



Quantitative estimation and evaluation of anti-inflammatory activity of macromolecules of *Boswellia serrata*

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The present study was aimed to isolate and perform qualitative analysis of macromolecules present in *Boswellia serrata* followed by evaluation of their anti-inflammatory activity by *in-vitro* IL-6 ELISA study. The oleo gum resin of *B. serrata* was pretreated with hexane, methanol and the obtained residue was extracted with water and the final dried powder was taken for macromolecule analysis. The macromolecules which comprise of polysaccharides, mucilages and proteins were quantitatively estimated and evaluated for their anti-inflammatory activity by *in-vitro* IL-6 ELISA study. From the current study, it was found that the macromolecules present in *B. serrata* majorly comprise of polysaccharides and mucilages with moderate quantity of proteins. The quantitative analysis of polysaccharides, mucilages and proteins were found 35.91%, 34% and 14.29%. From the *in-vitro* IL-6 ELISA study, it was found that the macromolecules showed 82.63% inhibition of IL-6. The identified macromolecules from *B. serrata* showed significant anti-inflammatory activity by inhibition of IL-6 and further need to confirm by *in vivo* study to qualify *B. serrata* macromolecules as a promising anti-inflammatory agent.

Keywords: Anti-inflammatory activity, *Boswellia serrata*, IL-6 ELISA, Macromolecules, MTT assay

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Biopolymers are the most common macromolecules in biochemistry such as nucleic acids, proteins, carbohydrates and lipids¹. Macromolecules are made up of monomers which have single units and form larger polymers by the joining of covalent bonds¹. They have several important pharmacological effects such as proteins help in providing structural support, storage, transport, cellular signaling, movement and defensive mechanism against foreign substances and carbohydrates play an important role in the energy production in human body.

Boswellia serrata belonging to Family Burseraceae, consists of the oleo-gum-resin is a very useful herb in the field of herbal medicine. It is a branching tree and widely cultivated in India, Northern Africa and Middle East^{2,3}. In India, the major commercial sources of *Boswellia* are Madhya Pradesh, Chhattisgarh, Andhra Pradesh, Gujarat and Jharkhand. The oleo-gum resin consists of 30-60% resin, 5-10% essential oils and polysaccharides such as galactose, arabinose and xylose^{4,6}.

There are six major boswellic acids reported namely, α and β -Boswellic Acids (BA, 10–21%), Acetylated α and β -Boswellic Acids (ABA, 0.05–6%), 11-keto- β -

Boswellic acid (KBA, 2.5– 7.5%) and 3-*O*-acetyl-11-keto- β -Boswellic acid (AKBA, 0.1–3%)⁷. Among them all, AKBA and KBA are the leading contenders, therefore the current study focuses on macromolecules found in *B. serrata* and their anti-inflammatory activity which is not well studied so far.

Therefore, the current study was planned to estimate the macromolecules present in *B. serrata* and their anti-inflammatory activity by the help of *in-vitro* IL-6 ELISA study.

Materials and Methods

Collection and authentication

Oleo gum resin of *B. serrata* was collected and authenticated by the Pharmacognosy Department, R&D center, The Himalaya Drug Company, Makali, Bangalore.

Standardization

Then oleo gum resin was standardized by the Pharmacognosy Department, R&D center, The Himalaya Drug Company, Makali, Bangalore.

Extraction

One kg of oleo-gum-resin was taken and refluxed with 3 litre of hexane for 1 h at 55°C and the same

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was repeated twice. The obtained residue was refluxed with methanol at 65°C for 3 h and the same was repeated twice. Then the material was extracted with water under agitations at 95°C for 4 h and filtered by 100 mesh muslin cloth. The filtrate was concentrated on a water bath and dried in oven at 95°C. The extract was obtained in powdered form and coded as BSM for further study.

Estimation of polysaccharides

Standard preparation

Weighed 0.15 g of glucose was dissolved in 50 mL of purified water in a 100 mL volumetric flask, the solution was sonicated for 3 min and the volume was made up with purified water.

Sample preparation

Weighed 0.2 g of BSM was taken and 10 ml of 1.5 M sulphuric acid was added. The mixture was heated for 1 h with occasional stirring and allowed to cool then added 12 mL of 10% sodium hydroxide. Thereafter it was mixed well and transferred into a 100 mL volumetric flask and make up the volume with purified water. The sample was filtered using Whatmann No-1 filter paper and used for further analysis.

