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### Effective Management of Allergy by a Siddha preparation- An In Vitro Study

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#### Abstract

Allergy or hypersensitivity is a common clinical condition encountered worldwide. Histamine and cytokines like interleukin (IL) 1 $\alpha$  and IL8 play a key role in inflammatory allergic disorders. Mast cells and basophils release histamine on sensitization. Moreover, keratinocytes synthesize and release IL1 $\alpha$  and IL8, which also activate basophils to release histamine. The present study demonstrates that G7, a Siddha medicine has properties in moderating the release of histamine, IL1 $\alpha$  and IL8 and therefore is a promising alternative for the management of allergic disorders.

#### Introduction

An allergic reaction of the skin is described in Siddha (a traditional system of medicine practiced in India for over 2000 years) as Kanakkadi. In Siddha medical practice, both topical and internal drug therapy are employed in the management of allergy. It is a major clinical entity encountered very often in clinical practice [1]. Allergy can manifest in various forms such as local or generalized itching, contact dermatitis, urticaria and eczema etc. [2]. The hypersensitive reaction may also lead to anaphylactic shock resulting in mortality [3]. Histamine is one of the preformed mediators synthesized by cells such as basophils and mast cells and is stored in granules [4,5]. On de-granulation of these cells,

histamine is released and its low molecular weight enables its rapid diffusion into tissues that contributes to itching and mediates inflammatory response [6].

Keratinocytes synthesize and secrete cytokines that mediate intercellular communication. When exposed to various allergens, the interleukins (ILs) released from these cells degranulate basophils to release histamine and also stimulate oxidative stress that form free radicals and other inflammatory mediators [2,7,8].

The present in vitro study reports the modulatory effect of G7 (a Siddha medicine) in release of histamine, IL1 $\alpha$  and IL8 involved in allergic and inflammatory responses.

## Materials and Methods

**Composition of G7:** Each 500 mg capsule contains extract of:

Phyllanthus emblica 50mg  
Smilax chinensis 100mg  
Withania somnifera 100mg  
Corallocarpus (Bryonia) epigaeus 75mg  
Clerodendrum inerme 100mg  
Processed Gandhakam 25mg  
Conch turbinella rapa parpam 25mg  
Linga chenduram 1mg  
Ponmimilai (copper pyrite) chenduram 1mg  
Excipients q.s.

### Effect of G7 in histamine release from mast cells:

Mast cells were isolated from peritoneal cavity of an albino rat after intra-peritoneal (I.P.) injection of thioglycolate solution and were maintained in RPMI 1640 (Roswell Park Memorial Institute medium- HiMedia, India). Substance P, a neuro-mediator, prepared at 3.10<sup>-5</sup>M was used as the mast cell de-granulating substance [1]. Out of 5 sets of cultured mast cells used in the study, set 1 served as a control and set 2 was treated with substance P. The sets 3, 4 and 5 were pre-treated with G7 at 20 $\mu$ g, 40 $\mu$ g and 100 $\mu$ g respectively before treatment with substance P. All the sets were incubated at 37°C for 20 minutes. After incubation, the reaction was stopped by reducing the temperature to 4°C. The reaction mixture was centrifuged and the supernatant was assayed for histamine using an Immunotech Kit (Beckman Coulter - Cat No.2562).

### Effect of G7 in release of IL1 $\alpha$ from keratinocyte culture:

Keratinocytes were maintained in KFSM medium (Gibco, BRL - France) at 37°C in an environment containing 5% CO<sub>2</sub>. The experiment was divided into four sets. In the set 1, the cultured keratinocytes were irradiated with UVA+UVB at 0.15 J/Cm<sup>2</sup>. In the set 2, 3 and 4, G7 was administered at 20 $\mu$ g, 40 $\mu$ g and 100 $\mu$ g respectively before irradiation. After exposure, the cells were incubated at 37°C for 24hrs. After incubation, the supernatant was collected and assayed for IL1 $\alpha$  by ELISA (Beckman Coulter Cat. No. IM0755).

**Effect of G7 in release of IL8 from keratinocyte culture:**

To study the release of IL8, the procedure followed was similar to the above using ELISA kit (Cat.No.IM2237). Besides irradiation, PMA (Pharbol-12-myristate 13-acetate) was also used as IL8 stimulant separately [7].

**Results**

The histamine release from the cultured mast cells lowered with increasing dose of G7 (**Table 1**).

The release of IL1 $\alpha$  was highly suppressed by G7 at 100 $\mu$ g concentration (**Table 2**).

G7 effectively suppressed the release of IL8 by the keratinocytes when irradiated by UV or induced by PMA (**Table 3**).

Set Nos	Experiment	Quantity of Histamine (nM)
1	Control ( Mast Cells)	248
2	Mast Cells treated with substance P	1457
3	Mast Cells treated with substance P + 20 $\mu$ g of G7	1300
4	Mast Cells treated with substance P + 40 $\mu$ g of G7	1216
5	Mast Cells treated with substance P + 100 $\mu$ g of G7	1080

**Table 1:** Effect of G7 in the release of histamine from cultured mast cells

Treatment sets	Variation of IL1 $\alpha$ Levels by the Test / Control %
G7 - 20 $\mu$ g	-21 $\pm$ 6
G7 - 40 $\mu$ g	-36 $\pm$ 8
G7- 100 $\mu$ g	-49 $\pm$ 5

**Table 2:** Effect of G7 in the release of IL1 $\alpha$  from cultured keratinocyte

Treatment sets	Variation of IL8 levels in the Test / Control % (UV as inducer)	Variation of IL8 Levels by the Test / Control % (PMA as inducer)
G7 - 20 $\mu$ g	-23 $\pm$ 3	-28 $\pm$ 6
G7 - 40 $\mu$ g	-44 $\pm$ 5	-30 $\pm$ 8
G7- 100 $\mu$ g	-69 $\pm$ 6	-48 $\pm$ 7

**Table 3:** Effect of G7 in the release of IL8 from cultured keratinocyte

## Discussion

Mast cells are granulocytic cells that contain histamine. When exposed to allergen, the mast cells de-granulate by active and sensitized IgE. On de-granulation, mast cells release histamine, a potent vasoactive amine. Histamine on its absorption into the skin induces itching and dilates the blood vessels. Therefore, histamine inactivation or mast cell stabilization is essential in the management allergic responses [8]. The present study clearly reveals that G7 is effective in preventing mast cell de-granulation event, which is evident from the recorded lower quantity of histamine released in vitro.

We also studied the effect of G7 in controlling the participation of keratinocytes in the allergic and inflammatory process. Keratinocytes synthesize the key interleukins such as IL1 $\alpha$  and IL8. These interleukins activate the basophil-mediated histamine release and other oxidative damages. We found that G7 effectively controls the release of these key interleukins and thereby effectively may help to manage allergic reactions in the skin.

## Conclusion

The formulation coded G7 is a known drug available in the Indian market (manufactured and marketed by Dr. JRK Siddha Research and Pharmaceuticals Pvt. Ltd., Chennai, India). The drug is of Indian Systems of Medicine origin. In the traditional Siddha literature, the ingredients used in this formulation have been indicated to have several medicinal properties including anti-allergic activity. The test drug is already available in the market for the last 10 years and there are no reported side effects. The study drug - G7, can therefore be used for the management of allergic conditions

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