Abstract

Breast cancer is the most frequent cancer among women and even with advancements in diagnosis and treatment, the number of new cases is alarmingly increasing worldwide. *Glycosmis pentaphylla* (Retz.) DC (Family-Rutaceae) has been used traditionally as anti-cancer medicine. Even though the plant has ethnopharmacological importance not much of studies were performed to explore the pharmacological activities of the plant in general and anticancer potential in particular. Our own preliminary studies on the plant showed that it was cytotoxic against cancer cell lines and cytotoxicity was more towards breast cancer cells. Hence we studied the plant for its potential against breast cancer both *in vitro* and *in vivo*.

In the present study various fractions from the leaf extracts of the plant was screened for cytotoxicity in a series of cancer and normal cell lines. Preliminary screening resulted in shortlisting of four fractions viz. PEG2, PEG5 (both obtained from petroleum ether extract), DCM2 (from dichloromethane extract) and EF1 (from ethanolic extract), for further studies. All four fractions were selectively cytotoxic to cancer cells, MCF-7 and MDA-MB-231, when compared to normal cell lines. The fractions were standardized by HPLC and HPTLC methods. The ability of the fractions to inhibit the proliferation capability of MCF-7 and MDA-MB-231 cells was studied by colony formation assay. The effect of fractions on apoptosis of breast cancer cells was studied by flow cytometry, nuclear staining, western blotting and ELISA methods. The fractions arrested MCF-7 cells at G0/G1 phase and MDA-MB-231 cells at G2/M phase. Apoptosis through intrinsic mitochondrial pathway was confirmed by Annexin V binding, caspase 3/7 activation, and increased levels of markers like p-53, Bax, cytochrome c, cleaved PARP and caspase-9. Anti-angiogenic and anti-metastatic potential of the fractions was showed by the decreased levels of CoCl2-induced HIF-1α expression and PMA-induced MMP-9 expression.

Acute toxicity studies (according to OECD 425 guidelines) of the fractions showed that the fractions were safe up to a dose of 2000 mg/kg. *In vivo* studies in DMBA-induced mammary tumor model in Sprague Dawley rats showed that the fractions PEG2, EF1 and DCM2 at a dose of 400 mg/kg, p.o. were able to significantly reduce the tumor weight and volume. The fraction PEG5 which has shown significant activity *in vitro* was unable to show a significant activity *in vivo* at 400 mg/kg, p.o. The animals treated with the fractions showed better anti-oxidant status.
compared to the DMBA-control group. In EAC model, the fractions were able to increase the survival and decrease the tumor load in Swiss albino mice.

Natural products due to their variety of chemical components have the property to act in a moderate way devoid of severe toxicities. They are also able to target and act through multiple signaling pathways. *Glycosmis pentaphylla* (Retz.) DC has shown promising results *in vitro* and *in vivo*, and maybe explored further.