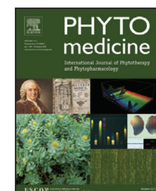
Phytomedicine 23 (2016) 901–913

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed



Original Article

Intestinal anti-inflammatory effects of total alkaloid extract from *Fumaria capreolata* in the DNBS model of mice colitis and intestinal epithelial CMT93 cells

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ARTICLE INFO

Article history:
Received 26 November 2015
Revised 4 May 2016
Accepted 10 May 2016

Keywords:
DNBS mouse colitis
Intestinal inflammation
Cytokine, Stylopine
protopine
Fumaria capreolata

ABSTRACT

Background: *Fumaria capreolata* L. (Papaveraceae) is a botanical drug used in North Africa for its gastro-intestinal and anti-inflammatory properties. It is characterized for the presence of several alkaloids that could be responsible for some of its effects, including an immunomodulatory activity.
Purpose: To test *in vivo* the intestinal anti-inflammatory properties of the total alkaloid fraction extracted from the aerial parts of *F. capreolata* (AFC), and to evaluate its effects on an intestinal epithelial cell line.
Study design and methods: AFC was chemically characterized by liquid chromatography coupled to diode array detection and high resolution mass spectrometry. Different doses of AFC (25, 50 and 100 mg/kg) were assayed in the DNBS model of experimental colitis in mice, and the colonic damage was evaluated both histologically and biochemically. In addition, *in vitro* experiments were performed with this alkaloid fraction on the mouse intestinal epithelial cell line CMT93 stimulated with LPS.
Results: The chemical analysis of AFC revealed the presence of 23 alkaloids, being the most abundants stylopine, protopine and coptisine. Oral administration of AFC produced a significant inhibition of the release and the expression of IL-6 and TNF-α in the colonic tissue. It also suppressed *in vivo* the transcription of other pro-inflammatory mediators such as IL-1β, iNOS, IL-12 and IL-17. Furthermore, AFC showed an immunomodulatory effect *in vitro* since it was able to inhibit the mRNA expression of IL-6, TNF-α and ICAM-1. Moreover, the beneficial effect of AFC in the colitic mice could also be associated with the normalization of the expression of MUC-2 and ZO-1, which are important for the intestinal epithelial integrity.
Conclusion: The present study suggests that AFC, containing 1.3% of stylopine and 0.9% of protopine, significantly exerted intestinal anti-inflammatory effects in an experimental model of mouse colitis. This fact could be related to a modulation of the intestinal immune response and a restoration of the intestinal epithelial function.

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Introduction

Inflammatory bowel disease (IBD) comprises different chronic inflammatory disorders of the gastrointestinal tract, mainly Crohn's disease and ulcerative colitis, which are characterized by remitting and relapsing episodes of intestinal inflammation. The most com-

Abbreviations: AFC, total alkaloid fraction extracted from the aerial parts of *Fumaria capreolata*; ANOVA, one-way analysis of variance; BPC, base peak chromatogram; DAD, diode array detection; DNBS, dinitrobenzenesulphonic acid; EIC, extracted ion chromatogram; ESI, electrospray ionization; GAPDH, 3-phosphate dehydrogenase; IBD, inflammatory bowel disease; ICAM, intercellular adhesion molecule; IFN-γ, interferon-γ; IL, interleukin; iNOS, inducible nitric oxide synthase; LC, liquid chromatography; LPS, lipopolysaccharide; MMP, metalloproteinase; MS, mass spectrometry; RT, retention time; TGF, transforming growth factor; TNFα, tumour necrosis factor α; TOF, time-of-flight; ZO, zonula occludens.

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http://dx.doi.org/10.1016/j.phymed.2016.05.003
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mon symptoms are intermittent abdominal pain, rectal bleeding, fever, weight loss, fatigue and diarrhoea, which seriously compromise the quality of life of these patients (Braus and Elliott, 2009). The precise aetiology of IBD has not been completely identified, but the chronic relapsing inflammation is thought to be the consequence of a genetic predisposition that triggers a deregulated and exaggerated immune response against the intestinal microbiota (Ardizzone and Bianchi Porro, 2005; Sanchez-Muñoz et al., 2008). This response results in a dysregulation of the synthesis and release of pro-inflammatory cytokines, such as tumour necrosis factor α (TNFα), interferon-γ, (IFN-γ), interleukin (IL)-1β, IL-6, and IL-12, and anti-inflammatory cytokines, including IL-10 or transforming growth factor (TGF)-β (Neurath, 2014), and an excessive production of reactive oxygen/nitrogen species that are not conveniently scavenged and lead to oxidative/nitrosative stress (Piechota-Polanczyk and Fichna, 2014). The final consequence of this unbalance is tissue damage together with lipid and protein modifications and DNA damage or apoptosis, which also contribute to the pathogenesis of the intestinal inflammation.

The conventional pharmacological treatments for human IBD include aminosalicylates, corticosteroids, immunosuppressants and biological agents, and although they show efficacy, in many patients they are not fully effective and can be associated with major adverse effects that limit their required chronic use (Siegel, 2011). These facts have promoted the development of emerging and alternative therapeutic strategies that may be useful for the management of chronic intestinal inflammation, including traditional plant–based remedies, which show immunomodulatory and antioxidant properties (Hur et al., 2012).

