OPEN ACCESS Freely available online

Journal of Clinical Toxicology

Research Article

Molecular Mechanism of Hepatotoxicity and Novel Therapeutic Targets

Abel Andualem[1*], Seid Mufti Hussen[1], Rehima Mohammed Endris[2], Birhanu Geta Meharie[3]

[1]Department of Pharmacology, School of Pharmacy, College of Medicine and Health Sciences, Wollo University, Amhara, Ethiopia; [2]Department of Pharmacy, College of Health Sciences, Selale University, Oromia, Ethiopia; [3]Department of Pharmacy, College of Medicine and Health Sciences, Debre Markos University, Amhara, Ethiopia

ABSTRACT

Introduction: Among pharmaceuticals that can induce liver injury or hepatotoxicity including anti-tubercular medications, general anesthetics, paracetamol, and some anti-cancer drugs. Liver can be damaged by certain chemicals known as hepatotoxins, such as galactosamine, alcohol and chloroform. Three pattern of injury are identified: Hepatocellular, cholestatic, and mixed. Mechanism of liver Injury caused by drugs may be direct (dose-dependent, intrinsic, and predictable), idiosyncratic or indirect injury. The classic example of intrinsic liver injury is acetaminophen intoxication.

Farnesoid X Receptor (FXR) is a regulator of hepatotoxicity and is used as therapeutic target in case of drug and alcohol induced hepatotoxicity. Many targets are also investigated as a potential therapeutic target in hepatotoxicity like CAR, LXR, PPARα and PXR. In addition, therapeutic strategies *via* inhibiting *NLRP3* inflammasome is the protective effects of the phytochemicals for drug induced hepatotoxicity.

In this review, updated researches on the molecular basis in hepatotoxicity of alcohol, drugs, food born mycotoxin and heavy metals were summarized followed by recent therapeutic targets associated with these agents.

Objective: The aim of this review is to offer a comprehensive review on molecular mechanism of hepatotoxicity, recent therapeutic target identified in clinical/preclinical setting and proposes new perspectives in the field of hepatotoxicity induced by alcohol, drugs, food born mycotoxin and heavy metals.

Keywords: Medications; General anesthetics; Paracetamol; Hepatotoxins; FXR

INTRODUCTION

The liver contributes a primary role in producing blood clotting factors, plasma proteins, and immune modulators in addition to metabolize nutrients, xenobiotics, alcohol, and drugs. Hepatocytes, endothelial cells, stellate cells, Kupffer cells, pit cells, and bile duct cells are responsible for performing these essential functions [1].

Liver damage can be triggered by various drugs, including anti-tubercular medications, general anesthetics, paracetamol, and some anti-cancer drugs. Toxic hepatitis, a severe adverse reaction to anti-tuberculosis drugs, often begins within the first few weeks of treatment and can lead to liver necrosis, which may progress to encephalopathy and even death. Alcoholic liver disease, characterized by cirrhosis (the formation of fibrous tissue in the liver) due to excessive alcohol consumption, is also common. Additionally, certain chemicals known as hepatotoxins, such as galactosamine and chloroform, can damage the liver. Over 900 drugs are known to cause hepatotoxicity, which has led to the withdrawal of some medications from the market. Liver toxicity can result from the direct effects of the primary compound, reactive metabolites, or immune-mediated responses, affecting hepatocytes, biliary epithelial cells, and liver vasculature. The severity of hepatotoxic reactions depends on factors such as the concentration of the toxic substance, the specific expression of enzymes, and the substance's concentration gradient in the blood covering the acinus.

Correspondence to: Abel Andualem, Department of Pharmacology, School of Pharmacy, College of Medicine and Health Sciences, Wollo University, Amhara, Ethiopia; E-mail: abel.andualem@wu.edu.et

Received: 17-Jan-2025, Manuscript No. JCT-25-36824; **Editor assigned:** 22-Jan-2025, PreQC No. JCT-25-36824 (PQ); **Reviewed:** 05-Feb-2025, QC No. JCT-25-36824; **Revised:** 12-Jun-2026, Manuscript No. JCT-25-36824 (R); **Published:** 19-Jun-2026, DOI: 10.35248/2161-0495.26.16.622

Citation: Andualem A, Hussen SM, Endris RM, Meharie BG (2026) Molecular Mechanism of Hepatotoxicity and Novel Therapeutic Targets. J Clin Toxicol. 16:622.

Copyright: © 2026 Andualem A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Despite numerous studies on the mechanisms of hepatotoxicity, the precise molecular processes by which drugs and other compounds cause liver damage remain unclear. Therefore, the objective of this review is to compile the latest discoveries regarding the molecular mechanisms involved in alcohol, drugs, heavy metal, and mycotoxin induced hepatotoxicity and identifies potential new targets for the development of innovative therapeutic strategies [2].

MATERIALS AND METHODS

In order to conduct this comprehensive review, outline and thematic analysis of the molecular mechanism of hepatotoxicity and recent therapeutic targets regarding alcohol, some selected drugs, heavy metals and food bore mycotoxin was prepared. First, different electronic databases were searched for studies regarding the molecular mechanism of hepatotoxicity and recent therapeutic targets; Google Scholar, PubMed, Embase, Midline and Cochrane library were among the most commonly searched databases. In addition, information was collected from New England Journal of Medicine, ResearchGate and Journal of Biomedical Science. The searching terms include drug induced liver injury, drugs and liver injury, drug induced hepatotoxicity, drugs and hepatotoxicity, molecular mechanism and hepatotoxicity, molecular mechanism and liver injury, hepatotoxicity and novel therapeutic targets, hepatotoxicity and new therapeutic targets, molecular mechanism and hepatotoxicity or liver injury, molecular mechanism and liver injury and therapeutic targets. By emphasizing and making advanced search, original articles were conducted and published from 2000 to 2024 were collected and reviewed to collect and analyze relevant literature focusing on the molecular mechanisms of hepatotoxicity and recent therapeutic targets. Both *in vivo* and *in vitro* research were included, along with relevant clinical trials and reviews [3].

Studies were excluded if they lacked mechanistic insights, therapeutic relevance, or clear methodologies. Finally, data were arranged based on the specific types of hepatotoxicity like alcohol-induced hepatotoxicity (ALD), Drug-Induced Liver Injury (DILI), heavy metal-induced hepatotoxicity and Food-borne mycotoxin-induced hepatotoxicity. Therapeutic interventions based on preclinical and clinical data were assessed and were grouped and summarized into categories based on their target mechanisms or pathways.

RESULTS

Molecular mechanism of hepatotoxicity

Alcohol induced hepatotoxicity:

Ethanol metabolism: Following oral ingestion, alcohol is mainly absorbed in the stomach (~22%) and small intestine (~75%), with feces and the esophagus account small percentage. About 95% the absorbed alcohol is metabolized in the liver with small portion is excreted in urine (~2-4%) or expelled as an exhaled gas (~5%) in its original form. Although the liver is the main organ for alcohol degradation, gastric mucosa also contributes to the metabolism of small amounts ethanol which contains Alcohol Dehydrogenase (ADH), in a process known as gastric First-Pass Metabolism (FPM) [4].

In the liver, three distinct enzymes are known to metabolize ethanol to acetaldehyde *in vitro*: Alcohol Dehydrogenase (ADH), the Microsomal Ethanol-Oxidizing System (MEOS) and catalase. In addition to being converted to acetaldehyde, ethanol metabolism leads to an elevation in Reactive Oxygen Species (ROS) levels and a reduction in the Nicotinamide Adenine Dinucleotide (NAD+)/NADH ratio. Within liver cells, ADH is located in the cytoplasm (cytosol) between various subcellular structures, MEOS is found in the endoplasmic reticulum, and catalase resides in the peroxisomes (also known as microbodies). ADH and MEOS are particularly relevant for hepatic alcohol metabolism *in vivo* but not catalase (Figure 1) [5].

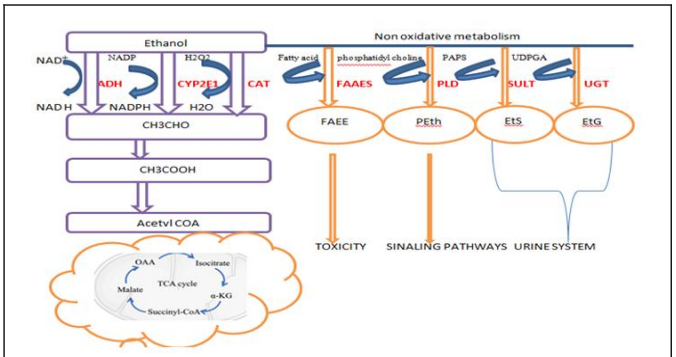


Figure 1: Ethanol metabolism pathways in the liver.

Alcohol Dehydrogenase (ADHs) metabolize the majority of ethanol (80-90%) under physiological conditions. Human liver ADH consists five classes-ADH1 through ADH5 each has distinct subunits. The main ADHs implicated in ethanol metabolism are *ADH1A*, *ADH1B*, *ADH1C*, and *ADH4*. Cytochrome P450 family 2 members E1 (CYP2E1) is responsible for the metabolism of the remaining 10%-20% of ethanol. Notably, ethanol consumption raises the expression level of CYP2E1 [6].

Aldehyde Dehydrogenases (ALDHs) oxidize acetaldehyde to acetic acid. This is a key rate-limiting step in the alcohol oxidative metabolism pathway, primarily mediated by *ALDH1A1*, *ALDH1B1*, and *ALDH2* in humans. Current studies have demonstrated that *ALDH2* metabolizes acetaldehyde to acetic acid in both the liver and cerebellar astrocytes. In the cerebellum, this can lead to mobility and balance disorder by raising Gamma-Aminobutyric Acid (GABA) levels.

Furthermore, enzymes such as FAEE Synthase (FAEES), Sulfotransferase (SULT), Acyl-CoA Ethanol O-acyltransferase (AEAT), Glucuronosyltransferase (UGT) and Phospholipase (PLD) can metabolize ethanol into non-oxidative metabolites [7].

Molecular mechanism of alcoholic liver disease

Patients with a history of prolonged alcohol abuse progresses through several stages of Alcoholic Liver Disease (ALD): Steatosis or Alcoholic Fatty Liver (AFL), Alcoholic Steatohepatitis (ASH), Alcoholic Hepatitis (AH), Alcoholic

Cirrhosis (AC) and Alcoholic Hepatocellular Carcinoma (AHCC).

