LEAF EXTRACTS AND ELLAGITANNINS FROM CHESTNUT (*CASTANEA SATIVA* MILL.) INHIBIT IL-8 RELEASE IN *H. PYLORI*-INFECTED GASTRIC EPITHELIAL CELLS

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Introduction: IL-8 plays a central role in the immune-pathogenesis of *H. pylori*-induced tissue injury, thus mediating the activation of neutrophils and macrophages in gastric mucosa. The inflammatory loop is sustained by a plethora of other pro-inflammatory mediators, such as TNFa, IL-6 and metalloproteases. *H. pylori* cagA positive strains are clinically related to more severe outcomes of gastritis, like ulcers and gastric cancer. Tannins from plants may exert anti-inflammatory and antibacterial effect, thus resulting of interest for nutraceutical approach against gastritis. Castanea sativa L. is a traditional source of tannins poorly investigated, with the exception of tannins from bark, mostly represented by the ellagitannins castalagin and vescalagin. The present work investigated the biological effect of tannins-containing extracts from fruits and leaves, using in vitro models of gastric inflammation.

Material and methods: Gastric epithelial cells (AGS and GES-1) were stimulated with TNFa (10 ng/mL) or co-cultured with different bacterial strains, obtained from clinical samples, to discover in vitro anti-*H. pylori* effect of chestnut extracts. For this purpose, we evaluated the inflammatory response (IL-8, IL-6) of AGS and GES-1 cells co-cultured with three clinical strains (#6, #39, MU-1) and one strain commercially available and fully characterized (ATCC-700392).

Results: Interestingly, despite all the strains similarly increased IL-8 and IL-6 release after 6 h, #6 and MU-1 were found cagA negative, thus suggesting that virulence factors other than cagA promote inflammation in gastric epithelial cells. Neither TNFa nor MMP-9 release was increased by the bacterial co-culture. Basing on our previous work on the inhibitory effect of chestnut fruit extracts (*Castanea sativa* Mill.) on TNFa-challenged IL-8 release, we tested the effect of chestnut in AGS co-cultured with H. pylori (ATCC strain). Episperm and pericarp fruit extracts, rich in condensed tannins, inhibited TNFa-induced IL-8 at 5 mg/mL, while *H. pylori*-induced IL-8 was inhibited at 25 mg/mL. Moreover, we investigated the effect of an extract from chestnut leaves, which have never been considered for a remedy against gastric inflammation but are rich in tannins as well. In addition, we evaluated the effect of ellagitannins castalagin and vescalagin, occurring in bark and leaves. Similar to the fruit, leaves extract inhibited TNFa and *H. pylori* induced IL-8 at 10 mg/mL and 50 mg/mL, respectively. Ellagitannins strongly inhibited TNFa-induced IL-8 with the same IC_{50}s (0.04 mM) whereas *H. pylori*-induced IL-8 was impaired at 50 mM.

Discussion and conclusions: Although the extracts exhibited a lower inhibitory potency in co-culture model then after TNFa induction of gastric epithelial cells, higher concentrations of extracts tested (25-50 mg/mL) may be easily achieved *in vivo* after oral consumption, thus suggesting the potential use of chestnut for nutraceutical or pharmacological purposes at the gastric level.