Chapter I

Introduction

1.1. Introduction

Besides being the primary source of energy in the form of food, plants relate to human life in many ways. They have been utilized as a source of useful chemicals since the dawn of civilization. Plants are the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide with new remedies. Plant based medicines, dispensed in the form of crude drugs now serve as the basis of novel drug discovery.

The use of plants in modern medicine started with the isolation of active compounds, beginning with the isolation of morphine from opium in the early nineteenth century. This was closely followed by the isolation of early drugs such as cocaine, codeine and quinine of which some are still in use. Isolation and characterization of pharmacologically active compounds from medicinal plants continue to this day.

The chance of finding new compounds from the plant kingdom is enormous. Till date, only 1% of the total tropical plants have been studied for their pharmaceutical potential. Drug discovery from plants has evolved to include numerous interdisciplinary fields and various methods of analyses. These involve random selection of plants followed by chemical screening or biological assays, follow up of biological activity reports and follow up of ethno medical use of plants etc.

1.2. Inflammation: Anatomy and Etiology

The inflammatory response represents a generalized response to infection or tissue damage and is designed to remove cellular debris, to localize invading
organisms and arrest the spread of infection. The inflammatory response is characterized by the following symptoms: Reddening of the localized area, swelling, pain and elevated temperature. Reddening results from capillary dialation that allows more blood to flow to the damaged tissue. Elevated temperature results from capillary dialation which permits increased blood flow through these vessels, with associated high metabolic activities of neutrophils and macrophages. The dialation of blood vessels is accompanied by increased capillary permeability causing swelling as fluid accumulates in the spaces surrounding tissue and cells. Pain in the case of inflammation is due to the lysis of blood cells that trigger the production of bradykinin and prostaglandins. The area of inflammation also becomes walled off as a result of the development of fibrinous clots. The deposition of fibrin isolates the inflamed area, cutting off normal circulation. The fluid in the inflamed area is known as inflammatory exudates, commonly called as pus. These exudates contain dead cells and debris in addition to body fluids. After the expulsion of the exudates, the inflammation may terminate and tissues may return to their normal state (Atlas, 1995).

Pain belongs to a basic sensory abnormality associated with inflammation. Pain develops when nerve fiber terminals of polynodal nociceptors become sensitized by mediators of inflammation. The pain producing inflammatory mediators are bradykinin, prostaglandins (PGE$_1$ and PGE$_2$) and leukotrienes, especially LTB$_4$. Pain becomes evoked by the synergistic action of bradykinin and prostaglandins (Antoni, 1991).

1.3. **Acute inflammation**

Acute inflammation is a short-term process which is characterized by the classic signs of inflammation which are: swelling, redness, pain, heat, and
loss of function due to the infiltration of the tissues by plasma and leukocytes. It occurs as long as the injurious stimulus is present and ceases once the stimulus has been removed, broken down, or walled off by scarring (fibrosis). The first four characteristics have been known since ancient times and are attributed to Celsus. Loss of function was added to the definition of inflammation by Virchow in 1870 (Vidya et al., 2001).

The process of acute inflammation is initiated by the blood vessels neighbouring to the injured tissue. It allows the exudation of plasma proteins and leukocytes into the surrounding tissue. The increased flow of fluid into the tissue causes the characteristic swelling associated with inflammation since the lymphatic system does not have the capacity to compensate for it, and the increased blood flow to the area causes the red color and increased heat. The blood vessels also get altered permitting the movement of leukocytes through the endothelium and basal membrane constituting the blood vessel. Once in the tissue, the cells migrate along a chemotactic gradient to reach the site of injury, where they can attempt to remove the stimulus and repair the tissue.