Steatosis is a mild stage characterized by the accumulation of fat in hepatocytes result from inhibition of fatty acid oxidation, enhanced fat uptake into the liver and increased fatty acid and triglyceride synthesis. About 8-20% of steatosis patient progress to steatohepatitis (moderate stage), fibrosis, and cirrhosis (advanced stage), with 3-10% eventually develop to Hepatocellular Carcinoma (HCC).

The oxidations of ethanol and acetaldehyde simulate lipogenesis and alter the cellular redox potential due to the up regulation of lipogenic enzymes and cytokines. Early growth response-1 (Egr-1) and Sterol Regulatory Element Binding Protein-1c (SREBP-1c) are transcription factors that gene of these enzymes and cytokine. Egr-1 regulates the expression of genes related to cellular stress responses and attaches itself to promoter regions of genes that are particularly essential to alcohol-induced liver damage. One of the most significant genes is Tumor Necrosis Factor alpha (TNF-α). Moreover, soon after ethanol administration, Egr-1 is activated. On the other hand, SREBP-1c is a member of transcription factors that control cholesterol metabolism in the liver and its expression is regulated by Egr-1 (Figure 2) [8].

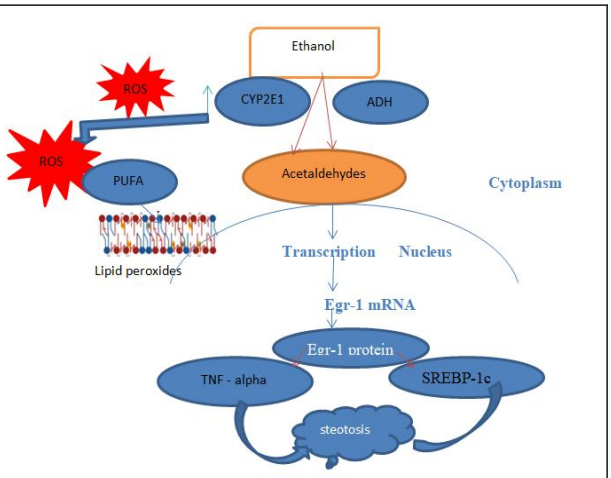


Figure 2: Mechanism by which ethanol oxidation regulates Early growth response-1 (Egr-1) and Sterol Regulatory Element Binding Protein-1c (SREBP-1c) to enhance lipogenesis.

Alcoholic hepatitis: Alcoholic hepatitis marked by hepatic inflammation and injury, along with steatosis, and can progress to fibrotic and cirrhotic disease states. A key factor in the development of alcoholic hepatitis is the involvement of macrophages, both resident and infiltrating immune cells, which play crucial roles in triggering liver inflammation. A primary trigger for the inflammatory response initiated by resident liver macrophages, known as Kupffer cells, is endotoxin or Lipopolysaccharide (LPS) [9].

Research have revealed that excessive consumption of ethanol causes increased intestinal permeability and encouraging bacterial proliferation which results in endotoxemia. This Lipopolysaccharide (LPS) interacts with Toll-Like Receptor 4 (TLR4) which activates hepatic stellate cells and Kupffer cells in the liver. Reactive Oxygen Species (ROS) and pro-inflammatory cytokines and chemokine are produced which contribute to hepatocyte damage. Alcohol-induced activation of various immune cells are additional factors that leads to hepatocyte damage and Damage-Associated Molecular Pattern (DAMP) molecules are also produced as the result of its effect on adipose tissue (Figure 3).

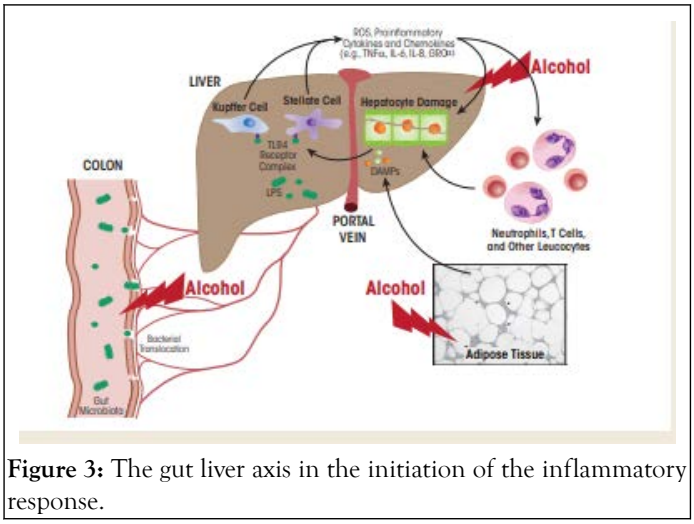


Figure 3: The gut liver axis in the initiation of the inflammatory response.

Alcoholic hepatitis fibrosis/cirrhosis: Fibrosis is a typical wound-healing response to liver injury, marked by the accumulation of extracellular matrix proteins, such as collagen, produced by activated Hepatic Stellate Cells (HSCs). Continuous activation of this wound-healing response can lead to cirrhosis of the liver, which may eventually progress to HCC.

Hepatic Stellate Cells (HSCs) are central to the development of liver fibrosis. The process begins with alcohol-induced damage to hepatocytes, leading to their apoptosis. This triggers Kupffer Cells (KCs) to release inflammatory cytokines such as TNFα, IFN-γ, TRAIL, and FasL. These cytokines attract immune cells like NK and NKT cells, which exacerbate inflammation. The activated HSCs respond by producing excessive extracellular matrix proteins, primarily collagen type 1, leading to the formation of scar tissue. Over time, this scarring can progress to cirrhosis, severely disrupting liver structure and function, impairing its regenerative capacity (Figure 4).

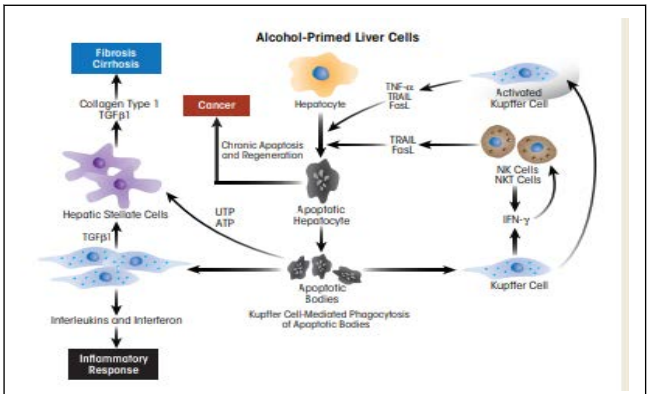


Figure 4: Schematic depiction of the role of Kupffer Cells (KCs) and Hepatic Stellate Cells (HSCs) in promoting alcohol-induced inflammatory changes and progression to fibrosis and cirrhosis.

Apoptotic signaling pathway and autophagy in ALD: Accumulated ROS, generated during alcohol metabolism primarily *via* CYP2E1 and NADPH oxidase, plays a critical role in mitochondria-dependent apoptosis. In hepatocytes, ROS accumulation inhibits the phosphorylation of AKT, leading to the downregulation of the G1 phase protein cyclin D1 by inhibiting the GSK3β/Wnt/β-catenin signaling pathway. This inhibition results in cell cycle arrest and triggers mitochondria-dependent apoptosis. Additionally, ROS can directly activate Apoptosis Signal-Regulating Kinase 1 (ASK1), NF-κB, and the JNK/P38 pathways, further promoting mitochondrial-dependent apoptosis. Both ROS and LPS stimulate the production of TNF-α and promote the expression of pro-inflammatory factors like IL-1β. They induce apoptosis through Endoplasmic Reticulum (ER) stress and activate the caspase cascade *via* the JNK/STAT3 and P53 pathways. Additionally, they cause Fas ligand-dependent apoptosis by recruiting neutrophils, further exacerbating liver.

Autophagy is a self-destructive process that degrades damaged proteins and organelles through lysosomes. Numerous studies have demonstrated that autophagy plays an inhibitory role in the progression of ALD by reducing steatosis (fat accumulation in the liver), suppressing the inflammatory response, and preventing hepatocyte apoptosis. Researchers have identified several pathways that can induce autophagy and help mitigate alcoholic liver damage, including the AMPK-Forkhead box O3 (FOXO3A) axis, Sorting Nexin 10 (SNX10)/chaperone, ALDH2, and cannabinoid receptor 2 (Figure 5).

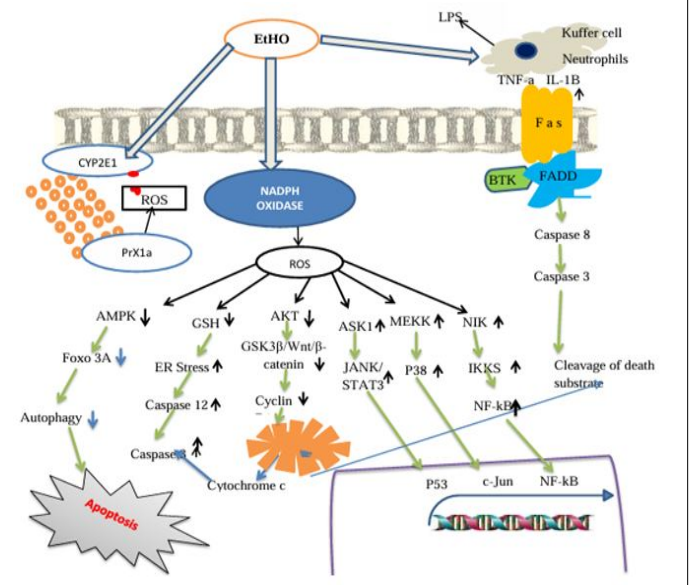


Figure 5: Molecular mechanisms of alcohol-induced apoptosis and autophagy in hepatocytes

Novel players in alcoholic liver disease pathogenesis

MicroRNA: Small noncoding RNAs known as microRNAs (miRNAs) inhibit RNA translation, promote RNA cleavage which regulate gene expression post-transcriptionally. Humans have found to have more than 2,000 miRNAs, together they regulate above 60% of the genome. The level of miRNA in the liver and circulatory system is influenced by excessive alcohol consumption which leads to Alcoholic Liver Disease (ALD) in mice. Numerous studies have frequently found that miR-21, miR-122, and miR-34a are involved in alcoholic liver damage. miRNA-21 is up-regulated in animal models and *in vitro* test after alcohol exposure which causes rise in Reactive Oxygen Species (ROS) production, β-oxidation, fibrosis, inflammation and carcinoma in the liver.

