Colon cancer cells treated with mastic essential oil release damage-associated molecular patterns (DAMPs) characteristic of immunogenic cell death

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Introduction

In recent years, there has been significant interest in the identification of plant-derived essential oils with health-promoting properties and potential as medicinal agents[1]. *Pistacia lentiscus* var. chia essential oil has been shown to exert a variety of health-beneficial effects[2,3]. The emergence of immunogenic cell death, a programmed cell death modality characterized by the emission of DAMPs capable of eliciting adaptive immune responses[4,5], has opened new possibilities on the potential therapeutic properties of plant-derived compounds.

Materials and Methods

Colon cancer cells of human (HT29) and mouse (CT26) origin were treated with **0.02%** (v/v) of *Pistacia lentiscus* var. chia essential oil and relative gene expression was estimated with **real-time PCR**, using the 2^{-ddCt} method

Cancer cells were treated with **0.02%** (v/v) of *Pistacia lentiscus* essential oil for up to 24 h. HMGB1 translocation out of the nucleus was evaluated with **Western blot** of nucleic proteins and **immunofluorescence microscopy.**

Tumor cells were treated with **0.02%** (v/v) of *Pistacia lentiscus* essential oil for 24 h. Calreticulin exposure on the outside of the cellular membrane was investigated with **flow cytometry** and **immunofluorescence microscopy.**

Results

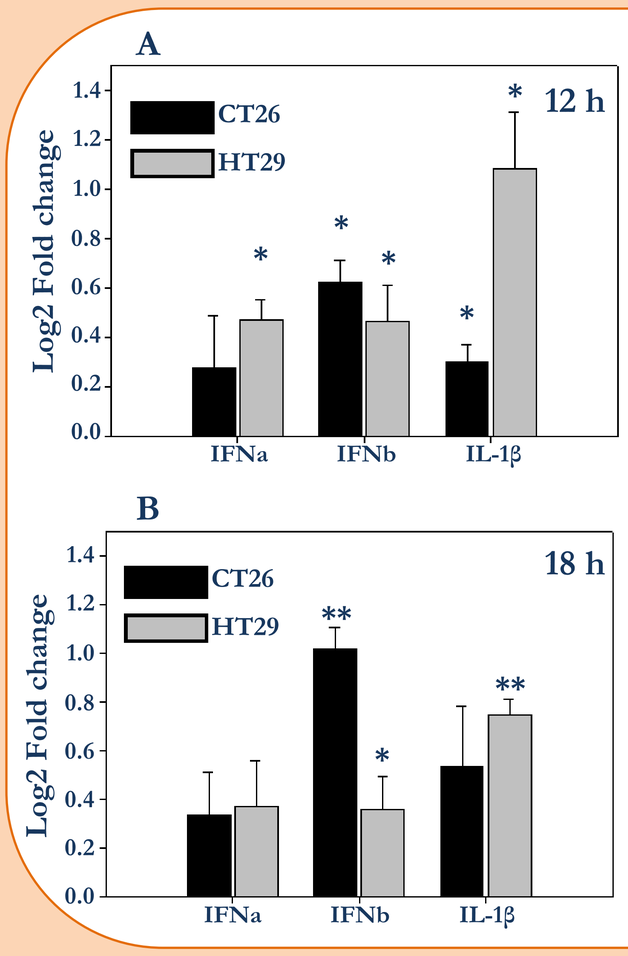

Figure 1: Gene expression analysis with real-time PCR revealed a significant up-regulation of **IFNa, IFNb** and **IL-1β** expression on both CT26 and HT29 cells treated with **0.02%** (v/v) *Pistacia lentiscus* var. chia oil for 12 h (**A**) and 18 h (**B**).

