Evaluation of extracts obtained from marine by-products using eutectic solvent systems for their therapeutic application in the dry eye disease

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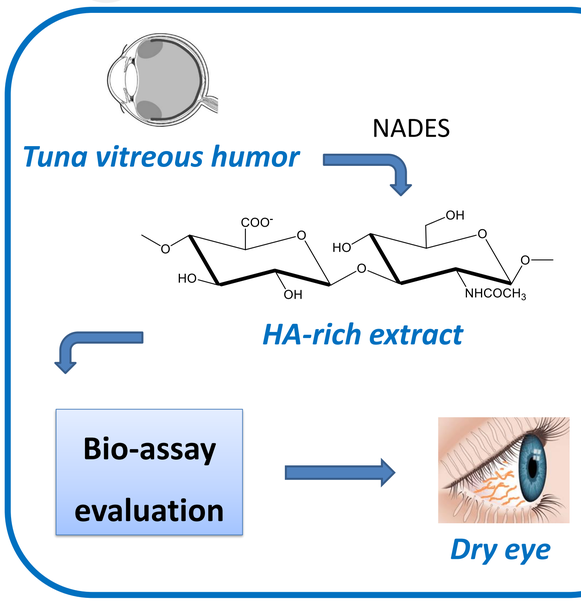
INTRODUCTION

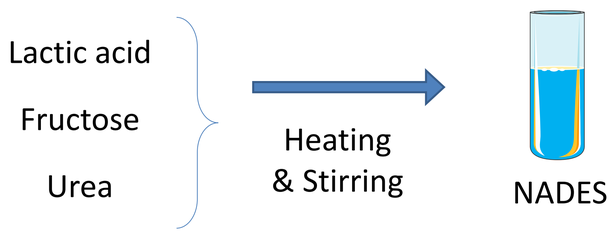
Hyaluronic acid (HA) is used in the treatment of the **dry eye disease (DED)** as it has shown improvements in the corneal epithelial barrier. In this work, HA is extracted from the **marine by-product** tuna vitreous humor (VH) using **natural deep eutectic solvents (NADES)** to replace toxic and costly conventional extractions. These solvents are prepared using molecules such as lactic acid, fructose and urea, and are used to **extract HA** from VH at specific conditions. The obtained isolated extract and the mixture of extract solved in the NADES are evaluated for their **potential application** in the treatment of the DED.

