# Antitumor effect of pinoresinol on human breast cancer cells

López-Biedma, A.; Sánchez-Quesada, C.; Gaforio, J.J.
Immunology division, Health sciences department, University of Jaén, Spain.
Center for Advanced Studies in Olive Grove and Olive Oils, Science and Technology Park Geolit, Spain.

## Introduction

There are scientific evidences linking the intake of Virgin Olive Oil (VOO) with a low incidence of breast cancer [1,2]. Among the minor components present in this VOO we can find polyphenols, that contribute to the protection against development and progression of cancer, diabetes, neurological and cardio-vascular diseases, etc. [3,4].

One of this polyphenols is pinoresinol, at which several health properties have been attributed. However, the effect of this compound on human breast cancer cells and the relation that it could have with a low incidence of this kind of cancer is unknown.

## Materials and methods

- **Pinoresinol**: minor component of virgin olive oil. Purity ≥ 95%.
- **Human breast cancer cell lines**: MDA-MB-231 and MCF7.
- **Citotoxicity assay**: cells were treated with different concentrations of pinoresinol for 24 hours. Cell survival, respect to the control without treatment, was measured fluorimetrically by CellTiter–Blue®.
- **Cell proliferation**: cells were treated with different concentrations of pinoresinol for 24 hours. Them, medium was replaced by fresh medium and cell viability was measured after 24, 48, 72 and 96 h.
- **Detection of Reactive Oxygen Species (ROS)**: the fluorescence emitted by the oxidation of the compound 2',7'-dichlorofluorescin diacetate (DCFH-DA) allows to measure the amount of ROS inside cells. H$_2$O$_2$ was added to assess if pinoresinol has a protective role against induced oxidative stress.

**Figure 1.** Effect of pinoresinol on cell survival of human breast cancer cell lines MDA-MB-231 and MCF7. * and t indicate statistically significant differences respect to the control (p≤0.05) for MDA-MB-231 and MCF7 respectively.

**Figure 2.** Intracellular ROS levels on human breast cancer cell lines MDA-MB-231 and MCF7 after treatment with pinoresinol for 24 h with H2O2-induced oxidative stress. * indicates statistically significant differences (p≤0.05) respect to the control.

## Results

**Citotoxicity**: pinoresinol shown citotoxic effect on both tumor cell lines at low concentrations studied. The effect was strong on the highly invasive breast tumor cells MDA-MB-231, being statistically significant from 0.001 to 1 µM (Figure 1).

**Cell proliferation**: cell survival was reduced on both cell lines at minor concentrations. This was in line with results obtained for citotoxicity assays (Figures 2a and 2b).

**Oxidative stress**: Figure 3 shows that pinoresinol tends to increase the oxidative stress inside tumor cells, because ROS levels were raised in a dosis-dependent way. This tendency was higher on MCF7 cells, being statistically significant at high concentrations.