ROS, resulting in LPO and inhibition of respiratory chain (complexes I and III) triggering apoptosis, which may explain the hepatotoxicity caused by statins (Figure 3) [143]. The mechanism of liver injury caused by most drugs has not been clearly studied, and the degree of drugs-induced damage to human varies greatly according to individual differences and drug dosage.

## Conclusion and future perspectives

DILI is a sustained area of immense clinical and research interest, especially the pathogenesis of DILI is still not completely determined, and the occurrence of DILI is the result of multiple factors. Oxidative stress, ER stress, and mitochondrial injury are the main etiological factors for DILI initiation and the



pathophysiological mechanism is complementary and participates in the initiation of immune response. The drug properties, host factors, and environmental conditions determine the susceptibility, phenotypic expression, and results of DILI. Especially with the rapid development of new drug research and the previously unidentified new mechanism of DILI, it is urgent to study the mechanism of DILI and improve the diagnostic efficiency. Continuous research on specific biological diagnostic markers and DILI development mechanisms will contribute to the detection of drug hepatotoxicity and side effects, but there is still a long way to reduce the incidence rate of DILI in clinical practice.

## Abbreviations

ALF: acute liver failure

APAP: acetaminophen

ASK1: apoptosis signal-regulated kinase 1

ATF6: activating transcription factor 6

BiP: binding immunoglobulin protein

CHOP: CCAAT/enhancer-binding protein homologous protein

Cyp2E1: cytochrome P450 isoenzyme 2E1

Cyp450: cytochrome P450

DILI: drug-induced liver injury

eIF2 $\alpha$ :  $\alpha$ -subunit of eukaryotic initiation factor 2

ER: endoplasmic reticulum

ETC: electron transfer chain

GRP78: 78-kDa glucose-regulated protein

GSH: glutathione

H<sub>2</sub>O<sub>2</sub>: hydrogen peroxide

iNOS: inducible nitric oxide synthase

IRE1: inositol-requiring enzyme 1

JNK: c-Jun N-terminal kinase

LPO: lipid peroxidation

MAPK: mitogen-activated protein kinase

MOM: mitochondrial outer membrane

mRNA: messenger RNA

mtDNA: mitochondrial DNA

NAPQI: *N*-acetyl-*p*-benzoquinoneimine

NO: nitric oxide

NOS: nitric oxide synthase

O<sub>2</sub><sup>-•</sup>: superoxide anion radical

OH<sup>•</sup>: hydroxyl radical

ONOO<sup>-</sup>: peroxynitrite

PERK: protein kinase RNA-like endoplasmic reticulum kinase

p-JNK: phospho-c-Jun N-terminal kinase  
PUFAs: polyunsaturated fatty acids  
RIDD: inositol-requiring enzyme 1 dependent decay  
RNase: ribonuclease  
RNSs: reactive nitrogen species  
ROSs: reactive oxygen species  
Sab: SH3 domain-binding protein  
sXBP1: spliced X-box binding protein 1  
UPR: unfolded protein response  
XBP1: X-box binding protein 1

## Declarations

### Author contributions

HW and XB equally contributed to Conceptualization, Investigation, and Writing—original draft. AHG: Validation, Resources, Investigation, and Formal analysis. YAN: Writing—original draft and Writing—review & editing. FJC: Supervision, Writing—original draft, and Writing—review & editing.

### Conflicts of interest

The authors declare that they have no conflicts of interest.

### Ethical approval

Not applicable.

### Consent to participate

Not applicable.

### Consent to publication

Not applicable.

### Availability of data and materials

Not applicable.

### Funding

This work was supported by the Ministerio de Ciencia e Innovación (MICINN) Proyectos de Generación de Conocimiento [PID2020-11782RB-I00, PID2020-117941RB-I00], all of which were co-funded with FEDER funds and Chief Office of Science and Technology (COST) Action [CA17112]. This project has received funding from the Horizon European's research and innovation programme HORIZON-HLTH-2022-STAYHLTH-02 under agreement [101095679]. The research group belongs to the validated Research Groups Ref. 970935 Liver Pathophysiology, 920631 Lymphocyte Immunobiology and IBL-6 (imas12-associated). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

### Copyright

© The Author(s) 2023.

