Chapter 1

1. INTRODUCTION

Drug-induced liver injury (DILI) is a formidable and multifactorial challenge that continues to impede both clinical management and pharmaceutical development. As the central organ for drug metabolism, detoxification, and biotransformation, the liver is particularly vulnerable to injury from xenobiotics, including prescription medications, over-the-counter drugs, herbal supplements, and environmental toxins. Among the multifaceted mechanisms implicated in the pathogenesis of DILI, mitochondrial dysfunction (MD) has emerged as a critical determinant of hepatocellular toxicity, oxidative stress, and liver failure [1]. The increasing incidence of hepatotoxic reactions due to mitochondrial perturbations highlights the urgent need for mechanistic elucidation and development of protective strategies to minimize liver-related adverse drug reactions.

Mitochondria are essential regulators of hepatocellular energy metabolism, redox homeostasis, apoptosis, and other biosynthetic functions. Their high density in hepatocytes is reflective of the liver's metabolic demands. Drugs that interfere with mitochondrial function can cause a spectrum of hepatic injuries, including steatosis, cholestasis, fibrosis, and ultimately hepatic failure [2]. These injuries are mediated through diverse mechanisms, such as inhibition of mitochondrial respiratory chain complexes, impairment of mitochondrial DNA replication and transcription, and uncoupling of oxidative phosphorylation (OXPHOS) [3]. Reactive oxygen species (ROS) generation, ATP depletion, and release of pro-apoptotic factors from damaged mitochondria serve as critical initiators of downstream hepatocellular death cascades.

The implications of MD in DILI have become increasingly evident, especially with the post-marketing withdrawal or restriction of numerous drugs. For instance, troglitazone, an antidiabetic agent, and nefazodone, an antidepressant, were both withdrawn due to hepatotoxic effects linked to mitochondrial dysfunction [4,5]. The U.S. Food and Drug Administration (FDA) has issued black-box warnings for several drugs, including valproic acid, linezolid, and amiodarone, based on accumulating evidence of mitochondrial-mediated liver injury [6]. Notably, mitochondrial toxicity is not always identified in preclinical studies due to limitations in standard hepatotoxicity models that do not recapitulate mitochondrial reliance.

Among the drug classes frequently associated with DILI, antibiotics rank prominently. Despite their indispensable role in managing infectious diseases, antibiotics contribute significantly to adverse hepatic events. Chloramphenicol, a prototypical broad-spectrum antibiotic, remains clinically relevant in resource-limited settings for the treatment of life-threatening infections such as typhoid, rickettsial diseases, and meningitis [7]. However, its therapeutic use is severely constrained by serious adverse effects, including dose-related bone marrow suppression, aplastic anemia, and hepatotoxicity [8]. Chloramphenicol is well-documented to impair mitochondrial protein synthesis by binding to the 55S mitochondrial ribosome, thereby mimicking its antibacterial mechanism of action on the bacterial 70S ribosome [9].

This mitotoxic mechanism inhibits mitochondrial translation, disrupts the electron transport chain (ETC), impairs ATP generation, and amplifies intracellular ROS accumulation, leading to hepatocyte stress and death. These effects are particularly evident in models that enhance mitochondrial dependency, such as galactose-adapted HepG2 cells, which rely more heavily on OXPHOS than glycolysis for energy production [10]. As such, chloramphenicol serves as an ideal model compound to explore mechanisms of antibiotic-induced mitochondrial dysfunction.

Despite the mechanistic clarity and clinical significance of chloramphenicol-induced mitochondrial toxicity, no specific regulatory measures such as black-box warnings currently exist for the drug in most regulatory jurisdictions. This regulatory gap underscores the need for revisiting risk assessments associated with mitochondrial liabilities. Furthermore, the search for pharmacological countermeasures to offset mitochondrial toxicity has gathered pace, with antioxidants emerging as promising therapeutic candidates.

Astaxanthin and Quercetin are two naturally occurring antioxidant compounds that have gained attention for their potent anti-inflammatory and free radical-scavenging activities. Astaxanthin, a xanthophyll carotenoid derived from marine sources such as microalgae (Haematococcus pluvialis), has demonstrated protective effects against mitochondrial membrane depolarization, oxidative stress, and apoptotic signaling in various models of oxidative liver injury [11,12]. Similarly, Quercetin, a widely distributed polyphenolic flavonoid found in fruits and vegetables, modulates mitochondrial pathways, enhances antioxidant enzyme expression, and reduces pro-inflammatory cytokines [13,14].

In the context of hepatotoxicity, both agents have shown potential to restore redox homeostasis and improve liver function in experimental models. However, limited studies have investigated their mechanistic effects in the setting of antibiotic-induced mitochondrial injury. The current

thesis aims to fill this gap by evaluating the protective efficacy of Astaxanthin and Quercetin in chloramphenicol-induced liver toxicity using a dual-model approach encompassing both in-vitro and in-vivo systems.