Inflammatory mediators, act in parallel to propagate and mature the inflammatory responses. These include the complement system, coagulation system and fibrinolysis system. Finally, down-regulation of the inflammatory response terminates acute inflammation. Removal of the injurious stimuli halts the response of the inflammatory mechanisms, which require constant stimulation to propagate the process. Additionally, many inflammatory mediators have short half lives and are quickly degraded in the tissue, helping to quickly cease the inflammatory response once the stimulus is removed.
1.4. Chronic inflammation

Chronic inflammation is a pathological condition characterized by concurrent active inflammation, tissue destruction and attempts at repair. Chronic inflammation is not characterized by the classic signs of acute inflammation listed above. Instead, chronically inflamed tissue is characterized by the infiltration of mononuclear immune cells (monocytes, macrophages, lymphocytes, plasma cells) tissue destruction and attempts at healing, which include angiogenesis and fibrosis (Vidya et al., 2001). Endogenous causes include persistent acute inflammation. Exogenous causes are varied and include bacterial infection, especially by *Mycobacterium tuberculosis*, prolonged exposure to chemical agents such as silica, tobacco smoke, or autoimmune reactions such as rheumatoid arthritis. In acute inflammation, removal of the stimulus halts the recruitment of monocytes
(which become macrophages under appropriate activation) into the inflamed tissue, and existing macrophages exit the tissue via lymphatics. However, in chronically inflamed tissue the stimulus is persistent, and therefore recruitment of monocytes is maintained, existing macrophages are tethered in place, and proliferation of macrophages is stimulated.

1.5. Molecular Etiology of inflammation

A. Inflammation mediatory compounds

Inflammation is produced by the release of chemicals, from tissues and migrating cells, induced by various reasons such as injuries. Most strongly implicated chemicals are the prostaglandins (PGs), leukotrienes (LTs), histamine and bradykinin. More recently, platelet-activating factor (PAF) and interleukin-1 also are included. Evidence for their involvement comes from studies with competitive antagonists for their receptors and inhibitors of their synthesis (Nicolaueu and Sorensen, 1996).

B. Eicosanoids

The regulated oxygenation of arachidonic acid leads to a large family of metabolites which have been termed as eicosanoids. The eicosanoids represent a class of molecules termed as “lipid mediators”, because they are the chemical messengers that carry information of cell activation from one cell to another. These cellular messenger molecules have a diverse set of important physiological and pathophysiological roles. They coordinate events between cells, so that proper tissue function can result. These molecules also play a central role in mounting important host defense reactions to protect tissues from adverse conditions, including bacterial infections.
The first step in eicosanoid biosynthesis, common to all the pathways including prostaglandin and leukotriene synthesis is the release of arachidonic acid from its storage site in the membrane phospholipids, by the enzyme Phospholipase A\textsubscript{2} (PLA\textsubscript{2}). The derivatives of the arachidonic acid are known as eicosanoids. The eicosanoids include prostaglandins (PG), thromboxanes (TX), prostacyclins (PGI), leukotrienes (LT), lipoxines and products generated by the action of P450 on fatty acids (Sardesai, 1997).

C. Prostaglandins

Prostaglandins (PG) are carboxylic acids containing twenty carbon atoms. They have the same basic carbon skeleton of the hypothetical parent compound, prostanoic acid. There are several PGs and all have a five membered ring, two aliphatic chains, a terminal -COOH group, a C-13: C-14 double bond and an -OH group on C-15 (Sardesai, 1997). Prostaglandins are like hormones and act as chemical messengers, but do not move to other sites. They work within the cells where they are synthesized. They are biochemically synthesized from the fatty acid, arachidonic acid. The synthesis is carried out by a membrane bound PG synthase complex, which has two components: the cyclo-oxygenase which catalyzes the cyclisation of C-8 and C-12 and peroxidase component which completes the formation of PG (Sardesai, 1997). The unique shape of the arachidonic acid is caused by a series of cis-double bonds helping to put it into position to make the five member ring. On the five membered rings there may also be double bonds, a ketone, or alcohol groups (Komoto \textit{et al.}, 2004). Arachidonic acid is produced by the action of the enzyme PLA\textsubscript{2} on phospholipids. Prostaglandins (PG) are produced by the action of cyclooxygenase (COX) enzymes on arachidonic acid (Komoto \textit{et al.}, 2006). PGs activate the inflammatory response, production of pain, and fever. Once formed, PGH is
acted upon by a series of enzymes that produce biologically active PG, TX and PGI (Samuelson and Bengt, 2001). All the metabolites of PGH are called prostanoids. PGH is converted to PGE, PGF or PGD by their individual isomerases (Sardesai, 1997). When tissues are damaged, white blood cells flood to the site to try to minimize tissue destruction; prostaglandins are produced as a result. Receptors mediating the action of PG and other prostanoids were recently identified (Sardesai, 1997). They are G protein coupled receptors with seven transmembrane domains. PGs are rapidly metabolized by a variety of tissues to compounds with little or no biological activity.

