"TREATMENT OF ALLERGIC DISORDERS BY PROPER HISTAMINE MANAGEMENT USING COMBINATION OF PLANT DERIVED HISTIDINE DECARBOXYLASE INHIBITOR AND ANTI-HISTAMINIC DRUG"

A Thesis Submitted to

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in Partial Fulfillment for the Award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

BY

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CERTIFICATE

This is to certify that the dissertation work entitled "Treatment of Allergic disorders by proper histamine management using a combination of histidine decarboxylase inhibitor and anti-histaminic drug" submitted by Ms. Anita K Bakrania with Regn. No. (11MPH203) in partial fulfillment for the award of Master of Pharmacy in "Pharmacology" is a bonafide research work carried out by the candidate at the Department of Pharmacology, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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DECLARATION

I hereby declare that the dissertation entitled "Treatment of Allergic disorders by proper histamine management using a combination of histidine decarboxylase inhibitor and anti-histaminic drug" is based on the original work carried out by me under the guidance of Dr. Snehal S Patel, Assistant professor, Department of Pharmacology, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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*"Gratitude unlocks the fullness of life. It turns what we have into enough, and more. It turns denial into acceptance, chaos to order, confusion to clarity. It can turn a meal into a feast, a house into a home, a stranger into a friend. Gratitude makes sense of our past, brings peace for today, and creates a vision for tomorrow"***.**

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Date: ***Anita K Bakrania***

Abbreviations

AA : Arachidonic acid

TcR : T-cell receptor

MHC : Major histocompatibility complex

APC : Antigen presenting cell

IL : Interleukin

IgE : Immunoglobulin E

DAG : Diacylglycerol

IP3 : Ionisitol triphosphate

PKC : Protein kinase C

gtp : Guanosine phosphate

Pip3 : Phosphatidylinositol 3,4,5-trisphosphate

PH : Pleckstrin homology

MAPK : Mitogen activated protein kinase

GDP : Guanosinediphosphate

ERK : Extracellular signal-regulated kinase

PLA2 : Phospholipase A2

PAF : Platelet activating factor

TNF : Tumor necrosis factor

INDEX

List of Instruments

INSTRUMENT	SOURCE
UV Spectrophotometer	SHIMADSU
Spectrofluorimeter	JASCO- FP-6500
Homogeniser	Remi Motors Pvt. Ltd.
Centrifuge	Remi Motors Pvt Ltd.
Electronic balance	Lab series C-3, Roy Electronics
pH meter	LI 127, Elico Ltd.
Micropipette	Accupette
Micro tips	Tarsons Ltd.
Aluminium foil	Nice chemicals Ltd.

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CHAPTER 1

ABSTRACT

ABSTRACT

Aim & Objective

Allergic and immune disorders have occurred since several decades and various new strategies have arisen in order to treat the various allergic and immune disorders. Having a glance at the treatment available for allergic disorders in the current scenario, it has not found to have complete satisfaction due to the presence of drawbacks such as CNS and CVS side effects, short duration of action which requires frequent dosing. Hence, research is more focused upon alternative treatment which may provide same efficacy with the absence of the side effects. The aim of the current study was to study the effect of catechin and the combination of catechin and anti-histaminic drug cetirizine in ovalbumin induced animal model of allergic conjunctivitis and allergic rhinitis.

Materials and Methods

Guinea pigs and rats were divided into 5 groups: 1) normal control, 2) disease control, 3) disease treated with catechin (100mg/kg), 4) disease treated with cetirizine, 10mg/kg, 5) disease treated with combination of catechin and cetirizine, 50mg/kg & 5mg/kg respectively. Sensitization was carried out by intraperitoneal injection of 100mcg ovalbumin and 20mg alum dissolved in 1 ml PBS on day 1 till day 14 in all 4 groups except normal control group. Simultaneously, catechin treatment was also started in group 3 and 5 while cetirizine was administered at the day of experiment. At the end of experiment, clinical scoring like redness, edema and tear discharge in allergic conjunctivitis model and sneezing, itching and mucous discharge in allergic rhinitis model was determined. Determination of mast cell histamine, blood histamine content, histidine decarboxylase activity from stomach was carried out in both models. Determination of vascular permeability was carried out by measuring the leakage of evans blue dye after secondary challenge of allergen.

Results

Treatment of allergic conjunctivitis and allergic rhinitis with catechin, cetirizine and the combination of catechin and cetirizine showed significant decrease in clinical scoring in allergic conjunctivitis and allergic rhinitis models. Similarly, catechin, cetirizine, and the combination of catechin and cetirizine showed significant ($P<0.05$) decrease in histamine content in mast cells as well as in blood in the disease treated groups of animals as compared to the disease control group. The treatment also showed significant ($P<0.05$) decrease in the enzyme activity when evaluated by the histidine decarboxylase enzyme assay using conversion of l-histidine to histamine, hence catechin is an efficient enzyme inhibitor. However, cetirizine group did not show any difference in enzyme activity and produced similar histamine content as disease control group in the enzyme assay hence it does not produce any enzyme inhibition. Vascular permeability was also found to decrease in all treatment groups as compared to disease control group measured using evans blue dye leakage. Histopathological examination also showed improvement in ulceration and decrease in edema and inflammation as compared to the disease control group.

Conclusion

From the present study carried out, we can conclude that catechin exhibits potent anti-allergic activity by histidine decarboxylase enzyme inhibition and the combination of catechin and cetirizine has also shown significant anti-allergic activity by both enzyme inhibition as well as inhibition of histamine receptors in both animal models of allergic conjunctivitis and allergic rhinitis as well as reduction of CNS side effects.

CHAPTER 2

INTRODUCTION

2. INTRODUCTION

Allergy was first defined by Clemens von Pirquet in 1906 in order to describe an altered reactivity to the immune system to foreign materials, irrespective of whether this results in immunity or a harmful effect. The foreign substances which provoke allergies are known as allergens. Allergens enter the body by inhalation, swallowing, injection, or contact with skin, eye, or airways. Atopic allergic conditions arise when individuals produce increased amounts of the allergic antibody immunoglobulin (IgE), which binds strongly to specific receptors on mast cells activating it, resulting into the release of several inflammatory chemicals such as histamine and leukotrienes. Allergic disorders include; allergic rhinitis, asthma, anaphylaxis, allergic conjunctivitis, urticaria, angioedema, atopic dermatitis, rhinosinusitis, food allergy, insect allergy, occupational allergy. The risk factors for allergic disorders include; Genetic factors, Environmental factors, indoor and outdoor pollution, Climate change and migration. However, sometimes disruption of the natural mechanisms occurs and this eventually results in ocular and nasal inflammation which leads to allergic conjunctivitis and allergic rhinitis respectively.

During any infection and/or trauma, natural mechanisms always exist by various body organs in order to defend itself against such infections. For example, tears lubricate the eye and enable cleansing away any foreign matter such as dust and microorganisms. Also, tears contain several substances such as lysosymes and interferons which also provide protection against infections. Similarly, eyelids and eyelashes enable protection to the ocular surface from the surrounding environment and helps maintain the moisture content of the surface of the eye. Similarly, there are several physiological, functional and immunological relationships between the upper (nose, nasal cavity, paranasal sinuses, pharynx and larynx) and lower (trachea, bronchial tubes, bronchioles and lungs) respiratory tracts. For example, both tracts contain a ciliated epithelium consisting of goblet cells that secrete mucous, which serves to filter the incoming air and protect structures within the airways. The submucosa of both upper and lower airways includes a collection of blood vessels, mucous glands, supporting cells, nerves and inflammatory cells which are provoked in the presence of allergens leading to a local inflammation (Small et al., 2007, Bourdin et al., 2007).

Allergic conjunctivitis refers to a diseases which affect the ocular surface and it is associated with type 1 hypersensitivity reactions. Two acute disorders exist; seasonal allergic conjunctivitis and perennial allergic conjunctivitis and 3 chronic diseases exist; vernal keratoconjuntivitis, atopic keratoconjunctivitis, and giant papillary conjunctivitis. The ocular inflammation leads to itching, tearing, lid and conjunctival edema, redness and photophobia during the acute phase. Allergic conjunctivitis is usually initiated when airborne allergens dissolve in our tear film and cause the conjunctiva to combine IgE antibiotics on the mast cells present on the conjunctiva. This eventually causes degranulation of mast cells and release of several chemical mediators including histamine. Hence, leads to signs and symptoms such as itching, tearing, photophobia, conjunctival hyperemia, mucous discharge, conjunctival papillary hypertrophy, keratitis and instability of the tear film (Abelson et al., 1994).

Allergic rhinitis refers an upper airway inflammatory disease associated with sneezing, itching, congestion, rhinorrhoea and disruption in the smelling sensation. The signs are due to IgE mediated activation of mucosal mast cells which exist on the nasal epithelia (Kawabori et al., 1985). Allergic rhinitis also includes the infiltration of mast cells, CD4 positive cells, B cells, macrophages and eosinophils into the nasal lining on exposure to allergens. This eventually leads to the release of cytokines which lead to the production of IgE. IgE further leads to the release of several mediators, such as histamine and leukotrienes which cause increased vascular permeability, arteriolar dilation, itching, rhinorrhoea, mucous secretion and smooth muscle contraction (Small et al., 2007; Dykewicz et al., 2010).

The current therapy for allergic conjunctivitis and allergic rhinitis includes; anti-histamines, mast cell stabilizers, non-steroidal anti-inflammatory drugs, corticosteroids and immunosuppressants, depending upon the type of allergic reaction. The drugs however have a quick onset of action to relieve allergic reactions but they have a short duration of action as well as cause various side effects (Mihaibisca, 1997). In order to get rid of the side effects, herbal medicines are brought into more focus, since they help prevail the various disorders with minimal or no adverse reactions.

Histamine is a fundamental mediator released during the immediate allergic response from tissue mast cells and during the late phase response chiefly from recruited basophils. H1 antagonists or antihistamines can be a choice in the treatment of allergic conditions by preventing histamine release by binding of histamine to the H_1 receptors.

Histamine is a primary amine synthesized from histidine by enzyme histidine decarboxylase present in mast cell which is an important mediator-secreting cell in allergic diseases. The histidine decarboxylase inhibitors are agent which inhibits the conversion of histidine to histamine, and so, is thought to be beneficial through reduction of potentially damaging, histamine-related local immune response in allergic diseases. Histamine interacts with H_1 receptors to induce smooth-muscle contraction, enhanced capillary permeability, and neuronal stimulation with multiple secondary effects. Cetirizine is a selective, second generation histamine H_1 receptor antagonist with rapid onset. The major drawback of Cetirizine is the sedative effects. In view of these drawbacks of currently available antihistaminic drugs, it is reasoned that the screening of plant based drugs in combination of H_1 antihistaminic drugs, which combine high selectivity with good efficacy and absence of CNS side effects, could constitute a major therapeutic improvement.

Several medicinal plants have been found to have a use in allergic disorders, such as; *Aervalanta, Ageratum conyzoides, Argemone Mexicana, Asystasiagangetica, Bacopamonnieri, Cassia sophera, Casuarinaequisetifolia, Clerodendrumserratum, Cnidiummonnieri, Crinum glaucum, Curculigoorchioides Gaertn, Eclipta alba, Euphorbia hirta, Ficusbengalensis, Gakani, Hemidesmusindicus, Amburanacearensis, Lepidiumsativum, Menthaspicata, Momordicadioica, Mucunapruriens, Myricaesculenta, Nyctanthesarbortristis, Oleaeuropea, Phymatodesscolopendria, Piper betel.* Some of the chemical constituents isolated from various plants also found to be useful in allergic conditions include: catechin, curcumin, withaferin, flavonoids such as; hesperetin, hesperidin and nobiletin.

Catechin is a flavan-3-ol that present in several plants such as *Acacia catechu, Camellia sinensis* (Green tea). It has found to have beneficial uses in various diseases and disorders

such as; Aging, parkinson's disease, stroke, obesity, diabetes, cancer, anti viral and anti-enzymatic actions. Catechin has anti-enzymatic actions upon enzymes such as B-lactamases, reverse transcriptase of HIV, collagenase, fatty acid synthase and histidine decarboxylase (Shimamura et al., 2007; Kawai et al., 2003).

Histidine decarboxylase enzyme is responsible for the conversion of amino acid l-histidine to histamine, a mediator which is responsible for allergy and inflammation processes. Hence, using Catechin, we can establish a target towards treatment of allergic conditions. However, catechin being an histidine decarboxylase inhibitor, it has a slow onset of action since it requires a steady process of enzyme inhibition which may lead to patient non-compliance and on the positive end it has a longer duration of action which is usually absent in the current therapies available for the treatment of allergic disorders. Therefore we explored possibility of catechin to be useful in treatment of allergic disorders and a combination of an herbal drug as well as a synthetic drug to obtain better efficacy as well as reduced side effects.

Therefore objective of our study is:

1. To study the effect of catechin in ovalbumin induced animal model of allergic conjunctivitis and allergic rhinitis.

1. To study the effect of the combination of catechin and anti- histaminic drug Cetirizine in ovalbumin induced animal model of allergic conjunctivitis and allergic rhinitis.

CHAPTER 3

Review of literature

3. REVIEW OF LITERATURE

3.1 Immune System

The immune system exists in order to protect its host by recognizing any foreign material in the body and responding to it by activation of various mechanisms which neutralize and degrade the foreign material (Goulding et al, 1993) This occurs by a complex series of interactions which prevent inappropriate stimulation of the system. Recognition of an immune response by the T or B lymphocytes is the main event which occurs in the initiation of an immune response. Both these cell types express a highly polymorphic receptor for the antigens which is known as the T cell receptor (TcR). This is a disulphide linked heterodimer, while the B cell uses a membrane associated immunoglobulin molecule. Each lymphocyte has only one specific antigen receptor. T lymphocytes, can be quantified by the expression of CD3 surface marker, recognize peptide fragments of foreign antigen which have been processed and presented to them. They are then encoded by genes within the major histocompatibility complex (MHC) and are an important part of the antigen recognition complex for T cells. Helper T cells (CD3+, CD8+) use structurally similar class I MHC molecules. However, B cells recognize antigens in a simpler manner by binding to epitopes on the whole protein antigen (Goulding et al., 1993).

3.2 Types of Immunity

3.2.1 Innate Immunity

This is also known as natural immunity which occurs naturally in every individual. It also includes the external defense mechanisms such as skin and mucous membranes i.e. membranes which line the nose, throat and gastrointestinal tract. These are also first line of defense in preventing foreign material from entering the body.

3.2.2 Adaptive Immunity

This is also known as active immunity. It involves the lymphocytes and it develops as the individual is exposed to diseases or immunized against diseases through vaccination.

3.2.3 Passive Immunity

This is immunity which has been borrowed from another source and it is short lived. For example, antibodies present in the mother's breast milk provide a baby with temporary immunity to diseases which the mother has been exposed to. This can also enable protection against infections during the early years of childhood.

3.3 How an immune response occurs

Initially, the foreign material is taken up by the body (antigen), processed and presented on the antigen presenting cells (APC). Specific helper T cells recognize and bind to the antigenic peptide class II MHC complex. Then, cytokines; Interleukin (IL)-1 and IL-6 produced by the APC are expressed at the cell surface and cause induction of IL-2 receptor expression and IL-2 secretion by the T cells. This promotes T cell proliferation and increases the number of specific antigen recognizing cells. T cells also produce several other cytokines such as IL-4, IL-5 and IL-6 (helper factors) which, along with the antigen, stimulate B lymphocyte proliferation and differentiation into plasma cells which secrete specific antibodies. Through antibody molecules, antigens are removed and broken down. The formation of the antigen-antibody complex leads to rapid phagocytosis of the foreign antigens (Goulding et al., 1993)

3.3.1 Lymphocytes – T cells and B cells

Lymphocytes are white blood cells which originate from the bone marrow and then migrate towards parts of the lymphatic system such as lymph nodes, spleen and thymus. There are mainly two types of lymphatic cells, T cells and B cells. The lymph vessels are responsible for the transport and storage of lymphocyte cells within the body. The lymphatic system feeds cells into the body and filters out dead cells and invades foreign material such as micro organisms.

The surface of lymphatic cells contains receptors which enable recognition of foreign material. These receptors are very specialized such that each receptor can match only one specific antigen. The lymphocytes can travel throughout the body until an antigen of the

exact specificity is found. To account for the specificity, the body produces so many different lymphocytes that the immune system can recognize all invaders.

3.3.2 T cells

There are two different types of T cells; Helper T cells and Killer cells. The name T cells originates from the word"Thymus", an organ situated under the breastbone. T cells are produced in the bone marrow and then migrate towards the thymus where they mature.

Helper T cells are the major regulators of the immune defense. They primarily activate B cells and killer T cells. However, the helper T cells themselves need to first be activated. This occurs when a macrophage or dendritic cell, which has eaten an invader, travels to the nearest lymph node inorder to present information regardung the captured pathogen.

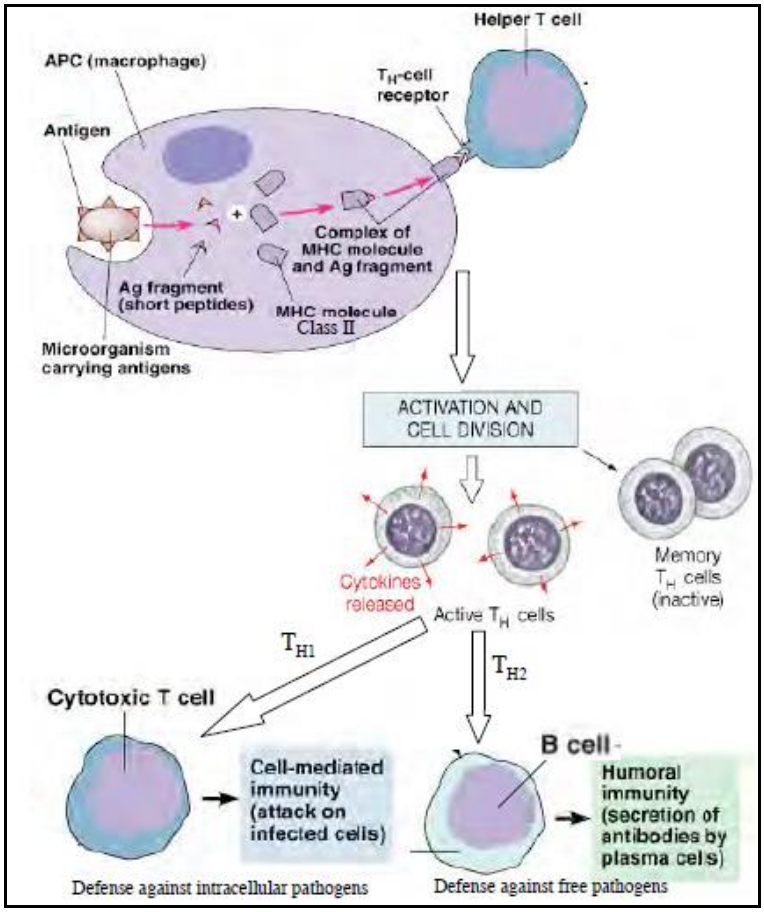


Figure 3.1 Normal functioning of immune system (Goulding et al., 1993)

The phagocyte would display an antigen fragment from the invader on its own surface. This process is known as antigen presentation. When the receptorof a helper T cell recognises the antigen, activation of the T cell occurs. After activation, helper T cells are able to divide and produce proteins which further activate B and T cells.

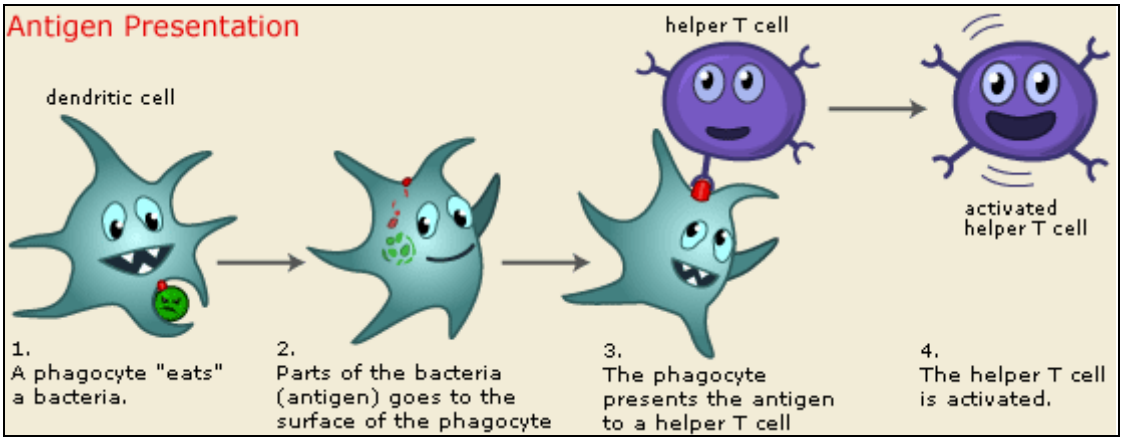


Figure 3.2 Activation of T cell (Goulding et al., 1993)

The killer cells are specialized in attacking the cells of the body which are infected y viruses and other microbes. Once a cell is infected, it is swiftly killed. The infected cells are recognized by the tiny traces of intruder, antigen which is found on their surface.

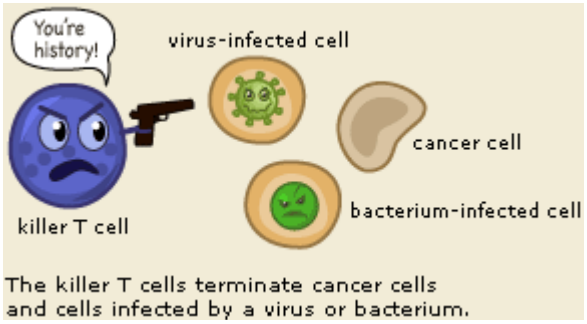


Figure 3.3 Action of T cell (Goulding et al., 1993)

3.3.3 B cells

The B cells search for antigens which bind to its receptors and bind to it. This causes the triggering of a signal and the B cells requires activation by the proteins produced by helper

T cells. After activation, B cell differentiates to produces clones of itself. During this process, two new cells are produced; plasma cells and B memory cells.

