Drug-induced liver injury (DILI) remains a major hurdle in drug development and a leading cause of post-market drug withdrawal. While non-animal models present ethically and scientifically attractive alternatives for studying DILI mechanisms, replicating the liver's complex cellular architecture and intercellular interactions in vitro remains a significant challenge. This talk will provide an overview of the current landscape of in vitro liver models—including 2D hepatocyte cultures, 3D spheroids, liver organoids, and liver-on-a-chip technologies—and their application in hepatotoxicity assessment. We will critically examine the strengths and limitations of these systems in capturing key aspects of liver function, metabolism, and immune responses. Special emphasis will be placed on emerging strategies that integrate multiple liver cell types and cross-organ interactions, such as gut-liver and liver-immune co-culture platforms, to better reflect systemic toxicity. Finally, we will discuss future directions for improving the physiological relevance and predictive power of these models through standardization, incorporation of human-specific endpoints, and validation with clinical data. Together, these advanced in vitro systems are poised to reshape DILI research and support safer, more efficient drug development.