D. Leukotrienes

Leukotrienes (LT) are naturally produced eicosanoid lipid mediators, which may be responsible for the effects of an inflammatory response (Bailey and Martyn, 1985). The name “Leukotriene” was introduced by the Swedish biochemist B. Samuelson, from the words leukocyte and three conjugated double bonds. The leukotrienes are formed by the transformation of Arachidonic acid into an unstable epoxide intermediate, Leukotriene A₄ (LTA₄) which is converted to LTB₄ by hydration. It gets converted to LTC₄ by the addition of glutathione. This LTC₄ is metabolized to LTD₄ and LTE₄ by the successive elimination of gamma glutamyl residue and glycine (Samuelson, 1983). LTB₄ is an important mediator of inflammation. It is a potent chemotaxin for neutrophils and increases leukocyte adhesion to the blood vessel walls (Samuelson et al., 1987). Both LTs and PAF share a number of common features. Both are lipid mediators produced through the arachidonic acid pathway. Both are synthesized and released from the mast cells and basophils following allergen challenge (Agarwal and Townley, 2000).
E. Histamine
Histamine is an important compound involved in many allergic reactions. It is formed by the decarboxylation of histidine. Allergies are caused by an immune response to a normally innocuous substance (i.e. pollen, dust) that comes in contact with lymphocytes specific for that substance, or antigen. In many cases, the lymphocyte triggered to respond is a mast cell. For this response to occur, a free-floating IgE (an immunoglobulin associated with allergic response) molecule specific to the antigen must first be attached to cell surface receptors on mast cells. Antigen binding to the mast cell-attached IgE then triggers the mast cell to respond. This response often includes the release of histamine. Histamine can cause inflammation directly as well as indirectly. Upon release of histamine by an antigen activated mast cell, permeability of vessels near the site is increased. Thus, blood fluids (including leukocytes, which participate in immune responses) enter the area causing swelling. This is accomplished due to the ability of histamine to induce phosphorylation of an intercellular adhesion protein called VE-cadherin found on vascular endothelial cells (Andriopoulou et al., 1999). That is how histamine is vasoactive. Gaps between the cells in vascular tissue are created by this phosphorylation, allowing blood fluids to seep out into extracellular space. Indirectly, histamine contributes to inflammation by affecting the functions of other leukocytes in the area. It has been suggested that histamine release triggers the release of cytokines and some inflammatory mediators (Dendofer et al., 2001). These chemicals in turn increase the inflammatory response.

F. Bradykinin
Bradykinin is a biologically active oligopeptide composed of nine amino acids, R P P G F S P F R. It is synthesized from a precursor protein kinninogen by the
enzyme kallikrein. It is a potent endothelium dependent vasodilator, causing the contraction of nonvascular smooth muscles that increases vascular permeability. It is also involved in the mechanism of pain. It raises the internal calcium level in neocortical astrocytes.

Bradykinin receptor is expressed only as a result of tissue injury and is presumed to play a role in the inflammatory process. Bromelain, a proteolytic enzyme from pineapple suppresses trauma induced swelling caused by the release of bradykinin into the bloodstream and tissues (Winter, 1990). Some other bradykinin inhibitors are found in Aloe species (Perez, Cobos and Cruz, 2004) and polyphenols found in wine and green tea (Richard et al., 2003). Bradykinin interacts with a variety of cells producing a broad series of biological responses. It activates two distinct membrane receptors, namely B₁ and B₂ receptors. The B₂ receptor is the classic bradykinin receptor that selectively binds bradykinin and kallidin and is constitutively present in most tissues. The B₁ receptor binds to kinin metabolites and is generally less abundant than B₂ receptor. (Willes and Coggeshall, 2004). Unlike the B₂ receptor which is constitutively expressed, the B₁ receptor is inducible and is involved in the sensitization of the peripheral nociceptors. Induction and binding of B₁ receptor can also lead to the production of pro-inflammatory mediators, including tumour necrosis factor α and Interleukin 1-β (Audette and Bailey, 2008).

