



EX-VIVO EVALUATION OF ANTI-HISTAMINIC POTENTIAL OF NOVEL SIDDHA FORMULATION SENGATHARI PATTAI KUDINEER BY USING ISOLATED CHICK ILEUM PREPARATION**D.Sowmiya^{*1}, S.Manju², V.Rani³, N.Manju hemamlini⁴**

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ABSTRACT

Atopic dermatitis (AD) is a chronic inflammatory skin disease affecting approximately 10 million people, who are also prone to experiencing relapses. AD is accompanied by pruritus, dry skin, eczema, and keratinization. Histamine is one of the important mediators of allergy, inflammation and bronchoconstriction, which were released after degranulation of mast cell by an antigen exposure. Targeting histamine, either prevention of its release from mast cell or use of histaminergic receptor antagonist becomes part of antihistaminic therapy in AD. Topical corticosteroids are not ideal because when used for a long time, they have side effects, such as cutaneous atrophy, immunosuppression, and thinning of the skin. Herbal medicines as an alternative therapy for AD have been widely used in many countries for decades. Clinical studies on the use of such remedies have reported favourable benefits to the patients reported with AD. Siddha system of medicine is one of the most renowned traditional practice that has survived and flourished since several centuries. Sengathari pattai kudineer (SPK) is a novel herbal formulation utilised in the form of decoction is known for anti-histaminic property as per the literature. Hence the present study aimed at screening the anti-histamine potential of SPK using isolated chick ileum assay. It was observed from the data's obtained from the present investigation that the height of response of concentration response curve of histamine before incubation with test drug ranges from 19 mm to 62 mm. There was a promising decrease in the height of the response curve after incubation with test drug SPK ranges from 15 mm to 41 mm. It was concluded that the siddha formulation SPK possess promising anti histamine property in the tested model and further studies need to be navigated to explore the mechanistic pathway by which the drug acts on the biological system.

KEY WORDS: Atopic dermatitis, Siddha, Sengathari pattai kudineer, Anti-histamine, Isolated chick ileum assay

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1. Introduction

Atopic dermatitis (AD) is an allergic skin disease characterized by complex symptoms such as drying and thickening of the skin, and scratch marks that are frequently associated with immunoglobulin-E (IgE) hyper-responsiveness to environmental allergens. The wrist, neck, face, and the crooks of the elbows and knees are among the most frequent locations of the lesions [1]. AD is mostly affecting children with onset before the age of five years [2]. Environmental (house dust mites and air pollution) and genetic factors considered the causes of AD. In addition, genetic predisposition accompanied by assorted peculiar immune symptom accounts for more than 50% of reported cases [3].

Inflammation limits the damage to the body cells after invasion by foreign organisms or mechanical injury, but if goes uncontrolled and untreated, it becomes a threat leading to other illnesses such as arthritis, cardiovascular disease, asthma, and cancer. Histamine is one of the most common inflammatory mediators; it causes symptoms of allergic reactions that mostly involve acute inflammation mediated by the H1 histamine receptor [4]. The histamine H1 receptor is mainly expressed in endothelial cells, smooth muscle cells and brain, and contributes to vasodilation, increase of vascular permeability [5] and pain [6] at the cellular level, while causing enhanced production of intracellular calcium (Ca²⁺) and nitric oxide (NO) [7] at the molecular level.

Recent studies have broadened the knowledge of immunological and molecular mechanisms involved in AD disease. For example, it is now known that infiltration of immune cells (e.g., T cells, mast cells, and dendritic cell subtypes) is increased in AD lesions, the serum IgE level is elevated in AD patients compared to that in patients affected by several inhaled allergens, and the increase of secreted Th2 helper cytokines is highly correlated with the disease [8]. The current common therapy is the anti-inflammatory treatment of visible skin lesions using steroids, including topical corticosteroids (e.g., glucocorticosteroids), topical calcineurin inhibitors (e.g., tacrolimus and pimecrolimus), or both [9]. Although these topical treatments are able to alleviate AD symptoms, reduce inflammation, and prevent flares, they are associated with long-term use side

effects. These include local cutaneous atrophy, striae formation caused by topical corticosteroids, and stinging upon application of topical calcineurin inhibitors [10]. Thus, there is a large unmet need for safe and effective AD therapeutics. Recently, there has been a growing appeal for alternative therapeutic agents for AD treatment, especially natural bioactive compounds from plants extracts [11].