## References

1. Fredriksson L, Wink S, Herpers B, Benedetti G, Hadi M, de Bont H, et al. Drug-induced endoplasmic reticulum and oxidative stress responses independently sensitize toward TNF $\alpha$ -mediated hepatotoxicity. *Toxicol Sci*. 2014;140:144–59.
2. Yu YC, Mao YM, Chen CW, Chen JJ, Chen J, Cong WM, et al.; Drug-induced Liver Injury (DILI) Study Group; Chinese Society of Hepatology (CSH); Chinese Medical Association (CMA). CSH guidelines for the diagnosis and treatment of drug-induced liver injury. *Hepatol Int*. 2017;11:221–41.
3. Roth RA, Ganey PE. Intrinsic *versus* idiosyncratic drug-induced hepatotoxicity—two villains or one? *J Pharmacol Exp Ther*. 2010;332:692–7.
4. Francis P, Navarro VJ. Drug-induced hepatotoxicity. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK557535/>
5. Garcia-Cortes M, Robles-Diaz M, Stephens C, Ortega-Alonso A, Lucena MI, Andrade RJ. Drug induced liver injury: an update. *Arch Toxicol*. 2020;94:3381–407.
6. Bernal W, Hyyrylainen A, Gera A, Audimoolam VK, McPhail MJW, Auzinger G, et al. Lessons from look-back in acute liver failure? A single centre experience of 3,300 patients. *J Hepatol*. 2013;59:74–80.
7. Reuben A, Tillman H, Fontana RJ, Davern T, McGuire B, Stravitz RT, et al. Outcomes in adults with acute liver failure between 1998 and 2013: an observational cohort study. *Ann Intern Med*. 2016;164:724–32.
8. Villanueva-Paz M, Morán L, López-Alcántara N, Freixo C, Andrade RJ, Lucena MI, et al. Oxidative stress in drug-induced liver injury (DILI): from mechanisms to biomarkers for use in clinical practice. *Antioxidants (Basel)*. 2021;10:390.
9. Kaplowitz N. Idiosyncratic drug hepatotoxicity. *Nat Rev Drug Discov*. 2005;4:489–99.
10. Andrade RJ, Chalasani N, Björnsson ES, Suzuki A, Kullak-Ublick GA, Watkins PB, et al. Drug-induced liver injury. *Nat Rev Dis Primers*. 2019;5:58.
11. Hillman L, Gottfried M, Whitsett M, Rakela J, Schilsky M, Lee WM, et al. Clinical features and outcomes of complementary and alternative medicine induced acute liver failure and injury. *Am J Gastroenterol*. 2016;111:958–65. Erratum in: *Am J Gastroenterol*. 2016;111:1504.
12. Zhou Y, Wang J, Zhang D, Liu J, Wu Q, Chen J, et al. Mechanism of drug-induced liver injury and hepatoprotective effects of natural drugs. *Chin Med*. 2021;16:135.
13. Iorga A, Dara L, Kaplowitz N. Drug-induced liver injury: cascade of events leading to cell death, apoptosis or necrosis. *Int J Mol Sci*. 2017;18:1018.
14. Roth RA, Ganey PE. Animal models of idiosyncratic drug-induced liver injury—current status. *Crit Rev Toxicol*. 2011;41:723–39.
15. Fredriksson L, Herpers B, Benedetti G, Matadin Q, Puigvert JC, de Bont H, et al. Diclofenac inhibits tumor necrosis factor- $\alpha$ -induced nuclear factor- $\kappa$ B activation causing synergistic hepatocyte apoptosis. *Hepatology*. 2011;53:2027–41.
16. Bradham CA, Plümpe J, Manns MP, Brenner DA, Trautwein C. Mechanisms of hepatic toxicity. I. TNF-induced liver injury. *Am J Physiol*. 1998;275:G387–92.
17. Tiegs G, Horst AK. TNF in the liver: targeting a central player in inflammation. *Semin Immunopathol*. 2022;44:445–59.
18. Grivennikov SI, Karin M. Inflammatory cytokines in cancer: tumour necrosis factor and interleukin 6 take the stage. *Ann Rheum Dis*. 2011;70:i104–8.
19. Qian H, Chao X, Williams J, Fulte S, Li T, Yang L, et al. Autophagy in liver diseases: a review. *Mol Aspects Med*. 2021;82:100973.
20. Almazroo OA, Miah MK, Venkataramanan R. Drug metabolism in the liver. *Clin Liver Dis*. 2017;21:1–20.

