Animal models accurately representing acute liver failure (Review)

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Received October 30, 2025; Accepted March 13, 2026

DOI: 10.3892/ijmm.2026.5820

Abstract. Animal models are essential for investigating disease pathogenesis and progression. Acute liver injury (ALI) precedes acute liver failure (ALF), establishing a crucial and close relationship between these conditions. Appropriate animal models are required in order to develop successful treatments for ALF. However, the inability to construct appropriate animal models that accurately represent the pathophysiological features of ALF has impeded research progress. The present review examined the pathophysiological mechanisms of ALF, evaluated the strengths and limitations of commonly used model organisms, and highlighted the advantages of mouse models in simulating the onset and progression of ALF. Furthermore, the review systematically summarized the varying drug and chemical dosages used in the development of drug-induced and chemical-induced ALF models in mice. In addition, whether ALI/ALF models constructed with different drug dosages accurately reflect disease progression has been a topic of critical discussion. Therefore, the present review proposed specific drug and chemical dosages for ALF model development and described future directions for developing optimal ALF animal models.

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Abbreviations: ALF, acute liver failure; ALI, acute liver injury; APAP, acetaminophen; Cyp2E1, cytochrome P450 2E1; JNK, c-Jun N-terminal kinase; NAPQI, N-acetyl-p-benzoquinone imine; CCl_4, carbon tetrachloride; AOM, azoxymethane; TAA, thioacetamide; D-GalN, D-galactosamine

Key words: acute liver failure, pathophysiological characteristics, animal models, drug/chemical induction, dosage

Contents

1. Introduction

Traditional cell lines and animal model systems have demonstrated notable success in biomedical research throughout the late twentieth and early twenty-first centuries, advancing the present understanding of cellular signaling pathways, identifying potential drug targets, and guiding therapeutic development for conditions including cancer and infectious diseases. The widespread adoption of these model systems in modern biomedical research validates their substantial contributions. The traditional pathway for studying disease mechanisms in animal models involves initial genetic screening in invertebrates, followed by verification of evolutionary conservation in mammalian systems, and ultimate translation to human clinical applications. This systematic approach has enabled a detailed mechanistic understanding of numerous human diseases.

Acute liver failure (ALF) represents a rare and complex clinical condition, with liver transplantation serving as the most effective treatment. However, limited donor availability restricts its clinical application (1-3). Novel therapeutic approaches, including hepatocellular cell transplantation, bioartificial liver support systems and stem cell transplantation,

require evaluation in animal models before clinical implementation (4-6). Consequently, animal models constitute a fundamental component in therapeutic development.

The ideal ALF model incorporates the following seven criteria: i) Reversibility: Some animals can survive the process if appropriate treatment is administered; ii) reproducibility: Death occurs at recognized time intervals and the extent of liver injury can be measured and standardized; iii) death due to liver failure: Complications arising from the injury need to accurately reflect a typical human clinical situation and death is a direct result of liver injury; iv) treatment window: Untreated animals should die from signs of progressive liver failure within a recognized time frame; v) appropriate animal size: Animals used need to be of a size that permits the collection of adequate blood and tissue samples during treatment; vi) minimal hazard to personnel: All methods used should represent the lowest possible health risk; and vii) conscious animal model: The use of a conscious animal model to assess the development of hepatic encephalopathy, which is an important component of ALF pathology (Fig. 1) (7).

The mouse serves as an effective experimental model, with developmental processes closely resembling human biology. Despite having a less comprehensive metabolic system compared with larger animal models, mouse models have gained widespread adoption and demonstrated notable utility (8). The C57BL/6J mouse strain from The Jackson Laboratory has emerged as a predominant choice in medical research, exhibiting gene regulation patterns similar to humans and strong concordance in immune and inflammatory pathways, thus providing a representative model of human disease (9). However, research has revealed substantial variations in drug and chemical dosages used in pharmacologically-induced and chemically-induced ALF models using C57BL/6J mice, impeding standardized research under consistent pathologic characteristics and hindering progress in ALF therapeutic development.

The present review examines C57BL/6J-derived acute liver injury (ALI)/ALF models induced by pharmacological and chemical injuries that demonstrate potential in modeling ALF onset, development and disease progression. While previous reviews have comprehensively described various model systems (10,11), the present analysis focuses on distinguishing between ALI and ALF models from existing research to establish uniform standards for drug/chemical usage in animal model development. The present review examines variations in drug and chemical applications for mouse models, assesses dosage similarities and limitations between animal models and human conditions, and highlights current dosage recommendations to promote standardized approaches in ALF pathophysiology research.

2. ALF

ALF is a rare but notable human health concern (12), and was initially defined in 1970 by Trey and Davidson (13) as a severe, potentially reversible liver injury without underlying liver disease, characterized by hepatic encephalopathy occurring within 8 weeks of initial symptom onset. The condition can be classified as hyperacute, acute or subacute, based on the time interval between initial symptoms and hepatic encephalopathy (13). Subsequently, international professional associations (European Association for the Study of the Liver (EASL) (14); American Association for the Study of Liver Diseases (AASLD) (15); Asian Pacific Association for the Study of the Liver (APASL) (16) refined this definition and published guidelines for ALF diagnosis and treatment, incorporating four characteristic clinical symptoms: i) Elevated serum aminotransferases; ii) elevated serum bilirubin; iii) impaired coagulation [International Normalized Ratio (INR) >1.5]; and iv) the presence of hepatic encephalopathy (17). The 2018 Guidelines for the Diagnosis and Treatment of Liver Failure by Chinese hepatologists (18) defined ALF as the presence of stage II or higher hepatic encephalopathy within 2 weeks, while the American League for the Study of ALF Group (ALFSG) (19) categorized ALF as hyperacute (<7 days), acute (7-28 days) or subacute (29 days to 6 months). The mortality rate correlates with the duration of delay, with longer delays associated with increased mortality (Fig. 2) (20).

ALF primarily results from poisoning, viral infection, autoimmune deficiency and hereditary diseases, predominantly affecting healthy adults that are ~30 years of age. The clinical manifestations include hepatic insufficiency, liver biochemical abnormalities and coagulation dysfunction (21). In developing nations, acute viral hepatitis remains the primary cause of ALF (20), whilst hepatitis B virus (HBV) infection constitutes the most common cause in Asian countries and the Mediterranean region (21,22). However, with increased HBV vaccination rates and declining HBV carrier populations, pharmacologic injury-induced ALF is becoming more prevalent (23), particularly in Western countries (Fig. 3).

Pathogenesis of ALF. ALF develops from rapid liver function loss triggered by various acute injuries, including hepatotoxic drugs, immune-mediated attacks or viral infection-induced rapid hepatocellular necrosis (24,25). The condition manifests when hepatocellular death exceeds the regenerative capacity of the liver, primarily through necrosis, apoptosis and necrotizing apoptosis (26). Apoptotic hepatocytes interact with Kupffer cells (KCs), newly infiltrated bone marrow-derived macrophages and hepatic stellate cells (HSCs). However, extensive hepatocyte death overwhelms the clearance capacity, preventing liver function restoration (27). The inflammatory response distinguishes necrotic from apoptotic cell death. Necrotic cell rupture triggers inflammation through the release of intracellular contents, notably the NLR family pyrin domain containing 3 (NLRP3) inflammasome. This well-established human inflammasome, when aberrantly activated, associates with various ALF types and induces different programmed cell death forms (Fig. 4) (28).

Mechanisms of ALF cell death caused by viral infections. The predominant mechanism of ALF cell death induced by viral infection involves the activation of death receptors. Blood samples from patients with ALF showed significantly elevated levels of death receptors and ligands, including CD95L, TNF-α and TNF receptor (29-32). Studies using *in vitro*-constructed TNF-α and anti-CD95 mouse models have demonstrated toxicity patterns similar to those observed in patients with ALF (33,34). Additionally, HCV-infected human livers demonstrated increased TRAIL receptor expression and enhanced susceptibility to TRAIL-induced apoptosis (31).

