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Regulation of basophil response in the management of allergic disorders

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Abstract
The present investigation describe the anti-allergic effect of a poly-herbal extract which was established by three distinct methods using KU812 cell lines and IL33 release inhibition by HaCaT cells. Basophil inactivation leading to suppression of histamine release, inhibition of PGD2 and the release inhibition of IL33 by HaCaT cells. All the three studies clearly show the effect of the poly-herbal exact in concentration dependent manner. The finding thus obtained from two separate cells lines that release different mediators responsible for allergic manifestation confirm the strong pharmacological effect of the poly-herbal extract and its possible medical value in the treatment of allergic disorders. Further, the no cytotoxic effect of the poly-herbal extract to both the cell lines by MMT was also established. The findings of the investigation is described in the article.

Keywords: Herbal preparation for skin allergy, basophil inactivation, PGD2 inhibition, itching, urticarial reaction

Introduction
Basophils are a group of white blood cells present in the fluid tissue in our body and they are otherwise called as leukocytes or granule containing cells [1]. Basophil play a significant role in the immune defense system. The granules, especially the histamine is released from basophils during allergic condition and the histamine would increase the blood flow and elicit inflammatory process. The increased level of basophils are medically referred as basophilia, an early indicator of allergic and inflammatory state [2]. In most allergic skin disorders such as atopic dermatitis and auto-immune condition, managing the response of basophils in critical and such an approach may offer effective treatment benefit to the allergic response [3].

Though mast cells are also involved in the clinical manifestation of allergic response, the role of basophils is more significant than mast cells as the mast cells are tissue resident cells while the basophils are in circulation as they are found in the fluid tissue (blood) and further, they play a major role in anaphylaxis [4]. Basophils play a significant role in the adaptive immune response, inflammation and the production of cytokines such as interleukin IL4 that modulate Th2 differentiation and development in allergic and inflammatory conditions.

The common clinical manifestation of allergic disorders are itching, urticarial reaction, oedema, inflammation and erythema. The allergic manifestations would recur with great frequency in those susceptible individuals and those who suffer from various auto-immune conditions.

In the conventional treatment, two lines of pharmacological preparations are used for the management such as use of anti-allergic/anti-histaminic drugs and steroids [5].

Most of the antihistamines can easily cross the blood-brain-barrier (BBB) and can block the histamine receptors in the central nervous system and thereby would results in sedative response [6]. Histamine in the brain region is the responsible for wakefulness and alert response. Besides that, some antihistamines also would antagonize cholinergic, serotonergic and alpha-andrenergic receptors and thereby cause sedation [7].

The prolonged usage steroidal preparations on the other hand, would cause severe side effects including long term, irreparable health complications.

The need for newer drugs and or drug combinations that are effective and safe for long term usage is imminent to mitigate the challenge. Only herbal preparations can offer some hope in this regard as herbal preparations by and large are safe, undergo rapid metabolic changes and cost effective.
In the present study, we report the possible treatment benefit of a herbal preparation in the management of allergic conditions by modulating the response pattern of basophils.

Materials and Methods

Details of Polyherbal extract

The Polyherbal extract is composed of the following herbs such as Withania somnifera, Phyllanthus embilica, Smilax chinensis, Corallocarpus epigaeus and Clerodendrum inerme and is formulated as tablet oral dosage form with the brand name Hista block tablet

Basophil activation inhibition assay in KU812 cell line

KU812 cell line was used for the present study. The cells were grown in RPMI 1640 with 10% FBS along with Penicillin and Streptomycin to prevent the microbial contamination [8].
KU812 cells were plated into 96-well plates at 1×10^5 cells/well in 100 µL RPMI-1640., and incubated for 30 min at 37 °C in 5% CO_2 incubator.
The polyherbal extract mixture Withania somnifera, Phyllanthus embilica, Smilax chinensis, Corallocarpus epigaeus and Clerodendrum inerme in 1% DMSO was prepared and the same was used. Anti-IgE was used as positive control to activate the basophils [9].
Histamine ELISA kit was used to detect histamine released by the cells and the same was read by microplate reader at 450 nano meter.

