ABSTRACT

Liver dysfunction results in impairment of toxin detoxification, leading ultimately to toxin accumulation in vital organs and consequently causing disseminated damage to them. Hence, it is imperative that a conscious effort be made to prevent exposure to these toxic substances, or where unavoidable, mitigate the associated liver injury by the use of plants or allopathic drugs known to possess universally accepted hepatoprotective potency. However, there is currently no allopathic drug that is effective for the treatment of liver diseases. On the other hand, Medicinal plants play significant role in the treatment/management of liver disorders and many of them have shown significant hepatoprotective potency. Modern science has examined numerous plant extracts for this purpose and has confirmed the traditional experience by discovering the mechanisms of action of these plants. In spite of the tremendous advances seen, very few significant and safe hepatoprotective agents are available in modern therapeutics.

Acetaminophen (APAP), which is a widely used over-the-counter drug and considered safe and effective as an analgesic and antipyretic drug, is associated with hepatotoxicity in overdoses. To study the effect of phytoextract for hepatoprotection, APAP-induced toxicity model was hence established in vitro using HepG2 cell line, which mimic parenchyma cells of human liver. Various concentrations of APAP were evaluated to establish the toxicity model. The assessment of hepatocyte dysfunction includes cytotoxicity assessment by MTT assay, various biochemical parameters namely AST, ALT, GGT and ALP as the indices of hepatic injury. Various antioxidant defence mechanisms operates within the hepatocytes as excessive generation of ROS play a central role in APAP toxicity that can be studied to evaluate the extent of injury and following reversal by the phytoextract. In the current study, the generation of ROS was detected directly by employing a sensitive and automated flow cytometric technique using an intracellular dye DCFDA. Further, the cell cycle analysis was performed to evaluate the effect of APAP on different phases of cell cycle and monitor the level of apoptosis occurring as a consequence of APAP toxicity. This work was also performed employing flow cytometer involving the use of propidium iodide (PI) as a fluorescent dye which enters the cell on
permeabilization and intercalates within the strands of the nuclear DNA quantitatively. Besides, the necrosis caused due to APAP induced hepatocyte injury was visualized by fluorescence imaging using EB/AO staining.

*Brassica juncea* Czern. and Coss., also known as Indian mustard, is an economically important plant that has been well known in India for centuries for its medicinal and nutritive values. The rich phytochemical profile of *B. juncea* and related species indicate the presence of various phytoconstituents reported to show varied pharmacological properties along with antioxidant potential. The beneficial effects of *Brassica* vegetables on health improvement have been partly attributed to their complex mixture of phytochemicals possessing antioxidant activity. Thus, the purpose of extraction was to isolate phytoconstituents from seeds responsible for maximum antioxidant activity. The broad spectrum of beneficial effects of the seeds observed in the earlier studies on *B. juncea* studies warrants further exploration of *B. juncea* seeds as a potential source for obtaining pharmacologically standardized phytotherapeutics, that could be potentially useful. The present study is one such attempt to rationalize the existing knowledge within the framework of modern science principles, practices and the techniques.

Subsequently, the elucidation of mechanism of action of the characterized extract in hepatoprotection at the cellular level was also studied. APAP and other nonsteroidal anti-inflammatory drugs have been evaluated extensively for their hepatotoxicity, and herbal extracts have been assessed for their protective nature against APAP toxicity. However, the mechanism of action of these extracts have been explored less thoroughly. The focus of the current investigation was to evaluate the mechanism of protection of BJHME against APAP-induced toxicity in HepG2 cell line. Our data demonstrate that BJHME suppresses the extensive generation of ROS that is caused by APAP, thus preventing the oxidant stress that appears to cause APAP toxicity. It is well established that, plant extracts with antioxidant potential can protect against the oxidative damage caused by APAP hepatotoxicity (Parmar et al. 2010). We confirmed this observation and demonstrated that BJHME substantially reduced the oxidative stress in HepG2 cell line and protected the membrane integrity as indicated by the enzyme release profile.