21. Andrade RJ, Robles M, Ulzurrún E, Lucena MI. Drug-induced liver injury: insights from genetic studies. *Pharmacogenomics*. 2009;10:1467–87.
22. Cubero FJ, Zoubek ME, Hu W, Peng J, Zhao G, Nevzorova YA, et al. Combined activities of JNK1 and JNK2 in hepatocytes protect against toxic liver injury. *Gastroenterology*. 2016;150:968–81.
23. Samuvel DJ, Nguyen NT, Jaeschke H, Lemasters JJ, Wang X, Choo YM, et al. Platanosides, a potential botanical drug combination, decrease liver injury caused by acetaminophen overdose in mice. *J Nat Prod*. 2022;85:1779–88.
24. Dong L, He J, Luo L, Wang K. Targeting the interplay of autophagy and ROS for cancer therapy: an updated overview on phytochemicals. *Pharmaceuticals (Basel)*. 2023;16:92.
25. Srinivas US, Tan BWQ, Vellayappan BA, Jeyasekharan AD. ROS and the DNA damage response in cancer. *Redox Biol*. 2019;25:101084.
26. Ye H, Nelson LJ, Gómez Del Moral M, Martínez-Naves E, Cubero FJ. Dissecting the molecular pathophysiology of drug-induced liver injury. *World J Gastroenterol*. 2018;24:1373–85.
27. Stoner MW, McTiernan CF, Scott I, Manning JR. Calreticulin expression in human cardiac myocytes induces ER stress-associated apoptosis. *Physiol Rep*. 2020;8:e14400.
28. Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *Eur Heart J*. 2012;33:829–37.
29. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: harms and benefits for human health. *Oxid Med Cell Longev*. 2017;2017:8416763.
30. Tell G, Vascotto C, Tiribelli C. Alterations in the redox state and liver damage: hints from the EASL Basic School of Hepatology. *J Hepatol*. 2013;58:365–74.
31. Kotiadis VN, Duchon MR, Osellame LD. Mitochondrial quality control and communications with the nucleus are important in maintaining mitochondrial function and cell health. *Biochim Biophys Acta*. 2014;1840:1254–65.
32. Mansouri A, Gattolliat CH, Asselah T. Mitochondrial dysfunction and signaling in chronic liver diseases. *Gastroenterology*. 2018;155:629–47.
33. Xiangsheng T, Long G, Yingying S, Xiao A, Ping Y, Mingsheng T. Personality traits predict regression of pelvic girdle pain after pregnancy: a longitudinal follow-up study. *BMC Pregnancy Childbirth*. 2021;21:353.
34. Cox AG, Winterbourn CC, Hampton MB. Mitochondrial peroxiredoxin involvement in antioxidant defence and redox signalling. *Biochem J*. 2010;425:313–25.
35. Larosche I, Choumar A, Fromenty B, Lettéron P, Abbey-Toby A, Van Remmen H, et al. Prolonged ethanol administration depletes mitochondrial DNA in MnSOD-overexpressing transgenic mice, but not in their wild type littermates. *Toxicol Appl Pharmacol*. 2009;234:326–38.
36. Nahon P, Sutton A, Rufat P, Charnaux N, Mansouri A, Moreau R, et al. A variant in myeloperoxidase promoter hastens the emergence of hepatocellular carcinoma in patients with HCV-related cirrhosis. *J Hepatol*. 2012;56:426–32.
37. Finkel T. Signal transduction by mitochondrial oxidants. *J Biol Chem*. 2012;287:4434–40.
38. Win S, Than TA, Zhang J, Oo C, Min RWM, Kaplowitz N. New insights into the role and mechanism of c-Jun-N-terminal kinase signaling in the pathobiology of liver diseases. *Hepatology*. 2018;67:2013–24.
39. Du K, Ramachandran A, Weemhoff JL, Chavan H, Xie Y, Krishnamurthy P, et al. Editor's highlight: metformin protects against acetaminophen hepatotoxicity by attenuation of mitochondrial oxidant stress and dysfunction. *Toxicol Sci*. 2016;154:214–26.
40. Lee KK, Imaizumi N, Chamberland SR, Alder NN, Boelsterli UA. Targeting mitochondria with methylene blue protects mice against acetaminophen-induced liver injury. *Hepatology*. 2014;61:326–36.