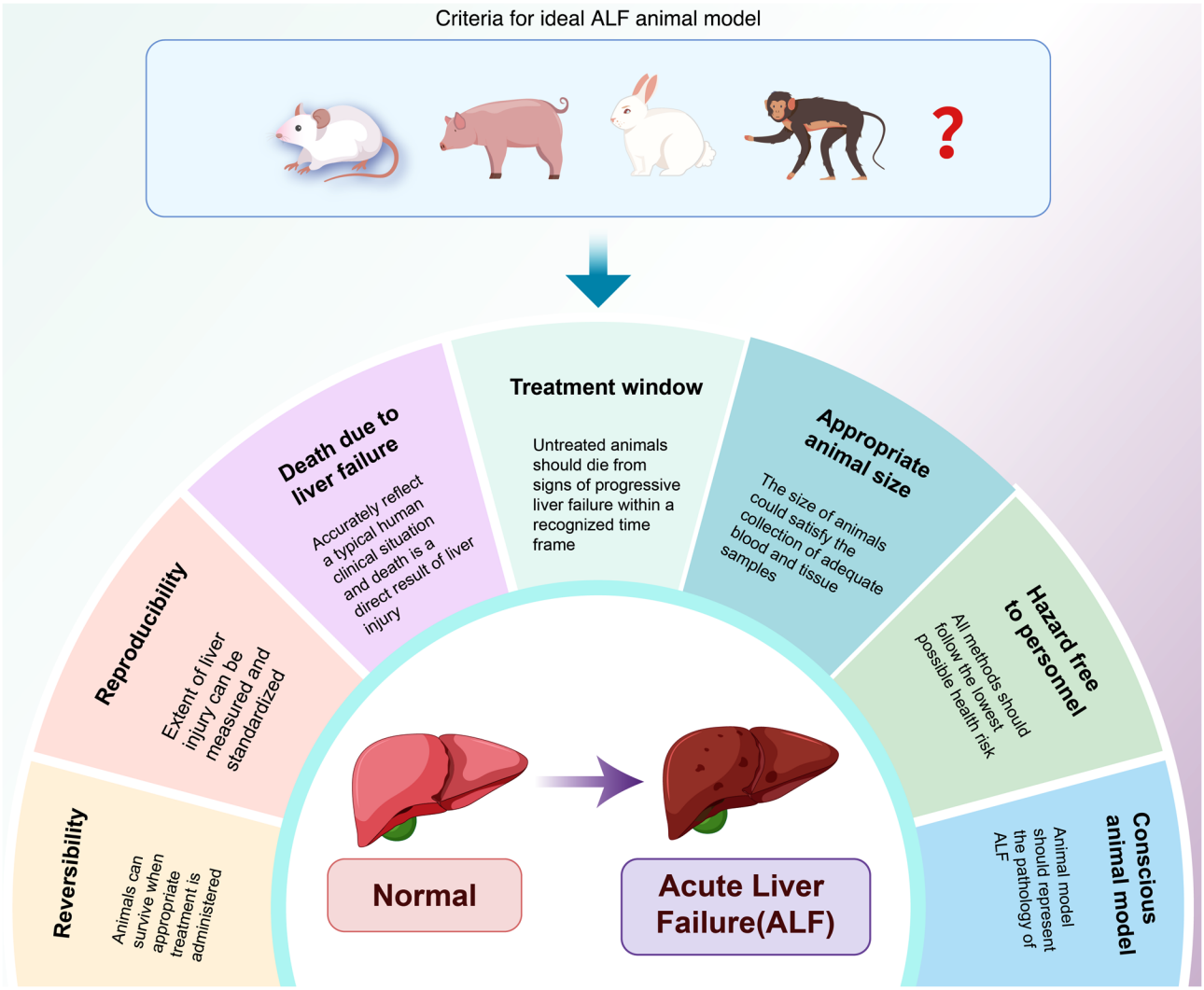

Figure 1. Criteria for ideal ALF model. The ideal ALF model incorporates the following seven criteria: i) Reversibility; ii) reproducibility; iii) death due to liver failure; iv) treatment window; v) appropriate animal size; vi) minimal hazard to personnel; and vii) conscious animal model. ALF, acute liver failure.

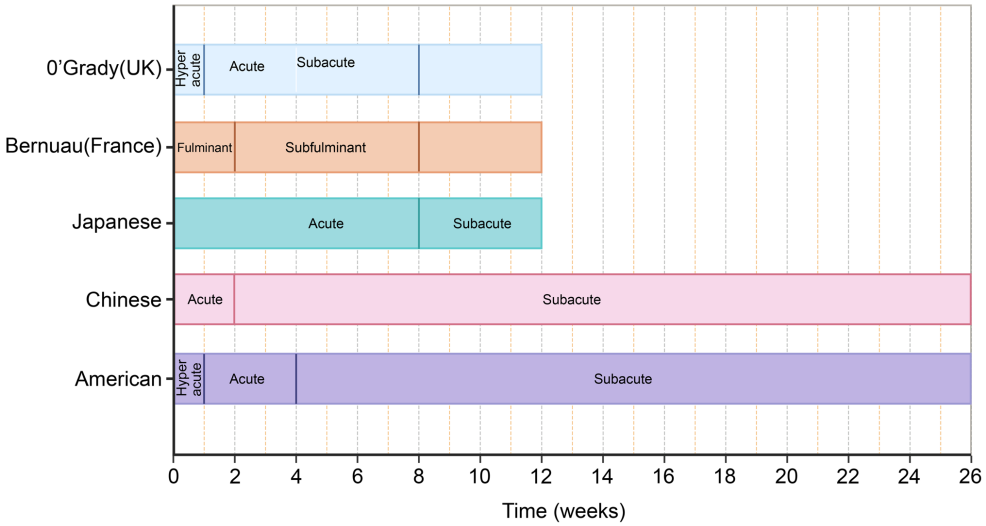

Figure 2. Classification systems for acute liver failure (24).

Mechanism of ALF cell death induced by drug-induced infections. Unlike viral infection, drug-induced liver injury primarily involves signaling pathways originating from mitochondria (35). The mechanism of acetaminophen (APAP)-induced ALF cell

