

Anti-inflammatory activity of tannins from *Fragaria* spp. at the gastric level

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Gastritis is a common inflammation of gastric mucosa caused for 90% of cases by the presence of the bacterium *Helicobacter pylori* (*H. pylori*). During *H. pylori* infection, macrophages release pro-inflammatory cytokines/chemokines, including TNF α , leading to the activation of the NF- κ B in epithelial gastric cells (Mai et al., 1991). NF- κ B is a transcription factor strictly involved in the expression of a variety of inflammatory genes, including IL-8, at the gastric level (Yasumoto et al. 1992).

Strawberries contain, in addition to anthocyanosides, great amount of tannins, a class of polyphenols with anti-inflammatory properties (Adams et al. 2006). Strawberry inhibits ethanol-induced gastritis in rats and this effect is due to the presence of anthocyanosides (Alvarez-Suarez et al. 2011). However, the inhibitory effect of tannins-enriched extracts (TEEs) from strawberries against gastric inflammation was not previously evaluated.

The aim of this study was to evaluate the ability of tannins, from two species of strawberry (*Fragaria X ananassa* and *Fragaria vesca*), to inhibit gastric inflammation, considering also the effect of an *in vitro* gastric digestion on the biological activity.

TEEs were prepared from *Fragaria* spp., strawberries were collected at maturity and the extraction was carried out with a mixture acetone/water (70/30 v/v); anthocyanosides were removed using a chromatography on Sephadex LH-20 resin obtaining extracts enriched in tannins. Digestion of TEE was performed following the protocol adapted from RIVM (Hagens et al. 2009). The extracts were assayed on human gastric epithelial cells (AGS) stimulated with TNF α (10 ng/mL). The NF- κ B driven transcription and IL-8 promoter activity were evaluated by transient transfection assays in human epithelial gastric AGS cells with reporter plasmids, while the NF- κ B nuclear translocation and IL-8 secretion were performed by an ELISA assay.

Both *Fragaria X ananassa* and *vesca* extracts were able to inhibit the TNF α -induced NF- κ B driven-transcription and nuclear translocation in a concentration-dependent way. Since IL-8, whose expression is strictly dependent on the NF- κ B activation, is widely involved in gastric inflammation, the following experiments were devoted to evaluate the effect of the extracts on the TNF α -induced IL-8 secretion activity in AGS cells; both the extracts inhibited IL-8 release and the effect was related to the IL-8 promoter impairment. To investigate if the effect observed with *Fragaria X ananassa* extract could be maintained at the gastric conditions, we subjected the extract to *in vitro* gastric digestion; digestion slightly reduced the activity of the extract; however, at the highest concentration tested (5 μ g/mL), digested extract completely abolished the effect of the pro-inflammatory stimuli. In order to associate the biological activity to the presence of a pure tannin, agrimoniin, the most abundant ellagitannin in the extract, and ellagic acid, representing the core of ellagitannins structure, were tested on the same parameters previously affected by the extracts; both pure compounds were able to inhibit the IL-8 secretion, promoter activity and the NF- κ B pathway, thus suggesting that these compounds could be responsible, at least in part, for the biological activity of the extract.

In conclusion, our results suggest that strawberry tannins possess anti-inflammatory activity at the gastric level even after *in vitro* gastric digestion; if the effect of strawberry tannins will be confirmed by *in vivo* studies, strawberry could be suggested as adjuvant to the treatment or prevention of gastric inflammatory diseases.

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