EVALUATION OF ANTI ASTHMATIC ACTIVITY OF *URTICA DIOICA (Linn.)* LEAVES AND ITS PHYTOCHEMICAL STUDIES

A Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI - 600 032

In partial fulfilment of the award of the degree of

MASTER OF PHARMACY

IN

BRANCH IV: PHARMACOLOGY

Submitted by

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OCTOBER – 2018


CERTIFICATE

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Internal Examiner **External Examiner**

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I do hereby declared that the dissertation **"Evaluation of Anti asthmatic activity of *urtica dioica* leaves and its phytochemical studies"** submitted to "**The Tamil Nadu Dr.M.G.R Medical University - Chennai",** for the partial fulfilment of the degree of **Master of Pharmacy** in **Pharmacology,** is a bonafide research work has been carried out by me during the academic year 2017-2018, under the guidance and supervision of **Mr. V.Venkateswaran, M.Pharm,** Assistant Professor, Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diplomo associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

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Dedicated to

Parents, Teachers &

My family

ACKNOWLEDGEMENT

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It is most pleasant duty to thank my beloved guide **Mr.V.Venkateswaran, M.Pharm,** Assistant professor, Department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Kumarapalayam for suggesting solution to problems faced by me and providing in dispensable guidance, tremendous encouragement at each and every step of this dissertation work. Without his critical advice and deep-rooted knowledge, this work would not have been a reality.

It is most pleasant duty to thank for our beloved Principal and my guide **Dr. R. Sambathkumar, M.Pharm., Ph.D.,** Professor & Head, Department of Pharmaceutics, J.K.K. Nattraja College of Pharmacy, Kumarapalayam for ensuring all the facilities were made available to me for the smooth running of this project.

My sincere thanks to **Dr. R. Shanmugasundaram, M.Pharm., Ph.D.,** Vice Principal & HOD, Department of Pharmacology, **Dr.CKalaiyarasi M.Pharm., Ph.D.,** Asst.proffessor **Mr.V. Venkateswaran, M.Pharm.,** Assistant Professor, **Mrs. M.Sudha, M.Pharm.,** Lecturer, **Mrs.R. Elavarasi, M.Pharm.,** Lecturer, **Mrs.M.Babykala, M.Pharm.,** Lecturer, Department of Pharmacology for their valuable suggestions during my project work.

My sincere thanks to **Dr.S.Bhama, M.Pharm.,** Associate Professor Department of Pharmaceutics, **Mr. R. Kanagasabai, B.Pharm, M.Tech.,** Assistant Professor, **Mr.K.Jaganathan, M.Pharm.,** Assistant Professor, **Mr.C.Kannan, M.Pharm.,** Assistant Professor, **Dr.V.Kamalakannan, M.Pharm.,** Assistant Professor, and **Mr.N.Subramani, M.Pharm.,** Lecturer, Department of pharmaceutics for the in valuable help during my project.

My sincere thanks to **Dr.N.Venkateswaramurthy, M.Pharm., Ph.D.,** Professor and Head, Department of Pharmacy Practice, **Mrs.K.Krishnaveni, M.Pharm.,** Assistant Professor, **Mr.R.Kameswaran, M.Pharm,** Assistant Professor, for their help during my project.

It is my privilege to express deepest sense of gratitude toward **Dr.M.Vijayabaskaran**, **M.Pharm.,** Professor & Head, Department of Pharmaceutical Chemistry, **Mrs. S. Gomathi, M.Pharm.,** Lecturer, **Mrs.B.Vasuki, M.Pharm.,** Lecturer and **Mrs. P.Lekha, M.Pharm.,** Lecturer, for their valuable suggestions and inspiration.

My sincere thanks to **Dr.V.Sekar, M.Pharm., Ph.D.,** Professor and Head, Department of Analysis, **Dr.I. Carolin Nimila, M.Pharm., Ph.D.,** Assistant Professor, **Mr.D.Kamalakannan, M.Pharm.,** Assistant Professor, Department of Pharmaceutical Analysis for their valuable suggestions.

My sincere thanks to **Mrs.Meena Prabha, M.Pharm.,** Assistant Professor, Department of Pharmacognosy and **Mrs. P. Seema, M.Pharm.,** Lecturer, **Mr. L.Kaviarasan, M.Pharm.,** Lecturer, Department of Pharmacognosy for their valuable suggestions during my project work.

I greatly acknowledge the help rendered by **Mrs.K.Rani,** Office Superintendent, **Miss.M. Venkateswari, M.C.A.,** Typist**, Mrs.V.Gandhimathi, M.A., M.L.I.S.,** Librarian, **Mrs.S. Jayakala B.A., B.L.I.S.,** and Asst. Librarian for their co-operation. I owe my thanks to all the technical and non-technical staff members of the institute for their precious assistance and help.

I am proud to dedicate my deep sense of gratitude to the founder, (Late) Thiru **J.K.K.Nattaraja Chettiar,** providing the historical institution to study.

My sincere thanks and respectful regards to our reverent Chairperson **Smt.N.Sendamaraai, B.Com.,** and Director **Mr.S. Omm Sharravana, B.Com., LLB.,**J.K.K. Nattraja Educational Institutions, Kumarapalayam for their blessings, encouragement and support at all times.

Last, but nevertheless, I am thankful to my lovable parents, all my friends for their co-operation, encouragement and help extended to me throughout my project work.

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1.0 INTRODUCTION

1.1 Antihistamine

Histamine, an autacoid, is widely distributed in plant and animal tissues. As it is widely spread in body tissues, it was named histamine, which means "tissue amine". It was first discovered in 1907 by Windos and Vogt. Its vasodepressor effect was reported in 1910 by Dale et.al. In 1927, it was first isolated from liver and lung and it is released in body usually, in response to tissue injury, inflammation and allergic or hypersensitivity reactions. Neurons located in tubomammillary nucleus of the posterior hypothalamus synthesize histamine1. Histamine is β-imidazolyl ethylamine, comprised of imidazole ringconnected to an amino group through ethylenebridge.

Allergy is one of the common diseases that affect mankind with diverse manifestations. The prevalence of allergy and asthma has risen in the recent years despite an improvement in the general health of the population. Allergic diseases are responsible for significant morbidity and have severe economic impact. Various epidemiological studies have identified the causes for an increase in the prevalence of upper and lower respiratory tract allergic diseases. Some of the postulated reasons are increasing environmental pollution and increased predisposition of individuals producing excessive IgE through a major change in the gene pool, changing lifestyles, and an increasing awareness of the disorders[1-10]

Intensive research during the last several decades has high-lighted the role of lymphocytes, immunoglobulins, mast cells, and various autacoids in the etiopathogenesis of allergic conditions. Inspite of the voluminous literature on the subject, the treatment of allergic diseases continues to be far from satisfactory. The available treatment options for upper and lower respiratory tract allergic diseases have major limita-tions owing to low efficacy, associated adverse events, and compliance issues.

Ayurveda, an Indian system of medicine, has described several drugs from indigenous plant sources for use in the treatment of bronchial asthma and allergic disorders. HK-07 is one such polyherbal formulation containing mainly the extracts of Curcuma longa, Zingiber officinale, Piper longum, Emblica officinalis, Terminalia belerica, Ocimum sanctum, Adhatoda vasica, and Cyperus rotundus. The dry rhizome of Curcuma longa contains curcumin, the main bioactive com-ponent, demethoxycurcumin, and bisdemethoxycurcumin. The traditional uses of turmeric or natural curcuminoids in folk medicine are multiple, and some are based on their antioxi-dant, antiinflammatory and antiallergic properties which have been confirmed by various experimental studies. Curcumin is also found to be a potent blocker of nuclear transcription factor (NF)-kB, which is linked to a variety of diseases in-cluding allergy and asthma. Ocimum sanctum has been dem-onstrated to protect against histamine, as well as pollen-in-duced bronchospasm in guinea pigs and inhibited antigen-in-duced histamine release from sensitized mast cells apart from its established antiinflammatory and antioxidant proper-ties. Adhatoda vasica is documented for its potent antiinflammatory, antiallergic, and antitussive activities. Piper longum has been shown to reduce the passive cutane-ous anaphylaxis in rats and protect guinea pigs against anti-gen-induced bronchospasm. Emblica officinalis was found to exhibit antiinflammatory, antitussive and antioxidant ac-tivities. Terminalia belerica demonstrated potent antiperoxidative activity and inhibited lipid peroxide forma-tion by scavenging hydroxyl and superoxide radicals in vitro. Zingiber officinale has been found to exert anti-inflammatory activity and is reported to be a potent inhibitor of inflamma-tory mediators such as prostaglandins and leukotrienes. Cyperus rotundus inhibited the nitric oxide and superoxide production in in vitro studies, which used murine macrophage cell lines. HK-07 was found to be clinically effective and safe in children suffering from upper and lower a respiratory tract infection which leads to asthma. Asthma is a disease of the lung's airways. It affects 155 million individuals in the world.

Its Prevalence and severity among children have increased significantly in the world over the past 40 year. It varies from 5–30 percent in different population [1, 2]. It has affected 14–15 million people in the United States, including estimated 4.8 million children. It is the most common chronic disease of childhood. It accounts for about 11 million hospital visits annually and the sixth most frequent reason for visits in ambulatory setting[11-15].

About 4, 70,000 patients are hospitalized and more than 5,000 patients die annually due to asthma [3]. Asthma closely correlates with the description of the disease "Tamak Shwasa" recorded thousands years ago by the sages and eminent scholars of Ayurveda [4].

Bronchial asthma is a chronic respiratory disorder affecting a large proportion of population throughout the world [5]. The plant is referred to as 'Jivanti' in Ayurvedic text and considered to be Rasayana (tonic) drug and is thus used to vitalize, nourish and rejuvenate the body [6]. Ethno medicinally the leaves and seeds are used in asthma and cough [7]. The major therapeutic claim is its galactogogue action, which has been proved in rats [8] along with the antimicrobial [9], anticarcinogenic [10] and hepatoprotective properties of plant [12, 13] in traditional system of medicine leaves of *L. reticulata* (Retz) Wight & Arn are mainly used for the treatment of cough, asthma, rheumatism [8, 14]. Many asthma attacks are triggered by allergens, such as dust, mould spores, mites, animal hair or feathers but the onset may equally be caused by cold air, or it may be preceded by an infection such as a cold. Certainly, stress and more specifically acute anxiety are known to be the immediate trigger for many attacks, and this can sometimes give rise to a vicious circle of asthma - anxiety about the asthma - further attacks. Thus a wide range of etiological factors can be involved in this all too common problem [16].

A number of different groupings can be applied:

Extrinsic asthma

Caused by allergic responses to house dust, animal fur, or various foods. Such causes 10-20% of adult asthma.

Intrinsic asthma

Caused by genetics, structural problems, infections, pollutants and stress - both physiological and psychological. Such causes 30-50% of adult asthma. The symptoms of people with asthma differ greatly in frequency and degree. Some have an occasional episode that is mild and brief; otherwise they are symptom free. Others have mild coughing and wheezing much of the time, punctuated by severe exacerbation's of symptoms following exposure to known allergies, viral infections, and exercise or nonspecific irritants. A series of stages have been characterized for describing the severity of an acute asthma attack:

Mild

Mild dyspnoea; diffuse wheezes; adequate air exchange.

Moderate

Respiratory distress at rest; hypernea, use of accessory muscles; marked wheezes.

Severe

Marked respiratory distress; cyanosis; use of accessory muscles; marked wheezes or absent breath sounds.

Respiratory failure

Severe respiratory distress; lethargy; confusion; prominent pulsusparadoxus. Use of accessory muscles[16, 17].

Medicinal Plants used in asthma

Asthma is a global problem. Many synthetic drugs are used to treat acute symptoms of asthma, but they are not completely safe for long term use. Hence search has been started once again to look back to traditional medicine which can be used to treat asthma. Some traditional plants with antiasthmatic potential are discussed in table 1.