Nuclear factor erythroid 2 (Nrf2): Nuclear factor erythroid 2 (Nrf2) is a key transcriptional and antioxidant factor that regulates redox mechanisms in ALD. Ethanol activates Nrf2 through the ECH-associated protein-1 (Keap1) protease activator. This activation leads to an imbalance in the redox reaction, characterized by increased ROS and deregulated antioxidants like SOD and GSH. This imbalance causes DNA damage and increases the level of tumor protein p53, promoting apoptosis and liver injury.

Additionally, mitochondrial damage occurs with elevated levels of cytochrome C and caspases 3, 6, and 9, which contribute to apoptotic processes and liver injury. Increased TNFα levels also contribute to inflammation, further exacerbating ethanol-induced hepatotoxicity and liver damage.

PPARs and downstream signaling factors: mPPAR-α is a transcriptional regulator of genes involved in mitochondrial oxidation and fatty acid metabolism. In alcoholic conditions, the RXR protein down regulates the AMPK pathway and subsequently reduces PPAR-α activity, contributing to liver steatosis. Agonists like Wy14643 and clofibrate can activate PPAR-α, helping to prevent alcohol-induced liver steatosis by enhancing PPAR-α signaling.

Toll-like receptor 4: TLR4 is a key component of the LPS recognition receptor complex, which includes co-receptors CD14 and MD-2, as well as lipid-binding proteins. TLR4 plays a role in recognizing Lipopolysaccharides (LPS) and mediating inflammatory responses in the liver. LBP is a soluble shuttle protein that binds LPS directly and facilitates the interaction of LPS with CD14.

The Mammalian Target of Rapamycin (mTOR): mTOR is a protein kinase that forms two functional complexes: mTORC1 and mTORC2. In the context of alcoholic liver disease, ethanol activation of mTORC1 leads to the activation of an S6K1-dependent negative feedback loop against IRS-1 and PI3K signaling. This results in reduced Akt activation. Studies indicate that mTORC1 activation, but not mTORC2, is linked to hepatic alcoholic steatosis.

Adipose Triglyceride Lipase (ATGL): In alcohol-induced mice, fatty acid released from adipose tissue was primarily elevated in combination with ATGL activation. For example, the ALD model showed, a decrease in fatty acid release which was confirmed by the suppression of ATGL gene expression. Also, omega-3 PUFA prevents inflammation by controlling TNFα and IL-1 beta in response to down regulated ATGL expression. 3-deoxyadenosine decreased the ATGL protein and mRNA expression to restore injury in the ALD mode.

PXR (Pregnane X Receptor): PXR regulation also promotes the building up of hepatic triglyceride which is a feature of ALD.

alcohol-induced elevation of Cyp2b10, which contributes alcohol induced hepatotoxicity was inhibited by PXR ablation.

Constitutive Androstane Receptor (CAR): Constitutive Androstane Receptor (CAR) is a detoxifier of xenobiotics and endobiotics. Rats lacking CAR were more susceptibile to alcohol induced liver injury, which was followed by increased apoptosis and fat accumulation. Pre-activation of CAR by TCPOBO however, greatly improved hepatic toxicity in chronic and acute alcohol use.

Drug induced hepatotoxicity

Drug-induced hepatotoxicity defined as an adverse toxic drug reaction resulting from exposure to drug causing liver injury. Drug-Induced Liver Injury (DILI) has been typically classified in to three phenotypes based on mechanism of action: Direct (intrinsic), idiosyncratic, and indirect injury. In addition, based clinical features (pattern of liver injury) DILI can be clinically divided into three phenotypes: Hepatocytic, cholestatic and mixed.

Intrinsic hepatotoxicity is dose-dependent hepatotoxicity that can be predictive in humans or animal models. It can be reproducible and related with the quantity of drug-exposure. Common example of intrinsic hepatotoxicity includes: High doses of Acetaminophen (APAP), together with aspirin, amiodarone, niacin, and methotrexate, due to the drug itself or by its metabolites. These drugs stimulate the release of DAMPs after hepatocyte injury which is detected by Kupffer Cells (KCs). This event causes the release of pro-inflammatory mediators, cytokines, and chemokines by activating innate immune receptors to initiate intracellular events which recruit neutrophils and monocytes to the blood.

Idiosyncratic DILI is host-dependent, an unpredictable injury that cannot be explained by the known pharmacological properties. Certain drugs that causes idiosyncratic DILI includes amoxicillin clavulanate, cephalosporins, diclofenac, fluoroquinolones, isoniazid, macrolide antibiotics and nitrofurantoin. Their ability to cause idiosyncratic injury is depending on the activation of the adaptive immune system. They covalently bind to proteins in the body to form drug/metabolite-protein adducts. This can be processed by antigen-presenting cells (dendritic cells) as semi-antigens and then recognized by T cells to elicit immune responses that may have a genetic or even metabolic basis.

Indirect drug induced liver injury is associated with medication like antineoplastic agents, monoclonal antibodies, risperidone, and haloperidol which is explained by indirect action of the drug on liver or immune system which leads to secondary form of liver injury.

The molecular mechanisms responsible for Drug Induced Liver Injury (DILI)

The basic mechanism behind drug induced liver injury include mitochondrial dysfunction, oxidative stress, endoplasmic reticulum stress, increased apoptosis and necrosis, bile duct injuries, and finally cell death by apoptosis or necrosis. The majority case of direct or intrinsic drug induced liver injury is caused by oxidative stress, mitochondrial dysfunction and bile duct injuries.

Oxidative stress: Imbalance between the body's ability to detoxify reactive oxygen species and their production can lead to oxidative stress. This Reactive Oxygen Species (ROSs) cause DNA damage and trigger the DNA Damage Response (DDR) which lead to cellular dysfunction and contributing to various disease like ALD. On the other hand, they have role in cell signaling and immune response in small amount. Furthermore, Reactive Nitrogen Species (RNSs) has a potential to cause oxidative stress.

Acetaminophen, amiodarone, methotrexate, isoniazid is known to cause DILI through oxidative stress mechanisms. In addition, troglitazone, flutamide, nimesulide, VPA, and diclofenac have noted to raise intracellular oxidants.

When these drugs enter the hepatocyte (liver cell) they undergo metabolism by cytochrome P450 enzymes, results in to the production of toxic metabolites. These toxic intermediates are unstable and cause synthesis of ROS such as hydrogen peroxide (H_2O_2), hydroxyl radicals (OH•), and superoxide (O_2•−). Long-branched fatty acids β-oxidation in the peroxisome is another source of H_2O_2. By oxidizing Ferrous (Fe^{2+}) to Ferric (Fe^{3+}) H_2O_2 produces highly toxic but the short-lived Hydroxyl radical (OH•). LPO results from OH•, damage mitochondrial metabolim by the production of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (mPTP) and mitochondrial DNA (mtDNA) mutation. It also raises the pro-apoptotic activity, and produce Malondialdehyde (MDA). In addition, chronic Fe^{2+} overload may also lead to Nuclear Factor-kappa B (NF-κB) activation which increases the production of inducible Nitric Oxide Synthase (iNOS). This events lead to the increasing of Nitric Oxide (NO), which react with the superoxide anion yielding peroxynitrite (ONOO−), which is a powerful oxidant and nitrifier (Figure 6).

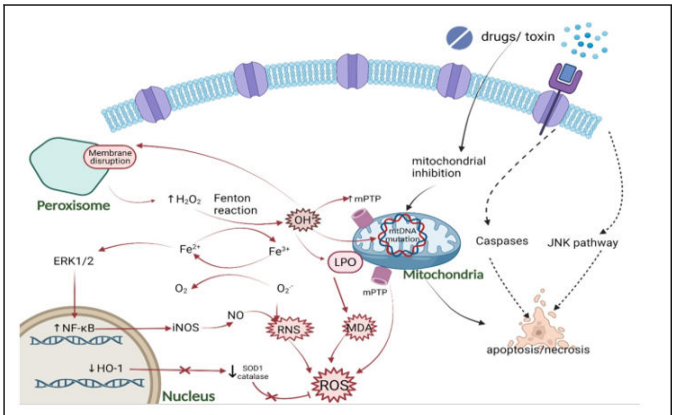


Figure 6: Oxidative stress induced by DILI.

Endoplasmic stress: When certain medications are exposed to the ER, they can cause the accumulation of misfolded or unfolded proteins which leads to ER stress and activation of an intracellular stress signaling cascade called Unfolded Protein Response (UPR). This activation is a defense mechanism and controlled by three ER-transmembrane proteins: Inositol Requiring Enzyme 1α (IRE1α), PKR-like eukaryotic initiation factor 2α kinase (PERK), and Activating Transcription Factor-6

(ATF6) and one chaperone protein (BiP/GRP78: binding immunoglobulin protein/78 kDa glucose-regulated protein).

The consequences of UPR activation includes the inhibition of translation, apoptosis if the UPR is strong enough and/or sustained, and stimulation of lipid synthesis by activation of different transcription factors such as Sterol Regulatory Element-Binding Proteins (SREBPs), CAAT/Enhancer-Binding Proteins (C/EBP) and Peroxisome Proliferator-Activated Receptor-γ (PPARγ).

Mitochondrial dysfunction, proteasome inhibition, and changes in key ER components are various mechanisms by which drugs can induce ER stress. They are transformed 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. Recent studies show that drugs such as Acetaminophen (APAP), Isoniazid (INH), and Valporic Acid (VPA) are known to induce ERS (Figure 7).

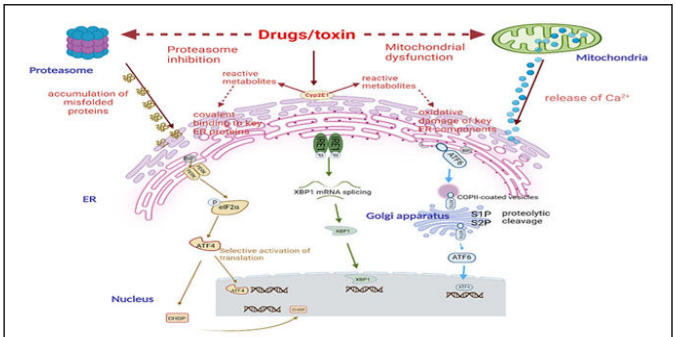


Figure 7: The potential mechanism of drug-induced ER stress.

