Journal of Ethnopharmacology 198 (2017) 226–234

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

A standardized methanol extract of Eclipta prostrata (L.) L. (Asteraceae) reduces bronchial hyperresponsiveness and production of Th2 cytokines in a murine model of asthma

Lucas Junqueira de Freitas Morel[a], Bruna Cestari de Azevedo[a], Fábio Carmona[b], Silvia Helena Taleb Contini[a], Aristônio Magalhães Teles[c], Fernando Silva Ramalho[b], Bianca Waléria Bertoni[a], Suzelei de Castro França[a], Marcos de Carvalho Borges[b], Ana Maria Soares Pereira[a,*]

[a] Departamento de Biotecnologia em Plantas Medicinais, Universidade de Ribeirão Preto, Av. Costábile Romano 2201, 14096-900 Ribeirão Preto, SP, Brazil
[b] Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes 3900, Monte Alegre 14049-900 Ribeirão Preto, SP, Brazil
[c] Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Campus Samambaia, Av. Esperança, 74690-900 Goiânia, GO, Brazil

ARTICLE INFO

Chemical compounds studied in this article:
Wedelolactone (PubChem CID:5281813)
Demethylwedelolactone (PubChem CID:5489605)
Oroboside (PubChem CID:44257298)

Keywords:
Eclipta prostrate
Wedelolactone
Demethylwedelolactone
Oroboside
Anti-inflammatory
Asthma

ABSTRACT

Ethnopharmacological relevance: *Eclipta prostrata* (L.) L. (Asteraceae) has been used in Brazilian traditional medicine to treat asthma and other respiratory illnesses.
Aims of the study: To investigate the effects of different doses of a standardized extract of *E. prostrata* using a murine *model* of *allergen* induced *asthma*.
Materials and methods: Balb/c mice were sensitized twice with ovalbumin (OVA) administered intraperitoneally and challenged over four alternate days with nasal instillations of OVA solution. The standardized methanol extract of *E. prostrata* was administered in doses of 100, 250 and 500 mg kg^{-1} concomitantly with nasal instillation over seven consecutive days. Control animals were treated with dexamethasone or saline solution. Bronchial hyperresponsiveness, production of Th1 and Th2 cytokines, allergen sensitization, airway and lung inflammation, mucous secretion and airway remodeling were assessed.
Results: The concentrations of chemical markers in the standardized methanol extract were 0.02% oroboside, 1.69% demethylwedelolactone and 1.71% wedelolactone. Treatment with 250 mg kg^{-1} of extract, which provided 0.745, 4.22 and 4.30 mg kg^{-1} day^{-1} of oroboside, demethylwedelolactone and wedelolactone, respectively, significantly reduced ($P < 0.05$) respiratory resistance and elastance. Such effects were comparable with those produced by dexamethasone. The total number of inflammatory cells and eosinophils in the bronchoalveolar lavage and the concentrations of interleukin (IL)-4, IL-5 and IL-13 in lung homogenate were significantly reduced ($P < 0.05$) by the methanol extract of *E. prostrata*.
Conclusion: The results presented herein demonstrate for the first time the *anti-inflammatory* activity of *E. prostrata* in a murine model of asthma, thereby supporting the ethnopharmacological uses of the plant.

1. Introduction

Asthma is a heterogeneous disorder characterized by inflammation of the airways, bronchial hyperresponsiveness (BHR) and variable airflow limitation (Bateman et al., 2008). The condition is associated with significant alterations in tissue structure, including goblet cell hyperplasia accompanied by mucus hypersecretion, subepithelial fibrosis, edema, (Bateman et al., 2008; Holgate, 2008; Roth et al., 2012) and lung inflammation with the presence of eosinophils (Woolnough and Wardlaw, 2015). These inflammatory responses are mediated by Th2 lymphocytes with activation of plasmocytes B and production of immunoglobulin E (IgE) and various cytokines including interleukin 4 (IL-4), IL-5 and IL-13 (Galli and Tsai, 2012; Galli et al., 2008).