Both B₁ and B₂ receptors activate the phospholipase C pathway leading to the metabolism of membrane phospholipid, phosphatidylinositol bis phosphate (PIP₂), and the release of two resulting fragments, inositol triphosphate (IP₃) and diacylglycerol (DAG). IP₃ interacts with IP₃ receptors to release calcium. DAG activates protein kinase C, which in turn phosphorylates a number of cellular targets. This pathway is important in sensitizing nociceptive neurons to other painful stimuli (Cesare, Gilabert and McNaughton, 1999).
G. Platelet-activating factor

Platelet-activating factor (PAF), also known AGEPC (acetyl-glyceryl-ether-phosphorylcholine) is a potent phospholipid activator and mediator of many leukocyte functions, including platelet aggregation, inflammation and anaphylaxis (Benveniste, 1974). PAF was the first intact phospholipid known to have messenger functions, in which the signalling results from the binding of the molecule. PAF is synthesized by a variety of cells, such as platelets, neutrophils, monocytes and macrophages. It is synthesized by a distinct membrane bound acetyl transferase which catalyzes the transfer of an acetyl residue from Acetyl CoA to lyso-PAF, generated by the action of PLA₂ on phosphatidylcholine. PAF is synthesized by the cells at low level, but much greater quantities are produced by inflammatory cells, when required in response to cell specific stimuli. The primary role of PAF is to mediate intercellular interactions. By binding to its specific receptor, PAF activates the cytoplasmic PLA₂ and phospholipase C. PAF has a number of pro inflammatory properties and in excess it has been implicated in the pathogenesis of a number of disease states, ranging from allergic reactions to stroke, myocardial infarction, colitis and atherosclerosis (Zen et al., 2002).

The administration of PAF can produce many of the symptoms observed in asthma, probably via the formation of leukotrienes as secondary mediators (Prescott et al., 2000). It is an important mediator of anaphylaxis in animals and interventions that block PAF, such as PAF acetylhydrolase and prevent fatal anaphylaxis (Vadas et al., 2008). The control of PAF is brought about partly by the tight regulation of its synthesis and partly by the action of specific acetylhydrolases, which remove the acetyl group from PAF, thus eliminating its biological activity (Snyder, 1995).
1.6. The management of inflammation

The treatment of patients with inflammation involves two primary goals
1. The relief of pain, which is often the presenting symptom.
2. The slowing or arrest of the tissue damaging process.

1.6.1. Classes of anti-inflammatory drugs

A number of compounds are known to interact with one or more of the
many steps involved in the formation of eicosanoids. The first step is the
formation of arachidonic acid by PLA₂. The steroidal anti-inflammatory
agents such as corticosteroids block PLA₂. Eg: dexamethasone, 
prednisolone, cortisol, corticosterone.
The nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, 
indomethacin and ibuprofen are potent inhibitors of cyclooxygenase (COX).
They block the synthesis of PGG and hence PGS, TX and PGI. Aspirin acts
by acetylating a serine residue in the active site of COX and irreversibly inhibits the enzyme. Other NSAIDs act reversibly to COX. Aspirin and other NSAIDs do not inhibit 5-Lipoxygenase and may facilitate the LT formation by making more of arachidonic acid available for metabolism (Sardesai, 1997). Three types of drugs are used presently for arresting the inflammatory process:

1.6.2. Steroidal drugs
Introduced in the 1950s, cortisone like drugs, called corticosteroids or glucocorticoids are synthetic mimics of stress response hormones produced in the adrenal glands. The best known corticosteroid is prednisone. When administered therapeutically, glucocorticoids have powerful anti-inflammatory and immunosuppressive effects. They inhibit both the early and late manifestations of inflammation. They affect all types of inflammatory reactions whether caused by invading pathogens, chemical or physical stimuli or by immune response. They are usually prescribed for autoimmune diseases such as rheumatoid arthritis, asthma and multiple sclerosis.

A. Mechanism of action
Glucocorticoids interact with intracellular receptors. The resulting steroid-receptor complexes dimerise and interact with DNA to modify gene transcription. Anti-inflammatory activity is due to the inhibition of transcription of genes for COX 2, cytokines, cell adhesion molecules and inducible NO synthase. Corticosteroids can be of great value when used to treat certain conditions, but they carry the hazard that they also suppress the necessary protective responses to infection and can decrease essential healing process.
B. Pharmaco kinetics
Most of these drugs are active when given orally. All of them can be administered systemically, either intramuscularly or intravenous route. The endogeneous glucocorticoids are carried in the plasma, bound to corticosteroid binding globulin and to albumin. They, being lipophilic molecules, enter their target cells by simple diffusion. Cortisone and prednisone are inactive until converted in vivo to hydrocortisone and prednisolone.

