A NEW NUTRACEUTICAL FORMULATION AS PROMISING AGENT AGAINST INFLAMMATORY BOWEL DISEASES: *IN VITRO* EVALUATION ON CACO-2TRANSWELL MODEL

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Introduction: Several evidence suggests that regular consumption of extra virgin olive oil (EVOO), the main source of fat in the Mediterranean diet, is associated with a reduced risk of developing chronic degenerative disorders such as cardiovascular diseases, type 2diabetes and cancer. The health effects of EVOO have been attributed, besides to the monounsaturated fats content, to the presence of unsaponifible fraction, of which polyphenols and α-tocopherol (Vitamin E) are the main components with recognized antioxidant, anti-inflammatory and immunomodulatory properties. In light of this, a hydroxytyrosol (HXT) and DL-alpha tocopheryl acetate (αTA)-based nutraceutical formulation could be useful to counteract the symptoms or to avoid the onset of inflammatory bowel diseases (IBDs) in predisposed patients. IBDs are chronic functional bowel disorders with an estimated global prevalence of 10-15%, characterized by periods of latency alternating with exacerbation phases of multiple gastrointestinal symptoms. The etiopathogenesis remains unknown. The prevalent hypothesis is an abnormal intestinal immunological reaction to an altered gastro-intestinal microbiota in genetically susceptible subjects, although different environmental factors as well as mental distress play a pivotal role in their aetiology. Several therapeutic approaches, including lifestyle and dietary modifications, psychological therapies as well as complementary and alternative medicine, were used in clinical practice. However, sometimes side effects occur, and some patients quickly become refractory to treatment requiring a surgery intervention.

Materials and methods: The aim of study was to test a new nutraceutical formulation (NF) as promising agent against inflammatory bowel diseases by a preliminary in vitro evaluation on Caco-2transwell model. A NF bulk solution containing HXT from a standardized olive extract (90 mM) and αTA (45mM), conveyed in extra virgin organic olive oil, and a superimposable placebo formulation, were employed. NF test solution (250 μL), opportunely diluted in cell culture medium (HXT 2μM and αTA 1μM), was applied on each Caco-2cell monolayer. A pre-assay quality control of the monolayer integrity was carried out by measuring the trans-epithelial electrical resistance. The absorption efficiency of bioactive compounds (HXT and αTA) was investigated by RP-LC-DAD-FLU analysis, while the IL-6and IL-8release, after the NF and placebo pre-treatment (4h) and LPS (10 μg/ml, 20 h)-induced inflammation, was evaluated by enzyme-linked immunosorbent assays. Cell viability evaluation as well as post-assay quality control of the barrier system were carried out by MTT and Lucifer yellow paracellular permeability assays, respectively.

Results: NF showed an excellent absorption efficiency of both bioactive compounds and an excellent safety profile without showing any statistically significant alteration of the cell viability and of the barrier system with respect to the negative control. The pre-treatment of Caco-2cells with NF decreases the LPS-induced IL-8release with respect to the negative control (34.44%, $P<0.001$) and placebo (28.30%, $P<0.001$). However, the best anti-inflammatory activity was observed monitoring the IL-6release. Indeed, the pre-treatment of Caco-2cells with NF decreases the LPS-induced IL-6release with respect to the negative control (53.83%, $P<0.001$) and placebo (43.92%, $P<0.001$).

Discussion and conclusion: Results showed that this new NF increases the absorption of HXT and αTA at the intestinal level. Furthermore, this study demonstrates, comparing anti-inflammatory activity results of NF with respect to the placebo that EVOO, used as formulation vehicle, synergizes the anti-inflammatory activity of the bioactive compounds. In conclusion, this new NF is able to counteract, in statistically significant manner, the LPS-induced intestinal inflammation, which makes it a potentially useful candidate, opportunely formulated as gastro-resistant liquid-filled capsule, to be clinically investigated in IBD patients.