Induction of Cell Death in Mouse Melanoma Cells by a Trypsin Inhibitor from *Mimosa regnellii* Seeds

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Introduction Cutaneous melanoma is an aggressive type of cancer with a poor prognosis for patients with advanced disease. The identification of several key molecular pathways implicated in the pathogenesis of melanoma has led to the development of novel therapies. In this work, a trypsin protease inhibitor was purified from the leguminosae *Mimosa regnellii* seeds (ITJ) and it was evaluated due to its ability to induce cell death via apoptosis in B16-f10 cell line.
Objectives Purify a trypsin inhibitor from *Mimosa regnellii* seeds and assess its ability to induce death in mouse melanoma cells.
Material and Methods The toxicity of B16-f10 cells was evaluated by MTT and BrdU assays; Flow cytometry techniques were applied to assess induction of cell death and the alterations on mitochondrial outer membrane permeabilization; immunofluorescence and confocal techniques were used to analyze caspase 3 activation.
Results and Discussion A reducing cell proliferation at a time and dose dependent manner was observed after ITJ incubation, with an IC_{50} of 0.65 µM. The hemolytic activity against peripheral blood cells and cytotoxicity against 3T3 cell lines were tested using several concentrations, indicating that ITJ is not toxic to normal cell lines. ITJ induces cell death via apoptosis after 72h of incubation, as shown in double staining to Annexin V-FITC/PI and cell cycle arrest at G0/G1 phase. Mitochondrial outer membrane permeabilization was also evaluated, indicating that an intrinsic cell death mechanism was activated to induce B16-f10 cells to apoptosis after 72h exposition with ITJ, with superexpression of caspase 3 in melanoma cells.
Conclusion The results showed that ITJ probably induces cell death by apoptosis activation, promoting mitochondrial membrane permeabilization, cell cycle arrest in G1 phase acting in a caspase dependent pathway, suggesting that ITJ has the potential to be investigated as drug treatment against cell death in melanoma cells.

Key words: Trypsin inhibitor; *Mimosa regnellii*; Apoptosis; Melanoma; B16-f10.

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