C. Structure-Activity correlations
Structure-Activity relationships of glucocorticoids are based on two natural hormones, cortisol and corticosterone. The characteristic structural features of these compounds are a conjugated 3-Ketone, 11-OH group and 17β Ketol side chain. Molecular modifications have been aimed at deriving compounds with glucocorticoid and anti inflammatory activity, but lacking in minaralocorticoid and other side effects. It has been noted that introduction of methyl group, double bonds and halogen substituents have a positive effect on activity (Nogrady, 1988).

D. Side effects
Prednisone causes a rounded “moon face” upon prolonged administration. An increase in abdominal obesity is also seen. Due to the dampening of immune response, they increase suscpctibility to infections. Corticosteroids also interfere with metabolism of key nutrients such as folic acid, vitamin B₆, B₁₂, potassium and zinc. They prevent bone development in young persons. Other common side effects include thinning of skin, high blood pressure, elevated blood sugar, cataracts, glucoma, male infertility and loss of muscle mass (Rang et al., 2003 & Challem, 2003).
1.6.3. Non Steroidal Anti-inflammatory Drugs (NSAIDs)

The origin of NSAIDs dates back to 1763, when sodium salicylate was introduced. The acetyl derivative of salicylic acid, called aspirin was used subsequently. Phenylbutazone, an indole acetic acid derivative was used from 1950s. This was the first nonsalicylate NSAID used. But due to bone marrow toxicity, it was abandoned. Indomethacin, another indole acetic acid derivative was developed in 1960s as a substitute for phenylbutazone.

Most NSAIDs are weak organic acids. Once absorbed, they get bound to serum albumin. Due to increased vascular permeability in localised sites of inflammation, this high degree of protein binding may result in the delivery of higher levels of NSAIDs. They are a chemically diverse group. A structural classification is given in table below (Higgins and Synder, 2006).

<table>
<thead>
<tr>
<th>No</th>
<th>Chemical Class</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Salicylates</td>
<td>Aspirin, sodium salicylate.</td>
</tr>
<tr>
<td>2</td>
<td>Quinolines</td>
<td>Cinchophen</td>
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<tr>
<td>3</td>
<td>2-aryl propionic acids</td>
<td>Carprofen, Ibuprofen, Naproxen, Ketoprofen</td>
</tr>
<tr>
<td>4</td>
<td>Anthranilic acids</td>
<td>Flunixin, Meclofenamic acid, Tolfenamic acid</td>
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<td>5</td>
<td>Indolines</td>
<td>Indomethacin, Eltenac, Tepoxalin</td>
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<td>6</td>
<td>Pyrazolones</td>
<td>Phenylbutazone, Oxyphenylbutazone</td>
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<tr>
<td>7</td>
<td>Oxicams</td>
<td>Meloxicam, Piromoxam</td>
</tr>
<tr>
<td>8</td>
<td>Sulphonamide derivatives</td>
<td>Nimesulide.</td>
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</table>

Table 1.1. The structural classification of NSAIDs.

The primary effect of NSAIDs is to inhibit COX enzyme, thereby blocking the transformation of arachidonic acid to prostaglandins, prostacyclin and
thromboxanes. These result in complex effects on vascular permeability and platelet aggregation, undoubtfully contributing to the overall clinical effects of these compounds.

COX 1, or prostaglandin synthase H, is a house keeping enzyme that regulates normal cellular functions and is stimulated by hormones and growth factors. It is constitutively expressed in most tissues and is inhibited by NSAIDs in varying degrees. COX 1 is important in maintaining the integrity of the gastric and duodenal mucosa and many of the side effects of NSAIDs on the gastrointestinal tract are attributed to its inhibition (Furst and Ulrich, 2007).

