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Inhibition of cyclooxygenase activity by standardized hydroalcoholic extracts of four Asteraceae species from the Argentine Puna

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Abstract

We determined the anti-inflammatory activity of standardized extracts of four medicinal plant species (*Baccharis incarum*, *B. boliviensis*, *Chuquiraga atacamensis*, *Parastrephia lucida*) that grow in the Argentine Puna (3800 m above sea level) and that are used to reduce oxidative stress and alleviate gout and arthritic pain. The extracts of plant aerial parts were standardized in terms of total phenolic compounds and flavone/flavanone content and free radical scavenging activity. All extracts showed high phenolic compound concentration (0.5-1.6 mg/mL), mainly flavones and flavonols (0.1-0.8 mg/mL). The extracts showed hydrogen donating ability (DPPH and ABTS) and reactive oxygen species scavenging activity ($O_2^{\bullet -}$, OH^-, H_2O_2). The ability of the extracts to inhibit cyclooxygenase enzymes (COX-1 and COX-2) was determined by calculating percent inhibition of PGE_2 production measured by enzyme immunoassay. All extracts inhibited both enzymes with IC_{50} values of 2.0 to 16.7 µg/mL. The anti-inflammatory activity of *B. incarum* and *C. atacamensis* extracts was higher than that of *B. boliviensis* and *P. lucida*. The IC_{50} values obtained for indomethacin were 0.11 and 0.78 µM for COX-1 and COX-2, respectively. The present results are consistent with the anecdotal use of these species in phytotherapic preparations.

Key words: *Baccharis incarum*; *Baccharis boliviensis*; *Chuquiraga atacamensis*; *Parastrephia lucida*; Anti-inflammatory activity; Cyclooxygenase

The Asteraceae family is represented by more than 1,100 genera and 25,000 species distributed in different kinds of habitat, mainly in the mountainous tropical areas of South America. *Baccharis incarum* (Wedd.) Perkins, commonly known as “tola” or “lejía”, *Baccharis boliviensis* (Wedd.) Cab. known as “tola”, “tola limón” or “tolilla”, *Chuquiraga atacamensis* Kuntze, known as “lengua de gallina” or “azafrán, “quebrolla” and *Parastrephia lucida* (Meyen) Cab., known as “romero”, “tola” or “chachakoa”, belong to the Asteraceae family and are used as ethnomedicinal plants, fodder plants and fuel wood. The leaves and stems of these plants maintained in ethanol are used as “rubbing” or topical preparation in order to treat rheumatism, fever and inflammation. Plant resin poultices are used in bruises and wounds and to consolidate luxations and fractures. The infusion or decoction of aerial parts of *Baccharis* are used also as a protective agent against live, prostate and stomach diseases, burns, skin wounds and ulcers, and as an antipyretic (1,2).

Some studies of the biological activity and phytochemistry of these plant species have been published (3-8), but a survey of the literature revealed that there are no studies on the validation of the anti-inflammatory capacity of polar extracts from these plants.

Cyclooxygenase enzymes (COXs, prostaglandin-endoperoxide synthases) catalyze two reactions, the first

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being a cyclooxygenase function consisting of the addition of molecular oxygen to arachidonic acid (AA) to form prostaglandin G_2 (PGG_2). The second is the conversion of PGG_2 to PGH_2 by a peroxidase function. Hence, this COX enzyme performs the critical initial reaction in the AA metabolic cascade leading to the formation of pro-inflammatory prostaglandins, thromboxanes and prostacyclins. Prostaglandins regulate smooth muscle contractility, blood pressure and platelet aggregation and mediate pain and fever. Inhibition of cyclooxygenase activity is the mechanism by which nonsteroidal anti-inflammatory drugs (NSAIDs) exert their analgesic, antipyretic, anti-inflammatory, and antithrombotic effects (9).

