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Intestinal anti-inflammatory effects of *Passiflora edulis* peel in the dextran sodium sulphate model of mouse colitis

Cinthia Baú Betim Cazarin [a,b,*], *Alba Rodriguez-Nogales* [a], *Francesca Algieri* [a], *M. Pilar Utrilla* [a], *M. Elena Rodríguez-Cabezas* [a], *José Garrido-Mesa* [a], *Eduardo Guerra-Hernández* [c], *Patrícia Aparecida de Campos Braga* [b], *Felix Guillermo Reyes Reyes* [b], *Mário Roberto Maróstica Jr* [b], *Julio Gálvez* [a]

[a] CIBER-EHD, Department of Pharmacology, ibs. GRANADA, CIBM, University of Granada, Spain
[b] School of Food Engineering, University of Campinas, Brazil
[c] Department of Nutrition and Bromatology, University of Granada, Spain

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ABSTRACT

Low dietary fibre intake has been associated with inflammatory bowel disease incidence. *Passiflora edulis* peel is considered to be a functional food because of its level of dietary fibre and polyphenols. Female C57BL/6J mice were assigned to three different groups: healthy, dextran sodium sulphate (DSS)-control, and *Passiflora edulis* treated. Treatment with *P. edulis* peel flour (8 mg/mL in the drinking water) started 2 weeks before colitis induction, which was performed by adding DSS in the drinking water (3%) for 5 days. *P. edulis* peel intake exerted an intestinal anti-inflammatory effect and attenuated the colonic damage caused by the DSS, as shown by the reduced disease activity index (DAI) values and after histological evaluation. Biochemical and molecular analyses revealed reduced expression of pro-inflammatory cytokine expression and enhanced intestinal protective barrier. Besides these effects, increases in short-chain fatty acid formation were observed, thus supporting a prebiotic effect.

1. Introduction

Inflammatory bowel disease (IBD) mainly comprises two related conditions – Crohn's disease (CD) and ulcerative colitis (UC) – which affect millions of people worldwide (Molodecky et al., 2012). The aetiology of IBD has not been fully elucidated; nevertheless, a combination of genetic and environmental factors is involved in its pathogenesis. They promote an abnormal exacerbated immune response in the intestine that generates an inflammation (Bamias, Nyce, De La Rue, & Cominelli, 2005). One of the key environmental factors involved in the increasing incidence of IBD is the lower intake of fibres (Hansen et al., 2011).

Dietary fibres are plant substances that resist the digestive tract hydrolysis and are fermented by colonic microbiota

* *Corresponding author.* School of Food Engineering, University of Campinas, Brazil. Fax: +55 19 3521 4060.
E-mail address: cinthiabetim@gmail.com (C.B.B. Cazarin).
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producing short-chain fatty acids (SCFA – acetic, propionic and butyric acids), which play an important role in the maintenance of colonic homeostasis, especially butyric acid, which provides the main fuel for colonocytes (Galvez, Rodriguez-Cabezas, & Zarzuelo, 2005), contributing, this way, to preserve mucosal integrity. In addition, any imbalance in the microbiota homeostasis can up-regulate the immune response leading to mucosal damage and impairment of the barrier function, and thus increasing translocation of antigens and intestinal inflammation (MacDermott, 1996). This barrier is formed by a monolayer of epithelial cells regulated by an apical intercellular junctional protein complex, essential to maintaining the barrier function (Bruewer, Samarin, & Nusrat, 2006).

Passiflora edulis is largely cultivated in Brazil for juice and pulp production. Since more than half of the fruit is peel, which is a great source of pectin (Pinheiro et al., 2008) and flavonoids (Zeraik, Yariwake, Wauters, Tits, & Angenot, 2012), a great amount of waste is generated. According to our previous works this by-product of food industry could be used to increase fibre intake, and modulate some inflammatory markers in ulcerative colitis (Cazarin et al., 2014a) and microbiota composition (da Silva, Cazarin, Bogusz Junior, Augusto, & Maróstica Junior, 2014). Therefore as our previous data suggest *P. edulis* peel could be considered a functional food source of fibres and polyphenols.

The aim of this study was to examine the preventative effects of *P. edulis* peel flour in the dextran sodium sulphate (DSS) model of experimental colitis. The DSS model was chosen because it reflects some clinical and histopathological features seen in human IBD, mostly UC (Dieleman et al., 1998; Gaudio et al., 1999; Kullmann et al., 2001), and exhibits good reproducibility. Special attention was given to the effects on the expression of some of the mediators involved in the inflammatory response, such as pro-inflammatory cytokines (tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-12 and IL-17), chemokines like monocyte chemotactic protein (MCP-1) and adhesion molecules (intercellular adhesion molecule (ICAM)-1), as well as different markers of epithelial integrity in the mucosa, like the mucins MUC-2 and MUC-3, tight junction proteins, occludin and zonula occludens (ZO)-1 and the matrix metalloproteinases (MMP) 2 and 9.

