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Anti-inflammatory activity of hydroalcoholic extracts of *Lavandula dentata* L. and *Lavandula stoechas* L.

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ABSTRACT

Ethnopharmacological relevance: Plants from genus *Lavandula* have been used as anti-inflammatory drugs in Mediterranean traditional medicine. Nowadays, there is a growing interest for complementary medicine, including herbal remedies, to treat inflammatory bowel disease (IBD).
Aim of the study: To test the anti-inflammatory properties of *Lavandula dentata* and *Lavandula stoechas* extracts in two inflammatory experimental models: TNBS model of rat colitis and the carrageenan-induced paw edema in mice, in order to mimic the intestinal conditions and the extra-intestinal manifestations of human IBD, respectively.
Material and methods: The extracts were characterized through the qualitative HPLC analysis. Then, they were assayed in vitro and in vivo. In vitro studies were performed in BMDMs and CMT-93 epithelial cells with different concentrations of the extracts (ranging from 0.1 to 100 μg/ml). The extracts were tested in vivo in the TNBS model of rat colitis (10 and 25 mg/kg) and in the carrageenan-induced paw edema in mice (10, 25 and 100 mg/kg).
Results: L. dentata and *L. stoechas* extracts displayed immunomodulatory properties in vitro down-regulating different mediators of inflammation like cytokines and nitric oxide. They also showed anti-inflammatory effects in the TNBS model of colitis as evidenced by reduced myeloperoxidase activity and increased total glutathione content, indicating a decrease of neutrophil infiltration and an improvement of the oxidative state. Besides, both extracts modulated the expression of pro-inflammatory cytokines and chemokines, and ameliorated the altered epithelial barrier function. They also displayed anti-inflammatory effects in the carrageenan-induced paw edema in mice, since a significant reduction of the paw thickness was observed. This was associated with a down-regulation of the expression of different inducible enzymes like MMP-9, iNOS and COX-2 and pro-inflammatory cytokines, all involved in the maintenance of the inflammatory condition.
Conclusion: L. dentata and *L. stoechas* extracts showed intestinal anti-inflammatory effect, confirming their potential use as herbal remedies in gastrointestinal disorders. In addition, their anti-inflammatory effect was also observed in other locations, thus suggesting a possible use for the treatment of the extra-intestinal symptoms of IBD.

1. Introduction

Nowadays, traditional remedies are the main medical treatment for two thirds of the world population, due to the limited availability and affordability of the standard pharmaceutical medicines used in developed countries (Kamboj, 2000; Pan et al.,

Abbreviations: BMDMs, Bone marrow-derived macrophages; COX-2, Cyclooxygenase-2; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GSH, glutathione; IBD, inflammatory bowel disease; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MPO, myeloperoxidase; TNBS, trinitrobenzene sulfonic acid

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2013). Besides, more and more people in the industrialized world choose natural remedies to treat their diseases, especially those with chronic functional gastrointestinal disorders (Comar and Kirby, 2005; Tillisch, 2007). The reputed efficacy of the medicinal plants in the treatment of different human diseases has been attributed to the presence of different active compounds, principally phenolic derivatives, such as flavonoids, phenylpropanoids, stilbenes and others. These compounds have been well-reported to exert antioxidant properties. They may prevent the damage caused by reactive oxygen species (Kahkonen et al., 1999), which have been proposed to display a prominent role in the pathogenesis of human disorders with an inflammatory background (Alfadda and Sallam, 2012).

Inflammatory bowel disease (IBD) is a group of chronic inflammatory pathologies of the gastrointestinal tract that comprises two major conditions: Crohn's disease (CD) and ulcerative colitis (UC). The etiology of IBD is probably related to a dysregulation of the mucosal immune response toward the resident microbiota in the intestinal lumen together with genetic and environmental factors (Liu et al., 2009). In consequence, there is an abnormal synthesis and release of different pro-inflammatory mediators, including eicosanoids, platelet-activating factor, cytokines and reactive oxygen and nitrogen metabolites, which lead to mucosal damage and result in the chronic tissue inflammation that characterizes human IBD (Strober et al., 2007). It is noticeable that oxidant-mediated injury also plays a key role in the pathogenesis of IBD (Karp and Koch, 2006). Oxidation and inflammation are reciprocally related; in fact, the generation of hydroxyl radicals, the most cytotoxic reactive oxygen species, takes place in inflamed tissues and up-regulates the expression of pro-inflammatory mediators, including cytokines like interleukin (IL)-1β, IL-6 or IL-17, nitrogen metabolites and chemokines. They all promote the pathogenic cascade and establish a vicious circle of inflammation in the gut that leads to functional alterations (Strober et al., 2007). Moreover, IBD is often associated with extra-intestinal symptoms so it could be also considered as a systemic disease. These symptoms can appear before, simultaneously or after the diagnosis of IBD and mean a deterioration of the quality of life of the patients (Veloso, 2011). In fact, it has been reported that the severity of some of these extra-intestinal manifestations parallels the activity of IBD, and some improve with the treatment of the underlying disease, although others require a specific treatment (Patil and Cross, 2013). The joint involvement is one of the most common extra-intestinal symptoms in human IBD, but other organs can be also affected (Huang et al., 2013; Salvarani and Fries, 2009).

