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# Effect of Eraippu Noi Chooranam (ENC) on Histamine and Acetylcholine Induced Isolated Guinea Pig Ileum

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**Abstract:** Eraippu Noi Chooranam (ENC) is a modified Siddha Poly Herbal formulation indicated for respiratory diseases in the text Siddha research pharmacopoeia. The indicated traditional claim enforced to evaluate its efficacy in the management of Bronchial asthma by evaluating its spasmolytic activity of ENC on histamine and acetyl choline induced isolated guinea pig ileum. The present study was designed to evaluate the antiasthmatic effect of Eraippunoi chooranam on Guinea pig ileum. In the present study, aqueous extract of ENC was evaluated for its antiasthmatic activity using histamine and acetylcholine-induced bronchospasm, in guinea pig at different dose levels. An assembly for isolated tissue consisting of the specified apparatus (Sherrington revolving drum and an organ bath) was used. Sub maximal doses of acetylcholine, histamine and different doses of ENC were administered. The responses were recorded on the kymograph. A dose response curve was obtained and then contraction inhibition percentage produced was recorded. The result obtained indicated that the test drug exhibited significant spasmolytic activity. The IC<sub>50</sub> value of ENC with acetyl choline was found to be 36.22% and IC<sub>50</sub> value of acetyl choline was found to be 13.24%. The IC<sub>50</sub> value of ENC with histamine was found to be 25.80% and IC<sub>50</sub> value of histamine was found to be 20.66%. It can be concluded that aqueous extract of ENC may be used in the management of asthma.

**Keywords:** Bronchial asthma, Siddha medicine, Poly Herbal formulation, Eraippu Noi Chooranam, Bronchospasm

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## Introduction

Every human race has its own distinct conventional medical system. The traditional medicines in our country are the oldest, richest,

and most varied in the entire globe. Many ethnic groupings in India use thousands of different plant species. Siddha medicine is a component of

the Indian medical system that is incredibly effective and distinctive in its own right since it offers healing for the body, mind, and spirit. The Siddha system, as put forward by the Siddhars, is a comprehensive and flexible system (Thirunarayanan, 2012).

The burden of asthma is becoming greater as communities adopt western lifestyles and become urbanised. Bronchial asthma is chronic inflammatory disorder of the respiratory tract, characterised by increased airway hyper responsiveness to provocative exposures and episodic airflow obstruction (Nadig and rajeshwari, 2016). The diverse disease of the airways known as asthma is characterised by bronchial and smooth muscle hyper-reactivity as well as persistent inflammation (Maurya *et al.*, 2013). It is characterised by a constriction of the airways, wheezing that occurs frequently, dyspnoea, chest tightness, waking up during the night, and coughing (Holgate *et al.*, 2008). The common symptom associated with these diseases is breathlessness, which is a most distressing symptom, affecting their day to day activities. Many causes, including allergens, respiratory infections, dust, cold air, exercise, emotions, workplace stimulation, certain drugs/chemicals, histamine, and inheritance, can cause asthma (Kavitha *et al.*, 2020). The production of interleukins (IL-4 and IL-5) and other inflammatory agents, such as eosinophils, neutrophils, cytokines and chemokines, which cause throat inflammation or obstruction, bronchial hyper responsiveness, and mucosal hypersecretion, are accelerated by these trigger factors (Sagar *et al.*, 2014).

Breathing exercise play a major role in controlling Asthma. Breathing exercises are useful and specific measures to increase the volume of air entering the lungs as well as being expelled from lungs. Despite a wide variety of antiasthmatic medications being readily available, the relief they provide is primarily symptomatic and exhibits a poor or absent response even at high doses with some or more side effects

(Annalakshmi, 2011). In order to produce new drugs that are safe and efficient for treating bronchial asthma, it is ideal to only use herbal sources. In this regard, a safe and effective herbal drug will be the choice of the individual to overcome the situation.

Though Siddha drugs are considered to be safe and effective, it is the utmost duty of the physicians to validate the formulation before trying out in humans. Siddha system of medicine enunciates the usage of herbs and herbal formulations since time immemorial (Sharma *et al.*, 2022). Poly Herbal formulation composed of multi components which produce synergistic effect apart from boost up body natural immunity. Eraippu Noi Chooranam is a modified Siddha Poly Herbal formulation mentioned in the text Siddha research pharmacopoeia. It is indicated for asthma, chronic bronchitis and flatulence. It is a poly herbal drug and all the ingredients included are very effective in curing kapha diseases. The indicated traditional claim enforced to evaluate its efficacy in the management of Bronchial asthma (Amala Hazel *et al.*, 2016). The main aim was to study the effect of Eraippunoi chooranam on Histamine and Acetylcholine induced Guinea pig ileum.

