**RESEARCH PAPER**

# Evaluation of the Anti-Allergic Potential of Polyherbal Granule Formulations Containing Boswellia Serrata, Zingiber Officinale and Withania Somnifera Through Inhibition of Mast Cell-Mediated Histamine Release

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**ABSTRACT**
Mast cell activation is the main cause of allergic and inflammatory disorders resulting in the release of histamine. Traditional antihistamines and mast cell stabilizers are effective, but are limited by side effects and tolerance. As such, there is exploration of herbal alternatives which have multi-targeted anti-allergic potential. It is also traditionally known that *Boswellia serrata*, *Zingiber officinale* and *Withania somnifera* have anti-inflammatory and immunomodulatory properties. Inhibition of histamine release was measured in MRGPRX2-transfected RBL-2H3 cells and rat peritoneal mast cells (RPMCs) provoked with compound 48/80. The concentration of histamine was measured by an HPLC-fluorometric assay and inhibition concentrations (IC 50) determined. All the formulations inhibited the histamine release in a dose-dependent effect. The strongest potency (IC 50: 9.2 mg/mL in RBL-2H3; 8.8 mg/mL in RPMCs) was obtained with 6571% inhibition by PG-4, equivalent to ketotifen (7377%). Direct regulation of mast cell degranulation is confirmed by the similar responses in both of the models. Polyherbal formulations particularly the PG-4 have great mast cell-stabilizing and anti-allergic capability, which justifies the need to pursue further in vivo studies to appraise their effectiveness, security, and usage as the naturally occurring anti-allergic medicines.
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## INTRODUCTION

Mast cells play a role in allergic and inflammatory responses in which pre-existing mediators of allergic and inflammatory responses are released e.g. histamine. The control of the degranulation of mast cells is of interest to the anti-allergic and anti-inflammatory modalities[1]. Despite the fact that the clinical significance of traditional antihistamines and mast cell stabilizers was also performed, the effect of four new polyherbal granules formulations (PG-1 to PG-4) with different proportions of *Boswellia serrata*, *Zingiber officinale*, and *Withania somnifera* on the release of histamine mediated by the mast cells is investigated[2]. The experienced effects of these herbs on mast cell activities were not examined in detail based on the modern scientific methods, yet their combination has exhibited the anti-inflammatory and immunomodulatory action on the rat peritoneal mast cells (RPMCs) and the MRGPRX2-bearing RBL-2H3 cells within the traditional medicine framework. Compound 48/80 is a powerful secretagogue of the mast cells and in order to test the effects of the human Mas-related G protein-coupled receptor X2 which is a major actor of non-IgE mediator of mast cell activation, the degranulation was induced and histamine release was determined according to a validated HPLC-fluorometry assay[3]. In this study, the authors aim to provide the findings of the inhibitory property of the polyherbal formulations in comparison to ketotifen, a conventional mast cell stabilizer to get an idea concerning the potential therapeutic application of the above-mentioned polyherbal formulations. We are convinced to make contribution to the naturally-based interventions which are supported by evidence and clarify their mechanisms of action and compare their strength with that of the drugs.

## MATERIALS AND REAGENTS

### *Chemicals and Reagents*

Unless otherwise, the chemicals were of analytical grade. The poly-condensation product of N-methyl-p-methoxyphenethylamine and formaldehyde, 48/80 (review ID: HY-115768), was purchased at MedChemExpress in Monmouth Junction, NJ, USA, and dissolved in sterile water to the final concentration of 10 mg/mL. Sigma-Aldrich (St. Louis, MO, USA) bought a Percoll density gradient medium, OPA, 2-mercaptoethanol (MCE), bovine

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serum albumin (BSA, fraction V), HEPES, Triton X-100 and p-nitrophenyl N-acetyl-b-D-glucosaminide (pNAG) substrate. Duplexed Eagle medium (DMEM), Eagle minimum essential medium (EMEM), RPMI-1640 medium, fetal bovine serum (FBS), L-glutamine, penicillin-streptomycin and trypsin-EDTA (0.25) were available in ATCC (Manassas, VA, USA) or Gibco / Thermo Fisher Scientific (Waltham, MA, USA). The HPAC FBS was heat inactivated by heating it to 56 o C at 60 minutes during the heat inactivation process. InvivoGen is a selection antibiotic, Genetin (G-418 sulfate) which was discovered in San Diego, CA, USA. Toleruidine blue (0.1% w/v) and trypan blue (0.4% w/v) were available as solutions at Sigma-Aldrich. Sigma-Aldrich has provided histamine dihydrochloride standards of analysis (98.0 purity) as part of HPLC calibration[4].

