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Anti-inflammatory effect of *Schinus terebinthifolius* Raddi hydroalcoholic extract on neutrophil migration in zymosan-induced arthritis

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ABSTRACT

Ethnopharmacological relevance: *Schinus terebinthifolius* is a species of plant from the Anacardiaceae family, which can be found in different regions of Brazil. *Schinus* is popularly known as aroeirinha, aroeira-vermelha, or Brazilian pepper. In folk medicine, *S. terebinthifolius* is used for several disorders, including inflammatory conditions, skin wounds, mucosal membrane ulcers, respiratory problems, gout, tumors, diarrhea and arthritis. According to chemical analyses, gallic acid, methyl gallate and pentagalloylglucose are the main components of hydroalcoholic extracts from *S. terebinthifolius* leaves. In the present study, we demonstrated the ability of a hydroalcoholic extract to inhibit cell migration in arthritis and investigated the mechanisms underlying this phenomenon.

Materials and methods: The anti-inflammatory effect of *S. terebinthifolius* hydroalcoholic leaf extract (ST-70) was investigated in a zymosan-induced experimental model of inflammation. Male Swiss and C57Bl/6 mice received zymosan (100 μg/cavity) via intra-thoracic (i.t.) or intra-articular (i.a.) injection after oral pre-treatment with ST-70. The direct action of ST-70 on neutrophils was evaluated via chemotaxis.

Results: ST-70 exhibited a dose-dependent effect in the pleurisy model. The median effective dose (ED_{50}) was 100 mg/kg, which inhibited 70% of neutrophil accumulation when compared with the control group. ST-70 reduced joint diameter and neutrophil influx for synovial tissues at 6 h and 24 h in zymosan-induced arthritis. Additionally, ST-70 inhibited synovial interleukin (IL)-6, IL-1β, keratinocyte-derived chemokine (CXCL1/KC) and Tumor Necrosis Factor (TNF)-α production at 6 h and CXCL1/KC and IL-1β production at 24 h. The direct activity of ST-70 on neutrophils was observed via the impairment of CXCL1/KC-induced chemotaxis in neutrophils. Oral administration of ST-70 did not induce gastric damage. Daily administration for twenty days did not kill any animals. In contrast, similar administrations of diclofenac induced gastric damage and killed all animals by the fifth day.

Conclusions: Our results demonstrated significant anti-inflammatory effects of ST-70, suggesting a putative use of this herb for the development of phytomedicines to treat inflammatory diseases, such as joint inflammation.

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1. Introduction

Schinus terebinthifolius Raddi (Anacardiaceae) is a native plant from South America. It has been used in folk medicine as teas, infusions or tinctures; as an anti-inflammatory, febrifuge, analgesic, and depurative agent; and to treat urogenital system illnesses (Medeiros et al., 2007). Through ethnopharmacological research, the gastroprotective properties of *S. terebinthifolius* are remarkably effective, especially in the treatment of gastritis and ulcers (Carlini et al., 2010). Previous reports have demonstrated that *S. terebinthifolius* extracts or fractions rich in polyphenols, display antioxidant, antibacterial, antifungal and anti-allergic activities in different experimental models (Cavalher-Machado et al., 2008; de Lima et al., 2006; Schmourlo et al., 2005; Velázquez et al., 2003). Despite its importance in popular medicine for the treatment of inflammatory disorders, few scientific studies have examined the biological activities and chemical composition of *S. terebinthifolius* extracts.

Inflammation is a complex physiological response that occurs in vascularized tissues in response to harmful stimuli, such as pathogens, damaged cells or irritants. The inflammatory process is coordinated by different chemical mediators that induce vasodilation, plasma leakage and leukocyte margination. However, when the inflammatory response becomes prolonged or chronic, the same process can become destructive and has been linked to a number of diseases. Chronic inflammation can result from a failure to eliminate harmful stimuli, an abnormal autoimmune response or the persistence of a low-intensity irritant that continually causes acute inflammation response (Medzhitov, 2010).

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease characterized by pathological changes, such as persistent synovitis, vascular proliferation, infiltration of inflammatory cells, and damage to cartilage and bone (Scott et al., 2010). A critical factor that contributes to joint damage is the excessive production of inflammatory mediators by resident or infiltrating inflammatory cells. Cytokines (TNF-α and IL-1β) and eicosanoids (leukotrienes and prostaglandins) are involved in the pathogenesis of arthritis and participate in pain, neutrophil accumulation and tissue damage (Brennan and McInnes, 2008; Guerrero et al., 2008). Recently, the importance of IL-17 has been studied in experimental arthritis, wherein the cytokine was detected during neutrophil accumulation and cartilage degradation and in hyperalgesic symptoms (Pinto et al., 2010). The recruitment of neutrophils contributes to the local production of cytokines and joint damage and appears to be important in the pathogenesis of human arthritis (Wright et al., 2014). In the last decade, the involvement of other cells, such as macrophages, synoviocytes, lymphocytes and mast cells, have been described, indicating that a wider variety of cells are also important in the perpetuating the articular inflammatory process (McInnes and Schett, 2011).