Group Description

- NC Negative Control (vehicle only)
- PC Positive Control (e.g., anti-IgE)
- The concentration of polyherbal extract mixture (H1-H4) used for the study were 0.1, 1, 10, 50 µg/mL.

The cells were incubated with the polyherbal extract mixture at the above concentrations for 60 min at 37 °C in 5% CO_2 incubator. After incubation for 60 minutes, the cells were challenged with IgE; except the cells in the negative control, for 1 hour. Then, the supernatant was collected by centrifugation at 300g for 5 minutes and the histamine released by cells under different treatment condition was measured by ELISA.

PGD_2 inhibition

The lipid mediator PGD_2 is released mainly by the mast cells, and the same is strongly involved in allergic and inflammation related medical problems.
For the present study, KU812 cell line was used and the measurement of PGD_2 was done by ELISA [10]. Calcium ionophore A23187 was used as positive control to stimulate PGD_2 release from KU812 Cells. As described, the cells were plated (KU812) in 96-well plates at 1×10^5 cells/well in 100 µL complete medium and Incubated overnight at 37 °C, 5% CO_2.
The polyherbal extract mixture at the pre-defined concentrations were treated with the cells along with separate negative control and incubated for 1 hr. Then Calcium ionophore A23187 (1-2 µM) was added to stimulate the release of PGD_2 and the cells were further incubated for 60 minutes at 37°C in 5% CO_2 incubator. After incubation, the mixture was centrifuged at 300 g for 5 minutes, the supernatant was collected and PGD_2 was read by ELISA at 450 nm by microplate reader.

Effect of the polyherbal extract mixture in inhibiting the release of allergen- IL 33 by KC

Keratinocytes are known to release IL-33 when stimulated/ exposed to environmental triggers and Pro-inflammatory cytokine- TNF-α + IL-1β [11].
HaCaT cell line was used for the present study. The cells were grown in DMEM + 10% FBS + antibiotics. Then the TNF-α -10 ng/mL + IL-1β 10 ng/mL were used to stimulate the allergic reaction by the cells. The release of IL-33 was then measured by ELISA.

Group	Description
NC	Negative control (unstimulated cells)
PC	Positive control (stimulated only)
H1-H4	the polyherbal extract mixture at increasing doses

The HaCaT cells were grown in 96-well plates at 5×10^4 cells/well in 100 µL in the cell culture medium and incubated overnight at 37 °C with 5% CO_2. After growth of the cells, the polyherbal extract mixture at the pre-defined concentration was treated separately and the cells were incubated further period of 1 hr. Then the cells under all treatment group except negative control were treated with TNF-α + IL-1β (10 ng/mL each and then incubated for 24 hours. The supernatant was collected and read by ELISA for the release of IL-33.

Results

Basophil activation inhibition assay in KU812 cell line
The polyherbal extract mixture treatment has significantly inhibited the release of histamine from KU812 cells and the effect showed perfect linearity with concentration of the polyherbal extract mixture tested.

Table 1

Sl. No.	Details	Con. µg/ml	Histamine µg/ml	% reduction in histamine release	Cell viability
1	Vehicle control	0	2.8	0	98
2	Anti-IgE	1	16.5	-	98
3	Polyherbal extract mixture	0.1	10.1	38.7	98
4		1	8.3	49.6	98
5		10	4.6	72.1	98
6		50	3.7	77.5	98

PGD2 inhibition

The polyherbal extract mixture exhibited strong inhibition of PGD_2 in a concentration dependent manner,

Table 2

Sl. No.	Details	Con. µg/ml	PGD_2 pg/ml	% inhibition
1	Negative control	0	38	-
2	Positive control	1	342	-
3	Indomethacin	10	30	91.2
4	polyherbal extract mixture	0.1	98	71.3
5		1	72	78.9
6		10	66	80.7
7		50	54	84.2

Effect of polyherbal extract mixture k in inhibiting the release of allergen- IL 33 by KC

The polyherbal extract mixture showed significant effect in inhibiting the release of IL33 and the effect showed marginal linearity with concentration,

Table 3

Sl. No.	Details	Con. µg/ml	IL-33 pg/ml	% inhibition
1	Negative control	0	58	-
2	Positive control	1	410	-
3	Dexamethasone	10	134	67.3
4	Polyherbal extract mixture	0.1	320	21.9
5		1	280	31.7
6		10	220	46.3
7		50	170	58.5

Discussion

The present investigation was conducted to understand the scope of a herbal preparation in the management of basophil response so the same polyherbal extract mixture can be further explored for the treatment of allergic conditions. We chose to study basophils over mast cells due to the relative dominance of basophils and their presence particularly in the fluid tissue that is blood [12]. Further, basophils also play a significant role in anaphylaxis, and other inflammatory mediations. Whereas the mast cells are tissue resident cells and at best elicit the response more localized.