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 (10). Prolonged use of NSAIDs is also associated with severe side effects such as gastrointestinal hemorrhage due to COX-1 inhibition (9). The new COX-2 selective drugs do not seem to be free of risk either since several COX-2 inhibitors have been found to cause cardiovascular problems (11). Steroids have an obvious role in the treatment of inflammatory diseases but, due to their toxicity, they can only be used over short periods of time except in very serious cases for which the risks are acceptable. Consequently, there is a strong need for natural products with minimum side effects.

The aim of the present study was to evaluate the anti-inflammatory potential of standardized hydroalcoholic extracts of *B. incarum*, *B. boliviensis*, *C. atacamensis*, and *P. lucida* using a COX inhibition assay in order to validate their popular use as phytomedicines.

B. boliviensis (Wedd.) Cab., *B. incarum* (Wedd.) Perkins, *C. atacamensis* Kuntze, and *P. lucida* (Meyen) Cab. were collected at 3800 m above sea level (Antofagasta de la Sierra, Catamarca, Argentina) in February 2005. The aerial parts of the plants were used. Voucher specimens (*B. incarum*: 607934 LIL, *B. boliviensis*: 607936 LIL, *C. atacamensis*: 607929 LIL, and *P. lucida*: 607923 LIL) were deposited at the Fundación Miguel Lillo herbarium, San Miguel de Tucumán, Tucumán, Argentina. These species were authenticated by Lic. Soledad Cuello, Fundación Miguel Lillo.

The species were dried at room temperature. Ground air-dried plant material was macerated in ethanol 80° (5 g dry tissue/100 mL) for 7 days with shaking (40 cycles/min) at room temperature. Next, the extracts were filtered through Whatman No. 1 paper and vacuum concentrated to dryness in a rotary evaporator at 40°C. The extracts were dissolved with DMSO to prepare stock solutions and called *B. incarum* (MI-1-3800), *B. boliviensis* (MI-2-3800), *C. atacamensis* (MI-3-3800), and *P. lucida* (MI-4-3800).

Total phenolic compound content was determined (12) and the results are reported as gallic acid equivalents. Flavone and flavonol content was measured by the method of Popova et al. (13) and quercetin was used as standard. Total flavanone and dihydroflavonol content was estimated using the colorimetric method for the DAB9 extract modified for propolis (13). Total flavanones and dihydroflavonols were calculated as naringenine from a calibration curve.

The ability of the extracts to inhibit the conversion of AA to PGH_2 by ovine COX-1 and human recombinant COX-2 was determined using a COX inhibitor screening assay kit (No. 560131; Cayman Chemical, USA). Cyclooxygenase catalyzes the first step in the biosynthesis of AA to PGH_2. $PGF_{2\alpha}$ produced from PGH_2 by reduction with stannous chloride was measured by enzyme immunoassay (14). This assay is based on the competition between PGs and a PG-acetylcholinesterase conjugate (a PG tracer) for a limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the wells, since the concentration of the PG tracer is held constant while PG concentration varies. This antibody-PG complex binds to an anti-IgG antibody previously attached to the well. The plate is washed with a buffer solution and Ellman's reagent, which contains the substrate of acetylcholinesterase, is added to the well. The yellow product of this enzymatic reaction is determined spectrophotometrically in a Microplate Reader (BioRad, Japan) at 415 nm. The assay for obtaining 100% COX activity was performed with and without DMSO as solvent control. The inhibitory assays were performed in the presence of extracts at different concentrations (1 to 16 µg total phenolic compound/mL) or of a commercial anti-inflammatory drug such as indomethacin. Indomethacin (Sigma, Germany) was chosen because it is an inhibitor of both enzymes (COX-1 and COX-2). Pre-incubation time between enzyme and inhibitor was 10 min with 2-min incubation in the presence of AA at 37°C. Enzyme control was performed with COXs that had been inactivated by placing them in boiling water for 3 min. The detection limit was 29 pg PG/mL. The intra- and interassay coefficients of variations were 5 and 10%, respectively.