Table No.1: List of Medicinal Plants Used in Asthma

Plant Name	Plant part used	Mechanism of action
Abutilon crispum (L.) Medicus.	Leaves	Antiasthmatic
Abutilon indicum (L.) Sweet.	Seed	Antiasthmatic
Aerva lantaLinn	Aerial parts	Antiasthmatic
Acalypha indica	Leaves, roots, stalk and flowers	Bronchodialator
Achillea mellifolium	flowers	Bronchodilator, Mast cell stabilizer
Acorus alamus	Rhizome	Mast cell stabilizer
Ailanthus excels	Leaves	Antiasthmatic, Antiallergic
Achyranthes aspera, Allium cepa	Fruit	Mast cell stabilizer
Ageratum conyzoides L	Leaves	Antiasthmatic

Plant Name	Plant part used	Mechanism of action
Adhatoda vasica Nees	Bulb	Mast cell stabilizer, Lipoxygenase inhibitor, PAF inhibitor, COX inhibitor
Albizzia *Lebbeck*	Bark	Bronchodilator Mast cell stabilizer
Asystasia *Gangetica*	Leaves	Bronchodilator Anti-inflammatory
Ammi visnaga	Seeds	Bronchodilator
Amburana cearensis	Bark	Bronchodilator
Allium cepa Linn.	Bulbs/Juice	Mast cell stabilizer,
Alstonia scholaris R. Br.	Leaves	Bronchodilator
Aquillaria agallocha Roxb.	Stem	Mast cell stabilizer & Antiallergic
Argemone Mexicana	Stem	Bronchodilator
Aristolochia indica L	Roots	Bronchodilator
Asclepias curassavica L	Roots	Antiasthmatic
Asystasia gangetica	Leaves	Antiasthmatic
Atropa belladonna	Seeds	Asthma, Bronchitis, Muscuar Pain
Azadirachta indica A. Juss	Leaves	Mast cell stabilizer

Plant Name	Plant part used	Mechanism of action
Azima tetracantha Lam	Leaves	Mast cell stabilizer
Bacopa monniera Linn.	Leaves	Mast cell stabilizer
Balanites roxburghii	Stem bark	Bronchodilator, Mast cell stabilizer
Benincasa hispida (Thunb.) Cogn.	Fruits	Bronchodilator
Boerhaavia diffusa Linn.	Root	Asthma, Bronchitis
Brassica camperstris Linn.	Seed	Bronchodilator
Biophytum nervifolium Thw	Leaves	Mast cell stabilizer
Cassia absus L	Leaves	Bronchodilator
Casuarina equisetofolia Linn	Bark	Antiasthmatic
Cedrus deodara	Wood	Mast cell stabilizer
Cnidium monnieri	Leaves	Bronchodilator
Curculigo Orchioides	Rhizomes	Antihistaminic Anti-inflammatory
Centipeda minima	Whole plant	Mast cell stabilizer
Clerodendron phlomidis	Leaves	Antihistaminic, Mast cell stabilizer
Casuarina equisetifolia Linn	Wood, Bark	Antiasthmatic
Chlorophytum laxum R. Br.	Tuber	Antiasthmatic

Plant Name	Plant part used	Mechanism of action
Cissus quadrangularis L	Stem	Antiasthmatic
Clematis smilacifolia Wall	Leaves	Antiasthmatic
Clerodendrum serratum Linn	Roots	Antiasthmatic
Coccinia grandis (L.) Voigt	Tuber	Antiasthmatic
Cynodon dactylon	Whole Plant	Antiasthmatic
Calotropis procera (Ait) R.Br.	Latex	Mast cell stabilizer & Anti-inflammatory
Cassia tora Linn.	Seeds	Mast cell stabilizer
Clerodendron serratum Linn. Moon.	Stem bark	Bronchodilator, Mast cell stabilizer
Cuminum cyminum Linn.	Roots	Bronchodilator
Curcuma longa Linn.	Rhizome	Mast cell stabilizer, Antiallergic & Anti Inflammatory
Cynodon dactylon Pers.	Rhizome	Mast cell stabilizer
Cassia sophera	Leaves	Bronchodilator, Antihistaminic
Dendrophthoe falcate L. f.	Bark	Antiasthmatic
Desmodium gangeticum	Roots	Cough, Asthma, Vomitting
Dhatura metel Linn.	Whole Plant	Asthma

Plant Name	Plant part used	Mechanism of action
Elaeocarpus sphaericus K. Schum	Fruits	Bronchodilator
Ephedra gerardiana	Stem	Bronchodilator
Eclipta alba Linn	Leaves	Antiasthmatic
Emblica officinalis	Fruits	Asthma, Bronchitis
Euphorbia hirta	Aerial parts	Antiasthmatic
Ficus bengalensis Linn	Bark	Antiasthmatic
Ficus exasperate Yahl	Root	Bronchodilator
Ficus racemosa Linn.	Latex	Antiasthmatic
Glycyrrhiza glabra	Roots	Antihistaminic, Antiallergic
Hemidesmus Indicus R.Br.	Roots	Antiasthmatic
Inula racemosa Hook. F.	Roots	Mast cell stabilizer & Antiallergic
Labisia Pumila	Leaf	Antiasthmatic
Leptadenia Reticulata	Leaves and Roots	Cough and AsthmaS
Lepidium sativum Linn.	Seeds	Bronchodilator
Lannea coromandelica Merr	Whole Plant	Antiasthmatic

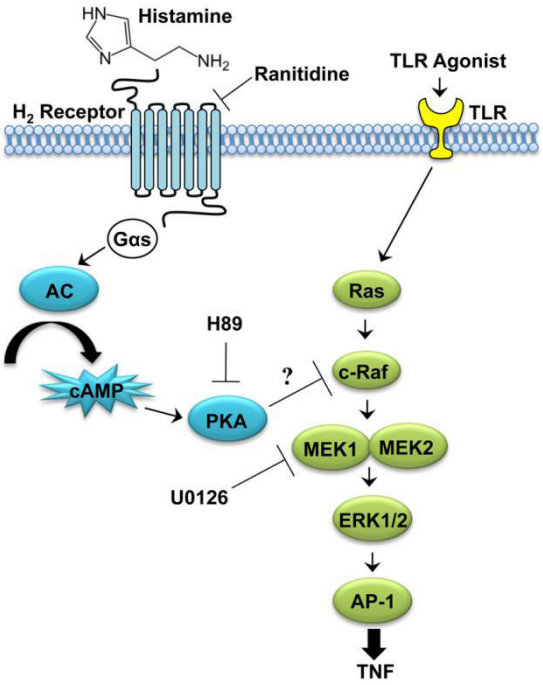
Plant Name	Plant part used	Mechanism of action
Leucas aspera (Willd.) Link	Leaves	Antiasthmatic
Mangifera indica Linn.	Seed & Bark	Asthma
Manilkara hexandra Dubard.	Leaves	Antiasthmatic
Mimosa pudica L	Leaves	Antiasthmatic
Mentha spicata Linn. Emend. Nethh.	Leaves	Leaves Mast cell stabilizer
Momordica dioica Roxb. Ex Wild.	Bulb	Mast cell stabilizer, Antiallergic
Moringa oleifera	Seed	Bronchodilator
Mucuna pruriens	Seed	AntiasthmaticS
Myrica esculenta Buch-Ham	Stem bark	Mast cell stabilizer, Bronchodilator
Nigella sativa	Seed	Bronchodilator
Nyctanthes arbortristis Linn.	Stem bark	Mast cell stabilizer, Bronchodilator
Ocimum sanctum	Leaves	Mast cell stabilizer
Ocimum tenuiflorum Linn	Leaves	Antiasthmatic
Ocimum sanctum	Leaf	Bronchitis, Cough

Plant Name	Plant part used	Mechanism of action
Olea	Ripe Fruits	Antiasthmatic
Orthosiphon rubicundus Benth	Leaves	Antiasthmatic
Oxalis corniculata L	Whole Plant	Antiasthmatic
Passiflora incarnate	Leaves	Bronchodilator & Histmine
Paederia foetida	Leaves	Bronchodilator
Phaseolus radiates	Seed	Asthma, Chronic Bronchitis
Physidis angulata Linn	Leaves	Mast cell stabilizer
Phymatodes scolopendria	Aerial parts	Bronchodilator
Piper betel Linn	Leaves	Bronchodilator
Pinus roxburghii	Whole Plant	Asthma, Chronic Bronchitis
Piper nigrum Linn.	Fruits	Bronchodilator
Picorrhiza kurroa	Roots	Mast cell stabilizer, Bronchodilator
Polygala elongataWilld	Roots	Mast cell stabilizer
Portulaca quadrifida L	Whole Plant	Mast cell stabilizer
Premna obtusifolia	Roots	Asthma, Bronchitis
Punica granatum Linn.	Seed	Asthma, Cough
Rauvolfia serpentina (L.)	Whole Plant	Bronchodilator

Plant Name	Plant part used	Mechanism of action
Benth.ex		
Rivea hypocratoriformis Choisy.	Leaves	Mast cell stabilizer
Sansevieria roxburghiana Schult.	Leaves	Antiasthmatic
Semecarpus ancardium	Fruits	Asthma, Cough
Solanum nigrum Linn.	Roots	Mast cell stabilizer
Solanum surattense Burm.f	Whole Plant	Asthma, Bronchospasm
Spondias pinnata Linn.f	Seeds	Antiasthmatic
Solanum xXanhocarpum	Roots	Mast cell stabilizer
Sphaeranthus indicus Linn.	Flowers	Mast cell stabilizer
Striga orobanchioides Benth	Whole Plant	Mast cell stabilizer, Antihistamine
Swertia Chirata	Leaves	Bronchial asthma
Tamarindus indica	Leaves	Bronchodilator, Antihistaminic, Anti-inflammatory
Taxus baccata Linn.	Leaf	Asthma, Bronchitis
Tephrosia purpuria	Aerial parts	Mast cell stabilizer, Bronchodilator
Terminalia belerica	Leaf galls	Asthma
Terminalia chebula Retz.	Fruits	Mast cell stabilizer & Antiallergic

Plant Name	Plant part used	Mechanism of action
Tinospora cardifolia Wild Mier ex Hook f.	Stem	Mast cell stabilizer
Trachyspermum ammi	Fruits	Asthma
Tylophora asthmatica (L.f.) Wight & Arn.	Leaves	Mast cell stabilizer & Anti inflammatory
Vitex negundo L.	Leaves	Bronchodilator, Antiallergic & Mast cell stabilizer
Zanthoxylem rhetsa (Roxb.) DC	Fruit	Antiasthmatic
Zingiber capitatum Roxb	Rhizomes	Antiasthmatic
Zingiber officinale Thw	Rhizomes	Antiasthmatic

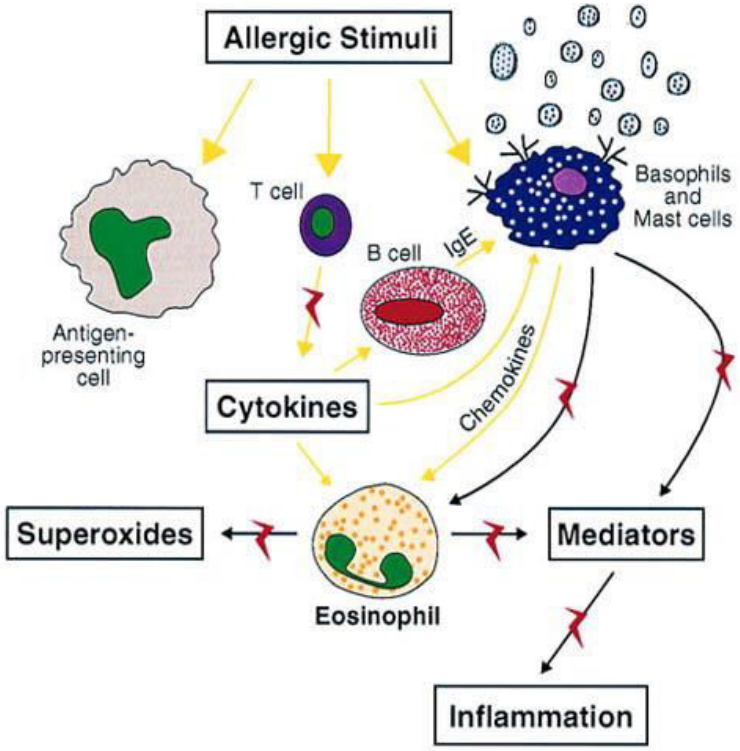
Figure No. 1: Antihistaminic Activity



1.2 Chemistry of histamine

Histamine (β-Aminoethyl imidazole or [2-(imidazol-4- yl)ethylamine]) is a hydrophilic molecule consisting of an imidazole ring with amino group connected by two methylene groups. Analogs of histamine activated the four classes of 4 histamine receptors (H1, H2, H3 and H4). 2-Methylhistamine and 4(5)-Methyl histamine have a preferential effect on H1 and H2 receptors respectively. A chiral analog of histamine with restricted conformational freedom (R)-α-methyl histamine is the preferred strong and weak agonist of the H3 and H4 receptors respectively[18-21].

