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Antiproliferative and apoptotic effects of polyphenol-rich crude methanol extract of *Gracillaria edulis* against human Rhabdomyosarcoma (RD) and breast adenocarcinoma (MCF-7) cell lines

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Abstract

Seaweeds are an important source of bioactive metabolites in the development of drugs and nutraceuticals. This study aimed to analyze the anti-proliferative and apoptotic activity of methanol extract (ME) of *G.edulis* on human Rhabdomyosarcoma (RD) and breast adenocarcinoma (MCF-7) cell lines. De-polysaccharide ME of *G.edulis* was extracted to determine the anti-proliferative and apoptotic activity. The cytotoxic activity was evaluated by MTT and neutral red uptake assay, while apoptotic activity was characterized by cellular morphology, DNA fragmentation and Caspase 3/7 assay. The results of cytotoxicity assay showed that the decrease of the percentage of cell viability in a dose-dependent manner as signified by cell death. The ME of *G.edulis* exhibited potent cytotoxicity activity against RD ($IC_{50METHANOL}$: 49.86±0.02µg/ml) and MCF-7 ($IC_{50METHANOL}$: 34.43±0.86 µg/ml) cells compared to the standard cycloheximide (IC_{50RD}: 36.17±1.78µg/ml; $IC_{50MCF-7}$: 28.76±0.55µg/ml) as confirmed by MTT assay. Further, potential cytotoxic activity of ME (IC_{50RD}: 33.47±2.25µg/ml; $IC_{50MCF-7}$: 35.13±0.95µg/ml) against RD and MCF-7 were confirmed by neutral red assay compared to the standard cycloheximide (IC_{50RD}: 32.78±0.91µg/ml; $IC_{50MCF-7}$: 27.84±0.33µg/ml). The apoptosis was induced by activating the Caspase 3/7 pathway both in RD and MCF-7 cells treated with ME after 24 hours. Highest Caspase 3/7 activity was observed after 4 hours in RD cells, whereas in MCF-7 high Caspase activity was observed after 3 hours of the treatment. Further, the DNA ladder pattern was observed in RD cells treated with ME of. *G.edulis* whereas MCF-7 cells did not show any typical ladder pattern. Besides, the morphological changes of apoptosis were examined using two concentrations which produce 50% cell inhibition (IC_{50}) and its half ($IC_{50/2}$). Most of the apoptosis morphological features were observed under the inverted microscope and when stained with fluorescence dye Hoechst stain. Thus, it can be concluded that the ME of *G.edulis* possesses anticancer activity via activating the Caspases pathway.

Keywords: G.edulis, Anti-cancer, Rhabdomyosarcoma, MCF-7, MTT, Neutral red.

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