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Abarema cochliacarpos reduces LPS-induced inflammatory response in murine peritoneal macrophages regulating ROS-MAPK signal pathway

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ABSTRACT

Ethnopharmacological relevance: *Abarema cochliacarpos* (Gomes) Barneby and Grimes (Fabaceae), known by the vulgar name of Babatenã, has been traditionally used in Northeast Brazil, as an anti-inflammatory remedy. Previous studies have demonstrated its anti-inflammatory and antiulcer effects in skin lesion, alcohol gastric ulcer and acute and chronic colitis.
Aims: The present study was designed to evaluate the antioxidant and anti-inflammatory effects of the butanolic fraction from *A. cochliacarpos* (BFAC) and its major flavonoid, (+)-catechin, in LPS-stimulated murine peritoneal macrophages. Moreover, we studied the role of mitogen-activated protein kinase (MAPK)s and NF-kB signaling pathways possibly involved in the beneficial effects.
Materials and methods: The quantification of the extract was carried out by ultra-performance liquid chromatography analysis. Cell viability was determined using SRB assay. Nitric oxide (NO) production was analyzed by Griess method and intracellular reactive oxygen species (ROS) by fluorescence analysis. In addition, cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS) expression, MAPK activation and IkappaBalpha (IKBα) degradation, were determined by Western blot.
Results: After BFAC characterization, (+)-catechin was revealed as its major constituent. Both BFAC and (+)-catechin, exerted significant anti-oxidant and anti-inflammatory effects inhibiting LPS-induced intracellular ROS and NO production in peritoneal macrophages. Additionally, the extract but also its major component reduced pro-inflammatory proteins expression probably through c-Jun N-terminal kinase and p38 MAPK signaling pathways.
Conclusion: These data suggest that the beneficial effects of BFAC might be mediated, at least in part, by the presence of (+)-catechin. Conclusively our findings confirm the potential of *A. cochliacarpos* as a new therapeutic strategy for the management of inflammatory and oxidative stress-related diseases.

1. Introduction

Inflammation is a complex biological response initiated by several factors that result in cell injury or death contributing to the pathogenesis of many diseases including rheumatoid arthritis, chronic inflammatory bowel disease neurodegenerative disorders, and septic shock syndrome (O'Byrne and Dalgleish, 2001; Kaminska, 2005; Altavilla et al., 2009). It is characterized by the release of cytokines, chemokines, and growth factors and by the transmigration of inflammatory cells, such as monocytes from the blood to the affected tissue, where the differentiation into macrophages and dendritic cells (DCs) occurs, contributing to host defense, tissue remodeling and repair (Gordon and Taylor., 2005; Shi and Pamer, 2011).

Macrophages are major inflammatory and immune effector cells closely related to phagocytic cells that cooperate during the onset, progression and resolution of inflammation (Soehnlein and Lindbom, 2010). These cells are activated by exposure to interferon-γ, pro-inflammatory cytokines, and bacterial lipopolysaccharides (LPS) (Xie et al., 1993; Zhang and Ghosh, 2000). Activated macrophages play pivotal roles in inflammatory diseases via excess production of inflammatory mediators such as nitric oxide (NO) and prostaglandin E_2 (PGE_2), as well as pro-inflammatory cytokines, to promote inflammatory responses (Vane et al., 1994; Marks-Konczalik et al., 1998). In addition, inducible nitric oxide

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synthase (iNOS) and cyclooxygenase-2 (COX-2) are important enzymes that regulate inflammatory processes (Chang et al., 2011). Moreover, it has been established that gene expression of these pro-inflammatory mediators is well-regulated by mitogen-activated protein kinases (MAPKs) and nuclear transcription factor-kappa B (NF-kB) pathways (Barton and Medzhitov, 2003; Qi and Shelhamer, 2005). On the other hand, the activation of some of these transcription factors may also be influenced by elevated levels of reactive oxygen species (ROS) through a signaling loop (Liu et al., 2005; Yang et al., 2007; Torres and Forman, 2003).

Today, many natural plant products and synthetic compounds have been evaluated for their capacity to modulate inflammatory responses. Among the active constituents of extracts of medicinal plants, polyphenols compounds like flavonoids, are a family of substances whose members have many interesting biological properties, including anti-cancer, anti-microbial, anti-viral, anti-inflammatory, immunomodulatory, and anti-thrombotic activities (Havsteen, 2002).

Abarema cochliacarpos (Gomes) Barneby and Grimes, Fabaceae, is an ornamental tree and native medicinal species in to Brazil. It is popularly known as "barbatimão" or "babatenã". Wildly it appears in the Atlantic Forest and in the Caatinga (dryland) region of Northeastern Brazil, Cerrado and Campo Rupestre which hosts many different ethnic communities (Albuquerque et al., 2007; Watkinson, 2012). The species was previously listed as Vulnerable (World Conservation Monitoring Center, 1998), however, it is now known to be more widely distributed than previously thought, currently being least concern. However, the Caatinga and Savanna habitat where this species is found is threatened and declining due to agricultural expansion (Watkinson, 2012), being the main threats to this species (The Nature Conservancy, 2010).