COX 2 or prostaglandin synthase H\textsubscript{2} is an inducible enzyme and is usually not detectable in most tissues. Its expression is increased during states of inflammation or experimentally in response to mitogenic stimuli. Its expression is inhibited by glucocorticoids. COX-2 is also inhibited by all of the presently used NSAIDs, to a lesser or greater degree. Thus, differences in the effectiveness with which a particular NSAID inhibits an isoform of COX may affect with its activity and its potential toxicity. It has been proposed that the ideal NSAID would inhibit the inducible COX-2 alone, without having any effect on COX-1 (Tsokos, 2002).

**A. Pharmocokinetics of NSAIDs**

In general, NSAIDs are absorbed almost completely from the gastrointestinal tract; tend not to undergo pre-systemic elimination. They are highly bound to plasma albumin. Their $t_{1/2}$ ranges from 1 to 60 hours. The vast majority of NSAIDs are weakly acidic drugs that localize in the synovial tissue of inflamed joints (Lawrence, Bennett and Brown, 1997).
B. Physiological effects

1. Analgesic action: The analgesic action of NSAIDs is exerted both peripherally and centrally. But peripheral actions predominate. Their analgesic action is usually associated with their anti inflammatory action and results from the inhibition of prostaglandin synthesis in the inflamed tissues.

2. Anti inflammatory action: The role of prostaglandins in inflammation is to produce vasodilation and increased vascular permeability. Inhibition of PG synthesis by NSAIDs attenuates rather than abolishes inflammation, because these drugs do not inhibit other mediators of inflammation. They do not alter the course of the disease responsible for inflammation.

3. Anti pyretic action: During fever, endogenous pyrogen called IL-1 is released from leukocytes and acts directly on the thermoregulatory centre in the hypothalamus to increase body temperature. This effect is associated with a rise in brain prostaglandins. Aspirin prevents the temperature raising effects of IL-1 by preventing the rise in brain prostaglandin levels.

C. Mechanism of COX inhibition

NSAIDs inhibit COX by several mechanisms. Aspirin acetylates a serine residue of the constitutive form of the enzyme, causing irreversible inhibition. This results from the steric hindrance of the access of the substrate to the oxygenase active site. In contrast, other NSAIDs, including salicylates are reversible competitive inhibitors of COX. The COX enzymes are bifunctional, having two distinct activities. The main action which gives PGG₂ and a peroxidase action which converts PGG₂ to PGH₂. Both COX-1 and COX-2 inhibitors block only the cyclo-oxygenation function. Both COX-1 and COX-2 are associated with membranes and the active site consists of a long channel with a bend at its end. The channel is wider in COX-2.
The traditional NSAIDs block both the enzymes halfway down the channel by hydrogen bonding to a polar residue, R120. Ion pairing of the carboxylic group of the inhibitor with R120 is also possible (Rowlinson et al., 2003). Most of them act reversibly, mainly by excluding arachidonate. But aspirin binds to and acetylates S530 causing irreversible inhibition. The crucial difference between the two COX enzymes is at position 523. Here, COX-1 has a bulky isoleucine, while COX-2 has valine which is smaller. This leaves a gap which gives access to a side pocket, which is the binding site for COX-2 selective agents. These COX-2 inhibitors are too bulky to fit into the COX-1 channel.

Crystal structure studies of the complex of diclofenac with murine COX-2 demonstrated that the ligand binds to COX-2 in an inverted conformation with its carboxylate group hydrogen bonded to T385 and S530. This has proved that carboxylate group of an acidic NSAID can bind to COX in an orientation that precludes the formation of a salt bridge with R120 (Rowlinson et al., 2003)

D. Adverse effects of NSAIDs

Side effects of NSAIDs are common, partly because the drugs may be given in high doses for a long time and partly they are widely used in elderly people who are more susceptible to side effects.

1. Gastro Intestinal tract: Damage to the mucosa of the GI tract seems to be mainly a consequence of the inhibition of PG synthesis, rather than a directly erosive action of the drug. Prostaglandins such as PGE2 and PGI2 inhibit gastric acid secretion, increase blood flow through gastric mucosa and have a cytoprotective role. By inhibiting PG formation, NSAIDs may cause ulceration by producing mucosal ischaemia and by impairing the
protective mucus barrier, thus exposing the mucosa to the damaging effect of acid.