Siddha system of medicine is one of the most renowned traditional practice that has survived and flourished since several centuries. With the enormous knowledge on medicinal herbs and related supplements, the relationship of physiology of biological system and their relevance and coordination in wellbeing of the individuals was well understood. Several aspects of siddha is still unexplored. Sengathari pattai kudineer (SPK) is a novel herbal formulation utilised in the form of decoction is known for anti-histaminic property as per the literature. Hence the present study aimed at screening the anti-histamine potential of SPK using isolated chick ileum assay.

2. Materials and Methods

2.1. Anti-Histamine evaluation using isolated chick ileum [12]

Chick ileum was purchased from local slaughter house in which the caecum part of the gut was lifted to identify the ileocaecal junction. About 2- 3cm of the ileum portion was cut and removed and immediately placed it in the watch glass containing physiological salt solution. Sufficient care was taken to avoid the damage to the gut muscle. Bath volume of about 25 ml was maintained, and the tissue was allowed to equilibrate for 30 min before adding test drug. Initial response on histamine induces the contraction in the ileal smooth muscles which were recorded on Kymograph by using frontal writing lever. Contact time of 30 sec, and 5 min time cycle was kept for proper recording of the responses. After measuring normal response, the ileal preparation were incubated with test drug (2ml) for brief period of time and the concentration response curved of histamine was then proceeded the height of response before and after incubation of test drug was measured for calculating the antagonist effect of the test drug.

3.Results

3.1. Effect of SPK on Dose response curve of isolated chick ileum preparation

Chick ileum preparation serves as remarkable tool for screening the anti-histamine property of the drug under investigation in recent times. Due to its simplicity and reliability the outcome of this study attains greater importance. In simple term the efficacy of the drug reclaims by it tendency to halt the contraction of the ileal smooth muscle even in the presence of potential agonist like histamine. This activity progressed as right shift from the conventional dose response curve. It was observed from the data's obtained from the present investigation that the height of response of concentration response curve of histamine before incubation with test drug ranges from 19 mm to 62 mm. There was a promising decrease in the height of the response curve after incubation with test drug SPK ranges from 15 mm to 41 mm. As show in table 1, figure 1&2.

Table 1: Effect of SPK on dose response of isolated chick ileum preparation

Dose in mcg	Initial Response in mm (Before Incubation)	Final response in mm (After incubation with Test drug SPK)
10	19	15
20	31	21
40	42	30
80	62	41

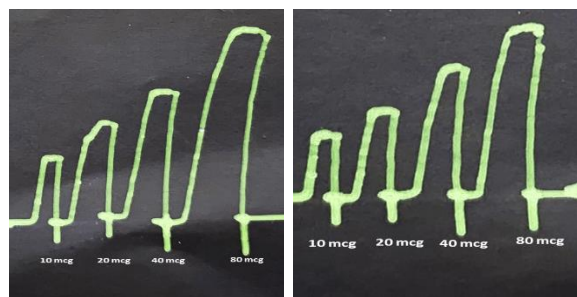


Figure 1: Concentration response curve of histamine in absence and Presence of sample SPK on Isolated chick ileum in optimized condition

