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Original article

Anti-inflammatory activity of bartogenic acid containing fraction of fruits of *Barringtonia racemosa* Roxb. in acute and chronic animal models of inflammation

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ABSTRACT

The fruits of *Barringtonia racemosa* are traditionally used in Indian medicine for the treatment of pain and inflammatory conditions. In this study, a fraction of ethyl acetate extract of fruits of *B. racemosa* (BREAF) was investigated for anti-inflammatory activity in experimental models of acute and chronic inflammation. Activity against acute inflammation was evaluated in inflammogens induced rat paw edema models. Whereas, effect in chronic inflammation was evaluated in cotton pellet granuloma and oxazolone induced delayed type hypersensitivity (DTH) model in mice. The BREAF exhibited dose dependent anti-inflammatory activity in both acute and chronic models at oral doses of 5, 10 and 20 mg/kg. BREAF inhibited both phases of carrageenan induced rat paw inflammation. The reduction in paw inflammation by BREAF was also evident in histamine and serotonin induced inflammation in rats. Effect of BREAF on DTH indicates inhibition of immune mediated inflammation. The reduction in cotton pellet granuloma by BREAF treatment shows inhibition of proliferative changes associated with chronic inflammation. Analysis of BREAF after chromatographic separations showed presence of bartogenic acid as a major constituent. Hence, it is proposed that anti-inflammatory effects of BREAF can be partially attributed to its bartogenic acid content. The minute doses at which this fraction shows anti-inflammatory effects emphasizes the need for further investigations on its efficacy in the immuno-inflammatory conditions.

1. Introduction

Inflammatory diseases are globally identified cause of morbidity among the population.[1] Inflammation is a natural protective response of the body to tissue injury caused by chemical, mechanical or thermal stimuli, trauma, microbial agents or autoimmune diseases.[1–3] Acute inflammatory response keeps the integrity of organisms through activation of immune cells.[4] Inflammatory response is a complex process mediated through variety of cellular pathways and activated complement factors. Although, acute inflammation is a protective response of the body, if unresolved, it leads to painful conditions, like rheumatoid arthritis, inflammatory bowel diseases, asthma, allergy, atherosclerosis, immune-inflammatory ailments and even neoplastic transformation.[5–7] Thus, persistent inflammation is vital factor in the development and progression of chronic diseases.[8]

Steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are globally practiced for the treatment of acute inflammatory disorders. However, their use is either associated with adverse effects or they are ineffective in the treatment of chronic inflammatory disorders, including rheumatoid arthritis.[1,9,10] The long term use of NSAIDs is associated with gastric ulcer, bleeding, renal dysfunction, kidney damage, bronchospasm, cardiac abnormalities, bone marrow depression, retention of salts and water etc.[3,9,11,12] Therefore, it is a clinical necessity to recognize the more efficacious and safer drugs for the prevention and treatment of

Abbreviations: BREAF, *Barringtonia racemosa* ethyl acetate fraction; DTH, Delayed type hypersensitivity; p.o., Per oral.

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inflammatory diseases.[12,13] In contrast to the limitations of NSAIDs, natural products have a more favorable pharmacological profile accompanied by lower toxicities. Additionally, natural products are biocompatible and cost effective alternatives for the treatment of various inflammatory diseases.[14–16] Anti-inflammatory drug discovery from plants is the most productive and rational strategy for the identification of novel drug candidates.[9] India has a great legacy of various medicinal plants which are useful as alternative medicines against various diseases. There is a great opportunity to develop novel anti-inflammatory drugs through the integration of traditional knowledge and indigenous resources.[1] Earlier evidence suggested that plant derived natural products exert their anti-inflammatory effects through the modulation of key inflammatory mediators, effects on pro-inflammatory molecule expression like cyclooxygenase (COX), nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-10 (IL-10) and other cytokines.[12,17]

The various parts of *Barringtonia racemosa (B. racemosa)* are known to possess multiple biological activities.[18] Extracts prepared from different parts of *B. racemosa* possess analgesic, antitumor and antimicrobial activities.[19–21] The aqueous bark extract of *B. racemosa* exerted significant and dose-dependent antinociceptive activity in experimental animals. This activity is attributed to the presence of opioids or opiodiomimetics as well as phenolics and steroidal constituents in *B. racemosa*.[22] Anti-oxidant and anti-inflammatory effects of *B. racemosa* leaves are attributed to its lycopene content.[23] This extract exerted *in vitro* nitric oxide synthase inhibitory and antioxidant activity in RAW cells.[23] Methanolic, ethanolic and boiling water extracts of *B. racemosa* leaves, sticks and barks at the concentration of 50 mg/mL were found to possess antifungal activity against *Fusarium* sp., *Tricoderma koningii*, *Penicillium* sp., *Ganoderma tropicum*, *Ganoderma lucidum*, *Aspergillus* sp. and *Rhizopus* sp.[24] The extracts obtained from the aerial parts of this plant demonstrated *in vitro* antioxidant activity.[25] *B. racemosa* leaves demonstrated higher antioxidant activities than the stems, owing to its antioxidant content. This plant is proposed as a potential source of natural antioxidants.[26] Previously, we reported the protective effects of bartogenic acid isolated from the fruits of *B. racemosa* in complete Freund's adjuvant induced arthritis model in rats.[27] Present study aimed to evaluate the anti-inflammatory activity of most active fraction of *B. racemosa* fruits in different models of acute and chronic inflammation. Recent review summarized the pharmacological activities of *B. racemosa*.[28] The crude ethanolic extract of *B. racemosa* (125, 250 and 500 mg/kg, p.o.) was evaluated in carrageenan and formalin induced paw edema model.[29] Another study reported the antioxidant activity of crude ethanolic bark extract of *B. racemosa* in DPPH assay, analgesic activity in acetic acid induced writhing model, anti-diarrheal activity in castor oil induced diarrhea model and antibacterial activity by disc diffusion assay. The extract was tested *in-vivo* at the oral doses of 250 and 500 mg/kg.[30] Although, these earlier reports highlighted the anti-inflammatory potential of *B. racemosa*, these studies were either performed using the crude extracts at higher doses or restricted to acute model of inflammation. As per our understanding, there is no systematic study reporting the *in-vivo* efficacy of characterized *B. racemosa* fraction in animal models of acute, chronic and immune inflammation.