Current clinical treatments for RA include steroidal and non-steroidal anti-inflammatory drugs (SAIDs and NSAIDs, respectively), disease-modifying antirheumatic drugs (DMARDs) and biological agents (Kalden, 2002). However, the prolonged use of SAIDS and NSAIDS has been associated with serious adverse effects, including gastrointestinal disorders, immunodeficiency and humoral disturbances (Roth, 2012), which are factors that have been attributed to treatment dropout.

In recent decades, the screening of safer and more potent anti-inflammatory drugs for clinical use has increased. In this context, plants with anti-inflammatory activities have shown promising effects against inflammatory diseases, such as arthritis (Lama and Saikia, 2011). A few reports have shown that a polyphenol from green tea extract displayed a protective effect in a model of inflammatory arthritis, largely through its ability to inhibit the production of key inflammatory mediators, such as IL-1β and IL-6, by RA synovial fibroblasts (Ahmed et al., 2006).

Considering the popular uses of teas and tinctures for medicinal purposes, we evaluated the anti-inflammatory effect of hydroalcoholic extracts from *S. terebinthifolius* Raddi to assess its ability to inhibit cell migration and inflammatory mediators in experimental arthritis. Furthermore, we explored the mechanisms involved in this phenomenon.

2. Materials and methods

2.1. Reagents

Zymosan serotype A, dexamethasone, potassium diclofenac, phosphate buffered saline (PBS), buffer perborate, *o*-phenylenediamine dihydrochloride (OPD), Bradford reagent, bovine serum albumin (BSA), ethylene diamine tetraacetic acid disodium salt (EDTA), RPMI 1640 medium and fMLP (*N*-formyl-methionyl-leucyl-phenylalanine) were all obtained from Sigma Chemical Co. (St. Louis, MO, USA). DMSO (for biological tests), ethyl ether, ethyl acetate, *n*-hexane, dichloromethane, methanol and acetone for chromatography were purchased from Vetec Química Fina, Ltda. (Xerém, RJ, Brazil). LTB_4 immuno-assay kit was obtained from Cayman Chemicals (Ann Arbor, Michigan, USA). Purified anti-murine TNF-α, CXCL1/KC, IL-6 and IL-1β mAbs; biotinylated anti-TNF-α, CXCL-1/KC, IL-6 and IL-1β mAbs; and recombinant TNF-α, CXCL-1/KC, IL-6 and IL-1β were all obtained from R&D Systems (Minneapolis, MN, USA).

2.2. Preparation and analysis of ST-70 extract

Leaves were collected from 10 individual of *S. terebinthifolius* plants in the Atlantic Forest Campus FIOCRUZ, Jacarepaguá, Rio de Janeiro, RJ, Brazil, and a voucher specimen was deposited into the Rio de Janeiro Botanical Garden Herbarium under number RB-451742.

The collected material were dried at 40 °C in an oven with air circulation, reduced to small fragments and extracted with 70% ethanol in a dynamic maceration for 24 h. Then, the extract was filtered, concentrated under reduced pressure and lyophilized, resulting in a hydroethanolic extract (ST-70) with a yield of 11.00%. These conditions were based on previous studies of extraction times.

The ST-70 extract was analyzed using techniques such as adsorption column chromatography, thin layer chromatography, partition chromatography (countercurrent chromatography), gas chromatography coupled to mass spectrometry, high performance liquid chromatography (HPLC) and crystallization by traditional methodologies.

Several different methodologies were employed to isolate compounds from *S. terebinthifolius.* Spectrometric and spectroscopic analyses led to the identification of luteolin, quercetin, kaempferol, agathisflavone, gallic acid (GA), methyl gallate (MG), 1,2,3,4,6-pentagalloylglucose (PG), epicatechin, α-amyrin, β-amyrin, and lupeol.

The main secondary metabolite constituents in the extract, namely GA and MG, were assigned by HPLC analyses to the respective standard substances based on the similarities of their UV spectra on a diode array detector (DAD) at 220–400 nm and their retention times (6.6 min for GA and 15.0 min for MG). The HPLC chromatograms of ST-70 and the standards are shown in Fig. 1. The samples and the standard compounds were prepared in methanol through conventional dilution, and 20 μL was injected for analysis. A Supelcosil LC-18 column (250×4.6 mm^2, 5 μm) coupled to a Supelcosil LC-18 (4.0 mm × 20 mm, 5 μm) guard-column (Supelco, Bellefonte) was used. The following gradient program of the