Revista Brasileira de Farmacognosia xxx (2017) xxx–xxx

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

In vivo anti-arthritic and antioxidant effects from the standardized ethanolic extract of *Moussonia deppeana*

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ARTICLE INFO

Article history:
Received 14 July 2017
Accepted 2 February 2018
Available online xxx

Keywords:
Anti-arthritic
Antioxidant
CFA-induced arthritis
Interleukins
Anti-inflammatory
Verbascoside

ABSTRACT

Moussonia deppeana (Schltdl. & Cham.) Klotzsch ex Hanst., Gesneriaceae, known as tlachichinole, is a Mexican medicinal plant used for treatment of chronic inflammation-related diseases such as arthritis. In this paper, the main metabolite verbascoside was quantified in ethanolic extract; anti-arthritic and antioxidant activities were also evaluated in Complete Freund's Adjuvant induced arthritis in mice, with complete hematological evaluation, and oxidative stress measure in edema and ganglionic tissues on day 28. In popliteal ganglion, $CD4^+$ lymphocytes and tumor necrosis factor alpha concentration were measured in addition to histological analysis. Ethanolic extract contained 79.2 mg of verbascoside/g extract, and this extract at 450 mg/kg generated an inhibition of 24% over paw edema development and increased body weight gain on final day. For hematological parameters, same dose decreased total leukocytes and lymphocytes, as well as decreased oxidation rate over biomolecules in edema and ganglionic tissues, and increased antioxidant enzyme activity. In ganglionic tissue, $CD4^+$ lymphocytes and tumor necrosis factor alpha level showed no differences at any tested dose compared to complete Freund's adjuvant untreated group. Histological analysis of popliteal ganglion revealed moderate reduction of follicular hyperplasia, leukocyte infiltration and lipid inclusions at 450 mg/kg dose. Ethanolic extract of *M. deppeana* possesses anti-edematous activity associated to a moderate reduction in follicular hyperplasia, with immune-modulatory and antioxidant effects during experimental arthritis in mice.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that affects the synovial membrane and leads to the degeneration of articular tissue and later to bone erosion, as a consequence of chronic inflammation (Smolen et al., 2016). It affects 2–4% of the world population (Wong et al., 2010), and its frequency varies depending on the ethnic group. In 2012, Mexico reported a prevalence of 59% in women and 41% in men (ratio, 3:1) and an incidence of 0.3% in Mexican population (González-López et al., 2013). When this disease progresses, the patient exhibits mobility disabilities; finally, 15–20% of patients will require surgery within a period of 5 years, giving this illness a high impact on the patients' quality of life. Cardiel et al. (2014) reported in Mexico an annual treatment cost approaching US$6000, of which US$2000 are provided by the Federal Government, and the remainder by the patient's relatives (representing 15% of their income). RA is characterized by increased levels of immune cells, such as macrophages and lymphocytes in the synovial space and a high concentration of free radicals (FR), mostly reactive oxygen species (ROS), which could irreversibly affect articular tissue through the oxidation of its biomolecules, contributing to progression of the disease (Lonkar and Dedon, 2011).

Anti-arthritic treatment is long and is focused on the inhibition of key mediators of the chronic inflammation process, such as interleukins (tumor necrosis factor alpha, TNF-α), cells (lymphocytes T), and enzymes (induced cyclooxygenase), with the aim of regulating these or of decreasing their degenerative impact on articular tis-

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https://doi.org/10.1016/j.bjp.2018.02.004

sue (Ventura-Ríos et al., 2012). Co-administration of non-steroidal anti-inflammatory drugs, gluco-corticosteroids, disease-modifying anti-rheumatic drugs, and biological therapies such as rituximab and infliximab (Covelli et al., 2010), is mostly prescribed. However, these prolonged therapies cause several adverse effects, such as gastrointestinal bleeding, cyto- and hepatotoxicity, immunosuppression or osteoporosis; and recurrent infectious disease (Scott et al., 2010; Toscano et al., 2010; Harboe et al., 2012; Ventura-Ríos et al., 2012).

Currently, in Mexican traditional herbal medicine, there are few plants with preclinical studies that showed anti-arthritic activity and a protective effect on chronic inflammation course. These include *Yucca schidigera*, *Dodonaea viscosa*, and *Sphaeralcea angustifolia*, this latter species the only one featuring a clinical trial for the treatment of osteoarthritis (García-Rodríguez et al., 2012; Salinas-Sánchez et al., 2012; Romero-Cerecero et al., 2013; Cheeke et al., 2016).

Moussonia deppeanna (Schltdl. & Cham.) Klotzsch ex Hanst., Gesneriaceae (commonly known as *tlachichinole*) is used in Mexican traditional medicine to treat several inflammatory diseases, kidney failure, rheumatic pain, and gastrointestinal diseases (Domínguez-Ortiz et al., 2010; Gutiérrez-Rebolledo et al., 2016). Recently, it has been described that its ethanolic (EtOH) extract from aerial parts showed a good anti-inflammatory activity in 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and carrageenan models, showing median effective dose (ED_{50}) = 1.5 mg/ear and 450 mg/kg, respectively, with a median lethal dose (LD_{50}) of >2 mg/kg by intragastric (*i.g.*) route. In the sub-acute toxicity test in healthy mice, the EtOH extract did not cause lethality and any alteration on biochemical and hematological parameters; also, histological analysis demonstrated no damage in liver, kidneys, and spleen. Phytochemical analyses revealed that the EtOH extract contains verbascoside (main compound), ursolic and oleanolic acids, apigenin, and hesperetin; these were identified by spectroscopic and spectrometric methods and high performance liquid chromatography (HPLC) (Gutiérrez-Rebolledo et al., 2016).