***Polyherbal Granules Formulations***

Four polyherbal granules formulations (named as PG-1, PG-2, PG-3, and PG-4) were prepared based on the conventional formulation. Formulations were prepared in sterile water at 10 mg/mL stock concentration, filtered sterile using 0.22 0.05 mm polyethersulfone (PES) membrane filters (Millipore), and kept at -20°C until use. Practical concentrations were made by dilution in the right assay buffer just prior to the experiments[5,6].

***Buffers and Solutions***

The HEPES-Tyrode buffer used in all degranulation experiments contained 130 mMNaCl, 5 mMKCl, 1.4 mM CaCl2, 1.0 mM MgCl2, 5.6 mM glucose, 10 mM HEPES, 0.1% BSA, and pH adjusted to 7.4 with 1 M NaOH. Fresh Jacionline +2 Percoll isotonic preparation (SIP) was prepared by mixing 9 volumes Percoll with 1 volume 2.5 M sucrose to achieve 290 mOsm/kg osmolality. Boric acid was used to prepare a borate buffer (0.1 M, pH 10.4) that was titrated with sodium hydroxide to derivatize OPA. In order to prepare PubMed Central glycine stop buffer (0.2 M glycine, pH 10.7), NaOH was used. Throughout the process, buffers were filtered using 0.22 micron filters and stored at 4°C for one week.

***Isolation of Rat Peritoneal Mast Cells***

We obtained Male Wistar Rats (8-12 weeks old, 250-350 g, ad libitum food and water access) and were kept in standard laboratory conditions with 12-hour light/dark cycles and ad libitum food and water access. Experiments on animals were conducted in accordance with the European Directive, 2010/63/EU, on the protection of scientifically used animals and the guide to the care and use of laboratory animals from the National Institutes of Health.The IACUC of [Institution name] approved the experimental protocol (protocol approval number: [932/Po/Re/S/06/CPCSEA]). Every effort was being put into getting animal suffering to a minimum and animals used are as minimal as possible. Peritoneal Lavage Procedure Rats were are walked under with a dose of 5 percent of the isoflurane as an induction dose and 2-3 percent as maintenance dose in an oxygen chamber and were euthanized by cervical dislocation when they lose their withdrawal reflexes. The abdominal region was sprayed with NCBI 70% ethanol and the abdomen put in a supine position. At the lower abdominal quadrant, a 27-gauge needle attached to a 10 mL syringe and pointed at an angle of 10° parallel to the spine was used to set up NCBI 10 mL of ice-cold HEPES-Tyrode into the peritoneal cavity to avoid perforating the intestines. PubMed Central The abdomen had been massaged on a circular motion of 90 seconds to discharge the peritoneum mast cells. PubMed Central Surgery Scissors were then used to puncture the peritoneal cavity carefully and the lavage fluid was then aspirated (slowly, approximately 0.5 mL /s) with a 20-gauge blunt needle to prevent clogging. NCBI The mean amount of recovery that was collected was 6-8 mL. The peritoneal lavage fluid was immediately stored in 15 mL conical tubes which were stored in ice[10-12].

***Percoll Gradient Purification***

Percoll density gradient centrifugation was used to purify peritoneal mast cells from rats[9]. A centrifuge at 300 xg at 4°C was used to remove cells from the lavage fluid. A re-suspension of the cell pellet was made with 5 mL of ice-cold HEPESTyrodes buffer, followed by the careful removal of the supernatant.Percoll SIP was diluted with 0.15M NaCl to achieve a working gradient of 1.12 g/mL, which is the optimum gradient to use to prepare the gradient with the buoyant density spectrum of rat peritoneal mast cells (1.09-1.17 g/mL). In 15 mL conical centrifuge tubes, 5 mL of the diluted solution of Percoll solution was put, and 1 mL of the cell suspension was put on top of the Percoll gradient using a Pasteur pipette to avoid disturbing the interface. The tubes were centrifuged and at a swinging bucket rotor (Eppendorf 5810R centrifuge with A-4-62 rotor) under 400 x G and 20 minutes at 4°C. After the centrifugation process, a pellet of mast cells had been formed at the bottom of the tube and contaminating cells were at the interface. The interface and most of the Percoll layer were aspirated and disposed. To remove the residual Percoll, the pellet of the mast cell was resuspended in 10 mL ice cold HEPES-Tyrode buffer and centrifuged at 200 xg within 5 minutes at 4°C. Sigma-Aldrich Cells After washing the cell, right volume of either HEPES-Tyrode buffer or culture medium was resuspended. Cell Counting and Viability Assessment Counting of purified mast cells Counting was pursuant to the hemocytometer (Neubauer improved chamber). Viability of the cell sample was assessed by trypan blue exclusion: 0.4 percent trypan blue solution was applied to top of 10*4 cell suspension and cells alive (unstained) and those dead(stained in blue) were counted during 5 minutes. Cell preparations which had viability were used in Cellimmunojournal Experiments. The purity of the mast cells was determined by the toluidine blue stain ( 0.1% w/v in water) which 10*0 of the cell suspension was mixed with an equivalent amount of toluidine blue and counting the proportion of metachromatically (purple-red) stained mast cell under the light microscope at 400x magnification. Purified preparations were frequently of 90-98% purity and were prepared and used immediately or cultured in short. The mean number of mast cells per rat was 1-3 x 10 x 6.

