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Mast cell degranulation inhibition activity of herbal extracts

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Abstract
Mast cell degranulation mediated by various allergen with subsequent cross linking of Antigen Antibody complex is the chief triggering mechanism of allergic inflammatory response. Among many mediators released, β-hexosaminidase have a leading role in propagating allergic reactions. The pharmacologic inhibition of this process would, therefore, have clear therapeutic potential. Luteolin formulations, alone or together with drugs that can selectively inhibit the release of pro-inflammatory mediators hold promise for the treatment of skin and brain inflammatory diseases. In this work, antiallergic properties particularly the inhibition of mast cell degranulation was studied with two of the samples A and B. Inhibition of β-hexosaminidase release by the samples have been studied.

Keywords: Mast cell degranualtion inhibition, β-hexosaminidase, *Boswellia serrata*, *Terminalia bellerica*

Introduction
Mast cells are derived from distinct precursors in the bone marrow or other hematopoietic tissues. They are well known for their role in allergic and anaphylactic reactions, as well as their involvement in acquired and innate immunity. Increasing evidence now implicates mast cells in inflammatory diseases where they are activated by non-allergic triggers, such as neuropeptides and cytokines. It is critical for the pathogenesis of inflammatory diseases, such as arthritis, atopic dermatitis, psoriasis, and multiple sclerosis [1]. Proteases released from mast cells could act on plasma albumin to generate histamine-releasing peptide that would further propagate mast cell activation and inflammation. However, unlike allergic reactions, mast cells are rarely seen to degranulate during inflammatory processes. The only way to explain mast cell involvement in non-allergic processes would be through "differential" or "selective" secretion of mediators without degranulation [2]. In allergic responses, immune cells such as mastocytes, eosinophils, basophils, and macrophages release several mediators including histamine and leukotrienes that are responsible for allergic symptoms [3].
In mast cells, large amounts of β-hexosaminidase are present in the granules. Exposure to allergens, harmless antigen, toxins etc., leads to mast cell degranulation. As a result, many of the mediators including β-hexosaminidase is released which spread through the tissues triggering typical allergic reactions [4]. Mast cells clearly participate in the induction and/or propagation of certain inflammatory diseases, through selective release of mediators. The pharmacologic inhibition of this process would, therefore, have clear therapeutic potential. Luteolin formulations, alone or together with drugs that can selectively inhibit the release of pro-inflammatory mediators hold promise for the treatment of skin and brain inflammatory diseases. In this work, antiallergic properties particularly the inhibition of mast cell degranulation was studied with two of the samples A and B. Inhibition of β-hexosaminidase release by the sampleshave been studied.

Materials and Methods
Cell Culture
Obtained P815, the murine-mastectomy cells from NCCS National center for cell science, Pune and cells were routinely maintained in Dulbecco's modified Eagle's medium (DMEM) containing 25 mM glucose, supplemented with 10% fetal calf serum, 2 mM L-glutamine.
Subculture and maintenance were performed as previously described. P815 cells presented in this study were at passages 20-30 (low passage) all assays used p815 cells are grown to 80-90% confluence unless otherwise stated.

Test compounds

1. **Sample A:** Hydroalcoholic Extract (90:10) of *Boswellia serrata*
2. **Sample B:** Equal Mixture of Hydroalcoholic Extract (90:10) of *Boswellia serrata* and *Terminalia bellerica*

Aqueous samples of A and B at 100 mg per ml were made by stirring the samples overnight in water. Stocks (10x) concentrations were made to get 500, 250, 100, 50, 25, 10 µg/ml of samples for the assays. Quercetin was used as positive control at 30, 75 and 150 µg /ml.

Stimulation of P815 cells

P815 cells were seeded at 3.0 x 10^5 cells/mL in 24-wells plate (1 mL/well), and assayed after 24h at near-confluent stage (90%). Calcium ionophore A23187, 500 ng/mL (1 mM) was used as a degranulation stimulus.

Released β-hexosaminidase quantification.