Asthma is considered the most common of chronic diseases and affects children and adults in all countries regardless of the level of development. Moreover, the prevalence of asthma has increased in the last 20 years (Belice and Becker, 2016; Martinez, 2002). It is estimated that 300 million individuals are affected by asthma worldwide (Global Initiative for Asthma, 2016) with considerable variation in prevalence among different countries (1–16%). In Brazil, for example, the

* Corresponding author.
E-mail address: apereira@unaerp.br (A.M.S. Pereira).

http://dx.doi.org/10.1016/j.jep.2016.12.008
Received 2 August 2016; Received in revised form 21 November 2016; Accepted 9 December 2016
Available online 10 December 2016
0378-8741/

prevalence of asthma in children aged 13 - 14 years was estimated at 9.4% in 2000–2003 (Lai et al., 2009). However, the exact incidence of the disease is difficult to assess because of the lack of a precise and universally accepted definition.

While the direct costs of asthma are high, indirect costs are incalculable since asthma attacks lead to loss of productivity, absenteeism from work or school, reductions in physical, emotional and social abilities, and even death (Bateman et al., 2008; Horak et al., 2016), all of which generate negative impacts on families and society. Although asthma cannot be cured, various quick-relief and long-term control medications with proven efficacy are available. However, some patients do not respond adequately to these drugs, while corticosteroids may have undesirable side effects, including reduced growth, osteopenia, osteoporosis, cataract and glaucoma (Bush and Saglani, 2010). For these reasons, the continued search for safer drugs is of utmost importance.

Various plant-derived natural products with *anti*-inflammatory properties have been screened for potential application in the treatment of asthma (Corrêa et al., 2008). In the traditional medicine of Brazil, *Eclipta prostrate* (L.) L.[1] is widely used to treat pulmonary disorders such as asthma, bronchitis and cough, diarrhea, syphilis, snakebites, filariasis and leprosy (Corrêa, 1984; Matos, 1997). In Asia, extracts of this plant are also used to treat asthma (Chichioco-Hernandez and Paguigan, 2010; Jahan et al., 2014; Savithramma et al., 2007) and other respiratory illnesses such as common colds and coughs (Sharma et al., 2012). In addition, *E. prostrata* exhibits immunomodulatory (Jayathirtha and Mishra, 2004), antidiabetic (Jaiswal et al., 2012), antitumor (Liu et al., 2012), anti-inflammatory (Tewtrakul et al., 2011), anti-hepatitis C virus (Manvar et al., 2012), and hepatoprotective (Tabassum and Agrawal, 2004) activities. Phytosteroids, flavonoids, triterpenoids, saponins and alkaloids constitute the major classes of secondary metabolites present in *E. prostrata* extracts (Fang et al., 2015; Han et al., 2015; Lee et al., 2008; Sawant et al., 2004), while the main bioactive constituents are reported to be the coumestans wedelolactone and demethylwedelolactone (Diogo et al., 2009; Wagner et al., 1986).

In consideration of the ethnopharmacological relevance and the recognized anti-inflammatory properties of *E. prostrata*, we standardized a methanol extract of the whole plant in order to evaluate its activity in a murine *model* of *ovalbumin (OVA)*-induced *asthma*. The effects of this methanol extract on the BHR, production of cytokines and OVA-specific *immunoglobulin E* (IgE) complexes, leukocyte migration, mucous secretion, lung inflammation and airway remodeling were investigated. This report is the first to focus on the *in vivo* effects of a standardized *E. prostrata* extract and represents an important advance towards the development of an alternative treatment for asthma.

2. Materials and methods

Details of the study were submitted to and approved by the local Ethics Committee on Animal Research (Protocol no. 221/2014). Procedures involving experimental animals were conducted at the Laboratory of Lung Pathophysiology and the Laboratory of Pathology at the Ribeirão Preto Medical School, University of São Paulo, following guidelines established by the National Animal Experimentation Control Board.

2.1. Plant material

Whole plants of *Eclipta prostrata* (L.) L. (The Plant List, 2013), a species known in Brazil as “erva-botão”, were harvested in March 2011 at the Medicinal Garden of Universidade de Ribeirão Preto (UNAERP; 21°11’54” S and 47°43’59” W) and identified by Dr. Aristônio Magalhães Teles (Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, GO, Brazil). A voucher specimen was deposited in the *Herbarium of Medicinal Plants* at UNAERP with voucher number HPMU-848.