Figure No. 2: Allergic Stimuli and consequences



1.3 Distribution

Histamine is widely distributed in animal kingdom and is presented in venoms, bacteria and plants. Almost all mammalian tissues contain histamine in amounts ranging from less than 1 to more than 100 µg/g. Particularly tissues having high concentration of histamine and that contain large number of mast cells such as

skin, bronchial tree mucosa and intestinal mucosa. 1.3 Synthesis, storage and metabolism of Histamine Histamine is a dibasic vasoactive substance formed from the decarboxylation of the amino acid histidine by the enzyme L-histidine decarboxylase.

1.4 Release and functions of endogenous Histamine

Histamine in the body tissues is found in mast cells. Mast cells are granulated cells of hematopoietic origin localized to tissues. Mast cells play a role in innate and adaptive defense to pathogens as well as in various inflammatory and immunoregulatory responses. Its liberation takes place during antigen-antibody reactions with prior sensitization of the organism with specific antigen. Mechanical stimuli like injury to the tissue, cold, heat and ultraviolet rays also cause release of histamine. Histamine establishes three species like at pH values between 6.5 and 8.5 the predominant species is the monocation, at pH values lower than 5, the predominant species is the dication and at pH values greater than 10, the predominant species is the free-base. When, histamine is a free base, it is bound to acid group like carboxyl, thiol and phosphate of the cellular proteins. Any free base species stronger than monocation and dication species, histamine, antigen, snake, spider, bee bites, bacterial toxins, acids, alkalies, proteolytic enzymes (trypsin), detergents, macromolecules like dextran and polyvinyl pyrrolidone displaces histamine from the bound form in plasma proteins. Drugs containing tertiary and quaternary nitrogen atoms particularly various compounds like d- 7 tubocurarine, morphine, codeine and atropine also cause the release of histamine in the body[22-24].

Asthma is a complex inflammatory disease causes airway narrowing and associated with changes in the levels of eosinophils, mast cells, lymphocytes, cytokines and other inflammatory cell products. It is well known that patients with asthma have high levels of specific IgE that binds to receptors of mast cells and other inflammatory cells. Interaction between IgE antibody and antigen results in the activation of a series of inflammatory cellular reactions, including the release of mediators such as histamines, prostaglandins and leukotrienes, which subsequently

lead to contraction of airway smooth muscle and bronchoconstriction. 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. Since 1970s, the global prevalence, morbidity, mortality, and economic burden of asthma have increased particular in children.

Medicinal plant used for the treatment of asthma should have anti-inflammatory, immunomodulatory, antihistaminic, smooth-muscle relaxants and allergic activity. According to Ayurveda anti-asthmatic drug should have properties such as anti-kapha and anti-vata. Antioxidant supplements are effective in reducing bronchoconstriction severity by inhibiting pro-inflammatory events as a result of neutralizing the effects of excess reactive oxygen species and reactive nitrogen species. Current asthma therapy lack satisfactory success due to adverse effect, hence patients are seeking complementary and alternative medicine to treat their asthma. Quercetin is one of the most widely occurring flavonoids ingested in food by humans. Quercetin inhibits mast cell degranulation and subsequent release of histamine.

1.4.1 Isoprenaline

Causes tachycardia.

1.4.2 Salbutamol

Muscle tremors (dose related), palpitation, restlessness, nervousness, throat irritation and ankle edema.

1.4.3 Theophylline

Convulsions, shock, arrhythmias, increased muscle tone, tachapnoea, (dose dependent) flushing, hypotension, restlessness, tremors, vomiting, palpitation, diuresis, dyspepsia, insomnia etc.

1.4.4 Anticholinergics

Dry mouth, difficulty in swallowing and talking, scarlet rash, photophobia, blurring of near (Atropine and its congeners) vision, palpitation, ataxia, delirium, hallucinations, hypotension, weak and rapid pulse, cardiovascular collapse with respiratory depression, convulsions and coma (in severe poisoning).

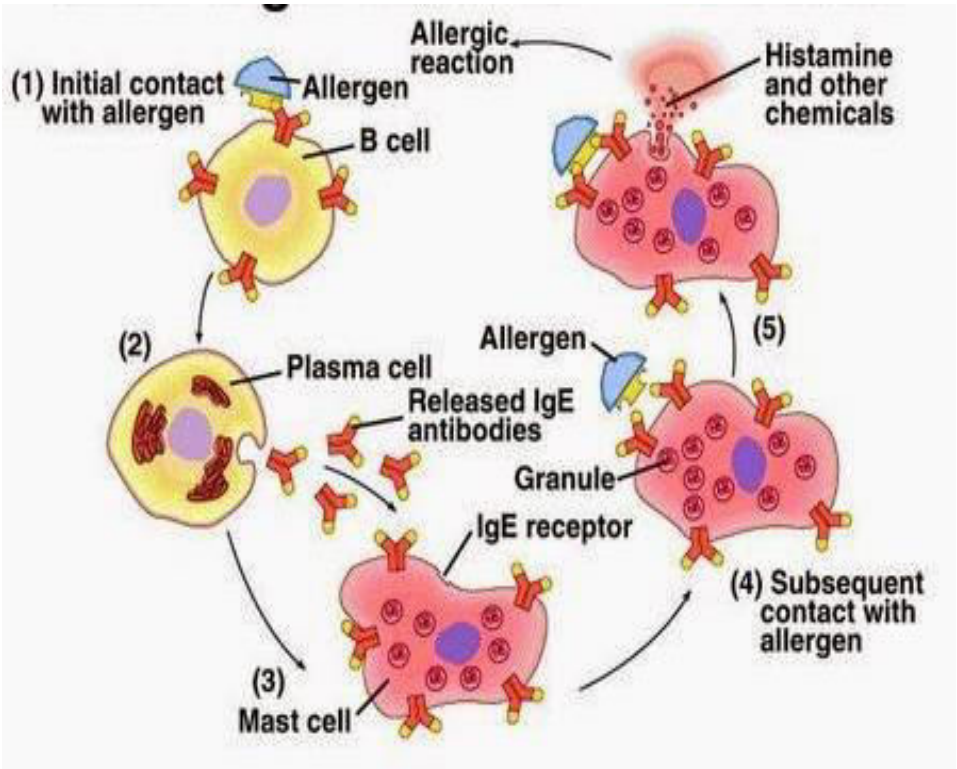
1.4.5 Ketotifen

Sedation, dizziness, dry mouth, nausea and weight gain.

1.4.6 Corticosteroids

Cushing's habitus, fragile skin, purple striae, hyperglycemia, muscular weakness, susceptibility to infection, delayed healing of wounds and surgical incisions, peptic ulceration, osteoporosis, glaucoma, growth retardation, psychiatric disturbances, suppression of hypothalamo-pituitary-adrenal (HPA) axis etc.

Figure No. 3: Scheme of Allergic reaction



As a consequence, the search for effective low-risk, non-drug strategies that provide a valuable adjunctive or alternative treatment in asthma management is clinically attractive and relevant. There is much interest in complementary and alternative medicine, and its use in the management and treatment of asthma is growing at a significant rate. Present review describes some plants that have been pharmacologically evaluated for those parameters involved in asthma[25-28].

1.4.7 Aerva lanta Linn (Amaranthaceae)

Aerva lanta (*A. lanta*) is an erect or prostrate herbaceous common wayside weed which is recognized by its white axillary bunches of small woolly flowers. It is abundant on the plains in the warmer parts of India. Ethanol extract of aerial parts of *A. lanata* at 100 µg/mL in the isolated goat tracheal chain preparation model and 30 and 60 mg/kg doses orally in clonidine-induced catalepsy and mast cell degranulation in mice possesses antiasthmatic activity.

1.4.8 Ageratum conyzoides L.

Ageratum conyzoides (*A. conyzoides*) is an erect, herbaceous annual plant from the family Asteraceae (Compositae), native to tropical America, but with a distribution range in tropical and subtropical areas around the world. Hydroalcoholic extract of leaves of *A. conyzoides* at doses of 250, 500 and 1 000 mg/kg shows antihistaminic activity by inhibiting clonidine induced catalepsy in mice.

1.4.9 Argemone mexicana

Argemone mexicana (*A. mexicana*) is common everywhere by road-sides and fields in India. It possesses antiallergic and antistress activity of aqueous extracts of *A. mexicana* stem at dose 50 mg/kg, *i.p.* using milk-induced leucocytosis and milk-induced eosinophilia.

1.4.10 Asystasia gangetica T. Adams (Acanthaceae)

Asystasia gangetica (*A. gangetica*) is used in many parts of Nigeria for the management of asthma. Akah, *et al.* evaluated hexane, ethylacetate, and methanol extracts of the leaves of *A. gangetica* for antiasthmatic activity using guinea pig trachea; rat stomach strip; guinea pig ileal preparation and egg albumin-induced acute inflammation. The results indicated that the extracts did not exhibit contractile or relaxant activity in isolated tissue preparations; however, they inhibited the contraction evoked by spasmogens.

1.4.11 Bacopa monnieri L. (Scrophulariaceae)

Bacopa monnieri: Samiulla, *et al*. evaluated petroleum ether, chloroform, methanol and water extracts of *B. monnieri* leaves at doses 10 µg/mL for mast cell stabilizing activity in rats. The result of investigation observed that all the extract significantly inhibits mast cell degranulation.

1.4.12 Cassia sophera (caesalpiniaceae)

Cassia sophera (*C. sophera*) is used in traditionally for treatment of asthma and bronchitis. Chloroform, ethyl acetate and ethanol fractions isolated from ethanol extract of leaves of *C. sophera* possesses significant antiasthmatic activity in carrageenan induced paw edema, histamine induced bronchoconstriction, clonidine and haloperidol induced catalepsy, milk induced leukocytosis, and eosinophilia and passive paw anaphylaxis animal models at doses 250, 500 and 750 mg/kg and this activity may be due to presence of flavonoids.

1.4.13 Casuarina equisetifolia Linn (Casuarinaceae)

Casuarina equisetifolia (*C. equisetifolia*) is evergreen tree; generally attain height up to 50 m, cultivated on Coastal regions from Gujarat to Orissa, some parts of West Bengal and in Andamans. The methanol extract of extracts of wood and bark possesses antihistaminic activity by inhibiting the histamine induced contraction of

trachea (10-80 mcg/mL), clonidine induced catalepsy and mast cell degranulation at doses 100 mg/kg.

