ejpmr, 2025, 12(8), 84-90

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

SJIF Impact Factor 7.065

Review Article

ISSN (O): 2394-3211

ISSN (P): 3051-2573

HEPATOTOXICITY: A COMPREHENSIVE REVIEW ON MECHANISMS, BIOMARKERS, AND THERAPEUTIC STRATEGIES

Aditi Rajaendra Waghmare*[1], Astikta Ashok Bhondave[2], Dr. Hemant V. Kamble[3] and Mr. S. R. Ghodake[4]

[1,2]Student, Department of Pharmacology, LSDP College of Pharmacy, Pune, Maharashtra.
[3]Principal, Department of Pharmacology, LSDP College of Pharmacy, Pune, Maharashtra.
[4]Professor, Department of Pharmacology, LSDP College of Pharmacy, Pune, Maharashtra.

***Corresponding Author: Aditi Rajaendra Waghmare**

Student, Department of Pharmacology, LSDP College of Pharmacy, Pune, Maharashtra.

Article Received on 05/06/2025 **Article Revised on 26/06/2025** **Article Accepted on 16/07/2025**

ABSTRACT

Hepatotoxicity, particularly drug-induced liver injury (DILI), represents a major challenge in pharmacology, toxicology, and clinical practice due to its unpredictable nature and potential severity. The liver's central role in detoxification makes it highly susceptible to various insults, including pharmaceuticals, herbal remedies, and industrial chemicals. This review provides an in-depth analysis of the molecular mechanisms of hepatotoxicity, such as oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, immune activation, and bile acid dysregulation. Emerging biomarkers, including microRNA-122, HMGB1, and cytokeratin-18, offer mechanistic and predictive insights beyond traditional markers like ALT and AST.[1,2] We explore both in vitro and in vivo models used to study liver injury and highlight therapeutic strategies ranging from antidotes like N-acetylcysteine to phytochemicals such as silymarin and trigonelline.[3,4] Emphasis is placed on novel therapeutic approaches targeting mitochondrial protection, inflammation modulation, and apoptosis inhibition. Future directions include the development of human-relevant models, personalized medicine, and integration of omics-based diagnostics to enhance hepatotoxicity prediction and management.

KEYWORDS: Hepatotoxicity, Drug-Induced Liver Injury (DILI), Oxidative Stress, Liver Biomarkers, Acetaminophen Toxicity, Experimental Models.

INTRODUCTION

The liver, a central organ in xenobiotic metabolism and detoxification, plays a crucial role in maintaining biochemical homeostasis. Due to its role in processing drugs, environmental agents, and dietary supplements, it is highly susceptible to injury. Hepatotoxicity, defined as chemical-induced liver damage, is a major concern in clinical pharmacology and toxicology, often resulting in acute liver failure, chronic liver diseases, or the need for transplantation.[1]

Drug-induced liver injury (DILI) is a predominant form of hepatotoxicity and contributes significantly to acute liver failure cases, particularly in developed nations.[2] DILI is categorized into intrinsic (predictable and dose-related, such as acetaminophen) and idiosyncratic (unpredictable and dose-independent, such as isoniazid) types.[3] The mechanisms involve reactive oxygen species (ROS) generation, mitochondrial dysfunction, bile acid accumulation, and activation of immune responses, which lead to apoptosis or necrosis of hepatocytes.[4]

Despite stringent preclinical screening and improved regulatory measures, hepatotoxicity remains a major hurdle in drug development. Many therapeutic agents fail during clinical trials or are withdrawn post-marketing due to liver-related adverse effects.[5,6] Conventional biomarkers like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin lack specificity and rise only after significant liver injury has occurred.[7] Recent research has identified novel biomarkers such as microRNA-122 (miR-122), high-mobility group box 1 protein (HMGB1), and keratin-18 fragments, which offer enhanced sensitivity and mechanistic relevance.[8] Therapeutic strategies now emphasize hepatoprotection using both synthetic drugs like N-acetylcysteine and natural compounds including silymarin, curcumin, and trigonelline.[9] Moreover, innovations in drug delivery—such as liver-targeted nanoparticles—aim to minimize hepatotoxicity and improve drug efficacy.[10]

This review presents a comprehensive analysis of hepatotoxicity, focusing on its molecular mechanisms, emerging diagnostic biomarkers, and current and evolving hepatoprotective strategies.