The release of β-hexosaminidase from stimulated P815 cells was measured as previously described Passante *et al*., 2009 [5]. In a 96-wells plate, 50 ml of substrats solution [p-nitrophénols N-acétyle-D-glucosamine 1.3 mg/ ml in citrate buffer (pH 4.5)] were added to 30 ml of supernatant**.**

The plate was incubated at 37 °C, during 1 h. The reaction was stopped by the addition of 80 ml of NaOH 0.5 M and the reaction product, p-nitrophenolate, was measured spectrophotometrically at 405 nm, in a microplate reader.

β -Hexosaminidase inhibitory release

Beyond avoiding β - hexosaminidase release, compounds may directly inhibit β - hexosaminidase enzymatic activity [6]. For this, the inhibition of β - hexosaminidase enzymatic activity by samples A and B and quercetin was evaluated in an assay similar to the one described above. Individual samples were incubated with supernatant of degranulated cells where β - hexosaminidase is present (25 µl of supernatant of cells treated with A23187), in presence of 50 µ l of substrate solution, during 1 h, at 37 °C. The determination was made at 405 nm, in a microplate reader.

Inhibition of β-hexosaminidase release = [1-(T-B)/(C-B) *100]

where T is the absorbance of the cells with stimulator and test compound, B is cells without stimulator and test compound and C is cells with stimulator alone and no compound.

Results

With A23187 stimuli, β - hexosaminidase release increased by 145.21% above basal value. In the A23187 assay, the positive control quercetin required a 10-fold higher concentration (30 µg/ml) to reduce β – hexosaminidase release by 37.88%. In a study by Pinho *et al*., 2014 [7], Quercetin was found to inhibit β - hexosaminidase release in rat basophilic leukemia cell line, RBL-2H3 cells by 58%. Sample A at 10 and 20 µg/ml did not have any effect on A23187 induced hexosaminidase release. Dose dependent inhibition of β - hexosaminidase release was noted in the range of 50-500 µg/ml. Sample B was not active until 100 µg/ml, at 250 and 500 µg/ml it inhibited hexosaminidase release by 16.7 and 27.3% respectively.

Table 1: Percentage inhibition of 0- hexosaminidase release

Concentration in µg/ml	Percentage inhibition ofl3- hexosaminidase release ± SD*		
	Sample A	Sample B	Quercetin
10	-	-	NT
25	-	-	NT
30	NT	NT	37.88 ± 2.1
50	19.69± 2.14	-	NT
75	NT	NT	65.15 ± 2.1
100	28.79± 10.71	-	NT
150	NT	NT	74 24+2 1
250	50± 10.71	16.67±2.14	NT
500	63.64± 4.29	27.27±4.29	NT

*NT - Not Tested, - No Activity

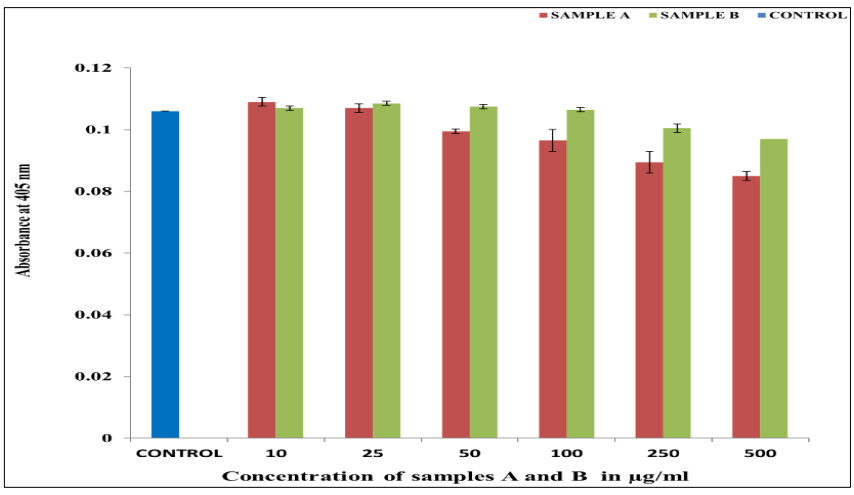

Fig 1: Inhibition of β-hexosaminidase release shown as absorbance at 405 nm for sample A & B