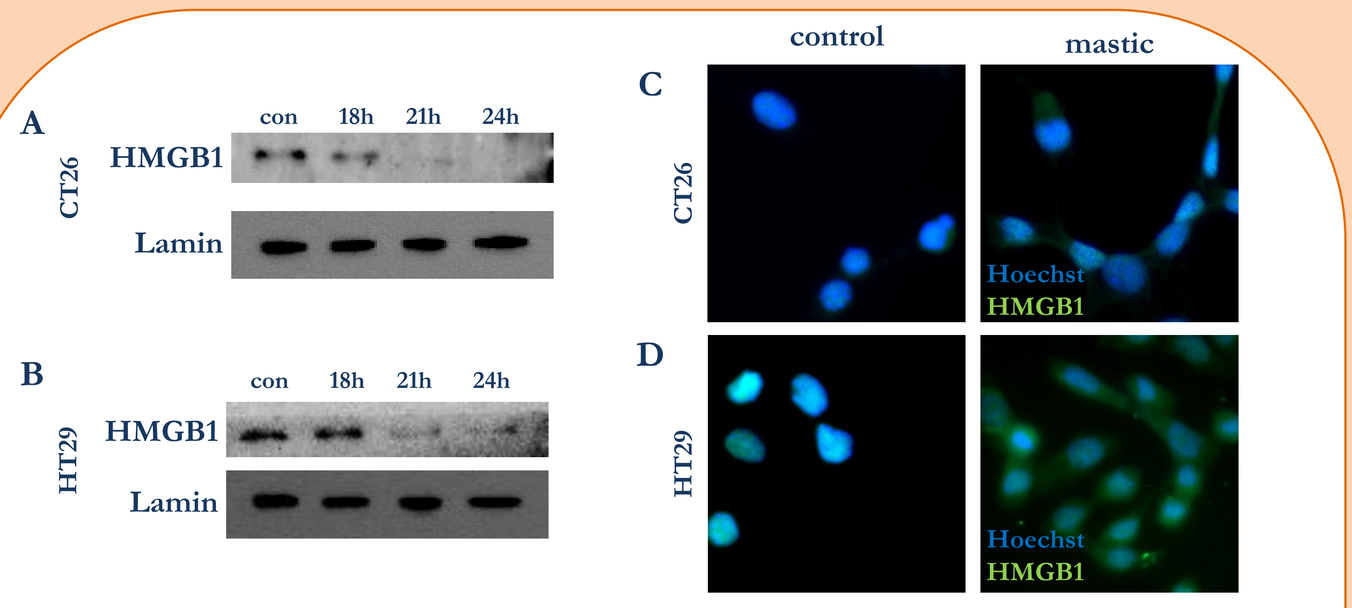

Figure 2: The nuclear **HMGB1** content of CT26 (**A**) and HT29 (**B**) cells was reduced following treatment with **0.02%** (v/v) of *Pistacia lentiscus* essential oil for up to 24 h as evident by Western blot analysis. This observations were confirmed at 24 h of treatment using fluorescence microscopy (**C, D**) and detecting reduced fluorescence in the nucleus and increased signal in the cytoplasm of treated cells.

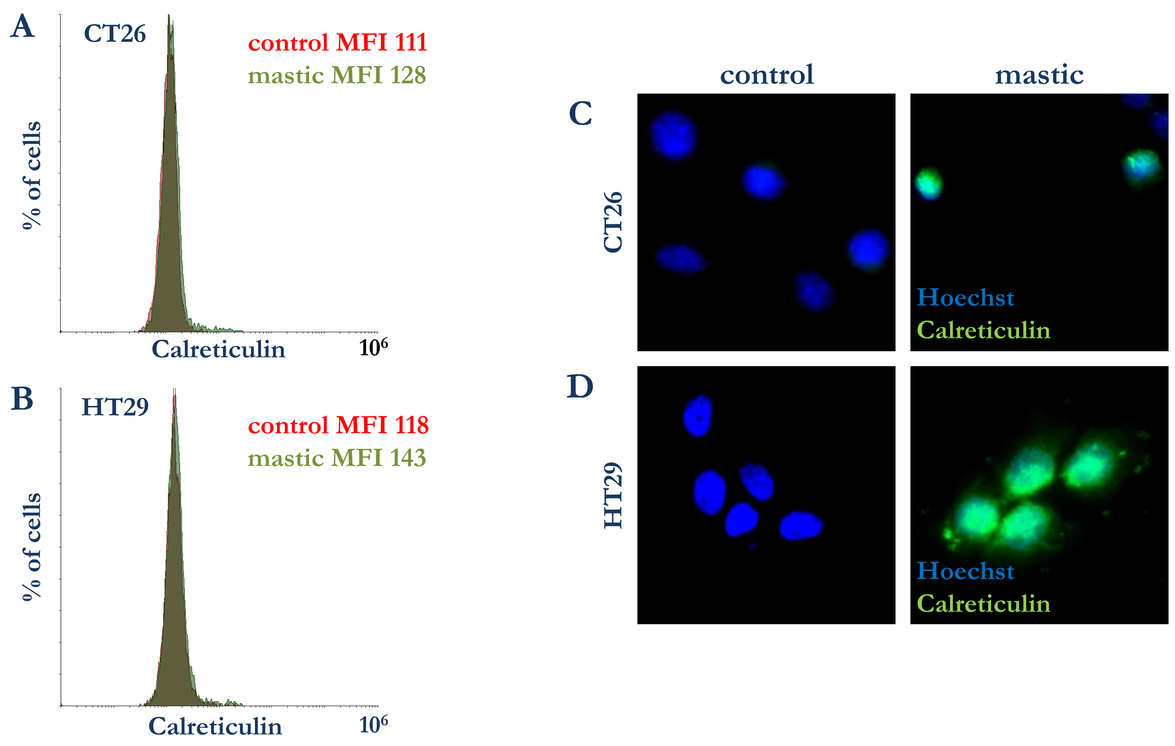

Figure 3: Calreticulin translocation on the cellular membrane was induced after treatment of CT26 (**A**) and HT29 (**B**) cells with **0.02%** (v/v) of mastic oil, as shown by flow cytometry. The presence of **calreticulin** on the outer layer of the membrane was confirmed using fluorescence microscopy (**C, D**) without permeabilization of the cells.

Conclusions

Our data support the conclusion that treatment of colon cancer cells with *Pistacia lentiscus* essential oil promoted the translocation of calreticulin on the cell membrane, the release of HMGB1 from the nucleus and an up-regulation in the expression of type I IFNs. This type of differential signaling has been associated with the phenomenon of immunogenic cells death. Further investigation is warranted to clarify if treatment with the essential oil can induce tumor-specific adaptive immune responses.

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