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Dentatin inspires apoptosis in MCF-7 cells through intrinsic pathway with involvement of release of cytochrome C and activation of caspase 9

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Ethno-pharmacologically, *Clausena excavata* Burm. F. has been used in folk medicines in eastern Thailand for the treatment of cancer. To investigate the apoptosis mechanism, Dentatin (DTN) was separated from this plant. DTN-induced cytotoxicity was observed with the MTT assay. Acridine orange/propidium iodide staining was used to detect cells in early apoptosis and High Content Screening (HCS) to observe nuclear condensation, cell permeability, Mitochondrial Membrane Potential (MMP) and cytochrome C release. Apoptosis was confirmed with a clonogenic assay, DNA laddering and caspase 3/7 and 9 assays. A significant increase in chromatin condensation in the cell nucleus was observed by fluorescence analysis. Apoptosis was confirmed by the reduced number of colonies in the clonogenic assay and the increased number of cellular DNA breaks in treated cells observed as a DNA ladder. Treatment of MCF-7 cells with DTN encouraged apoptosis with cell death-transducing signals that reduced MMP by down-regulation of Bcl-2 and up-regulation of Bax, triggering cytochrome C release from the mitochondria to the cytosol. The released cytochrome C triggered the activation of caspase 9 followed by the executioner caspase 3/7. DTN treatment significantly arrested MCF-7 cells at the G0/G1 phase ($p<0.05$) and ROS was significantly elevated. Moreover, DTN significantly blocked the induced translocation of NF-κB from cytoplasm to nucleus. Together, the results demonstrated that the DTN isolated from *Clausena excavata* inhibited the proliferation of MCF-7 cells, leading to cell cycle arrest and programmed cell death, which was confirmed to occur through the mitochondrial pathway with involvement of the NF-κB signalling pathway.

Biography

Ismail Adam Arbab Ishag completed his PhD from Putra University of Malaysia and one and half year Postdoctoral studies from the National University of Malaysia, School of Chemical Sciences and Food Technology. He has published more than 14 papers in reputed journals. He is currently continuoing his Postdoctoral studies in Herbal Cancer Research.

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