41. Jaeschke H, Williams CD, Ramachandran A, Bajt ML. Acetaminophen hepatotoxicity and repair: the role of sterile inflammation and innate immunity. *Liver Int.* 2012;32:8–20.
42. Begriche K, Massart J, Robin MA, Borgne-Sanchez A, Fromenty B. Drug-induced toxicity on mitochondria and lipid metabolism: mechanistic diversity and deleterious consequences for the liver. *J Hepatol.* 2011;54:773–94.
43. Pessayre D, Fromenty B, Berson A, Robin MA, Lettéron P, Moreau R, et al. Central role of mitochondria in drug-induced liver injury. *Drug Metab Rev.* 2012;44:34–87.
44. Larosche I, Lettéron P, Fromenty B, Vadrot N, Abbey-Toby A, Feldmann G, et al. Tamoxifen inhibits topoisomerases, depletes mitochondrial DNA, and triggers steatosis in mouse liver. *J Pharmacol Exp Ther.* 2007;321:526–35.
45. Teschke R, Méndez-Sánchez N, Eickhoff A. Liver injury in COVID-19 patients with drugs as causatives: a systematic review of 996 DILI cases published 2020/2021 based on RUCAM as causality assessment method. *Int J Mol Sci.* 2022;23:4828.
46. Teschke R, Uetrecht J. Mechanism of idiosyncratic drug induced liver injury (DILI): unresolved basic issues. *Ann Transl Med.* 2021;9:730.
47. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol.* 2004;55:373–99.
48. Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, et al. The role of oxidative stress and antioxidants in liver diseases. *Int J Mol Sci.* 2015;16:26087–124.
49. Adams L, Franco MC, Estevez AG. Reactive nitrogen species in cellular signaling. *Exp Biol Med (Maywood).* 2015;240:711–7.
50. Mohabati Mobarez A, Baseri N, Khalili M, Mostafavi E, Stenos J, Esmaeili S. Genetic diversity of *Coxiella burnetii* in Iran by multi-spacer sequence typing. *Pathogens.* 2022;11:1175.
51. Jaeschke H, McGill MR, Ramachandran A. Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. *Drug Metab Rev.* 2012;44:88–106.
52. Knight TR, Ho YS, Farhood A, Jaeschke H. Peroxynitrite is a critical mediator of acetaminophen hepatotoxicity in murine livers: protection by glutathione. *J Pharmacol Exp Ther.* 2002;303:468–75.
53. Cover C, Mansouri A, Knight TR, Bajt ML, Lemasters JJ, Pessayre D, et al. Peroxynitrite-induced mitochondrial and endonuclease-mediated nuclear DNA damage in acetaminophen hepatotoxicity. *J Pharmacol Exp Ther.* 2005;315:879–87.
54. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev.* 2007;87:315–424.
55. Zhao Y, Kuca K, Wu W, Wang X, Nepovimova E, Musilek K, et al. Hypothesis: JNK signaling is a therapeutic target of neurodegenerative diseases. *Alzheimers Dement.* 2022;18:152–8.
56. Seki E, Brenner DA, Karin M. A liver full of JNK: signaling in regulation of cell function and disease pathogenesis, and clinical approaches. *Gastroenterology.* 2012;143:307–20.
57. Johnson GL, Nakamura K. The c-jun kinase/stress-activated pathway: regulation, function and role in human disease. *Biochim Biophys Acta.* 2007;1773:1341–8.
58. Nguyen NT, Du K, Akakpo JY, Umbaugh DS, Jaeschke H, Ramachandran A. Mitochondrial protein adduct and superoxide generation are prerequisites for early activation of c-jun N-terminal kinase within the cytosol after an acetaminophen overdose in mice. *Toxicol Lett.* 2021;338:21–31.
59. Sharma M, Gadang V, Jaeschke A. Critical role for mixed-lineage kinase 3 in acetaminophen-induced hepatotoxicity. *Mol Pharmacol.* 2012;82:1001–7.
60. Jaeschke H, Williams CD, Farhood A. No evidence for caspase-dependent apoptosis in acetaminophen hepatotoxicity. *Hepatology.* 2011;53:718–9.
61. Kaplowitz N, Win S, Than TA, Liu ZX, Dara L. Targeting signal transduction pathways which regulate necrosis in acetaminophen hepatotoxicity. *J Hepatol.* 2015;63:5–7.

