



Immunological adjuvant effect of *Boswellia serrata* (BOS 2000) on specific antibody and cellular response to ovalbumin in mice

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ABSTRACT

In this study, the biopolymeric fraction BOS 2000 from *Boswellia serrata* was evaluated for its potential ability as adjuvants on the immune responses to ovalbumin (OVA) in mice. Balb/c mice were immunized subcutaneously with OVA 100 µg alone or with OVA 100 µg dissolved in saline containing alum (200 µg) or BOS 2000 (10, 20, 40 and 80 µg) on Days 1 and 15. Two weeks later, OVA specific antibodies in serum; concanavalin A (Con A), OVA stimulated splenocyte proliferation, CD4/CD8/CD80/CD86 analysis in spleen cells and its estimation of cytokines (IL-2 and IFN gamma) from cell culture supernatant were measured. OVA specific IgG, IgG1 and IgG2a antibody levels in serum were significantly enhanced by BOS 2000 (80 µg) compared with OVA control group. Moreover, the adjuvant effect of BOS 2000 (80 µg) on the OVA-specific IgG, IgG1, and IgG2a antibody responses to OVA in mice were more significant than those of alum. BOS 2000 significantly enhanced the Con A and OVA induced splenocyte proliferation in the OVA immunized mice especially at a dose of 80 µg ($p < 0.001$). However, no significant differences were observed among the OVA group and OVA/alum group. At a dose of 80 µg ($p < 0.001$), there was a significant increase in the CD4/CD8 and CD80/CD86 analysis in spleen cells and cytokine (IL-2 and IFN-gamma) profile in the spleen cell culture supernatant was observed. In conclusion, BOS 2000 seems to be a promising balanced Th1 and Th2 directing immunological adjuvants which can enhance the immunogenicity of vaccine.

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1. Introduction

Development of newer vaccines include highly purified subunit antigens that are weakly immunogenic in general. Vaccine formulations often require adjuvants for increased immunological efficiency and better vaccination schedules [1,2]. Adjuvants are used for improving the specific immune response to vaccine antigens and for induction of immunological memory [3]. The addition of adjuvants to vaccine formulations makes it possible to reduce the amount of antigen and the number of immunizations needed, while improving the magnitude and the duration of the specific immune response. Currently used adjuvants include aluminum hydroxide, aluminum phosphate, calcium phosphate, water-in-oil emulsions, products from bacteria and liposomes. Often there is compromise on level of adjuvanticity and acceptable level of safety. Other adjuvants such as mono-phosphoryl lipid A, ISCOMS, QS-21—a purified saponin from bark of *Quillaja saponaria* Molina and Syntex Adjuvant formulation (SAF) are being investigated for development of better and safer adjuvants [2]. Plant based immunomodulators are being considered as one option [4–8]. Previously we have reported various immunomodulators of Ayurvedic origin. The extracts and formulations prepared

from Ayurvedic medicinal plants including *Withania somnifera*, *Tinospora cordifolia* and *Asparagus racemosus* demonstrated significant immunostimulatory activity particularly at humoral level in experimental systems with or without induced immunosuppression [9–12]. Attempts to organize adjuvants in grouped categories in order to facilitate adjuvant selection have sometimes been difficult because of multiple and overlapping biological effects of many adjuvants. However, this approach can be useful for providing a prospective on the types of immunostimulators that are available.

Recently, substances for enhancing host defense responses were isolated from microorganisms, fungi [13] and plants [14,15]. Plant-derived polysaccharides are the best known and most potent immunomodulatory substances [14] and have been shown to be clinically therapeutic [16]. Their chemical properties and biological activities have been studied extensively. Plant polysaccharides have been shown to exhibit anti-inflammatory [17], anti-hypoglycemic [18], anti-bacterial [19] and anti-tumor [20] and anti-complementary activities. Indeed, the basic mechanism of the immunostimulatory, anti-tumor, bactericidal and other therapeutic effects of botanical polysaccharides are thought to occur via macrophage stimulation and modulation of the complement system [21]. Furthermore, modulation of these systems can significantly impact both humoral and cellular immune responses [22].

We propose that BOS 2000 from *Boswellia serrata* has the potential of being developed as a potent plant based immune adjuvant. BOS

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2000, isolated from *B. serrata* having anti-arthritic [23], anti-inflammatory [24–26], immunomodulatory [27] and anticancer activity [28–30] was considered for adjuvant testing in mice. A better understanding of the biology of non-conventional T cell subpopulations, T and B cell memory, regulatory T cells and mucosal immunity has profound implications for a modern approach to adjuvant screening and development. The future lies in the high throughput screening of synthetic chemical entities targeting well-characterized biological molecules. Used alone or in combination with such adjuvants will allow stimulation or modulation in a safe and efficient manner of strong effector, regulatory and memory immune mechanisms. The present study was undertaken to validate the immunoadjuvant effects of BOS 2000 against weak antigen ovalbumin.