The plasma cells are specialized cells which produce a specific protein known as an antibody which could respond to the same antigen that is specific to the B cell receptor. Antibodies are then released from the plasma cells in order to invade the intruders and destroy them. The antibodies also neutralize toxins and incapacitate viruses, preventing them from infecting new cells.

The memory cells have a prolonged life span and they memorise specific intruders. T cells can also produce memory cells with an even longer life span than B memory cells. The second time any intruder tries to invade the body, these memory cells help the immune system to get activated much faster and destroys the invaders before the individual feels any symptoms. In this manner the body has achieved immunity against the invader.

Antigen-Antibody binding leads to;

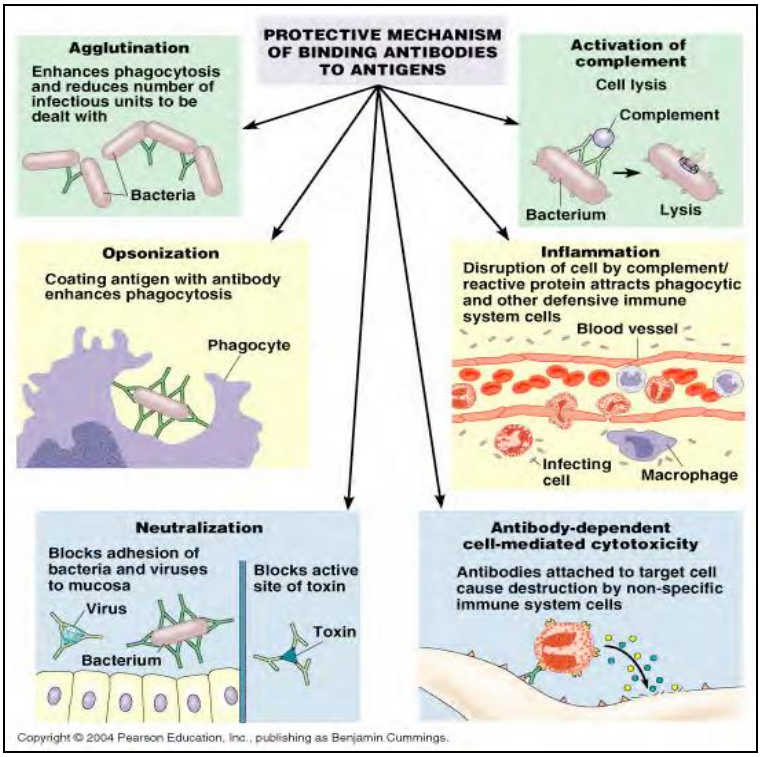


Figure 3.4 Protective mechanisms of binding antibodies to antigens (Barbara, 2007)

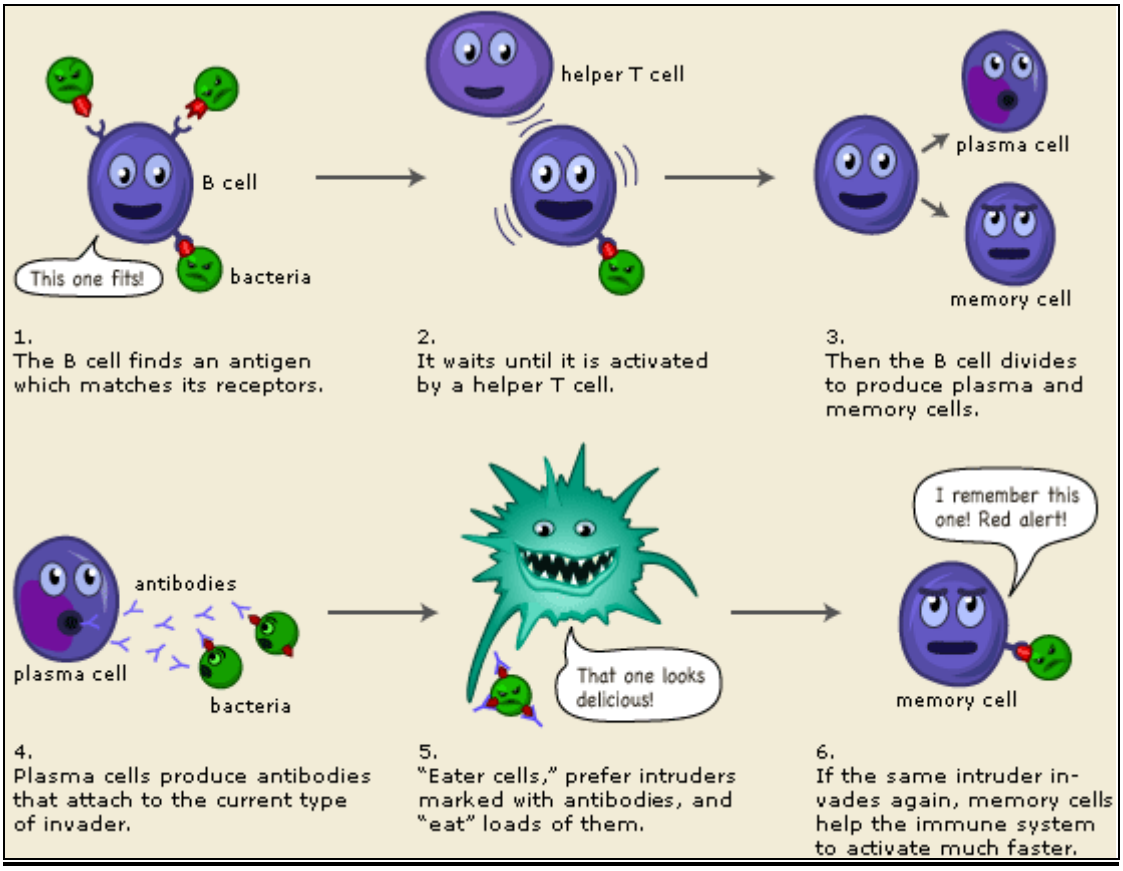


Figure 3.5 Process of immune response

(website; http://www.cabrillo.edu/~jtice/HSERV%20162/Immune%20System.pdf viewed on 7th april 2013).

3.4 Hypersensitivity reactions

Hypersensitivity refers to an immune-mediated response to a drug sensitized patient. (Riedl & Casillas, 2003; Shepherd, 2003; Barbara, 2007). The Gell and Coombs classification system of hypersensitivity reactions is used to describe the various types of hypersensitivity reactions. There exist four hypersensitivity reactions; Type I is an immediate, IgE-mediated reaction; Type II is an antibody-mediated reaction (IgG or IgM) that is cytotoxic in nature; Type III is an immune-complex mediated reaction; Type IV is a cell-mediated or delayed type reaction (Riedl & Casillas, 2003; Gobel, 2005; Thomas, 2004).

Gell and Combs classification of types of hypersensitivity reactions

Table No.3.1 Hypersensitivity classification

Type	Mechanism	Signs and Symptoms	Timing
Type I	Immunoglobulin E-mediated drug-immunoglobulin binds to mast cells with release of mediators of reaction (e.g. histamine)	Fever, rashes, nausea, vomiting, flushing, diarrhoea, dyspnea, urticaria, angioedema, bronchospasm, back pain, feeling of impending doom, anaphylaxis.	Minutes to hours after drug administration.
Type II	Antibody-mediated reaction (specifically IgG or IgM) resulting in antibody-antigen complexes. Complexes activate pathways in the immune system to cause inflammation.	Hemolytic anemia/ hemolysis, neutropenia, thrombocytopenia.	Variable
Type II	Drug-antibody mediated reaction through which immune complexes form in the circulation and deposit in various tissues, associated with an immune system response.	Fever, rashes, arthralglas, nephritis, vascultis, urticaria, lymphadenopathy, serum sickness.	1 to 3 weeks after drug administration

Type IV	Delayed reaction that involves the activation of T cells in the immune system. The T cells recognize the antigen and destroy the targeted cells.	Graft rejection, granuloma formation, allergic contact dermatitis.	2 to 7 days (or longer) after cutaneous drug exposure.

3.5 Signalling pathway; Allergic reactions

3.5.1 Mast cell signaling

Aggregation of IgE-occupied FceRI induces activation of the Src family tyrosine kinase Lyn, whereas stem cell factor–induced dimerization of KIT induces activation of its intrinsic kinase. Phosphorylation (indicatedby red circles) of tyrosine residues in the intracellular domains of each of these receptors recruits SH2 domain–containing signaling molecules. In the case of FceRI, Syk is recruited through FceRI by ITAMs contained in g chain cytoplasmic domains. Resulting activation of Syk leads to phosphorylation of LAT andLAT_2. These proteins then serve as scaffolds for multimolecular signaling complexes for the binding of cytosolic adapter molecules, such as Gads, Grb2, SLP76, and SHC; guanosine triphosphate exchangers, including Sos and Vav_1; and the signaling enzymes $PLCg_1$ and $PLCg_2$. PLCg catalyzes the hydrolysis of PIP2to yield diacylglycerol (DAG) and inositol trisphosphate (IP3), which result in the activation of protein kinase C (PKC) and the liberation of intracellular calcium. These signals lead to mast cell degranulation and eicosanoid generation and contribute to activation of transcription factors required for cytokineand chemokine production. In parallel, PI3K is activated after binding to Gab_2 on phosphorylation of this cytosolic adapter molecule by Fyn, Syk, or both; phosphorylation of the p85 adapter subunit of PI3K; and activation of the catalytic subunit by small guanosine triphosphate (GTP)–binding proteins. In the case of KIT, the p85 subunit

directly binds to the phosphorylated molecule. The subsequent formation of membrane-associated phosphatidylinositol 3,4,5-trisphosphate (PIP3) results in the recruitment of pleckstrin homology(PH) domain–containing signaling molecules, such as Btk and PLD. PI3K-regulated pathways serve to enhance or maintain LAT/PLCg regulated degranulation. KIT and FceRI-mediated activation of the Ras–Raf–mitogen-activated protein kinase (MAPK) pathway after Sos and Vav regulated guanosinediphosphate (GDP)–GTP exchange of Ras contributes to these processes. MAPK–extracellular signal-regulated kinase (ERK) signaling also regulates phospholipase A2 (PLA2) activation, which leads to the generation of eicosanoids. LAT2 can both upregulate and downregulate antigen-mediated responses and appears to be required for KIT to enhance FceRI-dependent degranulation. In addition to its role in mast cell mediator release, KIT signaling regulates mast cell proliferation, differentiation, survival, migration, and adhesion. JAK, Janus kinase; STAT, signal transducer and activator of transcription (Gilfilan and Rivera, 2009).

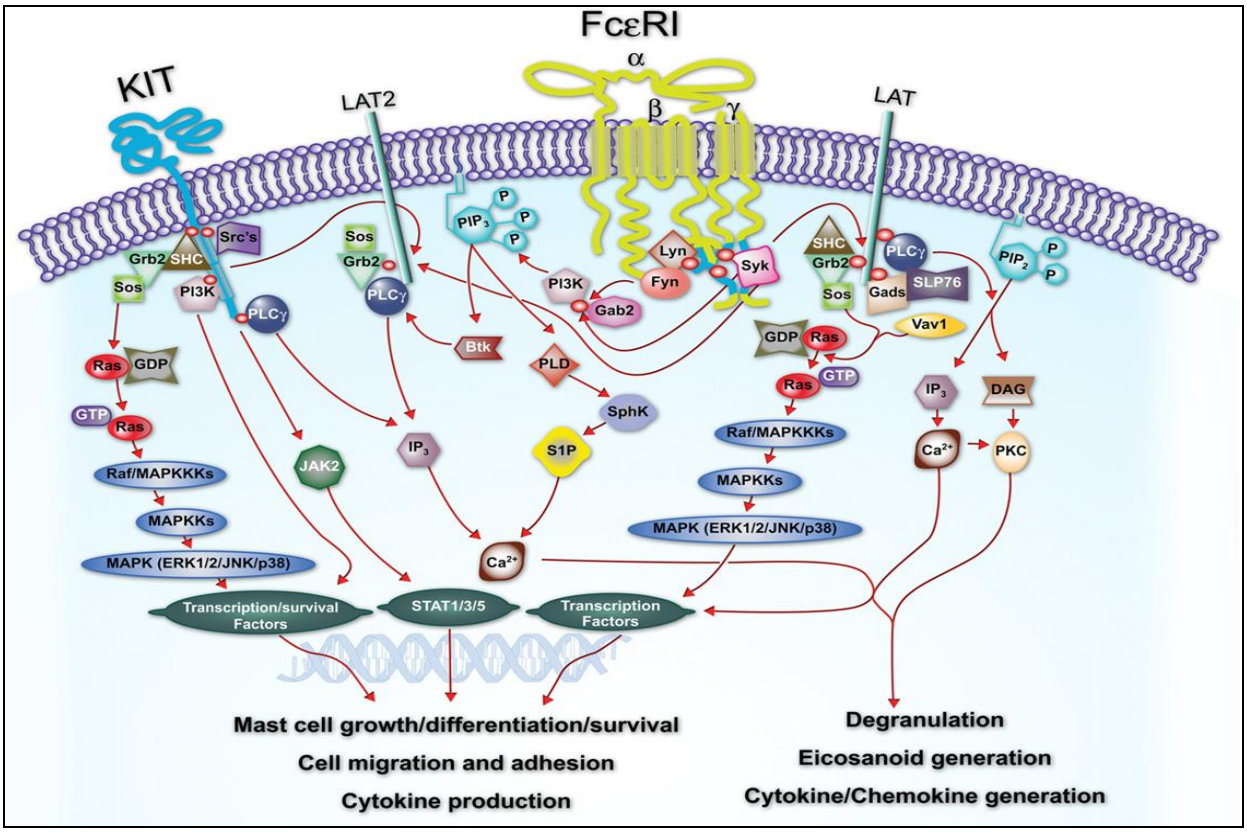


Figure 3.6.Signalling pathway of mast cells (Gilfilan and Rivera, 2009)

3.6 Pathophysiology of allergy

3.6.1 Basophils and mast cells

Basophils and mast cells were first identified by Paul Ehrlich. He called them "Mastzellen" (well fed cells) due to the presence of the large granules in their cytoplasm (Ehrlich, 1878; Ehrlich, 1879). Basophils and mast cells are the only cells with high affinity receptors for IgE and synthesise histamine. Their cytoplasmic granules contain glycosaminoglycans and they have metachromatic staining properties. The basophil has, on average, 1pg of histamine/cell. Mast cells purified from human lung, intestine, and skin contain 4pg of histamine/cell (Marone et al., 1989)

Table 3.2 Activators of basophils and mast cells

Basophils	Mast cells
Antigen	Antigen
Anti-IgE	Anti-IgE
Anti-IgG	Ca2+ ionophores (e.g. A23187)
Ca2+ ionophores (e.g A23187)	Hyperosmolarity
Hyperosmolarity	Gastrin (cutaneous mast cells)
C3a, C5a	Macrophage derived factors
Macrophage and monocyte derived factors	Maitotoxin
Lymphocyte and platelet derived factors (e.g. HRA)	Morphine (cutaneous mast cells)
Formyl-methionine peptide	
Eosinophil major basic protein	
Interleukin 3 (IL-3)	
Maitotoxin	
Major basic protein	
Pepstatin A	
Platelet activating factor (PAF)	

Protein A of staphylococcus aureus	
Phorbol esters	
Phhospholipase A	
Deuterium oxide	
5-Hydroperoxyeicosatetraenoic acid	
Prostaglandin D2	
Leukotriene B4	

3.6.2 Physiology of mast cells

Mast cells are derived from CD34+ bone marrow precursor cells and circulate in the blood as precursors. They are recruited into the peripheral tissues such as the dermis of the skin, lungs and the mucosa and submucosa of the intestines. Mast cells play a major role in inflammatory and immediate allergic reactions which are initiated by immunogolobulin IgE. Special cytoplasmic granules in the mast cells store several mediators such as histamine, heparin and proteases including tryptase. The release of these mediators occurs by degranulation of mast cells which can be induced by physical destruction (mechanical trauma, high temperature), chemical substances (toxins, venoms), endogenous mediators (proteins, tissue proteases) and immune mechanisms, including IgE-dependent and IgE-independent mechanisms.

IgE antibodies are generated by B lymphocytes after an exposure to a specific antigen. IgE molecules bind to the IgE receptors on the surface of mast cells. When IgE molecules are bridged by reintroduced antigen, mast cell degranulation occurs, releasing both preformed and newly synthesized mediators. Normally, the release of these mediators leads to a defensive acute inflammatory reaction. Degranulation triggered by the activation of C5areceptors on the mast cell surface. When there is massive release of these mediators under abnormal conditions, bronchoconstriction and vasodilatation predominate. Anaphylatoxins C5a, C3a and C4a are also formed during complement activation.

Mast cells have two types of inflammatory mediators; Preformed mediators (such as histamine, heparin, chondroitin sulphates and tryptase) are stored in secretory granules, and are released upon mast cell activation, Newly generated mediators (such as leukotrienes,

prostaglandins, TNF and interleukins IL-4, IL-5 and IL-6) are not present in the resting mast cell, but they are produced during IgE-mediated activation (Krishnaswamy et al., 2001; Payne &Kam, 2006).

3.7 Mediators in allergy

3.7.1 Histamine

Histamine (2-[4-imodazole]-ethylamine) was discovered as a uterine stimulant in different extracts more than 100 years ago. Its smooth muscle stimulating and vasodepressor action has been demonstrated. (Dale & Laidlaw, 1910; Akdis & Blaser, 2003). Histamine is synthesized by the decarboxylation of histidine by l-histidine decarboxylase (HDC), which is dependent on the do factor pyridoxal-5'-phosphate. Mast cells and basophils are the main source of histamine. Histamine is released during degranulation in response to various immunological and nonimmunological stimuli. HDC activity is modulated by cytokines, such as IL-1, IL-3, IL12, IL-18, GM-CSF, macrophage-colony stimulating factor, TNF-α and calcium ionophore (Schneider et al., 1987; Yoshimoto et al.,1999; Akdis & Blaser, 2003).

Table 3.3 Histamine receptors (Akdis & Blaser, 2003)

Histamine receptor	Expression	Activated intracellular signals	G proteins
HR1	Nerve cells, airway and vascular smooth muscles, hepatocytes, chondrocytes, endothelial cells, neutrophils, eosinophils, monocytes, T cells, B cells	Ca2+, cGMP, phospholipase D, phospholipase A2, NF-kB	Gq/11
HR2	Nerve cells, airway and vascular smooth muscles, hepatocytes, chondrocytes, endothelial cells, epithelial cells, neutrophils, eosinophils, monocytes, T cells, B	Adenylatecyclase. cAMP, c-Fos, C-Jun, PKC, p70S6K	Gαs

	cells		
HR3	Histaminergic neurons, eosinophils, monocytes low expression in peripheral tissues	Enhanced Ca2+, MAP kinase, inhibition of cAMP	Gi/o
HR4	High expression on bone marrow and peripheral hematopoietic cells, eosinophils, neutrophils, T cells, basophils, mast cells, low expressionin nerve cells, hepatocytes peripheral tissues, spleen, thymus, lung, small intestines, colon , heart	Enhanced Ca2+, inhibition of cAMP	Gi/o

3.7.2 Lipid mediators in inflammation

3.7.2.1 Prostanoids

Arichidonic acid is converted into two enzymes; cyclooxygenase and lipoxygenase enzymes. Cyclooxygenases yield prostanoids such as prostaglandins, prostacyclin and thromoxanes. Lipoxygenases convert arachidonic acid into leukotrienes and other hydroxylated metabolites. The major prostanoids used in inflammation include; Prostaglandin D2, prostaglandinE2, prostaglandin I2 and thromboxane A2.

3.7.2.2 Isoprostanes

These refer to the prostanoids which are formed non-enzymatically by free radical-induced conversion of arachidonic acid. They trigger adhesion of monocytes to endothelial cells, influence production of chemotactic factors such as IL-8 from macrophages, cause contractile response of airway smooth muscles. The effects are mediated byTXA2 receptors.

3.7.2.3 Leukotrienes

These are synthesized by the action of lipoxygenase enzymes upon arachidonic acid, followed by the action of various other enzymes leading to the production of other leukotrienes. They affect inflammatory cell recruitment, mucus formation, airways remodeling and pulmonary vascular leakage.

3.7.2.4 Linoleic acid-derived mediators

Linoleic acid is convertedto two hyrdroperoxy derivatives, 9- and 13-hydroperoxy octadecadienoic acid (9- and 13-HODE). These metabolites play a role in airway inflammation, such as pulmonary macrophages and tracheal epithelial cells. They also inhibit oxygen-centered radicals released from inflammatory cells (Nauta et al., 2008).

3.8 Allergic disorders

Allergic disorders include; Allergic rhinitis, Asthma, Anaphylaxis, Allergic conjunctivitis, Urticaria, Angioedema, Atopic dermatitis, Rhinosinusitis.

3.8.1 Allergic Conjunctivitis

Allergic conjunctivitis is an immediate type of hypersensitivity that develops minutes after exposure to antigen and lasts for a few hours. The symptoms of immediate hypersensitivity are itching, hyperemia, and edema accompanied by an increase in vascular permeability in the conjunctiva (Kamei et al., 1995; Calonge et al., 1990; Melamed et al., 2000).