2. Materials and methods

Analytical standards of vicenin, vitexin, isovitexin, orientin and isoorientin used for quantitation and identification of C-glycosyl flavonoids were purchased from Sigma Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile, methanol and ethanol were purchased from J.T. Baker (J.T. Baker, USA) and analytical grade acetic acid was obtained from Dinâmica Química Comtemporânea (Diadema, SP, Brazil). Ultrapure water used was obtained from a Milli-Q system from Millipore (Bedford, MA, USA). Polytetrafluoroethylene (PTFE) membranes (0.22 μm) were purchased from Analítica (São Paulo, SP, Brazil).

Dextran sodium sulphate (DSS) 36–50 kDa was purchased from MP Biomedicals (Ontario, USA). Analytical standards of 2-methylvaleric, acetate, propionate and butyrate used for quantitation of short chain fatty acids were purchase from Sigma Aldrich (St. Louis, MO, USA). Sodium bicarbonate ($NaHCO_3$), sulphuric acid (H_2SO_4), chloroform, sodium sulphate (Na_2SO_4), formalin, haematoxylin, and eosin used were of analytical grade from Sigma Aldrich (St. Louis, MO, USA). RNA later® was obtained from Sigma Aldrich (St. Louis, MO, USA); and Tri-Reagent® was obtained from Thermo Fisher Scientific (Invitrogen, USA). The oligo (dT) primers (Promega, Southampton, UK) and KAPA SYBRsFAST qPCR Master Mix (Kapa Biosystems) were used to perform the qPCR analyses.

2.1. P. edulis *flour*

The flour was produced from organic fruits harvested in June 2010 (Torre de Pedra, São Paulo, Brazil). Fruits were cleaned, and peels (flavedo and albedo) were separated from the pulp and dried in oven with air circulation at 50 °C (Marconi, Piracicaba, São Paulo, Brazil). The flour was prepared by milling the dried peels in a hammer mill (20 mesh). Dietary fibres, which were previously analysed by Cazarin, da Silva, Colomeu, Zollner, and Maróstica Junior (2014b), represented 65.22% of the peel.

3. Identification of phenolic compounds in *P. edulis* peel by UPLC–ESI-MS/MS

The *P. edulis* peel flavonoids were extracted by adding 5 mL of 50:50 (v/v) ethanol:water to 250 mg of sample. The mixture was rotated at room temperature for 60 min. Afterwards, the sample was filtered and the supernatant was stored in amber flasks at 4–8 °C to evaluate the presence of C-glycosyl flavonoids by UPLC–MS/MS and stored in amber flasks at 4–8 °C. This procedure was repeated 10 times until C-glycosyl flavonoid total extraction and the filtrates were combined in the same flask.

The analytical identification of C-glycosyl flavonoids from passion fruit peel was performed by LC–MS/MS. The equipment consists of an Acquity™ UPLC M-Class system coupled to a Xevo G2-XS QToF mass spectrometer (Waters Milford, MA, USA), with an electrospray ionization (ESI) source in negative mode. MassLynx™ software version 4.1 and TargetLynx™ were used for data acquisition and data processing, respectively. The identification of the C-glycosyl flavonoids was performed by MS^E method of data acquisition which collects information for both precursor and fragment ions in a single analysis. The low-energy collision (0 eV) provides ion spectra of the exact mass precursor, and a high-energy collision provides exact mass of the fragment ions. The data were acquired in centroid resolution mode, using a mass range from 100 to 700 Da. The Elemental Composition tool was applied to predict the molecular formulas for the ions, which were searched in the Chemspider browser to determine the possible structures. After that, the fragmentation pattern was achieved for all molecules using the MassFragment tool. The chromatographic separation was carried out on a Acquity UPLC® BEH C18 column (100 mm × 2.1 mm × 1.7 μm) (Waters, Milford, MA, USA) using a ternary mobile phase consisting of 0.2% acetic acid (A) and 55:45 v/v acetonitrile:methanol (B). The gradient used was: 0 min 82% A and 18% B; 10.00 min 75% A and 25% B; 10.01–11.00 min 10% A and 90% B; 11.01–13.00 min 82% A and 18% B. The elution