Chapter 1

Introduction

With advent in drug discovery, the clinically significant issues in the pharmaceutical development of new compounds from multicomponent synthetic as well as natural products are the knowledge on metabolism, the role and kinds of enzymes involved and the potential toxicity of these drug in their early stages. With regards to toxicity assessment, hepatotoxicity is most relevant, since on consumption, medicaments are transported to the liver for metabolism, biotransformation and elimination. Moreover, one of the major reasons of drug attrition is hepatotoxicity. Drug induced hepatotoxicity is a major concern related to health problem with increasing incidences, as the number of drugs being consumed has increased in the form of prescription and non-prescription medicines.

Acetaminophen (APAP), which is a widely used over-the-counter drug and considered safe and effective as an analgesic and antipyretic drug (Temple et al. 2007), is associated with hepatotoxicity in overdoses. Although, the exact incidence rate is difficult to estimate because of certain issues like under-reporting, lack of information about self-medication and drug-drug interactions. However, in 2009, the American Association of Poison Control Centers’ National Poison Data System reported 401 deaths caused by APAP or its combination products in U.S. alone (Hodgman & Garrard 2012). APAP overdose causes acute liver failure, reported widely in western countries with an increasing tendency (Fontana 2008). APAP undergoes extensive hepatic metabolism. The portion of APAP undergoes phase I metabolism by cytochrome P450 dependent mixed function monooxygenases (CYP450) forming an active intermediate, N-acetyl-p-benzoquinone imine (NAPQI), which binds glutathione (GSH) to form non-toxic
cysteine and mercapturate metabolites (Gelotte et al. 2007). Consequently, overdose of
APAP, results in the depletion of GSH, and excess NAPQI binds to cellular proteins
leading to the hepatocyte injury (McGill et al. 2012). As APAP-induced hepatotoxicity is
clinically most relevant, it is widely used hepatotoxicity model for assessment of
hepatoprotective effect of phytomedicines.

A mechanistic insight into the pathophysiology of APAP-induced liver injury
involves injury to parenchymal cells leading to the formation of reactive metabolites,
GSH depletion, mitochondrial membrane damage and protein-metabolite adduct
formation which results in extensive necrosis (Hinson et al. 2010). Substantial cell death
triggers further generation of oxidative stress and peroxynitrile formation, subsequently
collapsing mitochondrial potential and cessation of ATP generation that results in
mitochondrial swelling, further leaking intermembrane proteins. These events trigger
formation of cytokines and chemokines recruiting non-parenchyma cells including
kupffer cells, neutrophils and monocytes eliciting inflammatory response that may
modulate liver injury (Jaeschke et al. 2002; Hinson et al. 2010). Since oxidative stress is
postulated to be important in the propagation of APAP-induced hepatotoxicity,
suppression of the excessive generation of reactive oxygen species (ROS), antioxidants
could offer protection to counteract liver damage. Extensive literature revealed that
plants in the form of both undefined extract as well as isolated phytoconstituents
possessing strong antioxidant capacity demonstrate hepatoprotective activity (Panda et al.
products marketed as hepatoprotective agents have been derived from folk use of the
plants by indigenous cultures. The best known example ‘silymarin’, marketed as
Limarin® by Serum Institute of India ltd., Pune, India; is a mixture of flavonolignans
derived from Silybum marianum, and is also a gold standard for the assessment of
phytomedicines in hepatotoxicity studies (Schuppan et al. 1999). Many polyherbal
formulations like Liv-52 and HD-03 marketed by The Himalaya Drug Company,
Bangalore, India; Livex® marketed by Ban Labs ltd., Rajkot, India are other agents being
consumed by large part of Indian population as liver tonic and liver protectants because
of their chemical compositions (Girish et al. 2009). These pharmaceutical products act as
antioxidants as well as hepatoprotective agents. Few other plant extracts have also been
reported to possess these activities, including Andrographis paniculata (Koh et al. 2011;
Trivedi et al. 2007), Phyllanthus urinavia (Sarin et al. 2014), Tetrapanax papyriferum (Valan et al. 2010), Glycyrrhiza glabra (Nakagiri et al. 2003), Allium sativum (Vimal & Devaki 2004), Solanum nigrum (Gupta et al. 2009), Camellia sinensis (Duraisami et al. 2008), and Curcuma longa (Devasagayam et al. 2004), among others with their bioactive compounds being identified and characterized. However, there are numerous phytoextracts showing hepatoprotective activity, with uncharacterized and unidentified active pharmaceuticals offering a major challenge in the use of these phytomedicines to evaluate further as hepatoprotective drug. Moreover, the mechanism of action of the plant based drugs have not been studied.

Further, it has been suggested, that the damage caused due to APAP overdose and subsequent toxicity depends on the model used. Various models used for assessment of toxicity that have been employed, include animal models such as Wistar rats and albino mice (Kannan et al. 2013; Jothy et al. 2012) and in vitro models such as liver slices, primary hepatocytes, hepatocellular carcinoma cell lines, stem cell derived cultures, etc (Balabubramanian & Raghunathan 2012; Dvorak et al. 2007; Yong et al. 2013; Huang et al. 2011). However, it is reported that, the activity of rodent GSH-transferase is 10 to 20 times higher compared to humans, due to which the reactive intermediates gets immediately detoxified, limiting the use of these models because of the difficulties they offer in prediction of toxicity in humans (Akai et al. 2007). Hence, species differences in drug metabolism, target molecules and pathophysiology are important factors that must be considered in the interpretation of preclinical findings and in assessing their relevance to humans, emphasising the need for using human derived cellular system to study the APAP-induced toxicity.

In the view of the literature available on APAP-induced hepatotoxicity and lack of scientifically validated records on phytomedicines with relevance to their hepatoprotective activity, this work was undertaken to first establish the hepatotoxicity model based on APAP-induced liver damage using HepG2 cell line. Subsequently, the hepatoprotective potential of a well characterized phytoextract obtained from seeds of Brassica juncea Czern. and Coss. was investigated. The elucidation of mechanism of action of the characterized extract in hepatoprotection at the cellular level was also studied.