3.8.1.1 Types of Allergic conjunctivitis (Friedlaender, 1995)

1) Seasonal/ perennial allergic conjunctivitis
2) Vernal keratoconjunctivitis
3) Atopic keratoconjunctivitis
4) Giant papillary conjunctivitis
5) Contact allergy

Table 3.4 Types of Allergic conjunctivitis

Type	Characteristics	Clinical signs and symptoms	Diagnostic features
Seasonal/ perennial	Young (<30 years old). History of recurrent, seasonal onset. History of hay fever, allergy, asthma, or atopy.	Bilateral red, itchy, tearing, burning eyes. Stringy or ropy discharge. Milky, pale or pinkish conjunctiva.,	Eosinophils in conjunctival scrapings. Papillary hypertrophy of the tarsal conjunctiva.
Atopic keratoconjunctivitis	Typically in late teen years or early 20s. History of hay fever, allergy, asthma, or atopy.	Red, itching, burning, and tearing eyes with copious discharge. Eczematous eyelid changes. Papillary hypertrophy on lower and upper tarsal conjunctiva. Cornea1 vascularization, ulceration, and scarring.	Cornea1 signs include punctate epithelial keratitis, cataracts, cornea1 scarring, and vascularization.
Vernal keratoconjunctivitis	Occurs primarily in children, peak incidence between ages 11 and 13 years. History of hay fever, allergy, Asthma, or atopy.	Extremely itchy, sometimes painful eyes, photophobia, blurred vision, and blepharospasm. Stringy or ropy discharge. Occurs on a seasonal basis. Patients rub their eyes	Giant papillae (>I mm wide) on upper tarsal conjunctiva. Trantas' dots.

		vigorously.	
Giant papillary conjunctivitis	Patients usually wear contact lenses.	Mild itching on removal of lens with slight blurring of vision. May progress to mild hyperemia with presence of small strands of mucus and abnormal thickening of the conjunctiva. Opacification of the conjunctiva and development of large papillae (Xt.3 mm in diameter) may occur.	Macropapillae (0.3-l mm in diameter) and giant papillae (>l mm in diameter) may be seen on upper tarsal conjunctiva.
Contact allergy	History of repeated exposure to sensitizers (eg. ophthalmic medicationscosmetics, preservatives in solutions).	Severe itching, burning, and photophobia. Watery discharge and papillary responsemay be present.	Pronounced vasodilation, chemosis of conjunctiva. Fine epithelial punctate keratitis and possibly comeal opacities,

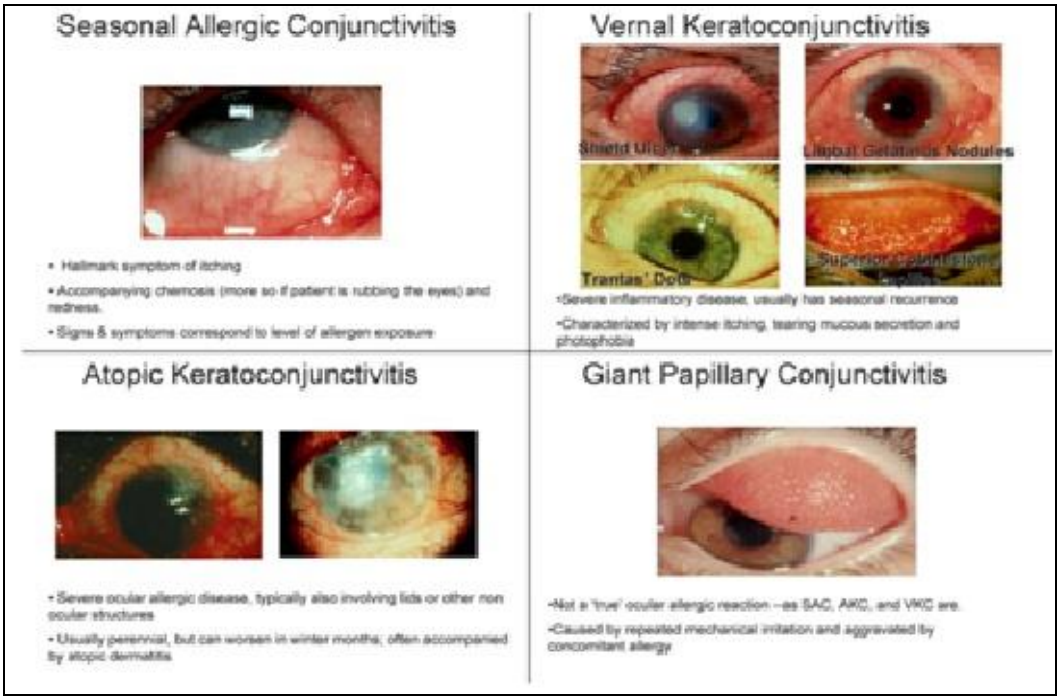


Figure 3.7 Types of allergic conjunctivitis (Friedlaender, 1995)

3.8.1.2 Mechanism:

1) Sensitisation process (allergen-induced IgE production and mast cell sensitization)

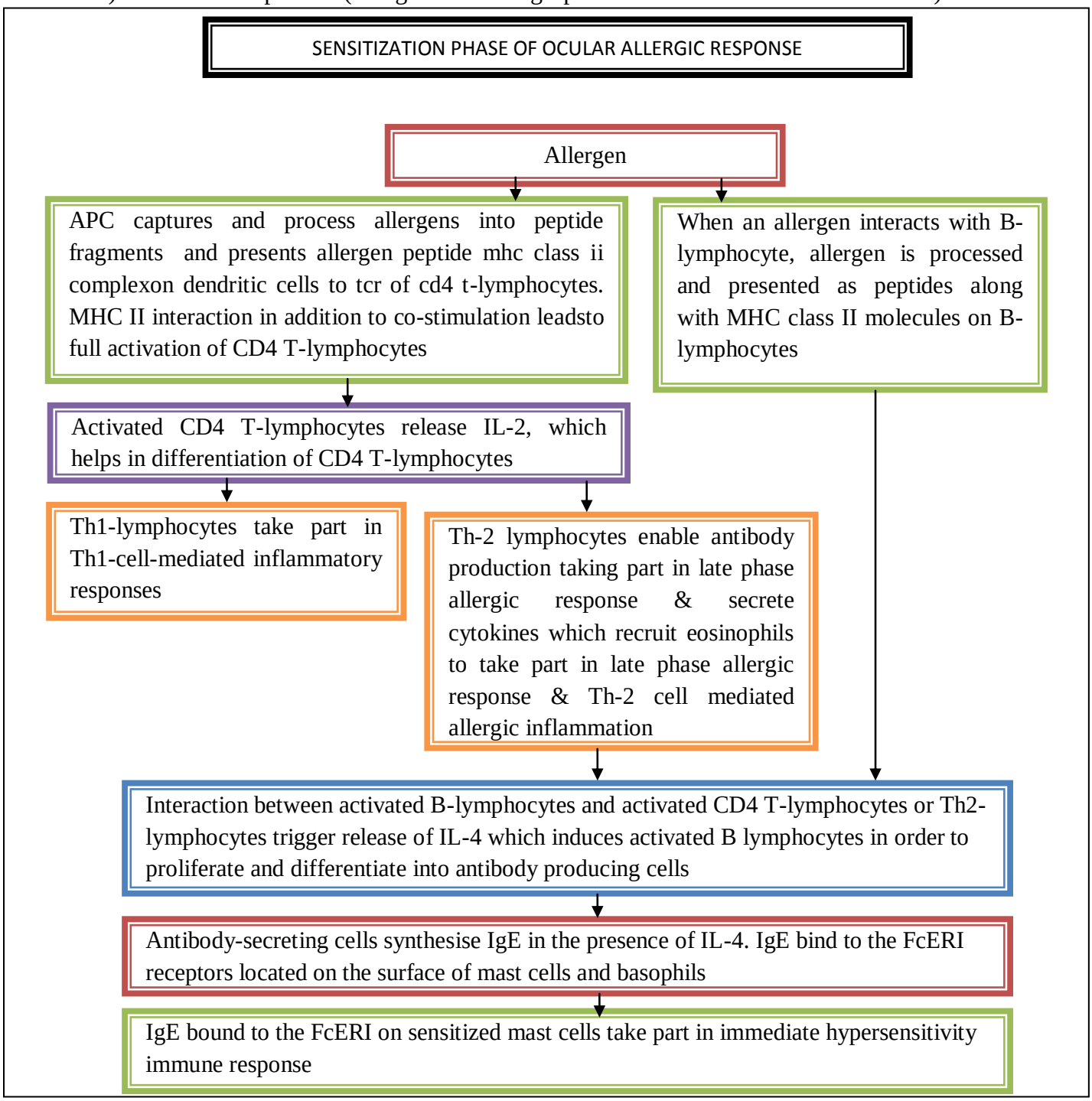


Figure 3.8 Sensitization phase of ocular allergic reaction

3) Early phase response (allergen-induced IgE-mediated mast cell degranulation and release of preformed mediators and chemotactic factors).

4) Late phase response (persistent mast cell activation and Th2-mediated DTH reaction involving actions of newly formed mediators and chemotactic factors which indicate the recruitment of additional inflammatory cells leading to allergen-induced late phase allergic response) (Chigbu, 2009).

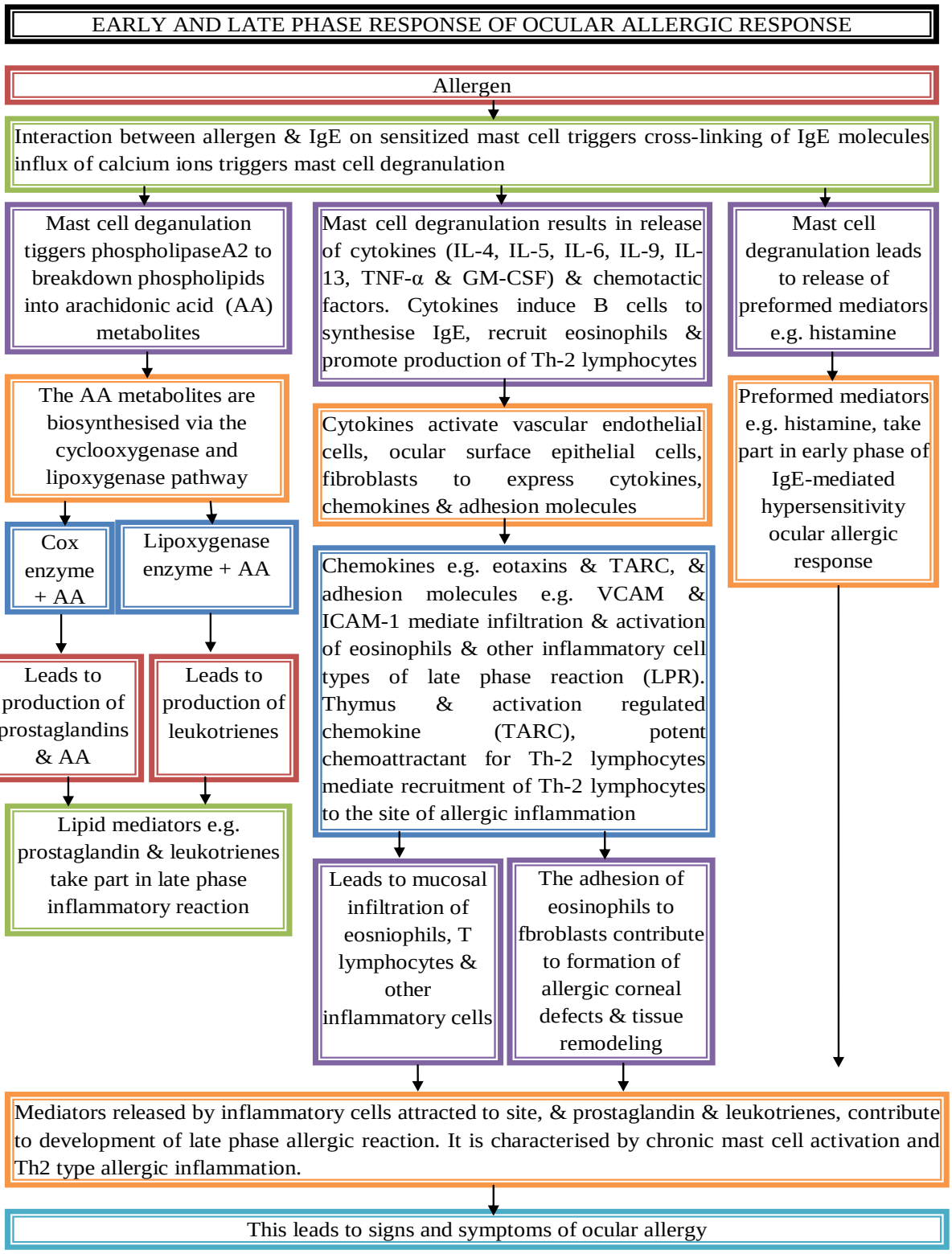


Figure 3.9 Early and late phase response of ocular allergic response

3.8.2 Allergic rhinitis

Allergic rhinitis is an inflammatory disorder of the upper respiratory tract. It is accompanied by sneezing, itching, congestion, rhinorrea and loss of sense of smell. These symptoms occur due to IgE-mediated activation of mucosal mast cells which are located on the epithelia of the nasal cavity (Kawabori et al., 1985).

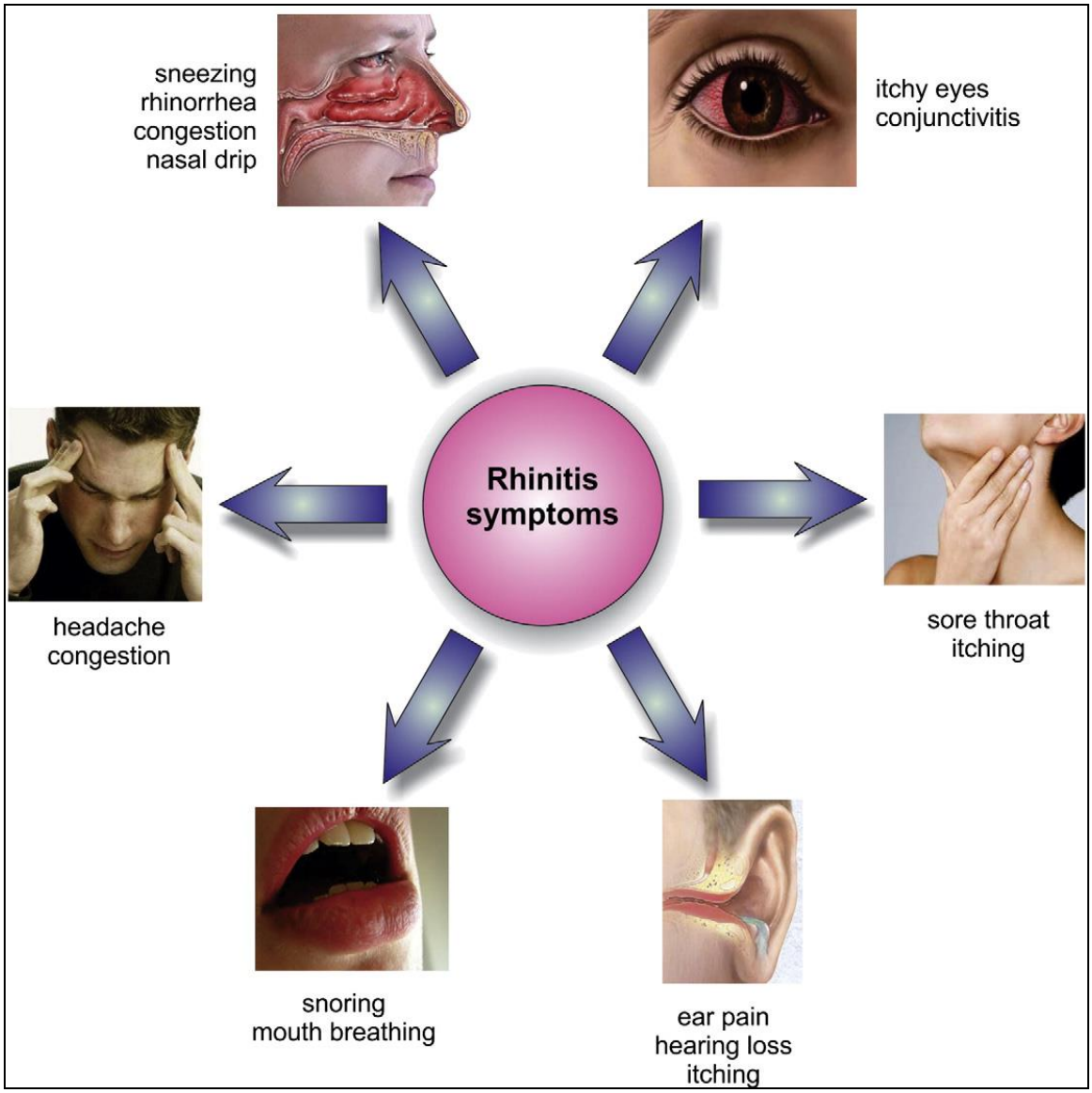


Figure 3.10 Symptoms of Allergic rhinitis (Kawabori et al., 1985)

Rhinitis is classified as; IgE-mediated (allergic), autonomic, infectious and idiopathic (unknown).

3.8.2.1 Etiological classification of rhinitis

Allergic rhinitis can further be classified as intermittent or persistent depending on the duration of the inflammation.

Intermittent allergic rhinitis is when the duration of the inflammation is less than 6 weeks while persistent allergic rhinitis is defined as the inflammation which continues throughout the year.

Table 3.5 Eitiological classification of Allergic rhinitis

Type	Decscription
Allergic	IgE mediated inflammation of the nasal mucosa, resulting in eosniphilic and Th2-cell infiltration of the nasal lining.
Autonomic	Drug induced (rhinitis medicamentosa), Hypothyroidism, Hormonal, Non-allergic rhinitis with eosinophilia syndrome
Infectious	precipitated by viral, bacterial or fungal infection
Idiopathic	Etiology cannot be determined

3.8.2.2 Pathophysiology of allergic rhinitis

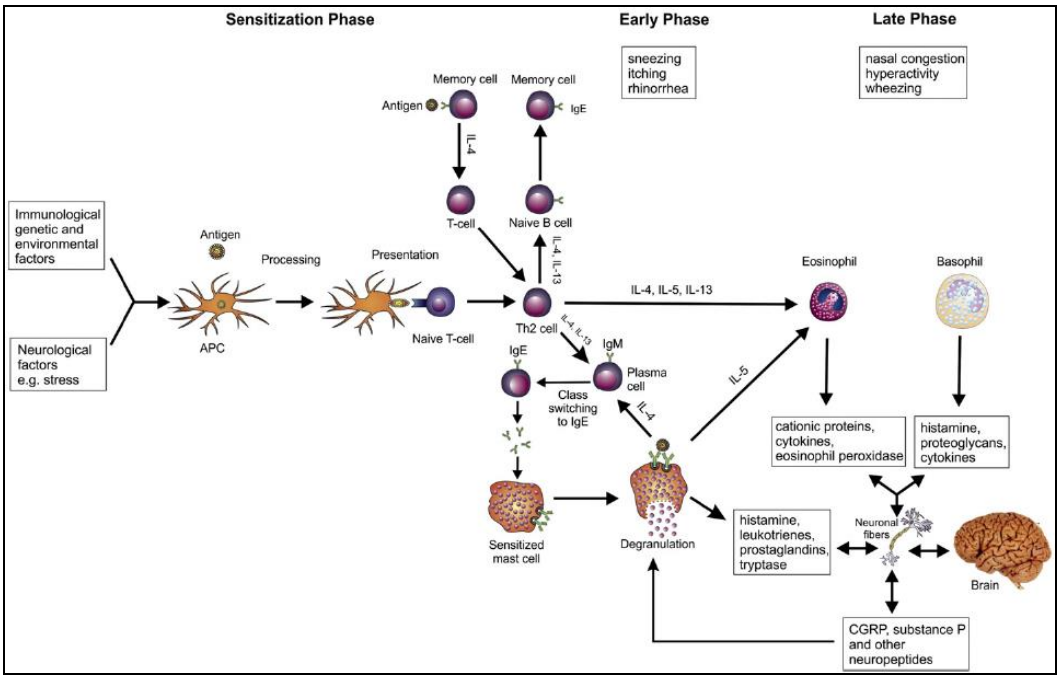


Figure 3.11 Pathophysiology of allergic rhinitis (Kawabori et al., 1985)

Sensitization is a preparation phase whereby APCs encounter allergen molecule process them and present the antigen to T-cells. B cells that have been exposed to antigen produce and secrete IgE, which binds to mast cell receptors. Memory B cells are formed from activated B cells which are specific to the antigen encountered during the primary immune response. On interaction with the antigen, cross linking of IgE results in degranulation of mast cells. Cytokines cause chemoattraction of other inflammatory cells such as eosinophils and basophils. Neurogenic component is strongly involved in the process of onset, persistence, amplification, and extension to accompanying symptoms such as stress.

3.9 Treatment - current therapy for allergic disorders

3.9.1 Antihistamines

The first generation anti-histamines; Pheniramine and Antazoline, have a long safety record and rapid onset of action but a short duration of action as well as they cause burning sensation upon instillation in case of ocular allergy. The second generation anti-histamines; Cetirizine, Levocabastine hydrochloride, Emedastine difumarate have a greater potency as well as duration of action of about 3-4 hours. Loratidine and Cetirizine have shown to also cause ocular dryness in ocular allergy as well as the sedative effects which are prominent with anti-histamines.

3.9.2 Mast cell stabilizers

Drugs available include; Cromolyn sodium, Nedocromil sodium, Lodoxamide tromethamine, Pemirolast potassium.These drugs inhibit mast cell degranulation hence prevents the release of histamine and other preformed mediators by preventing cell membrane disruption, blocks arichidonic acid cascade of prostaglandin and leukotriene formation. They hence inhibit chemotaxis, activation, degranulation and cytotoxicity of neutrophils, eosinophils and monocytes.

3.9.3 Non-steroidal anti-inflammatory agents (NSAIDs)

Ketorolac has been approved for use in allergic disorders mainly for the relief of ocular itching associated with seasonal allergic conjunctivitis. NSIADs inhibit te cyclooxygenase enzyme, hence blocking the synthesis of prostaglandins, especially PGE2.

3.9.4 Corticosteroids

These remain the last choice in treating allergic disorders and should be used in severe cases only. They work by both molecular and cellular levels, passing through the plasma membranes and binding to the steroid receptors on the cytoplasm. The drug-receptor complex then travels to the nucleus and reacts with mRNA, stimulating the synthesis of

specific proteins which regulate the anto-inflammatory response. On a cellular level, they decrease the migration of neutrophils to the inflamed site. They do not directly stabilize immune cell membranes and inhibit histamine release, but they modulate mast cell response by inhibiting mediators such as granulocyte-macrophage colony stimulating factor, IL-3, ICAM-1, IL-4 which induce proliferation and recruitment of mast cells. They are used only in chronic severe case due to the adverse effects produced such as cataracts, glaucoma, superinfection and corneal melting. However newer molecules have been developed for ocular allergy known as "soft" steroids which have very little systemic and corneal absorption compared to the stronger prednisolone and dexamethasone. "soft" steroids include; Fluorometholone and Medrysone, which have been steroids of choice with mild, minimally penetrating effect. Latest generation of "soft" steroids include; Loteprednol (Alrex; Bausch & Lomb) and Rimexolone have been used in allergic conjunctivitis.