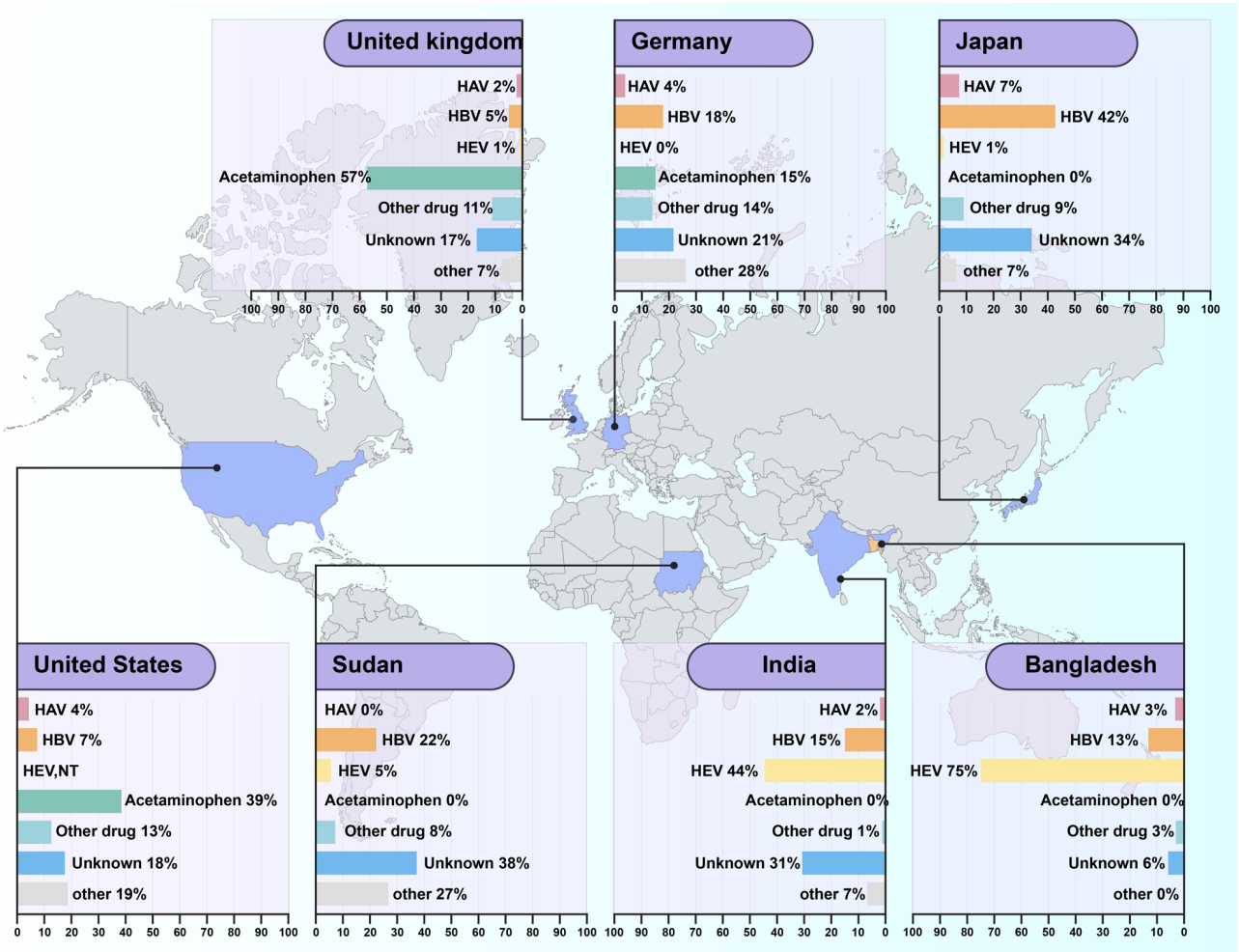

Figure 3. Worldwide causes of acute liver failure. HAV, hepatitis A virus; HBV, hepatitis B virus; HEV, hepatitis E virus; NT, not tested (24).

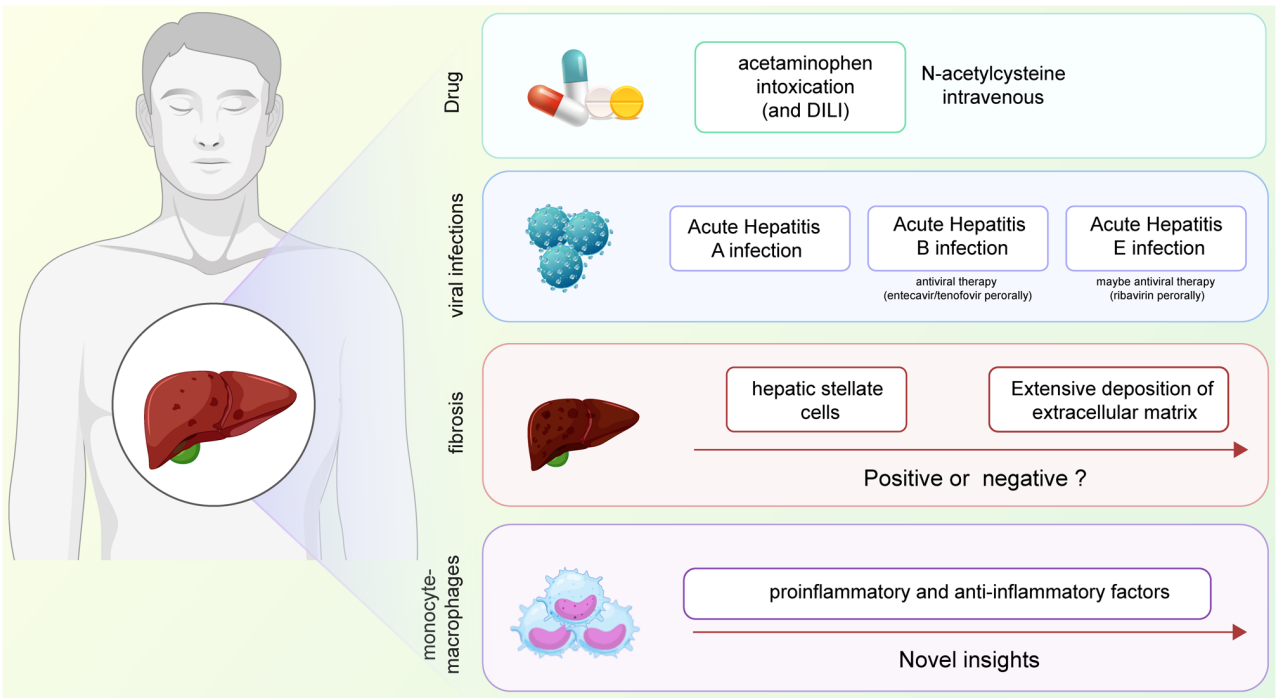

Figure 4. Schematic overview depicting the pathogenesis of ALF, including hepatotoxic drugs, immune-mediated attacks and viral infection-induced rapid hepatocellular necrosis. These causative elements may result in severe acute damage to liver tissue, ultimately leading to ALF. ALF, acute liver failure; DILI, drug-induced liver injury.

death primarily involves metabolic and adaptive mechanisms regulating toxicity in the cytochrome P450 system (36). Mitochondrial autophagy, a selective form of autophagy, eliminates damaged mitochondria through the interaction between mitochondrial and autophagic mechanisms. This process occurs through ubiquitin-dependent or ubiquitin-independent pathways, characterized primarily by PINK/Parkin-mediated and light chain 3 interacting region-domain receptor-mediated pathways, respectively (37).

Mitochondrial autophagy serves to attenuate local inflammatory responses and tissue necrosis by eliminating damaged or excess mitochondria, maintaining mitochondrial homeostasis, and inhibiting pro-inflammatory factor secretion triggered by inflammatory vesicles such as NLRP3 (38,39). During ALF onset, injured hepatocyte mitochondria release damage-associated molecular patterns and secrete inflammatory factors IL-1β and TNF-α. This leads to increased reactive oxygen species (ROS) production and ATP depletion, followed by mTOR activity inhibition and enhanced autophagic flux. Autophagic vesicles accumulate around necrotic foci, limiting necrosis expansion into normal hepatic areas. Mitochondrial autophagy proteins, activated by ectopic or ubiquitinated mitochondrial proteins, facilitate damaged mitochondria removal, ROS reduction, inflammation decrease and hepatocyte repair (40). However, insufficient activation of mitochondrial autophagy during disease onset impairs timely removal of damaged mitochondria and necrotic hepatocyte repair, resulting in increased local and systemic inflammation and ultimately irreparable massive liver parenchyma necrosis (41). In APAP-induced ALF, Parkin protein inhibition/knockdown significantly reduces mitochondrial autophagy, leading to NLRP3 inflammatory vesicle accumulation and increased APAP-induced hepatotoxicity (42). By contrast, mitochondrial autophagy activation removes ROS-damaged mitochondria, inhibits IL-1β release and alleviates APAP-induced ALF symptoms (43). This evidence demonstrates the crucial role of mitochondrial autophagy in liver protection and homeostasis maintenance, while its dysfunction leads to damaged mitochondria accumulation, disrupted mitochondrial homeostasis and ALF progression (Fig. 5) (41).