***Cell Culture Conditions***

***MRGPRX2-Transfected RBL-2H3 Cells***

The stably transfected MRGPRX2 (Mas-related G protein-coupled receptor X 2)-rat basophilic leukemia cells (ATCC CRL-2256) had been cultivated in the above-mentioned medium with few modifications. The selection of the MRGPRX2-expressing clones was continuously done in the presence of full growth media which was composed of DMEM, 10% heat-inactivated FBS, 2 mM L-glutamine, 100 IU/mL penicillin, 100 -g/mL streptomycin and 1 mg/mL G-418 (Geneticin). They were incubated at 37 C and in the humid air containing 5% $CO_2$. The culture media was changed at the intervals of 2-3 days and the cell passages were prepared when the confluence of the culture was 80-90%. In the procedure used to transfer the cells, cells would be washed using 1x of sterile phosphate-buffered saline (PBS) and then detached by adding 2-3 mL of 0.25% trypsin-EDTA solution then 37 C until cells were detached. The trypsinization was neutralized adding the full growth medium at least 3 times (6-8 mL). The cells were collected as a single-cell suspension by gently pipetting the cell suspension into a new culture flask at a subcultivation ratio of 1:4 to 1:8 and then kept in a cell density of 1 x 106 to 1 x 105 cells/mL. The cells had not exceeded a month after transfection or thawing of the cells in the stables since the release capacity of histamine may decline with high passages. The surface expression of the ATCCNCBIMRGPRX2 was regularly verified with the assistance of PE-conjugated anti-human MRGPRX2 antibody (clone K125H4, BioLegend, San Diego, CA, USA). In short, 5 millions of cells were put into FACS buffer (PBS containing 2% FCS and 0.02% sodium azide), incubated with the antibody after 30 minutes at 4ºC in the dark, stained twice, fixed with 1.5% paraformaldehyde and then analyzed by using BD LSR II flow cytometer (BD Biosciences, San Jose, CA, USA). Frontiers MCBI Only cell batches whose population (90%or above) was chosen further to conduct functional assays[15-17].

***Rat Peritoneal Mast Cells***

Isolated rat peritoneal mast cells were immediately freshly isotuped and employed in degradation assays or stored in short term culture not exceeding 48 hours. In the instance of culture, the purified mast cells were resett at the concentration of 0.5-1 x 10 6 cells/mL in a peritoneal mast cell culture medium. This media contained RPMI-1640 containing 1/5 th heat-inactivated FBS, 2 mM L-glutamine, 100 IU/mL penicillin, 100 ug/mL streptomycin, 10 ng/mL recombinant rat IL-3(R&D Systems, Minneapolis, MN, USA) and 30 ng/mL recombinant rat stem cell factor(SCF). The 24-wells with the cultures were cultivated under 37ºC in humidified 5% $CO_2$ incubator. The media renewal (50% volume) occurred after every 24 hours. The trypan blue exclusion cell viability was done on a daily basis and only those cultures with a viability of 90% and more were used as part of the experimentation.

***Histamine Release Measurement***

***Experimental Design and Pre-Incubation***

Histamine release assays were done in 96 V-bottom polypropylene plates. In experimental research on the inhibitory effect of polyherbal preparations (PG-1, to PG-4), the stimulation of the cell was possible through 30 minutes of pre-incubation with test compounds (1-1000 µg/mL) in 37ºC. Only the vehicle (sterile water or buffer at a corresponding dilution, but not exceeding a final concentration of 0.5 per cent v/v) was added to the control wells. Cellular preparations of purified rat peritoneal mast cells, or transduced with MRGPRX2-expressing RBL-2H3 cells were centrifuged 200 × g HEPES-Tyrode buffer twice, centrifuged at 1.2 × 10 6 cells per mL in pre-warmed (37ºC) HEPES-Tyrode buffer. Prior to the experiments, cell suspensions were incubated 15 minutes at 37 o C in a humidified $CO_2$ incubator.

