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Inhibition of arachidonic acid metabolism by the Andean crude drug *Parastrephia lucida* (Meyen) Cabrera

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ABSTRACT

Ethnopharmacological relevance: *Parastrephia lucida* (Meyen) Cabrera is used in the traditional medicine of Argentinean highlands as an antiseptic and anti-inflammatory medicinal plant. To give scientific support to the ethnopharmacological claim of *Parastrephia lucida* as an anti-inflammatory crude drug the effect of *Parastrephia lucida* extracts and fractions was assessed on key enzymes of the biosynthesis of pro-inflammatory eicosanoids mediators from arachidonic acid (AA).
Materials and methods: A bio-guided fractionation of the plant extract was carried out to find out the compounds or mixtures responsible for the anti-inflammatory effect. The extracts and fractions were tested *in vitro* for their ability to inhibit the enzymes cyclooxygenase (COX)-1 and COX-2, lipoxygenase (LOX) and phospholipase (sPLA2). Fractions were analyzed by HPLC–MS, HPLC–ESI-MS/MS and NMR to relate the effect with groups of secondary metabolites.
Results: *Parastrephia lucida* was more effective inhibiting COX and $sPLA_2$ than LOX. Assay-guided isolation led to the active fractions C and F which showed different effect on the selected enzymes. The fraction C was more effective inhibiting LOX while fraction F showed better activity against $sPLA_2$ and COX-2. Both fractions were further worked-up following the isolation of the anti-inflammatory agents with the selected enzyme assays. The main compounds identified in the most active fractions were 5,4′-dihydroxi-7-methoxyflavanone, apigenin, apigenin methyl ether and apigenin trimethyl ether, methyl and dimethyl ethers from quercetin, kaempferol and luteolin methyl ether, ferulic acid esters, cinnamic acid and vanillin.
Conclusions: *Parastrephia lucida* extract inhibit AA metabolism via several enzymes. The results give support to the traditional use of this plant for the treatment of inflammatory disorders.

1. Introduction

The Asteraceae *Parastrephia lucida* (Meyen) Cabrera is a shrub common in the highlands of Antofagasta de la Sierra, Provincia de Catamarca, Argentina. In traditional medicine, *Parastrephia lucida* is used as an anti-inflammatory agent, to treat toothache by applications of leaves. The plant resin in poultices is used for rapid healing of wounds, bruises and to consolidate bone dislocation and fractures (Villagrán et al., 2003). *Parastrephia lucida* has been widely used by Amerindian cultures living in the South American highlands from pre-Columbian times. It was incorporated into the Argentinian traditional medicine, largely without untoward incident, and is considered generally safe. In spite of the widespread use of *Parastrephia lucida*, the reputed anti-inflammatory effect of the plant was not yet supported by scientific studies. The underlying mechanisms that account for their anti-inflammatory activity and active components remain largely to be disclosed. The plant was reported to have acaricide, antifungal, antibacterial and antioxidant activities (Ayma et al., 1995; Sayago et al., 2006; Zampini et al., 2008, 2009; Rojo et al., 2009; D'Almeida et al., 2012).

The use of plants for the treatment of human diseases is associated with cultures and traditional medicine from different parts of the world. Inflammation and inflammation-related ailments such as rheumatism, muscle swelling, cut wound, accidental bone fracture, insect bites, pains and burn by fire and hot water are frequently treated with medicinal plants (World Health Organization

Abbreviations: AA, arachidonic acid; CAPE, caffeic acid phenethyl ester; COX, cyclooxygenase; 1,2dHGPC, 1,2-diheptanoilthio-glycerophosphocholine; DMSO, dimethyl sulfoxide; DTNB, 5,5′ dithiobis-2-nitrobenzoic acid; LTs, leukotrienes; LOX, lipoxygenase; NSAIDs, non-steroidal anti-inflammatory drugs; PAF, platelet activating factor; $sPLA_2$, phospholipase A_2; PGs, prostaglandins; ROS, reactive oxygen species; HAc, acetic acid; MeOH, methanol; EtOAc, ethyl acetate

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(WHO), 2001). Amongst the *in vitro* anti-inflammatory assays, the ones examining the effects of an extract or compound on arachidonic acid (AA) metabolizing enzymes are most commonly used. AA is the substrate of different oxygenases such as cyclooxygenases (COXs) and lipoxygenases (LOXs), key enzymes in the synthesis of eicosonoids. The release of AA from cell membrane phospholipids is the result of activation of phospholipases A_2 (PLA_2) by cell stimulation, which is a rate limiting step of the production of pro-inflammatory lipid mediators such as prostaglandins (PGs), leukotrienes (LTs), lipoxins, and platelet activating factor (PAF) (Khanum et al., 2005). These mediators are responsible for the maintenance of the inflammatory process. Physiological or acute inflammation is a beneficial host response to tissue damage, but when timely resolution is delayed, the overproduction of the inflammatory mediators may lead to many diseases, such as rheumatoid arthritis, inflammatory bowel disease, cancer and atherosclerosis. Thus, inhibition of the production of these inflammatory mediators may prevent or suppress a variety of inflammatory diseases.