62. Huo Y, Win S, Than TA, Yin S, Ye M, Hu H, et al. Antcin H protects against acute liver injury through disruption of the interaction of c-Jun-N-terminal kinase with mitochondria. *Antioxid Redox Signal*. 2017;26:207–20.
63. Win S, Than TA, Kaplowitz N. The regulation of JNK signaling pathways in cell death through the interplay with mitochondrial SAB and upstream post-translational effects. *Int J Mol Sci*. 2018;19:3657.
64. Szkolnicka D, Lucendo-Villarin B, Moore JK, Simpson KJ, Forbes SJ, Hay DC. Reducing hepatocyte injury and necrosis in response to paracetamol using noncoding RNAs. *Stem Cells Transl Med*. 2016;5:764–72.
65. Win S, Than TA, Fernandez-Checa JC, Kaplowitz N. JNK interaction with Sab mediates ER stress induced inhibition of mitochondrial respiration and cell death. *Cell Death Dis*. 2014;5:e989.
66. Win S, Than TA, Min RW, Aghajan M, Kaplowitz N. c-Jun N-terminal kinase mediates mouse liver injury through a novel Sab (SH3BP5)-dependent pathway leading to inactivation of intramitochondrial Src. *Hepatology*. 2016;63:1987–2003.
67. Win S, Than TA, Han D, Petrovic LM, Kaplowitz N. c-Jun N-terminal kinase (JNK)-dependent acute liver injury from acetaminophen or tumor necrosis factor (TNF) requires mitochondrial Sab protein expression in mice. *J Biol Chem*. 2011;286:35071–8.
68. Akakpo JY, Ramachandran A, Curry SC, Rumack BH, Jaeschke H. Comparing *N*-acetylcysteine and 4-methylpyrazole as antidotes for acetaminophen overdose. *Arch Toxicol*. 2022;96:453–65.
69. Akakpo JY, Ramachandran A, Kandel SE, Ni HM, Kumer SC, Rumack BH, et al. 4-methylpyrazole protects against acetaminophen hepatotoxicity in mice and in primary human hepatocytes. *Hum Exp Toxicol*. 2018;37:1310–22.
70. Alessio HM. Part III • Chapter 5 - Lipid peroxidation in healthy and diseased models: influence of different types of exercise. In: Sen CK, Packer L, Hänninen OOP, editors. *Handbook of oxidants and antioxidants in exercise*. Amsterdam: Elsevier Science B.V.; 2000. pp. 115–27.
71. Jaeschke H, Bajt ML. 9.21 - Mechanisms of acetaminophen hepatotoxicity. In: McQueen CA, editor. *Comprehensive toxicology (second edition)*. 2nd ed. Oxford: Elsevier; 2010. pp. 457–73.
72. Begriche K, Igoudjil A, Pessayre D, Fromenty B. Mitochondrial dysfunction in NASH: causes, consequences and possible means to prevent it. *Mitochondrion*. 2006;6:1–28.
73. Werner C, Wendel A. Hepatic uptake and antihepatotoxic properties of vitamin E and liposomes in the mouse. *Chem Biol Interact*. 1990;75:83–92.
74. Amimoto T, Matsura T, Koyama SY, Nakanishi T, Yamada K, Kajiyama G. Acetaminophen-induced hepatic injury in mice: the role of lipid peroxidation and effects of pretreatment with coenzyme Q<sub>10</sub> and  $\alpha$ -tocopherol. *Free Radic Biol Med*. 1995;19:169–76.
75. Knight TR, Fariss MW, Farhood A, Jaeschke H. Role of lipid peroxidation as a mechanism of liver injury after acetaminophen overdose in mice. *Toxicol Sci*. 2003;76:229–36.
76. Yamada N, Karasawa T, Kimura H, Watanabe S, Komada T, Kamata R, et al. Ferroptosis driven by radical oxidation of n-6 polyunsaturated fatty acids mediates acetaminophen-induced acute liver failure. *Cell Death Dis*. 2020;11:144.
77. Wendel A, Feuerstein S, Konz KH. Acute paracetamol intoxication of starved mice leads to lipid peroxidation *in vivo*. *Biochem Pharmacol*. 1979;28:2051–5.
78. Wendel A, Feuerstein S. Drug-induced lipid peroxidation in mice—I modulation by monooxygenase activity, glutathione and selenium status. *Biochem Pharmacol*. 1981;30:2513–20.
79. Amodeo GF, Solesio ME, Pavlov EV. From ATP synthase dimers to C-ring conformational changes: unified model of the mitochondrial permeability transition pore. *Cell Death Dis*. 2017;8:1.
80. Jaeschke H, Adelusi OB, Akakpo JY, Nguyen NT, Sanchez-Guerrero G, Umbaugh DS, et al. Recommendations for the use of the acetaminophen hepatotoxicity model for mechanistic studies and how to avoid common pitfalls. *Acta Pharm Sin B*. 2021;11:3740–55.