2. TYPES OF HEPATOTOXICITY

Hepatotoxicity refers to liver damage caused by chemicals or drugs. It can vary based on the type of toxin, amount, duration, and individual response. It is mainly divided into two types: intrinsic (predictable) and idiosyncratic (unpredictable). Clinically, liver injury is also classified into hepatocellular, cholestatic, or mixed types based on lab values and symptoms.[11]

2.1 Intrinsic Hepatotoxicity

This type is predictable, dose-dependent, and occurs shortly after exposure. A common example is acetaminophen (paracetamol), which forms a harmful metabolite (NAPQI) that causes oxidative stress and liver cell death.[12] It can often be treated with N-acetylcysteine, especially if given early.[13]

2.2 Idiosyncratic Hepatotoxicity

This type is unpredictable, not related to dose, and usually takes longer to appear. It may involve the immune system or genetics.[14] Drugs like isoniazid, diclofenac, amoxicillin-clavulanate, and valproic acid are common causes. It can present as hepatitis, cholestasis, or autoimmune-like liver injury.[1]

2.3 Clinical Patterns of Liver Injury

Liver injury is also classified by R-value, calculated as:
R = (ALT ÷ ULN) ÷ (ALP ÷ ULN)

- R > 5 = Hepatocellular
- R < 2 = Cholestatic
- R = 2–5 = Mixed[7]
- Hepatocellular: High ALT/AST. Seen with acetaminophen or viral hepatitis.[7]
- Cholestatic: High ALP and bilirubin. Caused by drugs like chlorpromazine or erythromycin.[15]
- Mixed: Both enzymes elevated. Linked to phenytoin, sulfonamides, etc.[16]

2.4 Special Types

- Autoimmune-like DILI: Caused by minocycline, nitrofurantoin
- Fatty Liver (Steatosis): Seen with methotrexate, tamoxifen
- Sinusoidal Obstruction Syndrome (SOS): From chemotherapy or certain herbs
- Granulomatous Hepatitis: Linked to hydralazine, diltiazem, allopurinol

3. MOLECULAR MECHANISMS OF HEPATOTOXICITY

Liver injury occurs due to multiple molecular events that damage hepatocytes. Both intrinsic and idiosyncratic hepatotoxicity often share common mechanisms such as oxidative stress, mitochondrial damage, immune activation, and bile acid imbalance. Understanding these helps in drug safety and treatment development.

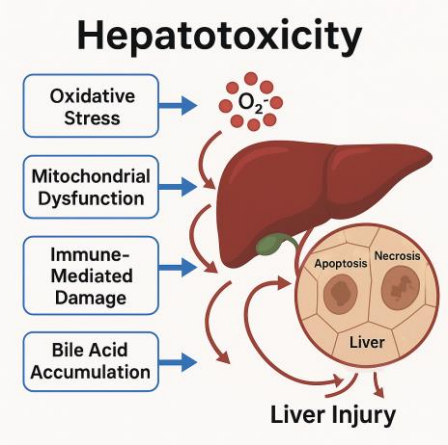

Figure 1: Mechanisms of Hepatotoxicity.

This diagram illustrates key interconnected pathways of hepatotoxicity.

- Oxidative stress via ROS production
- Mitochondrial dysfunction → ATP depletion and necrosis
- ER stress and misfolded protein response
- Bile acid accumulation leading to cholestasis
- Immune-mediated injury via cytokine release and T-cell activation

All of these mechanisms converge to produce apoptosis or necrosis in hepatocytes.

3.1 Oxidative Stress and ROS

Many toxic drugs increase reactive oxygen species (ROS) in the liver, leading to damage of lipids, proteins, and DNA. For example, acetaminophen reduces glutathione (GSH), increasing ROS and causing liver cell injury.[4,17]

3.2 Mitochondrial Dysfunction

Drugs like valproic acid and amiodarone damage mitochondria, reducing ATP and causing cell death. Mitochondrial pores open, releasing factors like cytochrome c, which activate apoptosis or necrosis depending on damage severity.[18,19]

3.3 Endoplasmic Reticulum (ER) Stress

Some drugs disrupt protein folding in the ER, activating stress responses (UPR). If prolonged, it can cause cell death via pathways involving CHOP and ATF4.[20]

