



**INVITRO AND INVIVO EVALUATION OF ETHANOLIC LEAF EXTRACT OF CITRUS LIMON IN ANTI INFLAMMATORY PROPERTY USING ASTHMA INDUCED GUINEA PIG MODELS**

P. Saranya\*, S. Gomathi, S. Ganesh, K. Gayathri, V. Ganapathi and S. Divya

Aadhibhagawan College of Pharmacy, Rantham, T.V. Malai, Tamilnadu.

\*Corresponding Author: P. Saranya

Aadhibhagawan College of Pharmacy, Rantham, T.V. Malai, Tamilnadu.

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### ABSTRACT

This study Citrus limon extract were used to treat asthmatic condition. The extract were prepared with ethanol and used based on the presence of anti-inflammatory property which can manage the asthmatic condition. The activity of the extract were bio screened (both invitro and invivo methods) using asthma induced guinea pig models. The present study was to reveals that the extract having Anti-inflammatory property for the purpose of treating asthmatic condition using guinea pig.

**KEYWORDS:** Citrus limon, Anti-inflammatory activity, Oedema.

### 1. INTRODUCTION

Asthma is a common disease that is rising in prevalence worldwide with the highest prevalence in industrialized countries. Asthma affect about 300 million people worldwide and it has been estimated that a further 100 million will be affected by 2025. Bronchial asthma is characterised by hyper responsiveness of tracheobronchial smooth muscle to a variety of stimuli, resulting in narrowing of air tubes, often accompanied by increased secretion, mucosal edema and mucus plugging.<sup>[2]</sup> (Or)

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Asthma (or Asthma bronchiale) is a disease that hurts the airways inside the lungs. It causes the tissue inside the airways to swell. Asthma also causes the bands of muscle around the airways to become narrow.

### SYMPTOMS

- Breathlessness
- Wheezing
- Sputum Production
- Difficulty in speaking
- Dyspnoea
- Tightness of Neck Muscle
- Coughing after physical activity
- Whistling Sound while breathing

- Frequent coughing
- Feeling Frightened, exhaustion
- Chest Tightness
- Greyish or bluish colouring of lips.<sup>[4]</sup>

Asthma is now recognized to be a primarily inflammatory condition: inflammation underlying hyper reactivity. An allergic basis can be demon strated in many adult, and higher percentage of pediatric patients. In others, a variety of trigger factors (infection, irritants, pollution, exercise, exposure to cold air, psychogenic) may be involved.

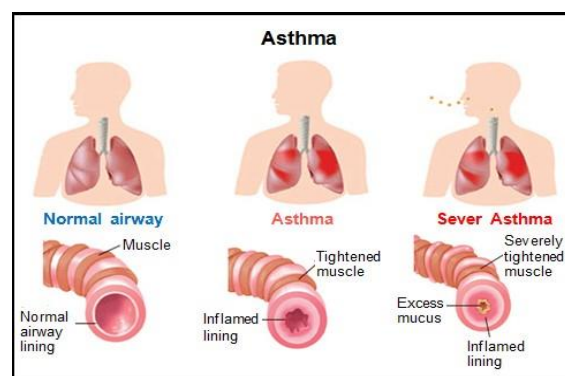


Fig No: 1 Types of asthma.

### TYPES OF ASTHMA

**Allergic Asthma (extrinsic asthma):** When the symptoms are induced by a hyper immune response to the inhalation of specific allergen. Type I (Immediate) hypersensitivity reaction is the basis of the IgE.

**Non-Allergic Asthma (Intrinsic asthma):** This type of asthma is triggered by the presence of irritants in the air that are not related to allergies. These irritants stimulate parasympathetic nerve fibers in the airways causing broncho-constriction and inflammation.

**Mixed Asthma:** Mixed asthma is the combination of both allergic and non-allergic asthma. This is the most common form of asthma.

**Cough-Variant Asthma:** This does not have the classic symptoms of asthma- such as wheezing and shortness of breath. Instead it is characterized by one symptom, a persistent dry cough.

**Exercise Induced Asthma:** Affects the person during or after physical activity.

**Nocturnal Asthma:** Characterized by symptoms that get worse at night. Those who suffer from nocturnal asthma can also experience symptoms any time of day.

**Occupational Asthma:** Induced by triggers that exist in person's workplace including textiles, farming and wood working.

#### COMMON ASTHMA TRIGGERS INCLUDE

- Animals (pet hair or dander)
- Dust mites
- Certain medicines (aspirin and other NSAIDs)
- Changes in weather (most often cold weather)
- Chemicals in the air or in food
- Exercise
- Mold
- Pollen
- Respiratory infections, such as the common cold
- Strong emotions (stress)

#### COMMON SYMPTOMS AND SIGNS INCLUDE

- Wheezing
- Coughing
- Breathing difficulty
- Tightness in the chest
- Worsening symptoms at night
- Worsening symptoms due to cold air
- Symptoms while exercising
- Symptoms after exposure to allergens

#### DIAGNOSING ASTHMA

- Physical Exam
- FeNO test
- Peak flow test
- Additional tests
- Imaging tests
- Allergy testing
- Nitric oxide test

## 2. BOTANICAL CLASSIFICATION OF LIMON

Table No: 1 Plant Profile.

KINGDOM	PLANTAE	PLANT
Subkingdom	Tracheobionta	Vascular plants
Super division	Spermatophyta	Seed plants
Division	Magnoliophyta	Flowering plants
Class	Magnoliopsida	Dicotyledons
Subclass	Rosidae	-
Order	Sapindales	-
Family	Rutaceae	Rue family
Genus	Citrus L.	citrus P



Fig No: 2 Plant of Citrus Lemon.