81. Adelusi OB, Ramachandran A, Lemasters JJ, Jaeschke H. The role of iron in lipid peroxidation and protein nitration during acetaminophen-induced liver injury in mice. *Toxicol Appl Pharmacol.* 2022;445:116043.
82. Conrad M, Pratt DA. The chemical basis of ferroptosis. *Nat Chem Biol.* 2019;15:1137–47.
83. Agarwal R, MacMillan-Crow LA, Rafferty TM, Saba H, Roberts DW, Fifer EK, et al. Acetaminophen-induced hepatotoxicity in mice occurs with inhibition of activity and nitration of mitochondrial manganese superoxide dismutase. *J Pharmacol Exp Ther.* 2011;337:110–8.
84. Campolo N, Bartesaghi S, Radi R. Metal-catalyzed protein tyrosine nitration in biological systems. *Redox Rep.* 2014;19:221–31.
85. Denicola A, Souza JM, Gatti RM, Augusto O, Radi R. Desferrioxamine inhibition of the hydroxyl radical-like reactivity of peroxyxynitrite: role of the hydroxamic groups. *Free Radic Biol Med.* 1995;19:11–9.
86. Jaeschke H, Adelusi OB, Ramachandran A. Ferroptosis and acetaminophen hepatotoxicity: are we going down another rabbit hole? *Gene Expr.* 2021;20:169–78.
87. Xia SW, Wang ZM, Sun SM, Su Y, Li ZH, Shao JJ, et al. Endoplasmic reticulum stress and protein degradation in chronic liver disease. *Pharmacol Res.* 2020;161:105218.
88. Halperin L, Jung J, Michalak M. The many functions of the endoplasmic reticulum chaperones and folding enzymes. *IUBMB Life.* 2014;66:318–26.
89. Kalinec GM, Thein P, Parsa A, Yorgason J, Luxford W, Urrutia R, et al. Acetaminophen and NAPQI are toxic to auditory cells via oxidative and endoplasmic reticulum stress-dependent pathways. *Hear Res.* 2014;313:26–37.
90. Rutkowski DT, Kaufman RJ. A trip to the ER: coping with stress. *Trends Cell Biol.* 2004;14:20–8.
91. Mannaerts I, Thoen LFR, Eysackers N, Cubero FJ, Batista Leite S, Coldham I, et al. Unfolded protein response is an early, non-critical event during hepatic stellate cell activation. *Cell Death Dis.* 2019;10:98.
92. Mozos A, Roué G, López-Guillermo A, Jares P, Campo E, Colomer D, et al. The expression of the endoplasmic reticulum stress sensor BiP/GRP78 predicts response to chemotherapy and determines the efficacy of proteasome inhibitors in diffuse large b-cell lymphoma. *Am J Pathol.* 2011;179:2601–10.
93. Schröder M, Kaufman RJ. ER stress and the unfolded protein response. *Mutat Res.* 2005;569:29–63.
94. Uzi D, Barda L, Scaiewicz V, Mills M, Mueller T, Gonzalez-Rodriguez A, et al. CHOP is a critical regulator of acetaminophen-induced hepatotoxicity. *J Hepatol.* 2013;59:495–503.
95. Nagashima Y, Mishiba K, Suzuki E, Shimada Y, Iwata Y, Koizumi N. Arabidopsis IRE1 catalyses unconventional splicing of *bZIP60* mRNA to produce the active transcription factor. *Sci Rep.* 2011;1:29.
96. Chen Y, Brandizzi F. IRE1: ER stress sensor and cell fate executor. *Trends Cell Biol.* 2013;23:547–55.
97. Hetz C, Glimcher LH. Fine-tuning of the unfolded protein response: assembling the IRE1 $\alpha$  interactome. *Mol Cell.* 2009;35:551–61.
98. Hetz C, Martinon F, Rodriguez D, Glimcher LH. The unfolded protein response: integrating stress signals through the stress sensor IRE1 $\alpha$ . *Physiol Rev.* 2011;91:1219–43.
99. Ali MMU, Bagratuni T, Davenport EL, Nowak PR, Silva-Santisteban MC, Hardcastle A, et al. Structure of the Ire1 autophosphorylation complex and implications for the unfolded protein response. *EMBO J.* 2011;30:894–905.
100. Korennykh AV, Egea PF, Korostelev AA, Finer-Moore J, Zhang C, Shokat KM, et al. The unfolded protein response signals through high-order assembly of Ire1. *Nature.* 2009;457:687–93.
101. Madhavan A, Kok BP, Rius B, Grandjean JMD, Alabi A, Albert V, et al. Pharmacologic IRE1/XBP1s activation promotes systemic adaptive remodeling in obesity. *Nat Commun.* 2022;13:608.