1.4.14 Clerodendrum Serratum Linn (Verbenaceae)

Clerodendrum Serratum (*C. serratum*), known as bharangi in ayurveda, is traditionally useful in treating pain, inflammation, rheumatism, respiratory diseases, and malarial fever. Ethanol extract of roots of *C. serratum* showed antiasthmatic activity using isolated goat tracheal chain preparation, clonidine induced catalepsy; Milk induced leucocytosis and eosinophilia in mice at doses 50,100 and 200 mg/kg.

1.4 15 Cnidium monnieri (Umbelliferae)

Cnidium monnieri (*C. monnieri*) in traditional medicine of China has been used for treatment of pain in female genitalia, impotence and suppurative dermatitis as an antipruritogenic agent. Matsuda *et al*. reported antiallergic activity of ethanol extract and Osthol a chromane isolated from ethanol extract of fruits of *C. monnieri* in passive cutaneous anaphylaxis in rats.

1.4.16 Crinum glaucum (Amaryllidaceae)

Crinum glaucum (*C. glaucum*) is popular in Yoruba of South West Nigeria. Traditional medicine practitioners reported it as an effective remedy in the relief of cough, asthma and convulsions. The aqueous extract of *C. glaucum* possesses antiallergic activity at dosed 100-400 mg/kg by reduction in area of dye leakage in passive cutaneous anaphylactic reaction, protecting degranulation of mast cell and histamine induced bronchoconstriction in the guinea pig.

1.4.17 Curculigo orchioides Gaertn (Amaryllidaceae)

Curculigo orchioides (*C. orchioides*) is a tiny herbal plant widely distributed in India, China, Malaya, and Japan. Alcoholic extract of *C. orchioides* rhizomes at doses (100-400 mg/kg) shows mast cell stabilizing and antihistaminic activity on

Compound 48/80-induced mast cell degranulation and systemic anaphylaxis. It also inhibited histamine-induced contraction in goat trachea, guinea pig ileum and bronchoconstriction in guinea pigs; egg albumin induced passive paw anaphylaxis in rats; milk induced leucocytosis and eosinophilia; clonidine induced catalepsy in mice.

1.4.18 Eclipta alba Linn (Asteraceae)

The 50% ethanol extract shows antianaphylactic and antihistaminic activity at doses 250 and 500 mg/kg on compound 48/80-induced degranulation of mast cell, egg albumin induced passive Cutaneous and paw anaphylaxis; bronchoalveolar lavage (BAL) study on gunea pig trachea; and determination of histamine.

1.4.19 Euphorbia hirta (Euphorbiaceae)

Popularly known as asthma weed, *Euphorbia hirta* is an herbaceous wild plant which grows in the hotter parts of India. Ethanol extract of whole aerial part of the plant at doses (100-1000 mg/kg) shows antihistaminic and antiallergic activity by inhibiting inhibited the passive cutaneous anaphylaxis and paw anaphylaxis reaction; protection of mast cell from degranulation.

1.4.20 Ficus bengalensis Linn (Moraceae)

*Ficus bengalensis*is (*F. bengalensis*) is a very large tree reaching about 30 m high and sending down many aerial roots from the branches. Ethyl acetate, ethanol and aqueous extracts as well as fractions isolated from aqueous extract of *F. bengalensis* bark possesses antihistaminic activity by inhibiting clonidine induced catalepsy in mice at dose 50 mg/kg. These activity may be due to presence of flavonoids.

1.4.21 Gakani

Gakani is a polyherbal drug contains *Cenchrus biflorus* Roxb. Gramineae *Olax subscorpioidea* Oliv. (Olacaceae), *Piper guineense* schum Thonn

(Piperaceae), *Psorospermum guineense* Hochr. (Hypericaceae), *Securidaca Iongipedunculata* Tresen (Polygalaceae), *Syziygium aromaticum* (L.) Merr. (Myrtaceae). The anti-asthmatic potential of *Gakani*, a popular herbal drug was investigated using guinea pig tracheal chain; guinea pig ileum preparation; on the rat stomach strip and egg albumin induced hind paw edema. Result indicates that the extract blocked the effects of histamine and isoprenaline on the guinea pig tracheal chain. It shows inhibition contraction of isolated guinea pig ileum and rat stomach strip, caused by histamine and 5-hydroxytryptamine (5-HT). The extract had good anti-inflammatory effect in rats.

1.4.22 Hemidesmus indicus R. Br. (Asclepiadaceae)

Hemidesmus indicus (*H. indicus*) is a twining shrub commonly found in India. Bhujbal *et al.* reported antiasthmatic activity of ethanol extract of *H. indicus* roots at doses 25, 50, 100 mg/kg using isolated goat tracheal chain preparation, passive paw anaphylaxis in rat and clonidine-induced catalepsy in mice.

1.4.23 Amburana cearensis (Fabaceae)

Amburana cearensis (*A. cearensis*) is a medicinal plant common to the Brazilian Northeastern "caatinga" (savannah), and popularly used in respiratory tract diseases including asthma. The flavonoid isokaempferide isolated from Trunk barks of *A. cearensis* shows significant relaxation of KCl induced contraction on guinea pig trachea.

1.4.24 Plants from Zinziberaceae

Tewtrakul *et al*. reported antiallergic activity of ethanol and water extract of some plants of Zinziberaceae family.

1.4.25 Lepidium sativum Linn (Cruciferae)

Commonly known as Asaliyo, it is an erect, glabrous annual herb cultivated as a salad plant throughout India. The ethanol extract and ethyl acetate, n-butanol and methanol fractions isolated from ethanol extract inhibit bronchospasm induced by histamine and acetylcholine.

1.4 26 Mentha spicata L

The four new flavonoids and three new glycosides isolated from ethyl acetate soluble fractions of *M. spicata* leaves shows antihistaminic activity by inhibiting antigen stimulated rat basophile.

1.4.27 Momordica dioica

Momordica dioica is climbing creeper plant. Its fruits and leaves are traditionally used as medicinal agent of asthma, leprosy, bronchitis, fever, tridosha. Methanol and aqueous extract of pulp possesses antihistaminic activity by inhibiting clonidine induced catalepsy in mice at dose 50 mg/kg; this activity may be due to polar constituents.

1.4.28 Mucuna pruriens

The L-DOPA isolated from methanol extract of seed possesses antihistaminic activity by inhibiting clonidine induced catalepsy and mast cell degranulation in mice at dose 50, 100 and 200 mg/kg.

1.4.29 Myrica esculenta Buch. Ham. (Myricaceae)

Myrica esculenta is commonly known as Kaiphal. It is used for treatment of asthma and broncititis in ayurvedic system of medicine. Patel *et al*. reported antiallergic and anti-inflammatory activity of ethanol extract of aerial parts using acetic acid induced vascular permeability and allergic pleurisy in mice methods at doses 75 and 150 mg/kg. Stem bark of this plant possesses bronchodilator and

antianaphylactic activity by inhibiting acetylcholine induced bronchospasm in guinea pigs, egg albumin induced anaphylaxis in guinea pigs at dose 75 mg/kg and by relaxing histamine and acetylcholine induced guinea pig trachea and ileum.

1.4.30 Nyctanthes arbortristis

It is used traditionally in the treatment of asthma. The petroleum ether extract shows antihistaminic activity by inhibiting clonidine-induced catalepsy in mice at dose 50 mg/kg.

1.4.31 Olea europea (Oleaceae)

It is a small evergreen tree, from 12 to 20 feet high, with hoary, rigid branches, and a grayish bark. Aqueous extract of ripe olives possesses antiasthmatic activity by inhibiting clonidine induced peritoneal mast cell degranulation in rats and catalepsy in mice at doses 4 and 8 mg/kg and also by protecting histamine induced contraction of goat trachea and guinea pig ileum at concentration of 100 µg/mL.

1.4.32 Phymatodes scolopendria (Burm.) Ching (Polypodiaceae)

Phymatodes scolopendria is a crawling fern growing in the sandy areas of the East coast of Madagascar. Ramanitrahasimbola *et al*. reported bronchodilator activity of 1, 2-benzopyrone (coumarin) isolated from ethanol extract of aerial parts by inhibiting histamine or carbachol pre-contracted guinea pig trachea.

1.4.33 Piper betel Linn

Piper betel is traditionally used to to cure cough, cold, pruritis, asthma and rheumatism. Ethanol and aqueous extract of leaves at doses 100 and 200mg/kg possesses antiasthmactic activity on histamine induced bronchoconstriction in guinea pig and histamine induced dose dependent contraction of guinea pig tracheal chain and isolated guinea pig ileum preparation.

1.4.34 Striga orobanchioides Benth (Scrophulariaceae)

Striga orobanchioides is a parasitic plant, lives on the roots of various plants. Ethanol and aqueous extracts of whole plant shows antihistaminic and mast cell stabilizing activity by inhibiting histamine-induced contractions of the guinea-pig ileum at the concentration 2.5-25 µg/mL in a dose-related manner and inhibiting degranulation of mast cells at dose 100 and 200 mg/kg]

1.4.35 Sphaeranthus indicus Kurz (Asteraceae)

Sphaeranthus indicus is a medicinally important plant used as folk medicine. The ethanol extract at the doses of 150, 300 mg/kg and its ethyl acetate extract at the dose of 100, 150 mg/kg and 300 mg/kg showed slightly better protection against sheep serum and Compound 48/80-induced mast cell degranulatiuon than the standard drug ketotifen

1.4.36 Cynodon dactylon (Poaceae)

Cynodon dactylon is one of the most commonly occurring perennial grass throughout India, commonly known as Dhub. The petroleum ether, chloroform and methanol extracts of whole plant and fractions isolated from chloroform extract possess antianaphylactic activity but fractions isolated possesses more potent activity at doses 10, 25, 50 and 100 mg/kg using compound 48/80-induced mast cell degranulation, determination of level of nitric acid in serum, compound 48/80-induced anaphylaxis.

1.5 Role in allergic responses

The main target cells of immediate hypersensitivity reactions are in mast cells and basophil.10 The allergic responses are caused by either an immune response to a normally innocuous substance, which comes in contact with lymphocytes specific for that substance or antigen or a freefloating IgE (an immunoglobulin associated with allergic response) antibodies. These are bind to the surfaces of mast cells and

basophils via high affinity Fc receptors, which are more specific for IgE. 1.6 Release of other autacoids Release of histamine in the human bodies provides a partial knowledge on all biological effects because of production of other autacoids that ensure from immediate hypersensitivity reactions. Stimulation of IgE receptors also activates phospholipase A2 (PLA2) leading to the production of a host mediators including Platelet-Activating Factor (PAF), metabolites of arachidonic acid and Leukotriene D4. Histamine generated in this way is a potent contractor of the smooth muscle of the bronchial tree[29-33].

1.5.1 Classification of antihistamines

The H1-antagonists are now commonly subdivided into two broad groups - the first generation8 or classical antihistamines and the second generation or "non-sedating" antihistamines – based primarily on their general pharmacological profiles. First generation H1-antagonist drug classes

A. Aminoalkyl Ethers (Ethanolamines): Diphenhydramine HCl, Bromo diphenhydramine HCl, Dimenhydrinate.

B. Ethylenediamines: Pyrilamine Maleate, Methapyrilene HCl , TripelennamineHCl or Citrate, Thonzylamine HCl , Antazoline Phosphate.

C. Piperazines(Cyclizines): CyclizineHCl or Lactate, Chlorcyclizine HCl, Meclizine HCl, Hydroxyzine HCl, Buclizine HCl. D. Propylamines (Monoaminopropyl Derivatives): Pheniramine Maleate, Brompheniramine Maleate, Dextrobrompheniramine Maleate, Chlorpheniramine Maleate, Dextrochlorpheniramine Maleate.

E. Phenothiazines: Promethazine HCl, Trimeprazine Tartrate, Methdilazine HCl

F.Dibenzocycloheptenes / heptanes: Cyproheptadine HCl (Dibenzocycloheptene),Azatadinemaleate (Dibenzocycloheptane). Second generation H1-antagonist drug classes.

