

Comparison of cytotoxicity between extracts of *Clinacanthus nutans* (Burm. f.) Lindau leaves from different locations and the induction of apoptosis by the crude methanol leaf extract in D24 human melanoma cells

Abstract

Background

Clinacanthus nutans (Burm. f.) Lindau leaves are widely used by cancer patients and the leaf extracts possess cytotoxic and antiproliferative effects on several human cancer cell lines. However, the effect of *C. nutans* leaf extract on human melanoma, which is the least common but most fatal form of skin cancer and one of the most common cancers diagnosed in both sexes worldwide, is unknown. There is also limited information on whether the bioactivity of extracts differs between *C. nutans* leaves grown in different geographical locations with varying environmental conditions.

Methods

The present study, for the first time, compared and demonstrated the cytotoxicity of the crude methanol extracts of *C. nutans* leaves from 11 different locations in Malaysia, Thailand and Vietnam, with diverse environmental conditions against D24 melanoma cells through WST-8 assay. The percentage of apoptotic cells following treatment with the most active extract was determined in a dose- and time-dependent manner by a cytofluorometric double staining technique. Biochemical and morphological changes in the treated and untreated cells were examined by fluorescence and transmission electron microscopy techniques, respectively, to further affirm the induction of apoptosis.

Results

The leaves of plants grown at higher elevations and lower air temperatures were more cytotoxic to the D24 melanoma cells than those grown at lower elevations and higher air temperatures, with the leaf extract from Chiang Dao, Chiang Mai, Thailand exhibited the highest cytotoxicity (24 h EC₅₀: 0.95 mg/mL and 72 h EC₅₀: 0.77 mg/mL). This most active crude extract induced apoptotic cell death in the D24 cells in a dose- and time-dependent manner. Typical biochemical and morphological characteristics of apoptosis were also observed in the treated D24 cells.

Conclusions

The results, showing the cytotoxicity of *C. nutans* and the induction of apoptosis in D24 cells, are significant and useful to facilitate the development of *C. nutans* as a potential novel chemotherapeutic agent for the management of skin melanoma.