The present study involved anti-inflammatory evaluation of fraction, isolated from an ethyl acetate extract of *B. racemosa* fruits (BREAF), at the lower oral doses of 5, 10 and 20 mg/kg in experimental models of acute and chronic inflammation. Acute anti-inflammatory activity was studied in carrageenan, histamine and serotonin induced paw edema models. The cotton pellet assay and oxazolone induced contact dermatitis model were executed to study the effects of BREAF in chronic inflammation and delayed type hypersensitivity respectively.

2. Materials and methods

2.1. Chemicals and biochemicals

Drug sample of diclofenac sodium (purity > 98%) was obtained as a gift from Kirti Pharmachem, Nasik, India. Serotonin (RM 1825-1G; purity ≥ 98%) and dexamethasone (RM 4185-1G; purity > 98%) were purchased from Himedia Laboratories Pvt. Ltd. Mumbai, India. Carrageenan (λ) (C-3889-5G), histamine (H-7375; purity > 98%), 4-ethoxymethylene-2-phenyloxazolone (oxazolone, E-0753-1G; purity ≥ 90%) and cyproheptidine (C-6022; purity ≥ 98%) were purchased from Sigma Aldrich, USA. Other chemicals and solvents used in the extraction, fractionation and chromatographic separations were of analytical grade.

2.2. Plant material

Fruits of *B. racemosa* Roxb. (Lecythidaceae), collected from the sea cost of Konkan, Maharashtra were purchased from the local vendor. The specimen was authenticated by the experts of Botanical Survey of India, Pune, India and the specimen was deposited (sample reference: voucher no. 74843). An authentic marker of bartogenic acid was generously supplied by Dr. Mangala Gowri, Senior Scientist, IICT, Hyderabad, India.

2.3. Bioassay guided extraction and isolation of BREAF

The BREAF was isolated according to the procedure described by Gowri *et al*[31] with some modifications. Dried fruits of *B. racemosa* were coarsely powdered and 500 g of material was defatted with petroleum ether (60–80°, 3 × 1 L), followed by extraction with methanol (3 × 1.5 L, each time) at room temperature for 3 days. For efficient extraction, the earlier solvent was replaced by an equal amount of fresh solvent at the end of each day. After filtration and concentration under reduced pressure, this process yielded 32.3 g of methanol extract. This extract was subjected to evaluation of anti-inflammatory activity in carrageenan induced rat paw edema model where it showed anti-inflammatory activity. Furthermore, methanol extract (15 g) was fractionated into n-butanol (6.2 g) and ethyl acetate (5.6 g) fractions and evaluated for the activity as mentioned earlier. Ethyl acetate extract displayed higher anti-inflammatory potency than methanol or n-butanol extracts. Hence, ethyl acetate extract (5 g) was subjected to further fractionation on silica gel column (3 × 90 cm, flow rate 2 mL/min), using ethyl acetate (1.4 L). Fractions of 100 mL were collected. Elution was monitored by TLC runs of each fraction using methanol as a solvent. The developed TLC plates were sprayed with p-anisaldehyde-sulfuric acid followed by heating at 110 °C for visualization. The fractions 8–15 were combined to get a triterpenoids enriched fraction called BREAF (0.9 g). BREAF was further methodically investigated for detailed anti-inflammatory activity. Part of this fraction (BREAF, 0.3 g) was subjected to chromatography on pre-coated reverse phase silica gel plate (Merck), using a mixture of acetonitrile: water (85:15, v/v) as eluent. High performance thin layer chromatography (HPTLC) of fraction revealed three spots, comprising of one intense blue spot having $R_f = 0.68$ resembling to bartogenic acid marker. To identify the composition and purity of BREAF, it was subjected to LC-ESI/MS analysis.

2.4. Experimental animals and drug administration

Healthy male Wistar rats (150–200 g) and male C57BL/6 mice (25–30 g) were used in the present study. Animals were maintained in polypropylene cages at 22 ± 2 °C with free access to food (commercially available standard pellet feed – Amrut Laboratory

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