AIM

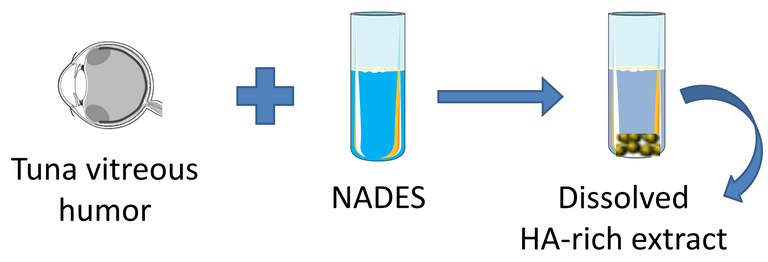


METHODS

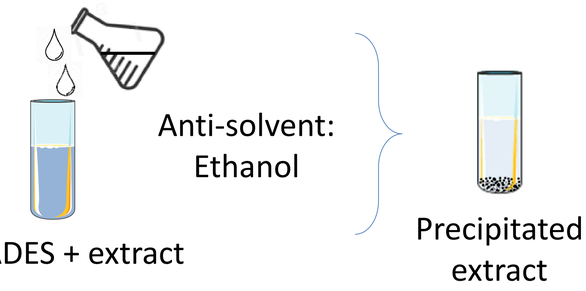
1. NADES preparation



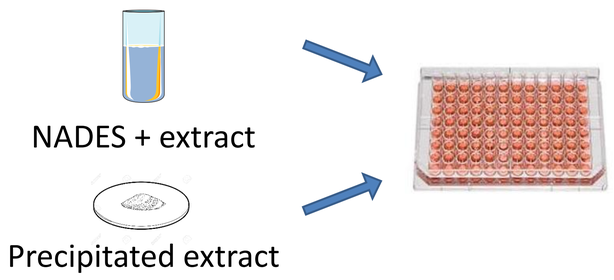
2. Extraction from marine by-product



3. Extract precipitation



4. Bio-assays on human corneal epithelial (HCE) cell line *

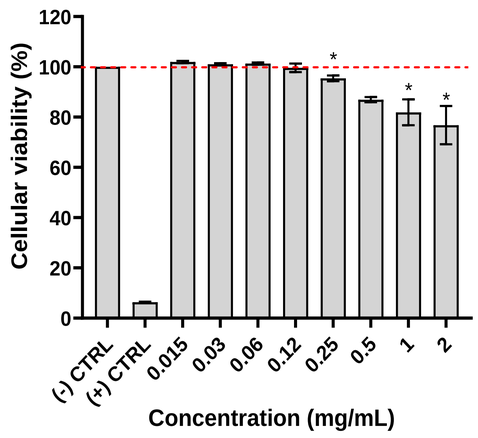


- Cytotoxicity → Cell viability measured using the XTT test
- Anti-oxidant activity → Reactive oxygen species (ROS) generation from UV-B exposure studied using H_2DCF-DA dye
- Anti-inflammatory effect → Expression levels of IL-6 and IL-10 studied after stimulation with TNF-α

* Araki-Sasaki, Kaoru, et al. Invest Ophthalmol Vis Sci. **1995,** *36*(3), 614–621.

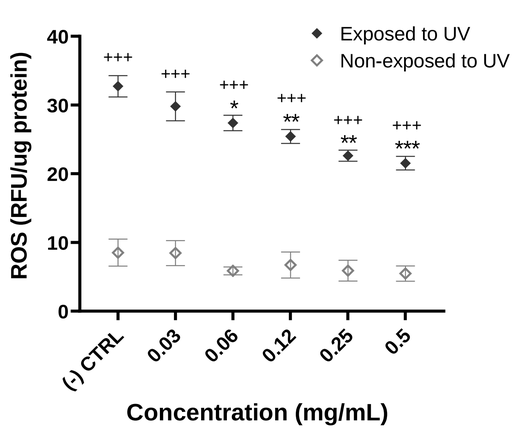
RESULTS – Extract obtained using lactic acid-based NADES

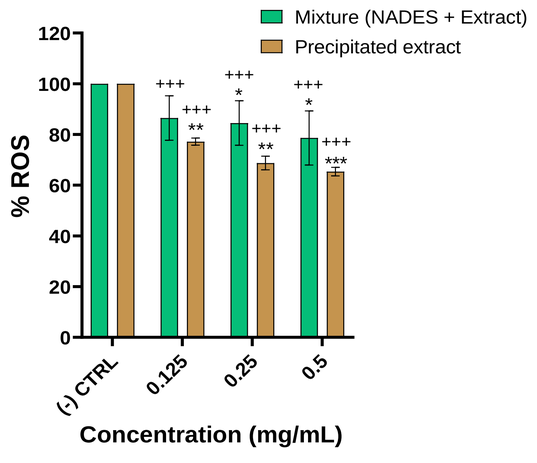
Cellular viability



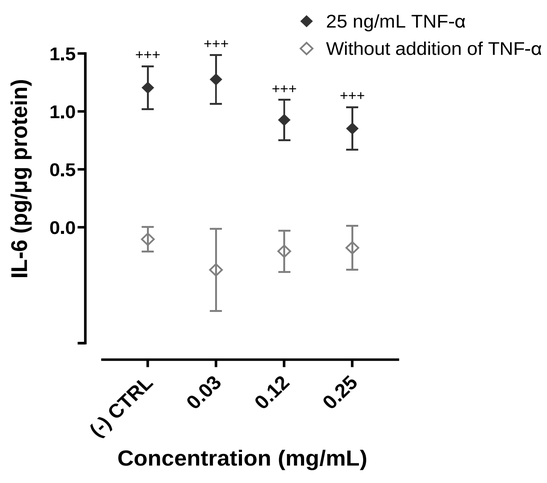
Highest viable concentration (> 90% viability) = 0.5 mg/mL

Anti-oxidant effect





Anti-inflammatory effect



In comparison to (-) CTRL cells exposed to stimulation (UV/TNF-α): **P* < 0.05, ***P* < 0.01, ****P* < 0.001; In comparison between stimulated (UV/TNF-α) and non-stimulated cells: +*P* < 0.05, ++*P* < 0.01, +++*P* < 0.001. Statistical analysis → SPSS software used for one-way analysis of variance (ANOVA) and Tukey's post hoc test or Games-Howell test for intergroup comparisons with n=3.

CONCLUSION

HA extraction from VH is done using green and novel NADES, as they have shown to be optimal in its isolation. The extracts, along with the mixture of the extract with the NADES have shown to have a therapeutic effect. Therefore, these compounds could be studied for their potential application in the treatment of the DED.

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