# Chapter 3

# 3. AIM AND OBJECTIVES

## 3.1 Rationale of the Study

Liver toxicity remains a major limitation in drug development and post-marketing drug safety, with mitochondrial dysfunction (MD) increasingly recognized as a central mechanism underlying drug-induced liver injury (DILI) [167]. A growing body of evidence suggests that mitochondrial liabilities account for the discontinuation of 3–4% of new chemical entities (NCEs) during clinical development [167]. Several widely prescribed drugs, including antibiotics, antivirals, and anticancer agents, have either been withdrawn or subjected to black-box warnings due to their off-target effects on mitochondrial function [167].

According to recent epidemiological data, DILI has a global incidence of approximately 13.9 per 100,000 individuals annually [168]. The liver's dense mitochondrial population and its role in biotransformation make it particularly vulnerable to compounds that impair oxidative phosphorylation, increase reactive oxygen species (ROS), or inhibit mitochondrial biogenesis [169].

Chloramphenicol, a broad-spectrum antibiotic historically used to treat life-threatening infections, is associated with serious hepatic side effects, including mitochondrial impairment. It exerts its toxicity by inhibiting mitochondrial protein synthesis through interaction with the 55S mitochondrial ribosome, leading to energy depletion, oxidative stress, and hepatocyte injury [169].

Given the lack of targeted therapies to counteract mitochondrial toxicity, antioxidant-based interventions have garnered interest. Antioxidants such as Astaxanthin and Quercetin have demonstrated mitochondrial membrane stabilization, ROS-scavenging, and mitochondrial biogenesis-promoting properties in various oxidative stress models [170,171]. These agents offer a potential strategy to mitigate hepatotoxicity while preserving therapeutic efficacy.

### 3.1.1 Rationale for Selecting Chloramphenicol

Among the list of drugs known for mitochondrial toxicity, Chloramphenicol is particularly relevant for in-depth mechanistic investigation. It remains an essential medicine in resource-limited countries, used to treat typhoid fever, meningitis, and rickettsial infections [172].

Chloramphenicol inhibits mitochondrial protein synthesis by binding to the 55S mitoribosome, impairing electron transport chain (ETC) complex assembly, reducing ATP production, and increasing ROS generation. These mitochondrial dysfunctions contribute to hepatocyte injury and systemic oxidative stress [172].

Its hepatotoxic effects are dose-dependent and well-characterized in both galactose-adapted HepG2 cell lines and Wistar rat models, making it ideal for translational studies. Unlike drugs such as troglitazone or perhexiline, chloramphenicol is not fully withdrawn but used under caution. Notably, despite the mechanistic clarity of its mitochondrial toxicity, it does not carry an FDA black-box warning, highlighting a regulatory gap [173].

Given these factors, chloramphenicol serves as a strategic model compound to investigate antioxidant-based therapeutic interventions aimed at restoring mitochondrial homeostasis and improving hepatic resilience. Co-administration with antioxidants such as Astaxanthin and Quercetin may preserve its clinical utility while minimizing its toxic potential [174,175].

The current study aims to investigate the mechanistic basis of chloramphenicol-induced mitochondrial toxicity and to evaluate the protective efficacy of Astaxanthin and Quercetin using a dual-model system, galactose-adapted HepG2 cells and Wistar rats. The integration of in-*vitro* and *in-vivo* models provides translational insights into the role of mitochondrial dysfunction in DILI and supports the therapeutic exploration of antioxidant co-treatment to restore mitochondrial integrity and hepatic function.

## 3.2 Objectives

1) **Identification of mitochondrial toxicity-related drugs:**

   - Review and document drugs that have been limited in use, withdrawn, or issued black-box warnings by the FDA due to mitochondrial dysfunction-mediated hepatotoxicity.

**2) *In-vitro* analysis of mitochondrial toxicity:**

- Evaluate the cytotoxic effects of chloramphenicol in HepG2 cells cultured in galactose media to simulate mitochondrial reliance.
- Quantify ATP levels, reactive oxygen species (ROS), and expression of mitochondrial genes (SOD2, NRF1, SURF1, TFAM, and UCP2).

**3) *In-vivo* assessment in Wistar rats:**

- Investigate the effect of chloramphenicol-induced hepatotoxicity by measuring biomarkers of oxidative stress (glutathione [GSH] and nitric oxide [NO]).
- Evaluate the protective efficacy of oral Astaxanthin and Quercetin in mitigating oxidative damage.

**4) Mechanistic exploration of antioxidant therapy:**

- Analyze the expression of key mitochondrial genes and oxidative stress markers in both models to delineate the protective mechanisms of Astaxanthin and Quercetin.

**4.**