3.9.5 Immunosuppressants

Cyclosporine is a potent immunosuppressant which interferes with T helper cell proliferation hence decreases interleukin-2 production. It also inhibits TNF-α. However, studies have shown that mast cell and IgE-mediated immune responses are not affected by Cyclosporine.

3.9.6 Leukotriene receptor antagonists

Montelukast and zafirlukast have been used in the treatment of allergic rhinitis but they are not as effective as antihistmines or nasal corticosteroids.

3.9.7 Allergen immunotherapy

This involves subcutaneous administration of gradually increasing quantities of the allergens until a dose is reached which is effective in inducing the immunological tolerance to the allergen. This has been seen to be effective in allergic rhinitis which has been caused

by dust mites and pollen, but it has limited use in treating mould and animal dander allergies (Small et al., 2007).

3.10 Medicinal plants used in allergic disorders & phytochemicals used in allergic disorders

As per WHO estimates, traditional, complementary, alternative, or non-conventional medicines are used by 70–95% of global population particularly in developing countries for their health- care (WHO, 2011). Traditional medicines vastly depend on the usage of plants, compared to other natural resources. India is one of the 12 mega bio-diversity zone covering 2.4% of world's area but with 8% of global biodiversity. It includes 15 agro-climatic zones containing about 47,000 plant species including nearly 15,000 medicinal plants (WHO, 2007). These plant species are used in different systems of medicine like in folk wisdom (44%), Ayurveda (19%), Siddha (12%), Unani (10%), Homeopathy (8%), Tibetan (5%) and modern medicine (2%).

The word Ayurveda is derived from the Sanskrit word 'Ayus' (all aspects of life from birth to death) and 'Veda' (knowledge or science) science of long life (Mukherjee, 2001). Ayurveda, the most ancient system of traditional medicine of the world, has been practiced in Indian subcontinent since 5000 BC (Dasgupta, 1992; Mukherjee and Wahile, 2006).

Ayurveda is a holistic approach towards life, health and disease management through medicinal herbs, minerals, diet, lifestyle and spirituality. It was developed through daily experiences and mutual relationship between people and nature, and thus not only cure diseases but also prevent disease, maintaining health and promoting longevity (Fradwley and Ranade, 2001).

3.10.1 Classification of dietary phytochemicals

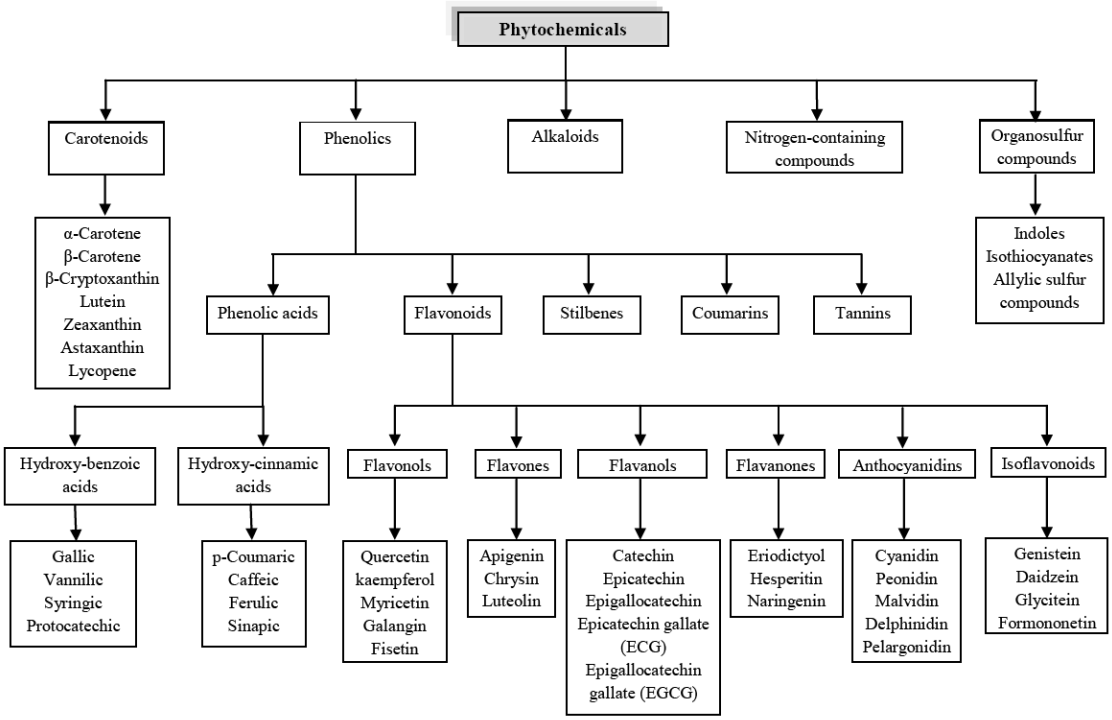


Figure 3.12 Classification of dietary phytochemicals (Fradwley and Ranade, 2001)

3.10.2 Pharmacological actions of phytochemicals

Table 3.6 Pharmacological actions of phytochemicals

Target pathway	Effects	Compounds	Mechanism of action
Antioxidant and radical scavenging activity	Promoting antioxidant enzyme activity	Quercetin, resveratrol, curcumin, hydroxytyrosol, catechin, luteolin	Increasing the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione *S*-transferase (GST), γ-

			glutamylcysteinesynthetase (γ-GCS) NADPH:quinone oxidoreductase-1 (NQO1) and heat shock proteins 70 (HSP70) expression
	Inhibiting pro-oxidant enzymes activity	Epigallocatechin, ECG, EGCG	Inhibiting lipoxygenase and cyclooxygenase
		Typheramide, alfrutamide, (−)-epicatechin, procyanidin	Inhibiting the activities of 5- lipoxygenase, 12-lipoxygenase and 15-lipoxygenase
		Curcumin, resveratrol, lupeol	Decreasing the activity of iNOS and myeloperoxidase (MPO) level
		Ellagic acid gallic, acid corilagin, luteolin	Inhibiting tyrosinase and xanthine oxidase
		Resveratrol	Inhibiting *O*-acetyltransferase and sulfotransferase activities
	Prevent free radical attacks	Epicatechin, rutin, mannitol	Scavenging hydroxyl radical (OH**.**)
		Ellagic acid gallic, acid corilagin, luteolin, β-carotene,	Scavenging superoxide radical (O2 **.**)

		tetrandrine	
		Quercetin, curcumin, lycopene	Decreasing MDA and lipoperoxidation
	Enhances endogenous antioxidant molecules	Quercetin, resveratrol, catechin, proanthocyanidin B4, β-carotene	Elevating cellular GSH content
Modulation of cellular activities of inflammation related cells	Inhibition of enzymes involved in signaling transduction and cell activation processes (T cell, B lymphocyte) or cytokine production	Genistein	Inhibition of tyrosine protein kinase inducing anti-proliferative effects on T cell, reducing IL-2 secretion and IL-2R expression
		Quercetin, kaempferol, apigenin, chrysin, luteolin	Inhibition of tyrosine protein kinase inducing anti-proliferative effects on M-CSF-activated macrophages
	Inhibition of arachidonic acid release from membranes (degranulation)	Quercetin	Inhibiting lysosomal enzyme release from stimulated neutrophil (elastase, β-glucuronidase)
		Rutin	Impairing lysosomal enzyme release from polymorphonuclear leukocytes

Modulation of arichidonicacidrelated enzymes	Inhibition of arichidonic acid metabolism	Quercetin, kaempferol, myricetin, hesperetin, naringenin, quercetagetin, kaempferol-3-galactoside, scutellarein, ochnaflavone, amentoflavone, ginkgetin, morelloflavone, bilobetin, triptolide, papyriflavonol A	Inhibition of PLA2 activity
	Inhibition of proinflammatory enzymes (COX, LOX and NOS) from different sources	Luteolin, 3',4'-dihydroxyflavone, galangin, morin, apigenein, chrysin, quercetin, myricetin, morusin, kuwanon C, sanggenon D, broussoaurone A, cycloheterophyllin, broussochalcone A broussoflavonol F, catechin, EGCG, resveratrol,	Inhibited COX activity

		xanthomicrol, cirsiliol, hypolaetin, diosmetin, tectorigenin, kuraridin, kurarinone, sophoraflavanone G, morusin, sanggenon B, kazinol B, rutaecarpine, 1,2-di-O-α-linolenoyl-3-O-β-galactopyranosyl-sn-glycerol (dlGG), curcumin, 4'-Me-gallocatechin, lonchocarpol A, tomentosanol D, catechins, catechinsgallate	
		Sophoraflavanone G, kenusanone A, kuraridin, papyriflavonol A, sanggenon B, sanggenon D, boswellic acid, diphyllinacetylapiosi	Inhibited 5-LOX activity

		de	
		Quercetin, kaempferol, fisetin, quercetagetin-7-*O*-glucoside, hibifolin, hypolaetin, sideritoflavone, 5,6,7-trihydroxyflavone (baicalein)	Inhibited 12-LOX activity
		Kaempferol, quercetin, myricetin, morin, cirsiliol, artonins	Inhibited 5-LOX and 12-LOX activity
		Quercetin	Inhibited eNOS activity
Modulation of the production of other proinflammatory molecules	Inhibition of proinflammatory cytokines from different sources	Formononetin	Inhibited iNOS activity
		Genistein, apigenin, quercetin, morin, wogonin, soyisoflavones, daidzein, glycitein, dlGG, paeonol	Inhibited NO production
		Genistein, quercetin, wogonin, baicalein, luteolin, nobiletin, paeonol, chlorogenic acid, hematein, aucubin, catalposide,	Inhibited cytokine production : IL-1β, IL-6, TNF-α

		tetrandrine, fangchinoline, colchicines, piperlactam S	
		Curcumin, amoradicin, genistein, silybin, quercetin, wogonin, rutin, luteolin, eriodictyol, hesperitin, EGCG, geraniin, corilagin, pinoresinol, woorenoside, lariciresinol glycoside, terpinen-4-ol, physalin B, triptolide, lupeol, [6]-shogaol, vitamin D, cepharanthine, fangchinoline, adenosine	Inhibited TNF-α production
		Apigenin, wogonin, bacalein	Inhibited IL-6 and IL-8 production
		Genistein, ilicic acid, inuviscolide acid, tryptanthrin	Inhibited LTB4 production
		Saikosaponins, masticaienonic acid,	Reducing LTC4 production

		masticadienolic acid, morolic acid	
		Chrysin, flavone, galangin, kaempferol, quercetin, salidroside, syringin, phillyrin, coniferin, tryptanthrin	Inhibited TXB2 production
		Lupeol, paeonol, quercetin, salidroside, syringin, phillyrin, tectorigenin, tectoridin, platycodin D, β-turmerone, ar-turmerone, rutaecarpine	Inhibited PGE2 production

3.10.3 Anti-allergic activities of phytochemicals (Bellik et al., 2013)

Table 3.7 Anti-allergic activities of phytochemicals

Target pathway	Effects	Compounds	Mechanism of action
Effect on IgE mediated Hypersensitivity (Type I)	Inhibition of chemical mediator release and cytokine	Luteolin, quercetin, baicalein	Inhibited the release of histamine, leukotrienes and prostaglandin D2 Inhibited IgE-

	production by mast, basophil or T cells		mediated TNF-α and IL-6 production
		Luteolin, quercetin, baicalein, Apigenin	Inhibited the p44/42 MAPK phosphorylation in response to crosslinkage of FcεRI
		Tetrandrine	Suppression of prostaglandin and leukotriene generation
		Coixol, pseudoephedrine, mallotophilippen A and B	Inhibited the release of histamine
		Apigenin, luteolin, 3.6-dihydroxy flavones, fisetin, kaempferol, quercetin, myricetin	Inhibition of the hexosaminidase release Suppression of cysteinyl leukotriene synthesis
		Flavone, quercetin	Inhibition of transport ATPase in histamine secretion
		Isoquercitrin	Inhibited carbachol and leukotriene D4 production

		Cirsiliol (3',4',5-trihydroxy-6,7-dimethoxy flavone)	Suppressed cysteinylleukotrienes release
		Ayanin, luteolin, apigenin, diosmetin, fisetin, ombuin, quercetin, kaempferol	Suppression of IL-4 synthesis
	Inhibition of Signal transduction and gene expression in mast, basophil or T cells Preventing allergic asthma	Mallotophilippen A and B	Inhibited iNOS gene expression
		Luteolin, apigenin, fisetin	Suppressed CD40 ligand expression
		Nobiletin	Reduced eotaxin expression
		Luteolin, apigenin, fisetin	Inhibited AP-1 and NFAT activation
		Dietary polyphenols	Interfer with activated T-helper 2
		Quercetin, provinol, flavin-7	Anti-inflammatory effects in experimental allergic asthma
Effect on cell-mediated Hypersensitivity (type IV)	Preventing contact dermatitis	Polyphenol (extract from the bark of Acacia mearnsii)	Inhibited itching in atopic dermatitis by preventing the skin from drying
		Polyphenols and anthocyanins	Improve atopic dermatitis disease in

		derived from Vaccinium uliginosumL	mice by reducing the Th2/Th1 ratio, IL-4 and IL-13 (as Th2 cytokines), IFN-γ, and IL-12 (as a Th1 cytokine) in spleens Decreased gene expression, such as IL-4, IL-5, CCR3, eotaxin-1, IL-12, IFN-γ, MCP-1, and IL-17, and suppressed Th 17
Attenuating autoimmune disorders	Improving multiple sclerosis (MS) disease	Dietary polyphenols, carotenoids, curcumin	Inhibited neuroinflammation in MS Inhibited the differentiation and expansion of Th17 cells in circulation induced by inflammatory cascade; Enhanced the expression of ZO-1; Down-regulated expression of CXC chemokines and

			receptor; Decreased Th17 cells to transmigrate across the blood brain barrier and the inhibition of autoreactive T cells transmigration can reduce neuroinflammation; Blocked IL17 and others, which lead to central system nervous tissue destruction in MS

3.11 Catechin

Catechins are basically flavan-3-ols. They are an important component of the daily diet. The main sources of catechins are green tea (800mg/l), chocolate (600mg/l), red wine (300mg/l), and fruits such as apricot or cherry (250mg/kg) (Arts et al., 2000; Chun & Chung, 2007; D' Archivio et al., 2007).

Biological source of catechin; Leaves of Camellia sinensis (Theaceae), Barks of Acacia catechu (Leguminosea).

Catechin are polyphenols that are grouped into different kinds: (1) epigallocatechin (EGC); (2) epigallocatechingallate (EGCG); (3) epicatechin (EC); (4) epicatechingallate (ECG); (5) gallocatechin (GC); (6) catechin (C), and (7) gallocatechingallate (GCG).

Epigallocatechingallate represents approximately 59% of the total catechins, Epigallocatechin 19%, Epicatechingallate 13.6% and epicatechin 6.4%.

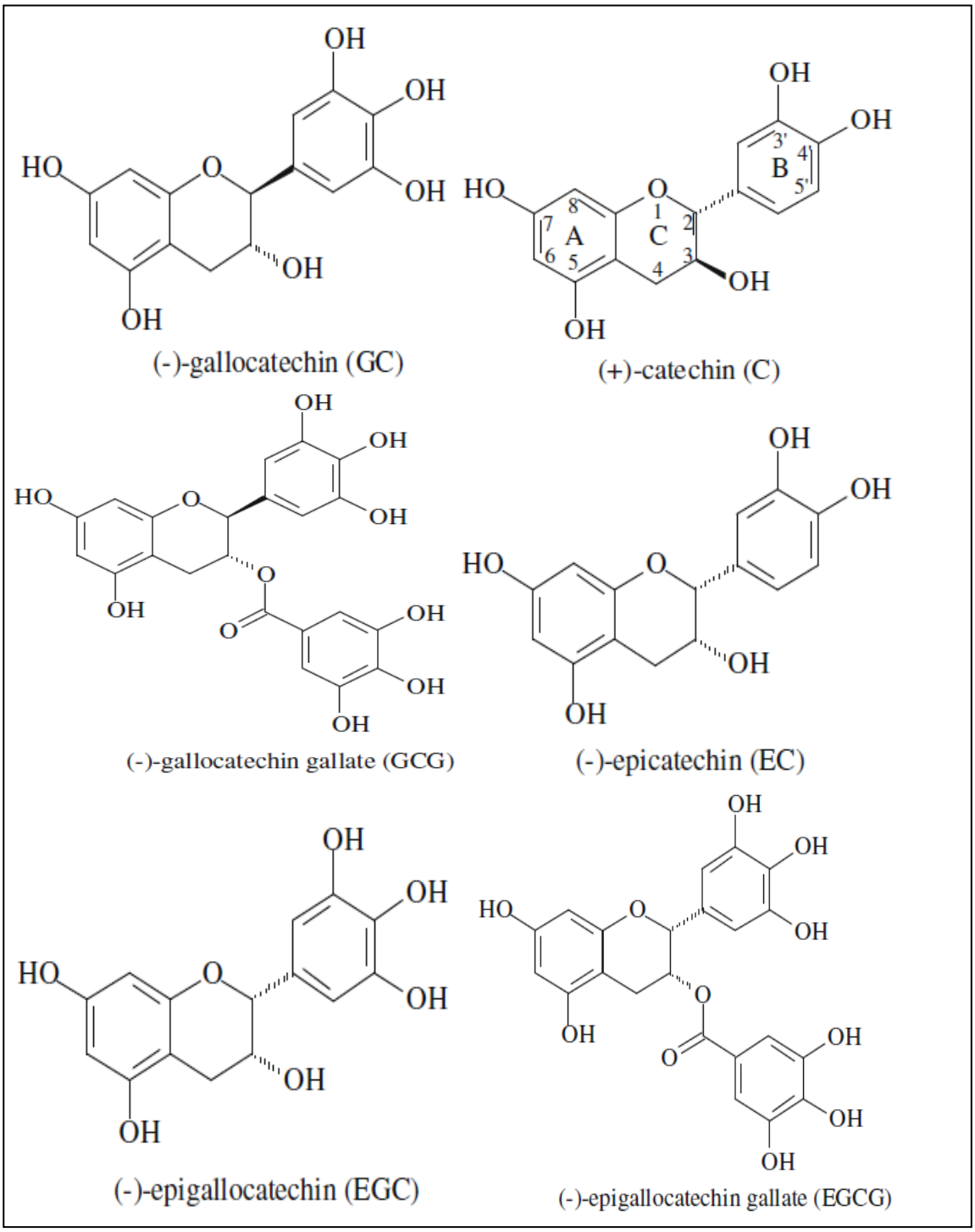


Figure 3.13 Different types of Catechins (Kofink et al., 2007)

After food processing, e.g. brewing green tea or roasting cocoa beans, catechins undergo conversion to suitable epimers such as; epigallocatechin (EGC) to gallocatechin (GC) and EGCG to gallocatechingallate (GCG) (Kofink et al., 2007).

3.11.1 Pharmacological actions of Catechins

3.11.1.1 Aging

Age associated detoriation and neurodegeneration mainly occur due to increased free radical generation and oxidative stress. In various animal model studies, it has shown that catechins, when administered in drinking water, it had a protective effect on the cognitive dysfunction and suppressed cerebral atrophy. It also decreased levels of 8-oxo-deoxyguanosine, which is a marker of oxidative DNA damage in the kidney, liver and cerebrum which suggests that catechins have a beneficial effect on the damage from the aging process (Unno et al., 2004).

3.11.1.2 Parkinson's disease

The pathogenesis of parkinson's disease suggests oxidative stress as a major factor (Olanow and Tatton, 1999). In various animal models, it has been seen that neurotoxins 1-methyl-4-phenyl-1-1,2,3,4-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) induce dopaminergic cell death and accumulation of lewy bodies. Catechins have shown to significantly prevent these pathologies in the animal models. They prevented the loss of dopaminergic neurons in the substantia nigra and preserved striatal levels of dopamine (Choi et al., 2002).

3.11.1.3 Alzheimer's disease

Animal studies treated with catechins have shown protection against beta-amyloid-induced neurotoxicity in cultured hippocampal neurons, reducing oxidative stress (Choi et al., 2001). Also, it has been shown that catechins regulate the processing of APP, through PKC activation, to the non-amyloidogenic soluble APP (sAPP), thus preventing the formation of the neurotoxic beta-amyloid (Levites et al., 2003). Similarly, Catechins have also shown to

inhibit beta-secretase enzyme (BACE1), which processes sAPP to beta-amyloid hence shows that Catechins have a potential in treating Alzheimer's disease (Jeon et al., 2003).

3.11.1.4 Stroke

When Catechins were administered immediately after ischemia in gerbils, it showed protection against neuronal damage. Also, Catechins showed a significant antioxidant effect in rats and protected against neurological deficit and infarction which occurred due to the focal ischemia, when administered 24 hours after a transient cerebral occlusion (Choi et al., 2004).

3.11.1.5 Cardiovascular diseases

Studies have shown that catechins have increased resistance to plasma LDL to oxidation in vivo, an effect which lowers the risk of atherogenesis (Miura et al., 2000). In apolipoprotein E-deficient mice model of atherosclerosis, Catechins when administered in drinking water, prevented atherosclerosis without affecting the plasma lipid or cholesterol levels (Miura et al., 2001). Also it has been studied that in spontaneously hypertensive rats, administration of Catechins has led to a reduction in the blood pressure (Negishi et al., 2004). Chronic administration of catechin to OLETF rats decreased blood pressure and ameliorated endothelium-dependent relaxations to acetylcholine. The acetylcholine-induced endothelium-dependent relaxations of aortic rings are mediated by nitric oxide, it implies that catechin restores the ability of endothelial cells to generate nitric oxide and prevent the degradation of nitric oxide (Ihm et al., 2009).