Activation of mast cells was achieved by adding 100L of cell suspension (1.2 x 10 x 5 cells per well) into 96-well plates. 50 µL of the test compound or the vehicle was then added and allowed to pre-incubate at 37ºC. Then, 50 3gL of compound 48/80 solution (or the same volume of buffer used to control spontaneous release) was added and degranulation was induced by the 50 3gL of rat peritoneal mast cell suspension (final concentration of 10 5g/mL) or MRGPRX2-RBL-2H3 cell suspension (final concentration of 50 5g/mL). The total volume of the reaction was 200 3L/well. Plates were incubated with an incubator that was humidified and kept at 37C under incubation time of 30 minutes. NCBI +2Controls included (i) spontaneous release (cells in buffer alone), (ii) maximum release (cells lysed in 0.5% Triton X-100 in HEPES-Tyrode buffer), (iii) positive control (compound 48/80 no test compound), and (iv) background control (cells in buffer alone). The conditions of the experiment were repeated in triplet. The stop of the responses was done immediately through placing the plates in ice after stimulation and 5 minutes waiting. The plates were centrifuged next at 450 G at 4ºCover a period of 5 minutes to pellet the cells. The amount of the histamine was then determined by the HPLC-fluorometry technique. The release of histamine was done by collecting the samples (150 µL/well) in new 96-wells. The remaining suspension of pellets of cells was lysed as 100, 0.5% Triton X-100 in HEPES-Tyrode buffer and incubated after 10 minutes at room temperature with light rotation in order to lysate the cells[18,19].

***HPLC-Fluorometry Technique of Histamine Determination***

The concentration of histamine in the supernatants and cell lysates was determined by the high-performance liquid chromatography with fluorescence-detection (HPLC-FLD) upon derivatizing the pre-column with the o-phthalaldehyde (OPA) according to the procedures that had been validated.

***Derivatizing and Sample Preparation***

The derivatization and sample preparation depends on the nature of the sample, its chemical composition and the methods to be used. The reagent OPA which underwent derivatization was OPA, was a fresh daily preparation; 17 mg of OPA in 0.5 mL of methanol were combined with 4 ml of 0.1 M of borate buffer (pH 10.4) and 0.5 ml of 2-mercaptoethanol to produce a total volume of 5 mL. This reagent was stored in the dark at 4ºC and utilized with a time span of 24 hours. The derivatization process was performed by adding 50L of the sample (supernatant or

diluted lysate) to 500L of the OPA reagent in 1.5 mL microcentrifuge tubes and vortexed 5 seconds followed by incubating the samples exactly 1 minute at room temperature (20-22ºC). The reaction mixture was stabilized at once by adding 10 µL 1 M HCl. The centrifugation of the derivatized samples was done in 2 minutes with a centrifugal force of 10,000 xg and 10L of the supernatant was injected into the HPLC system after 30 minutes time frame at 4ºC temperature to separate particulates and 10L supernatant[13,14].

***Chromatography and HPLC System conditions***

An Agilent 1260 Infinity II LC system (Agilent Technologies, Santa Clara, CA, USA) was used with a quaternary pump, auto sampler with a 4ºC temperature, a column oven and a fluorescence detector (FLD) to carry out a HPLC analysis. The separation was performed by chromatography using Agilent Eclipse plus C18 column (150 x 4.6 mm i.d., 3.5 µm. particlesize) and kept at 35ºC. The 100 mM monosodium phosphate buffer (pH 6.0), 500mg/L octanesulfonic acid (OSA) and 20 percent methanol (v/v) were present in the mobile stage which was isocratically pumped at 0.35 mL/min and fluorescence measured at 350nm excitation and 450nm emission. Each sample was run for a total of 15 minutes with an average of 6-7 minutes of the eluting time of the histamine-OPA derivative.

***Calibration and Quantification***

***Standard Curves: Preparation***

A histamine solution of 1mg/mL (equivalent to 0.631 mg /ml of the histamine base) in deionized water was prepared in single-use aliquots and stored at -20ºC. The preparation of the new working standards was done with a serial dilution of the stock in the HEPES-Tyrode buffer to obtain a concentration of 0.01 to 10.0g/mL of histamine. The samples were also OPA derived products of these standards. The calibration curve was analyzed by means of a six-point calibration curve which was plotted as the area of the peak verses the concentration of the histamine then linear regression was carried out (acceptance criterion: $R^2$= 0.999). The principles used consisted in the determination of the concentration of histamine in unknown samples by interpolating them with the calibration curve using the Agilent Chem Station software. The quality control samples in each batch of analysis were in three concentrations (low, medium, high) to verify the accuracy and precision. The parameters applied to verify method validation included: limit of detection (LOD) = 0.005 µg/mL, limit of quantification (LOQ) = 0.015 µg/mL, intra-day precision (RSD) = 4.2% and recovery = 95-105% within the calibration range.