Sample blank preparation

Weighed 0.2 g of BSM was taken and added 30 mL of purified water then sonication was done for 10 min. The solution was transferred into a 100 mL volumetric flask and make up the volume with purified water. The sample was filtered through whatmann No-1 filter paper and used for further analysis.

Procedure

Standard glucose solution (0.5 M), sample solution and sample blank solution (1 mL each) was taken in different test tubes. A reagent blank was prepared by the addition of 5 mL of DNS reagent and 2 mL of purified water. Heating of the test tubes was done on a water bath for 20 min then transferred into 25 mL volumetric flasks. The volume of each volumetric flask was adjusted to 25 mL using purified water and absorbance was measured at 540 nm⁸.

Estimation of protein content

Procedure

The protein content was estimated by Kjeldahl method. Weighed 0.5 g of BSM was taken in digestion tube and 12 mL of concentrated sulphuric acid, 0.8 g copper sulphate and 7 g of sodium sulphate were added. Thereafter digestion process was carried out at 42°C for 1 h followed by automatic steam distillation by 50 mL of

40% sodium hydroxide and 75 mL of purified water in to distillation tube. Then the distillate was titrated against 0.1 N standardized hydrochloric acid using 1% methyl orange and 1% bromocresol as indicator until the pink end point is achieved. The volume of acid consumed in the titration was noted and the blank titration also performed in the similar way omitting the BSM^{9,10}.

Estimation of mucilage content

Weighed 2.0 g of BSM was taken and added 100 mL of purified water. Then shaken for 1 h using mechanical shaker followed by stand for 6 h. Thereafter, filtered through ordinary filter paper and concentrated up to 10 mL using water bath. Then the concentrated solution was poured into 250 mL beaker containing 100 mL of 90% ethyl alcohol with continuous shaking, during this, precipitate was obtained as per method described earlier. The precipitate was collected in sintered glass crucible and drying was done at 105°C for 2 h¹¹.

In-vitro anti-inflammatory study of BSM

Cytotoxicity study by MTT assay

Procedure

The non-toxic concentration of BSM was determined by MTT assay. The THP-1 cells (Human leukemia monocytic cell line) were grown in 96 well plates in RPMI 1640 with 2% FBS and incubated for the assay. The stock solution of test product (10 mg/mL) was prepared and further diluted with 1:10 ratio by dissolving in RPMI 1640 with 2% FBS and from that sample subsequent dilutions were made to obtain the concentrations of 1000, 500, 250, 125, 62.5, 31.25 and 15.625 µg/mL. The dilutions (100 µL/well) were added to the THP-1 cells and the plate was incubated at 37°C with 5% CO₂. Cell control was also maintained. After incubation for 24 h, the morphological changes of the cells were observed under microscope and 10 µL of MTT (5 mg/mL) solution was added into each well and incubated for 4 h at 37°C. The cell supernatant was discarded and the cell bound dye was extracted by adding 100 µL of DMSO into each well and the absorbance was measured at 540 nm. The % cytotoxicity was calculated from absorbance values of treated and control groups. The CTC50 value for the sample was calculated from the dose response curves by linear regression analysis¹².

IL-6 ELISA study

Procedure

The preliminary anti-inflammatory activity of BSM was evaluated by IL-6 ELISA kit. The study was

carried out in THP-1 cells. Different concentrations of test samples were used for treating the cells and LPS (Lipopolysaccharides) (1 $\mu\text{g}/\text{mL}$) to induce inflammatory cytokines and incubated at 37°C with 5% CO_2 for 24 h. After incubation, the cell supernatant was separated by centrifugation. Quantitative determination of Human IL-6 Elisa was performed according to the assay procedure provided in Kit-Krishgen Biosystem ELISA kit catalog: KB1068. All incubation steps were performed at room temperature. Micro plate reader was used to measure the optical density at 450 nm¹².

Results

Quantitative Estimation of Macromolecules

Polysaccharide content in BSM obtained from the standard absorbance (0.474) and BSM absorbance (0.227) was found to be 35.91%. The protein content of BSM obtained from the titrimetric method was found to be 14.29%. The mucilage content of BSM obtained from the precipitate weight (0.68 g) was 34%.

In-vitro anti-inflammatory study of macromolecules

Cytotoxicity study by MTT assay

From the assay the CTC_{50} value of the sample BSM obtained was 829.68 $\mu\text{g}/\text{mL}$. The concentrations of the sample used for the IL-6 ELISA study were 500 and 250 $\mu\text{g}/\text{mL}$.

IL-6 ELISA study

From the study it was found that inhibition of IL 6 in BSM treated cells is 82.63 % at 500 $\mu\text{g}/\text{mL}$ concentrations. The morphology of the cells after treating with BSM was shown in Figure 1.