Relationship between fibrosis and the development of ALF. Previous evidence has suggested that extensive extracellular matrix (ECM) deposition and scarring from persistent HSC activation impairs tissue regeneration by hepatocytes (44). However, another study showed that preventing fibrosis through activated HSC depletion in ALF mouse models results in more severe liver injury and reduced survival (45). Fibrosis is an intrinsic injury response that maintains organ integrity during extensive necrosis or apoptosis, suggesting its beneficial role during acute tissue injury (46). Previous studies have proposed that ALF-related fibrosis may possess a protective function. The acute production of collagen and fibrosis onset in ALF may represent an intentional physiological process (47-49). Further investigation is needed to determine whether fibrosis formation could serve as an intervention target in ALF animal models.

Relationship between monocyte-macrophages and ALF development. From a clinical perspective, the primary cause of mortality in ALF is systemic inflammatory response syndrome, which results from excessive inflammatory responses, leading to concentrative renal anemia and multi-organ failure (50). Disruptions in the innate immune system constitute the principal mechanism leading to ALF (51). Notably, monocyte-macrophages, serving as crucial effector cells in both natural and intrinsic immunity, generate numerous proinflammatory and anti-inflammatory factors, playing a central role in ALF initiation and progression. As hepatic macrophages, both KCs and monocyte-derived macrophages regulate the hepatic inflammatory response. During ALF onset, hepatic KCs become activated by injury-associated molecular patterns released from damaged hepatocyte mitochondria, subsequently activating the NF-κB signaling pathway to produce inflammatory cytokines and reactive oxygen clusters (52). This leads to a substantial increase in hepatic KCs, partially through KC proliferation and division, and partially through circulating monocytes that migrate to injured hepatic areas via monocyte chemoattractant protein-1 receptor signaling. These monocytes differentiate into KCs, secreting additional inflammatory mediators and promoting neutrophil infiltration, thereby intensifying local inflammation and tissue necrosis (53). The resulting local and systemic inflammatory responses rapidly amplify, ultimately causing irreversible inflammation and tissue death. This evidence indicated that KCs represent a potential therapeutic target for ALF, where timely inhibition of KC activation may reduce liver injury and enhance liver regeneration. This area warrants further investigation, given its significance in ALF progression and outcomes (54,55).

3. Common biological models

Since the recognition of Mendel's work in the early 20th century, researchers have investigated numerous organisms in laboratories worldwide (56). However, relatively few have emerged as 'model organisms', which typically exhibit characteristics such as robustness, brief growth cycles, rapid production of numerous offspring and cost-effective laboratory cultivation (57). Established model organisms including *Saccharomyces cerevisiae* (brewer's yeast), *Drosophila melanogaster* (fruit fly) and mice possess extensive research histories and have notably influenced various biological fields. Subsequently, the development of novel model systems, such as nematodes, zebrafish and African clawed toads, has further expanded scientific understanding. For example, *Caenorhabditis elegans* demonstrates rapid growth and maintains its cell lineage, enabling precise analysis of cell fate determination mechanisms (58). Zebrafish larvae are particularly advantageous for studying vertebrate organ development due to their transparent bodies (59,60). The African clawed toad exhibits regenerative capabilities exclusively during its tadpole stage (through developmental stage, 50-54), losing this ability upon developing into a juvenile frog (stages, 56-66). The sole exception is the optic nerve, which retains lifelong regenerative capacity, offering valuable insights for regeneration research (61).

Model animals. The most commonly used model organisms currently include non-mammalian models (brewer's yeast,

