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Efficacy of boswellic acid on lysosomal acid hydrolases, lipid peroxidation and anti-oxidant status in gouty arthritic mice

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ABSTRACT

Objective: To evaluate the efficacy of boswellic acid against monosodium urate crystal-induced inflammation in mice. **Methods:** The mice were divided into four experimental groups. Group I served as control; mice in group II were injected with monosodium urate crystal; group III consisted of monosodium urate crystal-induced mice who were treated with boswellic acid (30 mg/kg/b.w.); group IV comprised monosodium urate crystal-induced mice who were treated with indomethacin (3 mg/kg/b.w.). Paw volume and levels/activities of lysosomal enzymes, lipid peroxidation, anti-oxidant status and inflammatory mediator TNF-α were determined in control and monosodium urate crystal-induced mice. In addition, the levels of β-glucuronidase and lactate dehydrogenase were also measured in monosodium urate crystal-incubated polymorphonuclear leucocytes (PMNL) *in vitro*. **Results:** The activities of lysosomal enzymes, lipid peroxidation, and tumour necrosis factor-α levels and paw volume were increased significantly in monosodium urate crystal-induced mice, whereas the activities of antioxidant status were in turn decreased. However, these changes were modulated to near normal levels upon boswellic acid administration. *In vitro*, boswellic acid reduced the level of β-glucuronidase and lactate dehydrogenase in monosodium urate crystal-incubated PMNL in concentration dependent manner when compared with control cells. **Conclusions:** The results obtained in this study further strengthen the anti-inflammatory/antiarthritic effect of boswellic acid, which was already well established by several investigators.

1. Introduction

Gouty arthritis is a metabolic disease manifestated by an increase in serum urate concentration and deposits of monosodium urate crystals with intense pain, reddening and swelling in joints[1]. Gouty arthritis is the most common form of arthritis seen in general practice in adults, with a prevalence of about 1.4%. The deposition of monosodium urate ctystals in synovium and cartilage can promote chronic inflammation that may lead to the damage to bone, cartilage, and other joint tissues such as tophaceous destruction or degenerative joint disease[2]. The primary pathologic hallmark of gout is neutrophil influx into the joint fluid. Neutrophils accumulate in both the joint fluid and the synovial membrane, where a small fraction of these cells actively phagocytose monosodium urate crystals resulting in membranolysis, generation of oxygen derived free radicals and the release of lysosomal enzymes, prostaglandin E2, leukotrienes and interleukin-1[3]. Gout has become more common and more clinically complex in recent years, particularly in older subjects. In addition, men with gout have an increased risk of death as a result of an elevated risk of cardiovascular diseases, particularly coronary heart diseases[4]. Nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids and colchicine have been used for treatment of crystal-induced inflammation for many years. However, although these agents are generally effective, they also present serious side effects such as gastrointestinal toxicity, renal toxicity, or gastrointestinal bleeding.

Recently, herbal medicines have been receiving attention as an alternative medicine and health supplement. Crude drugs prepared from plant materials are traditionally used and their active principles have been extensively studied from various view points. In particular, it was reported that boswellic acid, a mixture of triterpenic acids obtained from the oleo gum resin of *Boswellia serrata* has extensively been studied for a number of activities including anti-

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inflammatory, immunomodulatory, anti-tumor activities, and inflammatory bowel disease. It also belongs to a non-steroidal anti-inflammatory class of drugs with a different mechanism of action rather than those of the common NSAIDs[5]. Its anti-inflammatory properties were proved by inhibiting 5-lipoxygenase, human leukocyte elastase and the nuclear factor-_B pathway, without exerting the adverse effects known for steroids[6]. Among the six most important derivatives of boswellic acids, KBA and AKBA are the most potent inhibitors of 5-lipoxygenase[7]. Even though, several investigators reported the anti-inflammatory effect of boswellic acids. The scientific data supporting the use of boswellic acid in gouty arthritis are not available, therefore the present study was aimed to evaluate the efficacy of boswellic acid on monosodium urate crystal inflammation in mice which is an experimental model for gouty arthritis.