Discussion

The oleo gum resin of *B. serrata* is significantly used to treat many diseases in traditional medicine. In a clinical trial *B. serrata* showed fair to excellent anti-inflammatory results in 88% of the patients, with no adverse effects¹³. Our present study was particularly

focused on the extraction, quantitative estimation and determination of preliminary anti-inflammatory activity of macromolecules present in *B. serrata*. Not much research has been done on macromolecules of oleo gum resin of this plant. The aqueous fraction extracted from oleo-gum resin contains polysaccharides such as galactose, arabinose and D-glucuronic acid which were suggested to act as a potent enhancer of humoral and cell mediated immune response¹⁴. The anti-inflammatory activity of the polysaccharides was not explored yet¹⁵. From the quantitative analysis of macromolecules the amount of polysaccharides, mucillages and proteins present in *B.serrata* was found respectively 35.91%, 34% and 14.39%. The quantitative analysis revealed that the macromolecules of *Boswellia* majorly comprised of polysaccharides and mucillages.

In this study, the anti-inflammatory activity of the macromolecules was evaluated by the help of *in-vitro* IL-6 ELISA study. MTT assay was carried out to determine the non-toxic concentration of BSM. From the assay the non-toxic concentration obtained was 829.68 $\mu\text{g}/\text{mL}$. The study was carried out at different concentrations of 500 and 250 $\mu\text{g}/\text{mL}$.

IL-6 is a potent lymphoid cell growth factor that stimulates the growth and survivability of certain B cells and T cells. It plays an important role in host defense, acute phase reactions, immune response. IL-6 is expressed by T-cells, B cells, monocytes, fibroblasts, hepatocytes etc. It was found from the ELISA study that the macromolecules showed 82.63% inhibition of IL-6 and hence the macromolecules may act as a promising anti-inflammatory agent.

From the present study, it was found that the macromolecules of *Boswellia* are majorly comprised of mucilage's, polysaccharides and proteins. It was also found that these macromolecules showed anti-inflammatory activity by the inhibition of IL-6.

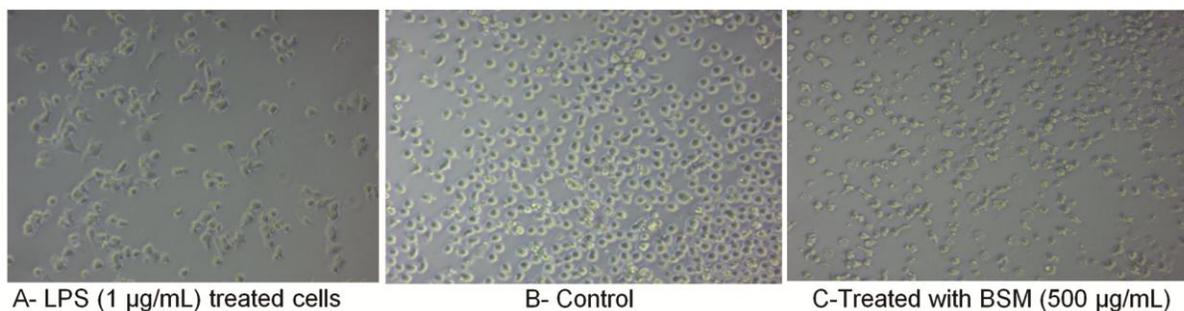


Fig. 1 — Anti-inflammatory effect of BSM on LPS induced Cells. Morphology of cells of LPS treated 1 $\mu\text{g}/\text{mL}$ (A), Cells control (B) and Cells treated with BSM 500 $\mu\text{g}/\text{mL}$ (C).

Conclusion

In the present study, *B. serrata* macromolecules are estimated quantitatively and evaluated for its anti-inflammatory activity using IL-6 ELISA. From this study it was concluded that BSM (*B. serrata* macromolecules) showed significant anti-inflammatory activity. Also BSM is a natural and safe anti-inflammatory agent compared to synthetic drugs. There lies a further need to perform *in vivo* study to qualify BSM is a promising inflammatory agent.

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Conflict of Interest

Authors declare that they do not have conflict of interest.

Authors' Contributions

S V conceived and designed the analysis, collected the data, performed the analysis, contributed data or analysis tools, N M wrote the paper, S P contributed data or analysis tools, D N performed the analysis, S S M performed the analysis, contributed data or analysis tools, U V B conceived and designed the analysis, R L conceived and designed the analysis, V P contributed data or analysis tools.

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