3.4 Inflammation and Immune Response

Toxic drugs activate Kupffer cells and immune cells, releasing cytokines like TNF-α and IL-6, which worsen liver damage. In idiosyncratic cases, the immune system attacks liver cells due to drug-protein complexes.[21]

3.5 Bile Acid Transport Disruption

Some drugs block bile transporters like BSEP, leading to bile buildup and cholestasis. This is seen with drugs like bosentan, cyclosporine, and estrogens.[22]

3.6 Apoptosis vs. Necrosis

- Apoptosis: Controlled cell death with caspase activation and no inflammation.
- Necrosis: Uncontrolled rupture and inflammation. Acetaminophen may cause both depending on dose and ATP levels.[23]

3.7 Genetic and Epigenetic Factors

Gene variations (e.g., in CYP2E1, GST) and epigenetic changes like DNA methylation or microRNAs affect how people respond to drugs and their risk for liver damage.[24]

4. DIAGNOSTIC BIOMARKERS OF HEPATOTOXICITY

Early detection of liver injury is essential to avoid progression to liver failure. Traditional liver function tests are widely used but have limitations—they are non-specific, may reflect injury late, and cannot identify the exact cause or mechanism.[25]

4.1 Conventional Biomarkers

- ALT (Alanine Aminotransferase): Found mostly in hepatocytes; a sensitive marker for hepatocellular injury. It increases early but cannot determine severity or cause.[26]
- AST (Aspartate Aminotransferase): Present in liver and other tissues (heart, muscle), making it less specific. Often elevated along with ALT.[26]
- ALP (Alkaline Phosphatase) and GGT (Gamma-Glutamyl Transferase): Elevated in cholestasis and bile duct injury. ALP is not liver-specific; GGT helps confirm hepatic origin.[27]
- Bilirubin (Total and Direct): Indicates liver's ability to process and excrete bile. Elevation usually occurs in severe or late-stage liver dysfunction.[11]

4.2 Novel Mechanistic Biomarkers

To address the drawbacks of conventional tests, several molecular biomarkers have been identified:

- HMGB1 (High-Mobility Group Box 1): Released from damaged or inflamed liver cells. Its different forms help distinguish between necrosis and immune-mediated injury.[28]
- miR-122 (MicroRNA-122): A liver-specific microRNA that increases rapidly during hepatocyte damage—earlier and more specific than ALT. Valuable in early diagnosis.[8]
- Keratin-18 (K18) Fragments: Structural proteins released during cell death.
  - Full-length K18 = necrosis
  - Cleaved K18 = apoptosis Used to determine the type of liver cell death.[29]
- GLDH (Glutamate Dehydrogenase): A mitochondrial enzyme that increases in mitochondrial injury. More liver-specific than AST.
- SDH (Sorbitol Dehydrogenase): Highly specific to liver cells, sensitive to acute injury. Limited by lack of routine clinical use.

4.3 Biomarker Panels

Combining biomarkers (e.g., ALT, miR-122, K18, HMGB1) improves diagnostic accuracy by:

- Detecting injury earlier
- Differentiating injury mechanisms (apoptosis vs necrosis)
- Predicting recovery or worsening Such panels are under evaluation in major global initiatives like FDA's DILI-SAFE-T and IMI SAFE-T (Europe).[30]

4.4 Challenges and Future Prospects

Despite their promise, novel biomarkers face barriers.

- Need for standardized assays
- High cost and limited availability
- Regulatory qualification still in progress Future tools aim to be non-invasive (e.g., blood, urine, or exosome-based) and incorporate multi-omics (genomics, proteomics, metabolomics) for personalized prediction and monitoring.

Table 1: Biomarkers of Hepatotoxicity.

Biomarker	Type	Significance
ALT/AST	Conventional	Hepatocellular damage (non-specific)
ALP/GGT	Conventional	Cholestasis marker
miR-122	Emerging	Highly specific liver injury marker
HMGB1	Emerging	Differentiates necrosis and inflammation
K18 fragments	Emerging	Distinguishes apoptosis vs necrosis

5. EXPERIMENTAL MODELS OF HEPATOTOXICITY

Experimental models are essential tools to understand how liver injury occurs, to test hepatoprotective drugs, and to evaluate safety during drug development. These models simulate liver damage either in vitro (outside the body) or in vivo (in animals). Choosing the right model depends on the research aim—whether it's mechanism discovery, drug screening, or biomarker validation.