2. Nephrotoxicity: Prostaglandins PGE$_2$ and PGI$_2$ are powerful vasodilators synthesized in the renal medulla and glomeruli respectively. They are involved in the control of renal blood flow and excretion of salt and water. Inhibition of renal PG synthesis may result in sodium retention, reduced renal blood flow and renal failure. In addition, NSAIDs may cause interstitial nephritis and hyperkalemia. Prolonged abuse of these drugs causes capillary necrosis and chronic renal failure (Neal, 2002).

3. Cardiovascular effects: NSAIDs can adversely affect cardiovascular function in many ways. In particular, they can cause or aggravate hypertension and interact negatively with anti hypertensive drugs. This problem is clinically relevant, since hypertension is common and is a major determinant of cardiovascular diseases, while NSAIDs are among the most commonly prescribed drugs (Favero, 2004).

1.7. Herbal Ayurvedic remedies for inflammation
The disease preventive and health promotive approach of ayurveda, which takes into consideration the whole body, mind and spirit while dealing with the maintenance of health, promotion of health and treating ailments is holistic and finds increasing acceptability worldwide.

According to ayurveda, most diseases connected with psycho-physiologic and pathologic changes in the body are caused by the imbalance of the three different doshas, namely, Vatha, pitta and kapha. The fundamental aim of ayurvedic therapy is to restore the balance between these three major body systems. Any imbalance can lead to inflammation called as ‘sopha’. Almost
seven types of inflammations have been described in ayurveda. The ayurvedic definition of pittaja sopha encompasses the modern concept of inflammation, which is defined as redness, pain, heat, loss of function and swelling (Garodia et al., 2007).

Several plants show anti inflammatory activity and are used in ayurvedic system of medicine, either singly or as mixtures. Many of them contain phytochemicals which inhibit PLA$_2$, COX etc which is responsible for their activity. Fermentative process is often employed for the preparation of ayurvedic medicines. This improves the palatability and prolongs the shelf life of the medicine due to self generated alcohol. It is not clear whether the fermentation improves the medicinal property of phytochemicals. Despite the complex structure of many phytochemicals such as alkaloids, microbes have the ability to transform or degrade them. Often, a small change in their structure causes an enormous change in potency or activity. The introduction of a hydroxyl group at fourteenth carbon of codeine dramatically increases the analgesic potency of codeine. The product is called 14β- hydroxy codeine. This biotransformation is brought about by *Pseudomonas putida* (Rathbone and Bruce, 2002). Some of the phytochemicals in plants which interfere with enzymes involved in the inflammatory pathway are given in the Table 1.2 (Majumdar, Govil and Singh, 2003).
<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Chemical Class</th>
<th>Biochemical target</th>
<th>Plant Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutacarpine</td>
<td>Alkaloid</td>
<td>COX-2</td>
<td>Erodia rutacarpea</td>
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<tr>
<td>Baicalein</td>
<td>Flavone</td>
<td>LOX</td>
<td>Scutellaria sps</td>
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<td>Brevifolin</td>
<td>Phenolic ketone</td>
<td>5-LOX, COX</td>
<td>Artemisia sps.</td>
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<td>Eugenol</td>
<td>Phenol</td>
<td>COX, PLA2</td>
<td>Eugenia sps.</td>
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<td>Gossypin</td>
<td>Flavanol</td>
<td>12-LOX</td>
<td>Gossypium indicum.</td>
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<td>Isoliquiritigen</td>
<td>Chalcone</td>
<td>COX, LOX</td>
<td>Glycyrrhiza glabra.</td>
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<td>Salicylic acid</td>
<td>Phenolic acid</td>
<td>COX-1, COX-2</td>
<td>Gaultheria procumbens.</td>
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<td>Linolenic acid</td>
<td>Unsaturated acid</td>
<td>5-LOX</td>
<td>Linum sps.</td>
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</table>

Table 1.2. Examples of anti inflammatory phytochemicals.

Triphala, an ayurvedic combination of three plant drugs has promising anti inflammatory activity comparable to NSAID indomethacin (Rasool and Sabina, 2007).

Muktasukthe bhasma, a compound formulation consisting of pearl, Aloe vera and vinegar inhibited acute and sub acute inflammation in albino rats induced by carrageenan. It was found to be a third as potent as acetylsalicylic acid (Mishra, 2004).