## **Materials and Methods**

### *Collection and Identification of plant materials:*

The plant materials were collected locally and identified at Department of Medicinal Botany, National Institute of Siddha, Chennai, India. The raw materials were processed and used.

### *Preparation of the drug Eraippu Noi Chooranam:*

#### *Ingredients:*

Leaves of *Acalypha indica* - 224 g; Leaves of *Mollugo lotoides* - 224 g; Leaves of *Eclipta prostrata* - 224 g; Leaves of *Azadiracta indica* - 224 g; *Piper nigrum* - 112 g; *Piper longum* - 112 g; *Withania somnifera* - 112 g; *Terminalia chebula* - 112 g; Cane sugar powder -392 g.

#### *Purification of raw drugs:*

The raw drugs were purified as per the methods mentioned in the Siddha literatures.

#### *Preparation of trial drug:*

All the ingredients were powdered separately and mixed together as per the mentioned composition and bottled up.

#### *Physicochemical Analysis:*

The most of the Traditional formulation are lacking in their defined quality control parameters and method of its evaluation (Jain and Dashora, 2012). Hence Preliminary Physicochemical evaluation of the test drug was done with the aqueous extract of ENC which revealed that it was of standard quality. Phytochemical analysis revealed the presence of phytosterols, flavanoides, aminoacids, carbohydrates, terpenoids, phenolic compounds and tannin.

#### *Toxicity Study of ENC:*

Single dose acute toxicity study revealed that Eraippu Noi Chooranam was safe and has no toxic effect at the dose of 2000 mg/kg.

The NOAEL for ENC in rats was found to be higher than 900 mg/kg/b wt. after repeated dose administration of ENC in a sub acute toxicity study. This is because no treatment-related histopathological changes were observed in any of the organs.

#### *Animals and Treatment:*

The experimental studies on animals were conducted at KMCH College of Pharmacy, Coimbatore (IAEC NO: KMCRET/MD(S)/10/2014-15).

#### *Isolated guinea pig ileum assay of histamine:*

##### *Drugs used:*

- (i) Eraippunoi chooranam (1mg/ml)
- (ii) Acetylcholine (1mg/ml)
- (iii) Histamine (10µg/ml)

*Experimental conditions:* Physiological salt Solution: Tyrode

#### *Composition of De Jalon solution:*

- (i) NaCl, (ii) KCl, (iii) NaHCO<sub>3</sub>, (iv) CaCl<sub>2</sub>, (v) Glucose, (vi). MgCl<sub>2</sub>, (vii) NaH<sub>2</sub>PO<sub>4</sub>

Temperature: 37°C; Tension of lever: 0.5 g; Magnification: 10 times; Aeration: air

#### *Preparation of test compound:*

The test compound ENC (1 mg/ml), was dissolved in distilled water.

#### *Effect of drugs:*

Dose response curves or isolated concentrations were performed with Ach (1 mg/ml) and Histamine (10 µg/ml).

#### *Procedure:*

The *in vitro* method was performed using guinea pig ileum using matching and interpolation method. As the ileum of Guinea pig ileum was very sensitive to acetylcholine- and histamine-induced contractions it was used for this study. The abdominal cavity was quickly opened and a piece of ileum was isolated. It was placed in a beaker containing Tyrode solution which was maintained at an average temperature of 37°C. Tissue was cut into pieces of 2-3 cm in length. The distal piece being the most sensitive to different spasmogens was most preferred and used in this study. An assembly for isolated tissue consisting of the specified apparatus (Sherrington revolving drum and an organ bath) was used. Spasms were induced using acetylcholine, histamine (Goyal *et al.*, 2004).

Sub maximal doses of acetylcholine and histamine were selected and different doses of ENC were administered. The responses were recorded on the kymograph. A dose response curve was obtained and then contraction inhibition percentage was recorded.

Data are expressed as mean ± SEM, n=4, Student t test was performed to observe statistical significance.