A. Piperidine Second Generation Antihistamines: Terfenadine, Fexofenadine Hydrochloride, Astemizole, Loratadine,

B. Piperazine Second Generation Antihistamines: Cetirizine, C. Pyrrolidine "Second Generation" Antihistamines: Acrivastine. "Dual-acting" antihistamines:- Pemirolast Potassium (Alamast), Azelastine HCl (Optivar),Ketotifen Fumarate, (Zaditor) Third generation H1-antagonist drug classes: Levocetirizine, Desloratadine, Fexofenadine.

1.5.2 Factors involved in sedative adverse effects.

The sedative symptoms caused by antihistamines include drowsiness, fatigue, lassitude, dizziness and weakness. At least two theories have been put forward to explain the apparent lack of sedative effects among the new generation H1-receptor antagonists. One is based on H1-receptor heterogenecity between central and peripheral systems. The non-sedative H1-receptor antagonists, like mequitazine and loratidine are therefore those with selective affinity for the peripheral H1- receptor. The other more commonly accepted explanation is that the non- sedative H1-antagonists, e.g., terfenadine and astemizole, do not readily penetrate into the CNS. A parameter reflecting the ionization of H1-receptor antagonists e.g., octanol/ water distribution coefficient at pH 4 (Doct 7.4) is necessarily to be considered. Log Doct lower than 0 or higher than 3 or higher hydrogen bonding capacity will cause poor brain penetration. To increase log D value, lipophilicity can be increased, basicity can be increased, hydrogen bonding can be increased if log Doct between 0-3 to avoid crossing Blood Brain Barrier[34-36].

1.6 List of Herbs having Antihistaminic activity

Histamine is a chemical, which is involved in our local immune response as well as regulating physiological function in the gut, acting as a neurotransmitter. During the time of seasonal allergies, the body's immune system sees pollen as an invader. In an allergic reaction, our body produces histamine, as a defense mechanism. This inflammatory chemical attaches the cells in our body and causes irritation. It is the deficiency of this enzyme that triggers an allergic reaction as histamines gathers in the synapses.

An antihistamine serves to reduce or eliminate the effects brought on by histamine, a chemical mediator released during allergic reactions. Antihistamines are commonly used for allergic rhinitis, allergic conjunctivitis, contact dermatitis, urticaria (hives), angioedema and pruritus (atopic dermatitis, insect bites). Basil (Ocimum sanctum): this great herb has a history in helping prevent stomach cramps, gas as well as constipation. A poultice of Basil leaves can can work as an antihistamine to draw out insect, bee wasp, or snake venom. It helps alleviate acne, heal abrasions and speeds healing when used on cuts. Chamomile (Matricaria chamomilla)**:** is rich in anti-histamine properties. The flowers can be crushed and used as a poultice for inflammatory swelling. Make a tea and drink 2-3 times a day. Chamomile can cause histaminic allergic reactions in some very sensitive people. If this occurs, simply discontinue.

Jewelweed (Impatiens aurea): contains a compound called "Lawsone" that treats uticaria. Jewelweed is used as a natural remedy for poison ivy, poison oak, okra spines, stinging nettle and acne treatment. Jewelweed is also used for heat rash, ringworm and many other skin disorders, as well as bug bites and razor burn. Papaya (Petroselinum crispum): inhibits the secretion of histamine. Papaya juice can be taken internally as well as applied topically to diffuse a histamine attack. Thyme (Thymus vulgaris): is a natural antihistamine, as well as having antiseptic properties to help purge infections. The essential oil has been shown to have

antimicrobial activity against a host of different bacteria and fungi. Wild Oregano (Origanum vulgare): aka Wild Marjoram, have at least seven different antihistaminic chemicals, therefore fights allergies as well as fungus and infection. Lemon balm (also known as melissa): has antihistamine action and is useful to treat eczema and headaches. This essential oil has antihistamine properties and helps with allergies[37,38].

2.0 LITERATURE REVIEW

Slinkard, K, et al., (2015)[39] revealed that orodispersible tablets (ODTs) have been successfully used in therapy for more than 20 years, there is still no compendia method of their disintegration time evaluation other than the pharmacopoeial disintegration test conducted in 800–900 mL of distilled water. Therefore, several alternative tests more relevant to *in vivo* conditions were described by different researchers. The aim of this study was to compare these methods and correlate them with in vivo results. Six series of ODTs were prepared by direct compression. Their mechanical properties and disintegration times were measured with pharmacopoeial and alternative methods and compared with the *in vivo* results. The highest correlation with oral disintegration time was found in the case of own-construction apparatus with additional weight and the employment of the method proposed by Narazaki et al. The correlation coefficients were 0.9994 ($p < 0.001$), and 0.9907 ($p < 0.001$) respectively. The pharmacopoeial method correlated with the in vivo data much worse ($r = 0.8925$, $p < 0.05$). These results have shown that development of novel biorelevant methods of ODT‟s disintegration time determination is eligible and scientifically justified.

Zhishen, J, et al., (2015)[40] reported that the Orodispersibletablets (ODTs) and orodispersible films (ODFs) are solid oral dosage forms disintegrating or dissolving rapidly when placed in the mouth. One of the main issuesrelated to their preparation is an efficient taste masking of a bitter drug substance. Therefore, the aim of this study was to prepare and evaluate the micro particles intended to mask a bitter taste of the prednisolone and use them in further preparation of two orodispersible dosage forms. Micro particles based on the Eudragit E PO or E 100 as a taste-masking agent were prepared with spray-drying technique. Tablets containing micro particles, co-processed ODT excipient Pharmaburst, and lubricant were directly compressed with single-punch tablet press. Orodispersible films were prepared by casting polymeric solutions of hydroxypropyl methylcellulose containing uniformly dispersed micro

particles. Physicochemical properties of micro particles were evaluated, as well as mechanical properties analysis, disintegration time measurements and dissolution tests were performed for prepared dosage forms. Both formulations showed good mechanical resistance while maintaining excellent disintegration properties. The dissolution studies showed good masking properties of micro particles with Eudragit E 100. The amount of prednisolone released during the first minute in phosphate buffer 6.8 was around 0.1%. After incorporation into the orodispersible forms, the amount of released prednisolone increased significantly. It was probably the effect of faster micro particles wetting in orodispersible forms and their partial destruction by compression force during tablet ting process.

Onay U, J, et al., (2006)[41] revealed that the quality by design (QbD) approach was applied for optimizing the formulation of extemporaneously prepared or odispersible films (ODFs) using Design-Expert Software. The starting formulation was based on earlier experiments and contained the film forming agents hypromellose and carbomer 974P and the plasticizer glycerol (Visser et al., 2015). Trometamol and disodium EDTA were added to stabilize the solution. To optimize this formulation a quality target product profile was established in which critical quality attributes (CQAs) such as mechanical properties and disintegration time were defined and quantified. As critical process parameters (CPP) that were evaluated for their effect on the CQAs the percentage of hypromellose and the percentage of glycerol as well as the drying time were chosen. Response surface methodology (RMS) was used to evaluate the effects of the CPPs on the CQAs of the final product. The main factor affecting tensile strength and Young‟s modulus was the percentage of glycerol. Elongation at break was mainly influenced by the drying temperature. Disintegration time was found to be sensitive to the percentage of hypromellose. From the results a design space could be created. As long as the formulation and process variables remain within this design space, a product is obtained with desired characteristics and that meets all set quality requirements.

Meir, S, et al., (2015)[42] reported that the Orodispersible films (ODFs) are promising drug delivery systems for customized small scale pharmacy preparations. The aim of the present study was to develop a versatile casting solution suitable for the extemporaneous production of ODFs to which active pharmaceutical ingredients (APIs) can be added. Different combinations of film forming agents and other excipients and different casting heights were tested for their suitability for production of ODFs. The best suitable casting solution contained hypromellose, carbomer, glycerol, disodium EDTA and trometamol. This casting solution was used to prepare ODFs containing water-soluble APIs (enalapril maleate and prednisolone disodium phosphate) and a poorly water-soluble API (diazepam) for which ethanol 96% was used as co-solvent. The water soluble APIs as well as ethanol influenced the viscosity of the casting solution, mechanical properties and disintegration time of the ODFs. All ODFs containing API met the requirements on uniformity of mass and uniformity of content set by the European Pharmacopoeia (2014) (Ph. Eur.) 8th edition. In conclusion, ODFs of good pharmaceutical quality can be prepared on small scale. Hereby opening the perspective of using ODFs for individualized pharmacotherapy.

Wood, LG, et al., (2006)[43] revealed that the There are no test procedures, definitions and specifications available how to determine mechanical strength of orodispersible or buccal films. Aim of the study was to develop an appropriate and discriminating method to feature the evaluation of marketed and newly developed film products covering well-known and new approaches. The limits for mechanical strength were set starting from a puncture strength of 0.06 N/mm 2 according to the obtained results from marketed products. Furthermore, elongation to break of the marketed films (1.03–6.54%) and prepared film samples (4.51–33.17%) offered information on the film properties. The developed mechanical strength test method was suitable for all film types without the need of a pre-defined specimen. A mechanical strength threshold could be specified for future orodispersible film development.

Benzie, FF, et al., (2014) revealed that the preformulation study of rotary spun hydroxypropyl cellulose fibers was carried out using the combination of textural characterization of gels in the concentration range of 42–60% w/w and optical microscopic evaluation of formed fibers. High adhesiveness values resulted in bead formation at lower polymer concentration, meanwhile fiber formation was hindered when high adhesiveness values were associated with high polymer content. The optimum gel concentration for fiber formation was given to 50% w/w. Drug loaded microfibers were prepared using a model drug of biopharmaceutical drug classification system class II. Fibers were milled, sieved and mixed with tabletting excipients in order to directly compress orodispersible tablets. Hardness, friability, *in vitro* disintegration time values complied with the pharmacopoeial requirements. *In vitro* dissolution profiles obtained from three distinct dissolution media (pH 1.0; 4.5; 6.8) were quite differentiated compared to the compressed physical mixture of the same composition. Difference and similarity factors confirmed that the drug dissolution from microfiber based formula was almost independent from the pH value of the media. X-ray diffraction patterns indicated that the drug embedded in microfibers was in amorphous state, and the decrease of o-Ps lifetime values suggested that fiber formation enabled the development of a more ordered fibrous system.

Ozen, T, et al., (2003)[45] reported that the orodispersible tablets or fast dissolving tablets dissolve or disintegrate immediately on the patients‟ tongue or buccal mucosa. This drug delivery system is suitable for drugs undergoing high first pass metabolism. It improves bioavailability, reduces dosing, and thereby minimizes the side effects and also makes the dosage form more cost-effective. In this study, polysaccharide isolated from the seeds of *Cassia tora* was investigated as a superdisintegrant in the orodispersible tablets. The model drug chosen was valsartan, an antihypertensive drug. Valsartan tablets were prepared separately using different concentrations (1%, 2.5%, 5%, and 7.5% w/w) of isolated *C. tora* seed polysaccharide (natural) and sodium starch glycolate (synthetic) as superdisintegrant by the direct

compression method. Evaluation of tablets was done for various pre- and post compression parameters. The stability studies were performed on optimized formulation F4. The disintegration time and *in vitro* drug release of the formulation F4 were compared with marketed formulations (conventional tablets).The drug excipient interactions were characterized by Fourier transform infrared studies. The formulation F4 containing 7.5% polysaccharide showed good wetting time and disintegration time as compared to a formulation prepared using a synthetic superdisintegrant at the same concentration level. Hence, batch F4 was considered optimized formulation. The present work revealed that *C. tora* seed polysaccharide has a good potential as a disintegrant in the formulation of orodispersible tablets. Because *C. tora* polysaccharide is inexpensive as compared to synthetic superdisintegrants, nontoxic, compatible, and easy to manufacture, it can be used in place of currently marketed superdisintegrants.

