Hops extracts (*Humulus lupulus* L.) as anti-inflammatory agents in gastric epithelial cells

E. Sangiovanni[1], M. Fumagalli[1], L. Santagostini[2], E. Colombo[1], G. Fico[3], M. Dell'Agli[1]

[1]Università degli Studi di Milano, Dipartimento di Scienze Farmacologiche e Biomolecolari. enrico.sangiovanni@unimi.it
[2]Università degli Studi di Milano, Dipartimento di Chimica. laura.santagostini@unimi.it
[3]Università degli Studi di Milano, Dipartimento di Scienze Farmaceutiche. gelsomina.fico@unimi.it

Gastritis and ulcers are very common inflammatory-based diseases caused by multiple factors, such as infections, chemical insult or immunological disorders. *Helicobacter pylori* (*H. pylori*) is the main aetiological agent of gastritis and its presence in the stomach is responsible for over 90% of gastric inflammation in the world. The bacterium induces immune cells to release a variety of pro-inflammatory mediators including TNFÎ±, which leads to the activation of NF-κB, a transcription factor deeply involved in the release of cytokines/chemokines by gastric mucosa. Moreover, NF-κB promotes the transcription of IL-8, the most abundant chemokine released by gastric epithelium during inflammatory processes. Histamine-2 receptor antagonists, proton pump inhibitors and antibiotics are currently included in the conventional therapy for gastritis or ulcer; however, repeated use of antibiotics could lead to drug resistance whereas the other drugs could exhibit side effects. The search for new therapies and nutritional approaches to treat gastric inflammatory diseases need to be carefully into consideration. Female hop flowers (*Humulus lupulus* L., hops) are widely used in the brewing and food industry as flavour agent; however, the literature ascribes several biological properties to hops, including anti-inflammatory [1-3] and anti-*H.pylori* [4] activities. Hops are able to inhibit the vacuolation caused by *H.pylori* cytotoxin VacA *in vitro* and *in vivo* [5], but no studies regarding the possible anti-inflammatory activity of hops at gastric level have been reported so far.

The aim of this study was to evaluate and compare the anti-inflammatory effect of the water (WE) and hydro-alcoholic (HE) extracts from hops in human gastric epithelial cells (AGS).

Fresh hops were firstly dried and mashed. The plant material was subjected to extraction twice at room temperature (1 g of drug in 10 mL of distilled water -WE- or ethanol-water -HE- in the ratio 50:50). Treatment with TNFÎ± (10 ng/mL) for 6 h was used as pro-inflammatory challenge. The NF-κB driven transcription and IL-8 promoter activity were evaluated by transfecting cells with two different reporter plasmids containing the luciferase gene, while IL-8 secretion was evaluated by ELISA assay. A preliminary characterization of HE was performed by HPLC-DAD and LC-MS.

Both WE and HE were able to inhibit TNFÎ±-induced IL-8 promoter activity (IC_{50}: 3.59 vs. 2.34 μg/mL, respectively) and the effect was confirmed on NF-κB driven transcription (IC_{50}: 2.25 vs. 2.44 μg/mL, respectively) where the extracts were similarly active; however, only HE inhibited IL-8 release (IC_{50}: 8.96 μg/mL) from AGS cells. Extracts showed a different inhibition profile when used as pre-treatment. Cells received HE or WE in absence of pro-inflammatory stimulus for 1 h, followed by TNFÎ± alone for 6 h. In this condition neither WE nor HE were able to inhibit IL-8 release, while HE showed an approximately 4 times higher inhibition of IL-8 promoter activity (IC_{50}: 34.91 vs. 9.60 μg/mL, respectively) and NF-κB driven transcription (IC_{50}: 36.82 vs. 8.11 μg/mL, respectively) than WE.

The present research firstly demonstrates the anti-inflammatory activity of hops at gastric level; further studies will try to elucidate the beneficial effect in the stomach with the aim to suggest hops as a possible new candidate to treat or prevent gastric diseases.

1. Bohr, G., et al., (2005) Journal of Natural Products, 68(10): p. 1545-1548.
2. Dorn, C., et al., (2010) Molecular Nutrition & Food Research, 54: p. S205-S213.
3. Dorn, C., et al., (2013) Experimental and Molecular Pathology, 94(1): p. 10-16.
4. Cermak, P., et al., (2015) Czech Journal of Food Sciences, 33(4): p. 302-307.
5. Yahiro, K., et al., (2005) Helicobacter, 10(3): p. 231-9.