#### *Results and Discussion*

The result obtained indicated that the test drug

Table 1: Evaluation of spasmolytic activity of ENC on histamine induced isolated guinea pig ileum

| Histamine                 |                      |            | Histamine + Eraippu noi Chooranam |                     |            |
|---------------------------|----------------------|------------|-----------------------------------|---------------------|------------|
| Conc. (µg/ml)             | Ht. of response (cm) | % response | Conc. (µg/ml)                     | Ht of response (cm) | % response |
| 0.1 (µg/ml)               | 73                   | 91.25%     | 0.1 (µg/ml)                       | 32                  | 40%        |
| 0.2 (µg/ml)               | 76                   | 95.00%     | 0.2 (µg/ml)                       | 36                  | 45%        |
| 0.4 (µg/ml)               | 70                   | 87.5%      | 0.4 (µg/ml)                       | 38                  | 47.5 %     |
| 0.8 (µg/ml)               | 80                   | 100%       | 0.8 (µg/ml)                       | 52                  | 65%        |
| IC <sub>50</sub> = 20.66% |                      |            | IC <sub>50</sub> =25.80%          |                     |            |

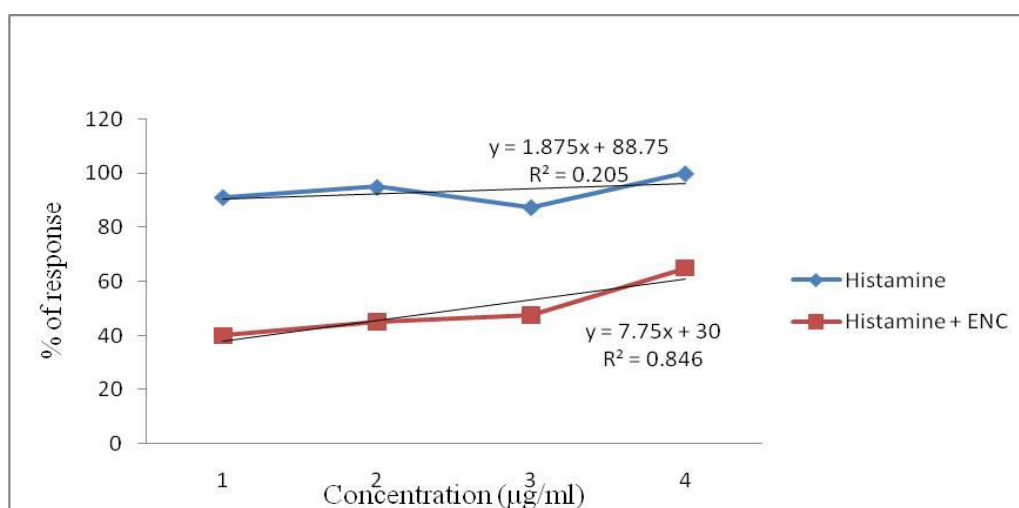


Fig. 1: Evaluation of spasmolytic activity of ENC on histamine induced isolated guinea pig ileum.

exhibited significant spasmolytic activity. The IC<sub>50</sub> value of ENC with acetylcholine was found to be 36.22% and IC<sub>50</sub> value of acetylcholine was found to be 13.24%. The IC<sub>50</sub> value of ENC with histamine was found to be 25.80% and IC<sub>50</sub> value of histamine was found to be 20.66%. The spasmolytic action of ENC on histamine is given in Table 1 and Figure 1 and acetylcholine is given in Table 2 and Figure 2.

The test drug ENC inhibits the contraction induced by histamine and acetylcholine. Muscarinic M3 receptor activation is the reason for Acetylcholine-induced spasms and H1 receptor activation mediates Histamine-induced spasms. Muscarinic M3 receptor activation, is a characteristic of vagal stimulation in the body and H1 receptor activation which is characteristic of allergy producing substances. Thus it may be

acting by antagonism of muscarinic M3 or histamine H1 receptors or it may inhibit the enzyme phosphodiesterase and thus inactivating Ca<sup>++</sup> channels which are responsible for spasms. Spasm induced in isolated guinea pig ileum by histamine and acetylcholine inhibited by ENC proves its spasmolytic activity.

Histamine and acetylcholine are both neurotransmitters which play significant roles in the regulation of bronchial smooth muscle tone and airway constriction. When discussing bronchospasm induced by these substances, it is essential to understand their mechanisms of action and how they contribute to respiratory distress.

Histamine is a potent inflammatory mediator released by various cells, including mast cells, in response to allergens, pathogens, or other stimuli.