Different species from genus *Fumaria* (Papaveraceae) have been traditionally used against quite diverse disorders. Thus, in Anatolian folk medicine, these plants have been reported to act as a blood purifier and as an anti-allergic agent, as well as in the treatment of some skin diseases (rashes or conjunctivitis) (Orhan et al., 2012). Furthermore, their beneficial effects as anti-hypertensives, diuretics or in hepatobiliary and gastrointestinal complaints have been also reported (Suau et al., 2002a). Typically, the biological activities associated with these plants have been related to the presence of isoquinoline alkaloids in their composition, such as aporphine, protoberberine, protopine and benzophenanthridine type (Suau et al., 2002a; Suau et al., 2002b; Grycova et al., 2007). Isoquinoline alkaloids are considered as a major group of pharmacologically important compounds, and some of them have demonstrated, among others, biological, antimicrobial, antibacterial, antifungal and antitumor properties (Dembitsky et al., 2014).

In a previous study, it was reported that the total alkaloid fraction from *Fumaria capreolata* L., in addition to exert antioxidant activity, was devoid of significant toxicity when administered orally to mice at doses up to 2 g/kg (Bribi et al., 2013). Moreover, it has been also recently described the antinociceptive and anti-inflammatory effects of this extract (Bribi et al., 2015). The aim of the present study was to evaluate the effects of a total alkaloid fraction from *F. capreolata* (AFC) in the dinitrobenzenesulphonic acid (DNBS) model of experimental colitis in mice, correlating its potential anti-inflammatory activity to the expression of some of the mediators involved in the intestinal inflammatory response, such as pro-inflammatory cytokines, like IL-6, IL-1β, TNF-α, IL-12 and IL-17, the chemokine intercellular adhesion molecule (ICAM)-1, the enzymes inducible nitric oxide synthase (iNOS) and the metalloproteinase (MMP)-9, as well as two markers of epithelial integrity in the mucosa, the mucin MUC-2 and the transmembrane protein zonula occludens (ZO)-1. Furthermore, some *in vitro* studies were performed to evaluate the impact of this alkaloid fraction on the mouse intestinal epithelial cell line CMT93.

Materials and methods

Drugs and chemicals

All the drugs and chemicals used were purchased from Sigma–Aldrich Chemical (Madrid, Spain), unless otherwise stated. The test substances were dissolved in distilled water and prepared fresh daily for administration to the animals. In addition, methanol, acetonitrile, ultrapure water of MS quality and formic acid were purchased from Fisher Chemicals (ThermoFisher, Waltham, MA, USA). Protopine hydrochloride was obtained from Sigma-Aldrich Chemical (Madrid, Spain) and a methanolic stock solution (1 mg/ml) was prepared.

Extraction of alkaloids

Aerial par*ts of F. capreolata were collected from Béjaia area, in the North East of Algeria in May 2013 when they were at the flowering and fruit setting stage. Dr Benabdesselam authenticated the plant and a voucher specimen* was deposited in a reference collection or the Herbarium of the Laboratory of Plant Biotechnology and Ethnobotany (University of Béjaia, Algeria) (Reference No. FC015). The alkaloid extract of *F. capreolata* (AFC) was obtained following the procedure previously reported (Soušek et al., 1999). Briefly, the aerial parts of the plant were dried in an oven at 40ºC overnight and ground into a fine powder using a grinder. The powder samples (1 kg) were extracted with ethanol in a Soxhlet apparatus for 8 h, then evaporated under reduced pressure, acidified with 2.5% HCl to pH 1–2 and filtered, and stored overnight at room temperature. The aqueous acid solution was adjusted to pH 9.5 with concentrated ammonium hydroxide and extracted with dichloromethane. The extracts were dried over magnesium sulphate and the solvent evaporated to afford a crude extract of total alkaloids. After evaporation, the yield of each fraction was calculated, and the AFC obtained was stored at 4ºC until use.

Characterization of the alkaloid fraction by liquid chromatography-coupled to diode array detection and high-resolution mass spectrometry (LC–DAD–MS)

Analyses were made with an Agilent 1200 series rapid resolution (Agilent, Palo Alto, CA, USA) equipped with a binary pump, an autosampler and a DAD. The mobile phases consisted of water with 0.2% formic acid (mobile phase A) and acetonitrile (mobile phase B). A multistep linear gradient was then applied: 0–5.5 min, 1–7% B; 5.5–11 min, 7–14% B; 11–17.5 min, 14–24% B; 17.5–22.5 min, 24–40% B; 22.5–27.5 min, 40–100% B; 27.5–28.5 min, 100–100% B; 28.5–29.5 min, 100–1% B. The latter value (99% A and 1% B) was held for 5.5 min to equilibrate the column to initial conditions before the next injection. The flow rate was set at 0.5 ml/min throughout the gradient. Separation was carried out with a Zorbax Eclipse XDB-C18 column (4.6 × 50 mm, 1.8 µm of particle size) (Agilent, Palo Alto, CA, USA) at 25ºC. The absorbance was monitored between 190 and 600 nm. The injection volume was 2 µl.

The effluent from the analytical column was reduced using a “T” type splitter before being introduced into the mass spectrometer (split ratio 1:3), a micrOTOF™ (Bruker Corporation, Bremen, Germany). This was equipped with an electrospray ionization (ESI) interface operating in positive ionization mode using a capillary voltage of –4.5 kV. The other optimum values of the ESI–TOF parameters were drying gas temperature, 190 ºC; drying gas flow, 7 L min−1, and nebulizing gas pressure, 1.5 bar. The detection was carried out considering a mass range of 50–1000 *m/z*. During the development of the HPLC method, external instrument calibration was performed using a 74900-00-05 Cole-Parmer syringe pump (Cole-Parmer, Vernon Hills, IL, USA), which was directly connected

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