Verbascoside has been isolated from some medicinal plants (*Verbascum*, *Buddleja*, *Striga* genus, and from species of the *Gesneriaceae* family) (Filho et al., 2012; Huang et al., 2013; Alipieva et al., 2014). The metabolite has anti-inflammatory properties: inhibition of paw edema formation in rat (Akdemir et al., 2011), inhibition of the induced nitric oxide synthase (NOS) enzyme (Marzocco et al., 2007), decreased ear edema formation in mice (Sánchez et al., 2013), inhibition of myeloperoxidase and nuclear factor kappa B (NF-κB) (Paola et al., 2011), decreased levels of interferon gamma (IFN-γ), interleukin 1β, TNF-α, and an increase in superoxide dismutase (SOD) activity (Hausmann et al., 2007; Mazzon et al., 2009; Esposito et al. 2010; Lenoir et al., 2011).

In this work, the anti-arthritic and antioxidant activities *in vivo* from the *M. deppeana* EtOH extract were evaluated in experimental arthritis induced by complete Freund's adjuvant (CFA) in mice; also, HPLC quantification of verbascoside was measured.

Materials and methods

Collection of plant material and crude extract preparation

Aerial parts of *Moussonia deppeana* (Schltdl. & Cham.) Klotzsch ex Hanst., Gesneriaceae, were collected in Coatepec, Veracruz, Mexico, in February, 2013 (GPS coordinates 19°32′34.0″ N 96°51′58.4″ W, voucher number 17139, deposited in FEZA UNAM Herbarium). EtOH extract was prepared following the procedure previously described (Gutiérrez-Rebolledo et al., 2016).

HPLC analysis of the EtOH extract

Verbascoside content in EtOH extract was quantified by HPLC utilizing a Waters 2695 separation module HPLC system equipped with a Waters 996 photodiode array detector and Empower Pro software (Waters Corporation, USA), as previously described (Cárdenas-Sandoval et al., 2015; Gómez-Aguirre et al., 2012). The following analytical conditions were employed: column ZORBAX Eclipse XDB-C18 (5 mm, 4.6 × 250 mm i.d.), with pre-column (Agilent Technologies); mobile phase linear gradient of 0.0125 N aqueous-acetic acid (eluent A) and CH_3CN (eluent B), starting from 95% A at 50% in 20 min, returning to 95% for 20–25 min; this was maintained for 35 min; the flow rate was 0.7 ml/min and the injection volume was 20 ml; peaks were detected at 280 nm. Retention time for verbascoside was 7.9 min (λ_{max} = 218, 247, 290, and 330 nm) and quantity was estimated by interpolation of peak areas in a calibration curve ($y = 22{,}144x - 16{,}1443$; $R^2 = 0.999$). *M. deppeana* EtOH extract and pure verbascoside standard were analyzed three times (triplicate), and results were expressed in mg of verbascoside/g dry extract weight. All chemical reagents were purchased from Sigma–Aldrich.

Animals' in vivo *assay*

Adult Balb/C male mice (25 ± 3 g) were obtained from Animal Vivarium of National Medical Center XXI Century, Mexican Social Security Institute, Mexico City, and were maintained under laboratory conditions. Project was approved by National Commission of Scientific Investigation and Bioethics with code CNIC-IMSS R-2013-785-053.

Monoarthritis induced with CFA

This experiment was carried out according to Rasool et al. (2006), with modifications. All groups (n = 7) were injected subcutaneously with 25 μl of CFA in the right hind paw on days zero and 14 (re-injection). Treatment groups were administered by *i.g.* route with phenylbutazone (PBZ, 100 mg/kg), and EtOH extract (200, 450, and 900 mg/kg) daily from day 7 to 27. All samples were solubilized in Tween 80:water (1:9); groups of healthy and un-treated arthritic mice received only vehicle. Paw edema was measured at different times (days 1, 4, 7, 14, 15, 21, and 28) (*Et*) with a digital micrometer (Mitutoyo model 293-831) and the value of day zero (*Eo*) was determined as baseline. Body weight (BW) gain was also registered on the same days compared to day zero. Inhibition percentage of edema development in each group was calculated from days 14 to 28 by comparison with CFA un-treated group as follows:

$$\%\,\text{inhibition} = \frac{(Et - Eo)_{\text{CFA group}} - (Et - Eo)_{\text{Treated group}}}{(Et - Eo)_{\text{CFA group}}} \times 100$$

Hematological and oxidative stress (OS) parameters

On day 28, blood samples were collected from each mouse for complete hematological analysis, which was performed in a Beckman Coulter Cell Counter. Later, mice were euthanized, hind paw with chronic edema and nearest popliteal ganglion tissues were obtained in cold to determine OS biomarkers. Tissue samples (500 mg) were homogenized in cold phosphate-buffered saline solution (2 ml at pH 7.4), and one ml of each homogenate was centrifuged at 10,500 × *g* and 4 °C/15 min; activity of SOD, catalase (CAT), and glutathione peroxidase (GSH-Px) was determined from the supernatants, while lipid peroxidation (LPO) and protein carbonyl content (PCC) were evaluated from un-centrifuged

Please cite this article in press as: Gutiérrez-Rebolledo, G.A., et al. *In vivo* anti-arthritic and antioxidant effects from the standardized ethanolic extract of *Moussonia deppeana*. Revista Brasileira de Farmacognosia (2017), https://doi.org/10.1016/j.bjp.2018.02.004