Pharmacological Underpinnings of Hepatotoxicity lead by Drugs

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Abstract: Hepatotoxicity leads to a major concern in clinical pharmacology and a leading cause of acute liver failure and drug withdrawal from the market. The pharmacological basis of hepatotoxicity involves complex interactions between drugs, their metabolites, and liver cellular mechanisms. Hepatotoxicity can arise from intrinsic (dose-dependent) or idiosyncratic (unpredictable) reactions, often influenced by genetic, environmental, and physiological factors. Key mechanisms include oxidative stress, mitochondrial dysfunction, disruption of bile acid homeostasis, and activation of immune-mediated responses. Metabolism by hepatic enzymes, particularly cytochrome P450 isoforms, can lead to the formation of reactive metabolites that bind covalently to cellular macromolecules, triggering hepatocellular injury. Additionally, variations in drug transporters and conjugation pathways may exacerbate toxicity. Biomarkers and advanced *In Vitro* models are increasingly used to predict hepatotoxic potential during drug development. Understanding the pharmacological and molecular underpinnings of drug-induced liver injury is essential for the development of safer therapeutics and effective risk mitigation strategies. This review emphasizes the need for a multidisciplinary approach combining pharmacokinetics, toxicodynamic, genomics, and systems biology to better predict, prevent, and manage drug-induced hepatotoxicity in clinical practice.

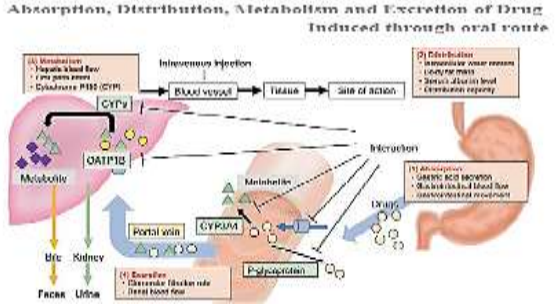
KEYWORDS: Idiosyncrasy, Hepatocellular Injury, P450, ALT, Acute Liver Failure, AST.

Introduction:

Liver damage brought due to drugs is a leading cause of acute liver failure and is responsible for a significant percentage of drug withdrawals and it is a disease of ongoing worldwide research [Gerussi et al, 2021; Liv et al, 2021]. Acute Liver Failure (ALF) is the resultant of Drug induced liver injury [Lee WM, 2013]. This arises due to direct toxic effects, metabolic bioactivation to reactive intermediates, immune-mediated responses, mitochondrial dysfunction, or idiosyncratic mechanisms [Jee et al, 2021]. The medications most frequently implicated in the pathophysiology of Drug Induced Liver Injury are listed in great detail, however new ones are being found in clinical practice [Katarey and Verma, 2016]. Understanding the pharmacological basis- how drug properties, metabolism, host factors, immune elements, and cellular pathways interplay is vital in predicting, preventing, and managing hepatotoxicity. [Skat-Rørdam et al, 2025]

Epidemiology and Clinical Presentation:

Drug induced Hepatotoxicity incidence is roughly ~10 per one lakh patient-years, though under diagnosis is common [Francis and Navarro, 2025].



It manifests in various patterns [Zhang et al, 2013]:

Hepatocellular injury: Elevated ALT/AST.

Risk Factors:

- Age and sex (some drugs more hepatotoxic in certain demographics)
- Genetic predisposition
- Pre-existing liver disease
- High-dose drug exposure

Clinical Presentation

Drug-induced liver injury presents in different patterns based on the type of liver damage [Bjornsson HK and Bjornsson ES, 2022].

Laboratory Findings:

Markedly elevated ALT and AST (alanine and aspartate aminotransferases).

ALT often >3x upper limit of normal (ULN).

Symptoms:

Fatigue, malaise, Nausea, anorexia, Jaundice in severe cases

Cholestatic Injury: Injury primarily affecting bile ducts or bile flow.

Laboratory Findings:

Elevated alkaline phosphatase (ALP) and bilirubin.

ALP typically >2x ULN.