The in-vitro component employs galactose-adapted HepG2 cells to simulate mitochondrial dependence and assess the cytotoxic impact of chloramphenicol. Biomarkers of mitochondrial function including ATP levels, ROS production, and expression of key mitochondrial genes such as SOD2 (superoxide dismutase 2), NRF1 (nuclear respiratory factor 1), SURF1 (surfeit locus protein 1), TFAM (transcription factor A, mitochondrial), and UCP2 (uncoupling protein 2) are quantified to delineate mitochondrial responses [17,26].

In parallel, the in-vivo studies utilize Wistar rats to assess systemic hepatic responses to chloramphenicol exposure. Biochemical markers such as glutathione (GSH) and nitric oxide (NO) serve as indicators of oxidative stress and redox imbalance. The co-administration of Astaxanthin and Quercetin is evaluated for its ability to attenuate these biomarkers and restore normal hepatic architecture.

This integrative approach, combining cellular and whole animal models, enhances translational relevance and facilitates a more comprehensive understanding of the mitochondrial basis of DILI. Furthermore, this study aligns with global regulatory priorities that emphasize the identification of mitochondrial liabilities early in drug development pipelines to prevent adverse hepatic outcomes.

In addition to the mechanistic and therapeutic evaluation, this study considers regulatory implications. Many existing regulatory guidelines, including those from the FDA, EMA, and ICH, have yet to fully integrate mitochondrial toxicity screening in preclinical drug development protocols. The absence of standardized screening tools for mitochondrial liabilities poses a risk to patient safety and increases the burden of post-market surveillance. Incorporating mitochondrial toxicity assessment into the safety pharmacology paradigm can offer earlier identification of hepatotoxic liabilities and reduce drug attrition rates during clinical development.

Moreover, the relevance of mitochondria in immunological responses and inflammation is also emerging as an important factor in DILI. Mitochondrial damage-associated molecular patterns (mtDAMPs), released during mitochondrial injury, can trigger innate immune responses,

activate Kupffer cells, and amplify hepatic inflammation. Exploring these immuno-mitochondrial interactions will contribute to a more comprehensive understanding of DILI pathogenesis and may inform the development of immunomodulatory therapies in combination with antioxidants.

Recent insights also underscore the importance of mitochondrial dynamics fusion, fission, and mitophagy in maintaining mitochondrial quality control under physiological and pathological conditions. Dysregulation of these processes has been associated with a heightened susceptibility to drug-induced toxicity. Therefore, studying the impact of chloramphenicol on mitochondrial dynamics, and whether antioxidants can normalize these perturbations, presents an important research frontier [14,16].

Additionally, the emergence of novel biomarkers for mitochondrial injury, such as glutamate dehydrogenase (GLDH), mitochondrial DNA (mtDNA) fragments, and circulating microRNAs like miR-122, is revolutionizing the diagnosis and monitoring of DILI. Incorporating such biomarkers in the preclinical evaluation may provide more sensitive and specific indicators of mitochondrial damage compared to conventional liver enzymes such as ALT and AST [15,16].

From a global health perspective, it is essential to note that drugs like chloramphenicol are still used extensively in low- and middle-income countries (LMICs), where cost-effective therapies for bacterial infections are limited. In such contexts, completely eliminating a drug with a narrow therapeutic index may not be feasible. Hence, finding strategies to retain its clinical utility while mitigating its hepatotoxic risks becomes particularly important. The approach adopted in this research of combining existing drugs with safe, naturally derived antioxidants aligns with the principles of global health equity and rational pharmacotherapy.

The economic burden of late-stage drug failure due to hepatotoxicity is another concern. Mitochondrial liabilities contribute to the attrition of drug candidates during clinical trials, with estimated costs running into billions of dollars annually. Improved early detection tools and intervention strategies, such as those investigated in this thesis, can help lower these costs and reduce the likelihood of post-marketing drug withdrawals.

Finally, advances in precision toxicology an emerging field integrating genomics, transcriptomics, and metabolomics into toxicity evaluation suggest that inter-individual variability in mitochondrial toxicity may be driven by genetic predispositions such as

polymorphisms in mitochondrial enzymes (e.g., POLG, SOD2). Understanding such genetic risk factors can help identify vulnerable subpopulations and tailor hepatoprotective strategies accordingly [15,16].

In summary, this thesis endeavors to:

Identify and document drugs with known mitochondrial hepatotoxicity, particularly those associated with regulatory warnings or market withdrawals, to underscore the clinical relevance and regulatory oversight gaps in detecting mitochondrial liabilities early in drug development.

Investigate chloramphenicol-induced mitochondrial toxicity in HepG2 cell lines under conditions that mimic physiological mitochondrial reliance, thereby offering a mechanistically relevant in-vitro model to assess energy metabolism, ROS generation, and gene expression changes.

Evaluate the hepatoprotective potential of Astaxanthin and Quercetin in mitigating oxidative and mitochondrial damage through both in-vitro and in-vivo models, leveraging their antioxidant, anti-inflammatory, and mitochondrial stabilizing properties.

Contribute to the mechanistic understanding of antioxidant-mediated hepatic protection, specifically in the context of antibiotic-induced mitochondrial stress, by assessing changes in key mitochondrial genes, ATP levels, oxidative biomarkers, and histological liver integrity.