102. McCullough KD, Martindale JL, Klotz LO, Aw TY, Holbrook NJ. Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state. *Mol Cell Biol.* 2001;21:1249–59.
103. Gyamlani GG, Parikh CR. Acetaminophen toxicity: suicidal vs accidental. *Crit Care.* 2002;6:155.
104. Gamal W, Treskes P, Samuel K, Sullivan GJ, Siller R, Srsen V, et al. Low-dose acetaminophen induces early disruption of cell-cell tight junctions in human hepatic cells and mouse liver. *Sci Rep.* 2017;7:37541.
105. Ye H, Chen C, Wu H, Zheng K, Martín-Adrados B, Caparros E, et al. Genetic and pharmacological inhibition of XBP1 protects against APAP hepatotoxicity through the activation of autophagy. *Cell Death Dis.* 2022;13:143.
106. Hur KY, So JS, Ruda V, Frank-Kamenetsky M, Fitzgerald K, Koteliansky V, et al. IRE1 $\alpha$  activation protects mice against acetaminophen-induced hepatotoxicity. *J Exp Med.* 2012;209:307–18.
107. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science.* 2011;334:1081–6.
108. Ni HM, McGill MR, Chao X, Du K, Williams JA, Xie Y, et al. Removal of acetaminophen protein adducts by autophagy protects against acetaminophen-induced liver injury in mice. *J Hepatol.* 2016;65:354–62.
109. Coelho DS, Domingos PM. Physiological roles of regulated Ire1 dependent decay. *Front Genet.* 2014;5:76.
110. Lebeaupin C, Vallée D, Rousseau D, Patouraux S, Bonnafous S, Adam G, et al. Bax inhibitor-1 protects from nonalcoholic steatohepatitis by limiting inositol-requiring enzyme 1 alpha signaling in mice. *Hepatology.* 2018;68:515–32.
111. Yan M, Huo Y, Yin S, Hu H. Mechanisms of acetaminophen-induced liver injury and its implications for therapeutic interventions. *Redox Biol.* 2018;17:274–83.
112. Matsumaru K, Ji C, Kaplowitz N. Mechanisms for sensitization to TNF-induced apoptosis by acute glutathione depletion in murine hepatocytes. *Hepatology.* 2003;37:1425–34.
113. Torres S, Baulies A, Insausti-Urkiá N, Alarcón-Vila C, Fucho R, Solsona-Vilarrasa E, et al. Endoplasmic reticulum stress-induced upregulation of STARD1 promotes acetaminophen-induced acute liver failure. *Gastroenterology.* 2019;157:552–68.
114. Gunawan BK, Liu ZX, Han D, Hanawa N, Gaarde WA, Kaplowitz N. c-Jun N-terminal kinase plays a major role in murine acetaminophen hepatotoxicity. *Gastroenterology.* 2006;131:165–78.
115. Liu Z, Lv Y, Zhao N, Guan G, Wang J. Protein kinase R-like ER kinase and its role in endoplasmic reticulum stress-decided cell fate. *Cell Death Dis.* 2015;6:e1822.
116. Locker N, Easton LE, Lukavsky PJ. HCV and CSFV IRES domain II mediate eIF2 release during 80S ribosome assembly. *EMBO J.* 2007;26:795–805.
117. Vattem KM, Wek RC. Reinitiation involving upstream ORFs regulates *ATF4* mRNA translation in mammalian cells. *Proc Natl Acad Sci U S A.* 2004;101:11269–74.
118. Wek RC, Cavener DR. Translational control and the unfolded protein response. *Antioxid Redox Signal.* 2007;9:2357–71.
119. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell.* 2001;107:881–91.
120. Liu X, Green RM. Endoplasmic reticulum stress and liver diseases. *Liver Res.* 2019;3:55–64.
121. Iurlaro R, Muñoz-Pinedo C. Cell death induced by endoplasmic reticulum stress. *FEBS J.* 2016;283:2640–52.
122. Nagy G, Kardon T, Wunderlich L, Szarka A, Kiss A, Schaff Z, et al. Acetaminophen induces ER dependent signaling in mouse liver. *Arch Biochem Biophys.* 2007;459:273–9.
123. Huang MY, Wan DW, Deng J, Guo WJ, Huang Y, Chen H, et al. Downregulation of RIP3 improves the protective effect of ATF6 in an acute liver injury model. *Biomed Res Int.* 2021;2021:8717565.



