

Immunomodulatory activity of boswellic acids of *Boswellia serrata* Roxb.

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Received 5 January 2003; revised 30 July 2003

Extract of gum resin of *B. serrata* containing 60% acetyl 11-keto beta boswellic acid (AKBA) along with other constituents such as 11-keto β -boswellic acid (KBA), acetyl β -boswellic acid and β -boswellic acid has been evaluated for antianaphylactic and mast cell stabilizing activity using passive paw anaphylaxis and compound 48/80 induced degranulation of mast cell methods. The extract inhibited the passive paw anaphylaxis reaction in rats in dose-dependant manner (20, 40 and 80 mg/kg, po). However, the standard dexamethasone (0.27 mg/kg, po) revealed maximum inhibition of edema as compared to the extract. A significant inhibition in the compound 48/80 induced degranulation of mast cells in dose-dependant manner (20, 40 and 80 mg/kg, po) was observed thus showing mast cell stabilizing activity. The standard disodium cromoglycate (50 mg/kg, ip) was found to demonstrate maximum per cent protection against degranulation as compared to the extract containing 60% AKBA. The results suggest promising antianaphylactic and mast cell stabilizing activity of the extract.

Keywords: Anti-anaphylactic activity, Boswellic acid, *Boswellia serrata*, Mast cell stabilizing activity

Boswellia serrata Roxb. (Family: Burseraceae) is a traditionally useful plant for the treatment of allergic disorders such as asthma and bronchitis¹. Contemporary studies have revealed acetyl 11-keto beta boswellic acid from *B. serrata* to inhibit the 5-lipoxygenase product formation from endogenous arachidonic acid in intact neutrophil and from exogenous substrate supernatants and highly enriched 5-lipoxygenase preparations². Mixed acetyl boswellic acids, pentacyclic triterpenes have been shown to inhibit the leukotriene (LT) B₄ and C₄ from intact human polymorphonuclear neutrophil leukocyte (PMNL)³. Recently it has been shown that acetyl 11-keto beta boswellic acid inhibited leukotriene biosynthesis by impairing the 5-lipoxygenase activity⁴. Additionally, acetyl 11-keto beta boswellic acid has been demonstrated to inhibit the enzyme human leukocyte elastase which might be the rationale for the putative anti-phlogistic activity of acetyl 11-keto beta boswellic acid⁵.

Allergic asthma is a chronic inflammatory process occurring due to exposure of inhalant allergen resulting in the activation of T-lymphocytes with subsequent cascade leading to release of cytokines

and inflammatory mediators such as histamine, leukotrienes LTC₄, LTD₄ and LTE₄⁶. In the classic immunologic model, asthma is a disease mediated by reaginic (IgE) antibodies bound to mast cells in the airway mucosa. On reexposure to an antigen, antigen antibody interaction takes place on the surface of mast cells, triggering mast cell degranulation; the central event of an allergic reaction^{7,8}.

The present study was undertaken to evaluate the immunomodulatory activity of acetyl 11-keto-beta boswellic acid, extracted from the gum resin of the plant *Boswellia serrata* against allergen (egg albumin) induced passive paw anaphylaxis and compound 48/80 induced degranulation of mast cells in order to verify the traditional claim.

Herbal preparation—Extract of the gum resin of the plant *Boswellia serrata* containing 60% acetyl 11-keto beta boswellic acid (AKBA) along with other constituents such as 11-keto β -boswellic acid (KBA), acetyl β -boswellic acid and β -boswellic acid was supplied by Ajanta Pharma Pvt. Ltd., India. Suspension of the extract was prepared in 0.1% (w/v) sodium carboxymethyl cellulose and used for the study.

Animals—Wistar albino rats (150-300 g) were housed in standard conditions of temperature (22° ± 2°C), relative humidity (60 ± 5%) and light (12 hr

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light/dark cycle) and allowed free access to food and water *ad libitum*.

Chemicals and drugs—Chemicals used were compound 48/80 (Sigma Aldrich Foreign Holder Company, Bangalore, India), egg albumin (Hi Media Laboratories, India). Dexamethasone (Cadila Healthcare Ltd., India) and disodium cromoglycate (Cipla Ltd., India) were used as reference drugs.

Effect on passive paw anaphylaxis⁴—Antiserum to egg albumin was raised in rats (150-200 g) using aluminum hydroxide gel as an adjuvant. Animals were given (sc) three doses of 100 µg of egg albumin adsorbed on 12 mg of aluminum hydroxide gel prepared in 0.5 ml of saline on 1st, 3rd and 5th day. On 10th day of sensitization, the animals were bled from the orbital plexus. The collected blood was allowed to clot and the serum was separated by centrifugation at 1500 rpm.