The decoction of stem bark of *A. cochliacarpos* has been used as an herbal remedy in this same region for wound-healing, as an analgesic and anti-inflammatory (Silva et al., 2006; da Silva et al., 2009). Ethnopharmacological studies have demonstrated that *A. cochliacarpos* is used in traditional medicine for many diseases, including gastrointestinal diseases, through the daily ingestion of this decoction or the tincture, made by placing the bark in white wine or "cachaça" (Silva et al., 2006; Santos, 2008).

Previous studies have shown analgesic and healing effects in skin lesions and antiulcer effects in experimental alcohol gastric ulcer (Santos et al., 2007; Silva et al., 2006; da Silva et al., 2010a). More recently, the butanolic fraction of the methanolic extract was tested on acute and chronic experimental colitis model induced by trinitrobenzene sulfonic acid (TNBS) demonstrating important anti-inflammatory effects (da Silva et al., 2010b, 2011).

Phytochemical screening of this fraction by electrospray ionization (ESI)/MS has been also performed showing a high content of polyphenols. Its major constituents were catechins, flavonoid-type compounds, and the minor were its dimers and trimers. Catechins refer to monomers of flavanols with similar composition such as catechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate that are particularly abundant in steam bark of the species of Fabaceae family (Santos, 2002). The structure of catechins was confirmed by means of ^{13}C Nuclear Magnetic Resonance analysis characterization, showing that (+)-catechin is the major constituent (da Silva et al., 2010b).

The mechanisms by which catechins achieve their beneficial effects are still not entirely clear; however, there is mounting evidence that they likely work through a combination of both anti-oxidant effect and alteration of intracellular signaling (Fraga and Oteiza, 2011).

With this background the aim of this study was to deepen in the anti-inflammatory effect of butanolic fraction from *A. cochliacarpos* (BFAC) and its major compound, (+)-catechins, in LPS-stimulated murine peritoneal macrophages.

2. Material and methods

2.1. Plant material and preparation of the extract

Collection and description of the bark of *A. cochliacarpos* (Gomes) Barneby and Grimes, Fabaceae, as well as preparation of the extract is described by da Silva et al. (2010b)

2.2. Sample preparation for UPLC-MS

Extract powder (1 mg) from butanolic fraction was dissolved in MeOH (10 mL). The solution was filtered using a 0.22 μm filter and then diluted with HPLC–MeOH to 100 ppm.

2.3. Electrospray ionization-mass spectrometry fingerprint (ESI-MS)

BFAC was diluted in a solution containing 50% (v/v) chromatographic grade methanol (Tedia, Fairfield, OH, USA) and 50% (v/v) deionized water and 0.5% of ammonium hydroxide (Merck, Darmstadt, Germany). ESI-MS fingerprints in the negative ion mode of butanolic fraction were acquired and accumulated over 60 s, and spectra were scanned in a range between *m/z* 100 and 1000, using a Micromass-Waters Q-TOF mass spectrometer (Waters, Manchester, England). Capillary and cone voltages were set at 3.00 kV and 30.00 V, respectively, with a source temperature of 150 °C and desolvation temperature of 350 °C. The collision energy was 30 eV. ESI-MS was performed by direct infusion with typical flow rate of 10 μL min^{-1} using a syringe pump (Harvard Apparatus, MA, USA). MS/MS data and spectrums of the standard compounds were compared with spectrums of the extract for confirmation.

2.4. Ultra-performance liquid chromatography (UPLC) analysis

UPLC was performed using a Waters UPLC system. Chromatographic separation was carried out on an ACQUITY TQD (1.7 μm, 50 mm × 2.1 mm i.d.) (Waters Corporation, Milford, MA, USA). Mobile phase A was water containing 0.1% formic acid. Mobile phase B was Acetonitrile. The column temperature was ambient. The UPLC flow rate was 0.25 mL/min. A sample solution of 5 μL was injected into the UPLC system. A mobile phase gradient was used with the percentage of B in A varying as follows: initial concentration, 30% B; 7 min, 50% B; 10 min, 50% B, 13 min, 30% B.

2.5. Animals

Swiss mice (6 or 10 weeks) were obtained from Harlan Interfauna Ibérica (Barcelona, España) and maintained under constant conditions (temperature: 20–25 °C; humidity: 40–60%; light/dark cycle: 12 h). Experiments followed a protocol observed by the Animal Ethics Committee of the University of Seville. All experiments were in accordance with the recommendations of the European Union regarding animal experimentation (Directive of the European Counsel 86/609/EC).

2.6. Isolation and culture of peritoneal macrophages

Mice were injected intraperitoneally with 1 mL of sterile 10% thioglycollate medium (Scharlau®, Barcelona, Spain). Three days later macrophages were collected by PBS intraperitoneal lavage (Alleva et al., 2002). After centrifugation the cells were resuspended in RPMI 1640 medium (PAA®, Pasching, Austria) supplemented with 10% heat-inactivated fetal calf serum (FCS) (PAA®, Pasching, Austria), L-glutamine (2 mM), glucose (4.5 g/L), and HEPES buffer (10 mM), in the presence of 100 mg/mL streptomycin and 100 U/mL penicillin (PAA®, Pasching, Austria) and seeded in