Potential of a flavonoid-rich extract from Bergamot juice as anticancer drug: preliminary data on its preventive effects in the Pirc rat (F344/NTac-Apc am1137), a genetic model of colorectal cancer

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Introduction: Colorectal cancer (CRC) is the second leading cause of cancer death in Europe. During past decades, polyphenolic-rich extracts have attracted attention as potential chemopreventive agents. Citrus bergamia Risso et Poiteau (Bergamot) is a typical tree of Calabria region (Italy). Its fruits are mainly used for the extraction of its essential oil from the peel, employed for the preparation of fragrances. Instead, bergamot juice (BJ) was considered a by-product with waste disposal problems. Recently, we chemically characterized the flavonoid-rich extract from Bergamot juice (BJe) and studied its antioxidant and anti-inflammatory activities, both in vitro and in vivo (Ferlazzo et al., 2016). Moreover, BJ and BJe have also shown to reduce the growth rate of diverse cancer cell lines through different molecular mechanisms depending on cancer types (Delle Monache et al., 2013; Visalli et al., 2014; Ferlazzo et al., 2016), as well as decrease the spontaneous neuroblastoma metastasis formation in the lung of SCID mouse (Navarra et al., 2014), suggesting its potential use also in cancer.

Object: Based on these considerations the aim of our study was to determine whether BJe administered to Pirc rats (F344/NTac-Apc am1137), may affect tumorigenesis when given mixed to the diet. The rat strain Polyposis in Rat Colon (Pirc rats) carries a germline mutation in Apc, the key genetic event in both familial adenomatous polyposis (FAP) and CRC (Femia et al., 2015). Notably, at variance with genetic models like ApcMin mice that develop tumours mostly in the small intestine, Pirc rats grow tumours also in the colon, thus resembling CRC and FAP and potentially standing as a robust model of colon cancer.

Methods: Pirc rats aged one month were fed for three months with: 1) a control diet (AIN 76), 2) the same diet supplemented with BJe at a dosage of 35 mg/kg b.w. (BJe 35) 3) the same diet supplemented with BJe 70 mg/kg b.w. (BJ 70). Three months later rats were sacrificed determining tumours and microscopic preneoplastic lesions (Mucin Depleted Foci- MDF) in the colon. We also performed Real-Time PCR in order to evaluate the expression of genes involved in apoptosis and inflammation.

Results: BJe causes a significant dose-related decrease in tumorigenesis (MDF/colon were: 133±8, 106±9, 74±4, in Controls, BJe 35 and BJ 70 groups, respectively; Means±SE). BJe did not alter colon proliferation in the normal mucosa or polyps. On the contrary, we found that apoptosis was significantly increased in the tumours of rats fed with BJe, suggesting that, at least in part, the protective mechanism involves an increase in apoptosis in these lesions. The evidence that BJe increased mRNA levels of p53 in the colon MDF, supported this finding. Moreover, BJe exerted

anti-inflammatory effects, as suggested by the reduced gene expression of inflammation markers, like iNOS and IL-1β.

Conclusions. The results obtained in this reliable model of colon carcinogenesis document that BJe is able to reduce colon tumorigenesis, indicating an alternative strategy to be exploited as preventive remedy in colorectal cancer.

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