Chapter 1

Introduction
1.1 Inflammation

Inflammation, a protective, pathophysiological reaction of the body to injury or to infections, allergy, or chemical irritants, is one of the common clinical presentations in most of the diseases / pathological states. Inflammatory condition of almost every organ has been described in the literature. However it occurs *per se* as well (Lee and Katayama, 1992). Although inflammation is the body's protective response to an injury, but if this response goes unchecked, it can end up doing more harm than good, which is what happens in a variety of inflammatory disorders. Inflammatory diseases are caused by abnormal immune responses such as immune system turning on itself to attack the very tissues it has evolved to protect. Seemingly unrelated disorders such as asthma, multiple sclerosis, inflammatory bowel diseases and rheumatoid arthritis all have common inflammatory elements that underlie the disease process. These cover a broad spectrum of conditions, including different types of chronic rheumatic diseases that are the major cause of morbidity all over the country. Common examples include:

- **Acute inflammatory conditions** – Acute appendicitis, conjunctivitis, glaucoma (close angle), rhinitis, skin reaction to a scratch or burn or insect bite or bruise, sore throat, stomatitis.

- **Chronic inflammatory disorders** – Alzheimer's disease, bronchiectasis, chronic obstructive pulmonary disease, glaucoma (open angle), glomerulonephritis, implanted foreign body in wound, inflammatory bowel disease (Crohn's disease), multiple sclerosis, pleurisy, pneumonia, rheumatic heart disease, rheumatoid arthritis, tuberculosis.

Although inflammation is the unifying factor, the specific treatment approach required for each type of inflammatory disease may be unique to that patient population. Many of the therapies available today are palliative rather than curative, directed to the symptoms rather than to the underlying causes of inflammation. Each inflammatory disease has distinct therapeutic needs that are not adequately served by current prevention and treatment strategies.
The search of screening and development of drugs for anti-inflammatory activity is an unending problem. There is much hope of finding active anti-inflammatory compounds from indigenous plants as these are still used in therapeutics despite the progress made in conventional chemistry and pharmacology for producing effective drugs (Farnsworth and Bingel, 1977).

Inflammation is body's beneficial, protective reaction to injury. In fact, it normally leads to removal of the inciting agent and repair of the injured site. This may involve temporary discomfort and loss of function, but in the end it is protective. Nevertheless, a large number of human diseases represent uncontrolled inflammatory reactions that may continue unchecked (rheumatoid arthritis), may induce permanent tissue destruction (emphysema) or may heal, but only by the appropriate deposition of collagen (Pulmonary or hepatic fibrosis) (Gary and Peter, 1983).

The systemic local effects of acute inflammation are usually clearly beneficial, for example the destruction of invading microorganisms; but at other times they appear to serve no obvious function, or may even be positively harmful.

Beneficial effects of inflammation
Both the fluid and cellular exudates may have useful effects; dilution of toxins, entry of antibodies, drug transport, fibrin formation that may impede the movement of micro-organisms and facilitating phagocytosis, delivery of nutrients and oxygen, stimulation of immune response by carrying soluble antigens through lymph to reach the local lymph nodes to stimulate the immune response.

Harmful effects of inflammation
The release of lysosomal enzymes by inflammatory cells may produce harmful effects also, such as; digestion of normal tissues, vascular damage, hypersensitivity reactions and swelling. Certain inflammatory responses like those seen in type I hypersensitivity reactions may be life threatening.
Even though inflammation is a protective mechanism, chronic inflammation breaks down the body and makes it older and more frail. The inflammatory process is both variable and extremely complex. The vascular response to injury is a common theme for inflammatory process, and it is variable and may also be reversible. The amount of variability and reversibility depends more upon the severity of injury than the kind of injury. Each site and stimulus may result in different mix, a different time course, and a different outcome. The inflammatory process is also influenced by the nutritional and hormonal status of the individual, as well as by genetic factors (Henry and Robert, 1989).

Early events in inflammation
When living tissues are injured, a characteristic series of changes follow in the small vessels. The reaction in the first few hours is more or less independent of the nature of the noxious agent, the response being very similar after widely diverse types of injury.