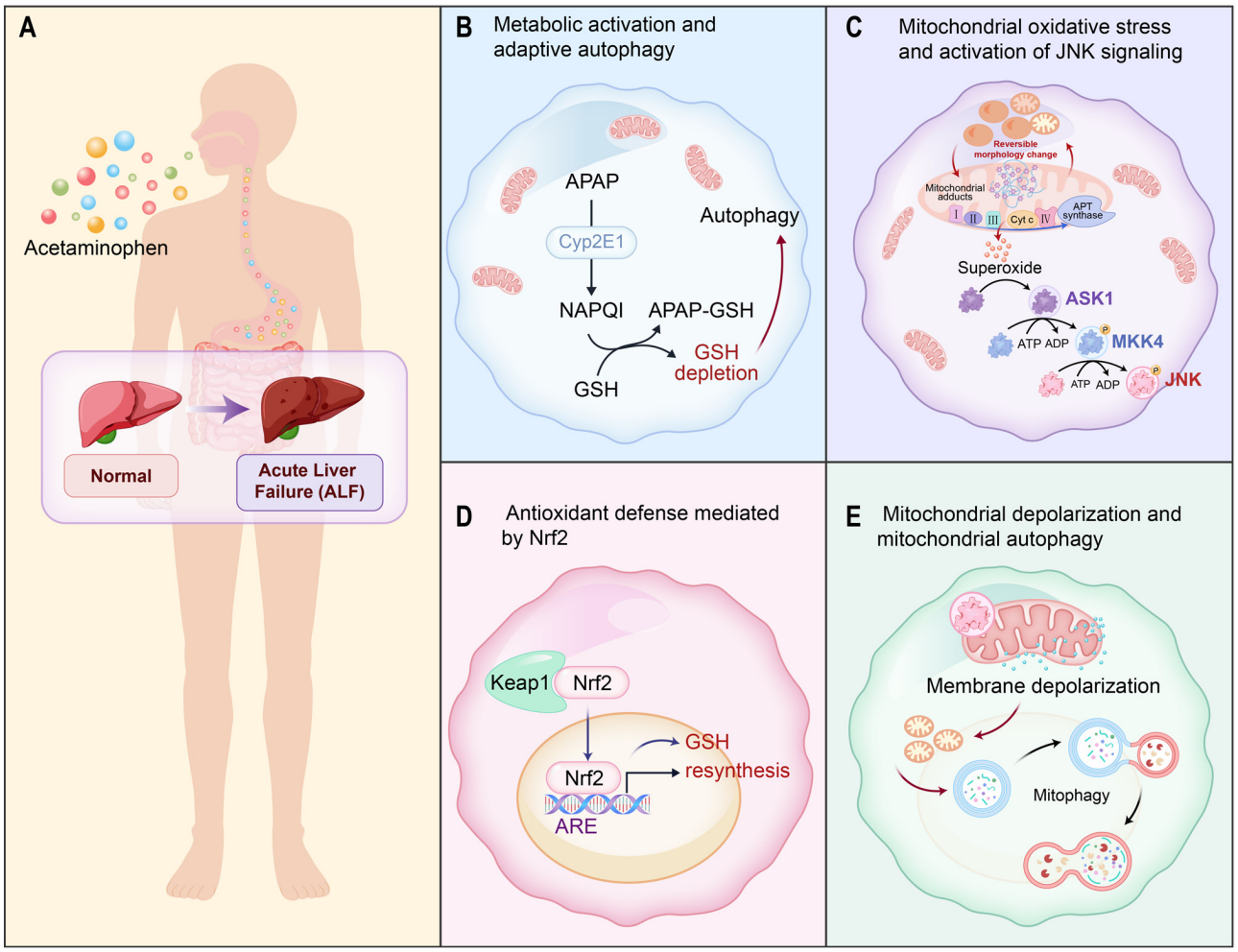

Figure 5. Adaptive responses after APAP overdose. Following an overdose, APAP hepatotoxicity predominantly impacts centrilobular hepatocytes (A). In these cells, Cyp2E1 produces the reactive metabolite NAPQI, which is neutralized by the glutathione reserves of the liver. This leads to a depletion of glutathione, enabling the formation of NAPQI protein adducts. This molecular alteration triggers autophagic pathways (B). These pathways function to eliminate the adducted proteins and strive to minimize cellular damage. When this adaptive mechanism is overpowered, the NAPQI adducts on mitochondrial proteins prompt the directional release of superoxide into the cytosol from respiratory complex III. This event sets off a MAP kinase cascade, ultimately resulting in the activation of JNK and its translocation to the mitochondria (C). Concurrently, there is a reduction in mitochondrial membrane potential, which causes a reversible change in mitochondrial morphology. The mild cytosolic oxidant stress likely also activates antioxidant responses mediated by Nrf2. Nrf2 dissociates from its binding partner Keap1, translocates to the nucleus and initiates the transcription of genes involved in glutathione resynthesis upon binding to the ARE (D). The translocation of JNK to the mitochondria also induces mitochondrial membrane depolarization and activates mitophagy. Mitophagy attempts to remove damaged organelles and preserves cellular function (E). APAP, acetaminophen; Cyp2E1, cytochrome P450 2E1; NAPQI, N-acetyl-p-benzoquinone imine; JNK, c-Jun N-terminal kinase; Nrf2, nuclear erythroid 2 p45-related factor 2; Keap1, kelch-like ECH associating protein 1; ARE, antioxidant response element; GSH, glutathione; ASK1, apoptosis signal-regulating kinase 1; MKK4, mitogen activated protein kinase kinase 4.

C. elegans, *Drosophila melanogaster* and *Danio rerio)*, and mammalian models *(Mus musculus)*. Patient-derived xenograft models and cell lines are also used in cancer research. In addition, *in situ* tissues and stem cell-derived organoids provide a new platform for basic research, each with unique advantages and limitations.

Non-mammalian models. C. elegans has served as a prominent research model since the mid-1970s (62), and its highly deterministic developmental process makes it an ideal model for cellular lineage analysis, through which numerous genes essential to the apoptotic program were initially identified (63).

The selection of zebrafish *(Danio rerio)* was primarily due to the near-complete transparency of its early embryo and robust reproductive capacity with manageable costs, enabling researchers to elucidate fundamental principles of early embryonic development (64,65). Large-scale genetic screening in zebrafish commenced in the 1990s with two concurrent extensive screens for mutations affecting various developmental processes in Tübingen and Boston (66-68).

However, these non-mammalian species exhibit notable differences from humans in their growth and development processes, including their physiological characteristics, genomes and disease pathogenesis.

Mammalian models. Among mammalian models, mice represent closer analogues to human genetics, development and disease compared with Drosophila or worms. The mouse serves as the preferred animal model for biomedical research, enabling researchers to identify interventions for neurological disorders (69,70), validate initial stem cell origin hypotheses (71,72) and advance emerging fields such as 'fertility preservation' (73) and reversal of epigenetic changes induced by assisted reproduction techniques (74). Researchers selected

the mouse as a model organism for three primary reasons: i) The mouse genome is more thoroughly characterized than other animal models, with sophisticated gene editing tools and germline totipotent embryonic stem cells, facilitating genetic modification through evolving tools for genetics, genotyping and phenotyping; ii) mouse populations can be readily expanded, enabling large-scale experiments to derive statistically significant conclusions regarding developmental and physiological mechanisms and systemic properties; and iii) feeding management and environmental conditions can be precisely controlled, allowing systematic multi-omics studies at molecular, cellular and organ levels (75-100). Although studies using identical mouse models may yield different findings, such variations often result from differences in technical analysis methods, a reproducibility challenge also present in human studies. This necessitates enhanced optimization of husbandry environments, stringent verification of mouse strain identity and standardization of basic experimental conditions, which, with appropriate mouse models, can provide accurate insights into human conditions, facilitate biological principle discovery, prioritize human studies and validate related hypotheses.

Cell-based organoid model. Prior to the emergence of organoid technology, various approaches to simulate human organ biology have been explored, including 2D human stem cell differentiation with or without 3D matrices, bio-3D printing of human cells and cell culture in microfluidic devices ('organ-on-a-chip') (101). Organoid generation from human patient-derived cells or human-induced pluripotent stem cells has demonstrated notable value in studying mechanisms related to differentiation, morphogenesis, pathogenesis and drug action, while also facilitating biomarker discovery for diseases at cellular and tissue levels (102).

However, organoids, being closed structures, lack tissue-tissue interfaces, vascular flow, circulating immune cells and physiologically relevant mechanical cues, limiting their capacity to fully capture organ-level responses or study drug effects under pharmacologically relevant conditions dependent on dynamic drug pharmacokinetic exposure profiles. The incorporation of self-organizing capillary networks in 3D organoid cultures (103) and immune cell addition to surrounding ECM gel may address some limitations (104). Nevertheless, substantial constraints persist, including the inability to control vascular structure and flow dynamics, and challenges in understanding chemical, inflammatory molecule, drug and immune cell movement within living vascularized organs. Due to their closed structure surrounded by dense ECM, organoids present difficulties in measuring nutrient, chemical or drug transport and uptake in epithelial tissues, sampling inner lumen contents, maintaining microbiome co-cultures and integrating sensors for on-line functional measurements (105).

Previous research indicates that organoids demonstrate notable potential in complementing existing model systems and advancing basic biology, medical research and drug discovery within physiologically relevant human environments (101). However, organoid technology remains in its early developmental stages compared with established cell lines and animal models, with numerous challenges yet to be addressed.

The utilization of distinct animal models, 2D human cell lines and 3D organoids throughout the past three decades has substantially enhanced the understanding of disease pathogenesis, while simultaneously revealing the limitations of these systems in replicating human pathophysiology. The mouse model maintains its position as the predominant model organism for disease research, with particular validation coming from disease-related genes and pathological features initially discovered in mouse studies being subsequently confirmed in humans (106,107), establishing the mouse model as the preferred animal model for biomedical research.

4. Role of mouse models in ALF-related studies

An ideal animal model is essential for disease research. Currently, to more accurately replicate the characteristics of ALF *in vivo*, the primary construction methods are categorized into APAP-based pharmacological injury and chemical injury-based fibrosis models (Fig. 6).

Drug-induced ALI/ALF animal model. Drug-induced liver injury serves as a key contributor to ALF (Fig. 6); consequently, researchers frequently establish drug-induced liver injury models for treatment or prevention studies in the early stages of the disease (108). Pharmacologic liver injury presents one of the most notable challenges for hepatologists due to several factors, including: i) The extensive range of drugs used in clinical practice; ii) the availability of potentially hepatotoxic herbs and dietary supplements; iii) the diverse clinical and pathologic manifestations of the disease; and iv) the absence of specific biomarkers, which renders diagnosis inherently complex. Current pharmacological models of ALI/ALF primarily utilize intraperitoneal injection of excessive APAP, a medication widely recognized as safe and effective for analgesic and antipyretic purposes at therapeutic doses. However, overdose results in liver injury and potentially progresses to ALF. Research has demonstrated that APAP notably contributes to the development of drug-induced ALI and ALF (31).

In 1973, Mitchell *et al* (109) discovered that rats exhibited resistance to APAP-induced hepatic injury following APAP injection, while C57BL/6J mice provided accurate indicators of hepatic injury status after APAP overdose. The C57BL/6J mouse remains the predominant animal model for APAP hepatotoxicity research, although other susceptible mouse strains, including ICR mice (110), C3Heb/FeJ mice (111) and B6C3F1 mice, have also been employed in ALI/ALF research (112).