5.1 In Vitro Models (Cell-Based)

In vitro models are useful for early-stage testing and mechanistic studies. They are cost-effective, ethically favorable, and suitable for high-throughput analysis, although they lack the complexity of a living organism.

a) Primary Human Hepatocytes (PHHs)

- Considered the gold standard because they retain important liver-specific functions like drug metabolism.

- Ideal for studying enzyme-mediated toxicity (e.g., CYP450s).
- Limitations: short lifespan, variability between donors, and limited availability.[31]

b) Hepatoma Cell Lines (e.g., HepG2, Huh7)
- Immortalized liver cancer cells that are easy to maintain and grow.
- Used for testing cytotoxicity, apoptosis, and oxidative stress.
- However, they express low levels of drug-metabolizing enzymes, limiting their predictive value.[32]

c) 3D Liver Organoids and Spheroids
- Cultures that mimic the 3D architecture of the liver, improving physiological relevance.
- Allow longer drug exposure and better simulate chronic toxicity.
- Useful for modeling fibrosis and long-term damage.[33]

d) Co-culture Systems
- Combine hepatocytes with non-parenchymal liver cells (e.g., Kupffer cells, stellate cells).
- These simulate inflammatory responses and provide a more complete picture of liver reactions to drugs.[34]

5.2 In Vivo Models (Animal-Based)

Animal models provide a whole-body system to evaluate drug metabolism, immune responses, and tissue repair—features not possible in cell cultures. Rodents are most commonly used.

a) Acetaminophen (APAP)-Induced Hepatotoxicity
- Mimics human liver damage caused by overdose.
- Involves oxidative stress, mitochondrial damage, and hepatocyte necrosis.
- Ideal for testing antioxidant and hepatoprotective agents.[4,35]

b) Carbon Tetrachloride (CCl_4) Model
- A classical model for chronic liver damage and fibrosis.
- Induces damage through lipid peroxidation and inflammatory response.
- Commonly used to evaluate anti-fibrotic drugs.[36]

c) Alcohol-Induced Liver Injury
- Mimics alcoholic liver disease (fatty liver, hepatitis, fibrosis).
- Requires long-term ethanol feeding; sometimes combined with binge models (NIAAA model).
- Reflects inflammation, steatosis, and oxidative damage.[37]

d) D-Galactosamine/Lipopolysaccharide (D-GalN/LPS) Model
- Induces immune-mediated liver injury, mimicking idiosyncratic DILI.
- Involves activation of Kupffer cells and a cytokine storm (TNF-α, IL-1β).
- Good for evaluating anti-inflammatory or immunomodulatory drugs.[38]

e) Bile Duct Ligation (BDL) Model:
- Mimics obstructive cholestasis and causes bile acid accumulation.
- Results in oxidative stress, inflammation, and fibrosis.
- Suitable for testing agents against cholestasis and bile acid toxicity.[39]

5.3 Limitations of Current Models
- In vitro models lack systemic interactions like immune response or blood flow.
- In vivo models have species differences—especially in CYP enzymes and immune function—making translation to humans difficult.
- Most animal models fail to predict idiosyncratic hepatotoxicity accurately.

5.4 Emerging Alternatives

To improve human relevance and predictive power.
- **Humanized Mouse Models:** Mice with human liver cells offer better mimicry of human drug metabolism.
- **Liver-on-a-Chip:** Microfluidic devices simulate liver microarchitecture, blood flow, and multi-cellular interactions.
- **Multi-Omics Approaches:** Combining genomics, proteomics, and metabolomics helps identify mechanisms and predictive biomarkers.

These innovations aim to reduce animal use and improve translation from bench to bedside.

6. THERAPEUTIC STRATEGIES IN HEPATOTOXICITY

Managing hepatotoxicity involves early detection, stopping the harmful agent, and supporting liver repair. Treatment options include general care, antidotes, natural protectants, antioxidants, and emerging molecular therapies. New research also focuses on personalized prevention and safer drug design.