124. Chakrabarti A, Chen AW, Varner JD. A review of the mammalian unfolded protein response. *Biotechnol Bioeng.* 2011;108:2777–93.
125. Korennykh A, Walter P. Structural basis of the unfolded protein response. *Annu Rev Cell Dev Biol.* 2012;28:251–77.
126. Yamamoto K, Sato T, Matsui T, Sato M, Okada T, Yoshida H, et al. Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6 $\alpha$  and XBP1. *Dev Cell.* 2007;13:365–76.
127. Rodrigues T, Ferraz LS. Therapeutic potential of targeting mitochondrial dynamics in cancer. *Biochem Pharmacol.* 2020;182:114282.
128. Zhang C, Zhao Y, Yu M, Qin J, Ye B, Wang Q. Mitochondrial dysfunction and chronic liver disease. *Curr Issues Mol Biol.* 2022;44:3156–65.
129. Yamada Y, Takano Y, Satrialdi, Abe J, Hibino M, Harashima H. Therapeutic strategies for regulating mitochondrial oxidative stress. *Biomolecules.* 2020;10:83.
130. Ding WX, Guo F, Ni HM, Bockus A, Manley S, Stolz DB, et al. Parkin and mitofusins reciprocally regulate mitophagy and mitochondrial spheroid formation. *J Biol Chem.* 2012;287:42379–88.
131. Umbaugh DS, Nguyen NT, Jaeschke H, Ramachandran A. Mitochondrial membrane potential drives early change in mitochondrial morphology after acetaminophen exposure. *Toxicol Sci.* 2021;180:186–95.
132. Mansouri A, Haouzi D, Descatoire V, Demeilliers C, Sutton A, Vadrot N, et al. Tacrine inhibits topoisomerases and DNA synthesis to cause mitochondrial DNA depletion and apoptosis in mouse liver. *Hepatology.* 2003;38:715–25.
133. Sorrentino V, Menzies KJ, Auwerx J. Repairing mitochondrial dysfunction in disease. *Annu Rev Pharmacol Toxicol.* 2018;58:353–89.
134. Grattagliano I, Russmann S, Diogo C, Bonfrate L, Oliveira PJ, Wang DQ, et al. Mitochondria in chronic liver disease. *Curr Drug Targets.* 2011;12:879–93.
135. Ong MMK, Latchoumycandane C, Boelsterli UA. Troglitazone-induced hepatic necrosis in an animal model of silent genetic mitochondrial abnormalities. *Toxicol Sci.* 2007;97:205–13.
136. Ramachandran A, Duan L, Akakpo JY, Jaeschke H. Mitochondrial dysfunction as a mechanism of drug-induced hepatotoxicity: current understanding and future perspectives. *J Clin Transl Res.* 2018;4:75–100.
137. Darr U, Sussman NL. Drug-induced liver injury in the setting of analgesic use. *Clin Liver Dis.* 2020;24:121–9.
138. Williams JA, Ding WX. Role of autophagy in alcohol and drug-induced liver injury. *Food Chem Toxicol.* 2020;136:111075.
139. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: drug-induced liver injury. *J Hepatol.* 2019;70:1222–61.
140. Hu J, Lemasters JJ. Suppression of iron mobilization from lysosomes to mitochondria attenuates liver injury after acetaminophen overdose *in vivo* in mice: protection by minocycline. *Toxicol Appl Pharmacol.* 2020;392:114930.
141. Moles A, Torres S, Baulies A, Garcia-Ruiz C, Fernandez-Checa JC. Mitochondrial-lysosomal axis in acetaminophen hepatotoxicity. *Front Pharmacol.* 2018;9:453.
142. Fromenty B. Alteration of mitochondrial DNA homeostasis in drug-induced liver injury. *Food Chem Toxicol.* 2020;135:110916.
143. Karahalil B, Hare E, Koç G, Uslu İ, Şentürk K, Özkan Y. Hepatotoxicity associated with statins. *Arh Hig Rada Toksikol.* 2017;68:254–60.