For ALI modeling in C57BL/6J mice, APAP doses typically range from 300 to 600 mg/kg, with 600-750 mg/kg considered the lethal dose for ALF modeling. However, a standardized dose for ALI/ALF modeling remains undefined, with researchers selecting different APAP doses for various mechanistic studies. APAP overdose triggers excessive production of reactive-free radicals and n-acetyl-p-benzoquinone imine (NAPQI) (113). NAPQI rapidly depletes cellular glutathione and forms complexes with biologically active molecules, inducing oxidative stress, causing mitochondrial damage and ultimately resulting in hepatocellular injury (114).

Reducing oxidative stress and hepatotoxicity with APAP doses. Herbal extracts demonstrate particular promise as

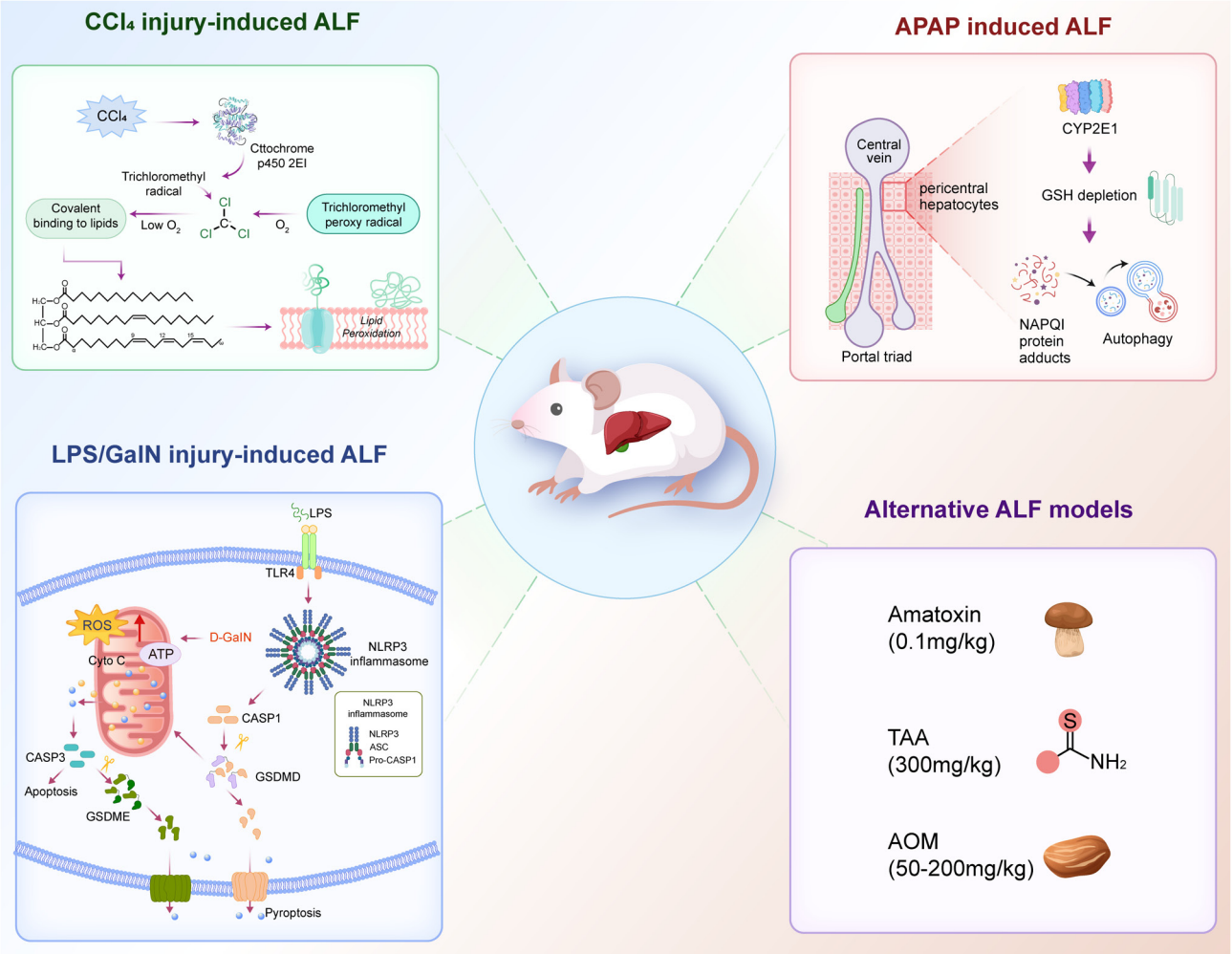

Figure 6. Schematic overview depicting the major pharmacological models of ALF, including CCl_4-induced ALF, APAP-induced ALF and LPS/GalN-injury-induced ALF. Amatoxin, TAA and AOM are also used to trigger the process of ALF in several studies. ALF, acute liver failure; CCl_4, carbon tetrachloride; APAP, acetaminophen; LPS, lipopolysaccharide; Gal, galactosamine; TAA, thioacetamide; AOM, azoxymethane; NLRP3, NLR family pyrin domain containing 3; ROS, reactive oxygen species.

therapeutic agents for ALI/liver failure, functioning through antioxidants. Platanoside (PTS), isolated from American sycamore (*Platanus occidentalis*), represents a potential novel tetramolecular phytopharmaceutical antibiotic effective against drug-resistant infectious diseases and exhibits antioxidant properties. A previous study demonstrated reduced inducible nitric oxide synthase expression and c-Jun-N terminal kinase (JNK) activation when APAP-overdosed mice (300 mg/kg) received PTS treatment (115). Atractylenolide I (AO-I), a phytochemical derived from *Atractylodes macrocephala* Koidz., demonstrates established antioxidant properties. Li *et al* (116) administered AO-I to C57BL/6 mice following 500 mg/kg APAP-induced hepatotoxicity, revealing that AO-I provided protection against APAP-induced hepatotoxicity through the TLR4/MAPKs/NF-κB pathway (117). Research utilizing an ALF mouse model constructed with high-dose APAP (700 mg/kg) demonstrated that mannose-binding lectin regulates CYP2E1 expression through the ROS-dependent JNK/SP1 pathway and mitigates APAP-induced hepatotoxicity (116).

Accelerating mitochondrial autophagy and endoplasmic reticulum (ER) autophagy. Mitochondrial damage plays a crucial role in APAP-induced hepatocyte necrosis and liver dysfunction. Research demonstrates that APAP activates mitophagy, evidenced by increased Parkin translocation to mitochondria and ubiquitination of mitochondrial proteins, along with concurrent sequestration of damaged mitochondria in autophagosomes and degradation of mitochondrial proteins in primary hepatocytes from mouse livers (118). In studies examining the relationship between mitochondrial autophagy and ALF occurrence, researchers predominantly established ALI models through intraperitoneal injection of 500 mg/kg APAP for 24 h (119-124). The initial implementation of the APAP-ALI model was conducted by Ni *et al* (125), who utilized the 500 mg/kg APAP C57BL/6J mouse model in 2012 and demonstrated that treatment with the autophagy inducer rapamycin inhibited APAP-induced hepatotoxicity. Subsequently, in 2015, the authors identified distinct responses to mitochondrial autophagy and hepatic injury in mice induced by chronic deletion of Parkin and acute knockdown of APAP using the same model (126). In 2019, their research revealed that PINK1 and dual deletion of Parkin compromised hepatic mitochondrial autophagy and intensified APAP-induced hepatic injury in mice (127). Studies investigating autophagy through

amelioration of inflammatory response and apoptosis-related research typically employ a lower dose of 300 mg/kg APAP (42,128). Chen *et al* (129) administered 400 mg/kg APAP coenzyme Q10 to C57BL/6J mice to activate mitochondrial autophagy and prevent APAP-induced hepatic injury. APAP doses >700 mg/kg are considered lethal, with C57BL/6J mice survival rates <60 h, paralleling ALF disease progression (130).