Mitochondrial dysfunction: Activation of mitochondria by certain drugs can trigger alteration in mitochondrial structure and functional abnormalities. Recent studies demonstrated that NSAIDs, antibiotics, antipyretics, antivirals, immunosuppressive drugs and others over 300 drugs cause mitochondria damage of hepatocyte. These drugs can lead to mitochondrial dysfunction by different mechanism such as Mitochondrial Permeability Transition Pore Opening (MPTP), Alteration of Oxidative Phosphorylation and Electron Transport Chain (OXPHOS and ETC), and alteration of fatty acid beta oxidation.

Various studies reported that drugs such as acetaminophen, alpidem, amiodarone, diclofenac, disulfiram, nimesulide, salicylic acid, troglitazone, and valproic acid to induce MPTP opening by different proposed mechanisms.

Certain medications like alpidem can directly interact with Peripheral Benzodiazepine Receptor (PBR) in order to cause permeabilization of mitochondrial membrane and subsequent cell death. By activation of JNK pathways, other medication like acetaminophen and troglitazone can indirectly cause MPTP opening. This enhance the cell's stress response cascade which promote mitochondrial translocation and causing cell death. Some drug like amiodarone, diasulfiram leads to the production of Reactive Oxygen Species (ROS) that can oxidize membrane protein thiol groups that regulate MPTP.

Other essential mechanism to induce MPTP opening is lysosome instability which allows ferrous iron translocation into mitochondria and increasing intracellular calcium levels most likely through the mitochondrial Ca^{2+}/Fe^{2+} uniporter which overloads the mitochondria. Excessive Fe^{2+} in the mitochondria can contribute to the production of Reactive Oxygen Species (ROS) through the Fenton reaction and Excess Ca^{2+} leads to MPTP opening and cell death.

Acetaminophen, alpidem, amiodarone, benzarone, benzbromarone, buprenorphine, diclofenac, disulfiram, ibuprofen, and nilutamid are reported to cause mitochondrial dysfunction by alteration oxidative phosphorylation and electron transport chain. In this case several mechanisms were distinguished.

One of these mechanisms is the OXPHOS uncoupling without the inhibition of ETC. Many cationic amphiphilic are frequently implicated. They are transported through the $\Delta\psi_m$ into the mitochondrial matrix after being protonated in the intermembrane space thereby dissipating the proton gradient generated during electron transport, which disrupts the OXPHOS and decreases ATP synthesis. Another mechanism involves OXPHOS uncoupling in conjunction with the inhibition of ETC and blocking of ETC by the direct inhibiting of ETC complexes without initial OXPHOS uncoupling (Figure 8).

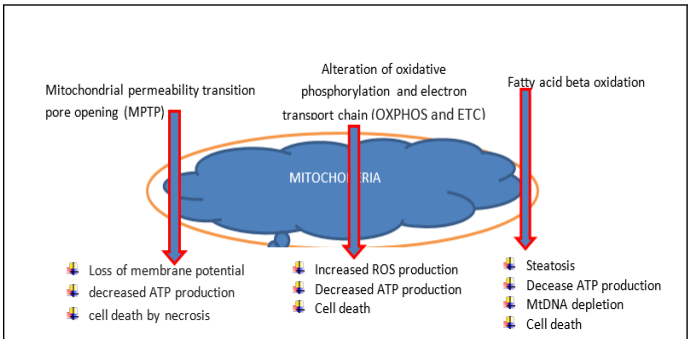


Figure 8: Mitochondrial dysfunction mechanism and consequences in DILI.

The molecular mechanisms of Acetaminophen-Induced Hepatotoxicity (AIH): APAP-induced hepatotoxicity is primarily caused by the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). Large number of studies shows that mitochondrial oxidative stress and dysfunction are the predominant molecular mechanism in APAP induced hepatic injury. Liver metabolism, sterile inflammation, Endoplasmic Reticulum Stress (ERS), autophagy, and microcirculatory dysfunction are also implicated in AIH.

During APAP overdose the production of NAPQI increases significantly, result in rapid depletion of GSH in the liver. Excessive NAPQI binds to the cysteine residues of various essential proteins in cells such as GSH peroxidase, Adenosine Triphosphate (ATP) synthase and interfere with complexes I and II in the mitochondrial Electron Transport Chain (ETC).

Recently several studies have found that, three major signaling proteins – including c-Jun N-terminal Kinase (JNK), Nrf2, and p53 are involved in mitochondrial oxidative stress induced by APAP.

One of the early results of APAP-induced mitochondrial oxidative stress is the activation of JNK in the cytoplasm. ROS is released into the cytosol by mitochondria as the result of NAPQI. These ROS can oxidize thioredoxins and separate the Apoptosis Signal-Regulating Kinase 1 (ASK-1) from them, which initiate the self-activation of ASK-1. Furthermore, the Mixed Lineage Kinase 3 (MLK3) proteins are activated by phosphorylation of Glycogen Synthase Kinase-3β (GSK-3β). Activated ASK1 and MLK3 stimulate Mitogen-Activated Protein Kinase 4/7 (MKK4/7), which further activates JNK. Thus cascade of events eventually lead to enhanced release of Reactive Oxygen Species (ROS) and dysfunction of the ETC. Additionally, activated JNK recruits cytoplasmic Bax, which triggers the opening of MPTP, which causes release of Apoptosis-Inducing Factor (AIF), Cytochrome c (Cyt C) and Endonuclease G (Endo G). These proteins are translocated to the nucleus, where they induce DNA fragmentation and cell death.

Through regulating the expression of protective genes, Nrf2 protects cells from oxidative stress. When oxidative stimulation occurs, Nrf2 dissociate from keap1, thus activates itself. Then Nrf2 translates to the nucleus, ultimately initiating the expression of Heme Oxygenase-1 (HO-1), NAD(P)H: Quinone Oxidoreductase 1 (NQO1), and Glutamate–Cysteine Ligase Catalytic (GCLC) subunit. These proteins are detoxification-related proteins that prevent the liver from damage by enhancing metabolic inactivation of APAP.

APAP model studies observed that p53 was activated in AIH mice and when p53 was inhibited, JNK phosphorylation was increased to induce more severe liver injury (Figure 9).

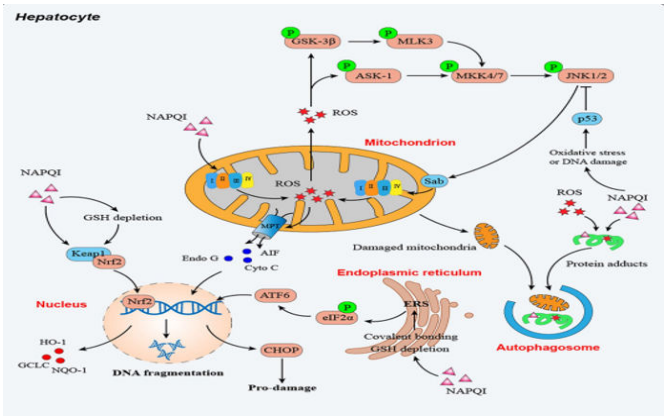


Figure 9: The major cellular events of acetaminophen-induced hepatotoxicity.

Molecular mechanism of methotrexate induced hepatotoxicity: Methotrexate enters hepatocytes *via* folate transporter. In hepatocytes, it is converted into MTX-Polyglutamates (MTX-PGs) by Folylpolyglutamate Synthetase (FPGS). The buildup of MTX-polyglutamate initiates oxidative stress, inflammation, steatosis, fibrosis, and apoptosis in hepatocyte. But several studies suggested that oxidative stress is the major molecular mechanism of MTX in the liver.

The MTX-PGs metabolite leads to lipid peroxidation that release ROS, NO, and other free radicals, which down regulate intracellular enzymatic and non-enzymatic antioxidants such as SOD, CAT, GSH, and GPx. furthermore, MTX-PG-generate ROS which suppress ARE and down regulates intracellular oxidant resistant regulators such as Nrf2, HO-1, and NQO1. There are also evidences suggesting MTX-PG activating MTHFR, which causes the accumulation of intracellular and plasma homocysteine which was related to the progression of oxidative stress, inflammation and fibrosis in liver tissue.

Methotrexate-PGs deplete hepatic folate levels and decreasing RNA and DNA synthesis in hepatocytes leads to cell death. MTX cause apoptosis by altering the homeostasis of bcl-2 and bax, respectively through the induction of pro-inflammatory markers, altering mitochondrial membrane potential ($\Delta\Psi_m$). Thus cause the release of cytochrome c from mitochondria to cytosol which forms apoptosome and activates caspase 3 that initiate hepatocyte death by apoptosis (Figure 10).

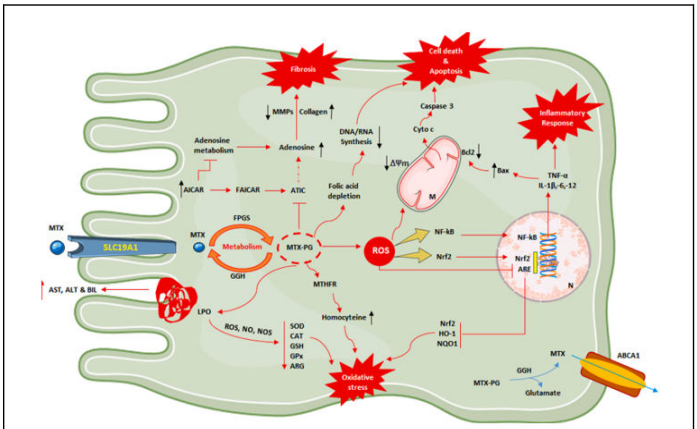


Figure 10: Mechanisms of Methotrexate (MTX)-induced liver injury.

Molecular mechanism antitubercular drugs induced hepatotoxicity: Many studies have demonstrated that possible molecular mechanisms of ATDs-induced liver injury involve: induction of oxidative stress, cholestasis, apoptosis, microvesicular steatosis, inflammation, and fibrosis.

Anti TB drugs cause oxidative stress-induced liver injury by accumulating lipid peroxidation byproducts, depleting cytoprotective antioxidants and downregulating Nrf-2 signaling. They also cause cholestasis by inhibiting bile acid and bile salt transporters and bilirubin effluence. For instance, NHI plus RIF administration decreased the expression of bile acid transporters such as the bile salt export pump and multidrug resistance-associated protein 2 and induced liver injury by sirtuin 1 and farnesoid X receptor pathway.

Anti TB drugs leads to intrinsic apoptosis by altering bcl-2 and bax homeostasis, dissipation of mitochondrial membrane potential, cytochrome c release and caspase 3 activation (Figure 11).

