

Identification of Methyl- α -D-glucopyranoside as the active compound from *Tulbaghia violacea* in the induction of apoptosis.

¹Lyantagaye SSL, ¹Meyer M, ²McKenzie J and ¹Rees DJG

¹Department of Biotechnology, University of the Western Cape, Private Bag X17, Bellville, 7535, South Africa. ²Department of Chemistry, University of Stellenbosch, 7600, South Africa

Tulbaghia violacea Harv is a medicinal plant indigenous to South Africa. It has traditionally been used to treat a variety of disease conditions including cancer of the esophagus, fever, colds, asthma, tuberculosis, and stomach problems. However the mechanisms of action against these diseases, and the active constituents of the plant are unknown. We have evaluated the pro-apoptotic activity of *T. violacea* in order to understand its medicinal properties.

We demonstrate the occurrence of morphological and biochemical changes, typical of apoptosis, in Chinese hamster ovary (CHO) cells treated with the aqueous extract from *T. violacea*. The shrinkage of the cells and the subsequent detachment from each other was observed. Depolarisation of mitochondrial membrane potential was detected by using TMRE and FACS, and the activation of caspase-3 was detected. Phosphatidyl-serine (PS) translocation from the inner to the outer surface of the cell membrane was detected by using both APOPercentageTM Apoptosis Assay and Annexin-V PE on FACS. Agarose gel electrophoresis revealed the fragmentation of chromosomal DNA. Overall these convincingly characterise the induction of apoptosis with the *T. violacea* extract.

We have purified three pro-apoptotic fractions from the *T. violacea* extract by using bioactivity-guided fractionation. Characterisation of these fractions by X-ray crystallography, NMR, MS, IR and microanalysis techniques showed the presence of methyl- α -D-glucopyranoside and fructofuranose as the major components. Methyl- α -D-glucopyranoside was confirmed to be active in the induction of apoptosis, using commercially sourced material, and we propose a mechanism of action involving hexokinase in the activation of the mitochondrial transition pore and generation of reactive oxygen species in inducing apoptosis. The identification of this novel apoptosis inducing secondary metabolite presents opportunities for novel anti-cancer therapies to be developed.