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DEATH EVALUATION OF TUMOR CELL LINES PROMOTED BY ANTHRAQUINONES FROM *Rhamnus sphaerosperma* var. *pubescens*

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Abstract: The genus *Rhamnus* includes species with a broad range of pharmacological potentials, which are widely used in folk medicine. *Rhamnus sphaerosperma* var. *pubescens* is a Brazilian native species popularly known as "fruto-de-pombo"[1]. Currently, there are no studies describing its biological properties. Thus, the aim of this study was to evaluate the cell death induced by anthraquinones emodin and physcion isolated from this species. The stems of *Rhamnus sphaerosperma* var. *pubescens* were collected in Curitiba-PR, Brazil, in May 2011. The dried plant was submitted to ethanol extraction and liquid-liquid partitioning in a modified Soxhlet apparatus[2]. After column chromatography of fractions, two crystalline samples were obtained and analyzed by Nuclear Magnetic Resonance spectroscopy of ^{1}H and ^{13}C. These compounds were identified as anthraquinones physcion (1,8-dihydroxy-3-methoxy-6-methylanthraquinone) and emodin (6-methyl-1,3,8-trihydroxyanthraquinone). After cytotoxic assays, the cell death of this compounds were evaluated by annexin-V, caspase-3 activity and fluorochromes exclusion assay[3]. All the protocols were adapted for IN Cell Analyzer 2000® (GE Healthcare). Emodin was evaluated, at concentrations of 46.25 to 185µM and physcion, of 43.75 to 175 µM, in SiHa, C33-A, HSC-3 and HaCaT cell lines. DMSO was used as a vehicle, and doxorubicin (90µM) and curcumin (100µM) were used as positive controls. After 24 hours of treatment with emodin (185µM), annexin-V and fluorochromes exclusion assay detected a selectivity for HSC-3 and SiHa (cell viability of 4.5±0.8% and 8.4±1.2%, respectively). The annexin-V assay detected the majority of cells on late apoptosis/necrosis. Therefore, the complementary assay showed cells in early apoptosis (31.9±4.6% in HSC-3 and 12.5±1.6% in SiHa), late apoptosis (3.6±0.4% in HSC-3 and 24.9±1.2% in SiHa) and necrosis (51.9±4.5% in HSC-3 and 31.2±1.2% in SiHa). After 24 hours of treatment, physcion at 175µM also promotes efficient cell death and selectivity for HSC-3 and SiHa; The cell viability was 10.5±1.5% and 19.8±1.8%, respectively. In Annexin-V assay, HSC-3 and HaCaT cells were shown in early apoptosis (36.5±2.8% and 28.5±2.1%, respectively). The data were confirmed in the complementary test. Considering SiHa and C33-A cell lines, the death occurred primarily by necrosis. In the caspase-3 activity assay, only cells treated with curcumin showed activation. In conclusion, emodin compared to physcion showed a slightly better capacity on cell death induction of all tested cell lines. Cell death in both treatments occurred by diverse pathways: early and late apoptosis and necrosis.

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