Evaluation of cytotoxic effect of Fucoxanthin and Phlorotannin on apoptosis

Abstract:

We aimed to investigate the effect of Fucoxanthin and Phlorotannin present in *Sargassum tenerrimum* on apoptosis mechanism by *in-vitro* analysis. We have to characterize the extracting compound Fucoxanthin and Phlorotannin by phytochemical analysis, UV-Visible spectrophotometer and FTIR respectively. Further docking by PYRX tool to evaluate the apoptogenic potential through stimulation of caspases-3, 8, 6, 9 and Bax, where Bax protein gets interaction at active site amino acid residue gives better binding affinity, which blocks the Bcl-2 for the upregulation of Bax protein to induce apoptosis. Further *in-vitro* analysis shows the cytotoxicity of fucoxanthin and phlorotannin crude extract in MCF 7 breast cancer cell line.

Introduction:

Sargassum tenerrimum species are tropical and sub-tropical brown macroalgae of shallow marine meadow. The derived bioactive compound Fucoxanthin and Phlorotannin have many biological activities such as anti-cancer, anti-coagulant, anti-oxidant and other activities. Breast cancer is the second leading cause of death in women worldwide, with nearly 1.7 million new cases in that 80% of breast cancer occur in women older than age 50. There are two major apoptosis signaling pathway, one is death receptor (extrinsic) pathway and the mitochondria (intrinsic) mediated pathway. Caspase 8,9 are initiators and Caspase 3, 7, 6 was executioners of apoptosis. The anti-apoptotic protein Bcl-2 blocks the mitochondrial outer membrane and inhibits apoptosis and the expression of pro-apoptotic Bax protein inhibit the Bcl-2 proteins might induce the apoptosis in cancer cells. We further demonstrated the apoptosis by *in-silico* analysis via extrinsic and intrinsic apoptotic signaling pathway through anti-apoptotic and pro-apoptotic proteins.

Materials and methods:

The Fucoxanthin and Phlorotannin were extracted by Solvent extraction method (chloroform: methanol: water in the ratio 4:2:1), after 3 days separate the phases and store at 4 ℃. The phytochemical screening of extracts for the presence of phenol, tannins, saponins were carried out. The pigment profile of the fucoxanthin was determined by using a double beam UV-Visible spectrophotometer at the wavelength range of 350-750 nm. The FTIR analysis was carried to evaluate the presence of fucoxanthin and phlorotannin through the functional groups respectively and then total phenolic content of phlorotannin crude extract was determined by the Folin-Ciocalteu method. For in-silico analysis, the proteins and ligands were obtained from PDB and PubChem respectively, then processed for the docking by using PYMOL. Then evaluate cytotoxicity by MTT assay with IC50 value and through western blotting the Caspase 3,6,8,9 and bax, bcl-2 proteins were treated with MCF7 cells to evaluate the morphological changes to confirm cytotoxic effects respectively.

Advantages:

Brown algae were abundantly available in Indian Coastal areas and the drug will be of low cost compared with available drugs. Further it may reduce most of the side effects.