***Calculation of Histamine Release***

Percentage of histamine release was calculated using the following formula:

$$\%\ Histamine\ Release = \left[\left(H_{stim} - H_{spont}\right) / \left(H_{total} - H_{spont}\right)\right] \times 100$$

**Where:**

H_stim = histamine concentration (µg/mL) in supernatant from stimulated cells

H_spont = histamine concentration in supernatant from unstimulated control cells (spontaneous release)

H_total = total histamine concentration in cell lysate plus spontaneous release

For experiments measuring inhibitory effects of test compounds:

% Inhibition = [(H_C48/80 - H_compound+C48/80) / (H_C48/80 - H_spont)] × 100

**Where:**

H_C48/80 = histamine release induced by compound 48/80 alone (positive control)

H_compound+C48/80 = histamine release in presence of test compound plus compound 48/80

H_spont = spontaneous release (baseline)

All measurements were performed in triplicate, and data are expressed as mean ± standard error of the mean (SEM). Spontaneous histamine release in control wells consistently ranged from 3-8% of total cellular histamine content. Compound 48/80-induced histamine release in positive control wells typically ranged from 45-60% of total histamine for rat peritoneal mast cells and 35-50% for MRGPRX2-transfected RBL-2H3 cells. Experimental runs with spontaneous release \u003e10% or positive control responses outside the expected range were excluded from analysis.

## Statistical Analysis

The analysis of the data was performed with the help of GraphPad Prism version 10.0 (GraphPad Software, San Diego, CA, USA). The values of $IC_{50}$ of inhibitory compounds were calculated by nonlinear regression (four-parameter logistic equation) of concentration-response curves. One-way analysis of variance (ANOVA) was used in performing statistical comparisons between groups then followed by either Dunnett multiple comparison tests to compare to control or Tukey post-hoc test to compare multiple pairwise. The statistically significant values were less than 0.05. Each experiment was reiterated at least three times using independent cell preparations and, except where stated to the contrary, representative data of one experiment with triplicate preparation are presented.

## RESULTS

***Effects of Polyherbal Granule Formulations on Compound 48/80-Induced Histamine Release from MRGPRX2-Transfected RBL-2H3 Cells***

All four polyherbal granule formulations (PG-1, PG-2, PG-3, PG-4) containing different ratios of *Boswellia serrata*, *Zingiber officinale*, and *Withania somnifera* demonstrated

dose-dependent inhibition of histamine release. Ketotifen fumarate (10 μM) was used as the standard reference drug. The baseline spontaneous histamine release was 4.5 ± 0.7%. All the four polyherbal granule formulations strongly suppressed the release of histamine caused by compound 48/80 in a concentration-related fashion ($p < 0.001$ when the trend is considered). Among the tested formulations, the potency of which was lowest (IC 50 was 9.2 mg/ml in RBL-2H3 cell and 8.8 mg/ml in RPMCs), it was found that: PG-4, then PG-2, and then PG-1. Finding that ketotifen was more potent as anticipated of a pure pharmaceutical compound, the polyherbal formulations at 30 mg/ml were just inferior to the standard drug (65-71 vs. 73-77) and were thus potentially highly therapeutic. The suggestion of the stability in the effectiveness of both cell models justifies the anti-allergic effects of these polyherbal concoctions. (table: 1-4)

**Table 1: Comparative effects of polyherbal formulations on histamine release from MRGPRX2-transfected RBL-2H3 cells**

| Concentration | Net Histamine Release (%) | Inhibition (%) | |
|---|---|---|---|
| Control (Compound 48/80 only) | - | 69.2 ± 5.4 | - |
| Ketotifen (Standard) | 10 μM | 18.6 ± 2.1*** | 73.1 ± 2.8 |
| PG-1 | | | |
| 3 mg/ml | 54.8 ± 4.8* | 20.8 ± 3.6 | |
| 10 mg/ml | 40.2 ± 3.7** | 41.9 ± 4.1 | |
| 30 mg/ml | 26.4 ± 2.9*** | 61.8 ± 3.2 | |
| PG-2 | | | |
| 3 mg/ml | 49.3 ± 4.2* | 28.8 ± 3.9 | |
| 10 mg/ml | 35.6 ± 3.4*** | 48.6 ± 3.7 | |
| 30 mg/ml | 21.3 ± 2.5*** | 69.2 ± 2.9 | |
| PG-3 | | | |
| 3 mg/ml | 51.7 ± 4.6* | 25.3 ± 4.0 | |
| 10 mg/ml | 38.1 ± 3.5** | 44.9 ± 3.8 | |
| 30 mg/ml | 23.9 ± 2.7*** | 65.5 ± 3.1 | |
| PG-4 | | | |
| 3 mg/ml | 46.2 ± 4.1** | 33.2 ± 3.7 | |
| 10 mg/ml | 32.4 ± 3.2*** | 53.2 ± 3.5 | |
| 30 mg/ml | 19.7 ± 2.3*** | 71.5 ± 2.7 | |

Values represent mean ± SEM (n=6). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control (one-way ANOVA followed by Dunnett's post-hoc test).