## 3. MATERIAL AND METHODS

### 3.1 Collection and authentication of Plant material

The leaves of *Citrus Limon* were collected from the natural habitat in and local area, Tamilnadu and the plant material were authenticated by Prof.Dr.P.Jayaraman Ph.D., The Director, Plant Anatomy Research Centre (PARC), West Thambaram, Chennai-45.

### 3.2 Extraction procedure

The collected leaves were cleaned, dried in the shade and ground into a fine powder from which 500g was extracted repeatedly with 2 liters of ethanol using Soxhlet extractor at 50°C for 72 h. The extracts were filtered using Whatmann filter paper (no.1) and concentrated in vacuum at 40°C using a rotary evaporator and the residues obtained were stored in a freezer at -80°C until further tests.

### 3.3 Methodology Animals

All animals were housed at ambient temperature (22±1°C), relative humidity (55±5%) and 12/12 h light/dark cycle. Animals had access to standard pellet diet and water given ad libitum. The study was approved by the Institutional animal ethical committee, CPCSEA.

### 3.4 Animal specification

Table No: 2 Animal Specification.

No. of animal required	48
Species	Albino
Gender	Eithersex
Weight	Rats(150-200gm), Guinea pigs(400-450gm)
Duration of study	45 days
Animal Will be housed	1 month
Disposal animals	Buried

### 3.5 Grouping of Animals

#### In vivo study

#### A) Anti inflammatory test

Table No: 3 Anti inflammatory test.

Group	No. of Animals	Treatment
I	6	Control(0.5% CMC) + Carrageenan 1% (0.1ml)
II	6	Diclofenac sodium(20mg/kg)(Std) p.o + Carrageenan 1%(0.1ml)
III	6	EECLI(200mg/kg p.o.) in 0.5% CMC + Carrageenan 1%(0.1ml)
IV	6	EECL II(400mg/kg p.o.) in 0.5% CMC + Carrageenan 1%(0.1ml)

#### B) Histamine and Acetylcholine induced bronchospasm in guinea pigs.

Table No. 4: Histamine and Acetylcholine Induced Bronchospasm in Guinea Pigs.

Group	No. of Animals	Treatment
I	6	Control(0.5% Ach, 0.25% Histamine)
II	6	Ketotifen (1 mg/kg)(std) orally
III	6	EECL I(200mg/kg) orally
IV	6	EECL II(400mg/kg) orally

proteins and amino acid, sterols, flavonoids, saponins, alkaloids, fixed oils and fats, gum and mucilage.

Table No: 5 Preliminary phytochemical test for EEPS Table.

S. No.	Phytochemical Tests	Results
1	Alkaloids	Present
2	Carbohydrates	Present
3	Glycosides	Present
4	Phenolic compound	Present
5	Tannins	Present
6	Proteins and amino acids	Present
7	Saponins	Present
8	Gums and mucilage	Absent
9	Sterols	Present
10	Fixed oils and fats	Absent
11	Flavonoids	Present

## 4. RESULTS AND DISCUSSION

### 4.1 PRELIMINARY PHYTOCHEMICAL STUDIES

Preliminary phytochemical analysis of ethanol extract of *Citrus limon leaves* revealed the presence of carbohydrate, glycoside, phenolic compound, tannins,

### 4.2 ANTI INFLAMMATORY TEST

Table No: 6 Anti inflammatory test.

Animal	Albinorats
Drugs	Carrageenan (prepare 1% w/v solution and inject 0.1 ml underneath plantar region) Diclofenac sodium(20mg/kg) Ethanol extract of <i>Citrus limon</i> (200 mg/kg and 400mg/kg)
Equipment	Plethysmograph

Table No. 7: Effect of EECL on Carageenan induced rat paw edema.

Treatment	Dose (mg/kg)	% Increase volume of paw (% anti inflammatory Effect) 1h	% Increase volume of paw (% anti inflammatory effect) 3h	% Increase volume of paw (% anti inflammatory Effect) 5h
Control		4.114±1.28	21.413±1.423	22.14±1.511
EECL	200	4.676±0.033*(17.75)	14.38±0.681*(20.64)	14.12±0.07**(42.35)
EECL	400	3.163±0.138*(46.86)	11.367±1.121**(39.27)	9.40±0.439**(63.79)
Diclofenac sodium	20	2.176±0.421**(54.21)	7.145±1.17**(64.14)	5.32±0.013**(75.9)

Values are expressed as mean ± SEM (n=6); Significantly different from control. \*P<0.05, \*\*P<0.01 Values in parenthesis indicates percentage inhibition of paw edema. Data were analysed by using ANOVA and Dunnett's t-test and expressed as mean ±SEM.

Table No. 8 Effect of EECL on Ach and Histamine induced bronchospasm in guinea pigs.

S.NO	Treatment % Increase in preconvulsion time	Acetylcholine	Histamine
1	Control(0.25% histamine, 0.5% Ach)	64.34±2.23	58.22±1.36
2	EECL I(200mg/kg p.o.)	53.47±4.24*	44.74±3.23*
3	EECL II(400mg/kg p.o.)	48.47±2.46*	39.13±3.57*
4	Ketotifen fumarate(1mg/kg p.o.)	34.81±1.22**	33.29±2.30*

Values are expressed as mean ± SEM (n=6);

Significantly different from baseline. \*P<0.05, \*\* P<0.01.

Data were analysed by using Dunnett's t-test and expressed as mean ±SEM.

## 5. DISCUSSION

In present study, significant increase in preconvulsion time was observed due to pretreatment with *Citrus lemon leaves extract*, when the guinea pigs were exposed to either Acetylcholine or Histamine aerosol. This bronchodilating effect of Citrus limon leaves extract was compared with control. Further studies needed for its exact mechanism of action.

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