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EVALUATION OF MAST CELL STABILIZING EFFECT OF ERAIPPU NOI CHOORANAM (ENC) ON RAT MESENTRY

Yoga

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ABSTRACT

Background: According to the Siddha research pharmacopoeia, Eraippu Noi Chooranam is a modified Siddha Poly Herbal formulation recommended for respiratory conditions. The suggested traditional claim was put into practise in order to assess its effectiveness in the treatment of bronchial asthma. **Aim:** The aim of this study is to evaluate the mast cell stabilizing effect of ENC on rat mesentry. **Materials And Methods:** Aqueous extract of ENC was tested in this study at various dose levels for its ability to stabilize mast cells in the rat mesentery. **Result:** The % protection offered by the standard drug ketotifen was found to be 70% and 32.5% , 50.5% with the trial drug ENC at the dosage of 270, 400 mg/kg respectively. **Conclusion:** The effectiveness of aqueous extract of ENC in the treatment of asthma may therefore be concluded.

KEYWORDS

Bronchial asthma, Poly Herbal formulation, Siddha medicine, Eraippu Noi Chooranam, Compound 48/80, mast cell

INTRODUCTION

Each race of people has its own traditional medical system[1]. The traditional medicine systems in our nation are the oldest, richest, and most varied in the entire world. Numerous 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 presented by the Siddhars, is a comprehensive and flexible system[2].

The complex disease of the airways known as asthma is characterised by bronchial and smooth muscle hyperreactivity as well as persistent inflammation. Airway narrowing, frequent wheezing, dyspnea, chest tightness, morning arousal, and night coughing are its hallmarks [3]. Numerous causes, including allergens, respiratory infections, dust, cold air, exercise, emotions, workplace stimulation, certain drugs/chemicals, histamine, and inheritance, can cause asthma. The production of interleukins (IL-4 and IL-5) and other inflammatory agents, such as eosinophils, neutrophils, -cells, cytokines, and chemokines, which cause throat inflammation or obstruction, bronchial hyperresponsiveness, and mucosal hypersecretions, are accelerated by these trigger factors[4].

Mast cells are crucial in the development of allergic asthma. A key mediator secreted from mast cells during allergic responses is histamine. Through the contraction of smooth muscles, bronchial secretion, and airway oedema, histamine contributes to airway blockage. Four different types of histamine receptors, including H1, H2, H3, and H4, have been identified in lung tissue and airways [5-8]. One of the most well-known physiologic effects of histamine on the respiratory system is the constricting of smooth muscle via H1 receptors. It was known for a long time that histamine caused human bronchi to constrict, and bronchoconstriction was the first biological effect of histamine to be recognized[9].

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 all side effects. 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 case, the choice of the individual will be a secure and efficient herbal medication to deal with the problem.

Despite the fact that Siddha medicines are thought to be safe and effective, it is the doctors' highest responsibility to validate the formulation before using it on humans. A modified Siddha Poly Herbal formulation named Eraippu Noi Chooranam is reported in the book Siddha Research Pharmacopoeia.It is prescribed for flatulence, chronic bronchitis, and asthma[10]. It is a multi-herbal medication, and each of the active ingredients is highly efficient in treating kapha disorders. The suggested traditional claim was put into practise in order to assess its effectiveness in the treatment of bronchial asthma.

AIM AND OBJECTIVES

The aim of this study is to evaluate the mast cell stabilizing effect of ENC on rat mesentry

MATERIALS & METHODS

Collection And Identification Of Plant Materials

Assistant professor of medicinal botany in National Institute of Siddha, Chennai verified the authenticity of the herbal constituents. The raw materials were processed using the techniques described in the literature.

Preparation Of The Drug Eraippu Noi Chooranam[11]:

Ingredients:

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

Purification Of Raw Drugs[12,13]:

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[14]

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[15]

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.w 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.