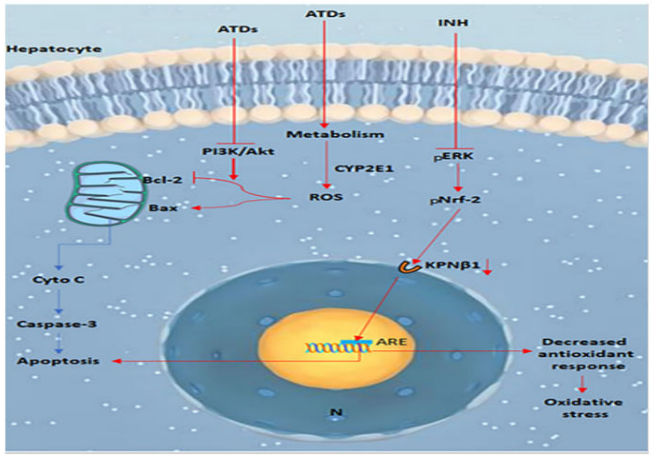


Figure 11: The apoptosis-inducing potential of the Antitubercular Drugs (ATDs).

These groups of drug also lead to stenosis by inducing expression of PPAR-c downstream signaling. For instance, it has been seen that RIF induces the expression of peroxisome proliferator-activated receptor-c and its downstream proteins and perilipin-2 by activating the pregnane X receptor in the liver to increase fatty infiltration into the liver. In addition, *via* activating NF-kB and *NLRP3* mediated signaling pathways they lead to inflammation.

Molecular mechanism doxorubicin induced hepatotoxicity: Recent studies has been observed that the major trigger for doxorubicin induced hepatotoxicity include oxidative stress, inflammation, apoptosis, and mitochondrial dysfunction.

The oxidative stress results from the generation of free radicals *via* Nrf-2/HO-1 pathway in which Nrf2 and HO-1 genes are down regulated responsible for antioxidant enzymes. Inflammation caused due to doxorubicin is *via* SIRT1/FOXO1/NF-κB signaling pathway. It has been seen to inhibit the SIRT1 activity there by activating of NF-κB signaling which leads to inflammation.

DNA fragmentation is the feature of apoptosis induced by doxorubicin which results from the activation of extrinsic and intrinsic signaling pathways. In the case intrinsic signaling pathway, the Bax initiates apoptosis through mitochondrial signaling which is activated by the release of apoptosis promoting factors such as cytochrome c, Apoptosis-Inducing Factor (AIF), and caspase activating factors which eventually lead to casp 3 activation resulting apoptosis. However, the extrinsic pathway is induced upon binding of Fas ligand to the Fas receptor which leads to trimerization and activation of caspase 8 which leads to Cas 3 activation results in apoptosis.

Doxorubicin causes mitochondrial dysfunction through deregulation of electron transport chain leads to decreased ATP/ADP ratio. Furthermore, mitochondrial biogenesis results from downregulation of PGC-1α and TFAM which is required for mitochondrial DNA replication.

DISCUSSION

Heavy metal induced hepatotoxicity

Heavy metals accumulation in the human body cause harm in the majority of organs especially in the liver. Mostly they induce oxidative stress in the hepatocyte which leads to an increase in the number of reactive oxygen species and a decrease in the level of antioxidants which *via* various mechanisms leads to cell death.

Several studies shown that exposure to heavy metals such as Pb, Cr, As, Hg, Ni, and Cd cause molecular as well as sub cellular and ultra-cellular structural changes. Data suggest that order of accumulation of heavy metals in the liver was Lead (Pb)>Cadmium (Cd)>Nickel (Ni)>Chromium (Cr).

Pb induces oxidative stress in the hepatocytes with the increase in free radicals along with lipid peroxidation and the decrease in various antioxidants (MDA, SOD, etc). In addition, it causes disruptions in the transcription of genes such as *NOX2* and *CYP2E1* and changes in the DNA, DNA methylation, mitochondrial stress and changes in the electron-transport chain and changes in the IRE1-JNK pathway and RER damage in the ER, nuclear pyknosis, inflammation and overexpression of NF-kB, and HMGR leading to changes in the cholesterol metabolism and hepatocytes necrosis.

Cr causes changes in transcription factors such as Nrf2 and mitochondrial dysfuction through a disruption in the ERK (ERK1/2) pathway, changes in PGC-1a, NRF-1 and TFAM genes, and changes in the transmembrane proteins ATF-6, IRE-1, and PERK as well as DNA cross link. It has also been seen that there has been a change in inflammatory factors such as IL-6 and IL-1b and generation of ROS that activate the p53 causes cell apoptosis.

As also causes a change in the transcription factors such as Nrf2 and Bach-1. It has shown to cause lipid peroxidation and produce reactive nitrogen and reactive oxygen species together with a decrease in the antioxidants CAT, GR, SOD, GPx. it leads to focal necrosis in hepatocytes and an increase in inflammatory cells, DNA hypomethylation and hypermethylation. It has been seen that through CHOP and PERK mechanisms arsenic causes apoptosis.

Mercury causes molecular changes in the liver incudes: Increase in the superoxides, reduced effects on the antioxidant system, lipid peroxidation, changes in the signaling of the Nrf2-Keap1 molecules as well as breaks in the DNA. Ultrastructural changes showed mitochondrial swelling, excess hydrogen peroxide formation, RER dilation, and changes in the phosphorylation of JNK and GRP78 protein activation. It has been seen that Hg toxicity leads to disruption in the level of cholesterol markers and promotes the process of apoptosis through downregulation in the expression of Bcl-2 proteins, activation of certain caspases, and the upregulation of expression of p53.

Similarly, cadmium and nickel cause hepatotoxicity through various molecular and ultrastructural changes in the liver such as Generation of ROS, changes in the antioxidant system, lipid

peroxidation, and genomic changes along with changes in the structure and function of mitochondria, ER, nuclear changes.

In addition, heavy metal exposure to the liver tissue causes Cell death *via* various molecular mechanisms. For instance, lead cause mitochondrial swelling, leading to cytc release and caspase activation and up regulation of Bax as well as Caspase8 and Caspase9 resulting apoptosis. Cr causes ROS activation which lead to activation of kinases enzyme which phosphorylate and activate p53 leading apoptosis. In case of Hg, increase in level of cytc, p53 overexpression, decrease in Bcl-2 expression and up regulation in the expression level of Bad lead to apoptosis in liver (Figure 12).

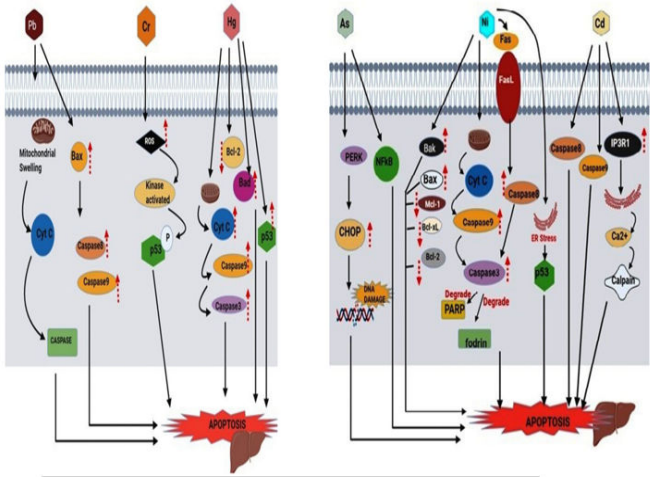


Figure 12: Molecular mechanism of heavy metal induced apoptosis in liver.

Food-born mycotoxins induced hepatotoxicity

Mycotoxins are toxic secondary metabolites produced by filamentous fungi and listed as major source of food-borne diseases by the World Health Organization. The most common mycotoxins found in food incude: Aflatoxins (AFs), Fumonisin B1 (FB1), Ochratoxins (OTs), Zearalenone (ZEN), Deoxynivalenol (DON), T-2 Toxin (T-2) and Patulin (PAT). Although the molecular mechanisms of hepatotoxicity induced by mycotoxins may be different for the different mycotoxins, it involve: Nuclear receptor regulation, effect on Cytochrome P450 (CYP450) and drug anitransporters, DNA damage, oxidative stress, inflammation, endoplasmic reticulum stress, mitochondrial dysfunction, autophagy, and change in intestinal-liver axis conduction.

The major mechanism of hepatotoxicity associated with AFB1 is stimulation of the upstream pathway Wnt/TGF-b/b-catenin to produce DNA damage (activation of CAR), oxidative stress (Nrf2/ARE/HO-1/GCLC), inflammation (IL-2, IFNc) and autophagy (beclin-1, ATG5, Dynein, LC3a, LC3b-I/II) and down-regulate p53 and mTOR, and induce hepatocyte apoptosis (FAS, TNFR1, XIAP and Bcl-2, Caspase-3).

Several literature shows that PAT causes hepatotoxicity mainly through the generation of oxidative stress (PIG3/CAT) that leads to DNA molecular damage (activation of PXR, p53 and MAPKs) induce autophagy-dependent apoptosis (LMP/LC3/CatB/Cyt-c/Casepase-3/PINK1). Similarly, the main mechanism of hepatotoxicity for DON is the oxidative stress (Nrf2/HO-1) leads to the activation of liver injury transcription factors (JUN, FOS, EGR1 and ATF3), inflammation (NF-kB) and cell apoptosis (ATF3, caspase-3, caspase-7).

Studies also shown that the main mechanism responsible for OTA hepatotoxicity is the activation of PI3K/AKT through DNA molecular damage (activation of AhR, imbalance of hhex, prox1 and miR-122 molecules) leading to oxidative stress Nrf2/Keap1 and induce mitochondrial-dependent apoptosis CCNG1/p53 pathway and Bcl-w/caspase-3).

The main mechanism FB1 induced hepatotoxicity is the inflammatory response (IFN-c, TNF-a and IL-12) results from the accumulation of sphingosine base through PP2A/PI3K/AKT/PTEN at the level of ceramide, endoplasmic reticulum stress IRE1a axis induced by ROS also leads to apoptosis (JNK, p38 MAPK, ERK and JNK phosphorylation) and autophagy (inhibition of mTOR and activation of LC3I/II).

