Abstract

Hepatocellular carcinoma (HCC) is the fifth leading cause of cancer-associated death worldwide. Current treatment modality for HCC is marginally effective. Plants belonging to Mistletoe family (Loranthaceae) have been used in chemotherapy for many years. *Elytranthe parasitica* (L.) Danser (EP), a hemiparasitic plant (Loranthaceae) is known for its potent anti-cancer properties. The aim of the present study was to isolate and characterize bioactive fractions/compounds from *Elytranthe parasitica* (L.) Danser (EP) and elucidate their effects on cancer cells. Stem parts of *Elytranthe parasitica* were standardized, extracted with methanol and sequentially fractionated with solvents petroleum ether, diethyl ether, ethyl acetate, butanol and water to obtain petroleum ether fraction (EP.PE), diethyl ether fraction (EP.DEE), ethyl acetate fraction (EP.EA), butanol fraction (EP.But) and aqueous fractions (EP.Aq) respectively. All fractions so obtained were screened for anti-cancer effects *in vitro* on various cancer cell lines by SRB (Sulphorhodamine B) assay. Active fraction of *Elytranthe parasitica*, EP.DEE exhibited potent cytotoxic activity in a dose dependent manner against HepG2 hepatocellular carcinoma cell line with an IC50 of 56.7 ± 7.8 µg/mL. The most bioactive EP fractions were further separated, and detailed mechanistic anti-cancer evaluation was carried out. The antitumor potential of the enriched fractions of EP stem parts was examined in human hepatocellular carcinoma cell line, HepG2. Accordingly, the anti-proliferative, clonogenic and anti-metastatic potential of EP enriched fractions was assessed by Sulphorhodamine B (SRB), colony formation and scratch wound assay respectively. The effects of EP enriched fractions on apoptosis/cell cycle arrest and on key markers of apoptosis/mitogen-activated protein kinase (MAPK) markers deregulated in HCC have been explored by flow cytometry and ELISA respectively. Bioactivity fractionation was performed via gel filtration chromatography and analytical TLC to isolate the phytochemical(s) exerting the anti-tumor activity in HepG2 cells. Activity guided fractionation led to the isolation of four phenolic compounds and two phytosterols: three flavanones (pinocembrin -7-O-glycoside, pinostrobin, chrysin), a phenolic acid (gallic acid), a triterpenoid (lupeol) and a phytosterol (β-sitosterol). Further, phytochemical composition of the most bioactive fraction as assessed by GC-MS, indicated the chief phytocompound present in the bioactive fraction as pinocembrin-7-O-glycoside. EP bioactive fractions significantly reduced cancer cell proliferation, induced apoptosis and caused cell cycle arrest in HepG2 cancer cells via upregulation of apoptotic markers, signifying the fraction to be a promising contender in the treatment of hepatocellular carcinoma.
Taken together, our findings reveal that EP enriched fraction-induced cell cycle arrest and apoptosis in HepG2 cells occurs via upregulation of apoptosis signaling and deactivation of MAPK pathways. Synergistic effects of the constituents in the EP fractions (acting on multiple sites and via diverse signaling pathways) may be responsible for its potent anticancer effects. These fractions can be further evaluated in vivo to comprehend the possible clinical implications.