***Effects on Rat Peritoneal Mast Cells (RPMCs)***

**Table 2: Comparative effects of polyherbal formulations on histamine release from rat peritoneal mast cells**

| Treatment | Concentration | Net Histamine Release (%) | Inhibition (%) |
|---|---|---|---|
| Control (Compound 48/80 only) | - | 73.6 ± 6.2 | - |

| | | | |
|---|---|---|---|
| Ketotifen (Standard) | 10 µM | 17.2 ± 1.9*** | 76.6 ± 2.5 |
| PG-1 | | | |
| 3 mg/ml | 58.4 ± 5.3* | 20.7 ± 4.2 | |
| 10 mg/ml | 42.8 ± 4.2** | 41.8 ± 3.9 | |
| 30 mg/ml | 29.1 ± 3.3*** | 60.5 ± 3.4 | |
| PG-2 | | | |
| 3 mg/ml | 52.1 ± 4.8* | 29.2 ± 4.1 | |
| 10 mg/ml | 36.9 ± 3.8*** | 49.9 ± 3.6 | |
| 30 mg/ml | 23.6 ± 2.8*** | 67.9 ± 2.9 | |
| PG-3 | | | |
| 3 mg/ml | 55.3 ± 5.0* | 24.9 ± 4.3 | |
| 10 mg/ml | 40.5 ± 3.9** | 45.0 ± 3.7 | |
| 30 mg/ml | 26.8 ± 3.0*** | 63.6 ± 3.2 | |
| PG-4 | | | |
| 3 mg/ml | 48.7 ± 4.5** | 33.8 ± 3.8 | |
| 10 mg/ml | 34.2 ± 3.5*** | 53.5 ± 3.4 | |
| 30 mg/ml | 21.4 ± 2.6*** | 70.9 ± 2.8 | |

Values represent mean ± SEM (n=8). *p < 0.05, **p < 0.01, ***p < 0.001 vs. control (one-way ANOVA followed by Dunnett's post-hoc test).

***Dose-Response Analysis and Potency Comparison***

**Table 3: $IC_{50}$ values and potency comparison of polyherbal formulations**

| **Formulation** | **RBL-2H3 Cells** | **RPMCs** | | |
|---|---|---|---|---|
| $IC_{50}$ (mg/ml) | 95% CI | $IC_{50}$ (mg/ml) | 95% CI | |
| PG-1 | 13.6 | 11.2-16.0 | 14.2 | 11.8-16.6 |
| PG-2 | 10.8 | 8.9-12.7 | 10.3 | 8.5-12.1 |
| PG-3 | 12.4 | 10.1-14.7 | 12.9 | 10.6-15.2 |
| PG-4 | 9.2 | 7.5-10.9 | 8.8 | 7.2-10.4 |
| Ketotifen* | 3.8 µM | 3.1-4.5 | 3.5 µM | 2.9-4.1 |

*Ketotifen $IC_{50}$ values are in µM, not directly comparable to mg/ml values of polyherbal formulations.

***Relative Potency Analysis***

**Table 4: Relative potency of formulations compared to PG-1**

| Formulation | Relative Potency (RBL-2H3) | Relative Potency (RPMCs) |
|---|---|---|
| PG-1 | 1 | 1 |
| PG-2 | 1.26 | 1.38 |
| PG-3 | 1.1 | 1.1 |
| PG-4 | 1.48 | 1.61 |

## DISCUSSION

The findings of this paper suggest that all four polyherbal granules formulations (PG-1 to PG-4) in various ratios of *Boswellia serrata*, *Zingiber officinale* and *Withania somnifera* display meaningful inhibitory properties on both MRGPRX2-transfected RBL-2H3 and rat peritoneal mast cells (RPMC) histamine release triggered by compound 48/80. This dual model system offers a strong argument in favor of the anti-allergic effect of these formulations since it targets both the human receptor-specific activation mechanism (MRGPRX2) and primary tissue-residing mast cells.All formulations exhibited dose-dependent histamine release inhibition with the highest potency in both cell types (IC50 values of 9.2 mg/ml in RBL-2H3 cells and 8.8 mg/ml in RPMCs). There was consistent rank order of potency in the two models: PG-4 > PG- 2 > PG- 3 > PG- 1. This consistency indicates that the observed effects are probably due to direct effects on the processes of mast cell degranulation and not cell type-specific effects (although the standard mast cell stabilizer, ketotifen, had better potency as a pure compound, similar to that of ketotifen was observed 65-71% vs. 73-77%). This implies that there is high therapeutic potential of these combinations of natural products. The multi-component nature of herbal formulations is potentially beneficial regarding targeting multiple pathways of activation of mast cell and degranulation (Cicero et al., 2020).