ZEN induced hepatotoxicity is through the activation of liver nuclear receptor PXR, oxidative stress (Nrf2/ARE/HO-1 NQO1) leads to autophagy of the endoplasmic reticulum stress pathway (GRP78 and CHOP, and eIF2a phosphorylation, beclin1 and LC3) and mitochondrial pathway-dependent apoptosis (PTPC-p53, Bcl-2).

New therapeutic targets for hepatotoxicity

New therapeutic targets in alcoholic liver disease: In recent years, investigational therapies for the treatment of ALD have focused on the aforementioned pathophysiologic mechanisms.

Experimental have been developed with the goal of inhibiting inflammatory pathways for instance, blockade of gut liver axis stimulation, inhibiting oxidative stress, stopping apoptosis, and encouraging liver regeneration. More recently, attention has increasingly focused in targeting metabolic pathways with the aim of inhibiting steatosis.

Inhibition of inflammatory pathways: IL1 inhibition: Canakinumab is a fully human monoclonal antibody which inhibits inflammation by targeting IL-1β and subsequently, the IL-6 signaling pathway. A multicenter Randomized Controlled Trial (RCT) examined the effect of canakinumab in 48 patients with biopsy confirmed AH. When Compared to the placebo group, receiving canakinumab showed a higher rate of histological improvement (58.3 versus 41.7%).

Anakinra, an IL-1 receptor antagonist inhibits IL-1α and IL-1β biologic activity. results from alcoholic steatohepatitis trial, multicenter trial, evaluating the efficacy of Anakinra in combination with zinc and pentoxifylline versus prednisolone in the treatment of severe AH, showed decrease death rate at 180 days.

Modulating gut–liver axis and inflammation: Fecal microbiota transplant: The possibility of using Fecal Microbiota Transplant (FMT) has been investigated to ameliorate gut dysbiosis in AH.

An open-label experimental trial evaluating 90-day survival in corticosteroids, pentoxifylline, nutritional therapy, or FMT in male patients with AH showed that the group treated with FMT had lower rate of death at 1 month and 3 months compared to all the other groups combined.

Phage therapy: Patients with AH showed to have higher fecal counts of cytolysin producing *E. faecalis* species, which is linked with increased severity and death rate in AH. In a recent study shows that, using a bacteriophage to target this cytolysin eliminate ethanol-induced liver disease in humaized mice.

Inhibition of oxidative stress: Antioxidants: N-acetylcysteine (NAC) is an antioxidant has been shown beneficial in AH when combined with corticosteroids. In animal model ethanol-induced glutathione depletion was prevented by metadoxine, a combination of pyridoxine and pyrrolidone carboxylate. An open label randomized control trial comprising seventy patients randomized to glucocorticoids alone versus metadoxine plus glucocorticoids demonstrated an improvement in mortality at 30 and 90 days, and a decrease in encephalopathy in the metadoxine-treated group.

Hepatic regenerating agents: Interleukin-22: IL-22 is a member of the IL-10 family that has pro-proliferative, antioxidant, antis teatotic, and antimicrobial properties. Treatment with IL-22 has previously been shown in murine models to ameliorate alcohol-induced liver injury.

Regulators of de novo lipogenesis: FXR receptor agonists have beneficial effects in reducing *de novo* lipogenesis. for instance, obeticholic acid is a semisynthetic FXR agonist has shown promise in the treatment of metabolic-associated fatty liver disease MAFLD by regulating homeostasis and reducing accumulation of toxic bile acids. However, a phase II RCT evaluating its role in ALD was terminated due to hepatotoxicity concerns.

PPAR agonists: PPARs-α, -δ, and -γ play primary role in the liver, muscle, and adipose tissues, respectively. Targeting these receptors leads to oxidative fatty acid metabolism and energy disposal. Bezafibrate and pioglitazone are PPAR-α and PPAR-δ agonists shown varying degrees of promise in MAFLD trials.

CYP2E1 inhibitors: Alcohol is metabolized by CYP2E1 and triggering ROS production which is directly related to the development of ALD. Inhibition of CYP2E1 effectively reduces alcohol-induced lipid peroxidation and lessens liver injury. The goal of Targeting CYP2E1 is to prevent the production of toxic acetaldehyde and the subsequent oxidative stress related with ALD. Chlormethiazole is a medication demonstrated to blocks CYP2E1 activity, possibly prevents the generation of acetaldehyde and oxidative stress in ALD models. CYP2E1 activity also blocked by silibinin, flavonoid, causes reduction oxidative stress and liver injury in ALD models, which may be responsible for its hepatoprotective properties.

TLR4: Recent studies show that recognition of LPS by TLR4 on the sinusoidal epithelial cells and HSCs, accelerated the development of ALD. targeting TLR4 by small-molecule inhibitors leads to blockage of TLR4 signaling and consequently prevented the generation of inflammatory cytokines that generate inflammation.

Eritoran is selective antagonists of the receptor TLR4, which have shown beneficial effects in preclinical models of ALD. curcumin, possesses TLR4 inhibitory properties and has been shown to inhibit TLR4 signaling and reduce liver inflammation in ALD models.

Apoptosis Signal-Regulating Kinase 1 (ASK1): Apoptosis Signal-Regulating Kinase 1 (ASK1) is a one well known mediator of fibrosis, inflammation, and cell death. preclinical studies show that the ability of a number of inhibitors to stop ASK1-mediated signaling pathways, lower inflammation, and lessens liver injury in models of ALD. Selonsertib (also known as GS-4997) is an investigational small-molecule inhibitor of ASK1. For instance, in various disease models NQDI-1, ASK1 inhibitor, has shown potential in ALD. ASK1 expressed in different tissues and acts as an upstream regulator of c-Jun N-terminal Kinase (JNK) and p38/Mitogen-Activated Protein Kinase (MAPK)-mediated diseases. Inhibition of p38/MAPK has shown promising results in reducing liver inflammation and fibrosis associated with ALD. SB203580 and BIRB 796 are p38/MAPK inhibitors shown beneficial effects in reducing liver injury and inflammation associated with ALD.

Cannabinoid receptor: CB1 and CB2 have been investigated as possible drug targets for ALD treatment. drugs that are both CB-1 and CB-2 agonists may protect against alcohol-induced liver damage through various mechanisms. SR141716A acts as a CB1 receptor inverse agonist, which inhibit CB1 receptor activity and has been investigated in various research studies focusing on the effects of CB1 receptor modulation in liver disease. JD5037 is a selective CB1 receptor antagonist/inverse agonist and has shown to lessen hepatic inflammation, oxidative stress, and fibrosis in experimental ALD. Otenabant (CP-945, 598) is a dual CB1/CB2 receptor modulator that has been investigated for its potential role in ALD treatment.

SCD1: SCD1 is involved in the production of oleic acid and other monounsaturated fats.

In preclinical models of ALD, A939572, a selective small-molecule inhibitor of SCD1, has shown efficacy in reducing inflammation and liver steatosis (fat accumulation).

Liver X Receptor: Liver X Receptor (LXR), a nuclear transcription factor, is essential for controlling cholesterol and lipid metabolism, which has been identified as a potential drug target for ALD. Through promoting the efflux of cholesterol and triglycerides from hepatocytes LXR activation can restore lipid homeostasis there by reducing hepatic steatosis (fatty liver). Various synthetic LXR agonists have been developed and evaluated in preclinical studies for their potential therapeutic benefits in ALD. For instance, T0901317 is a synthetic LXR agonist and has shown promising results in reducing hepatic steatosis, inflammation, and fibrosis in animal models.

MicroRNAs: Blockers of miRNA function or stabilization of specific miRNAs may be considered as the therapeutic targets for ALD treatment. Small molecules such as Aurintricarboxylic Acid (ATA) and NSC207895 have shown inhibitory effects on

miRNA activity by directly binding to miRNAs and interfering with their function but currently there are no widely established small molecule-based therapies for miRNA modulation in ALD (Table 1).

Table 1: Currently identifying agents and their target pathway in ALD.

Agents (Intervention)	Target pathway
FMT	Gut-liver-axis modification
NAC	Oxidative stress inhibition
Ademethionine	Oxidative stress inhibition
Bovine colostrum	Gut-liver-axis modification
TAK-242, Eritoran, Curcumin	Inflammatory pathway inhibition (TLR 4 signaling inhibitor)
DUR-298	Liver regeneration boosting
G-CSF	Liver regeneration boosting
VSL#3112.5 capsule	Gut-liver-axis modification
Cytosorb treatment	Inflammatory pathway inhibition
Ommaga fatty acid	Influencing metabolic pathway PPAR Y agonist
Chlormethiazole, Silibinin	CYP2E1 inhibitors
Selonsertib	Inhibitor of Apoptosis Signal-Regulating Kinase 1 (ASK1)
SB203580 and BIRB 796	Inhibition of p38/MAPK pathways
Otenabant, JD5037	Cannabonoid receptor modulator
A939572	inhibitor of SCD1

Recent therapeutic targets in drug induced hepatotoxicity

ATF4-ATF5-CHOP pathway: CHOP is a marker of ERS, and its inhibition can effectively reduce the consequences of ERS. recent studies have been observed that CHOP antibodies, and CHOP inhibitors are pivotal targets for DILI therapeutic approaches.

JNK signaling pathway and NF-κB pathway: ERS activates JNK which is inhibited by several compounds that used in preclinical as well as clinical practice to protect liver injury caused by various drugs. NF-κB is a central link between liver injury, fibrosis, and hepatocellular carcinoma and represents a target for the prevention or treatment of DILIs.

UPR pathway: This stress response can be suppressed by inhibiting the expression of three proteins namely; PERK, IRE1, and ATF6 which can cause different degrees of liver damage by mediating the phosphorylation of eIF2α. Studies found that many compounds like GSK2606414, PF-429242 and Ceapin that inhibit PERK, IRE1, and ATF6 respectively are currently used to regulate UPR.

Gut–liver axis: Numbers of studies have found that gut–liver axis dysfunction is involved in the onset and development of DILI and significant for the treatment of DILI. Recently several herbs and phytochemicals, probiotics, fecal microbial transplantation, postbiotics, bile acids, and Farnesoid X receptor agonists improve Gut–liver axis function and investigated as potential therapeutic strategies for DILI.

ChREBPα-FGF21 axis: Fibroblast Growth Factor 21 (FGF21) is hormone secreted by a hepatocyte that is important in the metabolism of glucose and lipids. It induces hepatic expression of the Nuclear Factor Erythroid 2 (NRF2) and peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), which are involved in the response to oxidative stress. FGF21 is one of the targets of Carbohydrate Response Element Binding Protein (ChREBP). Fructose reduces oxidative stress caused by the accumulation of APAP-protein adducts by activating antioxidative genes through the ChREBPα-FGF21 axis.