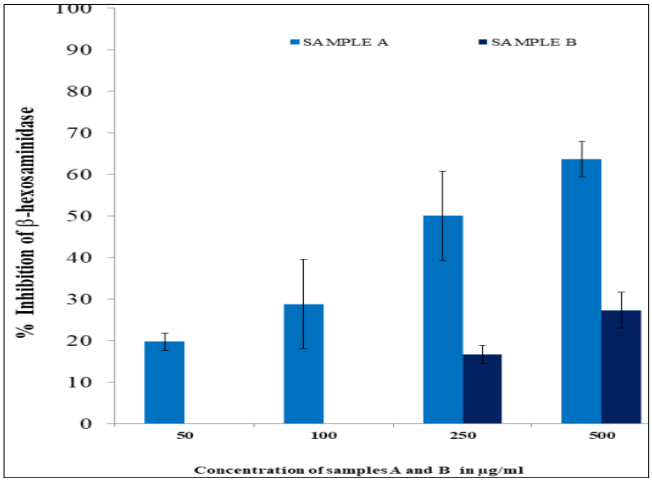

Fig 2: Inhibition of β-hexosaminidase release and activity by samples

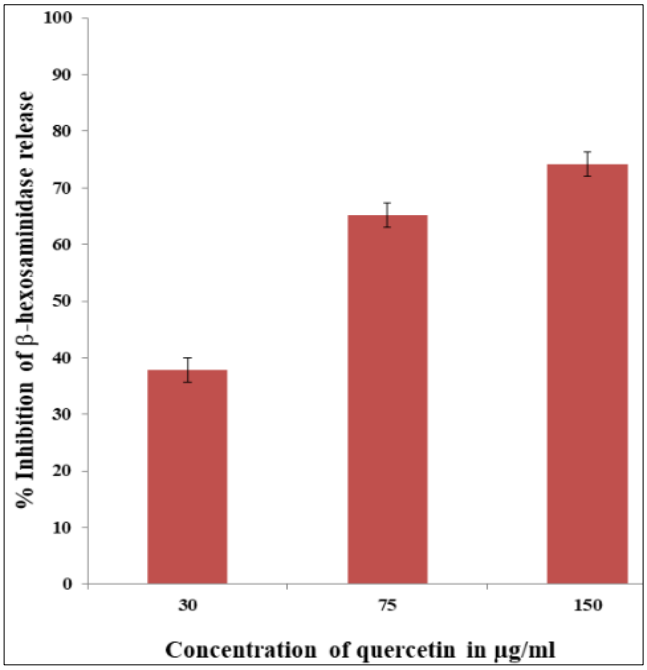

Fig 3: Inhibition of B - hexosaminidase release and activity by Quercetin

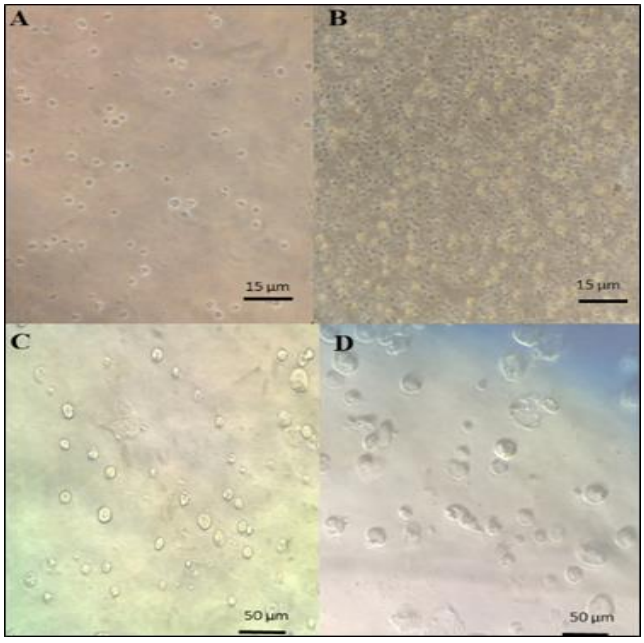

Fig 4: Mast cell p815 degranulation A. normal cells B cells stimulated by calcium ionophore C. cells treated with 250 Mg/ml of sample A and B respectively.

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