FORMULATION AND EVALUATION OF CONTROLLED RELEASE GASTRORETENTIVE IN SITU GEL-FORMING DELIVERY SYSTEMS OF PUNICA GRANATUM L. EXTRACT FOR THE THERAPEUTIC MANAGEMENT OF GASTRIC DISEASES

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Introduction: Gastritis is an inflammation of gastric mucosa mainly caused by the presence of the bacterium Helicobacter pylori (H. pylori). During H. pylori infection macrophages release pro-inflammatory cytokines, such as TNFα, triggering the expression of a variety of inflammatory genes, including IL-8, in epithelial gastric cells. Pomegranate, in the form of juice or extract, has been widely used in Europe and USA as a medicinal food since more than three decades. Pomegranate contains a great amount of ellagitannins (e.g. punicalagins and punicalin) exerting an in vitro direct activity against H. pylori and able to inhibit ethanol-induced gastritis in rats. Under physiological conditions ellagitannins are stable in the acidic gastric environment and may act locally. Gastroretentive drug delivery systems (GRDDS) show the ability to be retained in the stomach for a long period of time, enabling a controlled release of active principles and a better therapeutic efficacy in case of gastritis and peptic ulcer. In particular, raft-forming systems (RFS) are pourable sols which originate in situ floating gel raft upon contact with gastric fluids. Notably, a low pH value (3.5-5) of the formulation is crucial to maintain the phenolic concentration. The aim of this work was to formulate an RFS for the delivery of Punica granatum L. extract and evaluate its ability to achieve a prolonged release, considering also the effect of the extract in an in vitro model of gastric inflammation.

Materials and methods: RFS were prepared emulsifying aqueous mixtures of sodium alginate and pectin or carrageenins with light mineral oil, along with a calcium chelate slurry buffered at pH 4.5and a standardised pomegranate extract (40% punicalagins). The in vitro drug release was determined by dissolution test in simulated gastric fluid (SGF). At predetermined time intervals, samples of the dissolution medium were removed and assayed for extract content spectrophotometrically. Drug release data were analysed according to first order, Higuchi, Korsmeyer-Peppas, Weibull and Hixson-Crowell kinetic equations. The extract was also assayed in human gastric epithelial cells (AGS) stimulated with TNFα (10 ng/mL) and in co-cultures with H. pylori. IL-8secretion was assessed by ELISA assay.

Results: The formulations showed immediate gelation upon contact with SGF and buoyancy protracted over 24hours, suggesting, together with the strong consistence of the gel, the ability to achieve a prolonged gastric retention. The release was sustained for more than 6hours and its kinetic was in accordance with Korsmeyer-Peppas model (R^2=0.998), thus indicating a Fickian diffusional mechanism. In vitro assays showed that pomegranate extract was able to inhibit the TNFα-induced IL-8release, in AGS cells (IC_{50} 0.51μg/mL). Similar results were preliminarily obtained in a model of gastric cells-H. pylori co-culture.

Discussion and conclusions: GRDDS offer many advantages for the delivery of phytochemicals intended to act in the stomach, and alginate-based RFS are the most versatile. Sodium alginate is a biopolymer whose sol-gel transition is triggered by divalent cations, such as Ca^{2+}. Generally, $CaCO_3$is used with the dual function of Ca^{2+} source and gas-forming agent to enable floating in the gastric acidic environment. Unlike this case, pomegranate extract shows optimal stability only in acidic environment, therefore for the first time an RFS stable at pH 4.5relying on an oil emulsion for floatability and a pH-sensitive buffered calcium chelate as Ca^{2+} source was developed. The biological in vitro assays confirmed the ability of pomegranate extract to counteract gastric inflammation. In conclusion, liquid pourable formulations which showed immediate gelation and prolonged release behaviour in the gastric environment were produced, which would become a candidate as co-adjuvant treatment in gastric diseases.