6.1 General Management

Basic steps include
- Stopping the toxic drug or substance
- Monitoring liver function tests (LFTs), INR, and symptoms
- Providing fluid balance and nutrition
- Avoiding alcohol and liver-damaging medications
- Hospitalization in severe cases like acute liver failure (jaundice, confusion, INR > 1.5).[40]

6.2 Antidotes and Drug-Based Therapies

a) N-Acetylcysteine (NAC)

- Standard antidote for acetaminophen overdose
- Restores glutathione, reduces free radicals
- Works best within 8–10 hours of overdose.[41]
- Also useful in other liver injuries

b) Corticosteroids

- Used in immune-related liver damage (e.g., drug-induced autoimmunity)
- Helpful in selected cases, especially when inflammation is present.[42]

c) Liver Transplantation

- Needed if liver fails completely
- Guided by King's College Criteria or MELD score.[43]

6.3 Natural Hepatoprotective Agents

These are plant-derived compounds that protect the liver through antioxidant and anti-inflammatory effects:

a) Silymarin (Milk Thistle)

- Reduces oxidative damage and stabilizes liver cells
- Shown to lower liver enzymes in liver diseases.[44]

b) Curcumin

- Inhibits inflammatory markers (like NF-κB)
- Helps in chemical-induced liver injury.[45]

c) Trigonelline and Diosgenin

- Boost antioxidant levels and reduce inflammation
- Useful in acetaminophen and alcohol-induced liver injury.[46]

d) Glycyrrhizin (from Licorice Root)

- Lowers liver enzymes, prevents scarring
- Used in Japan for chronic hepatitis[47]

6.4 Antioxidants and Cytoprotective Agents

Agents like vitamin E, NAC, melatonin, and Coenzyme Q10.

- Reduce oxidative stress
- Support mitochondria and prevent liver cell death.[4,48]

6.5 Emerging Molecular Therapies

These therapies target cell death, inflammation, and liver regeneration.

a) Caspase Inhibitors

- Prevent programmed liver cell death
- Being tested in preclinical studies

b) Mitochondrial Stabilizers

- Prevent damage to mitochondria, a key step in liver injury

c) FXR Agonists (Farnesoid X Receptor)

- Regulate bile acid levels
- Help in cholestatic liver diseases and NASH

d) microRNA Modulators

- Target miRNAs like miR-122, miR-34a, miR-155
- Control inflammation and liver cell damage.[49]

6.6 Personalized and Preventive Approaches

- Genetic screening helps identify people at risk of drug-induced liver injury
- In silico testing and early lab screening reduce toxic drugs in development
- Biomarkers like miR-122 and K18 help detect early damage before symptoms worsen

7. CONCLUSION AND FUTURE DIRECTIONS

Hepatotoxicity is a serious global health concern caused by drugs, herbs, and toxins. Because the liver handles detoxification and metabolism, it is highly sensitive to damage, which can lead to liver failure, chronic disease, or even the need for a transplant. Despite better monitoring systems, drug-induced liver injury (DILI) remains a major reason for drug bans and patient harm.

This review explained how liver damage occurs through oxidative stress, mitochondrial problems, immune attacks, and bile buildup. It also showed the limits of traditional liver tests and the promise of new biomarkers like miR-122, HMGB1, and keratin-18 for early and accurate detection. Lab models like cell cultures, animal studies, and organoids help us understand liver damage and test new treatments.

Modern treatment is shifting from basic care to targeted therapies. Natural compounds such as silymarin, curcumin, trigonelline, and diosgenin have shown protective effects and could be part of future liver treatments. New technologies like omics tools, personalized medicine, and liver-on-a-chip systems are improving how we diagnose and treat liver injury.

Future research should focus on

- Making new biomarkers ready for routine clinical use
- Building better lab models that reflect human liver responses
- Studying genetic risks for liver injury
- Designing personalized treatment plans for high-risk patients
- Updating drug safety rules using molecular-level data

In summary, solving the problem of hepatotoxicity requires teamwork across biology, medicine, toxicology, and data science to protect the liver and reduce global harm.

REFERENCES

1. Andrade RJ, Lucena MI, Fernandez MC, Pelaez G, Pachkoria K, Garcia-Ruiz E, et al. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish Registry over a 10-year period. *Gastroenterology*. 2005; 129(2): 512–21.