Inhibitors of COXs are the main strays of current therapy aimed to modulate inflammation, pain, and to control fever. The constitutive form COX-1 is responsible for the maintenance of physiological prostanoid biosynthesis. In contrast, COX-2 is an inducible isoform linked to inflammatory cell types and tissues (Vane, 1994). Prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs) is also associated with severe side effects such as gastrointestinal hemorrhage due to COX-1 inhibition (Lee et al., 2003). Many COX-2 inhibitors have been developed as drugs to treat inflammation; however, some have been withdrawn from the market, indicating a need for inhibitors free of side effects (Viji and Helen, 2008). On the other hand, NSAIDs induced gastric inflammation has been associated with shunting of the AA to generate LTs from 5-LOX (Celotti and Durand, 2003).

Many compounds proved to be potent and selective inhibitors of LOX, but could not enter the pharmaceutical market due to severe side effects or inefficacy in clinical trials (Viji and Helen, 2008). Reports have appeared regarding so-called "dual inhibitors", agents that inhibit not only COX-1 and COX-2, but also 5-LOX-mediated AA metabolism (Celotti and Durand, 2003; Altavilla et al., 2009). These agents may be particularly effective for minimizing both gastric and cardiovascular side effects by balancing AA metabolism in the body (Altavilla et al., 2009).

Due to the role of reactive species in the inflammatory process (Halliwell et al., 1988), the hydrogen donating ability and the scavenging effects on reactive oxygen species (ROS) of *Parastrephia lucida* extracts (Zampini et al., 2008) could be considered relevant for the plant anti-inflammatory effect. Besides the importance of the scavenging activity against ROS, a putative direct anti-inflammatory activity may be relevant in the treatment of inflammatory affections by *Parastrephia lucida*. Thus, the aim of the present study was to investigate if *Parastrephia lucida* extracts can decrease the activity of COX, LOX and $sPLA_2$, key enzymes in the formation of pro-inflammatory eicosanoids mediators from AA, in order to support the traditional use of this plant as an anti-inflammatory crude drug. Furthermore, bio-guided fractionation of the extracts and active fractions was undertaken to get an insight into the chemical identity of the active anti-inflammatory agents.

2. Materials and methods

2.1. Chemicals

Soybean lipoxygenase-1, diphenylboric acid-β-ethylamino ester (NP), tannic acid, caffeic acid and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich (MO, USA). Linoleic acid and dimethyl sulfoxide (DMSO) were obtained from Merck (Germany). Triton X-100 was supplied from Fluka Chemical Corp. (USA). 1,2-diheptanoilthio-glycerophosphocholine (1,2dHGPC), secretory phospholipase A_2 ($sPLA_2$) from bee venom and 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) were obtained from Cayman Chemical Co. (MI, USA). Dichloromethane (DCM) and methanol (MeOH) were purchased from Cicarelli (Buenos Aires, Argentina). Analytical TLC was carried out using pre-coated plates (Kieselgel 60 F254, 0.2 mm, Merck, Germany). Other chemicals were purchased from local commercial sources and they were of analytical grade quality.

2.2. Plant material

The aerial parts of *Parastrephia lucida* (Meyen) Cabrera were collected from January to February 2006 at 3600 m over sea level (m.o.s.l) in Antofagasta de la Sierra, Provincia de Catamarca, Puna de Atacama. A voucher Specimen no. 607923/LIL, was deposited in the Herbarium of "Fundación Miguel Lillo", Tucumán, Argentina. The plant was authenticated by Dra. Soledad Cuello. The samples were dried at 40 °C. The parts used were leaves and stems (aerial parts), according to the traditional use.

2.3. Extraction and fractionation of bioactive compounds

The extraction and fractionation of bioactive compounds was performed according to D'Almeida et al. (2012) (Fig. 1). Briefly, the dried plant material was finely powdered and macerated with MeOH (250 g of dry plant material per liter) for 7 days at room temperature with gentle shaking or stirring (40 cycles/min). A liquid–liquid partition of the crude MeOH extract was performed with dichloromethane (DCM) and water (AQ). The DCM phase was more active as anti-inflammatory than the AQ phase and was further worked-up to disclose the active fractions. The DCM extract was permeated on Sephadex LH-20 and eluted with methanol. Seven fraction pools were obtained (A–G) based on TLC profiles revealed with NP reagent. Fractions C and F showed the highest enzyme inhibitory activities and were fractionated by silica gel column chromatography using a gradient of increasing polarity as follows.

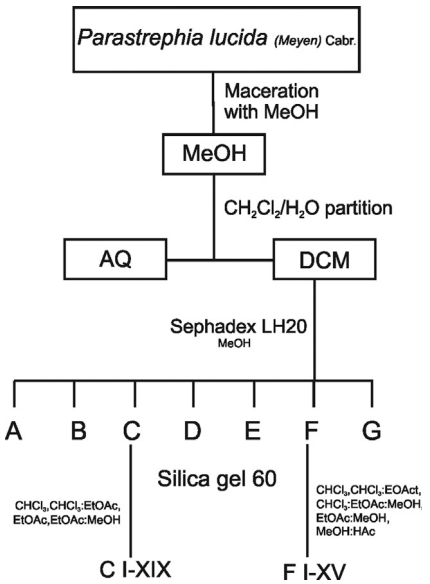

Fig. 1. Bio-guided fractionation of anti-inflammatory compounds from *Parastrephia lucida* aerial parts. Methanol (MeOH), aqueous (AQ) and dichloromethane (DCM) extracts.