More than 100 years ago, Addison, Waller, Cohnheim and others described in detail the essential features of the early stages of inflammation as seen in living tissue (Smith, 1978).
1. Changes in the caliber of and flow in small blood vessels.
2. Increased vascular permeability, which leads to the formation of protein rich exudate and local edema. There is a consequential increase in the volume and protein content of the lymph that drains from the injured area.
3. Escape of leukocytes from circulating blood into extra-vascular tissues. Erythrocytes may accompany the emigrating white blood cells. In milder injuries only a few red cells escape but after more severe stimuli there may be gross hemorrhage into the damaged tissues.

Once this initial reaction to injury has developed, subsequent changes within the area of damaged tissue depend upon the severity, nature and duration of action of the injurious agent. If this is of brief duration or is rapidly and successfully overcome by the defense mechanisms of the host, the inflammatory changes will either resolve completely or subside leaving a variable amount of scar tissue.
within the injured area. However, many irritant stimuli are of much longer duration and tissue injury may continue beyond the period necessary for full development of the initial stages of the inflammatory reaction. In these circumstances the later changes within the injured area depend upon the nature of the noxious agent. Some types of persistent stimulus lead to massive continued polymorph migration and to suppuration (i.e. pus formation) within the damaged area; with other types of long-acting stimulus the initial stages of inflammation are succeeded by a chronic granulomatous reaction.

As reviewed by Gary and Peter (1983), several mediators are involved in inflammatory process. Inflammation is accompanied by pain, edema, erythema and fever. The mechanisms of inflammatory process are complex and are controlled by the presence of group of substances called chemical mediators, each with a specific role at some definite stage of the inflammatory reaction. These mediators may be endogenous or exogenous in origin.

The inflammatory mediators include histamine, 5-hydroxytryptamine, slow reacting substance in anaphylaxis (SRS-A), bradykinin, lymphokines, complement, cyclooxygenase products (PGs), lipo-oxygenase products (LTs) etc. Some mediators may play a more prominent role than others in particular types of inflammation. For example, histamine is important in the type of response seen in urticaria but not in the rheumatoid arthritis (and H₁ receptor antagonists (anti-histaminics) are effective in the former but not later). Recently it has been understood that prostaglandins are intimately concerned with the inflammatory process and sensitize tissue and lower the pain threshold. The recognition of different mediators for different phases may have important implications for interpreting the effect of particular drugs.

Histamine: histamine is important in the initiation of the early phases of acute inflammation as it mediates the monophasic response of increased vascular permeability. It increases vascular permeability by causing endothelial cells to
contract, thus producing intercellular gaps. Its action is transient because it is rapidly inactivated.

Similarly the kinins are thought to be important in the early stages of the delayed phase of vascular permeability. They include peptides such as bradykinin and lysl-bradykinin. These are vasoactive compounds and induce arteriolar dilatation, increase permeability, increase gaps between endothelial cells.

Slow reacting substance of anaphylaxis (SRS-A) which is activated in mast cells during anaphylactic reactions is a mixture of three leukotrienes: LTC₄, LTD₄ and LTE₄.

In asthma and other allergic reactions, SRS-A (LTC₄, LTD₄, LTE₄) is an important mediator, as it can cause bronchospasm and stimulate mucous secretion as well as increase vascular permeability (it is more than 100 times as potent than histamine in causing bronchoconstriction).

In acute inflammatory reactions in which complement is involved, activation of C₃ of the Complement System is the most important event.

Polymorphonuclear neutrophils (PMNs): In acute inflammation these are the first leukocytes to emerge from the vessels in significant numbers.

1.2 Free Radicals in Inflammation

Superoxide (O²⁻) and hydroxyl (OH⁻) radicals are thought to be generated to subsequently initiate tissue destruction during both acute and chronic phases of inflammation. This superoxide radical is extremely reactive acts as a weak base and may act as an oxidizing agent. These hydroxyl and superoxide radicals considered the prime bioactive oxygen radical species, cause amplification of the inflammatory response by

1) lipid peroxidation with associated membrane dysfunction
2) formation of potent chemotactic factors derived from neutrophils
3) Increased microvascular damage and enhanced polymorph endothelial cell adhesion
4) Labilization of lysosomal membranes with possible consequences in release of phospholipases that causes arachidonic acid release from phospholipids and subsequent formation of inflammatory prostanoids and leukotrienes.

5) Depolymerisation of connective tissue hyaluronic acid, collagen, and other components of synovial fluid.

6) Enhancement of the release of anaphylactic mediators.

7) Scission of polymeric DNA

8) Suppression of the release of serum protease inhibitors.

