Induction of selective cytotoxicity and apoptosis in human T4-lymphoblastoid cell line (CEMss) by boesenbergin a isolated from boesenbergia rotunda rhizomes involves mitochondrial pathway, activation of caspase 3 and G2/M phase cell cycle arrest

ABSTRACT

Background Boesenbergia rotunda (Roxb.) Schlecht (family zingiberaceae) is a rhizomatous herb that is distributed from north-eastern India to south-east Asia, especially in Indonesia, Thailand and Malaysia. Previous research has shown that the crude extract of this plant has cytotoxic properties. The current study examines the cytotoxic properties of boesenbergin A isolated from Boesenbergia rotunda. Methods MTT assay was used to check the cytotoxicity of boesenbergin A. The morphological assessment of apoptosis was monitored using normal and fluorescence microscopy. The early and late phase of apoptosis was investigated using annexin V and DNA laddering assays, respectively. The mitochondrial membrane potential (MMP) was assessed by fluorescence microscopy. Human apoptosis proteome profiler assays were performed to investigate the mechanism of cell death. In addition, the protein levels of Bax, Bcl2 and HSP 70 were also analyzed using western blot. Assays of caspase =-3/7, -8 and =-9 were carried out in order to test for induction during treatment. Lastly, cell cycle progression was analyzed using flow cytometry. Results Boesenbergin A was found to have the highest toxicity towards CEMss cancer cells (IC50 = 8 μg/ml). The morphology of CEMss cells after treatment showed evidence of apoptosis that included blebbing and chromatin condensation. The annexin V assay revealed that early apoptosis is induced after treatment. The DNA laddering assay confirmed that DNA fragmentation had occurred during late apoptosis. The cell cycle analysis indicated that boesenbergin A was able to induce G2/M phase arrest in CEMss cells. The activity of caspases -3/7, -8 and -9 was increased after treatment which indicates both intrinsic and extrinsic pathways are induced during apoptosis. The involvement of mitochondria was established by increased mitochondrial membrane potential and up and down regulation of Bcl2 and Bax proteins as well as HSP70. Conclusion In conclusion, the results demonstrated that boesenbergin A induced apoptosis of CEMss cells through Bcl2/Bax signaling pathways with the involvement of caspases and G2/M phase cell cycle arrest. The current findings warrant further research on boesenbergin A as a novel chemotherapeutic agent for leukemia intervention including studies in animal models.

Keyword: Anticancer; Boesenbergia rotunda; Boesenbergin A; CEMss; Cytotoxicity.