The anti-inflammatory effect of the test compounds was evaluated by calculating percent inhibition of PGE_2 production. The test compound concentration causing 50% inhibition of PGE_2 release (IC_{50}) was calculated from the concentration-inhibition response curve by regression analysis. The selectivity indices (SI) of the reference compounds and extracts (SI = IC_{50} COX-2 / IC_{50} COX-1) were calculated. The values reported are the means of three separate experiments.

In the present study, the ethanolic extracts of plant species that grow in the Argentine Atacama Puna were chosen to validate their traditional use in folk medicine as anti-inflammatory solutions.

A phytochemical study of the extracts was conducted in order to obtain a standardized phytotherapic preparation of each plant species (Table 1). The phenolic compound concentration was 1.6, 1.5, 0.85, and 0.5 mg/mL for MI-2-

Table 1. Phenolic compound/flavonoid content and antioxidant activity of plant species extracts from the Argentine Puna (8).

Standardized extracts	Concentration (mg/mL)			IC_{50} (µg/mL)				IC_{25} (µg/mL)
	Phenolic compounds	Flavones and flavonols	Flavanones and dihydroflavonols	Free radical scavenging		ROS scavenging		
				ABTS	DPPH	$O_2^{\bullet -}$	OH^-	H_2O_2
MI-1-3800	0.8	0.6	0.07	4.0	35	15	13.5	30
MI-2-3800	1.6	0.8	0.07	3.3	31	20	6.0	47
MI-3-3800	0.5	0.1	0.15	3.5	4	20	*	55
MI-4-3800	1.5	0.4	0.05	2.9	5	12	27.0	82

*The MI-3 extract produced 33% inhibition of deoxyribose degradation at concentrations of 10 to 100 µg/mL. IC_{50} = 50% inhibition; ROS = reactive oxygen species; ABTS = 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid); DPPH = 1,1-diphenyl-2-picrylhydrazyl.

3800, MI-4-3800, MI-1-3800, and MI-3-3800, respectively. The extracts mainly contain flavones and flavonols (0.8 mg/mL for MI-2-3800, 0.6 mg/mL for MI-1-3800, and 0.4 mg/mL for MI-4-3800). MI-3-3800 mainly contains flavanones and dihydroflavonols (0.15 mg/mL).

The presence of terpenoids and flavonoids has been described in *B. incarum*, *B. boliviensis*, and *C. atacamensis* (3,15-19). There are no bibliographical data about chemical studies on *P. lucida.* The standardized ethanolic extracts showed hydrogen donating ability and reactive oxygen scavenging activity (Table 1), inhibiting the production of reactive oxygen species. Since these plant species contain xanthine oxidase inhibitors, they reduce both uric acid and $O_2^{\bullet -}$ production (IC_{50} of 10 to 20 µg/mL) (8). Numerous pathological events such as inflammation processes, aging phenomena and degenerative dysfunction are associated with the generation of reactive oxygen species. Thus, the effectiveness of these plant extracts used in folk medicine to suppress inflammatory responses may be due to their capacity to reduce oxidative stress and to their inhibitory activity on COX enzymes.

PGE_2 level increases markedly and provokes inflammation and pain in pathological events due to the activation of COX enzymes. Hence, the extracts were tested for their ability to inhibit COX-1 and COX-2. All extracts exhibited a significant anti-inflammatory activity *in vitro* by inhibiting PG production. The anti-inflammatory activities of all species were dose dependent in phenolic compound equivalents for COX-1 and COX-2 (Figure 1). At the same total phenolic compound concentration, the effects of the MI-1-3800 and MI-3-3800 extracts were higher than those of the MI-2-3800 and MI-4-3800 extracts. The greatest anti-inflammatory effect observed for MI-3-3800 could be due to a higher content of flavanones and dihydroflavonols.

