EFFECTS OF TWO ABIETANES ON MCF-7 BREAST CANCER CELL LINE

Madalena Pedro[a], Cristina Gaspar-Marques[b], M. Fátima A. Simões[b], Madalena Pinto,[a] Maria S. J. Nascimento[a]

[a]CEQOFFUP-Centro de Estudos de Química Orgânica, Fitoquímica e Farmacologia da Universidade do Porto, Faculdade de Farmácia da Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal

[b]CECF - Centro de Estudos de Ciências Farmacêuticas, Faculdade de Farmácia da Universidade de Lisboa, Av. Professor Gama Pinto, 1649-003 Lisboa, Portugal

A study on the effect of coleon U (**1**) and 7α-acetoxy-6β-hydroxyroyleanone (**2**), isolated from *Plectranthus grandidentatus*, on the metabolic activity of MCF-7 cells is now presented. We have evaluated if their tumour growth inhibitory effect was related with apoptosis. **1** and **2** had shown a potent growth inhibitory effect on different human tumour cell lines, including the breast cancer cell line MCF-7 [1].

The metabolic activity of MCF-7 cells was evaluated by MTT assay after exposition to a range of concentrations (0.02 to 50 μM) for different periods of time (12, 24 and 48 h). MCF-7 cells viability was measured by trypan blue exclusion assay. Fragmentation of the genomic DNA was evaluated by the *in situ* Cell Death Detection Kit Fluorescein-TUNEL assay (Boehringer Mannheim, Germany). Cell morphological analysis was accessed by fluorescent microscopy after DAPI staining.

The metabolic activity of MCF-7 cells was analysed based on their capacity to reduce MTT. At concentration below 3 μM compounds **1** and **2** did not affect significantly cellular metabolic activity (≥ 80 %) even after 48 h exposition. Only concentrations above 3 μM and expositions of 48 h caused an abrupt loss of activity (<50 %). Treatments of 12 and 24 h affected differently the metabolic activity of MCF-7 cells. While compound **2** did not affect significantly this activity**,** compound **1** increased the capacity of cells to reduce MTT**.** Further studies are needed to elucidate the cause of this unexpected increase MTT reduction capacity of cells.

MCF-7 cells were exposed to **1** or **2** (6.4 μM and 5.5 μM, respectively) for 48 h and evaluated for apoptosis. Treatment with coleon U (**1**) was associated with an increase of cells with abnormal nuclear condensation when compared with untreated control cells, but they still presented high values of viability (> 80%). This nuclear alteration was associated with DNA fragmentation, characteristic of apoptotic cells, when stained with TUNEL assay. The number of apoptotic cells reached 24.8 % after coleon U (**1**) treatment *versus* 4% on control cells. Treatment with compound **2** did not show an increase of apoptotic cells. These results suggest that coleon U exerts their growth inhibitory effect against MCF-7 cell line through the involvement of apoptosis while no relation could be established between compound **2** and this phenomenon.

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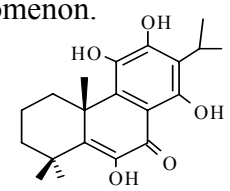

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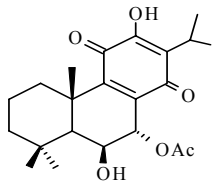

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[1] Gaspar-Marques C *et al*., Planta Med 2002; 68:839-840