Mast cell stabilizing activity on rat mesentry[16]

Animals

The study used female Wistar albino rats, which were bought from Sree Venkateshwara Enterprises Pvt. Ltd. in Bangalore. Five groups of rats were formed (n=5). All animals were kept in cages with a constant temperature of (22±1°C), a relative humidity of roughly 30–70%, and a 12/12 light–dark cycle. Animals were given a regular pellet meal from Sai Durga Feeds in Bangalore along with unlimited access to water. According to the CPCSEA's guidelines(Committee for the Purpose of Control and Supervision of Experiments on Animals) , the Institutional Animal Ethical Committee (IAEC) accepted the experiment's procedure CO_2 inhalation was used to kill the animals. The abdominal cavity was sliced open, exposing the intestines, and the fragments of mesentery were collected. The fragments of the mesentery were then put into a petri dish at 37^0 C Ringer Locke solution (CaCl2 0.24, NaHCo3 0.5, NaCl 9.0, KCl 0.42, and glucose 1 g/L in double distilled water; pH 7.4) and the following treatment protocols were applied.

Experimental Design

Petri dish no. 1 - Ringer Locke solution (Positive control) (CaCl2 0.24, NaHCo3 0.5 NaCl 9.0, KCl 0.42, and glucose 1 gm/L in double distilled water; pH 7.4)

Petri dish no. 2 - Ketotifen fumarate (10 µg/ml)

Petri dish no. 3 - ENC in distilled water (135 mg/ml)

Petri dish no. 4 - ENC in distilled water (270 mg/ml)

Petri dish no. 5 - ENC in distilled water (400 mg/ml)

At 37°C, each petri dish was incubated for 15 minutes. All petri dishes later received Compound 48/80 (0.1 ml, 10 g/ml) and were again incubated at 37°C for 10 minutes. All pieces were then placed in a 4% formaldehyde solution with 0.1% toluidine blue and left to sit for 20 to 25 minutes. Mesentery pieces were transported via acetone for 10 min., followed by xylene for 2 min., then mounted on slides after the mast cells had been dyed and fixed. Every component was examined using a powerful light microscope. The treatment groups' and the control groups' percentages of mast cell protection were computed. From a total count of at least 100 mast cells, the number of degranulated mast cells was determined. The percentage of protection was estimated using the formula after counting the number of intact and degranulated cells.

% Protection of mast cells = [1-(T / C)] x 100

Where, T denotes the number of degranulated cells of test.

C denotes the number of degranulated cells of control.

RESULTS

Following antigen exposure, the mast cells had considerable degranulation. Rat mesentric mast cell degranulation was significantly induced by antigen exposure with compound 48/80 (10 g/ml), a recognised mast cell degranulating agent (81.2± 2.73). A dose-dependent reduction in the Compound 48/80-induced mast cell degranulation was seen after exposure to ENC. A well-known mast cell stabilising drug, ketotifen fumarate, also significantly ($P< 0.01$) reduced the number of degranulating mast cells..Table.1 and Fig. 1 The % protection offered by the standard drug ketotifen was found to be 70% and the protection was 32.5% ,50.5% with the test drug ENC at the dose of 270, 400 mg/kg respectively fig .2

Table 1.effect Of Eraippu NOI Chooranam On Compound 48/80-induced Mast Cell Degranulation In Rats

Groups	Mast cells %		% protection
	Intact	Degranulated	
CONTROL	18.8± 2.73	81.2± 2.73	------
KETOTIFEN	75.6± 3.36**	24.4± 3.36**	70
ENC LD	24.8± 2.29	75.2± 2.29	7.4
ENC MD	45.2± 3.97**	54.8± 3.97**	32.5
V ENC HD	59.8± 3.01**	40.2± 3.01**	50.5

Results are expressed as Mean± S.E.M. where n=5,Statistical analysis done by using ANOVA followed by Dunnett's testwhere **p<0.01 Group II, IV, V compared with Group I.