Arulmozhi, S, et al., (2008)[46] showed that the present study aimed to formulateorodispersible tablets of flutamide (FTM) to increase its bioavailability. Orodispersible tablets were prepared by direct compression technique using three different approaches namely; super-disintegration, effervescence and sublimation. Different combined approaches were proposed and evaluated to optimize tablet characteristics. Sodium starch glycolate(SSG) was used as the superdisintegrant. The prepared powder mixtures were subjected to both pre and post compression evaluation parameters including; IR spectroscopy, micromeritics properties, tablet hardness, friability, wetting time, disintegration time and *in vitro* drug release. IR studies indicated that there was no interaction between the drug and the excipients used except Ludipress. The results of micromeritics studies revealed that all formulations were of acceptable to good flow ability. Tablet hardness and friability indicated good mechanical strength. Wetting and dispersion times decreased from 46 to 38 s by increasing the SSG concentration from 3.33 to 6.66%w/w in tablets prepared by super disintegration method. The F8 formulation which was prepared by combined approaches of effervescence and superdisintegrant addition gave promising

results for tablet disintegration and wetting times but failed to give faster dissolution rate. The incorporation of 1:5 solid dispersion of FTM: PEG6000 instead of the pure drug in the same formulation increased the drug release rate from 73.12 to 96.99% after 15 min. This increase in the dissolution rate may be due to the amorphization of the drug during the solid dispersion preparation. The presence of the amorphous form of the drug was shown in the IR spectra.

Soares, JR, et al., (2009) studied nettle (Urtica dioica) extract shows in vitro inhibition of several key inflammatory events that cause the symptoms of seasonal allergies. These include the antagonist and negative agonist activity against the Histamine-1 (H(1)) receptor and the inhibition of mast cell tryptase preventing degranulation and release of a host of pro-inflammatory mediators that cause the symptoms of hay fevers. Through the use of DART TOF-MS, which yields exact masses and relative abundances of compounds present in complex mixtures, bioactives have been identified in nettle that contribute to the inhibition of pro-inflammatory pathways related to allergic rhinitis. These results provide for the first time, a mechanistic understanding of the role of nettle extracts in reducing allergic and other inflammatory responses in vitro.

Sreena, KP, et al., (2011)[48] experimented Piper betel Linn. leaves were evaluated for its antihistaminic activity, commonly known as Pan, Vidyache Pan or Tamboli. P. betel has a long history of use in India being applied in multiple therapeutic activities like antibacterial, treating eczema, lymphangitis, asthma, treating rheumatism. So selected, the plant P. betel is effective in histaminic activity related diseases, but antihistaminic activity of P. betel is still not scientifically investigated. In the present study, the pharmacological evaluation of ethanolic extract and essential oil extract of leaves of P. betel Linn. has been done for their antihistaminic activity on guinea pig. In isolated guinea pig tracheal chain preparation, there was a right side shift of dose response curve (DRC) of histamine. Chlorpheniramine maleate was used as a standard drug. Moreover extracts of P. betel

disturbed histamine aerosol induce bronchoconstriction in whole guinea pig, where essential oil was more effective comparatively to ethanolic extract. Thus from the results obtained in the present investigation, it can be concluded that ethanolic extract and essential oil of P. betel Linn possess antihistaminic activity.

Kumar, TS, et al., (2008)[49] detailed The antianaphylactic activity was investigated in rats using the active anaphylaxis model. The effect on mast cell stabilization was performed by ex vivo challenge of antigen in sensitized rat intestinal mesenteries. Antihistaminic activity was studied in guinea pigs using histamine-induced bronchospasm where preconvulsive dyspnea was used as an end point following exposure to histamine aerosol. Dose response studies of HK-07 were conducted at 125, 250, and 500 mg/kg, p.o. in anaphylactic shock-induced bronchospasm in rats. The optimal dose level was used for the remaining experimental models.

3.0 PLANT PROFILE

Urtica dioica, often called **common nettle**, **stinging nettle** (although not all plants of this species sting) or **nettle leaf**, is a herbaceousperennial flowering plant in the family Urticaceae. It is native to Europe, Asia, northern Africa, and North America, and introduced elsewhere.[1][2] The species is divided into six subspecies, five of which have many hollow stinging hairs called trichomes on the leaves and stems, which act like hypodermic needles, injecting histamine and other chemicals that produce a stinging sensation upon contact ("contact urticaria").[3][4] The plant has a long history of use as a source for traditional medicine, food, tea, and textile raw material in ancient societies

Kingdom Plantae – Plants

Subkingdom Tracheobionta – Vascular plants

Superdivision Spermatophyta – Seed plants

Division Magnoliophyta – Flowering plants

Class Magnoliopsida – Dicotyledons

Subclass Hamamelididae

Order Urticales

Family Urticaceae – Nettle family

Genus *Urtica* L. – nettle

Species *Urtica dioica* L. – stinging nettle

Range Description The species is is widespread through Europe and North America, and also occurs in North Africa and parts of Asia (Kew 2015).

Countries occurrence

Andorra; Austria; Belgium; Bulgaria; Canada; China; Czech Republic; Denmark; Estonia; Finland; France; Greece; Hungary; Iran, Islamic Republic of; Ireland; Latvia; Liechtenstein; Lithuania; Luxembourg; Mexico; Morocco; Nepal; Netherlands; Norway; Peru; Poland; Romania; Slovakia; Spain; Sweden; Switzerland; Tunisia; United Kingdom; United States

Urtica dioica is a dioecious, herbaceous, perennial plant, 1 to 2 m (3 to 7 ft) tall in the summer and dying down to the ground in winter.[1] It has widely spreading rhizomes and stolons, which are bright yellow, as are the roots. The soft, green leaves are 3 to 15 cm (1 to 6 in) long and are borne oppositely on an erect, wiry, green stem. The leaves have a strongly serrated margin, a cordate base, and an acuminate tip with a terminal leaf tooth longer than adjacent laterals. It bears small, greenish or brownish, numerous flowers in dense axillary inflorescences. The leaves and stems are very hairy with non-stinging hairs, and in most subspecies, also bear many stinging hairs (trichomes or spicules), whose tips come off when touched, transforming the hair into a needle that can inject several chemicals causing a painful sting or paresthesia, giving the species its common names: stinging nettle, burn nettle, burn weed, or burn hazel[50-55].

3.1 Traditional medicine

U. dioica herb has been used in the traditional Austrian medicine internally (as tea or fresh leaves) to treat disorders of the kidneys and urinary tract, gastrointestinal tract, locomotor system, skin, cardiovascular system, hemorrhage, influenza, rheumatism, and gout.

As Old English *stiðe*, nettle is one of the nine plants invoked in the pagan Anglo-Saxon *Nine Herbs Charm*, recorded in the 10th century. Nettle was believed to be a galactagogue, a substance that promotes lactation.

Urtication, or flogging with nettles, is the process of deliberately applying stinging nettles to the skin in order to provoke inflammation. An agent thus used is known as a rubefacient(something that causes redness). This is done as a folk remedy for treatment of rheumatism. In Ecuador there are indigenous healers that use stinging nettles with the belief that they improve fatigue and circulation, by rubbing raw leaves or flogging the plant directly on the body.

4.0 AIM & OBJECTIVE

Synthetic drugs used for the treatment of asthma and allergy in India but more side effects are reported. Over the centuries, they are using medicinal herbs in daily life and approximately 6000 plants species are known to have medicinal properties in India. As per the literature survey will be expressed medicinal plants and traditional systems of medicines, Ayurveda, Yunani, Siddha and Homeopathy for the treatment of asthma and allergy but no scientific validation. Several literatures are indicated that the herbal drugs have lesser adverse effects when compared to synthetic drugs. The *Urtica dioica* is not scientifically validated and which was traditionally using herb. The work provides scientific validation for use of leaves against asthma by revealing the chemical compounds may be present in the plant. The present study is attempts to develop a novel plant based antihistamine work through antiastmatic drug which will be evaluated by *in vitro* and *in vivo*.

5.0 PLAN OF WORK

- Collection and authentication of leaves of *Urtica dioica*
- Extraction of leaves of *Urtica dioica*
- Performing the acute toxicity studies as per OECD guidelines
- Isolated guinea pig ileum preparation
- Histamine induced bronchoconstriction in guinea pig.

6.0 MATERIALS AND METHODS

6.1 Plant collection and authentication

The leaves of *urtica dioica* were collected from Anthiyur forest Tamilnadu and which was authenticated.

6.2 Preparation of plant extract

Cold maceration technique was used for the extraction of plant material and a total of 200 g of *Urtica dioica* leaves the coarse powder was used. During the process, 100 g of the coarse powder was soaked in an Erlenmeyer flask with 1 L of 50% of Ethyl Acetate and then placed on a shaker (Bibby Scientific Limited Stone Staffo Reshire, UK) tuned to 120 rpm with occasional shaking for 72 h at room temperature. The extract was filtered first using a muslin cloth and then Whatman grade No-1 filter paper and the marc was re-macerated for a second and third time by adding another fresh solvent. The filtrates were left overnight in a deep freezer and then lyophilized using freeze dryer. The dried plant extract was reconstituted with distilled water for oral administration.

6.2.1 Phytochemical Test

Chemical tests performed in the screening and identification of phytochemical constituents in the tested medicinal plants were carried out in extracts as well as powder specimens using the standard procedures.

6.2.2 Maeyer's reagent

0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water.

6.2.3 Dragendorff's reagent

Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water. Solution B: 16 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions (A and B) were mixed in1:1 ratio.

6.2.4 Test for alkaloids

About 0.5 to 0.6 g of the methanolic plant extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer's and Dragendorff's).

6.2.5 Test for steroids

About 0.5 g of the methanolic extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid.

6.2.6 Test for terpenoids

An aliquot 0.5 ml of methanolic extract was mixed with 2 ml of CHCl3 in a test tube. 3 ml of concentrated H2SO4 was carefully added to the mixture to form a layer.

6.2.7 Test for flavonoids

To the substance in alcohol, a few magnesium turnings and few drops of concentrated Hydrochloric acid were added and boiled for five minutes.

6.2.8 Test for tannins

The 0.5 g of powdered sample of each medicinal plant leaves was boiled in 20 ml of distilled water in a test tube and then filtered. The filtration method used here was the normal.

6.2.9 Test for Phytosterol

The extract (2 mg) was dissolved in 2 ml of acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulfuric acid was added along the side of the test tube.

6.2.10 Test for Phytosterol

1. Foam Test: 5 ml of the test solution taken in a test tube was shaken well for five minutes.

2. Olive oil test: - Added a few drops of olive oil to 2ml of the test solution and shaken well.

6.2.11 Test for glycosides

1.Keller -Killiani test: Added 0.4 ml of glacial acetic acid and a few drops of 5% ferric chloride solution to a little of dry extract. Further 0.5 ml of concentrated sulfuric acid was added along the side of the test tube carefully.

2. Hydroxyanthraquinone Test To 1 ml of the extract, added a few drops of 10% potassium hydroxide solution.

6.3 Preparation of oral suspension

500g of Carboxy Methyl Cellulose was mixed with 100ml of water 5% suspension prepared which are acts as suspending agent to mix with extract used for *In vivo* studies.

6.4 Experimental animals

The Adult female *S*wiss mice weighing between (20-30 g) were used to calculate LD50 and female and male guinea pigs with an average weight of 220-250 g were used antihistamine study. They were housed in clean polypropylene cages and maintained under standard conditions of light (12 hours with alternative day/night

cycles), relative humidity (60-70%) and temperature (26 ± 1 °C). The animals were fed daily with rodent pellet diet and tap water *ad-libitum* under strict hygienic conditions[58-60].

