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Title : MECHANISM OF ALOE EMODIN-INDUCED APOPTOSIS IN ER+-BREAST CANCER CELLS, MCF-7

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Aloe emodin, an anthraquinone exhibits higher cytotoxicity to hepatoma, prostate and cervical cancer cells through cell cycle arrest and apoptosis compared to normal cells. However, its underlying mechanism on ER+-breast cancer cell death remains unclear. Therefore, this study was done to investigate aloe emodin cytotoxicity and its mechanism on estrogen receptor (ER)-positive (MCF-7), ER-negative breast cancer cells (MDA-MB-231) and control breast cells (MCF-10A) in comparison with tamoxifen. Cytotoxicity was determined using WST-1 proliferation assay and Trypan blue exclusion test. Apoptosis mechanism was investigated using Annexin V-FITC/PI staining and DNA fragmentation assay. Both genes and proteins involved in the regulation of cell cycle (p53, p21, CDK1, CDK2, cyclin B1 and cyclin E1) and apoptosis (Fas, FADD, Caspase-3, Caspase-8, Caspase-9, Bax, Bcl-2, and Cytochrome c) in aloe emodin-treated MCF-7 were determined using Quantigene 2.0 Plex and protein ELISA assays respectively. Maximum treatment time was set up to 72 hours in all assays. Aloe emodin inhibited the proliferation of MCF-7 with IC_{50} of 80µM. No IC_{50} value was obtained on MDA-MB-231 and MCF-10A, even up to 150µM. In contrast, tamoxifen was non-selective to all cells with IC_{50} of 27µM, 19µM and 42µM, respectively. IC_{50} values obtained were used in all the other assays. Results from Trypan blue exclusion test were in concordance with the proliferation assay. Study on Annexin/PI staining showed the presence of early and late apoptosis (18.42% ± 3.53 to 29.25% ± 0.55; $p<0.05$, n=3 and 28.45% ± 2.36 to 30.22% ± 0.56; $p>0.05$, n=3, respectively) in aloe emodin and tamoxifen-treated MCF-7 cells. Accordingly, DNA fragmentation was observed. Aloe emodin and tamoxifen enhanced MCF-7 cytotoxicity through apoptosis. In cell cycle signalling, aloe emodin upregulated the expression of p53 and p21 proteins; while downregulating CDK1. Only CDK1 protein is in accordance with gene expression. In intrinsic apoptosis signalling, Bax, Cytochrome c and Caspase-9 proteins were upregulated; while no change observed in Bcl-2 protein. Except for Caspase-9, these results are in accordance with gene expression. In extrinsic apoptosis, Fas and Caspase-8 were upregulated, contrary to gene expressions. These findings indicate that aloe emodin cytotoxic action on MCF-7 cells is through G2/M arrest; both extrinsic and intrinsic apoptosis pathways. Its actions on G2/M phase arrest and activation of intrinsic apoptosis pathways were p53-dependent, while extrinsic apoptosis was p53-independent. Data obtained suggests (i) aloe emodin has potential as a selective apoptotic inducer in ER+-breast cancer management and (ii) and the present study could be used as a basic rationale for *in vivo* experiment setting.