[BIO04]
Eurycomanone exerts antiproliferative activity via apoptosis upon MCF-7 cells

Cheah Shiau Chuen, Azimahtol Hawariah Lope Pihie

School of BioSciences and BioTechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor Darul Ehsan, Malaysia.
E-mail: shiauchuen_chuen@yahoo.com

Eurycoma longifolia Jack is well known among the traditional medicine practitioners in Malaysia as a panacea for many diseases. Eurycomanone is a type of quassinoid extracted from *E. longifolia* Jack. Previous reports have noted its potential use as an antiproliferative agent. In this study, the purported efficacy of eurycomanone in inhibiting cell growth and its mechanism in eliciting cell death was evaluated on malignant breast cancer cells (MCF-7). These cells were treated with eurycomanone at increasing concentrations and viability was assayed by a dye inclusion method employing methylene blue. The antiproliferative effect of eurycomanone was evident only on MCF-7 cells, with an EC_{50} value of 2.2 ± 0.18 μg/ml. The reduced cell number suggested a possible cytotoxic effect, which was then confirmed by apoptotic cell death as evaluated by the TUNEL assay. High fluorescence intensity in the nuclear region of MCF-7 cells treated with eurycomanone was detected, depicting cell death via apoptosis compared with non-treated nucleus. Apoptosis levels were found to increase from >70% by 24-hrs, to >80% by 48-hrs, and then to >85% after 72-hrs. Nuclear fragmentation detected by nuclear staining methods revealed apoptotic cell death instead of necrosis. Western blotting demonstrated that the expression of (anti-apoptotic protein) BCL-2, decreased 2-hrs of post-treatment in contrast to the controls. However, pro-apoptotic protein, BAX remained at a basal level, thus suggestive of a possible shift in the Bax: Bcl-2 ratio, which favours apoptosis. The expression of p53 was not affected. Next, the processing of the initiator procaspase-8 and procaspase-9 was detected. Caspase-8 then in turn targeted Bid and caspase-7. Bid was cleaved and active caspase-7 then cleaved and inactivated PARP. Caspase-9 in turn targeted caspase-6. Caspase-6 then cleaved and activated lamin which is a important nuclear membrane protein in maintaining normal cell functions such as cell cycle control, DNA replication and chromatin organization. The use of *E. longifolia* Jack as a potential anticancer is bolstered by this study and holds great potentials for breast cancer treatment.