For basophil activation studies, we used KU812 cells. KU812 cell line is nothing but human pre -basophilic cell line obtained from chronic myelogenous leukemia and the cells can be induced to differentiate into mature basophils in laboratory condition. Due to the above advantage, KU812 is extremely useful for studying the allergic and inflammation related medical conditions at *in vitro* level. The KU812 cell line is an ideal surrogate for investigating how different stimuli, such as liposomes, cytokines like IL-33 and IL-18, or other agents, interact with basophil and activate them and induce degranulation [13].

The polyherbal extract mixture at dose dependent manner showed strong effect in preventing the release of histamine by the KU812 cells. We have used Anti-IgE as positive control to trigger the response. Though the molecular mechanism of action of the polyherbal extract mixture is unknown, but the specific effect of the polyherbal extract mixture in inhibiting the release of histamine is certain and hence the polyherbal extract mixture, prima facie assumes great pharmacological significance.

In order to understand further about the second level of confirmation for the polyherbal extract mixture having definite anti-allergic effect, preventing the release of PGD_2 by KU812 was studied by ELISA. Calcium ionophore A23187 was used to stimulate the release of PGD2 by KU812 cells and the inhibitory effect of Indomethacin was used as positive control for comparison.

The study findings clearly display the polyherbal extract mixture having definite effect in inhibiting the release of PGD2 and the effect was comparable with Indomethacin. After confirming the response pattern of KU812 cells to the polyherbal extract mixture, we further studied whether the similar anti-allergic effect the polyherbal extract mixture could exert when we use HaCaT cells as well. Using HaCat cells, we explored the release pattern of IL33, one of the pre-eminent inflammatory mediators in the allergic process. In order to trigger the release of IL33, we used the combination of TNF-α + IL-1β [14]. The polyherbal extract mixture surprisingly showed strong and consistent effect in inhibiting the release of IL33 by HaCaT cells despite the challenge by TNF-α + IL-1β.

We performed the three tests only to establish the anti-allergic effect of the polyherbal extract mixture, conclusively so the real pharmacological effect of the same can be understood correctly. During the study, the effect of the polyherbal extract mixture having any cytotoxic effect on PU812 and HaCaT cells were also studied by MTT. Up to 200 µg/ml concentration of the polyherbal extract mixture did not show any cytotoxic effect on either of the cells tested by us. Further, all the herbs used in the polyherbal extract mixture are considered safe based on their prolonged use in various paramedical and home remedy practices in Indian system of medicine.

The present investigation has offered immense proof on both the pharmacological effect and the possible target sites to prelude the possible mechanism of action of the polyherbal extract mixture viz., the models that we used in the present study such as PU812 and HaCaT cells, both leading to a same inference of basophil modulation.

The question of whether the polyherbal extract mixture by inhibiting histamine release do cause sedation is quite easy to answer. We do not assume the polyherbal extract mixture could cross BBB and would block histamine in the brain region to cause sedation [15]. A separate experiment was conducted to understand the direct effect of polyherbal extract mixture on histamine and which proved there is no effect between them. The findings suggest that the polyherbal extract mixture is effective at the level of cells, especially PU812 and HaCaT and may not be at histamine.

The rate of metabolism of the drug, its first pass effect and rate of clearance are though needed but the findings clearly prove the polyherbal extract mixture has definite effect on basophils at *in vitro* level, and hence, it is likely to have some pharmacological effect in the management of allergic conditions.