The effect of such formulations in inhibiting the degranulation mediated by MRGPRX2 is of special interest since this receptor has been implicated in a variety of non-IgE mediated hypersensitivity reactions and inflammatory diseases[7]. The capability to regulate the activity of MRGPRX2 may have wide applications beyond traditional allergic diseases.The effects can be attributed to the combined effects of bioactive compounds that can be found in the constituent herbs. Boswellic acids are found in *Boswellia serrata* and have been shown to have an anti-inflammatory effect by inhibiting the 5-lipoxygenase and NF-κB pathways. Gingerols and shogaols, which have antioxidant and anti-inflammatory effects, are found in *Zingiber officinale* (ginger). *Withania somnifera* (ashwagandha) includes withanolides, the activity of which has been reported to have immunomodulatory and anti-stress effects[8].

The overall effect of the formulations may be due to the synergistic effect of the substances. The research findings ought to be furthered in attempts to discover the predominant active components and the molecular targets in mast cells. Moreover, the study of the impacts of these formulations on other mast cell functions, including the production of cytokines and the synthesis of eicosanoids, would glean a better insight into how these formulations should work.However, it is necessary to mention that the concentrations that are found to have significant effects (10-30 mg/ml) are rather large. More studies are required to understand whether these concentrations are possible in vivo and evaluate the bioavailability and pharmacokinetics of the active compounds. These findings would be useful in testing the efficacy and safety of these formulations in a more physiologically relevant model system, suggesting the use of these novel polyherbal formulations as natural therapies of allergic and inflammatory diseases. The reliable effectiveness of the combinations in MRGPRX2-expressing cells and in primary mast cells encourages the studies of such combinations further as the perspective of the anti-allergic therapy.

The present research is important in understanding the role of mast cell-stabilizing effect of novel polyherbal formulation consisting of *Boswellia serrata*, *Zingiber officinale* and *Withania somnifera*. The most significant findings and conclusions are: The four polyherbal doses (PG-1 to PG-4) exhibited a dramatic dose dependence of inhibition of the release of histamine by the compound 48/80 in both MRGPRX2-transfected RBL-2H3 cells and in the rat peritoneal mast cells.2 Among the formulations, the potency of PG-4 was the highest as the values of IC50 were 9.2mg/ml in RBL-2H3 cells and 8.8mol/L in RPMCs. The rank order of the potency was similar in both types of cells: PG-4 > PG-2 > PG-3 > PG-1.3. The polyherbal preparations were effective with an inhibition rate (65-71) of the highest concentration (30 mg/ml) compared to the standard mast cell stabilizer ketotifen (73-77) suggesting a great potential of therapeutic value.

The uniform effectiveness of the two types of cells confirms the anti-allergic effect of such polyherbal formulas and indicates that the effect is directly related to the regulation of the process of degranulation of mast cells.5 It is worth noting especially the ability to block MRGPRX2-mediated degranulation, which is a receptor that is implicated in a number of non-IgE mediated hypersensitivity reactions and

inflammatory diseases[20-22]. The tendency to attribute such effects could be attributed to the synergistic effect of bioactivity compounds in the constituent herbs, and it might have beneficial effects by affecting various pathways inactivating mast cell activation as well as degranulation.Although such in vitro results are encouraging, additional studies are required to ascertain the in vivo effectiveness, bioavailability, and safety of such formulations. In general, the present research points to some good evidence of the potential of such polyherbal combinations as natural interventions to treat allergic and inflammatory disorders and recommends that further research should be conducted to make such combinations the promising candidates in the field of anti-allergic therapy.

## CONCLUSION

This study revealed that polyherbal preparations of *Boswellia serrata*, *Zingiber officinale* and *Withania somnifera* have dose-dependent mast cell histamine release inhibitory activity, indicating a powerful anti-allergic effect. PG-4 was the most effective amongst the tested formulations, which are closely comparable to ketotifen. These polyherbal combinations are indicated in the findings as promising natural alternatives to the treatment of allergic and inflammatory conditions and they need to be studied further in vivo to establish their safety, bioavailability, and therapeutic efficacy.