2. Reuben A, Koch DG, Lee WM. Drug-induced acute liver failure: results of a U.S. multicenter, prospective study. *Hepatology*. 2010; 52(6): 2065–76.
3. Kaplowitz N. Idiosyncratic drug hepatotoxicity. *Nat Rev Drug Discov*. 2005; 4(6): 489–99.
4. 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(1): 88–106.
5. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, et al. Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul Toxicol Pharmacol*. 2000; 32(1): 56–67.
6. Watkins PB. Drug safety sciences and the bottleneck in drug development. *Clin Pharmacol Ther*. 2011; 89(6): 788–90.
7. Temple RJ, Himmel MH. Safety of newly approved drugs: implications for prescribing. *JAMA*. 2002; 287(17): 2273–5.
8. Starkey Lewis PJ, Dear J, Platt V, Simpson KJ, Craig DG, Antoine DJ, et al. Circulating microRNAs as potential markers of human drug-induced liver injury. *Hepatology*. 2011; 54(5): 1767–76.
9. Flora K, Hahn M, Rosen H, Benner K. Milk thistle (Silybum marianum) for the therapy of liver disease. *Am J Gastroenterol*. 1998; 93(2): 139–43.
10. Hu Q, Li W, Hu X, Hu Y, Chen X. Development of liver-targeting drug delivery systems. *Int J Nanomedicine*. 2020; 15: 1907–27.
11. Zimmerman HJ. *Hepatotoxicity: The adverse effects of drugs and other chemicals on the liver*. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1999.
12. Jaeschke H, McGill MR, Ramachandran A. Mechanisms of acetaminophen hepatotoxicity and their translation to the human pathophysiology. *Clin Liver Dis*. 2014; 18(3): 507–18.
13. Lee WM. Acetaminophen toxicity: changing perceptions on a social/medical issue. *Hepatology*. 2007; 46(4): 966–70.
14. Uetrecht J. Immune-mediated adverse drug reactions. *Chem Res Toxicol*. 2009; 22(1): 24–34.
15. Björnsson E. Cholestatic injury: diagnostic and prognostic considerations. *Clin Liver Dis*. 2014; 18(3): 387–94.
16. Navarro VJ, Senior JR. Drug-related hepatotoxicity. *N Engl J Med*. 2006; 354(7): 731–9.
17. Ramachandran A, Jaeschke H. Acetaminophen hepatotoxicity. *Semin Liver Dis*. 2019; 39(2): 221–34.
18. Labbe G, Pessayre D, Fromenty B. Drug-induced liver injury through mitochondrial dysfunction: mechanisms and detection during preclinical safety studies. *Fundam Clin Pharmacol*. 2008; 22(4): 335–53.
19. Kon K, Kim JS, Jaeschke H, Lemasters JJ. Mitochondrial permeability transition in acetaminophen-induced necrosis and apoptosis of cultured mouse hepatocytes. *Hepatology*. 2004; 40(5): 1170–9.
20. Malhi H, Kaufman RJ. Endoplasmic reticulum stress in liver disease. *J Hepatol*. 2011; 54(4): 795–809.
21. Deng X, Luyendyk JP, Ganey PE, Roth RA. Inflammatory stress and idiosyncratic hepatotoxicity: hints from animal models. *Pharmacol Rev*. 2009; 61(3): 262–82.
22. Funk C, Pantze M, Jehle L, et al. Troglitazone-induced intrahepatic cholestasis in the human hepatocyte sandwich-culture model is due to inhibition of the canalicular bile salt export pump (BSEP). *Toxicol Sci*. 2001; 63(2): 190–7.
23. Gunawan BK, Liu ZX, Han D, et al. c-Jun N-terminal kinase plays a major role in murine acetaminophen hepatotoxicity. *Gastroenterology*. 2006; 131(1): 165–78.
24. Kacevska M, Robertson GR, Clarke SJ, Liddle C. Inflammation and CYP3A4-mediated drug metabolism in advanced cancer: impact and implications for chemotherapeutic drug dosing. *Expert Opin Drug Metab Toxicol*. 2008; 4(2): 137–49.
25. Senior JR. Alanine aminotransferase: a clinical and regulatory tool for detecting liver injury—past, present, and future. *Clin Pharmacol Ther*. 2012; 92(3): 332–9.
26. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. *CMAJ*. 2005; 172(3): 367–79.
27. Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N Engl J Med*. 2000; 342(17): 1266–71.
28. Antoine DJ, Jenkins RE, Dear JW, et al. Molecular forms of HMGB1 and biomarkers of cell death during acetaminophen hepatotoxicity. *Clin Pharmacol Ther*. 2012; 92(3): 444–52.
29. Schulz M, Schütte K, Link A, Malfertheiner P. Significance of cytokeratin-18 fragments in detecting cell death in liver diseases. *Expert Rev Gastroenterol Hepatol*. 2014; 8(5): 517–23.
30. Dragoi AM, Fu S, Zhang L, et al. Mechanistic biomarkers in drug-induced liver injury: recent advancements and challenges. *Front Pharmacol*. 2021; 12: 643642.
31. Gómez-Lechón MJ, Donato MT, Castell JV, Jover R. Human hepatocytes in primary culture: the choice to investigate drug metabolism in man. *Curr Drug Metab*. 2004; 5(5): 443–62.
32. Wilkening S, Stahl F, Bader A. Comparison of primary human hepatocytes and hepatoma cell line HepG2 with regard to their biotransformation properties. *Drug Metab Dispos*. 2003; 31(8): 1035–42.
33. Bell CC, Hendriks DF, Moro SM, Ellis E, Walsh J, Renblom A, et al. Characterization of primary human hepatocyte spheroids as a model system for drug-induced liver injury. *Toxicol Sci*. 2016; 155(2): 344–57.