References

1. Liu CB, Wu BF, Luo LQ, Wang JY, Hu WP, Zhang XT, Gao HZ. Elevated basophils in peripheral blood predict lower extremity deep venous thrombosis in non-surgical patients with spontaneous intracerebral hemorrhage. Sci Rep. 2025 Mar 3;15(1):7397. doi:10.1038/s41598-025-91851-x.
2. Siracusa MC, Kim BS, Spergel JM, Artis D. Basophils and allergic inflammation. J Allergy Clin Immunol. 2013 Oct;132(4):789-801; quiz 788. doi:10.1016/j.jaci.2013.07.046.
3. MacGlashan DW Jr, Savage JH, Wood RA, Saini SS. Suppression of the basophil response to allergen during treatment with omalizumab is dependent on two competing factors. J Allergy Clin Immunol. 2012 Nov;130(5):1130-1135.e5. doi:10.1016/j.jaci.2012.05.038.
4. He SH, Zhang HY, Zeng XN, Chen D, Yang PC. Mast cells and basophils are essential for allergies: mechanisms of allergic inflammation and a proposed procedure for diagnosis. Acta Pharmacol Sin. 2013 Oct;34(10):1270-1283. doi:10.1038/aps.2013.88.
5. Skröder C, Hellkvist L, Westin U, Sahlstrand-Johnsson P, Hansson K, Karlsson A, Dahl Å, Bjermer L, Cardell LO. Prednisolone versus antihistamine for allergic rhinitis: No significant difference found in randomized trial. Clin Transl Allergy. 2025 Jan;15(1):e70017. doi:10.1002/clt2.70017.
6. Simons FE, Simons KJ. H1 antihistamines: current status and future directions. World Allergy Organ J. 2008 Sep;1(9):145-155. doi:10.1186/1939-4551-1-9-145.
7. Church MK, Church DS. Pharmacology of antihistamines. Indian J Dermatol. 2013 May;58(3):219-224. doi:10.4103/0019-5154.110832.
8. Plant-Hately AJ, Eryilmaz B, David CAW, Brain DE, Heaton BJ, Perrie Y, Liptrott NJ. Exposure of the basophilic cell line KU812 to liposomes reveals activation profiles associated with potential anaphylactic responses linked to physico-chemical characteristics. Pharmaceutics. 2022 Nov 15;14(11):2470. doi:10.3390/pharmaceutics14112470.
9. Plant-Hately AJ, Eryilmaz B, David CAW, Brain DE, Heaton BJ, Perrie Y, Liptrott NJ. Exposure of the basophilic cell line KU812 to liposomes reveals activation profiles associated with potential anaphylactic responses linked to physico-chemical characteristics. Pharmaceutics. 2022 Nov 15;14(11):2470. doi:10.3390/pharmaceutics14112470. **(Duplicate kept as requested)**
10. Benyon RC, Robinson C, Holgate ST, Church MK. Prostaglandin D2 release from human skin mast cells in response to ionophore A23187. Br J Pharmacol. 1987 Nov;92(3):635-638. doi:10.1111/j.1476-5381.1987.tb11366.x.
11. Chen Z, Yang Y, Wang X, Xia L, Wang W, Wu X, Gao Z. Keloids and inflammation: the crucial role of IL-33 in epidermal changes. Front Immunol. 2025 Mar 31;16:1514618. doi:10.3389/fimmu.2025.1514618.
12. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. J Allergy Clin Immunol. 2010 Feb;125(2 Suppl 2):S73-S80. doi:10.1016/j.jaci.2009.11.017.
13. Hara T, Yamada K, Tachibana H. Basophilic differentiation of the human leukemia cell line KU812 upon treatment with interleukin-4. Biochem Biophys Res Commun. 1998;247(3):542-548. doi:10.1006/bbrc.1998.8816.
14. Lee JY, Park JY, Jeong Y, Kang CH. Anti-inflammatory response in TNFα/IFNγ-induced HaCaT keratinocytes and probiotic properties of *Lacticaseibacillus rhamnosus* MG4644, *Lacticaseibacillus paracasei* MG4693 and *Lactococcus lactis* MG5474. J Microbiol Biotechnol. 2023 Aug 28;33(8):1039-1049. doi:10.4014/jmb.2301.01028.
15. Leclerc M, Dudonné S, Calon F. Can natural products exert neuroprotection without crossing the blood-brain barrier? Int J Mol Sci. 2021 Mar 25;22(7):3356. doi:10.3390/ijms22073356.