3.11.1.6 Obesity and weight loss

Studies have shown that particularly EGCG (50-100mg/kg), but not other catechins, have significantly reduced or prevented any increase in body weight in lean and obese zucker rats. The effect was reversible and also showed a reduction in food consumption (Kao et al., 2000).

3.11.1.7 Diabetes

In a small study in human volunteers, it has shown that catechins have substantially increased oral glucose tolerance but did not affect basal blood glucose levels (Tsuneki et al., 2004). Also, in animal models, it has been observed that long term administration of Catechins increased insulin sensitivity (Wu et al., 2004a). Catechins have also shown to prevent development of insulin resistance, hyperglycemia and other metabolic defects in fructose fed rats (Wu et al.,, 2004b).

3.11.1.8 Cancer

Studies in animal models of carcinogenesis have demonstrated the preventive effects of Catechins against tumorigenesis in the breast, prostate, lung and skin. This evidence has led to the selection of green tea extract by the National Cancer Institute (NCI) for further development for treatment of cancer. Studies have shown tht catechins exhibit protection against all stages in cancerogenesis; initiation, promotion and progression (Chung et al., 2003).

3.11.1.9 Anti viral and Anti enzymatic activities

Antiviral activities have been shown against tobacco mosaic virus, influenza virus, rotavirus, enterovirus, adenovirus and Epstein barr virus. Anti-enzymatic activities have been observed against B-lactamases, reverse transcriptase of HIV, collagenase, fatty acid synthase, histidine decarboxylase, and various other enzymes (Shimamura et al., 2007). Recently, studies have shown that Catechins inhibit HIV-1 replication by inhibiting HIV reverse transcriptase and by interfering with the binding of the viral envelope. Catechins prevent the attachment of the HIV-1 virion,gp120, to the CD4 molecules on T helper cells, hence prevent the initial step in HIV-1 infection (Kawai et al., 2003).

3.11.1.10 Osteogenesis

Catechins have shown to increase ALP activity and mineralization in cultured mesenchymal stem cell line derived from bone marrow, hence leading to an increase in

osteogenesis. Although it cannot be concluded that the increased ALP levels by Catechins were through certain pathways, but the increase in these osteogenic markers show the contribution of Catechins in osteogenesis (Chung et al., 2005).

3.11.1.11 Apoptosis

Catechins have been shown to modulate apoptosis by altering the expression of antiapoptotic and proapoptotic genes. They have prevented the expression of proapoptotic genes Bax, Bad and Mdm2 and induced the anti apoptotic genes Bcl-2, Bcl-w and Bcl-xl to protect SH-SY5Y cells from 6-OHDA-induced apoptosis (Levites et al., 2002).

3.11.1.12 Prooxidant properties

Catechins have shown to increase the oxidative damage incurred after exposure of DNA to 8-oxo-7,8-dihydro-2'-deoxyguanosine. This occurs due to the generation of the hydroxyl radical and hydrogen peroxide in the presence of coppe r(II) and Iron (III). This suggests that the antioxidant mechanism of scavenging metals by Catechins in order to stop formation of free radicals could lead to prooxidant effects on DNA. The igh dose of Catechins induces an up-regulation of endogenous antioxidants such as SOD, catalase and glutathione, which are responsible for some of the cytoprotective actions of Catechins (Schroeter et al., 2002).

3.11.1.13 Anti-inflammatory effects

Through the NO synthase pathway, the neuronal NOS (nNOS) produces toxic effects through NO and Catechin inhibition of nNOS has shown to exhibit anti-inflammatory actions. Also, studies have shown that Catechins inhibit inducible NOS (iNOS) by preventing inhibitor kB disappearance, inhibiting nuclear factor kB (NF-kB) from binding to the promoter of the iNOS gene hence activating it, which also leads to anti-inflammatory effects. Endothelial NOS (eNOS), a vasodilator-inducing enzyme is activated and produces NO, which activates Guanylatecyclase to produce cyclic guanosine monophosphate and causes vasorelaxation by P13K, protein kinase A and Akt-dependent signaling pathways.

Topical administration of Catechins have shown to prevent immunosuppression, infiltration of cluster of differentiation (CD)11b+ leukocytes, including neutrophils and lymphocytes, and depletion of antigen-presenting cells such as dendritic cells and macrophages. Catechins also enhanced the production of IL-8 by decreasing the expression of adhesion molecules CD11b and CD18 on isolated leukocytes (Schroeter et al., 2002).

3.11.2 Catechin content in some common foods (Nutrient Data laboratory US dept, 2007)

Table 3.8 Catechin content in some common foods

Food	Catechin (mg/100g)	Epicatechin (mg/100g)	Epigallocatechin, EpicatechinGallate, & Epigallocatechingallate(mg/100g)
Apples	0.9	6.1	0.6
Blackberries	37.1	4.7	0.8
Black grapes	10.1	8.7	2.8
Brewed black tea	1.5	2.1	23.1
Brewed green tea	2.6	8.3	114.3
Cherries	1.3	7.0	0.4
Cocoa	0.00	26.2	0.00
Dark chocolate	12.0	41.5	0.00
Fava beans	8.2	7.8	4.7
Milk chocolate	2.1	6.3	0.00
Pears	0.3	3.8	0.8
Raspberries	1.6	4.1	1.0
Red table wine	7.0	3.3	0.1

3.11.3 Chemistry of Catechin- Oxidation of Catechin

(+) Catechin contains two different pharmacophores, the catechol group in ring B and the resorcinol group in ring A as well as a hydroxyl group at position 3 in ring C. Rings A and B are not conjugated and ionization of the OH groups of one ring system should not affect the ionization of OH groups of ring A are independent and different from ring B (Slabbert, 1977).

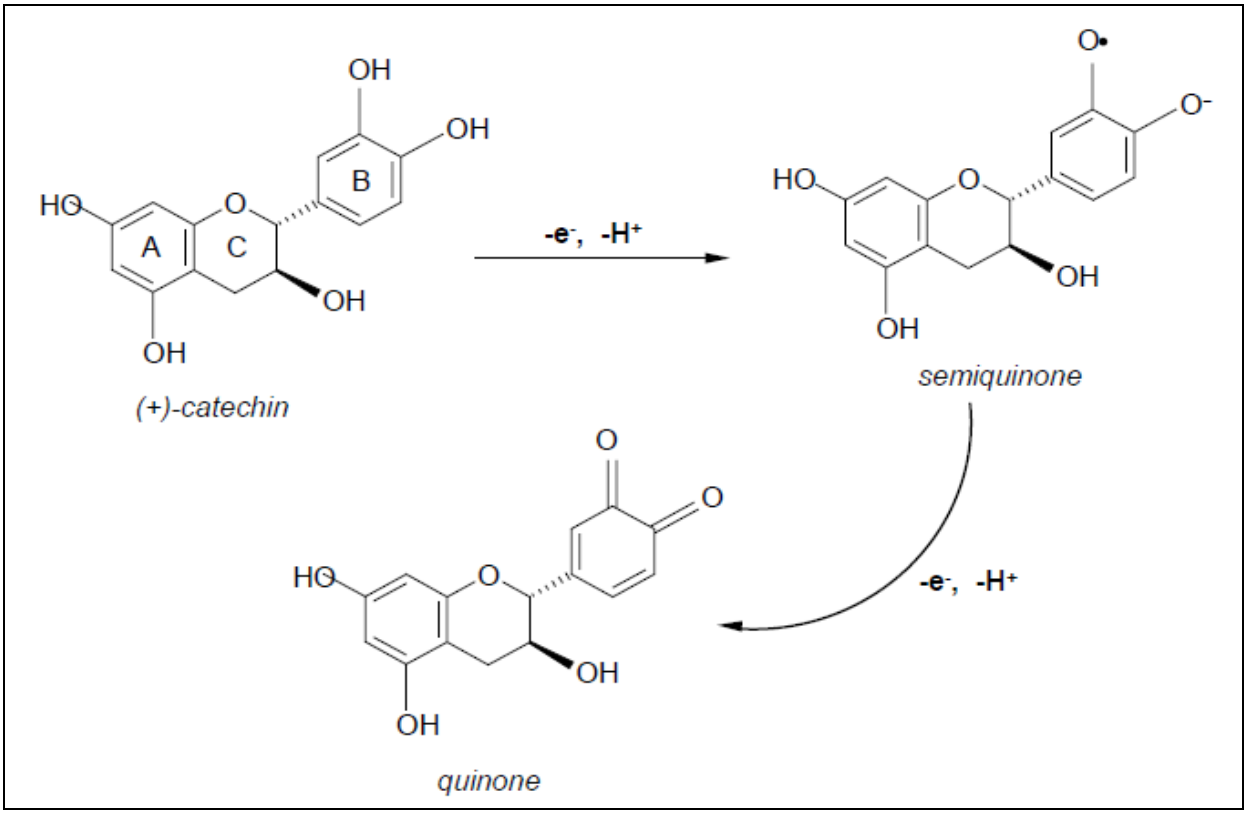


Figure 3.14 Chemistry of Catechin (Slabbert, 1977)

The first oxidation of (+) Catechin occurs at a very low positive potential indicating a high radical scavenging activity and a reversible reaction. The hydroxyl group oxidized later, undergoes an irreversible oxidation reaction.

The pH-dependent effect of (+) Catechin antioxidant activity occurs due to an increased radical scavenging activity upon deprotonation. Deprotonation enhances the antioxidant

action and since only the ionization potential, and not the bond dissociation energies, becomes lower during deprotonation. Hence, electron donation is the main mechanism of antioxidant action after deprotonation (Janeiro and Brett, 2004).

3.12 H1 Receptor blocker - cetirizine

Cetirizine is a second generation, long acting, selective peripheral histamine H1 receptor antagonist. It has shown good efficacy in treatment of several allergic disorders (Richards el at., 1990; Spencer & Noble, 1990; Curran et al., 2004))

3.12.1 Pharmacodynamic profile of Cetirizine

Cetirizine is a carboxylated metabolite of hydroxyzine and a racemic mixture of two enantiomers; Levocetirizine (R enentiomer) and dextrocetirizine (S enantiomer) (Wang et al., 2001).

3.12.2 Antihistaminic effects

Cetirizine is a selective H1 receptor antagonist, with an IC50 (concentration producing 50% inhibition of H1 receptors) value of 0.65μmol/l and low affinity for calcium channel, α1-adrenergic, dopamine D2, serotonin 5-HT2 and muscarinic receptors (all IC50> 10μmol/l) (Davies, 1991; Day et al.,2001; Curran et al., 2004). It has shown decreased histamine-induced wheal and flare formation, protection against histamine-induced bronchospasm or bronchoconstriction, decreased nasal airway resistance in allergic rhinitis.

3.12.3 Anti-allergic and anti-inflammatory effects

Cetirizine reduced wheal and flare responses to various inflammatory mediators including platelet activating factor and compound 48/80.

It has shown increased conjunctivital reaction threshold in conjunctivitis, decreased inhibition of eosinophil chemotaxis induced by inflammatory mediators, decreased expression of cell adhesion molecules including ICAM-1 on endothelial or epithelial cells, decreased PAF induced hyper adherence, decreased histamine induced eotaxin production

and gene expression, decreased serum tryptase release from mast cells, decreased proinflammatroy cytokine or PMA-induced expression of IL-8 from epithelial cells, decreased antigen-induced release of histamine, decreased IL-4, IL-5 and interferon-γ gene expression in nasal lymphoid tissue, decreased PGD2 and histamine production, increased release of PGE2 from peritoneal macrophages.

3.12.4 CNS effects

Current evidence shows that the recommended therapeutic dosages of Cetirizine does not have significant CNS activity although it is able to cross the blood brain barrier and bind to approximately 30% of the H1 receptors in the cerebral cortex (Jinquan et al., 1995, Patat et al.,1995). It has shown few effects drowsiness, no impact on cognitive function, behavior or learning seen, high sedation effects relative to terfenadine or loratadine at therapeutic doses, no effect on EEG of healthy volunteers, it does not potentiate CNS depressant effects of ethanol in healthy adult volunteers.

3.12.5 Cardiac effects

Cetirizine has not been associated with any adverse effects when administered either as monotherapy or in combination with agents that are metabolized by the cytochrome P450 system.

It shows no ECG abnormalities with no effect on QT interval, any ventricular tachycardia or torsades de pointes, little or not effect on potassium channels in vitro.

3.12.6 Drug interactions

Cetirizine hardly has any drug interactions since it is only minimally metabolized by the liver. Cetirizine is therefore unlikely to interact with agents which are metabolized by the CYP isoenzyme system; CYP1A2, CYP2C9, CYP2C19, CYP2D6 or CYP3A4 isozymes.

3.12.6 Place of Cetirizine in the management of selected allergic disorders

Allergic disorders significantly impair the quality of life of affected patients. The pharmacological intervention in allergic disorders has generally focused on blocking the effect of a major mediator which is involved in the allergic response- histamine.

The first generation H1 receptor antagonists e.g. Chlorpheniramine, Diphenhydramine or Hydroxyzine have been associated with adverse effects which has limited their use. They lead to sedation, impaired cognitive function and short half life which requires more frequent administration (Walsh et al., 2001). The second generation H1 receptor antagonists include; Cetirizine, Loratidine, Fexofenadine, Mizolastine and Ebastine. These drugs also show similar anti allergic efficacy as the first generation drugs, but with minimal sedative effects when given at the therapeutic dosages. This is due to the greater receptor selectivity and reduced blood brain barrier penetration (Rosenwasser, 2002). Oral or intranasal H1 receptor antagonists are regarded as the first line pharmacotherapy in patients with mild intermittent allergic rhinitis (Bousquet et al.,2002). Cetirizine has a well established role in the treatment of symptoms of SAR (seasonal allergic rhinitis), PAR (perennial allergic rhinitis) or CIU (chronic idiopathic urticaria) in adult, adolescent and pediatric patients. It has shown to possess a corticosteroid sparing effect and reduced the risk of developing asthma in infants with atopic dermatitis.

CHAPTER 4

Materials & Methods

4.0 MATERIALS AND METHODS

4.1 Study effect of catechin, cetirizine and combination of catechin and cetirizine in ovalbumin induced animal model of allergic conjunctivitis

4.1.1 Materials and chemicals

Catechin (Sigma Aldrich Pvt. Ltd., India), cetirizine (Balaji drugs Ltd.) Ovalbumin, Histamine hydrochloride (Sigma Aldrich Pvt. Ltd., India), o-phthalaldehyde, Compound 48/80, l-histidine, other chemicals were of analytical grade.

4.1.2 Experimental Animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Institute of Pharmacy, Nirma University, Ahmedabad as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of social justice and empowerment, Government of India. Protocol number is IPS/PCOL/MPH12-13/1009. Male and female Healthy Guinea pigs (500-800g) were procured from Zydus Cadila Pvt Ltd, Ahmedabad & Animal vaccination institute, Ahmedabad. Animals have been housed in groups of 6 animals in the animal house of Nirma University, Ahmedabad under controlled conditions of temperature 23±2°C, relative humidity 55±5%, and photo schedule (12hr light and 12hr dark).

4.1.3 Experimental protocol

Male and female Hearty guinea pigs were divided into 5 groups- normal control group, disease control group, disease treated with catechin (100mg/kg), disease treated with cetirizine (10mg/kg), disease treated with combination of catechin and cetirizine (50mg/kg & 5mg/kg respectively). Allergic sensitization was induced by intraperitoneal injection of 100mcg ovalbumin and 20mg alum dissolved in 1 ml PBS on day 1 till day 14 in the disease control, disease treated with catechin, disease treated with cetirizine and disease

treated with catechin & cetirizine groups. Simultaneously, catechin treatment was also started in both diseases treated with catechin and disease treated with combination of catechin & cetirizine groups at a dose of 100mg/kg and 50mg/kg p.o. while the normal control group was administered PBS intraperitoneal. On the 14th day, 10mg/kg and 5mg/kg was administered to the disease treated with cetirizine and disease treated with combination of catechin & cetirizine groups respectively. At the end of experiment, determination of mast cell histamine, blood histamine content, histidine decarboxylase assay using stomach was carried out.

4.1.4 Effect of catechin, cetirizine & combination of catechin and cetirizine on clinical scoring for physical characteristics in allergic conjunctivitis (Yanni et al., 1994)

Feature	Characteristic	Clinical scoring
Conjunctiva redness	Normal	0
	Pink	1
	Red	2
	Dark red	3
Eyelid edema	No edema	0
	Lower lid edema	1
	Upper & lower lid edema	2
	Swollen, everted eye lids	3
	Swelling of both lids and side of face	4
Discharge	No discharge	0
	Glazed, glassy appearance	1
	Moist lids and surrounding hair	2
	Moist lids and surrounding hair, thicker mucous like	3

4.1.5 Effect of catechin, cetirizine & combination of catechin and cetirizine on vascular permeability in allergic conjunctivitis

4.1.5.1 Standard curve of evans Blue

Various concentrations of evans blue were prepared using Phosphate buffer saline (2.5g sodium dihydrogen phosphate, 2.523g disodium hydrogen phosphate & 8.2g sodium chloride dissolved in 1000ml distilled water) in the range 1ng/ml to 1µg/ml. The absorbance was measured at 620nm spectrophotometrically.

4.1.5.2 Estimation of vascular permeability

After 10 minutes, the animal was anesthetized using Diazepam and Ketamine combination and surgical procedure performed. Fur was then removed from the cervical region and the Jugular vein was traced and injected with 0.4ml evans blue dye. Immediately 0.1ml 0.3% ovalbumin is administered in the conjunctiva tissue. After 30 minutes, the conjunctival tissue is excised and immersed in a solution of 3ml 0.5% sodium sulphate and 7ml acetone kept at room temperature. After 24 hours, the solutions were centrifuged at 300rpm for 10 minutes and absorbance taken at 620nm using U V spectrophotometer (Eugenia et al., 2008).

4.1.6 Effect of catechin, cetirizine & combination of catechin and cetirizine on mast cell histamine

4.1.6.1 Standard curve of Histamine

Various concentrations of histamine were prepared using distilled water in the range 1ng/ml to 1µg/ml. 1ml of the histamine solution was reacted with 0.1ml of ice-cold o-pthalaldehyde (OPA). The reaction was incubated at room temperature for 4 minutes and terminated by addition of 0.6ml 3N HCl. Fluorescence of the conjugate was assayed at 450nm emission excited at 360nm.

4.1.6.2 Preparation of peritoneal mast cells

10ml of Phosphate buffer saline (2.5g sodium dihydrogen phosphate, 2.523g disodium hydrogen phosphate & 8.2g sodium chloride dissolved in 1000ml distilled water) was injected intraperitoneal into the animals. After thorough massage, the animal was anesthetised and the PBS collected and the peritoneal cavity was washed with 5ml of PBS. This PBS collected was then centrifuged at 2000rpm for 5 minutes at 4°C. The pellet formed was washed twice and finally resuspended into 2ml PBS.

Measurement of histamine release

1.8ml of the cell suspension was pre-incubated for 5 minutes at 37°C followed by stimulation with 0.1ml compound 48/80 for 10 minutes. Histamine release was terminated by cooling the cells in ice. Cells and solution was separated by centrifugation (100 X g, 10minutes, and 4°C).equal volume of 0.8N $HClO_4$ and twice volume of 0.4N $HClO_4$ was added to supernatant and pellet respectively. To 1ml of sample, a mixture of 125µl 5N NaOH, 0.4g NaCl and 2.5ml n-butanol was added. Then, the samples were centrifuged at 200 X g for 1minute at room temperature. To 1ml of the lower aqueous phase, 0.1ml of 10N NaOH was added. The histamine-o-pthaldehydye (OPT) reaction was carried out by incubation with 0.1ml of OPA (10mg/ml Methanol) for 4 minutes at room temperature. Fluorescence of the conjugate was assayed at 450nm emission excited at 360nm.

4.1.7 Effect of catechin, cetirizine & combination of catechin and cetirizine on blood histamine content

2ml Blood samples were drawn and immediately poured into equal volumes of ice-cold 0.6N perchloric acid and mixed. After centrifugation at 4000rpm at 4°C for 20 minutes, 1ml of supernatant was saturated with K_2HPO_4 to yield pH 8.5-8.8.histamine was then extracted into 5ml isoamyl alcohol by shaking for 10 minutes. After centrifugation for 10 minutes at 4°C at 2000rps, the supernatant was washed with 2ml alkaline salt (NaCl) saturated $CHCl_3$. The washed extract was then shaken with 5ml heptanes and 2.5ml 0.1N

HCl. Histamine was measured fluorimetrically in 1ml of the final acid extract with 0.1ml OPA for 4 minutes at room temperature and 450nm emission excited at 360nm.

4.1.8 Effect of catechin, cetirizine & combination of catechin and cetirizine on histidine decarboxylase enzyme

The histidine decarboxylase enzyme assay was performed using the stomach of the SD rats. The stomach of the animal was removed, cut open and washed in ice cold saline, blotted on filter paper and immediately stored at^{-} 20°C. Each tissue sample of 250mg weight was put into a tube containing 2.5ml ice-cold 0.02M phosphate buffer of pH 6.2 containing 1µg/ml pyridoxal-5'-phosphate and 1µg/ml dithiothreitol and then homogenized. After homogenization, the homogenate was centrifuged at 1000rpm for 5 minutes and the supernatant obtained was used as the enzyme solution. 1ml of the enzyme solution was incubated at 37°C for 4 hours with 0.1µg/ml l-histidine. The enzyme reaction was terminated using $HClO_4$. The histamine formed is measured fluorimetrically using 1ml of the final acid extract with 0.1ml OPA for 4 minutes at room temperature and 450nm emission excited at 360nm.

4.2 Study effect of catechin, cetirizine and the combination of catechin and cetirizine in ovalbumin induced animal model of allergic rhinitis

4.2.1 Materials and chemicals

catechin (Sigma Aldrich Pvt. Ltd., India), cetirizine (Balaji drugs Ltd.), Ovalbumin, Histamine hydrochloride (Sigma Aldrich Pvt. Ltd., India), o-phthalaldehyde, Compound 48/80, l-histidine, other chemicals were of analytical grade.

