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Anti-inflammatory effects of Sicilian pistachio (*Pistacia vera* L.) nut in an *in vitro* model of human intestinal epithelium

C. Gentile, A. Perrone, A. Attanzio, A.M. Pintaudi, L. Tesoriere, M.A. Livrea

Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

Intestinal epithelial cells play an important role in the mucosal inflammatory response. These cells synthesize and secrete inflammatory mediators, and selectively modulate the permeability of the epithelial monolayer thus exposing immune cells to antigens. Although intestinal inflammatory response is crucial to maintain gut structural integrity and function, alteration and dysregulation of inflammatory pathways contribute to tissue damage and ulceration, and are thought to be pivotal factors in the pathogenesis of different inflammatory gut diseases.[1] The limited efficacy of conventional pharmacological therapy in the intestinal inflammatory conditions has fostered research on alternatives and, at the same time, stresses the importance of prevention. In this context, the influence of dietary components, becoming in a physiological close proximity to intestinal cells and then to inflammatory processes within the intestinal mucosa, appears of nutritional and clinical interest.[2] Among natural preventive and complementary approaches to improve inflammatory symptoms, dietary polyphenols represent potential candidates and proanthocyanidins are particularly interesting.[3] For their relatively high concentration in a number of edible plants and their high digestive stability and limited intestinal adsorption,[4,5] proanthocyanidins reach the colon at relatively high concentrations and may have direct effects on the intestinal mucosa through their interaction with the intestinal epithelial cell membranes.[6,7]

The edible pistachio nut has been ranked among the first 50 food products highest in antioxidant potential.[8] A number of data show that the pistachio nut consumption has positive effects in human serum lipid profile and cardiovascular disease (CVD) risk factors[9,10] and significantly improves oxidative status and reduces circulating inflammatory biomarkers.[11] Our previous research provided evidence that a hydrophilic extract from Sicilian pistachio nuts (HPE) contains substantial amounts of polyphenols, including proanthocyanidins, and possesses radical scavenging and antioxidative properties in *in vitro* models of lipid oxidation.[12] Moreover we also demonstrated that HPE has anti-inflammatory activities in lipopolysaccharide (LPS)-activated macrophages interfering with the NF-*k*B activation, and that the high molecular weight proanthocyanidin fraction (PF) can play a major role as the bioactive component of HPE.[13]

In the present study we investigated the activity of HPE, and of its polymeric proanthocyanidin fraction as well, in an *in vitro* model of intestinal inflammation, consisting of Caco-2 cells differentiated into epithelial intestinal cells and exposed to the inflammatory actions of interleukin (IL-1β). Our results clearly show that HPE effectively inhibits the inflammatory response in intestinal epithelial cells, and that highly polymeric proanthocyanidin components exhibit qualitative and quantitative effects substantially comparable to those of whole extracts when tested at the same concentration found in the extracts. The protective effects are expressed through a marked decrease in release and expression of inflammatory mediators and occur in parallel with a reduced activation of the nuclear factor-1β. Moreover, our results clearly show that HPE can partially prevent the IL-1β-induced gap formation with perturbation of the monolayer integrity, as shown by a limited IL-1β-induced increase of paracellular permeability. Finally we provide evidence that HPE treatment increases transepithelial electrical resistence of Caco-2 cells monolayer, demonstrating that protective effects of HPE under our conditions occur in parallel with molecular interaction of nut components with the epithelial cells membranes.

To assess the physiological relevance of the tested concentration it is worth noting that a single serving of pistachio nut (28.34 g) (USDA National Nutrient Database for Standard Reference) contains around 62,34 mg polymeric proanthocyanidins (cyanidin equivalents). Once diluted in a gastrointestinal volume of 600 mL, this result in a 3.5 μM concentration (104 μg/mL) (as cyanidin equivalents) of polymeric proanthocyanidins which represent a plausible concentration in the human gut.[14] This concentration is one order higher than the concentrations selected in our cell model and this suggests that our results might be physiologically relevant in the gastrointestinal tract.

Data here presented may further remark the potentially beneficial health effects that may arise by daily intake of small quantity of pistachio nut. Widely available, inexpensive and frequently consumed, this nut for its favorable fatty acid profile and high content in bioactive antioxidant compounds, positively influences the plasma lipid parameters and oxidative status, and elicits antinflammatory properties. In this respect high content in large proanthocyanidins consumption of pistachio nut can exerts locally significant beneficial effects to physiology of gastrointestinal tract.

Correspondence: Carla Gentile, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, viale delle Scienze, Ed. 16, 90127 Palermo, Italy.
E-mail: carla.gentile@unipa.it

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