The water soluble portion of alcoholic extract of Nyctanthes arbartristis inhibited acute inflammatory edema produced by carrageenan, formalin, histamine and turpentine oil in the hind-paw of rats. It also inhibited inflammation in adjuvant induced arthritic models. In sub acute models, this extract was found to check granulation in tissue formation significantly in granuloma pouch and cotton pellet test. Acute and chronic phases of
formaldehyde induced arthritis were significantly reduced (Mishra, 2004). Similarly, the chloroformic extract of *Semicarpus anacardium* nut was found to significantly reduce acute inflammation and was also active against the secondary lesions of adjuvant induced arthritis in rats (Mishra, 2004).

The anti inflammatory activity of ethanolic and acetone extracts of *Bahunia varigata* was investigated. Carrageenan induced rat paw edema and cotton pellet granuloma in albino rats. Acetone extract showed higher activity (Seshadri *et al.*, 2009).

Turmeric, (*Curcuma longa*) appears to modulate inflammation via downregulation of NF-κB and subsequent suppression of COX-2, 5-LOX, TNF-α, IL-1β, IL-6, IL-8 etc. (Khanna *et al.*, 2007). It can be compared favorably to ibuprofen and phenylbutazone (Deodhar, Sethi and Srimal 1980). The lack of negative side effects, especially gastric distress makes it attractive as a therapeutic choice (Khanna *et al.*, 2007).

Ginger (*Zingiber officinale*) contains 6-shogaol which appears to be one of the multiple chemicals responsible for its anti inflammatory activity (Levy *et al.*, 2006). Ginger has also been demonstrated reduction in pain and improvement in mobility in humans (Srivastav and Musthafa, 1989).

Guggul, (*Commifera mukul*) use correlates with significant reduction of pain, stiffness and improved function without side effects in subjective and objective measures for older people with osteoarthritis (Singh *et al.*, 2001).

### 1.8. Naturopathic management of inflammation

The nature cure school regards germs not as a primary cause, but as a secondary manifestation of disease. Naturopaths base their contention that inflammation is a healing process, that all acute disorders, being merely the
efforts of nature for cleansing the system. These are self limiting and self curative, if inflammation is left unchecked until the cleansing process is completed. Nature cure practitioners realize of course that there is a danger point beyond which inflammation and fever may not go with impunity, and that their efforts are to be directed only to keep these processes within safe limits, never to suppress them nor even suddenly check them, until they have run their natural course to a healing climax and abatement.

This is the basic and all important difference between naturopathic and allopathic conception of disease. What allopath calls disease, meaning thereby the superficial signs of trouble, the naturopath regards these as evidence of nature’s efforts to cure. A cleansing or healing diet may be prescribed to eliminate the toxins. Hydrotherapy may be applied to stimulate metabolism and circulation and reduce inflammation. Massage may be applied to provide similar effects. Spinal manipulative treatment may be employed to remove congestion and obstructions to the free flow of blood and nerve force into the organs and tissues (Stanley Lief, 2010).

1.9. Homoeopathic treatment

Homoeopathy is a form of alternative medicine that attempts to treat patients with highly diluted preparations. Preparations which cause certain symptoms in healthy individuals are given as the treatment for patients exhibiting similar symptoms. Most of the homoeopathic remedies are derived from plants, minerals and animal products. By principle, substances eliciting inflammatory response such as poison ivy (Rhus toxicodendron) are used in medicine against inflammation.
1.10. Need for developing new anti inflammatory drugs

Most of the currently used anti inflammatory drugs either steroidal or non steroidal have undesirable side effects ranging from gastrointestinal irritation to cardiovascular effects. So, natural compounds derived from plants or other sources are especially important to be developed into anti inflammatory drugs. More diverse chemical structures are found in plants which would be absolutely specific in inhibiting a particular enzyme. Such compounds may contain chemical groups susceptible to chemical or biological transformation. Their derivatives may exhibit novel properties which may be advantageous in the pharmacological view.

1.11. Aims and Objectives of this study

A. To identify the chemical substance responsible for the inhibition of PLA2, hence, acting as anti-inflammatory agent in Cardiospermum halicacabum (Balloon vine), which is known to be anti-inflammatory in traditional medicine.

B. To enhance the inhibition of PLA2 by biotransformation of the anti inflammatory compound of A

C. To assess the PLA2 inhibitory activity of the biotransformed derivatives formed.