34. Wang Y, Zhang L, Wang H, et al. Role of hepatic non-parenchymal cells in liver injury and regeneration. *World J Gastroenterol*. 2014; 20(46): 15498–506.
35. McGill MR, Jaeschke H. Mechanistic biomarkers in acetaminophen-induced hepatotoxicity and acute liver failure: from preclinical models to patients. *Expert Opin Drug Metab Toxicol*. 2014; 10(7): 1005–17.
36. Weber LW, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit Rev Toxicol*. 2003; 33(2): 105–36.
37. Bertola A, Mathews S, Ki SH, Wang H, Gao B. Mouse model of chronic and binge ethanol feeding (the NIAAA model). *Nat Protoc*. 2013; 8(3): 627–37.
38. Leist M, Gantner F, Naumann H, Bluethmann H, Volk HD, Tiegs G, Wendel A. Tumor necrosis factor-induced apoptosis during the poisoning of mice with hepatotoxins. *Gastroenterology*. 1997; 112(3): 923–34.
39. Tag CG, Sauer-Lehnen S, Weiskirchen S, et al. Bile duct ligation in mice: induction of inflammatory liver injury and fibrosis by obstructive cholestasis. *J Vis Exp*. 2015; (96): e52438.
40. Bernal W, Wendon J. Acute liver failure. *N Engl J Med*. 2013; 369(26): 2525–34.
41. Prescott LF. Paracetamol overdosage: pharmacological considerations and clinical management. *Drugs*. 1983; 25(3): 290–314.
42. Andrade RJ, Lucena MI, Kaplowitz N, et al. Outcome of acute idiosyncratic drug-induced liver injury: long-term follow-up in a hepatotoxicity registry. *Hepatology*. 2006; 44(6): 1581–8.
43. O'Grady JG, Alexander GJ, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. *Gastroenterology*. 1989; 97(2): 439–45.
44. Abenavoli L, Capasso R, Milic N, Capasso F. Milk thistle in liver diseases: past, present, future. *Phytother Res*. 2010; 24(10): 1423–32.
45. Banerjee A, Kunwar A, Mishra B, Priyadarsini KI. Concentration-dependent antioxidant/pro-oxidant activity of curcumin: studies from AAPH-induced hemolysis and EPR spectroscopy. *Toxicol Appl Pharmacol*. 2008; 226(2): 192–200.
46. Al-Asmari AK, Al-Shehri FS, Athar MT. Protective effect of Trigonelline and Diosgenin against acetaminophen-induced hepatotoxicity. *J Pharm Res Int*. 2021; 33(40B): 212–20.
47. van Rossum TG, Vulto AG, de Man RA, Brouwer JT, Schalm SW. Review article: glycyrrhizin as a potential treatment for chronic hepatitis C. *Aliment Pharmacol Ther*. 1998; 12(3): 199–205.
48. McGill MR, Sharpe MR, Williams CD, et al. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. *J Clin Invest*. 2012; 122(4): 1574–83.
49. Bala S, Marcos M, Kodys K, Szabo G. Up-regulation of microRNA-155 in macrophages contributes to increased tumor necrosis factor α (TNFα) production via increased mRNA half-life in alcoholic liver disease. *J Biol Chem*. 2011; 286(2): 1436–44.