

Unfermented freeze-dried leaf extract of Tongkat Ali (*Eurycoma longifolia* Jack.) induced cytotoxicity and apoptosis in MDA-MB-231 and MCF-7 breast cancer cell lines

ABSTRACT

The present study was conducted to determine the cytotoxicity effect of *Eurycoma longifolia* (Jack.) leaf extracts and also its possible anticancer mechanism of action against breast cancer cell lines: non-hormone-dependent MDA-MB-231 and hormone-dependent MCF-7. The leaves of *E. longifolia* were processed into unfermented and fermented batches before drying using freeze and microwave-oven drying techniques. Obtained extracts were tested for cytotoxicity effect using MTT assay and phenolic determination using HPLC-DAD technique. The most toxic sample was analyzed for its apoptotic cell quantification, cell cycle distribution, and the expression of caspases and apoptotic protein using flow cytometry technique. Fragmentation of DNA was tested using an agarose gel electrophoresis system. The results determined that the unfermented freeze-dried leaf extract was the most toxic towards MDA-MB-231 and MCF-7 cells, in a dose-dependent manner. This extract contains the highest phenolics of gallic acid, chlorogenic acid, ECG, and EGCG. The DNA fragmentation was observed in both cell lines, where cell cycle was arrested at the G_2/M phase in MCF-7 cells and S phase in MDA-MB-231 cells. The number of apoptotic cells for MDA-MB-231 was increased when the treatment was prolonged from 24 h to 48 h but slightly decreased at 72 h, whereas apoptosis in MCF-7 cells occurred in a time-dependent manner. There were significant activities of cytochrome c, caspase-3, Bax, and Bcl-2 apoptotic protein in MDA-MB-231 cells, whereas MCF-7 cells showed significant activities for caspase-8, cytochrome c, Bax, p53, and Bcl-2 apoptotic protein. These results indicate the ability of unfermented freeze-dried leaf extract of *E. longifolia* to induce apoptosis cell death on MDA-MB-231 and MCF-7, as well as real evidence on sample preparation effect towards its cytotoxicity level.