Symptoms:

Jaundice, Pruritus (itching), Dark urine, pale stools

Blood reports shows elevated ALT, AST, ALP, and bilirubin

Table 1: Key Aspects of Hepatotoxicity associated with Drugs (Kobayashi et al, 2023; Gafar et al, 2019)

Sr. no.	Categories	Details	Duration of cause Hepatotoxicity
1.	Incidence	• ~0.2% cases in one lakh persons annually in the general population • ~1 in about 10,000 prescription • Main reason of acute liver failures	Drugs vary from hours to months to cause Hepatotoxicity. Majority of symptoms recovered through withdrawal of drugs. At the same times observes liver enzymes when indicated. Avoiding high-risk drugs in vulnerable populations
2.	Population/community at risk	• Adults > elderly more commonly affected • Women may be at slightly higher risk • Patients with polypharmacy, underlying liver disease • - Genetic predisposition (e.g. HLA alleles)	
3.	Usually, drug causing Hepatotoxicity	• Acetaminophen (dose-dependent toxicity) • Antibiotics (e.g., amoxicillin-clavulanate, isoniazid) • Antiepileptics (e.g., valproate, phenytoin) • Herbal & dietary supplements	
4.	Type of liver injury	• Hepatocellular (Elevate ALT > AST) • Cholestatic (Increases ALP, bilirubin) • Mixed pattern	

Clinically, it can range from asymptomatic enzyme elevations to acute liver failure. Timing varies to some drugs cause rapid injury (acetaminophen within hours to days), others appear after weeks or months. Risk factors span dosage, lipophilicity, metabolic pathways, patient genetics, age, sex, comorbidities (fatty liver) and comedications [Juanola et al, 2021].

Pharmacokinetics & Drug Properties [Kaplowitz N, 2004; Uyen et al, 2005]

1. Dose & Duration

High doses and prolonged therapy often raise Liver Injury risk. With acetaminophen, for example, toxicity increases above ~4 g/day.

2. Lipophilicity & Tissue Penetration

Drugs with high lipophilicity accumulate in hepatocytes, increasing potential for local toxicity.

3. Chemical Structure & Bioactivation

Drug structure (e.g., aromatic rings) determines propensity for conversion into electrophilic reactive metabolites that form adducts with proteins/nucleic acids.

Hepatic Metabolism & Bio-activation:

1. Phase I Reactions

Cytochrome P450s (CYP1A2, CYP2E1, CYP2C9, CYP3A4) oxidize drugs to potentially reactive intermediates. For instance, Acetaminophen → NAPQI: Overdose shifts conjugation to CYP-mediated formation of toxic NAPQI. Aflatoxin/isoniazid similarly undergo bioactivation yielding reactive moieties [Mazaleuskaya et al, 2015].

2. Phase II Conjugation

Glutathione (GSH), glucuronic acid, and sulfate conjugate drug metabolites for excretion. With depletion or saturation (e.g., GSH depletion by NAPQI), reactive intermediates accumulate.

3. Detoxification & the Balance

Equilibrium exists between formation of reactive metabolites and detoxification capacity. Genetic variability or overconsumption can shift this balance toward injury.

Mechanisms of Hepatocyte Injury [Kumar et al, 2024]

1. Direct Cytotoxicity

Excess reactive metabolites bind cell macromolecules, impairing mitochondrial function, oxidizing lipids, and compromising cell viability.

2. Oxidative Stress & Mitochondrial Injury

Reactive oxygen species (ROS) from mitochondrial damage or CYP enzymes provoke lipid peroxidation, DNA damage, and activate apoptosis via cytochrome c release. Drugs like valproic acid disrupt β-oxidation and cause mitochondrial permeability transitions directly.

3. Endoplasmic Reticulum (ER) Stress

Misfolded proteins, due to covalent binding by reactive metabolites, accumulate in the ER, triggering stress pathways (PERK, ATF6, IRE1), leading to apoptosis [Chen et al, 2023].

4. Cholestasis and Bile Salt Accumulation

Some drugs inhibit transporters such as BSEP or MRP2, causing intrahepatic bile stasis and hepatocyte injury.

5. Steatosis

Weakened mitochondrial fatty-acid $\mathscr{B}$-oxidation (e.g., by tamoxifen, amiodarone) leads to triglyceride accumulation and macro/microvesicular steatosis, eventually causing injury.

Some examples of drug associated Acute liver Infection

Acetaminophen

Its Overdose saturates GSH conjugation, shunting to NAPQI. After oxidation of hepatic GSH stores, NAPQI binds proteins → centrilobular necrosis. Diagnosed via acetaminophen blood levels and Rumack–Matthew nomogram; treated with N-acetylcysteine [Lee HM, 2017].