A concentration of 8 µg/mL of total phenolic compounds in the standardized extracts produced a COX-1 inhibition of 80, 70, 51, and 45% for MI-3-3800, MI-1-3800, MI-2-3800,

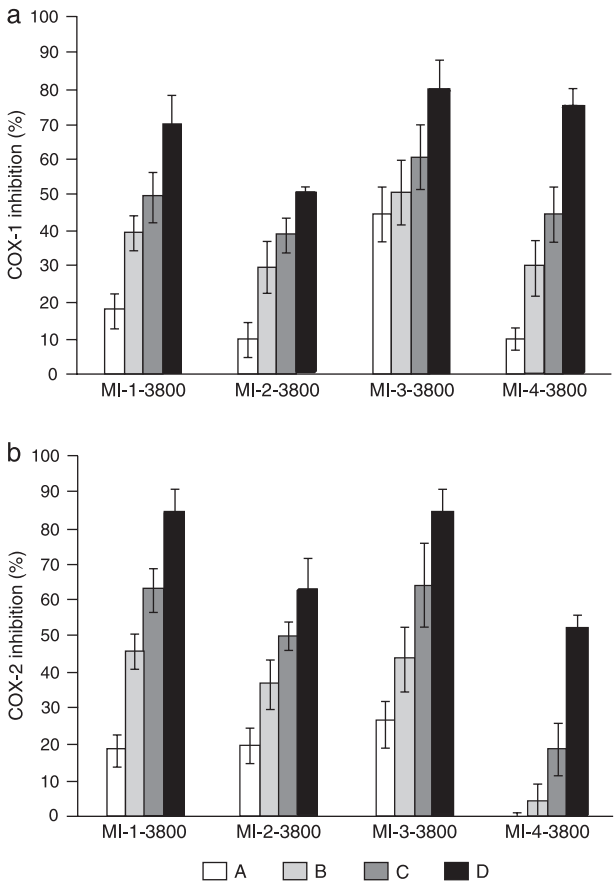

Figure 1. Effect of different phenolic compound concentrations (reported as µg/mL of gallic acid equivalent) of the standardized extracts on PGE_2 release by *a*, COX-1 and *b*, COX-2 (reported as inhibition percentage). The concentrations assayed for MI-1-3800, MI-2-3800, MI-3-3800 were 2, 4, 6, and 8 µg/mL and for MI-4-3800 were 2, 4, 8, and 16 µg/mL (A, B, C and D, respectively). The results (means ± SD, N = 3) of three separate experiments are shown. PGE_2 = prostaglandin E_2; COX = cyclooxygenase.

and MI-4-3800, respectively. Percent COX-2 inhibition was 84% for MI-1-3800 and MI-3-3800, and 63 and 19% for MI-2-3800 and MI-4-3800, respectively.

The IC_{50} values obtained for COX-1 were 5.7, 7.6, 2.0, 10.0 µg/mL and the IC_{50} values obtained for COX-2 were 4.9, 6.0, 4.7, and 16.7 µg/mL for the standardized extracts MI-1-3800, MI-2-3800, MI-3-3800, and MI-4-3800, respectively. The selectivity indices of the extracts were 0.86, 0.79, 2.35, 1.67, respectively. The IC_{50} values obtained for indomethacin were 0.11 and 0.78 µM for COX-1 and COX-2, respectively. Classical NSAIDs such as ibuprofen, naproxen and indomethacin have a selectivity ratio >1. This accurately reflects their ability to inhibit both COX-1 and COX-2 to a similar extent, or to inhibit COX-1 more selectively. All species extracts have similar inhibiting power over COX-1 and COX-2. Hence, their SI would indicate their non-selective anti-inflammatory ability.

The preliminary results presented in this paper provide some scientific basis for the traditional use of these plant species for managing inflammatory pains. There are no data in the literature on the anti-inflammatory compounds from the species included in this study, whereas data have been reported on the anti-inflammatory activity of terpenoids and flavonoids isolated from other *Baccharis* species (20). Further analysis, including additional purification of the extracts and chemical characterization of isolated compounds, should permit the identification of compounds possessing specific activities. These investigations, along with further studies in order to determine the effect of these extracts on these and other inflammatory parameters, are currently being conducted in our laboratory.

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