BIOLOGICAL EVALUATION AND MOLECULAR CHARACTERIZATION OF *Annona crassiflora* Mart. EXTRACT AS POTENTIAL ANTINEOPLASTIC THERAPY ON CERVICAL CANCER CELL PANEL

Silva, L.R.V.¹; Silva V.A.O.¹; Rosa M.N.¹; Morais T.H.¹; Tansini A.¹; Ribeiro R.I.M.A.², Reis R.M.¹,³

¹Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, S. Paulo, Brazil. ²Federal University of São João Del Rei (UFSJ), Divinópolis, Minas Gerais, Brazil. ³Life and Health Sciences Research Institute (ICVS), University of Minho, Braga, Portugal.

Introduction and objectives: Cervical cancer (CC) is the third most commonly diagnosed cancer and the fourth leading cause of cancer death in females worldwide. Therapeutic options for CC patients remain very poor making it imperative the development of new drugs. The use of new anticancer agents of natural sources have revealed efficacy, offering a large field for scientific research. *Annona crassiflora* Mart. is used in traditional medicine as antimicrobial and anticancer. However, little is known about its anticancer properties. To evaluate the antitumor effect of one crude extract of leaves derived from *A. crassiflora* in a panel of human cervical cancer cell lines (CCCL).

Material and Methods: The *in vitro* cytotoxicity and proliferative ability of *A. crassiflora* extract was assessed using MTS assays and colony formation assay on 7 human CCCL (HeLa, HtTA-1, Siha, HR5, HR5-CL11, BuTK-25 and Caski). Apoptosis was quantified by AnnexinV/PI and flow-cytometry. The biological effect of *A. crassiflora* extract on migration were evaluated by wound healing assay. Additionally, we assessed the global protein changes by Western blotting to identify potential extract targets.

Results: *A. crassiflora* extract exhibited dose-dependent cytotoxic effects on CCCL. Most importantly, the plant extract treatment effectively reduced colonies formation and migration on Siha and HtTA-1 cells. Flow cytometry further revealed an increase of the number of late apoptotic and necrotic cells. Likewise, PARP cleavage was markedly increased, suggesting apoptotic signals. Furthermore, protein profile analysis showed up-regulated activity of H2AX, an early sign of DNA damage. *A. crassiflora* extract treatment induced ERK1/2 activation and phosphor-AKT downregulation suggesting a pro-apoptotic effect. These preliminary results suggest.

Conclusions: *A. crassiflora* extract as potential antitumor and DNA-damaging agent, which may induce apoptosis *in vitro*, supporting that it may play an important role in cervical cancer treatment.

Keywords: cervical cancer cell, cytotoxic activity, natural compounds.

Financial Support: FINEP(MCTI/FINEP/MS/SCTIE/DECIT-01/2013,FPXII-BIOPLAT) and FAPEMIG.