Antitumor effect of pinoresinol on human breast cancer cells

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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.

Objectives

The aim of the study was to assess the effects of pinoresinol on viability, proliferation and reactive oxygen species formation on human breast cancer cells, in order to know if this compound could be responsible, at least in part, for the minor incidence of breast cancer associated to consumption of virgin olive oil.

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 assay**: 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 -diclorofluorescin diacetate (DCFH-DA) allows to measure the amount of ROS inside cells. H_2O_2 was added to assess if pinoresinol has a protective role against induced oxidative stress.

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 shown that pinoresinol tended 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.

Fig. 1

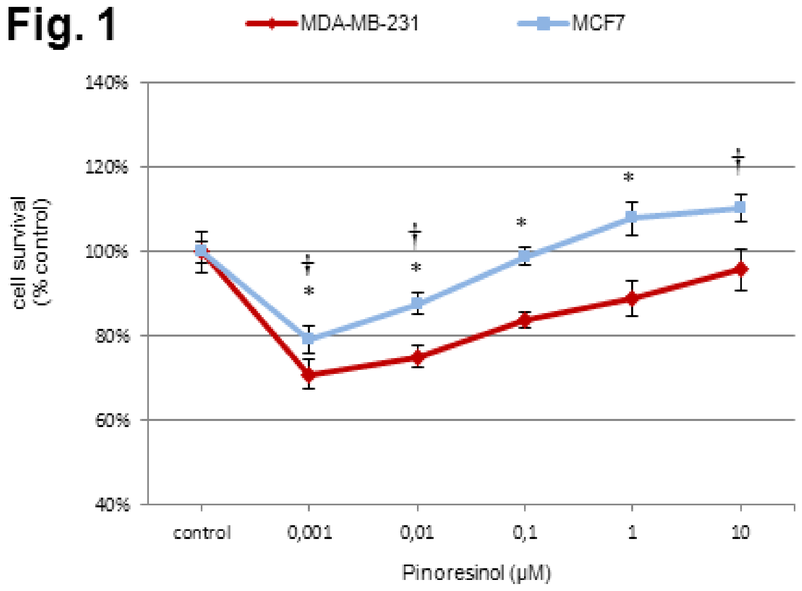


***Figure 1**. Effect of pinoresinol on cell survival of human breast cancer cell lines MDA-MB-231 and MCF7. * and † indicate statistically significant differences respect to the control (p≤0.05) for MDA-MB-231 and MCF7 respectively.*

Fig. 2. A

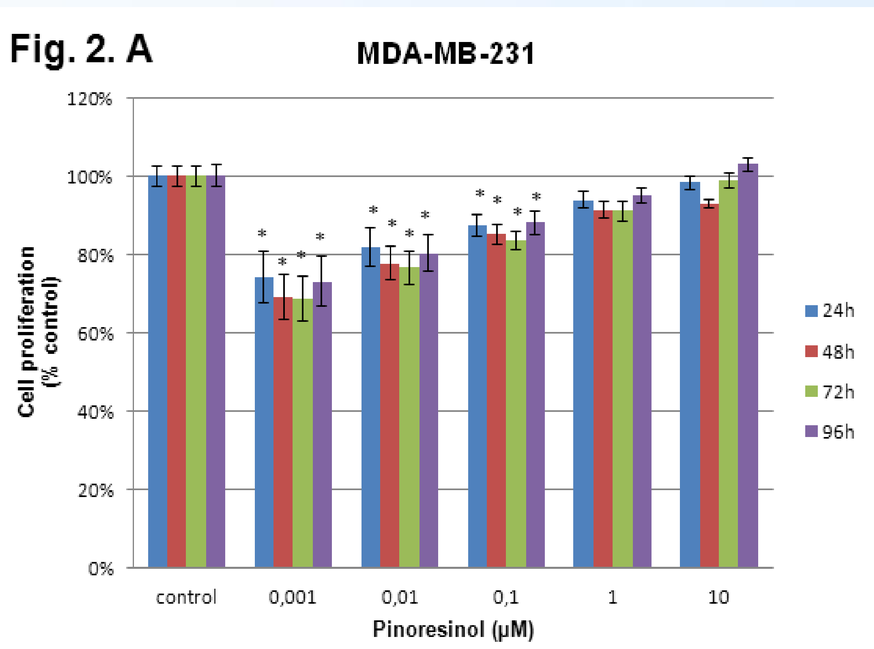
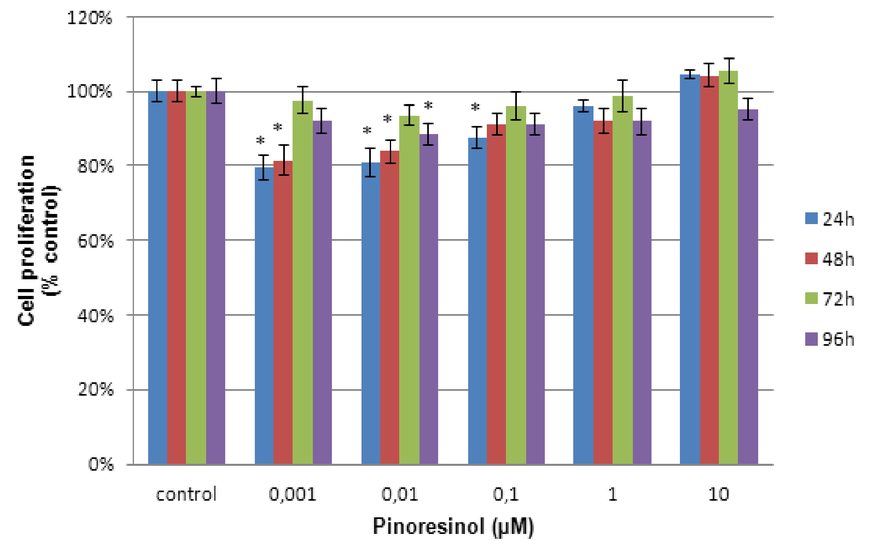


