

BIOACTIVE EXTRACTS FROM MARINE SOURCES AND AGRI-FOOD WASTE REGULATE ETosis MECHANISMS

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ETosis is an evolutionary-conserved immunological defense strategy present in vertebrates and invertebrates. This process involves a specific cell death pathway where inflammatory cells expel nuclear chromatin to form extracellular traps (ETs) designed to sequester and neutralize invading microorganisms. However, under conditions of dysregulation, it may lead to the onset of several disease conditions.

This investigation evaluated the efficacy of natural products in the modulation of ETosis and associated inflammatory responses. Murine RAW 264.7 macrophages underwent a 2-h PMA stimulation with or without exposure to extracts from *Posidonia oceanica* leaves (GLE) or rhizomes (RE), or polyphenols obtained from olive mill wastewater (OMW) for 24 h. The dosages of GLE, RE and OMW were established at the peak concentrations that preserved cell viability and effectively inhibited NO production under inflammatory conditions. The formation of ETs in control conditions and after treatment with PMA with or without supplementary extracts was conducted through confocal microscopy analysis of citrullinated H4 histone in the presence/absence of DNase I, spectrophotometric measurement of extracellular DNA and dot blot quantification of total and citrullinated H4 histone components released in the culture media. Additionally, Western blot analysis was conducted to determine the role of major intracellular signaling pathways in the extract-mediated modulation of ETosis.

Microscopic analysis revealed that all treatments significantly reduced ET formation, consistent with results derived from dot-blot assays and extracellular DNA assessment. Western blot data revealed significant alterations in the activation profiles of the AGC kinase AKT and the MAP kinases JNK, ERK and p38, upon exposure to the preparations.

Overall, the current results establish a robust foundation for further molecular investigations into the beneficial effects of these natural extracts on ETosis and inflammation, supporting their prospective integration into sustainable biomedical practices.

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