2. Materials and methods

2.1. Chemicals and reagents

The organic solvent exhausted material (0.5 kg) of the plant *B. serrata* was used in this study. Ammonium chloride, potassium bicarbonate, o-phenylenediamine, hydrogen peroxide (H_2O_2), citrate-phosphate buffer and disodium ethylene diamine tetraacetic acid (EDTA) were purchased from Merck, India. Medium RPMI 1640 (Himedia, Mumbai, India), 96-V-well microtitration plates and microtissue culture plates (96-U-wells) from Tarson, trypan blue (Microlabs, Mumbai), fetal calf serum (FCS), lipopolysaccharide (LPS, *Escherichia coli* 055 B5), dimethylsulphoxide (DMSO), bovine serum albumin (BSA), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl 2,5-dimethyltetrazolium bromide (MTT), Hank's balanced salt solution (HBSS), HEPES, 2-mercaptoethanol, penicillin, streptomycin, streptavidin-horse radish peroxidase, rabbit anti-mouse IgG peroxidase conjugate, goat anti-mouse IgG1 and IgG2a peroxidase conjugate (Sigma), fluorescein isothiocyanate (FITC)-labeled anti-CD4/CD80 (B7-1) and phycoerythrin (PE) labeled anti-CD8/CD86 (B7-2) mAbs (BD Biosciences) were used.

2.2. Preparation of biopolymeric fraction BOS 2000

The organic solvent exhausted material (0.5 kg) of the plant *B. serrata* was air-dried and extracted twice with water using 700 ml of water for the first extraction and 200 ml for the subsequent extractions. The combined aqueous extracts were clarified by centrifugation and to the clear solution (600 ml) alcohol (1.2 l) was added. The light brown solid, which separated on keeping, was collected by filtration and purified by dissolving in water and precipitating with alcohol. The purification process was repeated once more to get the biopolymeric fraction BOS 2000 (Fig. 1) as white solid (70 g).

2.3. Hydrolysis of biopolymeric fraction (BOS 2000)

The biopolymeric fraction BOS 2000 (1.0 g) was suspended in 50 ml of aqueous 2 N-TFA and then refluxed (120 °C) for 2.5 h. The reaction mixture was concentrated under reduced pressure on a film evaporator and then kept in a desiccator containing NaOH, overnight. Paper chromatography of the hydrolyzed fraction BOS 2000 in comparison with reference monosaccharides revealed the presence of arabinose, glucose and galactose.

2.4. Quantitative analysis of monosaccharides in the hydrolyzed BOS 2000 by HPLC

HPLC grade water was prepared from Milli-Q water purification system. All the four monosaccharides, i.e. D-glucose, D-xylose, D-galactose and D-arabinose were procured from Aldrich chemicals of purity $\leq 98\%$ (HPLC).

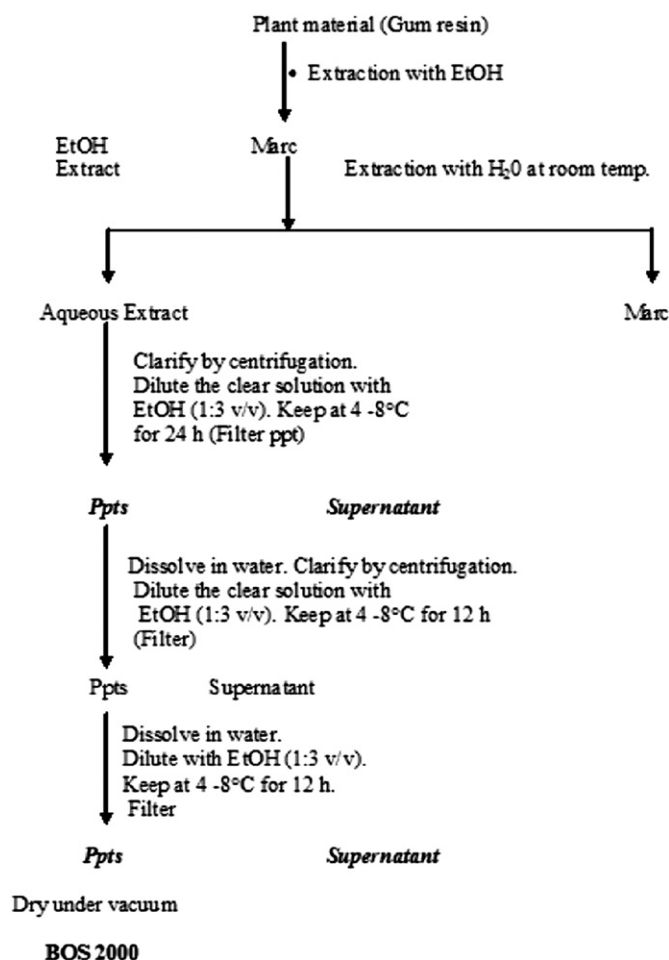


Fig. 1. Flowsheet of biopolymeric fraction BOS 2000 isolated from *Boswellia serrata*.

2.5. Chromatography

Monosaccharides were separated and quantified by using Shimadzu HPLC system consisting of Pump LC-10 ATVP, an automatic sampling unit (Autosampler), SIL-10 ADVP, a column oven CTO-10 ASVP, RI detector and System controller SCL-10 AVP version 5.4. Shimadzu Class VP software version 6.1 was used for data analysis and data processing. The samples were analyzed at 80 °C on a Phenomenex Rezex RPM-monosaccharide Pb++ (8%) column (300 mm × 7.80 mm) by RI detector using a gradient mobile phase of HPLC grade water.

2.6. Sample preparation

The accurately weighed quantity of the dried hydrolysate of BOS 2000 was dissolved in known volume of HPLC grade water. The samples were filtered through millipore microfilter (0.45 μm) and then injected into the HPLC system.

2.7. Preparation of stock solutions and samples

Stock solutions of the pure reference compounds were prepared in HPLC grade water and stored in a refrigerator at 4 °C. From the stock solutions working solutions for each reference compound were prepared by dilution with HPLC grade water. These working solutions of all the reference compounds were mixed together in equal volumes for further analysis.

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