Table 2: Evaluation of spasmolytic activity of ENC on Ach induced isolated guinea pig ileum

| Acetylcholine             |                      |            | Acetylcholine + Eraippu noi Chooranam |                     |            |
|---------------------------|----------------------|------------|---------------------------------------|---------------------|------------|
| Conc. (µg/ml)             | Ht. of response (cm) | % response | Conc. (µg/ml)                         | Ht of response (cm) | % response |
| 0.1(µg/ml)                | 52                   | 89.65%     | 0.1(µg/ml)                            | 16                  | 27.58%     |
| 0.2(µg/ml)                | 56                   | 96.55%     | 0.2(µg/ml)                            | 18                  | 31.03%     |
| 0.4(µg/ml)                | 43                   | 94.13%     | 0.4(µg/ml)                            | 22                  | 37.93%     |
| 0.8(µg/ml)                | 58                   | 100%       | 0.8(µg/ml)                            | 34                  | 58.62%     |
| IC <sub>50</sub> = 13.24% |                      |            | IC <sub>50</sub> = 36.22%             |                     |            |

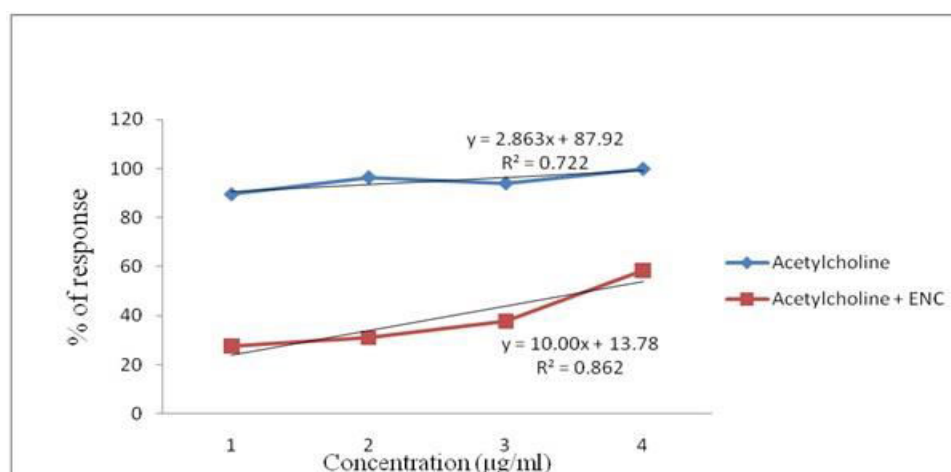


Fig. 2: Evaluation of spasmolytic activity of ENC on histamine induced isolated guinea pig ileum.

In the lungs, histamine acts on specific receptors, particularly the H1 receptors present on the smooth muscle cells of the bronchi. Activation of these receptors leads to bronchoconstriction, increased mucus secretion, and heightened vascular permeability, all of which contribute to the characteristic symptoms of asthma and allergic reactions.

On the other hand, acetylcholine is a neurotransmitter released by parasympathetic nerves that innervate the bronchial smooth muscle. Its primary role is to induce bronchoconstriction through activation of muscarinic receptors. In conditions such as asthma or chronic obstructive pulmonary disease (COPD), there is often an exaggerated response to acetylcholine due to increased parasympathetic tone or heightened sensitivity of the bronchial

smooth muscle to its effects.

In both cases, the end result is bronchospasm, which refers to the sudden and excessive contraction of the smooth muscle lining the bronchial airways. This contraction narrows the air passages, making it difficult for air to flow in and out of the lungs. Clinically, bronchospasm manifests as wheezing, shortness of breath, chest tightness, and coughing.

When discussing treatments for bronchospasm induced by histamine or acetylcholine, it is essential to target the underlying mechanisms. Antihistamines, such as cetirizine or loratadine, block the action of histamine at H1 receptors, thereby reducing bronchoconstriction and other allergic symptoms. Similarly, bronchodilators like beta-agonists (e.g.

albuterol) or anticholinergics (e.g. ipratropium bromide) counteract the effects of acetylcholine by relaxing bronchial smooth muscle and widening the airways.

In severe cases, systemic corticosteroids may be used to reduce inflammation and prevent further release of histamine from mast cells. Additionally, patients with chronic conditions like asthma may benefit from long-term controller medications, including inhaled corticosteroids or leukotriene modifiers, to maintain airway stability and prevent exacerbations triggered by histamine or acetylcholine.

Overall, understanding the roles of histamine and acetylcholine in bronchospasm is crucial for developing effective therapeutic strategies to manage respiratory conditions characterized by airway hyperreactivity and constriction. By targeting these pathways, healthcare providers can alleviate symptoms and improve the quality of life for patients with asthma, allergies, or other related disorders.

## Conclusion

It is essential to comprehend the functions of histamine and acetylcholine in bronchospasm in order to devise efficient treatment plans for respiratory disorders marked by hyperreactivity and constriction of the airways. Evaluation of spasmolytic activity of *ENC*, carried out through histamine and ACH induced isolated guinea pig ileum proved that *ENC* possess significant anti-asthmatic activity.

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