Crude ethyl acetate extract of marine microalga, Chaetoceros calcitrans, induces Apoptosis in MDA-MB-231 breast cancer cells

ABSTRACT

Background: Marine brown diatom Chaetoceros calcitrans and green microalga Nannochloropsis oculata are beneficial materials for various applications in the food, nutraceutical, pharmaceutical and cosmeceutical industries. Objective: This study investigated cytotoxicity of different crude solvent extracts from C. calcitrans and N. oculata against various cancer cell lines. Materials and Methods: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was carried out to screen the cytotoxic effects of hexane (Hex), dichloromethane (DCM), ethyl acetate, and methanol extract from C. calcitrans and N. oculata toward various cancer cell lines. Flow cytometry cell cycle was used to determine the cell cycle arrest while the mode of cell death was investigated through acridine orange/propidium iodide (AOPI) staining, Annexin V-Fluorescein Isothiocyanate (FITC) and Terminal deoxynucleotidyl transferase-mediated d-UTP Nick End Labeling (TUNEL) assays. Expression profile of apoptotic and proliferative-related genes was then determined using the multiplex gene expression profiler (GeXP). Results: Crude ethyl acetate (CEA) extract of C. calcitrans inhibited growth of MDA-MB-231 cells, with IC 50 of 60 μg/mL after 72 h of treatment. Further studies were conducted to determine the mode of cell death at various concentrations of this extract: 30, 60 and 120 μg/mL. The mode of cell death was mainly apoptosis as shown through apoptosis determination test. The expression data from GeXP showed that caspase-4 was upregulated while B-cell leukemia/lymphoma 2(Bcl-2) was down regulated. Thus, caspase-4 induction endoplasmic reticulum death pathway is believed to be one of the mechanisms underlying the induction of apoptosis while Bcl-2 induced S and G2/M cell cycle phase arrest in MDA-MB-231 cells. Conclusion: CEA extract of C. calcitrans showed the highest cytotoxicity on MDA-MB-231 via apoptosis.

Keyword: Apoptosis; Chaetoceros calcitrans; Crude ethyl acetate extract; Gene expression profiler; MDA-MB-231