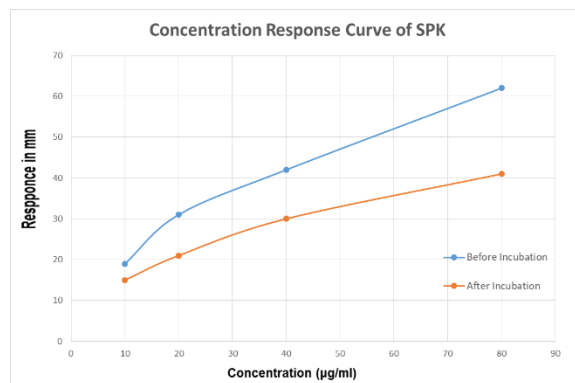


Figure 2: Concentration Dose response curve of SPK

4.Discussion

Atopic dermatitis (AD) is an allergic skin disease characterized by complex symptoms such as drying and thickening of the skin, and scratch marks that are frequently associated with immunoglobulin-E (IgE) hyper-responsiveness to environmental allergens. The wrist, neck, face, and the crooks of the elbows and knees are among the most frequent locations of the lesions. AD is mostly affecting children with onset before the age of five years. Environmental (house dust mites and air pollution) and genetic factors considered the causes of AD. In addition, genetic predisposition accompanied by assorted peculiar immune symptom accounts for more than 50% of reported cases [13]. In spite of the profound side effects caused by topical steroids and oral anti-histamines, these drugs are commonly used to treat AD. The binding of antigen activates infiltrated cells to secrete chemical mediators such as histamine, proteases, cytokines, and chemokines that are essential in the progression of dermatitis [14].

Histamine contracts the muscle of guinea pig, goat, horse, dog, chick and humans. Chick ileum assay found easy to prepare; it is also much more sensitive than guinea pig tracheal chain. Therefore, the dose-relative contractile responses of agonist like histamine can be observed in isolated chick ileum preparation. In this assay there was right side shift of DRC of histamine in the presence of SPK, which indicate antihistaminic activity.

Topical corticosteroids are important anti-inflammatory drugs that alleviate redness, itching, and inflammation, and have been the mainstay of AD treatment for the past few decades [15]. Topical corticosteroids are not ideal because when used for a long time, they have side effects, such as cutaneous

atrophy, immunosuppression, and thinning of the skin [16]. Tacrolimus, an immunosuppressive agent, is an alternative to topical corticosteroids, as a calcineurin inhibitor [17]. It is useful for treating thin-skin areas, such as the face and flexures. However, tacrolimus has side effects, such as skin burning, hypertension, nephrotoxicity, and renal injury [18].

Considering the 3R policy suggested by the CPCSEA on reduction, refinement and replacement the chick ileum assay offers potential biomedical application in availing the slaughter house waste as one of viable research tool. Preliminary screening of compounds in order to ascertain the anti-histaminic efficacy shall be easily screened in this ex-vivo model. Being a slaughter house waste this methods doesn't even requires the ethical approval. Histamine receptors are diversely distributed throughout the intestinal flora of the chick ileum upon contact with the agonist like histamine it began to contract and the tracing were been noted in the kymograph. Percentage shit in dose response curve determines the anti-histamine potential of the study drug. It was observed from the data's obtained from the present investigation that the height of response of concentration response curve of histamine before incubation with test drug ranges from 19 mm to 62 mm. There was a promising decrease in the height of the response curve after incubation with test drug SPK ranges from 15 mm to 41 mm.

5. Conclusion

Atopic dermatitis (AD) is a common, highly pruritic, chronic inflammatory skin disease. Various antihistamines are used in the treatment of allergic disorders due to their H1-antagonism. In spite of the availability of abundant antihistamines in the market, the search continues for the development of novel antihistaminic agents with reduced sedation, anticholinergic and cardiovascular effects Herbal plant extracts are promising agents in AD treatment because they have fewer side effects and are safer to use compared to synthetic derivatives. It was concluded from the outcome of the present study that the siddha formulation SPK possess promising anti histamine property in the tested model and further studies need to be navigated to explore the mechanistic pathway by which the drug acts on the biological system.

Acknowledgement

We wish to acknowledge my thanks to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India and The Noble research solutions, Chennai, Tamil Nadu, India for their support.

6. References

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