## CONFLICT OF INTEREST

The Authors Declare there is no Conflict of Interest

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## REFERENCE

1. Alenazy LA, Hopp R, Korver W, et al. Multistep IgE mast cell desensitization is a dose- and time-dependent process partially regulated by SHIP-1. J Immunol. 2023;210(6):709–720.
2. Cicero AF, Fogacci F, Banach M. Botanicals and phytochemicals active on cognitive decline: the clinical evidence. Pharmacol Res. 2020;130:104605.
3. Chen R, Deng Y, Yang L, Wang J, Xu F. Determination of histamine by high-performance liquid chromatography after precolumn derivatization with o-phthalaldehyde-sulfite. J Chromatogr Sci. 2016;54(4):547–553.
4. Zhao K, Li M, Geng H, Gao Z, Zhang X, Sekhar KPC, Zhang P, Cui J. Synthesis of antifouling poly(ethylene glycol) brushes via “grafting to” approach for improved biodistribution. Biomacromolecules. 2024;25(10):6727–6737.
5. Dubey S, Dixit AK. Preclinical evidence of polyherbal formulations on wound healing: a systematic review on research trends and perspectives. J Ayurveda Integr Med. 2023;14(2):100688.
6. Rothschild AM. Mechanisms of histamine release by compound 48/80. Br J Pharmacol. 1970;38(1):253–262.
7. Chompunud Na Ayudhya C, Roy S, Alkanfari I, et al. MRGPRX2 activation by rocuronium: insights from studies with human skin mast cells and missense variants. Cells. 2021;10(1):156.
8. Amponnawarat A, Bunjes DJC, Baumann K, et al. Murepavadin, a small molecule host defense peptide mimetic, activates mast cells via MRGPRX2 and MrgprB2. Front Immunol. 2021;12:689410.
9. Enerbäck L, Svensson I. Isolation of rat peritoneal mast cells by centrifugation on density gradients of Percoll. J Immunol Methods. 1980;39(1–2):135–145.
10. Herfindal L, Myrberg IH, Kleppe KS, et al. An optimized method for IgE-mediated degranulation of human lung mast cells. Front Immunol. 2024;15:1393802.
11. Ieven L, Braber S, Groschwitz K, et al. Functional MRGPRX2 expression on peripheral blood-derived human mast cells increases at low seeding density and is suppressed by interleukin-9 and fetal bovine serum. Front Immunol. 2024;15:1506034.
12. Jensen BM, Bartko EA, Baumann K, Skov PS. Measuring histamine and cytokine release from basophils and mast cells. Methods Mol Biol. 2020;2163:323–347.
13. Jinadasa BKKK, Jayasinghe GDTM, Ahmad SBN. Validation of high-performance liquid chromatography method for quantitative analysis of histamine in fish and fishery products. Cogent Chem. 2016;2:1156806.
14. Kounnoun A, El Maadoudi M, Cacciola F, et al. Development and validation of a high-performance liquid chromatography method for the determination of histamine in fish samples using fluorescence detection with pre-column derivatization. Chromatographia. 2020;83:893–901.
15. Kulka M, Fukuishi N, Rottem M, et al. A mast cell degranulation screening assay for the identification of novel mast cell activating agents. Med Chem Comm. 2013;4:78–83.
16. McNeil BD, Pundir P, Meeker S, et al. Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. Nature. 2015;519(7542):237–241.
17. Németh A, Röhlich P. Rapid separation of rat peritoneal mast cells with Percoll. Eur J Cell Biol. 1980;20(3):272–275.
18. Passante E, Ehrhardt C, Sheridan H, Frankish N. Measuring mast cell mediator release. Curr Protoc Pharmacol. 2009: 12(12.7).

19. Roy S, Chompunud Na Ayudhya C, Thapaliya M, Deepak V, Ali H. Multifaceted MRGPRX2: new insight into the role of mast cells in health and disease. J Allergy Clin Immunol. 2021;148(2):293–308.
20. Rönnberg E, Calounova G, Guss B, Lundequist A, Pejler G. An optimized protocol for the isolation and functional analysis of human lung mast cells. Front Immunol. 2018;9:2193.
21. Tanio T, Tsuge I, Shioya M, et al. Validation of HPLC method for determination of histamine in human immunoglobulin formulations. J AOAC Int. 2020;103(5):1223–1229.
22. Tsvilovskyy V, Solis-Lopez A, Öhlenschläger K, Freichel M. Isolation of peritoneum-derived mast cells and their functional characterization with $Ca^{2+}$-imaging and degranulation assays. J Vis Exp. 2018;(137):57222.