Fig. 2. B



***Figures 2.A y 2.B**. Cell proliferation on MDA-MB-231 (2.A) and MCF7 cells (2.B) after treatment with pinoresinol for 24 h, followed by proliferation times of 24, 48, 72 and 96 h. * indicates statistically significant differences respect to the control (p≤0.05).*

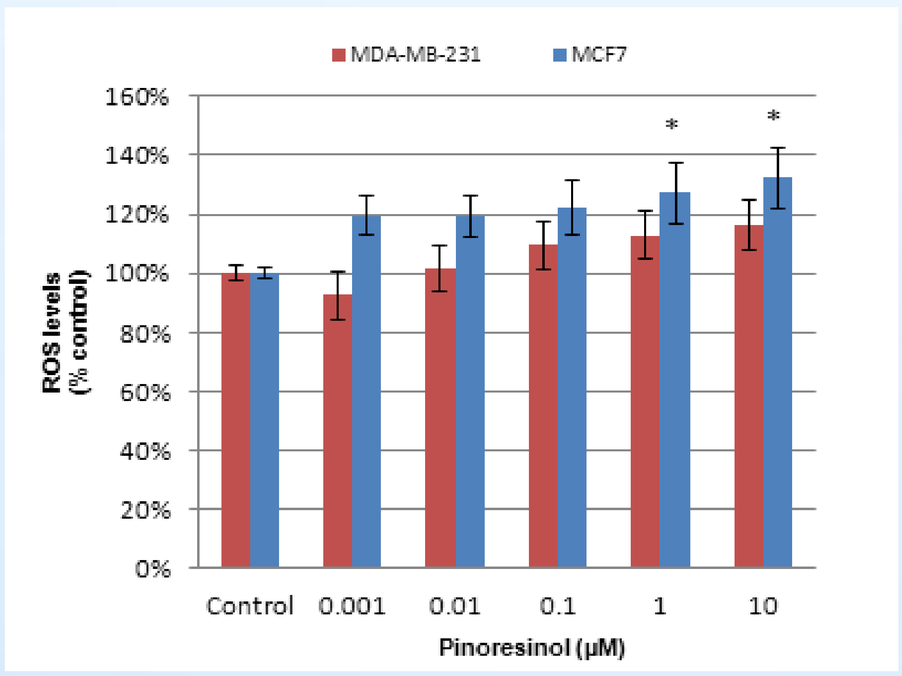


***Figure 3**. Intracellular ROS levels on human breast cancer cell lines MDA-MB-231 and MCF7 after treatment with pinoresinol for 24 h with H2O2-induced oxidative stress. * indicates statistically significant differences (p≤0.05) respect to the control.*

Conclusions

The minor compound pinoresinol, found in Virgin Olive Oil (VOO), presents citotoxic, antiproliferative and prooxidant activity on human breast tumor cell lines. Reduction of cell viability and proliferation after treatment with pinoresinol suggests that this compound possesses antitumor activity.

Moreover, it is known that tumor cells present higher intracellular ROS levels than non-tumorigenic cells. Many of the commonly used chemotherapies are based on this feature: they increase oxidative stress above a toxic threshold level that kills cancer cells, while non-tumorigenic cells bear these levels [5]. In this sense, pinoresinol could be adjuvant of pro-oxidative cancer therapies, because it increases ROS levels after H_2O_2-induced oxidative shock and it could promote the death of tumor cells.

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