Potential therapeutic use of olive leaf extracts obtained from the olive tree (Olea europaea) against Helicobacter pylori infection

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INTRODUCTION

Helicobacter pylori (H. pylori) is one of the major human pathogens infecting approximately 50% of the world’s population [1]. H. pylori could be implicated in the pathogenesis of gastritis, peptic ulcer disease, gastric carcinoma, and gastric lymphoma. Its treatment is based on the combined use of different antibiotics, however, in last years, the number of antibiotic resistant strains have been increased [3]. Therefore, new alternative therapies to antibiotic are required for H. pylori treatment. Moreover, there is increasing evidence that H. pylori infection can induce oxidative stress in host cells and an inflammatory process that conditions an immunological response both local and systemic. These events may represent an important mechanism leading to epithelial injury in H. pylori infection. The aim of the present work was to evaluate the antibacterial, anti-inflammatory and antioxidant effect of two olive leaf extracts (OLE) against antibiotics resistant H. pylori strains.

MATERIALS & METHODS

- **Bacterial strains, growth media and culture conditions**
  - Seven H. pylori strains isolated from gastric mucosal biopsy from symptomatic patients were used in the present study.
  - Selective growth media: Brucella Broth (BB) and Mueller-Hinton agar supplemented with 5% sheep blood (MHB).
  - Growth conditions: strains reactivation into MHB and incubation for 72h at 37°C, microaerophilic atmosphere in VAIN workstation (85% N2, 10% CO2, 5% O2).

- **Olive leaf extracts (OLEs)**
  - OLE1: enriched in hydroxytyrosol (10%)
  - OLE2: enriched in oleuropein (20%)

- **Antibacterial activity**
  - Assay:
    - 1 mL of extracts dissolved in BB (or only BB for control growth)
    - 4 mL of BB
    - 100 μL bacterial inoculum (1×10⁵ CFU/mL)
      - Incubation for 48h at 37°C, 150 rpm, in microaerophilic atmosphere (VAIN)
      - Serial decimal dilutions of mixtures were plated onto fresh MHB agar
      - Incubation microaerobically for 72h at 37°C microaerophilic atmosphere (VAIN)
      - Antibacterial activity and MCI determination by CFU counting

- **Cell culture conditions**
  - Human gastric epithelial AGS cell line were used.
  - Seed cells in 24-well plates (~5x10⁴ cells/well), incubation for 24h at 37°C, 5% CO2

- **Antioxidant activity against intracellular reactive oxygen species (ROS)**
  - AGS cells were pre-incubated with olive leaf extracts (1 mg/mL) 2h, 37°C, 5% CO2
  - Wash cells with PBS and incubation with fluorescent probe (DCFDA) 30 min
  - Wash cells with PBS and infection with H. pylori strains for 3h
  - Fluorescence measure: λex= 485 nm, λem= 530 nm

- **Anti-inflammatory response on infected-AGS cells**

RESULTS

- **Antibacterial activity against H. pylori strains**
  - Table 1. Antibacterial activity of OLEs on the viable counts of different H. pylori strains and MIC values after 48h of treatment at 2 mg/mL. Results are expressed as log CFU/mL ± standard deviation (n=3).

- **Antioxidant activity against intracellular reactive oxygen species (ROS) production on H. pylori infected-AGS cells**
  - Figure 1. Protective effect of olive leaf extracts (1 mg/mL) on intracellular ROS production.

- **Anti-inflammatory activity on H. pylori infected-AGS cells**
  - Table 2. Effect of OLEs (1 mg/mL) on IL-8 production in AGS cells infected by H. pylori strains. Values of IL-8 production are expressed as pg/mL (mean ± standard deviation) (n=3).

CONCLUSIONS

- OLE1 completely inhibited the bacterial growth of all tested strains following a 48h exposure to 2 mg/mL, suggesting a broad antibacterial activity. Minimal inhibitory concentration (MIC) of OLE1 ranged from 0.5 to 2 mg/mL.
- OLE2 showed bactericidal activity (after 48h at 2 mg/mL) against three of the seven (3/7) tested strains (Hp44, Hp58, and Hp19449), and reduced 1 and 2 log CFU the bacterial growth of Hp59 and Hp48 strains, respectively, with controls growth.
- Both extracts reduced up to 33% the production of intracellular reactive oxygen species (ROS) in human gastric AGS cells infected by H. pylori, being the antioxidant activity of the OLE2 extract higher than OLE1 in all cases.
- All H. pylori strains induced IL-8 production from AGS cells in a strain-dependent manner.
- OLE1 and OLE2 showed anti-inflammatory activity, reducing IL-8 pro-inflammatory factor secretion by infected-AGS cells in a range around 20-74% and 71-93%, respectively.
- Therefore, the olive leaf extracts could be consider as a potential new candidate for H. pylori treatment, providing an alternative for the 20% of infected people with symptoms for whom antibiotic treatments are not effective. Furthermore, the recycling of olive industry by-products could also contribute to its revalorization, reducing also the environmental impact.

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