4.2.2 Experimental Animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Institute of Pharmacy, Nirma University, Ahmedabad as per the guidelines of committee for the purpose of control and supervision of

experiments on animals (CPCSEA), Ministry of social justice and empowerment, Government of India. Protocol number is IPS/PCOL/MPH12-13/1009.

Male and female SD rats (250-400g) were procured from Zydus Cadila Pvt Ltd., Ahmedabad. Animals have been housed in groups of 6 animals in the animal house of Nirma University, Ahmedabad under controlled conditions of temperature 23±2°C, relative humidity 55±5%, and photo schedule (12hr light and 12hr dark).

4.2.3 Experimental protocol

Male and female SD rats were divided into 5 groups- normal control group, disease control group, disease treated with catechin (100mg/kg), disease treated with cetirizine (10mg/kg), disease treated with combination of catechin and cetirizine (50mg/kg & 5mg/kg respectively).Allergic sensitization was induced by intraperitoneal injection of 100mcg ovalbumin and 20mg alum dissolved in 1 ml PBS on day 1 till day 14 in the disease control, disease treated with catechin, disease treated with cetirizine and disease treated with catechin & cetirizine groups. Simultaneously, catechin treatment was also started in both disease treated with catechin and disease treated with combination of catechin& cetirizine groups at a dose of 100mg/kg and 50mg/kg p.o. while the normal control group was administered PBS intraperitoneal. On the 14^{th} day, 10mg/kg and 5mg/kg was administered to the disease treated with cetirizine and disease treated with combination of catechin& cetirizine groups respectively. At the end of experiment, physical characteristics, determination of mast cell histamine, blood histamine content, histidine decarboxylase assay using stomach was carried out.

4.2.4 Effect of catechin, cetirizine & combination of catechin and cetirizine on clinical scoring for physical characteristics in allergic rhinitis

After half an hour of drug administration in all groups, 10µl of 10% ovalbumin was administered into the nostrils of all the animals except the normal control group. The animal was then observed for nose scratching and nasal discharge. Animal behaviour was observed for 15 minutes and scoring done accordingly (Xu et al., 2012).

Feature	Characteristic	Clinical scoring
Nasal itch	None	0
	Scratching nose lightly one to two times	1
	Scratching the nose and face constantly	2
Sneeze	None	0
	One to three times	1
	Four to ten times	2
	11 or more times	3
Nasal discharge	None	0
	Secretions flow to anterior nostril	1
	Secretions surpass anterior nostril	2
	Secretions cover the face	3

4.2.5 Effect of catechin, cetirizine & combination of catechin and cetirizine on vascular permeability in allergic rhinitis

4.2.5.1 Standard curve of evans blue dye

Various concentrations of evans blue were prepared using Phosphate buffer saline (2.5g sodium dihydrogen phosphate, 2.523g disodium hydrogen phosphate & 8.2g sodium chloride dissolved in 1000ml distilled water) in the range 1ng/ml to 1µg/ml. The absorbance was measured at 620nm spectrophotometrically.

4.2.5.2 Estimation of vascular permeability

After 10 minutes, the animal was anesthetized using Diazepam and Ketamine combination and surgical procedure performed. Fur was then removed from the cervical region and the Jugular vein was traced and injected with 0.4ml evans blue dye. Immediately after the administration of the dye, the trachea was surgically exposed and cannulated so that the

animal can breathe spontaneously through the tracheal cannula. A polyethylene cannula was then inserted into the nasopharynx from the side of the larynx and the other end of the cannula was connected to an artificial infusion pump. The oral cavity was filled with glycerine soaked absorbent cotton in order to interrupt perfusate flow across the oral cavity. PBS was then perfused for 10 minutes to wash the nasal cavity at a flow rate of 0.25ml per minute. PBS was then again perfused for 10 minutes and the perfusate droppings collected from the nostrils. This was considered as period 1.Ovalbiumin (0.3% w/v) was then perfused through the nasal cavity for 10 minutes and the perfusate was collected (Period 2).PBS was again perfused continuously for 40minutes and the perfusate was collected for 4 intervals of 10 minutes each (Period 3,4,5,6). The perfusate samples were then centrifuged at 1700rpm for 10 minutes and evaluation of samples for vascular permeability was carried out (Shirsaki et al., 1998).

4.2.6 Effect of catechin, cetirizine & combination of catechin and cetirizine on mast cell histamine

4.2.6.1 Standard curve of histamine

Various concentrations of histamine were prepared using distilled water in the range 1ng/ml to 1µg/ml. 1ml of the histamine solution was reacted with 0.1ml of ice-cold o-pthalaldehyde (OPA). The reaction was incubated at room temperature for 4 minutes and terminated by addition of 0.6ml 3N HCl. Fluorescence of the conjugate was assayed at 450nm emission excited at 360nm.

4.2.6.2 Preparation of peritoneal mast cells

10ml of Phosphate buffer saline (2.5g sodium dihydrogen phosphate, 2.523g disodium hydrogen phosphate & 8.2g sodium chloride dissolved in 1000ml distilled water) was injected intraperitoneal into the animals. After thorough massage, the animal was anesthetised and the PBS collected and the peritoneal cavity was washed with 5ml of PBS. This PBS collected was then centrifuged at 2000rpm for 5 minutes at 4°C. The pellet formed was washed twice and finally resuspended into 2ml PBS.

4.2.6.3 Measurement of histamine release

1.8ml of the cell suspension was pre-incubated for 5 minutes at 37°C followed by stimulation with 0.1ml compound 48/80 for 10 minutes. Histamine release was terminated by cooling the cells in ice. Cells and solution was separated by centrifugation (100 X g, 10minutes, 4°C).equal volume of 0.8N $HClO_4$ and twice volume of 0.4N $HClO_4$ was added to supernatant and pellet respectively. To 1ml of sample, a mixture of 125µl 5N NaOH, 0.4g NaCl and 2.5ml n-butanol was added. Then, the samples were centrifuged at 200 X g for 1minute at room temperature. To 1ml of the lower aqueous phase, 0.1ml of 10N NaOH was added. The histamine-o-pthaldehydye (OPT) reaction was carried out by incubation with 0.1ml of OPA (10mg/ml Methanol) for 4 minutes at room temperature. Fluorescence of the conjugate was assayed at 450nm emission excited at 360nm.

4.2.7 Effect of catechin, cetirizine & combination of catechin and cetirizine on blood histamine content

2ml Blood samples were drawn and immediately poured into equal volumes of ice-cold 0.6N Perchloric acid and mixed. After centrifugation at 4000rpm at 4°C for 20 minutes, 1ml of supernatant was saturated with K_2HPO_4 to yield pH 8.5-8.8.histamine was then extracted into 5ml Isoamyl alcohol by shaking for 10 minutes. After centrifugation for 10 minutes at 4°C at 2000rps, the supernatant was washed with 2ml alkaline salt (NaCl) saturated $CHCl_3$. The washed extract was then shaken with 5ml heptanes and 2.5ml 0.1N HCl. Histamine was measured fluorimetrically in 1ml of the final acid extract with 0.1ml OPA for 4 minutes at room temperature and 450nm emission excited at 360nm.

4.2.8 Effect of catechin, cetirizine & combination of catechin and cetirizine on histidine decarboxylase enzyme

The histidine decarboxylase enzyme assay was performed using the stomach of the SD rats. The stomach of the animal was removed, cut open and washed in ice cold saline blotted on filter paper and immediately stored at^{-} 20°C. Each tissue sample of 250mg weight was put into a tube containing 2.5ml ice-cold 0.02M phosphate buffer of pH 6.2 containing 1µg/ml

pyridoxal-5'-phosphate and 1µg/ml dithiothreitol and then homogenized. After homogenization, the homogenate was centrifuged at 1000rpm for 5 minutes and the supernatant obtained was used as the enzyme solution. 1ml of the enzyme solution was incubated at 37°C for 4 hours with 0.1µg/ml l-histidine. The enzyme reaction was terminated using $HClO_4$. The histamine formed is measured fluorimetrically using 1ml of the final acid extract with 0.1ml OPA for 4 minutes at room temperature and 450nm emission excited at 360nm.

4.3 Safety pharmacological evaluation of cetirizine

Safety pharmacological evaluation of cetirizine was performed by observing the locomotor activity of mice using photoactometer. Overnight fasted mice were divided into 3 groups; 1) Normal control, 2) cetirizine treated (5mg/kg), 3) cetirizine treated (10mg/kg). One hour after treatment with the respected dose of cetirizine, each animal was placed in the photoactometer and number of 'cut offs' was recorded for 30 minutes.

4.4 Statistical analysis

Results are represented as mean ± S.E.M. Statistical analysis was performed using Graph pad prism 5 statistical software. Statistical differences between the means of various groups were evaluated using one way analysis of variance (ANOVA) followed by turkey's test. Data were considered statistically significant at $P<0.05$.

CHAPTER 5

RESULTS

5.0 RESULTS

5.1 Study effect of catechin, cetirizine & combination of catechin and cetirizine in ovalbumin induced animal model of allergic conjunctivitis

5.1.1 Effect of catechin, cetirizine & combination of catechin and cetirizine on clinical scoring for physical characteristics

Ovalbumin challenge showed significant increase in clinical score in disease control group as compared to normal control group, while the disease treated with catechin, cetirizine and combination of catechin and cetirizine showed significantly ($P<0.05$) decreased clinical score compared to disease control group of animals. (Figure 5.3; Table 5.1)

5.1.2 Effect of catechin, cetirizine & combination of catechin and cetirizine on mast cell histamine

5.1.2.1 Standard curve of Histamine

The histamine standard curve plotted showed linearity with positive correlation and coefficient 0.951.

5.1.2.2 Effect on histamine release from mast cells

Ovalbumin challenge with 100µg/kg intraperitoneal for 14 days showed significant ($P<0.05$) increase in Histamine concentration in mast cells as compared to normal control group, while the disease treated with catechin, cetirizine and combination of catechin and cetirizine showed significantly decreased histamine concentration compared to disease control group of animals. (Figure 5.4; Table 5.1)

5.1.3 Effect of catechin, cetirizine & combination of catechin and cetirizine on blood histamine content

Ovalbumin challenge with 100µg/kg intraperitoneal for 14 days showed significant ($P<0.05$) increase in histamine concentration in blood samples as compared to normal control group, while the disease treated with catechin, cetirizine and combination of

catechin and cetirizine showed significantly decreased histamine concentration compared to disease control group of animals. (Figure 5.5; Table 5.1)

5.1.4 Effect of catechin, cetirizine & combination of catechin and cetirizine on histidine decarboxylase enzyme

Ovalbumin challenge with 100µg/kg intraperitoneal for 14 days showed significant ($P<0.05$) increase in enzyme conversion of l-histidine to histamine as compared to normal control group, while the disease treated with catechin, cetirizine and combination of catechin and cetirizine showed significantly decreased enzyme conversion of l-histidine to histamine compared to disease control group of animals. (Figure 5.6; Table 5.1)

5.1.5 Effect of catechin, cetirizine & combination of catechin and cetirizine on vascular permeability

5.1.5.1 Standard curve of evans blue dye

The standard curve of evans blue showed positive correlation with coefficient 0.972.

5.1.5.2 Estimation of vascular permeability

Ovalbumin challenge showed significant increase in vascular permeability to evans blue dye in disease control group as compared to normal control group, while the disease treated with catechin, cetirizine and combination of catechin and cetirizine showed significantly ($P<0.05$) decreased vascular permeability compared to disease control group of animals. (Figure 5.7; Table 5.1)

Table 5.1 Effect of catechin, cetirizine & Combination in Allergic Conjunctivitis

Parameters	NC	DC	DT-CAT	DT-CET	DT-CAT + CET
Clinical scoring of conjunctiva	0.5 ± 0.341	5.5 ± 0.428 *	3.0 ± 0.516 #	1.5 ± 0.428 #	2.667 ± 0.421#

Histamine release from mast cells (ng/ml)	36.06 ± 9.183	135.8 ± 2.602*	94.05 ± 4.333#	103.1 ± 6.551#	100.7 ± 14.66#
Histamine release in blood (ng/ml)	4.186 ± 2.831	70.26 ± 11.56*	36.06 ± 3.797#	62.19 ± 10.46#	38.87 ± 10.15#
Histidine Decarboxylase assay	25.12 ± 4.726	94.12 ± 7.591*	59.99 ± 8.752#	85.05 ± 3.741#	57.41 ± 9.170#
Vascular permeability (ng/ml)	161.7 ± 8.809	768.9 ± 107.7*	404.4 ± 23.14#	276.1 ± 14.49#	402.2 ± 28.68#

5.1.6 Effect of catechin, cetirizine & combination of catechin and cetirizine on histopathology of conjunctiva

In histopathology of conjunctivae tissue, normal control group of animals shows normal structure with no ulceration, inflammation or tissue damage. Ovalbumin challenge showed significant increase in the ulceration, infiltration of inflammatory cells and edema in the subcutaneous region. Treatment with catechin, cetirizine, and combination of catechin and cetirizine showed improvement in ulceration, infiltration of inflammatory cells and edema as compared to disease control group.

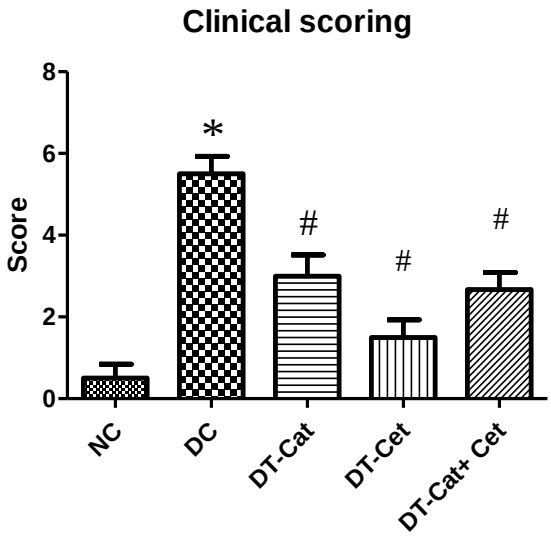


Figure 5.3 Clinical scoring in Allergic Conjunctivitis

*Significantly different from normal control ($p<0.05$), # significantly different from disease control ($p<0.05$), each group consists of 6 animals. Values are expressed as Mean ± SEM, NC- Normal control, DC- Disease control, DT- Cat – Disease treated with catechin (100mg/kg/day), DT-Cet – Disease treated with cetirizine (10mg/kg, p.o), DT- Cat + Cet – Disease treated with combination of catechin & cetirizine (50mg/kg/day, p.o & 5mg/kg, p.o).

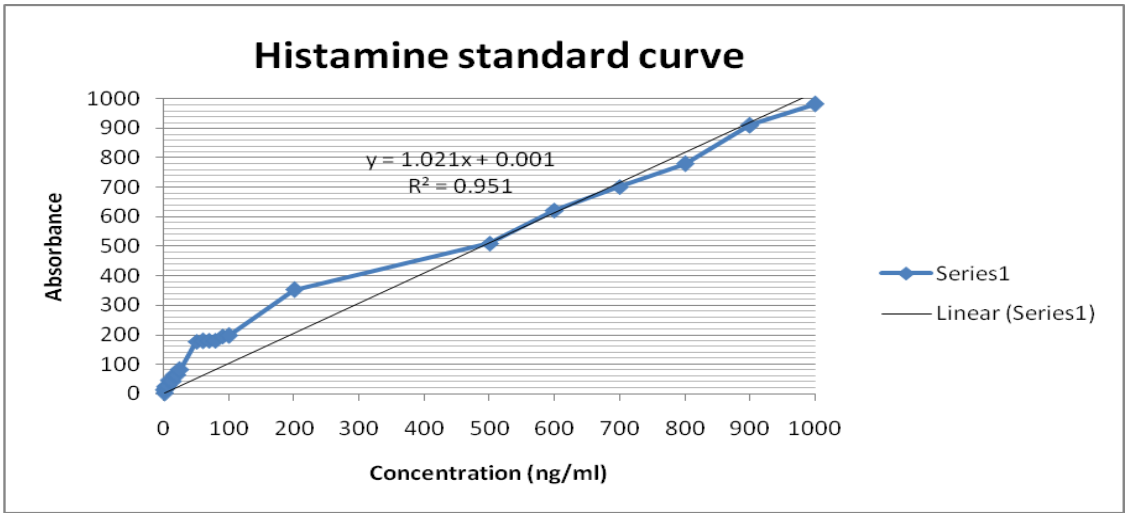


Figure 5.1 Histamine standard curve

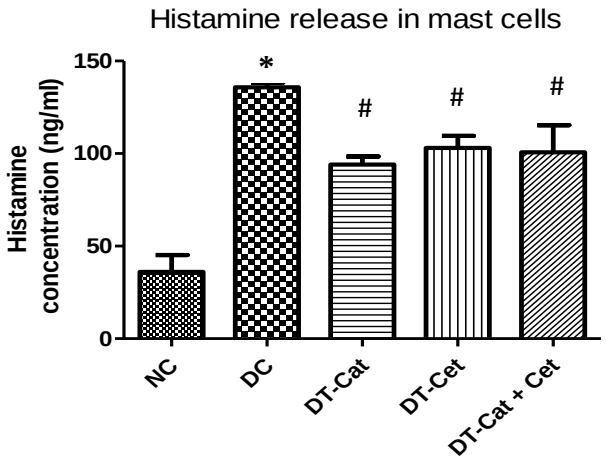


Figure 5.4 Histamine release in mast cells in Allergic Conjunctivitis

*Significantly different from normal control ($p<0.05$), # significantly different from disease control ($p<0.05$), each group consists of 6 animals. Values are expressed as Mean ± SEM, NC- Normal control, DC- Disease control, DT- Cat – Disease treated with catechin (100mg/kg/day), DT-Cet – Disease treated with cetirizine (10mg/kg, p.o), DT- Cat + Cet – Disease treated with combination of catechin & cetirizine (50mg/kg/day, p.o & 5mg/kg, p.o).

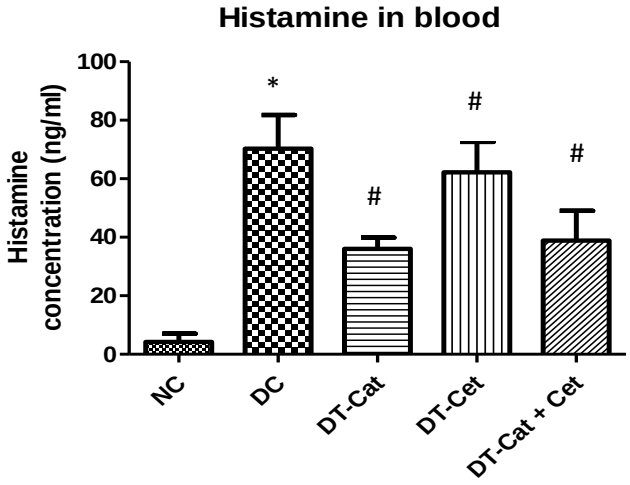


Figure 5.5 Blood histamine content in Allergic Conjunctivitis

*Significantly different from normal control (p<0.05), # significantly different from disease control (p<0.05), each group consists of 6 animals. Values are expressed as Mean ± SEM, NC- Normal control, DC- Disease control, DT- Cat – Disease treated with catechin (100mg/kg/day), DT-Cet – Disease treated with cetirizine (10mg/kg, p.o), DT- Cat + Cet – Disease treated with combination of catechin & cetirizine (50mg/kg/day, p.o & 5mg/kg, p.o).

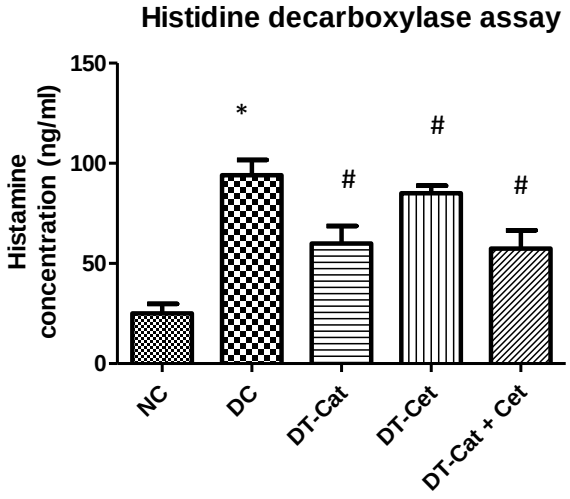


Figure 5.6 Histidine decarboxylase assay in Allergic Conjunctivitis

*Significantly different from normal control (p<0.05), # significantly different from disease control (p<0.05), each group consists of 6 animals. Values are expressed as Mean ± SEM, NC- Normal control, DC- Disease control, DT- Cat – Disease treated with catechin (100mg/kg/day), DT-Cet – Disease treated with cetirizine (10mg/kg, p.o), DT- Cat + Cet – Disease treated with combination of catechin & cetirizine (50mg/kg/day, p.o & 5mg/kg, p.o).