6.5.1 Acute oral toxicity study

Weigh the mice range 18-22gms and marked. Animals were divided in to two groups each group containing six animals and also fasted 3hrs period of fasting. Three hours later, based on the animals weight test substance were administered orally different dose levels of 2000 mg/kg and 5000 mg / Kg for divided groups. After the administration of plant extract, animals on special observed for 4hrs and normal observed daily up to 14 days. The observed parameter is mortality

6.5.2 Isolated guinea pig ileum preparation

The guinea pig 200 – 250 g was deprived of food overnight. After the fasting animal was dissected terminal pieces of ileum isolated from guinea-pig suspended in krebs solution at 50 ml. Pieces 4 to 6 cm long was cut from the guinea-pig ileum about 5 cm above the ileo-caecal valve. A piece of ileum was suspended in a 50 ml. bath containing Krebs solution at 37° C, gassed with 95% 0_2 and 5% $C0_2$ for stabilization period 45 mins. To be record the DRC of histamine, after the incubation of standard drug perform the DRC of histamine and also followed by above procedure with aqueous ethanolic extract of *Urtica dioica* were incubated 2 mins later observe the response. Tissue bath, connected through aerator which are maintaining the aeration (3 Bubbles per min) for tissue survival. The movements of the ileum were recorded magnified frontal-writing lever with Sherrington rotating drum.

6.5.3 Histamine induced convulsion by using histamine chamber

Animals with nearly same pre convulsion time were selected and randomly divided into three groups of six animals each

GROUP I – Asthmatic control-0.5% Histamine HCL aerosol.

GROUP II – Standard treatment 0.5% Histamine HCL aerosol with Mepyramine (8mg/ kg,p.o)

GROUP III - High dose (200mg/kg) of aqueous ethanolic extract of *urtica dioica.*

The experimental animals were kept in a closed chamber and exposed to an aerosol of 0.5% of histamine dihydrochloride and preconvulsion time was measured two hours after the above drug treatment, animals were exposed to histamine aerosol and pre convulsion time was noted. As soon as dyspnea occurs, it leads to the appearance of convulsion. animals were removed from the chamber and placed in fresh air to recover.

Percentage protection = {1-T1/T2} x 100 where, T1 = time in second for PCD before treatment; T2 – time in send for PCD after treatment.

6.6 Statistical Analysis:

Statistical analysis was done by using GRAPHPAD PRISM 5.0. All the values of biochemical parameters and body weight were expressed as mean ± standard error mean (SEM). The values were analyzed for statistical significance using one-way analysis of variance (ANOVA), comparison was done by using Dunnett's t test. P values < 0.05 were considered as significant, P value < 0.01 were considered as very significant, P values < 0.001 were considered as highly significance and ns were considered as not significant.

7.0 RESULTS AND DISCUSSION

7.1 Appearance and percentage yield of Extract

Urtica dioica were a light semisolid brownish color extract and the percentage yield was found to be 16.35%

7.2 Phyochemical Analysis

The phytochemical screening results revealed that the after which it was observed whether the alkaloids were present by the indication of turbidity and/or precipitate formation. The colour changed from violet to blue or green in some samples indicated the presence of steroids. An interface with a reddish brown coloration was not formed in the absence of terpenoids, as positive result. Red coloration identifies the presence of flavonoids (Shinado's test). A colour change was observed in the test tube, which indicated in the presence of tannins. A brown ring formation at the junction and the turning of the upper layer to dark green color confirmed the test for the presence of phytosterols. Below two observation indicated presence of Saponins Formation of stable foam confirmed the test The formation of a soluble emulsion confirmed the test.The formation of blue colour in acetic acid layer confirmed the test. The Formation of red color confirmed the test. Above two observation indicated presence of glycosides.

Table No.2: Phyochemical Analysis

S. No	Phytochemicals	Inference
1	Alkaloids	+
2	Steroids	+
3	Terpenoids	-
4	Flavonoids	+
5	Tannins	+
6	Phytosterol	+
7	Saponin	+
8	Glycosides	+

+, Presence of the compound

-, Absent

7.3 Toxicity Study

5000mg/ kg is LD50 value, which are used fix as doses such as 125, 250 & 500 mg / Kg for antidepressant activity. 15 days observation report was given below:

Method: OECD423

Animal: Mice

Total No. of animals used: 18

Body weight: 18-22 gms

Sex : Female

Table No.3: Toxicity Study

S. No	Parameters	Inferences
1.	**Mortality**	2000 mg/ kg:1& 5000mg/ kg: No death
2.	**Percentage of Mortality**	0%
3.	**Alive Percentage**	100%
4.	**Convulsion**	Not found all the groups
5.	**Locomotion**	Absent
6	**Sniffing**	Special observation (4 Hrs Period) were found the character but remaining other days absent
7.	**Rearing**	Special observation (4 Hrs Period) were found the character but remaining other days absent
8.	**Grooming**	Special observation (4 Hrs Period) were found the character but remaining other days absent
9.	**Hair loss**	**No**
10.	**Excess urination**	**Nil**
11.	**Excess Feces elimination**	**Absent**

7.4 Isolated guinea pig ileum preparation for Antihistamine activity

Guinea pig ileum preparation is suitable for screening of the antihistaminic activity of a drug are demonstrated in Spasmogen such as histamine produces dose dependent contraction of Guinea pig ileum preparation. The Guinea pig ileum has H1 receptors. The stimulation of H1 receptors causes contraction of ileum. In the present study, ethanolic extract and essential oil of EAEUC (100 µg/ml) significantly inhibited the histamine-induced contraction of isolated. Guinea pig ileum preparation was indicating antihistaminic activity. Guinea pig ileum is used for screening of antihistaminic activity. The stimulation of H1 receptors produces graded dose related contraction of isolated guinea pig ileum. In the present study, EAEUC (100 µg/ml) significantly inhibited the histamine-induced contraction of isolated guinea-pig ileum preparation indicating H1 receptor antagonistic activity shown in Table 2.

Table No.4: Results obtained from guinea pig illeum preparation.

S.No	Dose of Histamine (100 µg / ml)	Concentration of Histamine	Log molar concentration	CRC of Histamine (%)	% of Standard response	% of Extract response
1.	0.1	10	0.002	21.42±1.6	9.9± 1.3***	15.2±1.2**
2.	0.2	20	0.3010	47.6±1.5	23.9±1.5***	36.02±1.6**
3.	0.4	40	0.6021	61.4±2.1	37.5±2.0***	42.02±2.7**
4.	0.8	80	1.202	76.120±1.5	38.12±1.0***	49.16±2.0**
5.	1.6	160	2.002	88.320±2.4	36.08±1.8***	51.08±1.0**
6.	3.2	320	4.060	100.230±1.4	35.06±2.4***	50.06±1.2**

Figure No. 4: Results obtained from guinea pig illeum preparation of Standard

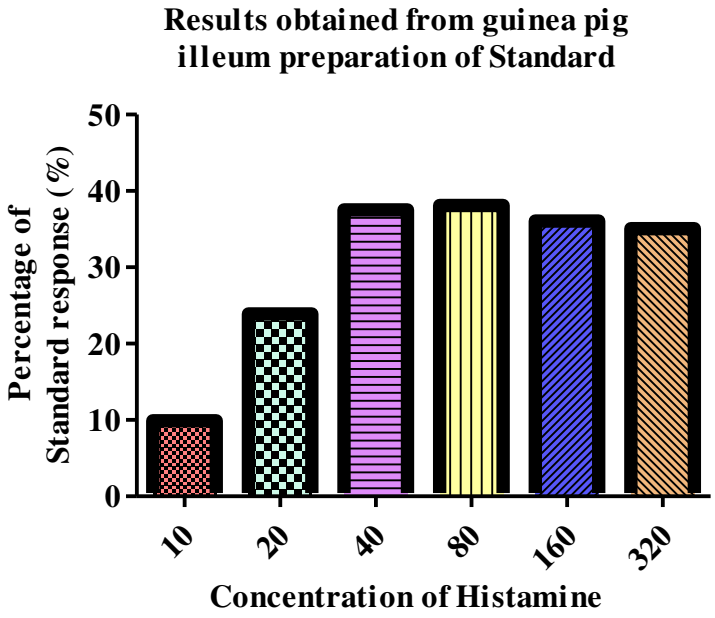


Figure No. 5: Results obtained from guinea pig illeum preparation of leaf extract

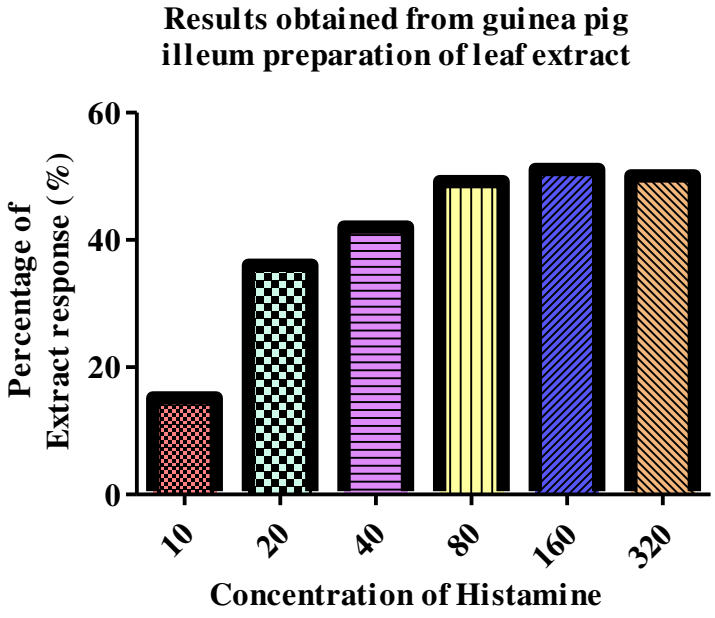


Figure No. 6: Six times Repeated Dose Response Curve of Histamine

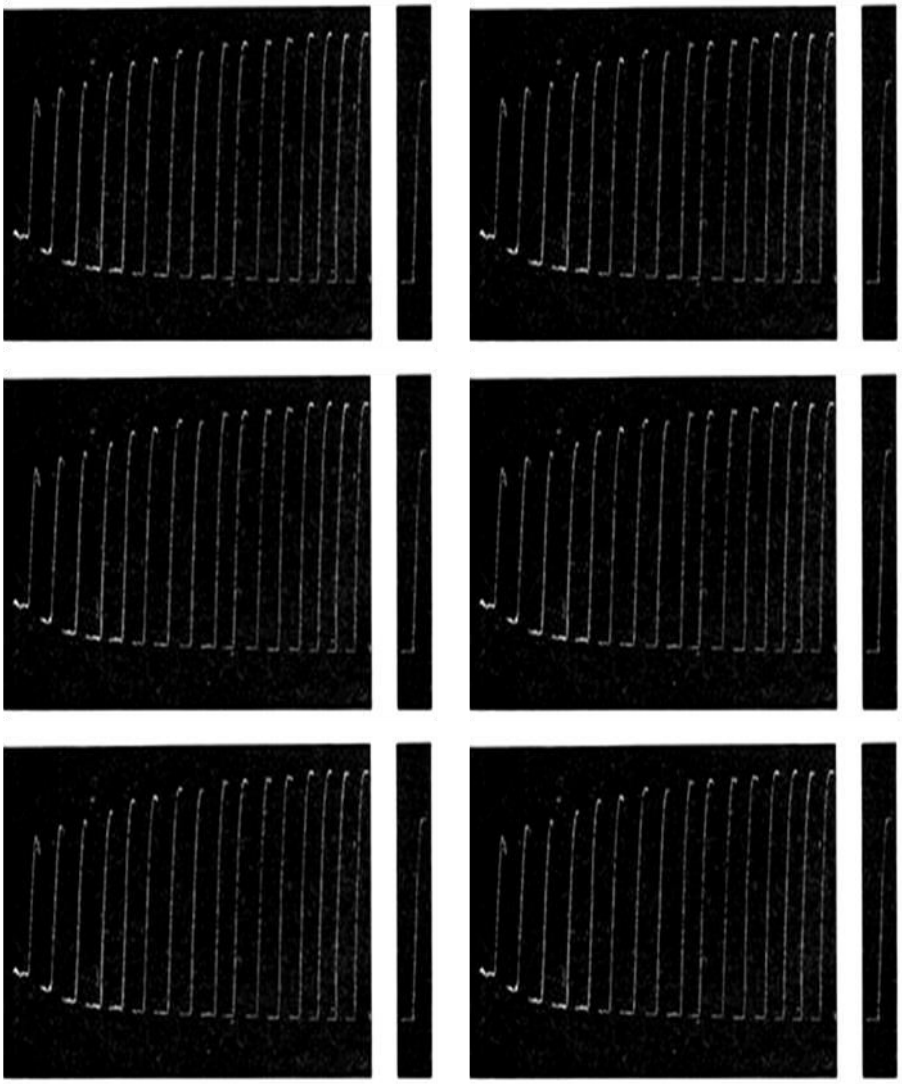


Figure No. 7: After the incubation of extract Expression of Dose Response Curve of Histamine