2.2. Preparation and fractionation of the methanol extract of E. prostrata

Whole plants were dried in a circulating-air oven at 45 °C for 72 h, comminuted and passed through a *40 mesh sieve*. The resulting powder (4.5 kg) was macerated with 23 L of methanol for 7 days, filtered through filter paper and concentrated at the rotary evaporator to yield 155 g of crude dry extract. An aliquot (100 g) of crude extract was dissolved in methanol: water (8:2; v/v) and partitioned sequentially against hexane and ethyl acetate to yield, after solvent removal, 17.68 g of hexane fraction, 11.49 g of ethyl acetate fraction, and 36.84 g of remaining aqueous fraction.

The ethyl acetate fraction was applied to a Sephadex LH 20 column (43×2.5 cm i.d.; GE Healthcare Life Sciences, São Paulo, SP, Brazil) and submitted to isocratic elution with methanol to yield 89×15 mL fractions. These fractions were combined according to the similarity of their chromatographic profiles to yield five main fractions designated EA1 through EA5. Fraction EA3 was re-applied to the Sephadex LH 20 column and submitted to isocratic elution with acetone: water (7:3; v/v) to yield 59×10 mL fractions that were subsequently combined into five subfractions designated EA3.1 through EA3.5.

Subfractions EA3.3 and EA3.4 were submitted separately to preparative reverse-phase high performance liquid chromatography (RP-HPLC) using a Shimadzu (Kyoto, Japan) LC-10 AVP chromatograph coupled to a SPD-M10A photodiode array detector (PAD) and fitted with a Supelco C_{18} column (250×10 mm i.d.; 4.6 µm particle size; Sigma Aldrich, St Louis, MO, USA). Samples containing 50 mg mL^{-1} of the subfraction were injected onto the column and eluted with water (solvent A) and methanol (solvent B) supplied at a flow rate of 20 mL min^{-1} in the form of a linear gradient from 40% to 100% B between 0 and 100 min. The effluent was monitored by PAD at 355 nm and collected as 30 mL fractions. The separated constituents were submitted to ^{1}H- *and* ^{13}C-*nuclear magnetic resonance* (NMR), heteronuclear single-quantum correlation (HMQC) and heteronuclear multiple-bond correlation (HMBC) spectroscopic analysis, and subsequently identified as wedelolactone and demethylwedelolactone in subfraction EA3.4, and as oroboside (orobol 7-O-glucoside) in subfraction EA3.3 (Fig. 1).

2.3. HPLC-mass spectrometric (MS) analysis of the methanol extract of E. prostrata

The identities of the three flavonoid-derivatives present in the methanol extract of *E. prostrata* were confirmed by HPLC-MS-MS analysis using a Waters (Milford, MA, USA) Acquity UPLC H-Class system equipped with a PAD and a Waters Xevo TQ-S tandem quadrupole mass spectrophotometer and fitted with a Sigma-Aldrich Ascentis Express C_{18} column (100×4.6 mm i.d.; 2.7 µm particle size). A sample containing 10 mg mL^{-1} of extract in methanol was passed through a 0.45 µm pore size syringe filter and an aliquot (5 µL) injected onto the column. Elution was performed using a mixture of 0.1% ammonium acetate (solvent A) and acetonitrile (solvent B) supplied at a flow rate of 0.5 mL min^{-1} in the form of a linear gradient from 40% to 90% B between 0 and 5 min and from 90% to 40% B between 5 and 16 min. The *Z-spray ionization source* was maintained at 150 °C with capillary voltages of 3.0 and −2.5 kV in the positive and negative ionization modes, respectively. Additional operating parameters were: cone voltage 30 V, source offset 60 V, nitrogen desolvation gas temperature 300 °C with gas flow rate of 600 L h^{-1}, and mass scan range 150–600 m/z in the full-scan mode.

[1] Synonym: *Eclipta alba* (L.) Hassk. (The Plant List, 2013)