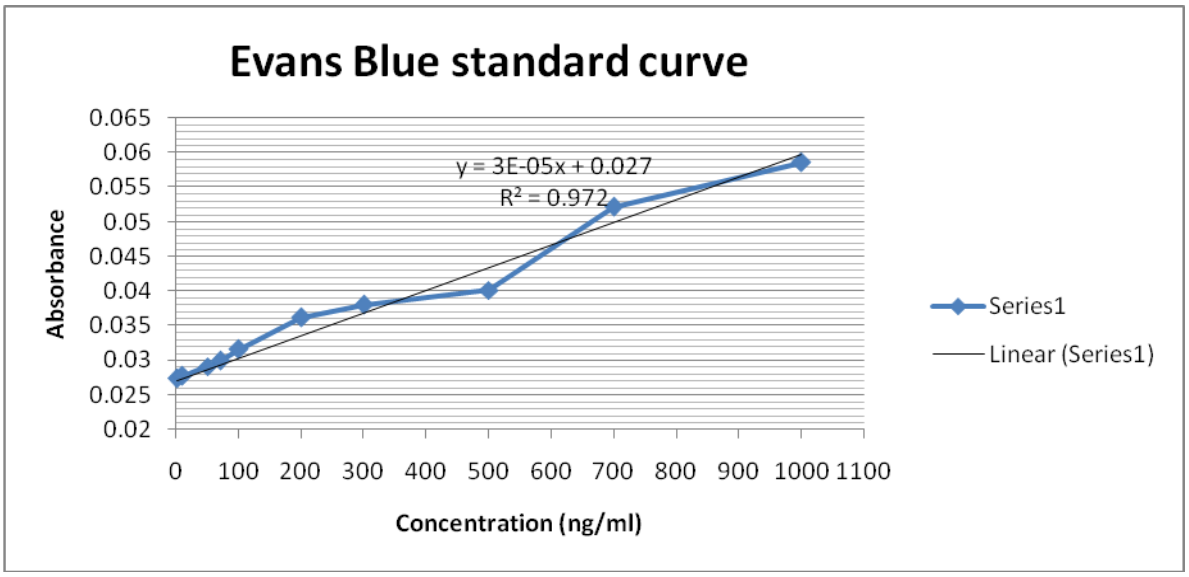


Figure 5.2 evans Blue standard curve

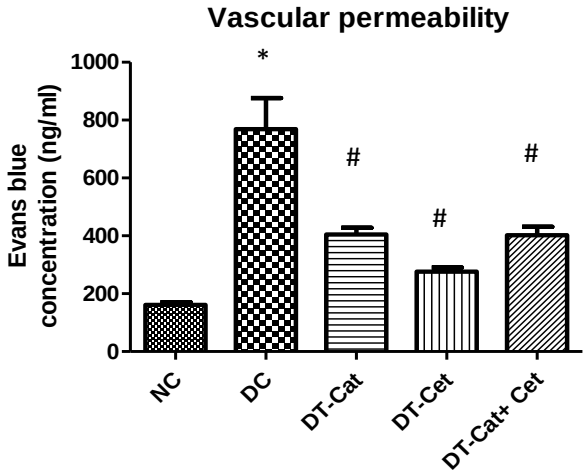
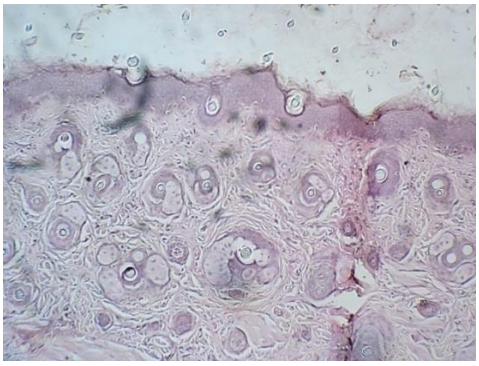
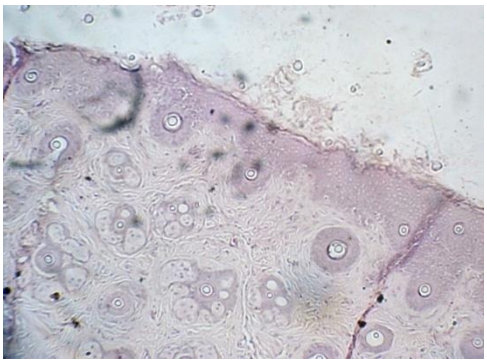


Figure 5.7 Vascular permeability in Allergic Conjunctivitis

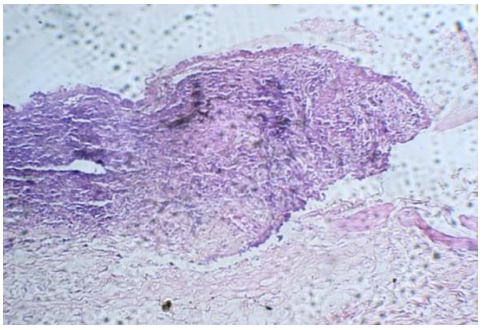
*Significantly different from normal control ($p<0.05$), # significantly different from disease control ($p<0.05$), each group consists of 6 animals. Values are expressed as Mean ± SEM, NC- Normal control, DC- Disease control, DT- Cat – Disease treated with catechin (100mg/kg/day), DT-Cet – Disease treated with cetirizine (10mg/kg, p.o), DT- Cat + Cet – Disease treated with combination of catechin & cetirizine (50mg/kg/day, p.o & 5mg/kg, p.o).

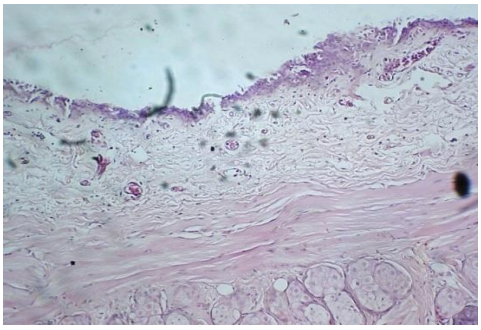




(a) **(b)**

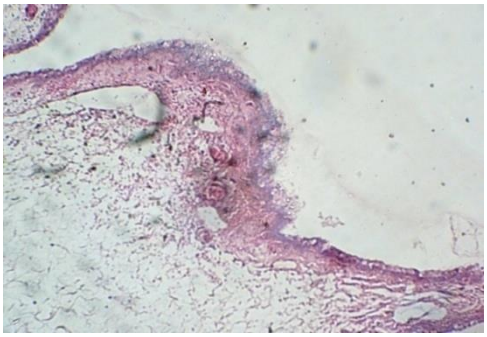
Normal control conjunctivae

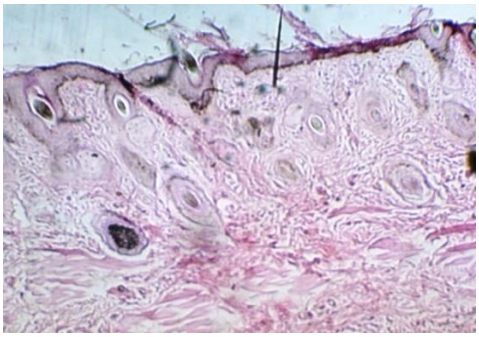




(c) **(d)**

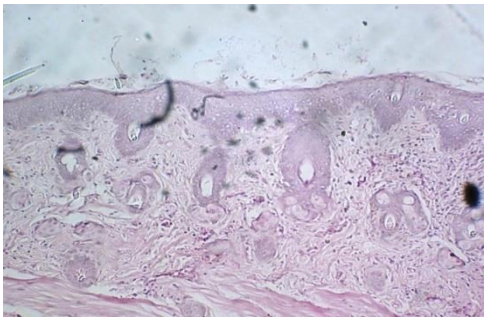
Disease control conjunctivae

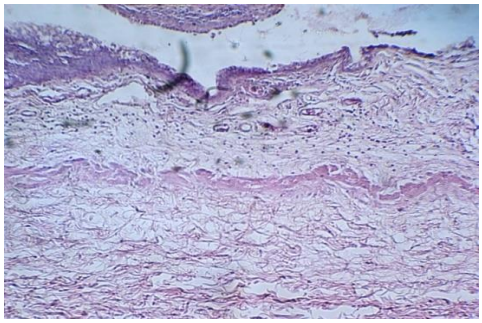




(e) **(f)**

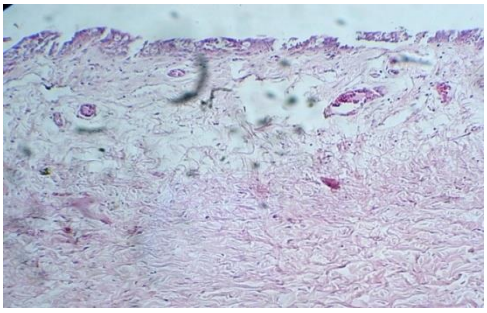
Disease treated with catechin conjunctivae

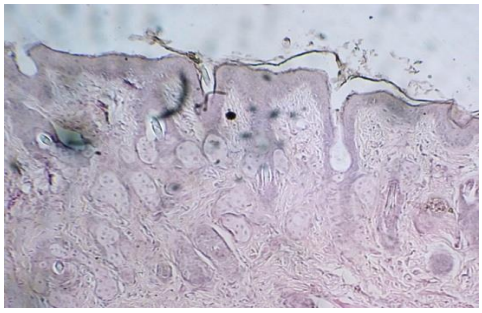




(g) **(h)**

Disease treated with cetirizine conjunctivae





(i) **(j)**

Disease treated with combination of catechin and cetirizine conjunctivae

Figure 5.16 Histopathology of conjunctivae

5.2 Study effect of the combination of catechin, cetirizine & combination of catechin and cetirizine in ovalbumin induced animal model of allergic rhinitis

5.2.1 Effect of catechin, cetirizine & combination of catechin and cetirizine on clinical scoring for physical characteristics

Ovalbumin challenge showed significant (P<0.05) increase in clinical score in disease control group as compared to normal control group, while the disease treated with catechin, cetirizine and combination of catechin and cetirizine showed significantly decreased clinical score compared to disease control group of animals. (Figure 5.6; Table 5.2)

5.2.2 Effect of catechin, cetirizine & combination of catechin and cetirizine on mast cell histamine

5.2.2.1 Standard curve of Histamine

The histamine standard curve plotted showed linearity with positive correlation and coefficient 0.951.

5.2.2.2 Effect of histamine release from mast cells

Ovalbumin challenge with 100µg/kg intraperitoneal for 14 days showed significant (P<0.05) increase in Histamine concentration in mast cells as compared to normal control group, while the disease treated with catechin, cetirizine and combination of catechin and cetirizine showed significantly decreased histamine concentration compared to disease control group of animals. (Figure 5.7; Table 5.2)

5.2.3 Effect of catechin, cetirizine & combination of catechin and cetirizine on blood histamine content

Ovalbumin challenge with 100µg/kg intraperitoneal for 14 days showed significant (P<0.05) increase in Histamine concentration in blood samples as compared to normal control group, while the disease treated with catechin, cetirizine and combination of catechin and cetirizine showed significantly decreased histamine concentration compared to disease control group of animals. (Figure 5.8; Table 5.2)

5.2.4 Effect of catechin, cetirizine & combination of catechin and cetirizine on histidine decarboxylase enzyme

Ovalbumin challenge with 100µg/kg intraperitoneal for 14 days showed significant (P<0.05) increase in enzyme conversion of l-histidine to histamine as compared to normal

control group, while the disease treated with catechin, cetirizine and combination of catechin and cetirizine showed significantly decreased enzyme conversion of l-histidine to histamine compared to disease control group of animals. (Figure 5.9; Table 5.2)

5.2.5 Effect of catechin, cetirizine & combination of catechin and cetirizine on vascular permeability at period 3

5.2.5.1 Standard curve of evans blue dye

The standard curve of evans blue showed positive correlation with coefficient 0.972.

5.2.5.2 Effect on vascular permeability

Ovalbumin challenge showed significant ($P<0.05$) increase in vascular permeability to evans blue dye in disease control group as compared to normal control group, while the disease treated with catechin, cetirizine and combination of catechin and cetirizine showed significantly decreased vascular permeability compared to disease control group of animals. (Figures 5.12, 5.13, 5.14; Table 5.2)

5.2.6 Effect of catechin, cetirizine & combination of catechin and cetirizine on vascular permeability at period 4

Ovalbumin challenge showed significant ($P<0.05$) increase in vascular permeability to evans blue dye in disease control group as compared to normal control group, while the disease treated with catechin, cetirizine and combination of catechin and cetirizine showed significantly decreased vascular permeability compared to disease control group of animals. (Figure 5.15; Table 5.2)

Table 5.2 Effect of catechin, cetirizine & Combination in Allergic Rhinitis

Parameter	NC	DC	DT-CAT	DT-CET	DT-CAT + CET
Clinical scoring of symptoms	0.666±0.210	5.0±0.365*	2.66 ± 0.4216#	1.5 ±0.428 #	3.5 ± 0.428 #
Histamine release in mast cells	24.22 ±2.480	60.66 ± 2.847*	40.26 ± 4.088#	53.47 ± 5.626 #	44.82 ± 2.61 #
Histamine in blood	18.42 ± 14.75	38.31 ± 3.754*	20.94 ± 3.234#	29.86 ± 4.023 #	27.07 ± 5.170#
Histidine decarboxylase assay	10.69 ± 2.173	66.57 ± 3.572*	50.73 ± 5.254#	64.19 ± 5.706 #	59.89 ± 6.408#
Vascular permeability at period 3 (µg/ml)	0.309 ± 0.088	7.331 ± 2.885*	0.997±0.039#	0.483 ± 0.153 #	0.744 ± 0.071#
Vascular permeability at period 4 (µg/ml)	0.678 ± 0.162	9.324 ± 3.021*	0.746 ±0.148 #	0.222 ± 0.061 #	0.493 ± 0.112#

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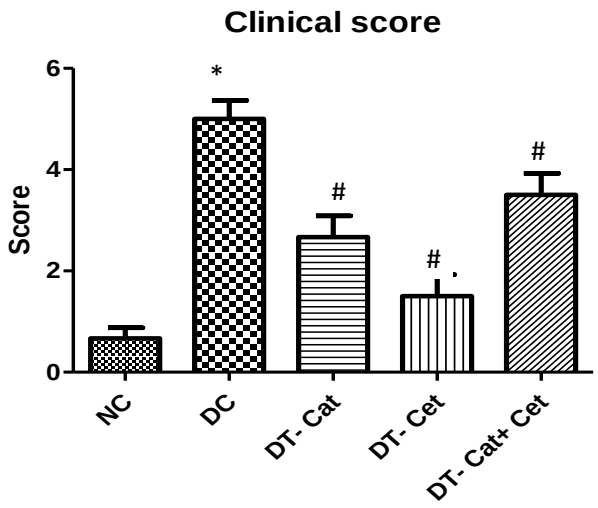

Figure 5.8 Clinical scoring in Allergic Rhinitis

*Significantly different from normal control ($p<0.05$), # significantly different from disease control ($p<0.05$), each group consists of 6 animals. Values are expressed as Mean ± SEM, NC- Normal control, DC- Disease control, DT- Cat – Disease treated with catechin (100mg/kg/day), DT-Cet – Disease treated with cetirizine (10mg/kg, p.o), DT- Cat + Cet – Disease treated with combination of catechin & cetirizine (50mg/kg/day, p.o & 5mg/kg, p.o).

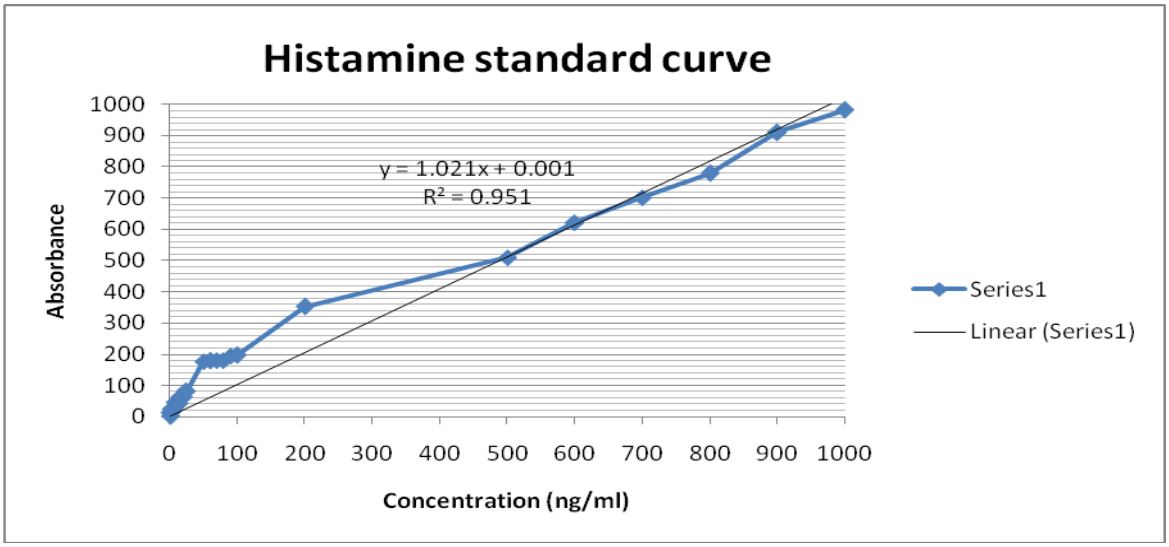

Figure 5.1 Histamine standard curve

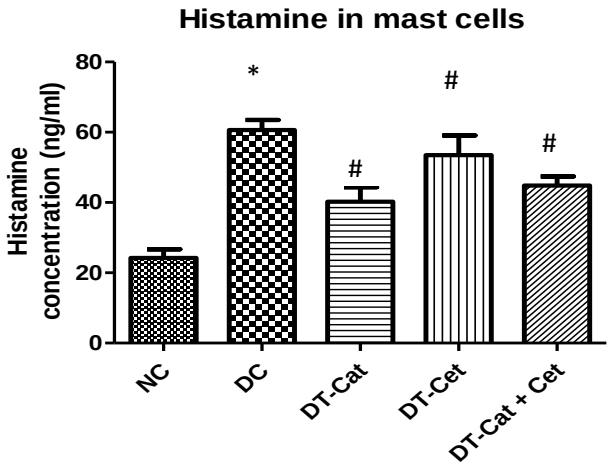

Figure 5.9 Histamine in mast cells in Allergic Rhinitis

*Significantly different from normal control ($p<0.05$), # significantly different from disease control ($p<0.05$), each group consists of 6 animals. Values are expressed as Mean ± SEM, NC- Normal control, DC- Disease control, DT- Cat – Disease treated with catechin (100mg/kg/day), DT-Cet – Disease treated with cetirizine (10mg/kg, p.o), DT- Cat + Cet – Disease treated with combination of catechin & cetirizine (50mg/kg/day, p.o & 5mg/kg, p.o).

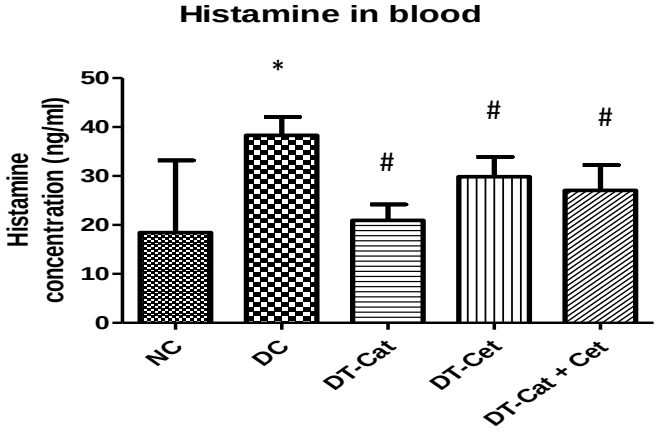

Figure 5.10 Blood histamine content in Allergic Rhinitis

*Significantly different from normal control (p<0.05), # significantly different from disease control (p<0.05), each group consists of 6 animals. Values are expressed as Mean ± SEM, NC- Normal control, DC- Disease control, DT- Cat – Disease treated with catechin (100mg/kg/day), DT-Cet – Disease treated with cetirizine (10mg/kg, p.o), DT- Cat + Cet – Disease treated with combination of catechin & cetirizine (50mg/kg/day, p.o & 5mg/kg, p.o).

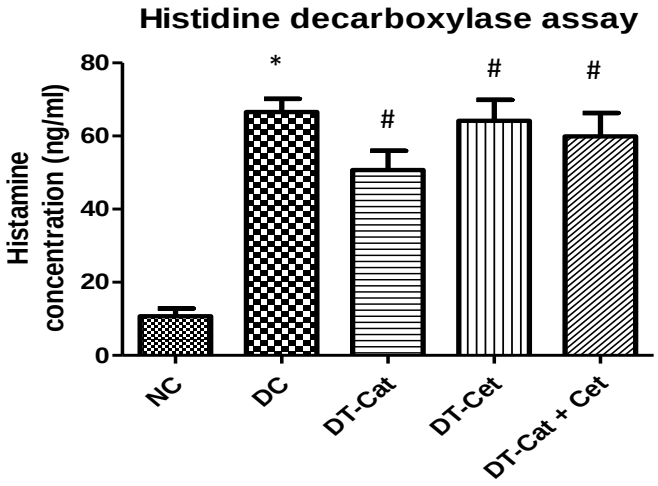

Figure 5.11 Histidine decarboxylase assay in Allergic Rhinitis

*Significantly different from normal control (p<0.05), # significantly different from disease control (p<0.05), each group consists of 6 animals. Values are expressed as Mean ± SEM, NC- Normal control, DC- Disease control, DT- Cat – Disease treated with catechin (100mg/kg/day), DT-Cet – Disease treated with cetirizine (10mg/kg, p.o), DT- Cat + Cet – Disease treated with combination of catechin & cetirizine (50mg/kg/day, p.o & 5mg/kg, p.o).