The ER facilitates a specialized intracellular environment for protein processing, folding and sorting for intracellular and extracellular transport. ER stress (ERS) occurs when the ER structure sustains damage or when protein synthesis exceeds the functional capacity of the ER (131). Transient ERS serves as a protective mechanism for normal cellular function under stress. However, prolonged ERS may trigger apoptosis, potentially compromising organ function (132). Sustained ERS can enhance ROS production, intensifying oxidative stress (133), or initiate an inflammatory response, further aggravating cellular damage (134). In 2019, Torres *et al* (135) first established that APAP induces ALI through ERS, utilizing C57BL/6J 300 mg/kg APAP for ALI modeling. By contrast, studies examining rhodiola rosea glycosides for APAP-induced ALI prevention employed C57BL/6J 500 mg/kg APAP (136). This dosage also revealed the transcription factor CHOP as a key regulator of APAP-induced hepatotoxicity (137). The investigation of hepatotoxicity alleviation through ERS remains in its early stages, leaving uncertainty about future optimal APAP model development.

Chemical-induced ALF animal model

Carbon tetrachloride (CCl_4)-induced ALI/ALF animal model. CCl_4 constitutes a classical hepatotoxicant that induces hepatocellular damage through direct or indirect hepatocyte stimulation, closely resembling clinical human ALI (138). CCl_4 hepatotoxicity relies on reductive dehalogenation catalyzed by cytochrome P-450 in liver cell ER. Research demonstrates that CCl_4 metabolism initiates a cascade of secondary mechanisms responsible for ultimate plasma membrane disruption and cell death (139). While CCl_4 has been employed to model ALI/hepatic failure features across various mouse models, with C57BL/6J mice being the predominant choice, the absence of standardized dosage protocols for ALI/ALF model construction presents a notable challenge for researchers (Fig. 6).

The primary administration method for the ALI/ALF model in C57BL/6J mice involves mixing CCl_4 solution with peanut, corn or olive oil at specific ratios, followed by intraperitoneal injection for 24 h. Based on varying mixing ratios and intraperitoneal injection doses, the constructed models are primarily categorized into ALI and ALF models, with some researchers defining specific lethal doses of CCl_4 injection. For ALI model construction (Table I), CCl_4 is mixed with co-solvents at ratios of 0.3-50%, with injection doses ranging from 0.5-10 ml/kg. At this dose, extensive hepatocyte injury and intense inflammatory responses occur, triggering repair pathways while preserving sufficient residual hepatocytes and regenerative potential. Animals survive severe injury and undergo gradual repair. This model is suitable for studying liver injury, inflammatory responses, repair initiation and early reversible lesions such as drug-induced liver injury and acute hepatitis (140-150).

Table I. Construction of a C57BL/6J-ALI model induced by CCl_4.

Dilution (CCl_4:oil)	I.p. (ml/kg)	Time (h)	Survival rate (%)	(Refs.)
1:3	1	24	100	(140)
1:19	0.5	24	100	(141)
1:1	1	24	100	(142)
1:1	2	24	100	(143)
1:1	3	24	100	(144)
1:200	10	24	100	(145)
3:1,000	10	24	100	(146)
No dilution	0.5	24	100	(147,148)
1:9	5	24	100	(149,150)

I.p. (ml/kg) refers to the volume of the CCl_4-oil mixture injected per kg body weight. Survival rate (%): Survival rate within 7d after CCl_4 administration. CCl_4, carbon tetrachloride, I.p. intraperitoneal.

By contrast, ALF model construction (Table II) utilizes CCl_4 mixed with co-solvents at ratios of 10-50%, with injection doses primarily ranging from 4-10 ml/kg. The lethal dose of CCl_4 is typically administered through intraperitoneal injection of 2.5% CCl_4 (10 ml/kg, dissolved in olive oil) (151). Alternatively, the lethal dose can be administered using CCl_4 in a 1:1 dilution with corn oil, at a final concentration of 2.6 ml/kg of body weight (140). Furthermore, a novel method for constructing acute-on-chronic liver failure involves inducing chronic liver disease over 10 weeks through intraperitoneal injection of CCl_4 in C57BL6 mice (6-8 weeks; ~20-24 g body weight), followed by APAP and lipopolysaccharide (LPS) injection for acute injury (152). Due to the use of high drug doses, this approach directly induces extensive hepatocyte necrosis and functional failure, rapidly inducing lethal or near-lethal liver injury within 24-72 h, typically resulting in a mortality rate >50%. The mouse model established using CCl_4 mixed with co-solvents at ratios of 50%, with injection doses of 5 ml/kg, closely mimics the clinical characteristics of patients with ALF, including onset and progression, aiding research into the terminal processes of complete liver function collapse, high mortality, impaired liver regeneration and multi-organ failure (153-155).

Research indicates that severe ALI may progress to ALF with notable mortality rates if not properly treated (156). While most current studies refer to the constructed model as 'ALI' rather than 'ALF', the ALI model has not successfully replicated ALF characteristics in terms of survival or disease progression. Consequently, additional research is necessary to analyze the effects of varying CCl_4 doses on mouse survival and liver pathology, aiming to establish reference standards for ALF research.

LPS/D-GalN-induced ALF animal model. LPS/D-galactosamine (D-GalN)-induced ALF represents a well-established animal model extensively utilized to investigate the pathogenesis of fulminant hepatitis in humans (Fig. 6) (157). LPS (also termed endotoxin), a macromolecule present in the outer membrane of Gram-negative bacteria, stimulates

Table II. Construction of a C57BL/6J acute liver failure model induced by CCl_4.

Dilution (CCl_4:oil)	I.p. (ml/kg)	Treated time (h)	Survival rate (%)	(Refs.)
1:1	2.6	48	20	(140)
1:1	4	24	10	(153)
1:10	7.5	24	40	(154)
3:10	4	24	50	(155)

I.p. (ml/kg) refers to the volume of the CCl_4-oil mixture injected per kg body weight. Survival rate (%): Survival rate within 7d after CCl_4 administration. CCl_4, carbon tetrachloride, I.p. intraperitoneal.

Table III. Construction of a C57BL/6J-ALF model induced by LPS/D-GalN.

LPS (μg/kg)	D-GalN (mg/kg)	Treated time (h)	Survival rate (%)	(Refs.)
10	250	24	80	(159)
250	250	24	0	(160)
2.5	300	24	60	(161)
5	300	24	40	(162)
10	300	24	20	(163)
50	400	24	20	(164)
100	400	24	10	(165,166)
5	500	24	0	(167)
10	500	24	0	(168-170)
100	700	24	0	(171)
50	800	24	0	(172)
100	800	24	0	(173,174)

Survival rate (%): Survival rate within 12 h after LPS/D-GalN administration. LPS, lipopolysaccharide; D-GalN, D-galactosamine.

inflammation. D-GalN molecules, when metabolized through the galactose pathway in the liver, induce severe metabolic alterations and hepatic necrosis by depleting various intracellular uridine mediators (11). Additionally, D-GalN heightens the sensitivity of the liver to the lethal effects of LPS and hepatocellular cell death (158). The ALF model developed using LPS/D-GalN demonstrates pathophysiological characteristics more closely aligned with the progression of ALF triggered by ALI. However, current studies lack a uniform standard, and even when utilizing the same animal model, C57BL/6J, for ALF modeling, the dosages employed by different researchers exhibit considerable variation (159-174) (Table III). Notable variations in LPS dosage exist across different studies, ranging from 2.5 to 250 μg/kg. However, independent studies across various LPS dosage groups consistently demonstrate different degrees of liver injury. For instance, serum AST and ALT levels both reached 2,000 U/l within 12 h, with a 60% mortality rate in ALF models following administration of 300 mg/kg D-GalN and 5 μg/kg LPS to C57BL/6J mice (162). However, mice injected with high doses of drugs such as 800 mg/kg D-GalN and 100 μg/kg LPS exhibited biochemical indicators similar to those observed at lower doses, yet their mortality rate reached as high as 100% (174). To date, existing studies cannot conclusively determine whether a dose-dependent effect exists when LPS and D-GalN are combined. However, current research indicates that the ALF induced by the combination of LPS and D-GalN is primarily used to study the hepatoprotective effects of drugs.