Animals were divided into five groups each comprising six animals. Animals belonging to group I served as control and were administered only the vehicle. Animals belonging to groups II, III and IV received the extract orally at doses of 20, 40 and 80 mg/kg respectively and group V received the standard drug dexamethasone, 0.27 mg/kg. Rats were passively sensitized into the left hind paw with 0.1ml of the undiluted serum. The contralateral paw received an equal volume of saline. Test drugs were administered 24 hr after sensitization. After 1 hr of drug administration, the rats were challenged in the left hind paw with 10 µg of egg albumin in 0.1ml saline. The hind paw was measured using a micrometer. The difference in the reading prior to, and after antigen challenge represented the edema volume and per cent inhibition of edema was calculated⁹.

Effect on mast cell degranulation¹⁰—Rats (250-300 g) were divided into five groups of six animals in each group. Rats in group I received the vehicle and served as control. Groups II, III and IV rats were administered (po) the extract in 3 doses: 20, 40 and 80 mg/kg. Group V received the reference drug disodium cromoglycate: (50 mg/kg, ip). Drugs were administered 1 hr before separation of mast cells. One-hour post administration of drugs, normal saline (10 ml) was injected into peritoneal cavity of rats. After gentle massage, the peritoneal fluid was collected and transferred into siliconised test tubes containing 7-10 ml RPMI 1640 medium (pH 7.2-7.4). Mast cells were then washed thrice by centrifugation at a low speed (400-500 rpm) by discarding the supernatant and taking the pellet of mast cells into the

medium. These cells were challenged with compound 48/80 (0.5 µg /ml), incubated at 37°C in a water bath for 10 min and then stained with 1% toluidine blue and observed under microscope and per cent protection against degranulation was calculated.

Statistical analysis¹¹—Statistical analysis was performed using Dunnett's 't' test followed by Student's 't' test.

Passive paw anaphylaxis—The extract at doses of 20, 40 and 80 mg/kg per oral produced dose-dependant inhibition of edema of about 7.86, 16.84 and 26.96% respectively (Table 1). However, dexamethasone (0.27 mg/kg) produced maximum inhibition of edema of about 59.55%.

Mast cell degranulation—The extract at a dose of 20 mg/kg failed to offer any protection. However, 40 and 80 mg/kg doses exhibited significant dose-dependant inhibition of mast cell degranulation, thus demonstrating mast cell stabilizing activity. However, Disodium cromoglycate provided maximum protection (63%; Table 2).

The present findings reveal the plant extract to exert protective effect against allergen-induced passive paw anaphylaxis and compound 48/80 induced degranulation of mast cells. Thus, the antigen and antibody reaction taking place on the surface of mast cells leading to the release of mediators⁷ seem to have been modulated. Degranulated mast cells release

Table 1—Effect of boswellic acids on passive paw anaphylaxis in rats

[Values are mean ±SE of 6 animals in each group]

Group	Treatment	Dose (mg/kg; po)	Edema at 30 min	% Inhibition of edema at 30 min
I	Control	—	0.89 ± 0.034	—
II	Boswellic acids	20	0.82 ± 0.31	7.86*
III	Boswellic acids	40	0.74 ± 0.44	16.85**
IV	Boswellic acids	80	0.65 ± 0.57	26.96**
V	Dexamethasone	0.27	0.36 ± 0.005	59.55**

**P < 0.01; *P < 0.02

Table 2—Effect of boswellic acids on mast cell degranulation induced by Compound 48/80

[Values are mean ±SE of 6 observations]

Group	Treatment	Dose (mg/kg, po)	% Protection of mast cells
I	Control	—	—
II	Boswellic acids	20	0
III	Boswellic acids	40	20.16 ± 0.83*
IV	Boswellic acids	80	36.00 ± 0.85*
V	Disodium cromoglycate	50 (ip)	62.66 ± 0.80*

*P < 0.01

a number of mediators like leukotrienes, platelet-activating factor, eosinophilic chemotactic factor and eosinophil-derived neurotoxin⁴. The prevention of degranulation process by the extract indicates a possible stabilizing effect on the biomembrane of mast cells⁹. This justifies the rationale behind the use of the plant *Boswellia serrata* in the treatment of asthma known to have an immunological basis. The study paves way for further evaluation of the potential of acetyl 11-keto beta boswellic acid and other boswellic acids for allergic disorders known to involve immune-mediated reactions. Allergic immunological mechanisms have revealed up regulation of cytokines such as IL-4 and IL-5 and more so IL-5 which is selective growth factor for terminal and activation of eosinophils seen in allergic asthma¹². These cytokines serve as molecular markers which can be considered for further study.

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