Different pharmacological strategies are used to control the inflammation or reduce the symptoms of IBD, including aminosalicylates, corticosteroids, immunosuppressants like azathioprine or methotrexate, as well as biologicals, mainly the tumor necrosis factor (TNF)α monoclonal antibodies infliximab and adalimumab (De La Rue and Bickston, 2006). Although most of them have shown efficacy in human therapy, in some cases this is limited and/or can be associated with serious adverse effects, such as opportunistic infections, diabetes mellitus, hypertension, ocular effects (glaucoma and cataracts), psychiatric complications (Curkovic et al., 2013), which can restrict their chronic use (Dignass et al., 2010). For this reason it is important to search for other safer and effective therapeutic agents for this disease. In this regard, the use of medicinal plants with anti-inflammatory and anti-oxidant properties could be a good therapeutic strategy that could also treat the associated extra-intestinal symptoms.

The Mediterranean region has been long considered as an important source of medicinal plants, and one of the most interesting families that provides a greater number of species to the catalog of popular herbal medicine for the treatment of inflammatory disorders is Lamiaceae. Among these, *Lavandula dentata* L. and *Lavandula stoechas* L. are common in the Spanish heliophilous scrubs. The first one is typically located in coastal areas on basic substrates, reaching up to 700 m, whereas the second is preferably found on acid soils from sea level to 1700 m. Both species are frequently used in traditional medicine for the treatment of digestive disorders, such as liver and intestinal inflammatory conditions (González-Tejero et al., 1992), but also for other inflammatory disorders such as arthritis (González-Tejero et al., 1992), rheumatism (Benitez et al., 2010). The medicinal importance of these plants is well documented; in fact, different Pharmacopeias include the medicines prepared from them, mostly as infusions (Goren et al., 2002).

The aim of the present study was to evaluate the effects of hydroalcoholic extracts of the aerial parts of *Lavandula dentata* L. and *Lavandula stoechas* L. in two inflammatory experimental models that resemble histological and biochemical features of the human IBD and its extra-intestinal symptoms: the trinitrobenzenesulfonic acid (TNBS)-induced rat colitis and the carrageenan-induced paw edema in mice, respectively (Jurjus et al., 2004; Mukherjee et al., 1996).

2. Material and methods

This study was carried out in accordance with the 'Guide for the Care and Use of Laboratory Animals' as promulgated by the National Institute of Health. The protocol was approved by the Ethic Committee of Laboratory Animals of the University of Granada (Spain) (reference number CEEA-2010-286).

2.1. Reagents

All chemicals were purchased from Sigma Chemical (Madrid, Spain), unless otherwise stated. Solvents used for extraction and analysis were of analytical and HPLC-MS grades, respectively. Methanol, ultrapure water, acetonitrile, and glacial acetic acid were purchased from Fisher Chemicals (ThermoFisher, Waltham, MA, USA).

2.2. Plant material and preparation of the extracts

The aerial parts of *Lavandula dentata* and *Lavandula stoechas* were collected in the area of Calahonda, 36° 42′ 29.35″ N and 3° 24′ 8.75″ W (Granada, Spain), and in the area of Lujar, 36° 46′ 2.97″ N and 3° 25′ 6.44″ W (Granada, Spain), respectively, in June 2012. Both plants were identified and authenticated by Dr. M. R. González-Tejero, Dr. J. Molero-Mesa and Dr. M. Casares-Porcel from the department of Botany of the University of Granada, Spain. The voucher specimens corresponding to *Lavandula dentata* L. (GDA 60249) and *Lavandula stoechas* L. (GDA 60250) were deposited in the herbarium of the University of Granada (Granada, Spain).

The plant extracts were prepared as described previously (Algieri et al., 2013). Briefly, 5 g of ground plant material were mixed with washed sea sand (Panreac Química S.A.U., Spain) and extracted with 30 ml of methanol 50% (v/v) at 1500 PSI and 80 °C for 10 min in a ASE200 extraction system (Dionex Corporation, USA). After two cycles of extraction, liquid extracts were pooled and the solvent evaporated under vacuum at 60 °C. Extraction efficiency (expressed as percentage w/w) was 20% for *Lavandula dentata* and 14% for *Lavandula stoechas*. The polyphenolic content of the extracts was determined by the Folin-Ciocalteu method, as previously described (Delgado-Torre et al., 2012) using acid gallic as standard; and the results were expressed as equivalent to grams of gallic acid per 100 g of extract (%); these values were 15.6% for *L. dentata* and 18.8% for *L. stoechas.*