Table IV. Construction of a C57BL/6J-ALF model induced by AOM, TAA and a-Avantin.

Chemical	I.p. (mg/kg)	Treated time (h)	Survival rate (%)	(Refs.)
AOM	100	24	NA	(177-179)
TAA	300	24	12.5	(189)
a-Avantin	0.25	24-48	100	(192,193)
	0.35	24-48	20	(192,193)
	0.45	24-48	20	(192,193)

Survival rate (%): Survival rate within 7d after AOM, TAA and a-Avantin administration. AOM, azoxymethane; TAA, thioacetamide; NA, not applicable.

5. Alternative ALF models

Azoxymethane (AOM) is a chemical compound found in the nuts of the Guam soursop palm, that exhibits hepatotoxic and carcinogenic properties (175). Matkowskyj *et al* (176) demonstrated that intraperitoneal injection of AOM in mice at doses ranging from 50-200 mg/kg induced ALF and hepatic encephalopathy. In studies of ALF hepatic encephalopathy using C57BL/6J mice as a model, researchers administered a single intraperitoneal injection of AOM at a uniform dose of 100 mg/kg (177-179) (Table IV). However, the AOM mouse model is unique as it is a progressive liver injury model that does not spontaneously recover; the survival rate of this mouse model remains unclear.

Thioacetamide (TAA) is a sulfur-containing compound that induces hepatocellular necrosis through monooxygenase biotransformation (180). This compound is frequently utilized in the development of induced fulminant hepatic failure models (181). The primary mechanism of TAA-induced hepatic injury involves the generation of ROS, triggering oxidative damage (182). Consequently, TAA-based ALF mouse models are widely employed to investigate antioxidant protective effects (183), free radical scavenging in damaged tissues (184) and pro-hepatic tissue regeneration (185-189). The model construction in C57BL/6J mice or other mouse strains typically involves a single intraperitoneal injection of 300 mg/kg TAA for 24 h, resulting in a 3/24 survival rate (Table IV).

Amatoxin is a peptide derived from highly toxic mushrooms (10). Following ingestion, it is completely absorbed by hepatocytes, where it exerts toxic effects by inhibiting RNA polymerase II, including its largest subunit, RNA polymerase B1, the bridge helix and the trigger loop. Furthermore,

α-muscarinic acid operates through the enterohepatic circulation and transport system, causing recurrent hepatocyte toxicity primarily through apoptosis, oxidative stress and autophagy (190). Amatoxin is predominantly utilized in establishing ALF models in large animals, demonstrating notable therapeutic effects. For instance, in *Macaca mulatta*, intraperitoneal injection of 0.1 mg/kg amatoxin dissolved in 50 ml saline resulted in a 49-h survival rate (10), while intravenous injection of 0.1 mg/kg in a porcine model yielded an 8-h survival rate (191). *In vivo*, α-amanitin caused dose-dependent liver injury in mice, with 0.25 mg/kg (i.p., once) considered a low-dose, 0.35 mg/kg (i.p., once) considered a medium-dose and 0.45 mg/kg (i.p., once) considered a high-dose. Significant mortality was reported within 72 h in mice that received medium and high doses of α-amanitin (Table IV) (192,193).

6. Current challenges and future perspectives

As animal models constitute the primary research subjects in ALF studies, establishing a standardized method for ALF construction would notably advance ALF research. Current models predominantly focus on developing ALI models. However, not all ALIs progress to ALF, limiting precise therapeutic interventions. In the APAP-induced pharmacological C57BL/6J mouse hepatocyte injury model, doses >600 mg/kg resulted in a 48-h survival rate. Similarly, single intraperitoneal doses of CCl_4 at ≥5 mg/kg (1:1) demonstrated a 48-h survival rate. While these survival cycles may reflect ALF progression more accurately, establishing uniform ALF standards requires additional pathological and physiological characterization. Recent advances in single-cell RNA sequencing and spatial transcriptomics enable precise tracking of disease onset and progression, potentially facilitating the assessment of disease feature remodeling efficiency by various drug doses or chemical inducers. For instance, after obtaining spatial transcriptomic data, by calculating 'cell adjacency relationships', whether the probability of cytotoxic T cells directly adjoining dying hepatocytes in pathological regions significantly increases with rising drug doses can be quantified, thereby measuring the 'spatial intensity' of immune attacks. Similarly, by defining 'niche composition', the co-localization ratio of pro-reparative macrophages, activated stellate cells and hepatic progenitor cells within a 100-μm radius of regenerating nodules can be analyzed. This ratio serves as an indicator of 'regenerative microenvironment health'. Ultimately, these mathematical vectors extracted from spatial coordinates will form the objective, quantitative foundation for comparing whether tissue microenvironments undergo 'orderly remodeling' or 'disruptive chaos' under different dose treatments.

Although LPS/D-GalN offers an alternative approach for ALF model construction, the dosing protocols lack standardization and exhibit greater variability compared with APAP and CCl_4 protocols, despite utilizing the same C57BL/6J mouse strain. The existing literature does not adequately explain these dose variations in ALF studies, necessitating further consultation with investigators to understand the rationale behind the different dose combinations crucial for ALF model development.

Notably, ALF may exhibit patient-specific heterogeneity, which could potentially explain the current lack of harmonization in animal models. Addressing this challenge requires enhanced collaboration between hepatologists and biologists. Tools such as humanized mouse models or patient-derived organoids offer a promising path toward accurately capturing the diversity of human ALF. By inducing liver injury and implanting a mixture of human fetal liver cells with hepatic progenitor cells and liver stem cells, humanized mice with human-mouse liver chimeras (human-reconstructed liver mice, HEP mice) have been successfully established. These mice can be used to study human hepatotropic pathogens (such as hepatitis B and C virus infections) and liver diseases, providing a research platform for replicating the human immune system (194). On the other hand, patient-derived liver organoids hold immense potential for reproducing disease characteristics, offering a unique platform for identifying biomarkers of disease progression and inferring personalized treatment strategies. For instance, iPSC-derived liver organoids retain innate immune responses and maintain hepatocyte polarity, reproducing the natural entry process of HBV and HCV and enabling their intercellular transmission, faithfully recreating host-virus interactions (195). Constructing 'immunized' or 'vascularized' organoids that better reflect the *in vivo* microenvironment by introducing HSCs, endothelial cells, and immune cells may aid in reproducing the pathological features of human ALF (196,197).

7. Conclusions

Model organisms provide essential support for analyzing human health and disease mechanisms. The precise development of model organisms with standardized feeding environments, genetic backgrounds and biological interventions facilitates both the collection of biological samples from cases and controls throughout disease progression and the establishment of a comprehensive understanding of molecular, cellular, developmental and physiological properties, representing fundamental elements of organismal biology research.

Acknowledgements

Not applicable.

Funding

The present review was supported by grants from the Science and Technology Support Project of Yinchuan City (grant no. 2024SF045), and the Special Talent Introduction Project of Ningxia Autonomous Region Key R&D Programs (grant no. 2024BEH04106).

Availability of data and materials

Not applicable.

Authors' contributions

SL designed the scope and structure of the review and wrote the manuscript. FW and XH performed structured literature

searches. YJ performed the literature review and designed the figures. DL and BT provided expert knowledge and critically revised the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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