PRECLINICAL STUDY OF ANGELICA ARCHANGELICA: CYTOTOXIC ACTIVITY AGAINST BREAST ADENOCARCINOMA CELLS AND CATHEPSINS INHIBITORY PROPERTIES

Garcia, D.M.1; Oliveira, C.R.1,2; Spindola, D.G.1,2; Bechara, A.1; Erustes, A.G.1; Palmeira-dos-Santos, C.1; Peixoto-da-Silva, J.1; Lima, C.S.1; Hinsberger, A.1,2; Antunes, A.A.3; Tomaz, S. L.4; Júdice, W.A.3; Rodrigues, E.G.4; Smaili, S.S.1; Bincoletto, C.1

1Departamento de Farmacologia, EPM, Universidade Federal de São Paulo, SP, Brazil; 2Grupo de Fitocomplexos e Sinalização Celular, Universidade Anhembi Morumbi, SP, Brazil; 3Centro Interdisciplinar de Investigação Bioquímica, Universidade de Mogi das Cruzes, SP, Brazil; 4Departamento de Microbiologia, Imunologia e Parasitologia, EPM, Universidade Federal de São Paulo, SP, Brazil.

Introduction and objective: Anti-tumor therapy is considered one of the greatest challenges of medicine. Herbs began to be studied and used for anticancer purposes. In this context Angelica archangelica has been popularly used due to its anti-inflammatory, carminative, and analgesic actions. This study aimed to evaluate its antitumour activity against two breast adenocarcinoma cell lines (MCF-7 and 4T1) and its inhibitory activity upon human cathepsins (B and L). Materials and Methods: Angelica archangelica ethanolic extract (AAEE) was provided by Laboratório Almeida Prado, SP-Brazil. Cells viability was evaluated after AAEE exposure for 24h by MTT assay. Cell death studies were evaluated by Annexin-V-FITC/PI and Hoechst staining by flow cytometry and fluorescence microscope analysis. Cell cycle and ROS generation were analyzed by PI and DCFDA, and mitochondrial membrane potential (ΔΨm) was analized in cells loaded with TMRE. Bax and Bcl-2 expression were evaluated by Immunoblotting after AAEE exposure (IC50/24h). Cathepsins inhibitions were carried out by spectrofluorimetric method (λEx =360nm/λEm=480nm) using the fluorescent substrate Z-FR-AMC. In vivo assays were performed by injection of 4T1 cells into the mammary gland of host BALB/c mice treated for 30 days with AAEE by oral inoculation. Results: The AAEE were cytotoxic to the MCF-7 and 4T1 cells (IC50 250μg/mL). AAEE increased Sub-G1 fraction and AnnexinV-FITC/PI analysis evidenced that AAEE induces cell death in both cell lines. Hoechst staining revealed the apoptotic nuclei. These events were accompanied by ROS generation, Bax overexpression and decreased Bcl-2 expression leading to reduction of ΔΨm. AAEE administered orally (250–500mg/kg) protects mice against primary mammary adenocarcinoma reducing tumor volume. AAEE has a 54-fold higher affinity to cathepsin-B than to cathepsin-L. Conclusion: AAEE induced cell death, mainly by apoptosis in MCF-7 and 4T1 cells in a similar way. As MCF-7 cells are caspase-3 deficient we suggest that other cell death modalities, besides apoptosis, are involved in AAEE cytotoxicity. Acknowledgements: FAPESP, CAPES and CNPq. KeyWords: antitumour; cell death; cysteine protease.