Explore emerging concepts such as mitochondrial dynamics, immuno-mitochondrial signaling, precision toxicology, and novel biomarkers (e.g., mtDNA, GLDH, miR-122), to enrich the mechanistic depth and translational potential of the findings, and to inform strategies for early detection, risk stratification, and therapeutic intervention [15,16].

The novelty of this thesis lies in its integrated experimental approach that bridges cellular, molecular, and systemic assessments of mitochondrial dysfunction, its focus on a clinically relevant yet underregulated antibiotic (chloramphenicol), and its exploration of natural antioxidants as cost-effective, mechanistically grounded interventions. The findings of this research are anticipated to:

Provide foundational insights into the role of mitochondrial dysfunction in DILI pathogenesis.

Inform future development of targeted antioxidant-based therapies;

Support incorporation of mitochondrial endpoints in preclinical screening;

Promote rational use of essential but hepatotoxic drugs in resource-limited settings.

Ultimately, this thesis aims to advance the broader goal of mitigating drug-induced hepatotoxicity through mechanistic clarity, translational modeling, and therapeutic innovation.

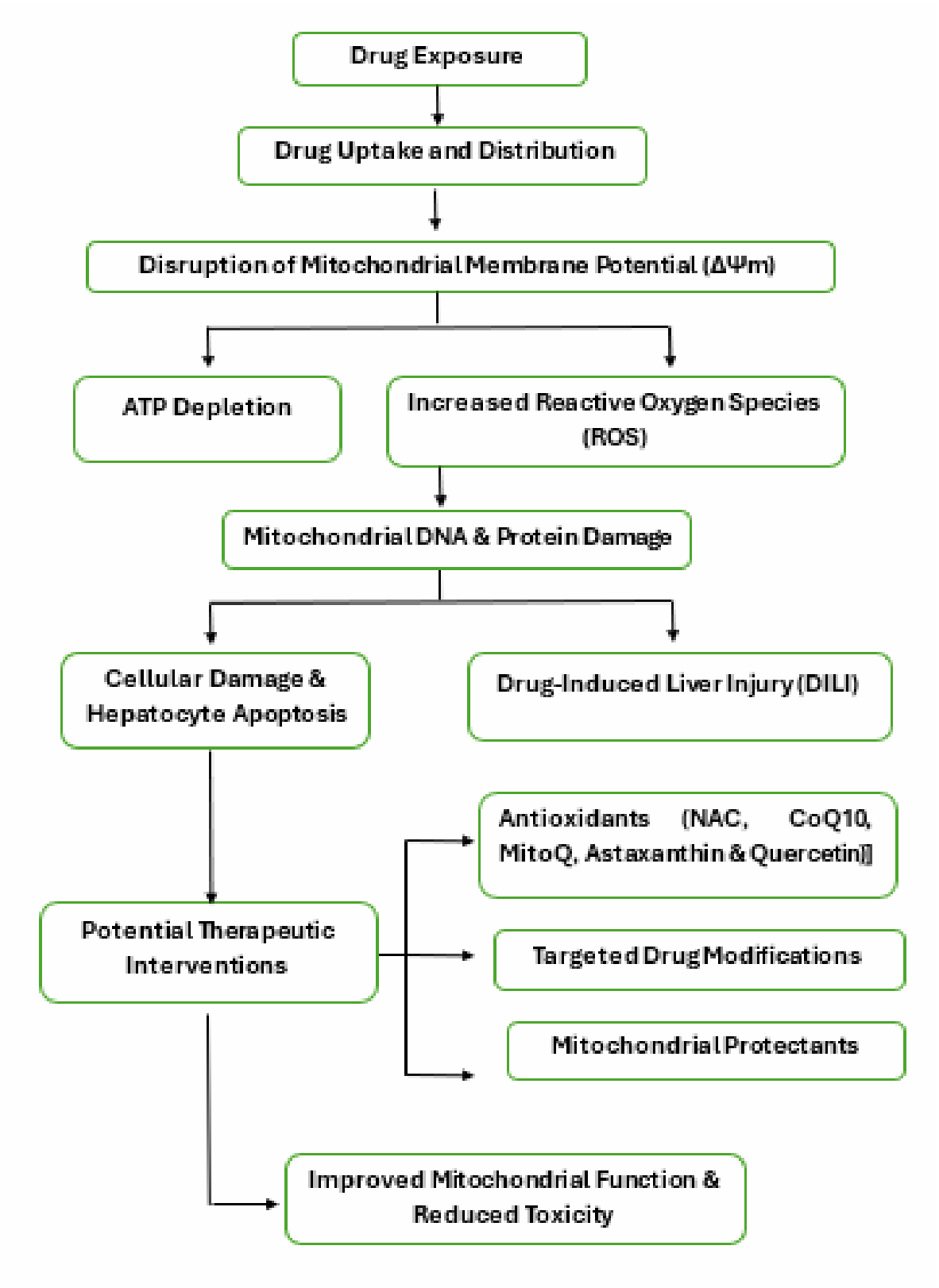

Figure 1: Common Mechanistic Routes Linking Drug Exposure to Liver Damage