Isoniazid

It is a prodrug and firstly Converts to hydrazine and acetyl-hydrazine via CYP2E1. Slow acetylators accumulate hydrazine, causing oxidative stress and immune-mediated injury.

Flucloxacillin

Flucloxacillin strongly associated with cholestatic liver injury and immune-mediated injury via reactive metabolites and HLA-B*57:01.

Amiodarone

It belongs to Class III antiarrhythmic agent have high lipophilicity and mitochondrial toxicity causing steatosis and fibrosis after chronic use.

Risk Factors [Rordam et al, 2025]

1. Patient-specific

- Genetic polymorphisms in metabolic enzymes or HLA types.
- Older age, female sex (for cholestatic & Drug related liver Infection).
- Pre-existing liver disease: Non-alcoholic fatty liver disease (NAFLD) can magnify risk.
- Alcohol consumption: synergistic or enzyme-inducing effect (e.g., with isoniazid).
- Nutritional or antioxidant status affecting GSH reserves.

2. Drug-specific

- High daily dose (>50 mg) and high lipophilicity are associated with greater DILI risk.
- Drugs known for mitochondrial toxicity (valproate, amiodarone).

- Drugs forming reactive metabolites (halothane, tienilic acid).

3. Drug–drug Interactions

Inhibition or induction of CYPs alters metabolic rates and accumulation of reactive forms. Eg. Isoniazid inhibits CYP2E1 which raising risk when co-administered with rifampin.

Biomarkers and Diagnosis

Diagnosis involves lab work, history, and exclusion of other causes:

Liver function tests: ALT, AST, ALP, GGT, bilirubin. The Roussel Uclaf Causality Assessment Method (RUCAM) is commonly used to estimate the likelihood of a drug causing liver injury [Danan and Teschke, 2019]. Emerging biomarkers such as glutamate dehydrogenase (GLDH), microRNA-122 (miR-122), and high mobility group box-1 protein (HMGB1) offer potential for earlier and more specific detection. Genetic testing (e.g., HLA genotypes) may help identify susceptibility in some cases.

Prevention & Mitigation

1. Preclinical Screening

- Use of *In Vitro* human hepatocyte assays, microsomes, S9 fractions for reactive metabolite detection.
- Mitochondrial toxicity testing (e.g., Seahorse assay).
- Computational (in silico) modelling for structure safety prediction.

2. Clinical Monitoring

- Periodic monitoring of liver enzymes, especially for drugs with known risks (e.g., isoniazid and methotrexate).
- Patient education to recognize symptoms: jaundice, pruritus and dark urine.
- Dose adjustment for high-risk patients (elderly, fatty liver, polypharmacy).

3.. Genetic & Personalized Medicine

- Pharmacogenetic screening offered where strong HLA associations are demonstrated (e.g., testing for HLA-B*57:01 before abacavir therapy).
- Monitoring relevant enzyme polymorphisms (e.g., NAT2 slow acetylators on isoniazid).

4. Hepatoprotective Adjuncts

- Use of N-acetylcysteine for acetaminophen overdose and as prophylaxis in certain high-risk scenarios.
- Ursodeoxycholic acid in cholestatic injury.
- Antioxidants/vitamin E investigated for mitochondrial or steatotic drug induced hepatocytes injury.

Emerging Research & Challenges

1.. Organoid and micro physiological systems

Human “liver-on-a-chip” platforms simulate 3D architecture and multi-cellular interactions to better predict DILI.

2. Multi-omics integration

Transcriptomics, proteomics, metabolomics identifies early perturbations, immune signatures, and mechanistic pathways.

3. Regulatory harmonization

ICH and FDA guidance emphasize reactive metabolite identification and transporter interaction.

The main challenge is to striking a balance between patient safety and innovation without incurring undue expenses and delays.

Conclusion

Liver damage due to drugs, results from complex interactions between drug properties, metabolism, immune activation, and host variability. Key mechanisms include reactive metabolite formation, mitochondrial dysfunction, oxidative and ER stress, transporter inhibition, and immune-mediated idiosyncrasy. Advances in *In-vitro* models, biomarkers, and pharmacogenetics are refining our ability to predict and prevent hepatotoxicity. Continued research across bench, clinical, and computational domains is vital to reduce Drug Induced liver injury incidence, safeguard patients, and sustain therapeutic innovation.

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