G Protein-Coupled Receptor 116 (GPR116): GPR116 inhibits the Binding immunoglobulin Protein (BiP) *via* its interaction with β-arrestin1, thereby mitigating ER stress during the early stage of AILI. FNDC4 suppresses ER stress *via* activation of

GPR116 has been shown to confer a protective effect against early hepatotoxicity caused by APAP in murine model.

GADD45α: Growth Arrest and DNA Damage-inducible 45α (GADD45α) showed preventive effect against APAP-induced liver injury and alleviated the accumulation of small lipid droplets *in vitro* and *in vivo*. Studies found that GADD45α cause the activation of AMP-activated protein kinase α and induced fatty acid beta-oxidation. GADD45α interact with Ppp2cb, serine/threonine phosphatases, can bind directly with AMPKα leading to the dephosphorylation/activation of AMPK (Figure 13).

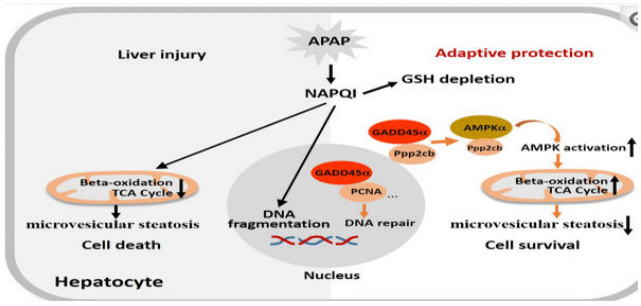


Figure 13: GADD45α allivavte paracetamol induced hepatotoxicity *via* promoting AMPK activation.

NLRP3 inflammasome: Nucleotide-binding Oligomerization Domain (NOD)-like receptor family pyrin domain containing 3(NLRP3) inflammasome is an intracellular protein complex that induces cellular injury by assembly of NLRP3, apoptosis-Associated Speck-like protein (ASC), and pro-caspase-1. It is involved in the generation and reduction of drug-induced liver toxicity through multiple signaling pathways (Figure 14).

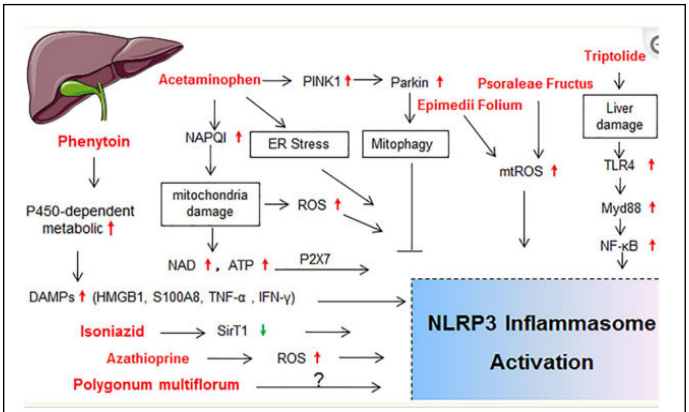


Figure 14: Molecular mechanism of NLRP3 activation in DILI.

Acetaminophen-induced hepatotoxicity in mice require P2X7R activation provoked by ATP and NAD and it initiate stimulation of PTEN-induced Putative Kinase 1 (PINK1)/Parkin-mediated mitophagy in mouse liver. Protective effects of the phytochemicals through inhibiting NLRP3 inflammasome has been shown to be promising therapeutic strategies in several literatures (Table 2).

Table 2: Summary of recent therapeutic targets with their promising compounds to be used for hepatotoxicity.

Chemicals/ Drugs causing DILI	Recent target molecule/pathways	Investigating inhibitor compounds	Proposed mechanism
	JNK pathway	SP600125	JNK inhibitor
		GS-444217	ASK1 inhibitor
	NF-κB pathway	MG-132	Protease inhibitors
	UPR pathway	GSK2606414	PERK inhibitors
		PF-429242	IRE1 inhibitors
		Ceapin	ATF6 inhibitors
		BIX	Bip inducer
Acetaminophen Methotrexate and Antituberclosis	Gut-liver-axis	Wolfberry	Upregulate tight junction expression, activating Nrf2 pathways
		Oridonin	
		MgIG	Inhibiting toTLRs/NF-κB pathways
Valporic acid, Pyrazinamide, Methaphetamine	FXR receptors,	Obticholic acid	FXR agonist,
	BA hemostasis		Improve BA homeostasis
Acetaminophen	FXR receptor	Kampeferol-7-rhaminoside, Ginsenoside	Up regulate FXR receptor gene expression
Tripterygium-Glycoside	PKM2 regulated oxidative stress	Glycyrrhizic acid	PKM2 inhibitor

Cadmium	Signaling pathway modulating oxidative stress, inflammation, and apoptosis	*Opuntia dillenii* Polysaccharide	Nrf2 activation NF-κB inhibition, NLRP3 inflammasome suppression: Bcl-2/Bax regulation, Caspase-3, Caspase-9 inhibition MAPK (JNK, p38) pathway modulation, ER stress reduction PI3K/AKT pathway activation
Triptolide	SIRT1/HIF-1α signaling pathway	Catalpol	Activating SIRT overexpression, mitochondrial dysfunction and reduced glucose metabolism disorder and oxidative stress
Acetaminophen	GPR116	FNDC4	inhibition of ER stress *via* the β-arrestin1-BiP signaling pathway
Methotrexate	TGF-β/NF-κB and JAK/STAT3 pathways	Araucaria Heterophylla Resin (AHR)	Elevation in the antioxidant's markers (SOD, HO-1, and NQO1) ihibit the TGF-β/NF-κB signaling pathway as well as the downstream cascade (IL-6, JAK, STAT-3, and cyclin
CCl_4, acetaminophen methotraxate	Pro-fibrotic and pro-inflammatory agents	*Levisticum officinale* extract (apigenin and silibinin)	Decrease pro-fibrogenic and pro-inflammatory mRNA expression such as TNF-α, TGF-β1, IL-6, COX-2, and MCP-1
Acetaminophen	ChREBPα-FGF21 axis, CYP2E1 and GSH gene	Fructose	Increase the expression of antioxidant genes decrease the rate of conversion of APAP to NAPQI
Phyntoin Acetaminophen Isoniazid Azathioprine Triptolide *Psoraleae fructus* *Epimedi folium*	NLRP3 inflammasome	Rapamycin (paracetamol)	Inhibit the activation of NF-κB, NLRP3 inflammasome, and the production of IL-1β
Bosutinib and nilotinib	NLRP3 inflammasome	MCC950, N-acetyl-l-cysteine	Inhibit the effects of these drugs on the NLRP3 signaling pathway
Methotrexate	TLR4/NF-κB pathway and NLRP3 inflammasome/caspase 1 axis	Dihydromyricetin	Inhibit cytokine formation of these interleukins through suppressing TLR4/NF-κB pathway and NLRP3 inflammasome/caspase 1 axis
Acetaminophen	Liver metabolism	Zileuton	Suppressing CYP3A11 by inhibiting 5-LO
		Tea polyphenols	Reducing CYP2E1 and CYP1A2 expression
	Mitochondrial oxidative stress and dysfunction	Metformin	Inhibiting JNK phosphorylation
		Docosahexaenoic acid and fenofibbate	Activating PPARα

	Sterile inflammation	Benzyl alcohol	Reducing the release of IL-1 and IL-18 in TLR4-dependent way
	Endoplasmic reticulum stress	4-Phenylbutyric acid, simastatin, Ozagrel hydrochloride	Inhibiting/downeulating CHOP expression
		Guanabenz	Reducing ERS by inhibiting the proteolysis of ATF6
Chemotherapeutic agents (Cisplatin, doxorubicin, cyclophosphmide) Methotrexate	miRNAs	(Curcumin, resveratrol, quercetin, and silymarin)	Modulating miRNAs (Reduces oxidative stress, inhibits apoptosis, promotes liver regeneration),
		Ginkgo biloba extract	Modulation of the IL-6/STAT3 signaling pathway and the downregulation of the miRNA-21 hepatic expression
Cyclophosphamide	PPAR-γ/Nrf-2/NF-κB signaling pathway	Metformin plus hesperidin	Upregulation of Nrf-2, PPAR-γ, Bcl-2 expression, hepatic GSH content, and marked suppression of TNF-α and NF-κB expression
Aflatoxin B_1	JNK pathway NF-κB signaling pathway apoptotic pathways Mitochondrial apoptotic pathway	Baicalin Luteolin (LUTN) Curcumin	Inhibit AFB1-induce c-Jun-N-terminal Kinase (JNK) activation and cell apoptosis Lowering the production of Malondialdehyde (MDA) and Reactive Oxygen Species (ROS), inhibit NF-κB activation inhibits caspase-3 and caspase-9 regulation of p53, caspase-3, Bax, caspase-9, Bcl-2 and cytochrome-C
T-2 toxin	Nrf2 pathways	Resveratrol (Res)	Modulated Nrf2-mediated antioxidant pathway and glutathione synthesis inhibition
CCl_4	Cystine/glutamate antiporter in the Xc- system	Luteolin (LUTN)	Suppressing ferroptosis regulating *via* SLC7A11 expression
Alcohol	NRF1-TFAM signaling pathway	Nobiletin	Restored the reduction of hepatic Mitochondrial Transcription Factor A (TFAM) at both mRNA and protein levels reinstated the levels of hepatocellular NRF1 restored the decrease of Sirt1/Bcl-2 expression, restrained the elevation of caspase3, cleaved caspse-3, p53 and ac-p53 expression
	Sirt1/p53 signaling pathway	Vitexin	

Various chemicals, including alcohol, drugs, heavy metals, and foodborne mycotoxins cause hepatotoxicity through shared molecular pathways as well as distinct molecular mechanism. Oxidative stress, mitochondrial dysfunction and inflammation are common mechanism among different agents. For instance, oxidative stress, occurs when the regulation mechanisms that control the cellular levels of ROS break down, is caused by alcohol and acetaminophen (APAP) metabolism leading to inflammation and apoptosis. Drugs also activate inflammatory pathways, such as NF-κB and JNK signaling which lead to inflammation. Similarly alcohol and heavy metals induce immune responses that lead to liver inflammation.