2. Materials and methods

2.1. Animals

Swiss albino mice, (25-30 g), of either sex were obtained from Tamil Nadu Veterinary College, Chennai, India. They were acclimatized for a week in a light and temperature -controlled room with a 12 h dark-light cycle and fed with commercial pelleted feed from Hindustan Lever Ltd. (Mumbai, India) and water was freely available. The animals were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, Ministry of Culture, Chennai. The experimental protocol was approved by our departmental ethics committee.

2.2. Drugs

The commercially available boswellic acid (pentacyclic triterpenoid acid mixture ($\alpha+\beta$) isolated from gum resin of *Boswellia serrata* Roxb., Family: Burseraceae, a fine white crystalline powder, >95% purity by HPLC, Lot no: T7P011) was purchased from Natural Remedies Ltd., Bangalore, India and stored at -20 ℃. Indomethacin was purchased from Tamil Nadu Dadha Pharmaceuticals Ltd., Chennai, India. A homogenous suspension of boswellic acid and indomethacin was made with 0.5% carboxy methyl cellulose in phosphate buffered saline. Fresh solution was prepared before each experiment. All other reagents used were standard laboratory reagents of analytical grade and were purchased locally.

2.2.1. Dosage

Based on our preliminary studies with different dosages (10 mg, 20 mg, 30 mg) of this boswellic acid, it was found that 30 mg/kg b.w. dosage produced significant anti-inflammatory effect by reducing paw swelling in monosodium urate crystal-induced animals. Hence, 30 mg/kg b.w. dosage was considered for this study. The dosage of standard drug indomethacin (3 mg/kg b.w.) used in this study was selected based on our previous reports[8,9].

2.3. Synthesis of monosodium urate crystals

About 4 g of uric acid was dissolved and heated in 800 mL H_2O with NaOH (9 mL/0.5 N), adjusted to pH 8.9 at 60 ℃; cooled over night in a cold room; washed and dried. Needle-like crystals were recovered and were suspended in sterile saline (20 mg/mL)[8].

2.4. Monosodium urate crystal-induced inflammation in mice

The mice were divided into four groups with six animals in each group. Group I served as a control group. In group II, inflammation was induced by intradermal injection of 0.2 mL (4 mg) of monosodium urate crystal suspension into the right foot pad[8]. Group III comprised monosodium crystal-induced mice who were treated with boswellic acid (30 mg/kg b.w., i.p.) and group IV consisted of monosodium crystal-induced mice who were treated with indomethacin (3 mg/kg b.w., i.p.). Boswellic acid and indomethacin were suspended in 0.5% carboxy methyl cellulose in phosphate buffered saline and administered intraperitoneally, 1 h before the monosodium urate crystal injection and which was repeated for 3 more days on a daily basis.

2.4.1. Assessment of inflammation

The inflammation was quantified by measuring the thickness of the paw with a vernier scale at different intervals for 3 days. At the end of the experimental period (72 h), the mice were killed by cervical decapitation. Blood from each animal was collected for serum separation. The liver and spleen were immediately dissected out and homogenized in ice-cold (0.01 M), Tris HCL buffer (pH 7.4) to give a 10% homogenate. The tissue homogenate of spleen, liver and serum was used for assaying the lysosomal enzymes, lipid peroxidation, antioxidant status and inflammatory mediator tumour necrosis factor- α.

2.4.2. Effect of boswellic acid and indomethacin on lysosomal enzymes

The activity of acid phosphatase was assayed by the method of King[10]. β-glucuronidase was determined by the method of Kawai and Anno[11] and β-galactosidase by the method of Rosenblit[12]. The method of Marhun[13] was followed for the determination of N-acetyl glucosaminidase and the protein content was measured by the method of Lowry *et al*[14].

2.4.3. Effect of boswellic acid and indomethacin on lipid peroxidation and antioxidant status

Lipid peroxidation in plasma was estimated by the method of Ledwozy *et al*[15]. Spleen and liver lipid peroxidation was carried out by the procedure of Hogberg *et al*[16] using thiobarbituric acid as the colouring agent. Malonaldehyde (MDA) produced during peroxidation of lipids served as an index of lipid peroxidation. MDA reacts with TBA to generate a colour product, which absorbs at 532 nm.

Superoxide dismutase (SOD) activity in spleen and liver was determined by the method of Marklund and Marklund[17]. The degree of inhibition of the auto-oxidation