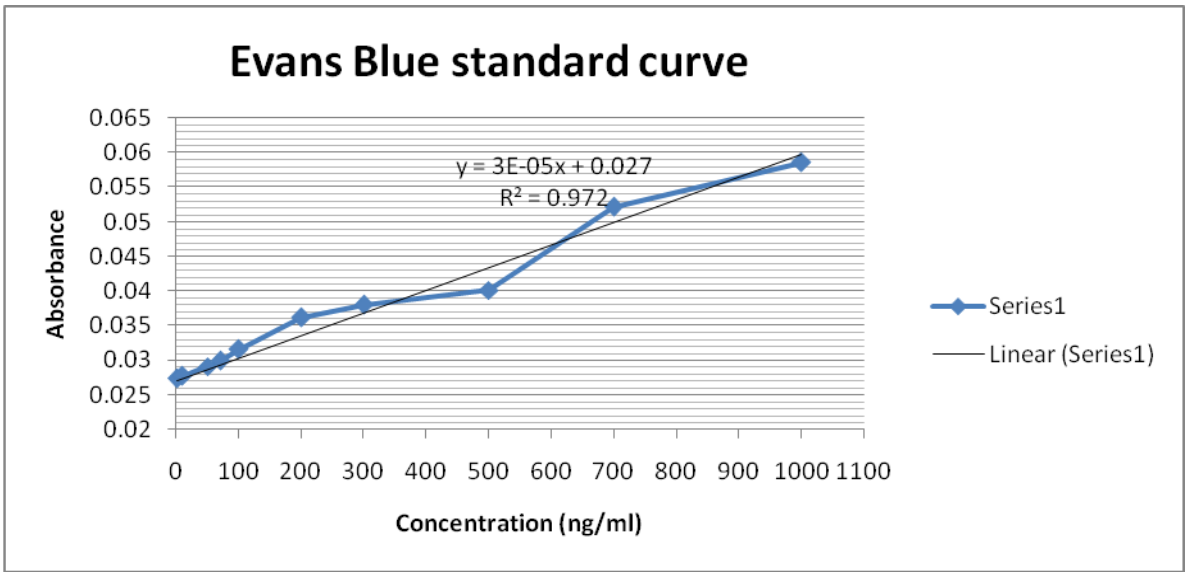

Figure 5.2 Evans blue standard curve

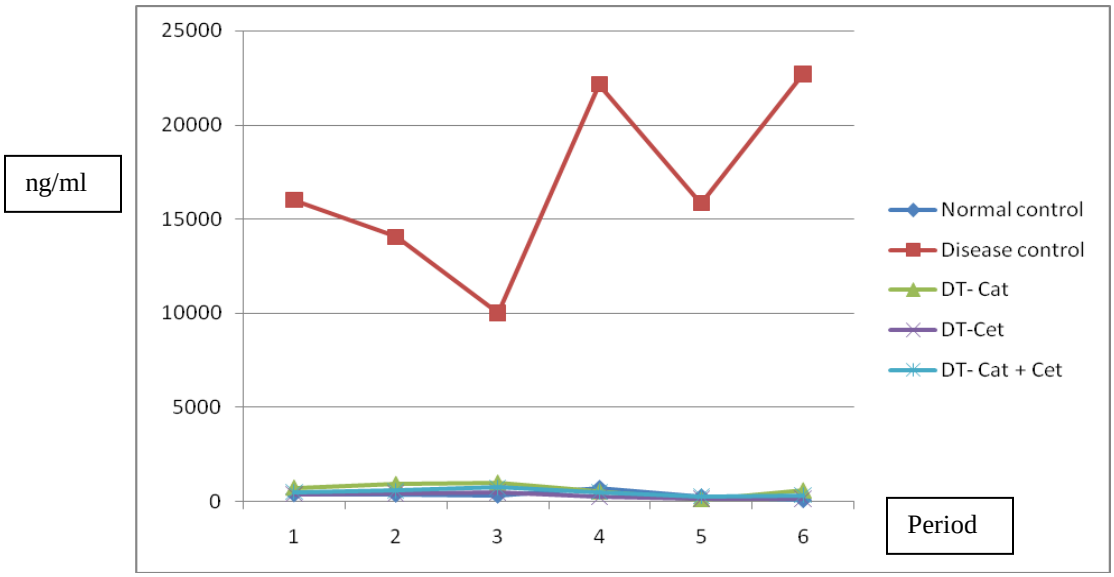

Figure 5.12 Vascular permeability curve in allergic rhinitis

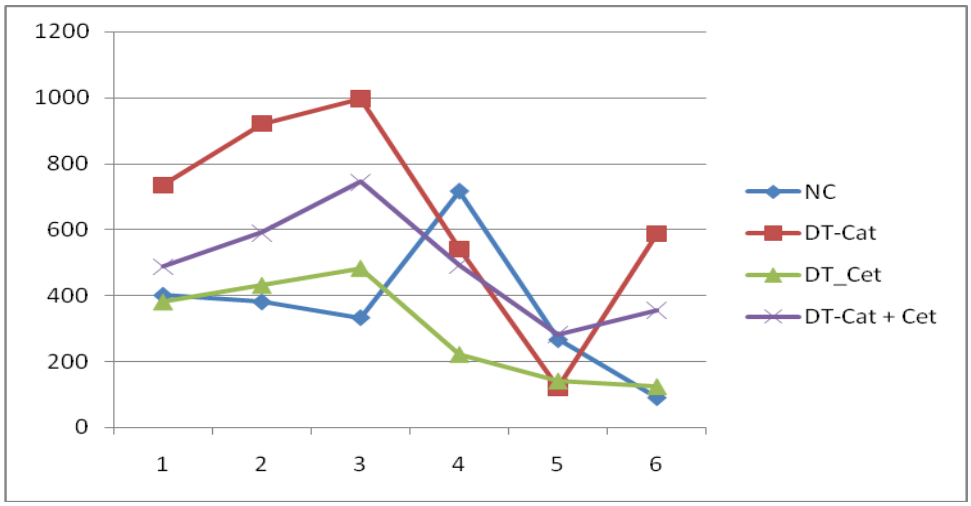

Figure 5.13 Vascular permeability in allergic rhinitis (Expanded)

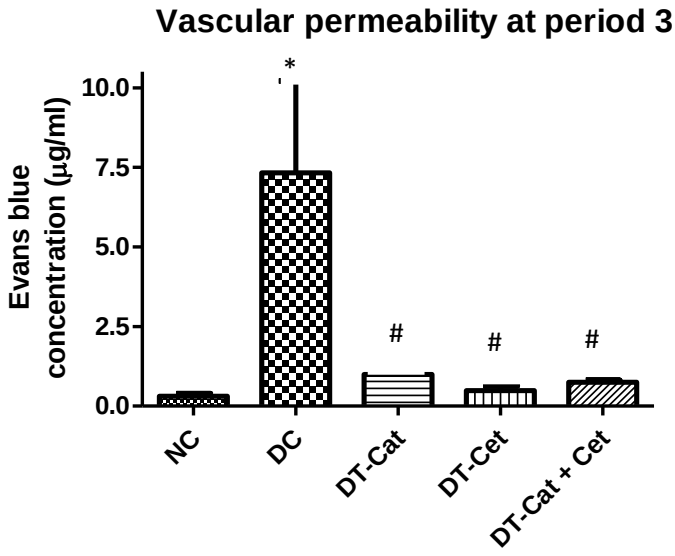

Figure 5.14 Vascular permeability at period 3 in Allergic Rhinitis

*Significantly different from normal control ($p<0.05$), # significantly different from disease control ($p<0.05$), each group consists of 6 animals. Values are expressed as Mean ± SEM, NC- Normal control, DC- Disease control, DT- Cat – Disease treated with catechin (100mg/kg/day), DT-Cet – Disease treated with cetirizine (10mg/kg, p.o), DT- Cat + Cet – Disease treated with combination of catechin & cetirizine (50mg/kg/day, p.o & 5mg/kg, p.o).

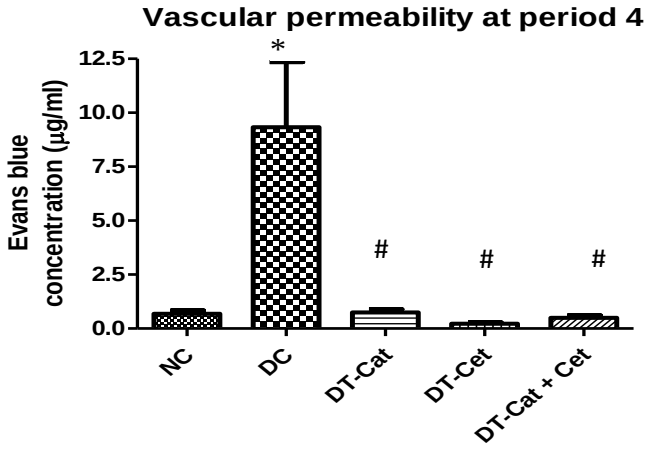

Figure 5.15 Vascular permeability at period 4 in Allergic Rhinitis

*Significantly different from normal control ($p<0.05$), # significantly different from disease control ($p<0.05$), each group consists of 6 animals. Values are expressed as Mean ± SEM, NC- Normal control, DC- Disease control, DT- Cat – Disease treated with catechin (100mg/kg/day), DT-Cet – Disease treated with cetirizine (10mg/kg, p.o), DT- Cat + Cet – Disease treated with combination of catechin & cetirizine (50mg/kg/day, p.o & 5mg/kg, p.o).

5.3 Safety pharmacological evaluation of cetirizine

During the safety pharmacological evaluation of cetirizine, it was observed that sedation produced in the cetirizine treated animals was not significant as compared to the normal control group (Figure 5.16, Table 5.3).

Table 5.3 safety pharmacological evaluation of cetirizine

Group	Mean ± S.E.M
Normal control	108 ± 9.324
Cetirizine treated (5mg/kg)	99.67 ± 4.232
Cetirizine treated (10mg/kg)	101.3 ± 9.773

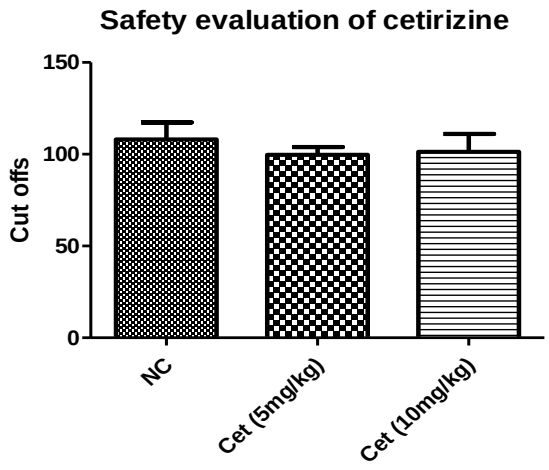

Figure 5.16 Safety pharmacological evaluation of cetirizine

CHAPTER 6

DISCUSSION

6. DISCUSSION

Allergies have occurred since decades in developed countries. However, recent estimates show that about a third of the population will develop symptoms due to allergy at some point in their life. Allergy is not a disease but a mechanism which plays a role in a number of disorders. Allergy can either be atopic or non-atopic. Atopic allergy results when individuals produce increased amounts of the allergic antibody immunoglobulin IgE, which binds strongly to specific receptors on mast cells resulting into the release of inflammatory mediators such as histamine and leukotrienes. These mediators are then responsible for the various symptoms of allergy such as sneezing, spasm of airways, itching, rash and tissue swelling. It is associated with disorders such as hay fever, allergic asthma and eczema. Non-atopic allergy is not IgE mediated and it involves an abnormal immune response to a wide variety of external environmental substances (Lebrec et al., 1999).

Current therapies for allergic disorders involve antihistamines, mast cell stabilizers, anti leukotrienes, etc. Despite availability of a wide range of these drugs, effect produced is short lived having lots of side effects. Currently, H1 antihistamines are the drug of choice for allergic disorders. But, all the first generation & many second-generation antihistamines produce central nervous system (CNS) side effects due to their CNS depressant properties, adverse cardiovascular events and majority of these first generation compounds have a limited duration of action. CNS side effects are comparable to placebo in clinical studies with second-generation antihistamines but with the exception of cetirizine that has shown significantly more sedation, somnolence and drowsiness than placebo, and may be similar to the first-generation antihistamines.

In view of these drawbacks of currently marketed drugs, it is reasoned that the screening of plant derived products, which combine high selectivity with good oral efficacy and absence of CNS side effects, could constitute a major therapeutic improvement in treatment of allergic diseases (Mainardi & Bielory, 2011).

Catechin, a member of the flavanol group of polyphenols, is the best known biologically active component of green tea; *Camellia sinensis* (Theaceae) and *Acacia catechu* (Leguminosae). Catechin is known as a strong antioxidant. It has long been consumed primarily in Asian countries including China and Japan (Graham, 1992). Consumption of tea in these cultures has been associated with prevention of many diseases including cancer and heart disease and cataracts (Lambert & Yang, 2003). Catechin has also been reported to have protective mechanisms in age-dissociated diseases such as cancer, parkinson's disease, Alzheimer's disease, cardiovascular diseases and diabetes due to their antioxidant properties and free radical scavenging activities (Polidori, 2003; Junqueira et al., 2004). Catechin has been reported to produce inhibition of metastases induced by B16F-10 melanoma cells (Menon et al., 1999).

Catechin is a specific histidine decarboxylase inhibitor, which has shown effectiveness in allergic disorders in several studies carried out (Polidori, 2003). Catechin also significantly reduced gastric tissue histamine levels as effectively as Cemetidine in reducing gastric acid secretion (Parmar, 1984). Catechin being an enzyme inhibitor of histidine decarboxylase enzyme, it acts upon the synthetic pathway of conversion of the amino acid l-histidine to histamine. Histamine is one of the inflammatory mediators which produce various symptoms of allergy such as itching, sneezing, spasm of airways, tissue swelling, etc. Hence, targeting the enzyme would decrease the production of histamine due to enzyme inhibition and produce anti-allergic activity. Similarly, cetirizine, H1 histaminic blocker also produces inhibition of effect by directly blocking the receptors hence reducing the allergic symptoms. Cetirizine has been an excellent drug in allergic conditions; however it has a short duration of action as well as sedative effects. Catechin has a slower onset of action but a longer duration of action and hasn't been reported to produce sedative effects. By the virtue of these properties, a combination of catechin and cetirizine may be beneficial in allergic conjunctivitis and allergic rhinitis. We have carried out evaluation of catechin, cetirizine, and the combination of catechin and cetirizine in the animal models of allergic conjunctivitis and allergic rhinitis.

Ovalbumin induced allergic conjunctivitis leads to symptoms such as redness of the conjunctiva, eyelid edema, and tear discharge. Among the experimental animals used, the guinea pig is often used for allergic conjunctivitis because it is easy to observe the symptoms of allergic conjunctivitis such as hyperemia and edema (Takada et al., 2000; Kamei et al., 1991; Kamei et al., 1995). Histamine is well established as a classical itch-producing substance that can produce sensations of pruritis when applied locally to human skin (Davies et al., 1981). It can therefore be reported that the physical symptoms such as hyperemia and edema observed in sensitized animals is mainly due to the histamine released by an antigen-antibody reaction (Kamei & Minami, 2004). It was observed that increased physical score existed in the disease control group as compared to normal control group. Treatment with catechin, cetirizine and the combination showed significant reduction in the physical score compared to disease control group. This effect may be due to the inhibition of histamine synthesis by catechin and blocking of histamine receptors by cetirizine which also leads to decreased histamine activity hence absence of vasodilatation and release of inflammatory cells into the conjunctivae.

In ovalbumin induced allergic rhinitis model, physical symptoms of itching, sneezing and mucous discharge were observed. It was observed that these symptoms were increased in the disease control group of animals compared to normal control group after sensitization with ovalbumin. Symptoms such as sneezing, itching, congestion, rhinorrhoea and loss of sense of smell are caused by IgE mediated activation of mucosal mast cells that are located on the epithelia of the nasal cavity (Kawabori et al., 1985). The activation of mast cells leads to the release of various mediators such as histamine, leukotrienes, prostaglandins, platelet activating factor and cytokines which in turn recruit other inflammatory cells, trigger release of further inflammatory mediators and stimulate afferent nerves (White, 1990; Naclerio et al., 1991; Sugimoto et al., 1994; Miadonna et al., 1996.). Several investigations have indicated that sneezing and itching symptoms are due to stimulation of histamine H1 receptors on sensory nerve endings (Ohtsuka and Okuda, 1981; Raphael et al., 1989; Shelton and Eiser, 1994; Wang et al., 1997). The catechin, cetirizine and combination treated groups showed decreased physical score as compared to disease

control group. Decreased physical score in catechin treated groups may be due to the inhibition of histidine decarboxylase enzyme and in cetirizine treated group H1 receptor blockaded. The combination group showed beneficial effect by decreased histamine synthesis and H1 blocking activity. The decrease in physical score in treated groups may be due to the inhibition of histamine synthesis or receptor blocked which decreases release of other inflammatory mediators causing inhibition of afferent nerves.

Evaluation of histamine content in mast cells and basophils in allergic conjunctivitis and allergic rhinitis animal models showed an increase in the histamine concentration in disease control group of animals as compared to the normal control group. Treated groups with catechin, cetirizine and combination showed a decrease in histamine concentration as compared to the disease control group. catechin being a histidine decarboxylase inhibitor, it inhibits histamine synthesis hence decreasing the histamine concentration. cetirizine acts as a H1 receptor blocker and it also inhibits histamine activity. The combination of catechin and cetirizine however, acts by both mechanisms of enzyme inhibition as well as receptor blocked producing decreased histamine activity. It has been reported that ovalbumin challenge results in the activation of transcription factor NF-kB and an increase in the expression of NF-kB dependent genes TNF-α and increase in histamine levels (Spilsbury, 2012). Both mast cells and basophils contain histamine. It is well known that mast cells exist in conjunctiva and basophils infiltrate into the conjunctiva in allergic conjunctivitis, therefore high affinity to IgE receptors express on their surface (Hamid et al., 2003; Kamei & Minami, 2004). It is also reported that in ovalbumin induced guinea pig allergic conjunctivitis model, physical irritation induced by ovalbumin was almost inhibited by H1 receptor antagonists; therefore, in present investigation, treatment showed beneficial effect by decrease in histamine release and blockade of H1 receptor.

Histidine decarboxylase enzyme assay was performed in the presence of l-histidine and the conversion to histamine was taken as the indicator which can be measured fluorimetrically. In both allergic conjunctivitis as well as allergic rhinitis animal models, histamine concentration was found to be significantly higher in disease treated group as compared to

the normal control group in the enzyme assay. Catechin treated groups and combination group showed significantly reduced histamine concentration, while cetirizine treated group showed similar histamine concentration as disease control group of animals. Catechin being a histidine decarboxylase enzyme inhibitor, it reduces the synthesis of histamine from l-histidine hence there is decreased histamine concentration. cetirizine however, does not show any enzyme inhibition and it acts by blocking histamine receptors therefore it had no significant decrease in histamine release in the enzyme assay. The combination of catechin and cetirizine showed decrease in histamine concentration due to the presence of catechin which inhibited the histidine decarboxylase enzyme hence decreasing histamine synthesis. Histidine decarboxylase enzyme seems to have a highly restrictive and hydrophobic catalytic site. It only binds to histidine or imidazole containing analogues such as α-fluoromethyl histidine, histidine methyl ester, and natural polyphenols such as catechins by inducing a similar conformation change of the enzyme bound substances (Hayanshi et al., 1993; Olmo et al., 2002; Brtoldi et al., 2002; Rodri-guez-Caso et al., 2003).

Vascular permeability was assessed for, in the allergic conjunctivitis animal model. The evans blue dye concentrated in the conjunctiva was measured spectrophotometrically and results showed that there was increased vascular permeability in the disease control group of animals as compared to the normal control group. Previous studies have shown the presence of mast cells and basophils in the conjunctivae and hence histamine release occurs simultaneously in the conjunctivae (Hamid et al., 2003). The catechin, cetirizine and combination treated groups showed comparatively reduced vascular permeability by measuring the amount of evans blue dye extracted. In disease control group, there is enhanced histamine release due to ovalbumin sensitization. Histamine leads to vasodilatation hence produces an increase in vascular permeability. Therefore, as a measure of the vasodilatation occurring due to histamine release, we have measured the vascular permeability of evans blue dye. Catechin inhibits histamine synthesis by enzyme inhibition hence there is a decrease in vasodilatation which eventually decreases the vascular permeability. Cetirizine blocks H1 histaminic receptors preventing vasodilatation leading to decreased vascular permeability. The combination treated group shows decreased vascular

permeability by the action of two different mechanisms; both histidine decarboxylase enzyme inhibition by catechin as well as H1 receptor blockade by cetirizine.

In allergic rhinitis animal model, vascular permeability was measured for one hour in which samples of the PBS injected were collected every ten minutes at 6 time intervals and ovalbumin solution was injected at period 3. It was observed that the evans blue leakage was increased in disease control group as compared to normal control group. The treated groups with catechin, cetirizine and combination showed significant decrease in evans blue leakage. It was observed that the permeability to the evans blue dye increased abruptly at period 3 during the injection of ovalbumin sensitizing agent and then gradually declined. This curve was observed for all the groups of animals. It has been reported that ovalbumin challenge leads to release of histamine, kinins and leukotrienes C4 and PAF into nasal lavage fluid and increased eosinophil infiltration into nasal mucosa following nasal antigen challenge (Narita et al., 1992; Shirasaki et al., 1992; Shirasaki et al., 1990). Treatment with catechin, cetirizine and the combination showed a decrease in vascular permeability which could be due to decrease in histamine release. catechin acts as a histidine decarboxylase enzyme inhibitor decreasing histamine synthesis while cetirizine blocks H1 histaminic receptors hence decreased histamine activity and a combination works by both mechanism

Histopathology studies of the conjunctivae were performed and it showed significant increase in the ulceration, infiltration of inflammatory cells and edema in the subcutaneous region as compared to the normal control group of animals. This can be due to decrease in corneal epithelial and basement-membrane damage caused by the release of eosinophil-derived major basic and cationic proteins, neurotoxin, peroxidase, and collagenase has been seen in allergic conjunctivitis models. Also, degranulation of mast cells releases preformed heparin, histamine, and neutral proteases, increasing the allergic inflammatory process (Abelson et al., 1993; Bacon et al., 1993). The catechin, cetirizine and combination treated groups showed improvement in the ulceration, infiltration of inflammatory cells and edema compared to disease control animals. This protective effect may be due to inhibition of release of cationic proteins and histamine which increases allergic inflammatory process.

Safety pharmacological evaluation of cetirizine was performed. It showed no significant change in the number of cut offs between the normal control group and the cetirizine treated group. This could be due to the absence of CNS side effect such as sedation hence there was no reduction in the locomotor activity at treatment dose in the animals.

CHAPTER 7

Conclusion

7. CONCLUSION

The a fore going analysis upon the experimental work performed and results obtained suggest that Catechin has significant anti allergic activity in disorders such as allergic conjunctivitis and allergic rhinitis. The combination of Catechin and Cetirizine also has significant anti-allergic activity in allergic conjunctivitis and allergic rhinitis with similar efficacy by improving allergic symptoms, inhibiting histamine release from mast cells as well as basophils, reduced histidine decarboxylase enzyme activity, decrease in vascular permeability and less or no side effects.

CHAPTER 8

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