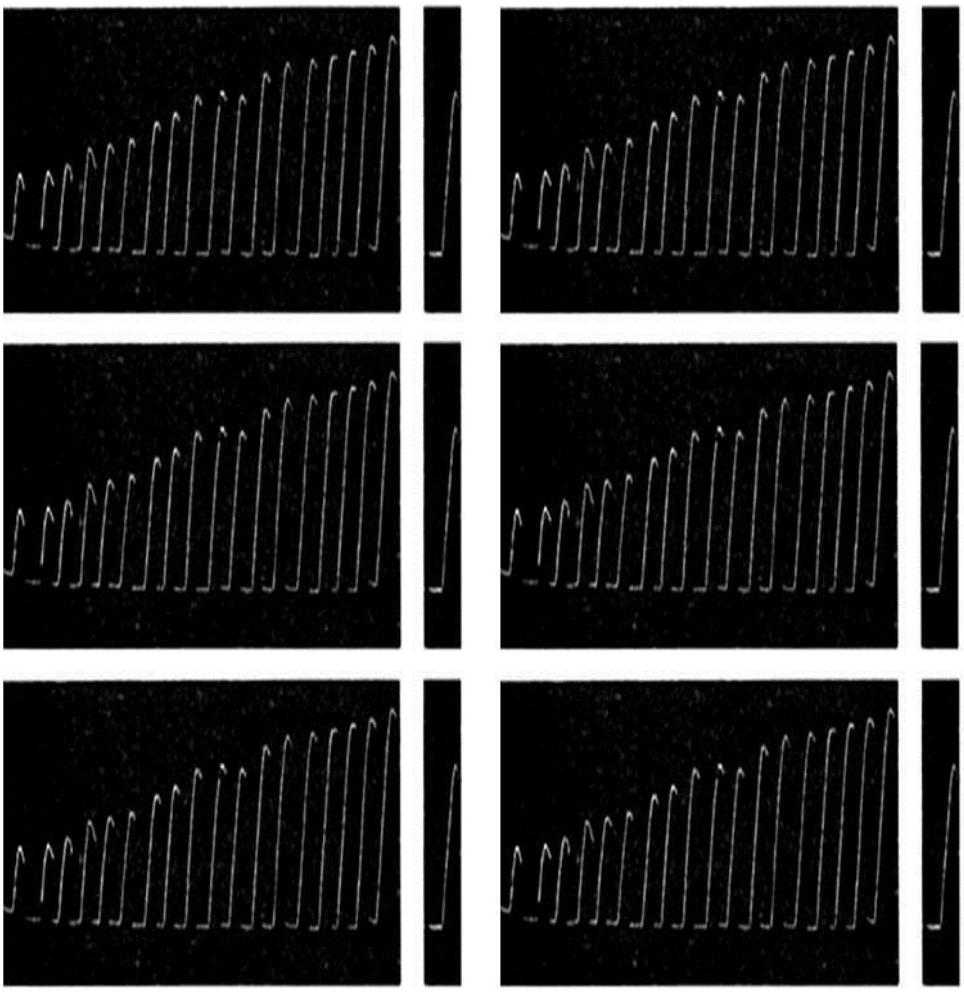
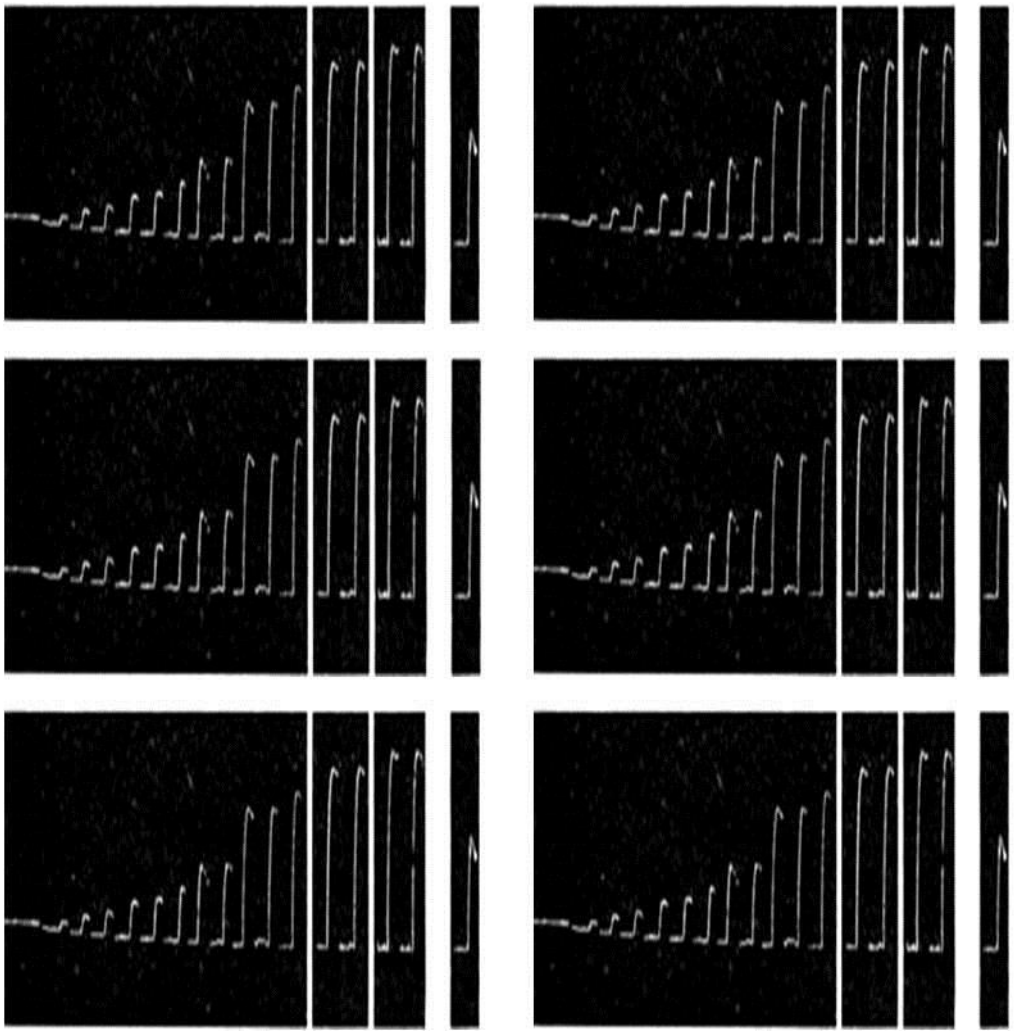


Figure No. 8: After the incubation of Standard drug Expression of Dose Response Curve of Histamine (Six Times)

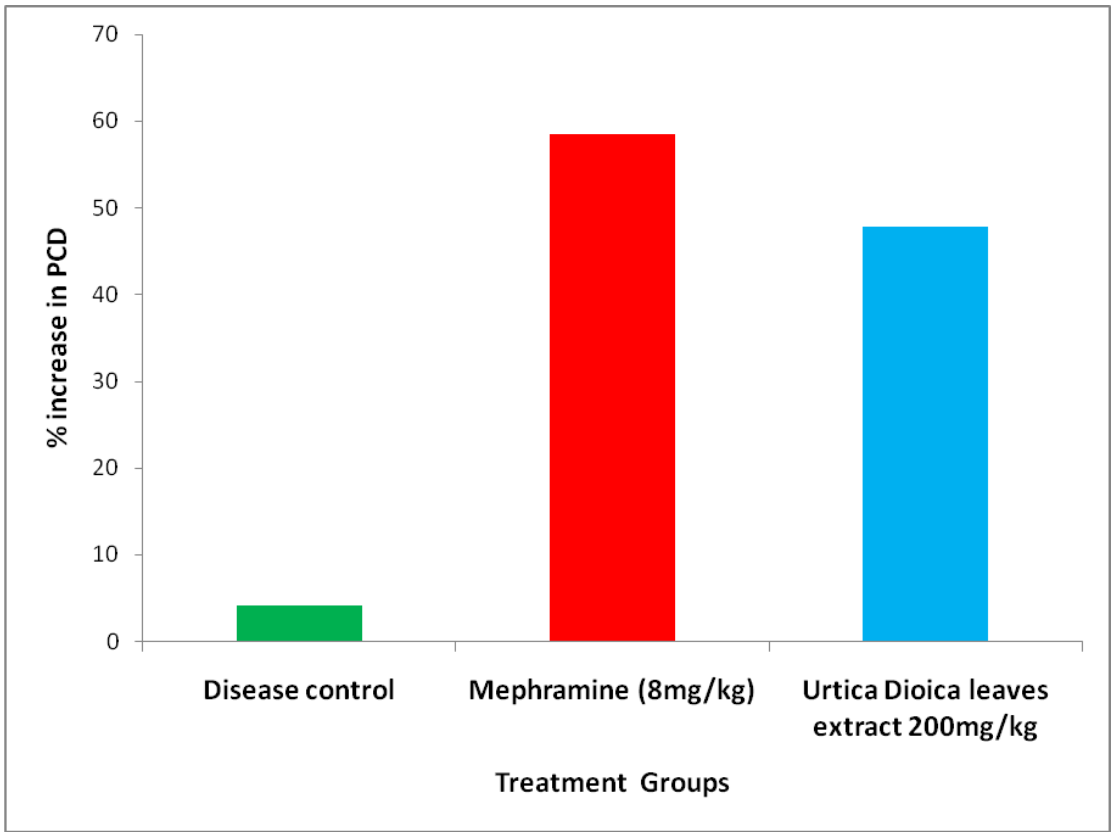


7.5 Histamine induced convulsion by using histamine chamber

Table No. 5

S.No.	Treatment Groups	% increase in PCD
1.	Disease control	4.16 ± 0.83
2.	Mephramine (8mg/kg)	58.5 ± 0.22 *
3.	*Urtica Dioica* leaves extract 200mg/kg	47.83 ± 0.47 *

When the animals were exposed to the aerosol of 0.5% histamine, there was a bronchospasm seen in form of Pre-Convuslion Dyspnoea (PCD) at day 0. Pre-treatment with standard drug Mepyramine 8mg/kg p.o.) and L. *Urtica Dioica* leaves extract (200 mg/kg. p.o.) given 2 hour before aerosol exposure to guinea pigs significantly increased Preconvulsion dyspnoea Time at day 15.



8.0 CONCLUSION

This work will be useful to find new anti asthamatic drug with help of in vitro and in vivo models. Ethanolic extract will be possess highly substantial anti-asthmatic activity by significantly inhibited the histamine induced broncho constriction of guinea pig representing its H1 receptor antagonistic activity and support the plants by its anti-asthmatic properties. The results will be obtained in the study to be provide basic data for further progress and application of plant.

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