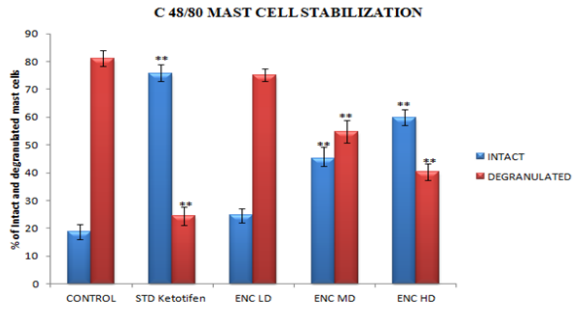

Fig.1 Effect of Eraippu NOI Chooranam on Compound 48/80-induced mast cell degranulation in rats

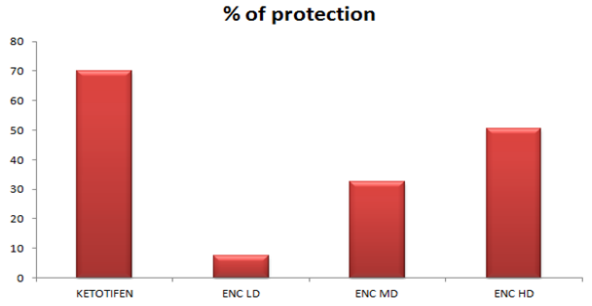

Fig.2 % Of Protection Offered By ENC C 48/80- Induced Mast Cell Degranulation In Rats

DISCUSSION

Mast cells are crucial in the development of Type I hypersensitivity-mediated illnesses, such as allergic rhinitis and asthma. The development of numerous physiological changes during anaphylactic reaction and allergic reaction depends heavily on the mast cells. Antibodies called immunoglobulin-E attach to mast cell surface receptors. Histamine, heparin, proteases, and other mediators are released by allergen-IgE interaction on mast cells. Leukotrienes and prostaglandins are also produced and secreted during this process. These products have pro-inflammatory effects such as bronchoconstriction, altered blood vessel tone, increased vascular permeability, and others. Mast cell functions can be altered for therapeutic purposes by controlling their activity with the right medications. Constituents with a plant origin may have an impact on the architecture, chemical content, or development of mast cells. Additionally, it might affect the production of IgE molecules or IgE binding to mast cell surface. The lifespan of mast cells may potentially be shortened by the plant medicine. The medicine mentioned in the text significantly shielded the mast cells. However, the impact was less than that seen with the commonly prescribed medication, ketotifen fumarate. The degranulation of mast cells and basophils, which is followed by the release of mediators including histamine, leukotrienes, and prostaglandins from these cells, has been used to explain the pathophysiological mechanism involved in Type-I allergies. Mast cells degranulate in response to immunological stimuli, with the antigen-antibody interaction on the cell surface dominating. Owing to its capacity to restrict the release of mediators from mast cells and its ability to affect the course of disease by preventing the negative effects brought on by the released mediators, the current analysis suggests that the test drug ENC is active in the Type-I allergic diseases.

The results of the preliminary phytochemical studies indicated that the aqueous extract of ENC contained flavonoids. By preventing the release of neutrophil beta glucuronidase, basophil and histamine, plant flavonoids are known to have in vivo antiallergic effect. The release of histamine caused by C 48/80 is likewise to be inhibited by flavonoids. Plants containing flavonoids have been reported to have antihistaminic, antiallergic, and mast cell degranulation effects. These findings could lead to the conclusion that ENC stabilises mast cells in rats mesentery.

CONCLUSION

The findings of this study imply that the trial drug ENC has a strong

dose-dependent mast cell stabilising action and can be utilised to manage bronchial asthma.

Ethics Approval :
The experimental studies on animals were conducted at KMCH college of Pharmacy (IAEC NO: KMCRET/MD(S)/10/2014-15)

Consent To Participate And Consent For Publication:
Not Applicable

Competing Interests:
The Authors have no competing interests to declare

Authors Contributions:
Conceptualization, supervision, Methodology, Data interpretation, Original Data preparation, Writing, reviewing, editing done. All the Authors read and approved the final manuscript.

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We declare that all the data generated are included in this study.

Conflict Of Interest:
The authors declare that there is no conflict of interest.

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