Active oxygen species such as superoxide radicals and hydroxyl radicals play an important role in the inflammation process after intoxication by carrageenin. These radicals react with cell membranes, induce lipid peroxidation and have been implicated as important pathologic mediators in many clinical disorders, which have been widely investigated (Ginsburg and Kohen, 1995).

Cells that play an important part in the inflammatory response are; mast cells, eosinophils, lymphocytes, epithelial cells, platelets and polymorphonuclear leukocytes. Each of these cell types can contribute mediators and cytokines to initiate and amplify both acute inflammation and the long-term pathologic changes described as pathophysiology of inflammation. The mediators released – histamine, bradykinin, the leukotrienes C, D, and E, platelet-activating factor, and prostaglandins E₂, E₂α and D₂. The epithelial cells are both the target of, and a contributor to, the inflammatory cascade (Haynes and Fauci, 2003).

1.3 \( \gamma \) GTP

The enzyme gamma glutamyl transpeptidase (\( \gamma \) GTP) is a membrane bound enzyme. It catalyses the transfer of \( \gamma \) - glutamyl groups from \( \gamma \) - glutamylpeptides to other peptides, to L- amino acids and to water. \( \gamma \)-GTP is a sensitive marker for liver disease. However, elevation is not confined to single disease category. In the
majority of liver disorders the incidence of serum $\gamma$-GTP elevation approaches to maximum levels, the test is therefore useful in screening for liver disease. It has been postulated now, that the determination of $\gamma$-GTP is an additional liver function test. This is inspite of the fact that the concentration of $\gamma$-GTP in kidney has been shown to be approximately ten times that found in liver. It may also be raised in pancreatitis and myocardial infarction. Grossly elevated $\gamma$-GTP values found in the absence of jaundice are reported as being an indication of liver metastases (Szasz, 1976; Hanign et al., 1994).

1.4 Control of Inflammation

The following are examples of control mechanisms:

1. Rapid enzymatic destruction of activators (e.g., histaminase degrades histamine, kininases degrades bradykinin, superoxide dismutase degrades superoxide anion)

2. Natural inhibitors of inflammatory mediators.

3. Relative balance between intracellular levels of cyclic AMP and cyclic GMP may influence the intensity of inflammatory reactions. An increase in cyclic AMP tend to intensify the response, cyclic GMP dampens it. Prostaglandins E1 and E2 and catecholamines are cyclic AMP stimulants.

4. Inhibitors of complement system.

Treatment of disease also aims at the control of inflammation, which may be achieved successfully by interfering with the synthesis, release or action of the inflammatory mediators. Many drugs available today interfere with the synthesis, release or action of some receptor-type mediators such as histamine or prostaglandins. But these drugs, although of great benefit to the patient, modulate symptoms, apparently without affecting the ultimate progression of the disease. Furthermore, all these drugs have side effects, which frequently limit their usefulness.
The future in anti-inflammatory therapy may lie in the discovery of:

a) agents capable of minimizing the side effects of presently existing drugs;
b) development of similar anti-inflammatory agents but devoid of side effects;
c) agents which can block the evolution of chronic inflammatory diseases either by blocking the effects of the trauma or by acting on some step of the inflammatory reaction which is responsible for irreversible lesions, such as the release or action of the enzymes responsible for the deterioration of tissue structure (Vane and Ferreira, 1978).

Although inflammation is the unifying factor among several diseases, the treatment approach required for each type of inflammatory disease may be unique. Each inflammatory disease has distinct therapeutic needs that are not adequately served by current prevention and treatment strategies. Many of the therapies available today are palliative rather than curative, directed to the symptoms rather than to the underlying causes of inflammation.

Most of the over-the-counter and prescribed anti-inflammatory drugs temporarily mask the symptoms of inflammation, not treat its underlying causes. Worse, the side effects of these drugs can often be extraordinarily dangerous, causing weight gain, severe stomach pain, bone deformities, and heart failure. No substantial progress has been made in achieving a permanent cure. The search of screening and development of drugs for anti-inflammatory activity is an unending problem. There is much hope of finding active antirheumatic compounds from indigenous plants as these are still used in therapeutics despite the progress made in conventional chemistry for producing effective drugs (Farnsworth and Bingel, 1977).