Different agents affect distinct pathways for causing liver injury. For instance, acetaminophen results significant oxidative stress *via* toxic metabolite (NAPQI) while methotrexate cause hepatotoxicity primarily through mitochondrial oxidative stress

and inflammatory pathways. Alcohol mainly causes steatosis and inflammation by enhancing lipid synthesis and promoting alcoholic steatosis and inflammation *via* activating CYP2E1 pathway.

Heavy metal cause liver injury through direct interaction with biomolecules via pathways distinct from those activated by other drugs while as myocotoxins like aflatoxins, cause liver injury predominantly through mechanisms involving DNA damage and disruption of normal cellular signaling pathways, such as Wnt/β-catenin.

Based on regulatory factors, a number of studies suggested possible therapeutic interventions for alcoholic liver injury. Alcohol induced oxidative stress or lipid peroxidation induces inflammation and apoptosis which leads to the production cytokines and chemokine. For instance, an IL-1β receptor agonist and a chemokine chemo attractant cell receptor-2/5 antagonists like anakinumab and cenicriviroc respectively have been investigated for clinical use.

Promoting the expression of anti-inflammatory factors is another advance in treatment of ALD. Researches indicate that IL-22 has been shown to prevent the liver against oxidative stress and fibrosis. Furthermore, the therapeutic effect of recombinant IL-22 protein drug, F-652, the on AH was established. Studies also found that suppressing NF-κB by kahweol, acanthoid acid, clomethiazole, and fucoxanthin can effectively decrease inflammatory response and liver injury induced by alcohol.

Another therapeutic option in treating alcoholic liver disease is to regulate the balance between oxidants and antioxidants level. Many Findings have showed that induction of NRF2 which regulate antioxidant system is target for the prevention or treatment of ALD. Bardoxolone, Omaveloxolone, Oltipraz, Liraglutide, Resveratrol, Ursodiol, and Curcumin are promising potential drugs target and currently investigating in pre-clinical and clinical studies. Currently, all of these drugs are testing in phase II and below except Ursodiol which is in phase IV studies. Studies indicate that with the administration of Ursodiol there was improvement in LFT, levels of antioxidant proteins, and hepatic expression levels of both total and phosphorylated Nrf2.

Targeting hepatocyte proliferation and apoptosis is another novel therapeutic strategy for ALD. Inhibiting p53 and other apoptosis-related signaling pathway activation can Ethanol-induced apoptosis of liver cells. For instance vitexin, flavonoids compound, decreased ethanol induced liver damage through restoring the decrease of Sirt1/Bcl-2 expression, restraining the elevation of caspase3, p53 and ac-p53 expression *in vitro* and *in vivo*.

Another novel therapeutic target for alcohol induced liver injury is NRF1-TFAM signaling pathway. Hepatic Mitochondrial Transcription Factor A (TFAM) is one of the most abundant DNA-binding protein found inside mitochondria that regulate mtDNA replication, transcription, and packaging. NRF1 is a crucial nuclear transcription factor responsible for positively regulating TFAM genes. Numerous studies have demonstrated that alcohol-induced reduction of hepatic TFAM levels leads to ALD and hepatocyte-specific over expression of Nrf1 protect against alcohol-induced hepatic TFAM reduction. According to one study Nobiletin (NOB), flavone sourced from citrus fruit peels, had the ability to protect chronic-plus binge alcohol-induced mitochondrial dysfunction and liver injury by restoration of hepatic TFAM through regulating Nrf1 activation.

For the treatment of ethanol-induced hepatocyte apoptosis, targeting miR-155 and miR-122 are therapeutic option. For instance, one study discovered that in mice, ethanol-induced liver injury is reduced by gene therapy that induced miR-122 activity. In both human livers and murine hepatocytes, chronic alcohol intake reduces MIR122 levels and that this reduction contributes to, steatosis, inflammation and liver damage in ALD. According to this study, a novel mechanism of alcohol-induced inhibition of MIR122 is the overexpression of grainyhead like transcription factor 2 (GRHL2). Furthermore, MIR122 appears to prevent the liver from ethanol-induced damage by lowering levels of hypoxia inducible factor 1 alpha (HIF1A) which were raised in patients and mice with ALD.

NLRP3 inflammasome has been demonstrated in drug-induced hepatotoxicity toxicity of paracetamol, azathioprine, phenytoin, isoniazid, triptolide and others through inducing different forms of cell death and inflammatory responses. Moreover, Liver injury is mediated by the secreted inflammatory factors (mainly IL-1β) and recruitment of inflammatory cells.

Blockage of NLRP3, NF-κB, P2X7R, caspase-1, TLRs, IL-1β, and IL-18 by natural products inhibit NLRP3 inflammasome activation. For example MCC950 which is the most effective inhibitor, kaempferol, quercetin, and chicoric acid shown to have beneficial effects on drug-induced liver toxicities.

Current therapeutic option for hepatotoxicity lacks specificity for a particular molecular target, on the other hand targeted therapy like JNK and ASK1 inhibitors reduce apoptosis and inflammation but non-hepatic tissues toxicity is a problem. Other limitations in Current therapeutics is that they are more effective in early stages of liver disease, such as steatosis or mild inflammation, but show limited efficacy in advanced state.for example inhibitors of CYP2E1 or NF-κB pathways have failed to reverse extensive fibrosis or cirrhosis.

Owing to multiple pathways involved in hepatotoxicity combining drugs that target these pathways is important but lack of robust clinical data supporting the efficacy and safety of such combination therapies.

However, the complex molecular pathways in the liver due its central role in metabolism, detoxification and immune regulation pose a significant challenge in identifying specific targets without disrupting the liver's functions. Another challenge is inconsistent of translation from animal model to human particularly drugs targeting pathways like JNK or Nrf2. they demonstrated promising preclinical results but human trials have not showed similar benefits.in addition to this, conducting clinical trials create ethical challenge for conducting clinical trials in patients with liver disease that delays in translating promising preclinical findings into clinical trial. For some therapeutic target, innovative delivery systems are required to achieve effective concentrations in the liver without causing systemic toxicity. They are recent research area, but their

translation to clinical use create problem due to long-term safety, cost, and other issues.

CONCLUSION

Liver is essential organ for detoxifying xenobiotic and chemicals. However, molecules that can cause liver injury through various pathways are created during this biotransformation. The molecular mechanism of DILI is quite complex. The mechanism of alcohol induced hepatotoxicity ranging from simple stenosis to hepatocellular carcinoma which is linked to acetaldehyde as well as o cytochrome P450 2E1. hepatocytes, stellate cells, Kupffer cells, and liver sinusoidal endothelial cells are target of Reactive Oxygen Species (ROS) produced by *CYP2E1* like the superoxide radical HO_2, Hydrogen Peroxide (H_2O_2), and Hydroxyl radical (HO), and their signaling mediators induce liver injury.

Recent evidences suggest ROS and endoplasmic reticulum stress generate lipid peroxides, can initiate and perpetuate alcoholic liver injury. There are also recent evidences indicating the role of endotoxins that originate from intestinal bacteria and the leaky gut in the pathogenesis of alcoholic liver injury.

More recent studies focus on suppression lipid metabolism, protein expression and inflammatory responses through regulation of nuclear genes of Sirtuin-1 (*SIRT1*) related with alcohol. I have identified recent therapeutic targets in alcohol hepatotoxicity in including liver X receptor, microRNAs, cannabinoid receptors, *ASK1*, PPAR agonist and others.

DILI is a potentially fatal adverse reaction to a drug with complex molecular mechanism. Based on the pattern of injury, it is classified as hepatocellular, cholestatic, and mixed. Based on mechanism it can be classified as direct (dose-dependent, intrinsic, and predictable, idiosyncratic (largely dose-independent and unpredictable) and indirect DILI.

Recent important advances in the understanding of general molecular hepatotoxic mechanisms are the foundation for further studies on mechanisms for specific hepatotoxic drugs. Regulation of downstream events of injury may be used as promising new therapeutic targets. For instance specific JNK inhibitor, inhibition of protective cytokines and Bcl-2 proteins, specific caspase inhibitors to suppress apoptosis have been developed.

With the focus on decreasing adverse effect with current treatment option, future research should give an emphasis on developing pharmacological agents that directly target these molecular pathways.in addition, knowing how the gut-liver axis contributes to the development of drug and alcohol induced liver disease may open new insight for probiotics or microbiome-based therapies.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

COMPETING INTERESTS

The author(s) declare(s) that there are no conflicts of interest regarding the publication of this paper.

FUNDING

No funding was received for this study.

AUTHORS' CONTRIBUTIONS

All authors contributed to the search for relevant literature and the composition of the manuscript. Each author critically reviewed and approved the final draft of the manuscript.

REFERENCES

1. Schulze RJ, Schott MB, Casey CA, Tuma PL, McNiven MA. The cell biology of the hepatocyte: A membrane trafficking machine. J Cell Biol. 2019;218(7):2096–2112.
2. Pandit A, Sachdeva T, Bafna P. Drug-induced hepatotoxicity: A review. J Appl Pharm Sci. 2012;2:233–243.
3. Deng X, Luyendyk JP, Ganey PE, Roth RA. Inflammatory stress and idiosyncratic hepatotoxicity: hints from animal models. Pharmacol Rev. 2009;61(3):262–282.
4. Kedderis GL. Biochemical basis of hepatocellular injury. Toxicol Pathol. 1996;24(1):77–83.
5. Yan C, Hu W, Tu J, Li J, Liang Q, Han S. Pathogenic mechanisms and regulatory factors involved in alcoholic liver disease. J Transl Med. 2023;21(1):300.
6. Caballeria J, Frezza M, Hernandez-Munoz R, DiPadova C, Korsten M, Baraona E, et al. Gastric origin of the first-pass metabolism of ethanol in humans: effect of gastrectomy. Gastroenterology. 1989;97(5):1205–1209.
7. Frezza M, di Padova C, Pozzato G, Terpin M, Baraona E, Lieber CS. High blood alcohol levels in women: the role of decreased gastric alcohol dehydrogenase activity and first-pass metabolism. N Engl J Med. 1990;322(2):95–99.
8. Hall PdlM, Lieber CS, DeCarli LM, French SW, Lindros KO, Jarvelainen H, et al. Models of alcoholic liver disease in rodents: a critical evaluation. Alcohol Clin Exp Res. 2001;25:254S–261S.
9. Teschke R. Alcoholic steatohepatitis (ASH) and alcoholic hepatitis (AH): cascade of events, clinical aspects, and pharmacotherapy options. Expert Opin Pharmacother. 2018;19(8):779–793.