Inflammatory diseases including different types of chronic rheumatic diseases are the major cause of morbidity all over the country. The understanding of pathophysiology of inflammation has advanced so much in the recent years and new parameters are suggested for its diagnosis and evaluation. Many chemical compounds (including Non-steroidal anti-inflammatory drugs – NSAIDs) have
been introduced as anti-inflammatory drugs. While number of them produce
dramatic symptomatic relief in rheumatic diseases but could not prevent the
progress of disease process and many of them shared gastro intestinal irritations
and gastric ulcer problems – the most serious side effect. Therefore, there is an
ever increasing need for efficacious, economic and safer (free from serious side
effect i.e. gastric ulcer) anti-inflammatory agent.

Screening of Indian Medicinal Plants for anti-inflammatory and related activities
has been attempted by various researchers. Literature survey reveals that the
species of 96 genera belonging to 56 families contain anti-inflammatory agents
(Handa et al., 1991). There is a general revival of interest in alternative systems of
medicine partly because of inadequacies of the modern westernized system of
medicine and their hazards includes the adverse reactions and the costs (Kukarni,

1.5 *Lawsonia inermis* Linn.

*Lawsonia inermis* Linn. commonly known as ‘Henna’ is found throughout India.
The ancient and modern literatures reveal the usefulness of the plant in various
diseases. Literature on various uses of Lawsonia, in traditional system of
medicine, is reported to be having following *Pharmacological* actions; abortifacient, analgesic, anti-allergic, anti-microbial, anti-fungal, anti-inflammatory,
anti-oxidant, aphrodisiac, astringent, blood purifier, brain tonic, desiccation of
ulcers, diuretic, hepatoprotective and nootropic (Nadkarni, 1976; Ali; 1996).

**Unani medicinal uses** - Over 27 uses are reported in the literature (Nadkarni,
1976).

**Use in Western herbal** - Lawsonia is official in British Pharmacopoeia 1962. It is
used in certain allergic, infective and inflammatory conditions (Khare, 2004).

**Marketed preparations** - Over 20 Unani, 4 Ayurvedic, and several Cosmetic
marketed preparations containing Lawsonia are available in market (Ali, 1996).
Active principles and pharmacology - Different parts of the plant, in the form of extract and isolated oil, have been studied. But there are not many detailed scientific studies reported on the anti-inflammatory actions of the different extracts of the plant leaves.

The herb contains coumarins, napthaquinones (including lawsone), flavonoids, sterols, saponins and tannins. Lawsone (2-hydroxy-1,4-napthaquinone), xanthones, laxanthones I, II, III, beta-ionone of the essential oil impart specific medicinal properties to the herb (Ali, 1996).

Lawsonia has been reported to possess severe hemotoxicity, evident in the form of severe anaemia. It also has nephrotoxic effects. Literature also reports inflammatory disorders to be associated with anaemia (Kandil et al., 1996; Hazra, 2002).

1.6 Current investigation

The current investigation has therefore been carried out on the extracts of *Lawsonia inermis* and the same includes anti-inflammatory action, general behaviour and toxicology studies. In first phase, anti-inflammatory, analgesic activities of the Extracts were studied along with some biochemical parameters related to anti-inflammatory actions, specially \( \gamma \)-GTP in serum. The effect of oral pretreatment of the extracts were studied on acute inflammation induced by different phologistic agents and compare with respective standard drugs, which could possibly guide the probable mechanism of anti-inflammatory action of *Lawsonia inermis*. In the second phase its Toxicity especially hemolytic effect, ulcerogenic and hepatotoxic effects were investigated. The general behavioral effects were observed. In the last phase, the physicochemical characteristics were determined. Finally, attempts were made to outline the possible actions that contribute to its anti-inflammatory activity and compare it with that of the existing standard anti-inflammatory agent. The cause and pathogenesis of many of the chronic diseases affecting man remain much of a mystery. It has been necessary
therefore to model such conditions in experimental animals to gain insight into their nature and to search for effective therapies.

Carrageenin induced edema of the rat foot is widely used as a working model of inflammation in the search for new anti-inflammatory agents. Apart from other phlogistic agents to induce hind paw edema, carrageenin method achieved popularity because of its simplicity and economic feasibility.

It has been shown that different models of paw edema in rats showed varied responses to various anti-inflammatory drugs, each drug showing a different profile of activity against different type of edema. However, a fairly good correlation was observed between the responses of carrageenin edema to various anti-inflammatory drugs. Analgesic effect of the extracts was evaluated.

Toxic effects of therapeutic dose of the extracts were observed on; blood, gastric ulcer, kidney and liver.

Phytochemical standardization of the extracts of leaves was carried out.